MADHA
Expertise | Empathy | Excellence
ENGINEERING COLLEGE

DEPARTMENT OF BIOTECHNOLOGY

BT3352-MICROBIOLOGY

LECTURE NOTES

REGULATION 2021

II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
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UNIT – I

PART – A

1. Write any 2 differences between prokaryotic and Eukaryotic cells? Give one example in each?

S.No	Parameter	Prokaryotic	Eukaryotic
1	Nucleus	No nuclear membrane bound the nuclear material.	Nuclear membrane bound the nuclear material
2	Chromosomes	One circular chromosome present. No Histone proteins present in chromosomes.	More than one chromosomes present. Histone proteins present in chromosomes.
	Example	Bacteria	Algae, Fungi, Protozoa, plants & animals.

2. Write the contribution of Antony Van Leeuwenhoek?

Antony van Leeuwenhoek invented the simple microscope by grinding the lenses and his microscope has a single lens system. He observed the microorganisms in his microscope from stored rain water and spoiled material. He called the invisible protozoa and microbes as very little "Animalcules".

Later he made several microscopes with magnification of 50X to 300 X.

3. What is the theory of spontaneous generation?

Aristotle (384-322 B.C) reported that animals might be originated spontaneously from the soil, plants or other unlike animals. The creatures originated spontaneously include microbes, small reptiles and flies. This theory of invention of living things is called as spontaneous theory of generation.

4. What is fermentation? Give suitable example?

The breakdown or decomposition of complex organic substances into simple molecules or substances by the action of micro organisms is explained as fermentation. The fermentation was first reported by Louis Pasteur from fruits and fruits juices to alcohol.

E.g. Fermentation (or) conversion of sucrose into alcohol by yeast.

5. Write the contribution of Louis Pasteur?

Louis Pasteur was the professor of chemistry studied the process of fermentation. He found the fermentation of fruits and Grains into alcohol by the action of microorganisms is involved is

fermentation. Fermentation converts the complex organic molecules or substances to simple substances or products called as resulting products.

6. What is meant by pasteurization?

Louis Pasteur adopted the method of heat sterilization of fermenting product called pasteurization. Pasteurization is a type sterilization method, which was established by Louis Pasteur. This is applicable to milk and milk products, fruit juices and certain liquid beverages. In this process, the liquids are heated at 62.8°C (145°C) and held for 30 minutes period or 71.7°C (161°F) for 15 seconds.

7. What is Germ theory of diseases?

The Bacteria and other microorganism were able to cause diseases on animals and man. The organism is transmitted, from diseased organism to healthy organism and spreading of disease occur in healthy organisms. The study of above concept was explained as Germ theory of diseases.

8. Write Koch's postulates?

The series of guidelines to identify the causative agent of an infectious disease is called Koch's postulate. These are,

1) Specific organism can always be found in association with a given diseases.
2) The organism can be isolated and grown in pure culture in the laboratory.
3) The pure culture will produce the disease when inoculated into a susceptible animal.
4) It is possible to recover the organism in pure culture from the experimentally infected animals.

9. Define pure culture?

In order to study the characteristics features of a particular species, it is necessary to separate one species from all other species. Laboratory procedures have been developed that make it possible to isolate microorganisms representing each species and to grow (cultivate) each of the species separately. A growth of mass of cells of the same species in a laboratory vessel (such as test tube) is called a pure culture. Joseph Lister (1878) first obtained pure culture.

10. Write the contribution of Joseph Lister?

Joseph Lister (1878) obtained pure cultures of many microbes and adopted a pure culture technique. He made a serial dilution technique for isolation of single kind of organism from

sample mixture. The isolated single kind of bacterium from milk and named as ***Bacterium lactis.*** He further isolated single kind of bacterium from various patient samples and injected parts.

11. Write short notes on Vaccines?

The substances called antibodies produced by the attenuated cultures (having decreased virulence) in host stimulate & protect against disease causing (or) virulence organisms. These organisms (or) attenuated cultures called **vaccines** and the administering the vaccines to biological organisms are called as **Vaccination.**

e.g., Hepatitis B vaccine.

12. Write the contribution of Edward Jenner?

Edward Jenner is the medical microbiologist who first worked on vaccines and vaccination. Edward Jenner applied attenuated cow pox virus ion 1798 to immunize people against small pox. He further worked on prevention of anthrax disease by vaccination. The organism causedis *Baccills anthracis.*

13. Name any 2 microorganisms and it diseases on human?

1. Bacillus anthracis – Anthrax disease
2. Salmonella typhi - Typhoid fever
3. Plasmodium sp. - Malaria
4. Mycobacterium *tuberculi* – Tuberculosis
5. Mycobacterium leprae - Leprase

14. Write any 4 major characteristics of microorganism, which are useful for classification?

1. Morphological characteristics
2. Chemical composition (or) characteristics
3. Cultural characteristics
4. Metabolic characteristics.

15. Define metabolic characteristics of microorganisms?

The life process as growth and metabolism of microbial cell with a complex integrated series of chemical reactions collectively referred to as metabolism.

E.g. Synthesis of energy, cell components etc. through series of chemical reaction. As the reactions involved are oxidation, reduction etc. by using protein such as enzymes.

16. Define strains?

The individual species construct a taxa called **strains**. It is made up of all the descendents of a pure culture. It is usually a succession of cultures derived from an initial colony. Each strain has a specific history and designation.

17. What is mean by type strain?

The type strain is designated to be the permanent reference specimen for the species. It is the strain to which all other strains must be compared to see it they resemble it closely enough to belong to the species. So, the type strains are important and special attention to be given to their maintenance and preservation.

18. Write about Binomial Nomenclature?

It is the rule of naming the individual species of microorganism. The name must be contain Latin (or) Latinized two words must follows certain rules of Latin grammar. The first word of binomial in the genus name and it is always capitalized. The second word is the specific epithet (species) and it is not capitalized. Both the genus and species name should be italics (or) underlined.

19. What is neo-type strain?

The neo-type strain is equal to the type strain. When the original specimen of type strain preserved is lost due to fire or natural calamities, then the closely similar strain are preserved in the place of type strain. The later strain is called **neo-type strain**.

20. What are different microscopes used in examination of microorganism?

1. Light microscope of
    i) Bright field microscope
    ii) Dark field microscope
    iii) Fluorescence microscope
    iv) Phase contrast microscope

2. Electron microscopy
    (i) Scanning Electron microscope
    (ii) Transmission electron microscope.

21. Define resoling power? (RP)

The resolving power (RP) is the ability to distinguish two adjacent points as distinct and separate. The RP of the microscope is a function of the wavelength of light used and the numerical aperture (NA) of the lens system. The greater the RP of a microscope, more detail we can see in specimen.

22. What is numerical aperture (NA)?

The combined measurement of lens size and use of immersion oil is called Numerical Aperture_(NA). It is a mathematical constant that describes the relative efficiency of lens in bending light rays.

$NA = n \sin \theta$
I.e. half operative angle.

The magnitude of this angle is expressed as a sine value. The sine value of the half operative angle in multiplied by the refractive index of the medium filling the space between the front lens and the cover slip glass gives the numerical aperture.

23. Write the application of Dark field microscope?

In this microscope, the dark background is produced and the objects are brilliantly illuminated. This is done by with special kind of condenser lens. This is applicable for examination of unstained microorganisms suspended in fluid – wet mount and hanging drop cultures.

24. Write the principle involved in fluorescent microscope?

Fluorescent microscope depends on a specimen who absorbs light of short wave length and gives off (or) emits the higher wave length light and glowing brightly against dark background. This microscope his special attachment with barrier filters which allow green light to pass through and reach eye. Other light rays will be filtered through special filter. Flourescent dye chemicals also used in this application. The specimen will appear in furiousence light.

25. Explain phase contrast microscopy?

It is useful in studying living unstained cells. It has phase contrast objective and a phase contrast condenser.

In principle, this technique is based on the fact that light passing through one material and into another material of a slightly different refractive index and / or thickness will undergo a change in phase. These differences in phase (or) wavelength irregularities are translated into variations in brightness of the structures and hence are detectable by the eye.

26. Give any 4 differences between light microscope and Electron microscope?

S.No.	Light microscope	Electron microscope
1.	Light source is lamp. Tungston (or) Tutorium	Electron gun is used as light source.
2	Optical lens are used as a condenser & objectives lens	Magnetic coils are used as condenser and objective lens
3	Live specimen can be observed.	Only Dead specimens can be observed.
4	Images are seen through naked eyes.	Images are captured through video camera.
5	Less expensive	More experiment.
6	Sample preparation is easier	Sample preparation are more cumbersome.

27. What is mean by Negative staining?

An electron dense material such as phosphotungstic acid can be used as a stain to outline the object. The electron-opaque phosphotungstate does not penetrate structures but forms thick deposits in crevices find details of object such as viruses (or) bacterial flagella can be seen by this technique.

Other stain used in this type is Nigrosin and india ink.

28. Write the difference between simple and differential staining?

In simple staining, only one type of stain (dye) is used for sample preparation. This is uncomplicated procedure.

In differential staining method, two different stains (dyes) are used as the primary dye and counter stain dye to distinguish between cells type (or) parts.

29. Define Gram staining?

The name of the stain after the scientist Christian Gram, who developed the procedure for universal technique for bacteria. In which the cells differentiated into categories based up on the color reaction. The Gram positive, cells strain purple, and gram negative which stain pink.

30. Write short notes on acid fast staining?

This strain used to differentiate the acid fast bacteria from non- acid fast bacteria. This stain mainly developed to detect Mycobacterium tuberculosis in specimens. This bacterial outer wall

holds fast to the dye (control fuchsine) even when washed with a solution containing acid (or) acid alcohol. This is used in medically important leprosy bacteria & for Nocardia an alert of using skin infection.

31. Explain Endospore staining?

It is similar to the acid fast strain method, but the dye is forced by heat into spores or endospores. Thin stain is designed to distinguish between spores and the cells that they come from. It is significance in medical microbes as gram positive, spore-forming members of the genus Bacillus.

32. Explain the method of capsular staining?

Capsule staining is a method to observe a microbial capsule an unstructured protective layer covering the cells of some bacteria. The India ink is used for this method, because the capsule not stained with any stains so, it is negatively stained. India ink give stained on the crevice of capsule and not absorbed.

33. Define Protista? Give 2 examples for higher protista?

Hackel (1886) suggested the classification of lower organisms i.e microorganisms as 5 kingdom protista which include those unicellular microorganisms that typically neither plants or animals. Protista include bacteria, algae, fungi and protozoa. Two examples for higher protests are Algae & fungi .

34. What are the limits of resolution in light microscopy?

The limits of resolution in LM are;

1. **Resolving power.** (RP) which is the ability to distinguish two adjacent points as distinct and separate. The RP of the microscope is a function of the wavelength of light used and the numerical aperture (NA) of the lens system.

2. **Numerical Aperture (NA):** The angle θ subtended by the optical axis and the outermost rays still covered by the objective is the measure of the aperture of the objective. It is half aperture angle. The sine value of the half aperture angle multiplied by the refractive index 'n' of the medium filling the space between the front lens and the cover slip gives the numerical aperture (NA): $NA=n \sin \theta$.

35. Describe the difference between prokaryotes and Eukaryotes with respect to I) Chemical compositionand ii) Cytoplasm

Prokaryotes	Eukaryotes
(i) Peptidoglycon (murein (or) mucopeptide) as a chemical component.	Peptidoglycon is absent. Chitin, cellulose are present
(ii) Cytoplasmic streaming is absent. Cytoplasmic gas vacuoles can be present	Cytoplasmic streaming is present.
Endoplasmic Reticulum Absent.	Eendoplasmic Reticulum present
Cell organelles absent.	Cell organelles present.

36. Describe Whitaker's 5 kingdom classification?

R.H.Whitaker (1969) proposed five kingdom group of classification, which is evolved based on cellular organization of organism.

The five kingdom are;

Kingdom 1. Monera (Bacteria)
Kingdom 2 Protista (Protozoa)
Kingdom 3 Fungi (Yeast and mold)
Kingdom 4 Plantae (Plants)
Kingdom 5 Animalea (Animals)

37. Compare the microorganisms as seen by dark field microscope and a phase contrast microscope?

1. **Dark field microscope:** The microorganism (or) microbial cell will appear bright on the dark background. It is valuable for examination of unstained microorganism, wet mount & hanging drop cutters.

2. **Light microscopes:** It is also useful for studying living unstained cells and is widely applied & theoretically in microbiological studies. The special optical system distinguishes unstained structures with in a cell, which differ only slightly in their refractory indices (or) thicknesses.

38. Is it possible to convert a bright field microscope to dark field microscope? If so explain?

Yes. It is possible to convert bright field microscope to dark field microscope.
This is accomplished by equipping the light microscope with a special kind of condenser that transmits a hollow cone of light from the source of illumination.

39. List out the differences between the TEM & SEM?

TEM	SEM
1. Useful in study of detailed structure of cells.	Useful for study of three-dimensional structures.
2. The electrons will transmit through the specimen.	The electrons bombards on the surface of the specimen & scanned over the surface.
3. The finest details of structures can be seen.	3-D structures can be studied.
4. higher magnification (4,00,000 x)	Lower magnification (100,000 x)

40. Describe Auto-radiography?

It is a cytochemical method in which the location of a particular chemical constituent in a cell or specimen is determined by observing the site at which radioactive material becomes positioned. In practice, the specimen prepared for microscopic examination is covered with a layer of photographic emulsion and stored for a period of time. The ionizing radiation emitted during the decay of radio active substance produces latent images in the emulsion. The developed image is seen as grains of silver in the preparation.

PART – B

1. Brief the history of microbiology introduction:

In the development of microbiology, the outstanding names are often of those who convinced the world who developed a technique, a tool on a concept that was generally adopted, or who explained their findings so clearly or dramatically that the science grew and prospered.

Antony van Leeuwenhoek's lucid reports on the ubiquity of microbes and the discovery of microscope are made the foundations. Based on these reports, Louis Pasteur 200 years later to discover the involvement of these creatures (microbes) in fermenting reactions and allowed Robert Koch, Theo bald smith, Pasteur and many others to discover the association of microbes with disease. Koch is remembered for his isolation of the bacteria that cause anthrax and tuberculosis and for the rigid criteria he demanded before a specific bacterium he hold as the cause of a disease.

Paul Erlich's discovery of a chemical compound that would destroy the syphilis spirochete in the human body. For this, the Nobel prize was awarded in 1908 with Elie Metchnikoff.

Though, the relatively short duration, the history of microbiology is filled with thrilling achievements. We have won many battles with micro- organisms and have learned not only to make them work for us but also to control some of those that work against us.

Anton van Leeuwenhoek and Microscope

Though the reports were available in earlier days as the minute creatures are occur on decaying bodies, meat, milk and diarrheal secretions, which are invisible to our naked eye. There is no accurate proof for these. Anton van Leeuwenhoek (1632 to 1723) was the first person that reports his observation with accurate description and drawings with help of simple and primitive microscope, which were made by him. Anton van Leeuwenhoek made more than 250 microscopes consisting of home- ground lens mounted in brass and silver, the most powerful of which would magnify about 200 to 300 times. Leeuwenhoek described his observation as "very little Animalcules "which we recognize as free-living protozoa bacteria & fungi and sent to Royal society of London. This observation of findings laid foundation for invention of modern microscope and study of micro organizations.

Spontaneous Generation versus Biogenesis

As for human beings were concerned, the Greek explanation that the goddess age was able top creates people from stones and other inanimate objects. Aristotle (384-322 BC) taught that animals might originate spontaneously from the soil, plants or other unlike animals.
Virgil (70-19 BC) gave direction for the artificial propagation of bees. In 1665 Redi did an experiment with set of covered & uncovered meat. So, Redi disproved the spontaneous theory. Lazzaro spallanzani (1729-1799) boiled beet broth for an hour and then he sealed the franks. There is no microbes appeared.

Ten the debate was over after the Pasteur's experiment with Goose neck flaks and sterile beef broth did not allow to form microbes spontaneously.

Finally Tyndall (1820-1893) conducted experiments with designed Box with high provision. The air passed through the height showed dust particles, which gave microorganism & the absence of dust particles in the air, not gave microorganism then the spontaneous theory was ignored & Abiogenesis theory was put full top.

Fermentation theory

Louis partner (1822-1895) found that microbes brought the fermentation of fruits & Grains resulting into alcohol. The observed many types of microorganism involve in fermentation as good (or) beneficial organism and bad (on) Harmful microorganism involve in fermentation, which give ununiform product formation.

Pasteur removed the undesirable or harmful micro organization by heating the liquid (or) ferment at 62.8^{o}C (145^{o}F) for 60 min, the phenomena was called as pasteurization.

Pasteur conducted fermentation trials in many kinds of microorganisms and produced alcohols and organic acids, which are economically, value.

The germ theory of diseases

Fracastera of Verona suggested that the diseases might cause by invisible organism, which are transported from one person is another. In 1762, von Planciz stated that different grimes were responsible for different diseases Oliver Vendell Holness (1809-1894) insisted that the disease of child birth was contagious and that it was caused by a germ carried form one mother it another. Joseph Lister (1890) known the importance of antisepsis to control the injection and disease severity in human.

After fermentation aspect, Louis pastuer invented the silk worm disease by microbe Later Pasteur tackled the problem of Anthrox in cattle human beings.

Later Pasteur tackled the problem of Anthrox in cattle and human beings.

Mean while **Robert Koach** (1843-1910) isolated the anthrax bacteria and injected in health animals, which were observed, with symptom of anthrax. This was later studied as Koch's postulates in which the infection microorganism was isolated in pure culture form and injected in healthy animals and produced disease symptoms.

Lab theories & pure cultures

Later based on the invention of Robert Koch, Laboratory practices were developed for isolation, culturing of microorganisms and preservation of micro trial cultures in the laboratory for further studies. The culture of individual species in called **pure cultures** Joseph Lister first isolated pure culture of Bacteria called Bacterium lactis from milk.

The importance of pure cultures technique and other laboratory practices were recognized as over to scientist for further contribution in for field like marine microbiology, rumen microbiology, microbiology of intestinal tract etc.

Development various and immunity

Edward Jenner in 1798 immunized people against small pox virus by use of cowpox virus. Pasteur studied the Anthrax bacterium and developed vaccines from attenuated culture, which were explored in the laboratory. Then the term vaccines, was coined form attenuated (or) Non-virulence culture of microorganisms.

Then may various were invented based on the findings of Louis Pasteur and Edward Jenner to cure many diseases. Modern vaccines were developed in the modern days as DNA vaccines, Subunit vaccines, and cocktail vaccines.

Modern day microbiology

Based on the above developments in the field of microbiology the following access were emerged to study extensively about the application of microbes. There are,

Medical microbiology
Agricultural microbiology
Industrial microbiology
Food microbiology
Marine microbiology
Animal microbiology

And the modern microbiology was related to molecular biology.

2. Write an essay on Nomenclature and classification of microorganisms?

Introduction:

Classification is a means of bringing order to the bewildering variety of organism in nature. Once we learn the characteristics of an organism to discover similarities and differences. The human mind tends to arrange similar thing together in groups and they distinguish these groups form one another.

Once the characteristics of microorganisms have been determined and appropriately catalogued, the process of classification begin.

About Nomenclature, each species of microorganism has only one efficiently accepted Name, by international agreement. The system provides precise communication, i.e., naming of the organism. The scientific naming of any organism called binomial Nomenclature.

Classification: In microbiology, taxa are initially constructed from strains. A strain in made up of all the descendants of a pure culture. It is usually a succession of culture derived form an initial colony, each strain has a specific history and designation.

Taxonomic group:

Strain is a individual species of microorganism which first isolated from particular source and preserved in the collection centre.

The basic taxonomic group (taxon) is the species. i.e. a collection of strain having similar characteristics. Bacterial Species consists of a special strain called the type strain. Type strain is a original species (or) strain of organism permanently preserved in collection centre and consider as reference specimen or compare with new organism.

Bacterial species is composed of similar strains, a bacteria, genus is composed of collection of similar species. One of the species is designated the type species and this serves as the permanent example of the genus.

Taxonomic groups of higher rank has genus are listed below, and the same considerations about subjectivity apply here as well;

Species – A group of individual strains
Genus – A group of similar species
Family – A group of similar genera
Order – A group of similar families
Class – A group of similar orders
Division – A group of similar classes
Kingdom – A group of similar divisions

Goals of classification

Taxonomists strive to make classifications that have the following two qualities;

1. **Stability.** Classification that are subject to frequent radical changes lead to confusion. Every attempt should be made to devise classifications that need only minor changes as new information becomes available.

2. **Predictability:** By knowing the characteristics of one member of a taxonomic group, it should be possible to assume that the other members of the same group probably have similar characteristics. If this cannot be done, the classification has little value.

General method of classifying Bacteria

There are 3 methods are used for arranging bacteria into taxa:

1. The intuitive method:

A microbiologist who is thoroughly familiar with the properties of the organisms he or she has been studying for several years may decide that the organisms represent one or more species or genera. The trouble with this method is that the characteristics of an organism that seem

important to one person may not be so important to another, and different taxonomists may arrive at very different groupings. However, some classification schemes based on the intuitive method have proved to be quite useful.

2. Numerical Taxonomy:

In an effort to be more objective about grouping bacteria, a scientist may determine many characteristics
(Usually 100 to 200) for each strain studied, giving each characteristic equal weight then using a computer he or she calculates the % similarity (%s) of each stain to every other strain. For any two strains, this is;

$$\%S = \frac{NS}{NS + ND}$$

Where, NS is the number of characteristics that are the same (positive or Negative) for the two species, and ND is the number of characteristics that are different those strains having a high %S to each other are placed into groups; those groups having a high % S to each other are in turn placed into larger groups, and so on, the degree of similarity needed to rank a group as a species, genus or other taxon is a matter of judgment on the part of the taxonomist. This method of classification has great practical usefulness as well as being relatively unbiased in its approach; it also yields classifications that have a high degree of stability and predictability.

3. Genetic Relatedness:

The most reliable method of classification is based on the degree of genetic relatedness between organisms. This method is mot objective of all and is based on the most fundamental aspect of organisms, their hereditary material (DMA). The molecular biology provide techniques by which the DNA of one organism could be compared with that of other organism could be compared with that of other organisms. The molecular %G+C values are taken for crude comparison. The closely, related organism will have similar mol% G+C values however, different organism also may have similar mol% G+C values. Therefore, the DNA of organism were compared for components of nucleotides for its sequence. The sequence is most fundamental characteristic of an organism.

The basic principles can be described briefly as follows:

1. DNA homology experiments:

When the DNA molecules of closely related organism were heated to convert as single stands and cool them, the heteroduplexes will form otherwise the organism were assumed to be unrelated.

(ii) Ribosomal RNA homology experiments and ribosomal RNA oligonucleotide cataloging.

Ribosomes, the small granular- appearing structures with in the cell which manufacture proteins, are composed of proteins and RNA. The ribosomal RNA (tRNA) is coded for by only a small fraction of the DNA molecules, the rRNA storms, In all bacterial so for studied, the nucleotide sequence of these tRNA genus has been found to be highly conserved.

RNA homology experiments and RNA oligonucleotide cataloging are two modern methods used to determine the degree of similarity better the tRNA cistrons of difficult organisms. The techniques are complex and are being used by only c few laboratories.

Nomenclature:

Each species of microorganism has only one officially accepted name, by international agreement. This system provides for precise communication. If an organism were to be called Escherichia coli in one country and Coprobacterium intestinale in another country. It would be difficult to know that the same organism was being studied. One rule in bacteriological nomenclature is that a name must be written as a later (or) Latinized binomial (two words) and must be follow certain rules of Latin grammar.

The international code of nomenclature of Bacteria was developed with reference to the much earlier established International codes of Zoological and Botanical Nomenclature. All of these codes incorporate certain common principles as listed below

1. Each distinct kind of organism is designated as species.
2. The species is designated by a Latin binomial to provide a characteristic international label.
3. Regulation is established for the application of names.
4. A law of priority ensures the use of the oldest available legitimate name.
5. Designation of categories is required for classification of organisms.
6. Requirements are given for effective publication of new specific names, as well as guidance coining new names.

3. Write an Essay on various characteristics of microorganism used in classification?

Major characteristics

1. Morphological characteristics:

Cell shape, size, structure cell arrangements, occurrence of special structures and development forms; staining relations, and mobility and capsular arrangement.

2. Chemical composition:-

The various chemical structures of cells.

3. Cultural characteristics

Nutritional requirement and physical condition required for growth, and the manner in which growth occurs.

4. Metabolic characteristics

The way in which cells obtain and use their energy carry out chemical reactions and regulate these reactions.

5. Antigenic characteristics

Special large chemical components (antigens) of the cell, distinctive for certain kinds of microorganisms.

6. Genetic characteristics

Characteristics of the hereditary material of the cell (CDNA) and occurrence and function of other kinds of DNA that may be present such as plasmids.

7. Pathogenecity

The ability of cause disease in various plants or animals or even other microorganism.

8. Ecological Characteristics

Habitat and the distribution of the organism in nature and the interactions between and among species in natural environments.

I . Morphological characteristics

Determination of morphological features requires studying individual cells of a pare culture. Microorganism are very small and their size is usually expressed in micrometers (μm). Routine examination of microorganisms also requires high power microscope, a magnification about 1000 diameters.

The use of EM provides magnification of thousands of diameters and makes it possible to see fine details of cell structure.

II . Chemical characteristics

Microbial cells commit of wide variety of organic compounds. Each kind of microorganism is found to have a characteristic chemical composition. For example, the occurrences of lipopolysachariде in cell walls is characteristics Gram – ve bacteria but not gram +ve bacteria in other hand, many Gram +ve bacteria have cell walls that contain techioic acids, compounds not present in Gram +ve bacteria. Fungal and algal cell walls are very different in composition from those of Bacteria. A major distinction among viruses is made on the bares of the kind of nucleic acid they posses, namely ribonucleic acid (RNA) or deoxyribonucleic acid (DNA),

III. Cultural characteristics

Each kind of microorganism has specific growth requirements. Many microorganism can be grown in or on a **culture medium.** Some microorganisms can grow in a medium containing only **in organic compounds,** whereas others require a medium containing **organic compounds** (amino acids, sugars, purines, pyrimidines, enzymes (or) co – enzymes), Some require **Complex substances** (peptone, yeast autolysate, blood cells (or) blood serum) and some can not grow on artificial media which require living cells (or) host.

In addition to nutrients requirements each kind of organism also requires specific physical conditions for growth. For example, some bacteria grow best at high temperatures and can not grow below $40^{o}C$; others grow

IV. Metabolic characteristics:

The life processes of the microbial cell are a complex integrated series of chemical reactions collectively referred to as metabolism. The variety of these reactions affords many opportunities to characterize and differentiate various groups of microorganisms. For instance, some organism may obtain energy by absorbing light, others by oxidizing various organic or in organic compounds and others by redistributing the atoms within certain molecules so that the molecules become less stable. Organisms also differ in the ways in which they synthesize their cell components during growth. The various chemical reactions of an organism are catalyzed by proteins called enzymes, and the complement of enzymes possessed by one kind of organism, as well as the ways in which those enzymes are regulated, can differ significantly from that of other organisms.

V. Antigenic characteristics

Certain chemical compounds of microbial cells are called antigens. Antigenic characterization of a microorganism has great practical importance. It microbial cells enter the animal body, the animal responds to their antigens by forming specific blood serum best in the cold and can not grow above 20°C; still others, such as bacteria pathogenic to humans, require a temperature close to that of the human body (i.e.37°C).

The gaseous atmosphere required for growth is also important; for instance, some bacteria require **oxygen** is lethal to others and they can grow only in its absence. **Light** may be another important physical condition; certain bacteria such as cyanobacteria, require light as source of energy, whereas others may be indifferent to light or may even find it deleterious to their growth.

Each kind of microorganism grows is a characteristic manner. For example, growth in a liquid medium may be abundant or sparse; it may be evenly dispersed throughout the medium. On solid media, microbes grow as colonics distinct, compact of maser of cells that are macroscopically visible the DNA molecule is made up of base pairs; guanine – cytosine and adenine –thymine. Of the total number of nucleotide bases present in the DNA, that percentage represented by guanine plus cytosine is termed the moles % G+C value (or briefly more mole % G + C). values for various organisms range from 23 to 75. some examples are:

Spices	Moles % G+C content DNA.
Azospirillum brasilens	70-71
A. spoferum	69-70
Pseudomonas aeruginosa	67
P. cichoni	59

2) Sequence of nucleotide base in the DNA:

This sequence is unique for each kind of organism and is the most fundamental of all the characteristic of an organism; consequently classification.

Proteins called antibodies which find to the antigens. Antibodies are highly specific for the antigens that induce their formation. Because different kinds of microorganisms have different types of antigen, antibodies are widely used as tools for the refried identification of particular kinds of microorganisms.

If me know the identity of one part of the system (antigen (or) antibody) we can identify the other. For example, if we take typhoid bacterium antibody and mix it with a suspension of unknown bacterial cells, and a positive reaction occurs, we can conclude that the cells are those of the typhoid organism. If no reactions occurs, then these bacterial cells are some species other than the typhoid bacterium.

VI. Genetic characteristics

The double – standard chromosomal DNA of each kind of microorganism has certain features that are constant and characteristic for that organism and useful for its classification.

1. DNA base composition:

It is important to note that

Ecological characteristics:

The habitat of a microorganism is important in characterizing that organism. For example, microorganisms normally found in marine environments generally differ from those is freshwater environments. The microbial population of the oral cavity differs from that of the intestinal tract. Some kinds of microorganisms are widely distributed in nature, but offers maybe restricted to a particular environment. The relation of an organism to its environment is often complex and may involve special characteristics of the organism that are not yet known.

In addition to chromosomal DNA, plasmid DNA may sometimes be present in microbial cells. Plasmids are circular DNA molecules that capable of autonomous replication within bacterial cells, and their presence can confer special characteristics on the cells that contain them, such as the ability to make toxins (toxigenicity), to become resistant to various antibiotics, or to use unusual chemical compounds as nutrients.

Pathagenicity:

The ability to cause disease (or) pathogenicity, of some microorganisms is certainly a dramatic characteristic and it stimulated much of the early work with microorganisms. Although we know that the relatively few species of microorganisms cause disease, certain microorganisms are pathogenic for animals (or) plants and some microorganisms may cause disease in other microorganisms. For example, bacteria known as Bdellovibrios are predatory on other bacteria, and viruses called bacterio- phages can infect and destroy bacterial cells.

4. Write the principles and functions of Light Microscopy?

Introduction:

The microscope is the instrument most characteristic of the microbiology laboratory. The magnification it provides enables us to see microorganisms and their structures otherwise invisible to the naked eye. The magnification attainable by microscopes range from X100 to X40 000 Different kinds of microscopes available and many techniques have been developed to examine the microorganisms.

Microscopes are two categories as,

1. Light microscopy in which optical height is used
2. Electron microscopy in which electron beam is used.

Light microscopy

In light microscopy, the magnification is obtained by a system of optical lens using light waves, includes,

1. Bright – field
2. Dark- field
3. Fluorescence and
4. phase contrast microscope

In a light microscope, there are 2 set of lens are used for viewing cells.

1) **Objective lens:** which forms an image of the specimen with in the body tube of the microscope? The transmitted light enters into the objective lens.

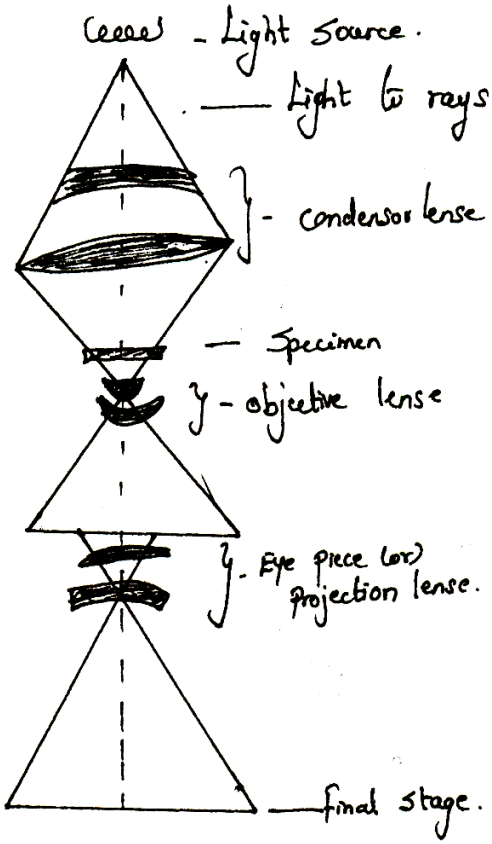
2) **The ocular lens:** (or) eye piece lens which is further magnifies the image with in the body tube, and projects it to the last lens in, the series – the one with in the eye. The lens of the eye forms an image on the retina.

3) **Condensers:** Condensers may be defined as a series of lens for illumination to the object on the stage of microscope by transmitted light.

Magnification: The microscope is used to achieve magnification (enlargement of the image) of the invisible object like microorganisms. Any convex lens (thicker in the centre than at the edge) can magnify the image. By refraction, it bends parallel rays of light from an object so that they meet at a single print.

Magnification can be increased by making the lens more convex or by bringing the object closer to the lens. Magnificent is essential but it is not enough. The two main factors necessary to achieve these goals are contrast and resolution.

Contrast: Contrast refers to a difference in light intensity. Such differences with in the field of view of microscope are essential for us to see an image of the specimen. Contrast is created by light absorption sometimes, the contrast in increased by addition of stains in the microorganism.



Diagramatic scetch showing the function of Light Microscope

Resolution:

Resolution is also another requirement for obtaining a useful image is the ability to distinguish detail within an image. For example, a television set with high resolution has a clear picture; one with poor resolution has fuzzing picture.

Resolving Power:

It is defined as the power of an object able to separate distinctly two adjacent points. The resolving power of a microscope is a function of the wavelength of light used and the numerical aperture (NA) of the lens system. The larger the NA, the greater the resolving power of the objective and finer the detail obtained.

Numerical Aperture (NA)

The angle subtended by the optical axis and the outer must rays still covered by the objective is the measure of aperture of the objective. It is half – aperture angle. The magnitude of this angle is expressed as a sine value.

The sine value of the half –aperture angle multiplied by the refractive index (n) of the medium gives the numerical aperture.

Hence NA=n sine θ

Limit of Resolution:

It is the smallest distance by which two objects can be separated and distinguished as two separate objects. The greater resolution in light microscopy is obtained with the shortest wavelength of visible light and an objective with maximum NA. The relationship define

NA and resolution can be expressed as

$d = \frac{\lambda}{2NA}$ when d=resolution

λ=wavelength of light

NA= Numerical Aperture

Function:

The optical light is absorbed and reflected by condenser lens is regulated and concentrated to specimen (or) object. The light rays are further transmitted by object through objective lens and reach to eye piece (or) ocular lens. The light image of the specimen is further focused for its contrast by ocular lens and final image in obtained through on naked eye. There, the optical light rays are transmitted from the light source and reaches to naked eye through microscopic body tube.

5. Write the principles and function of Electron microscope?

Introduction:

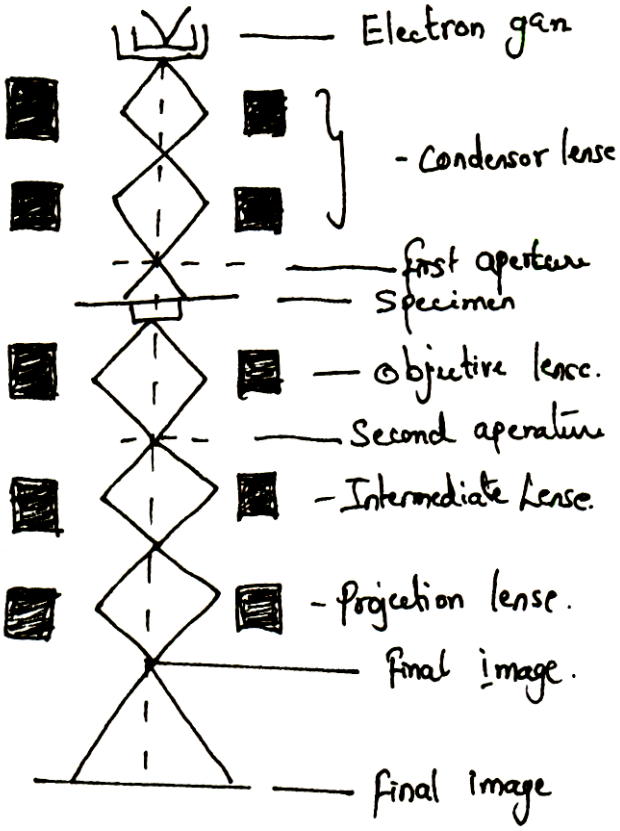
Electron microscope differs marked by and in many respects from the optical microscopic techniques. The electron microscope provides tremendous useful magnification, because of much higher resolution obtainable with the extremely short-wavelength of the electron beam used to magnify the specimen. On electron microscope electron beams are used to produce image.

Electron microscope

The electron microscope employing 60-80kv electrons the wavelength is only 0.05A^{o} (1A^{o} is equal to 1/100,000,000(10^{-8}). It is possible to resolve objects as small as 10A^{o}. the resolving power of the electron microscope is more than 100 times of the light microscopes and it produces useful magnification up to x n 400,000.

Function:

In electron microscopy the specimen to be examined is prepared as an extremely this dry film or small screens. The specimen is introduced into the instrument at a point between the magnetic condenser and the magnetic objective. This point is comparable to the stage of the light microscope. The magnified image on a photographic plate by a camera built into the instrument.



Schematic diagram of working of transmission electron microscope

Numerous techniques are required (or) available for use with electron microscopy which extend its usefulness in characterizing cellular structure and details these are given below:

1. **Shadow casting:** This technique involves depositing an extremely thin layer of metal of an oblique angle on the organism so that the organism produces a shadow on the uncoated slide. The shadowing technique produces a topographical representation of the surface of the specimen.

2. **Negative staining:** An electron dense material such as phosphotungstic acid can be used as a "stain" to outline the object. The electron-opaque phosphotungstate does not penetrate

structures but form thick deposits in crevices. This is useful in studying fine details of specimen.

3. **Ultra thin sectioning:** In order to make observations of intracellular structures, the material for examination must be extremely thin. To provide this, the material is embedded in plastic material and made into ultra thin section that in up to 60mm. For these thin sections, a special stains such as uranium and lanthanum salts and used.

4. **Freeze-Etching:** This is useful to prevent the restoration of specimen during chemical processing. The specimen is sectioned while containing frozen block. Carbon replicas of these exposed surfaces are then prepared for revealing.

Localization of cell constitutes and Localization of enzymes in this sections.

Special technique has been developed to make it possible to locate the chemical constituents and enzymes of the call.

i.e. labeling specimen with Ferritin – labeled antibody.

Auto radio grapy:

It is a cytochemical method in which the location of a particular chemical constituent in a specimen is determined by observing the site at which radioactive material becomes positioned. The cells are first exposed to the radioactive substance to permit its uptake. Un practice, the specimen prepared for microscopic examination in covered with a layer of photo graphic emulsion and stored in the dark for a period of time the ionizing radiation emitted during the decay of the radio active substance produces latent images in the emulsion and after photographic processing, the developed image is seen as grains of silver in the preparation.

Scanning Electron microscope

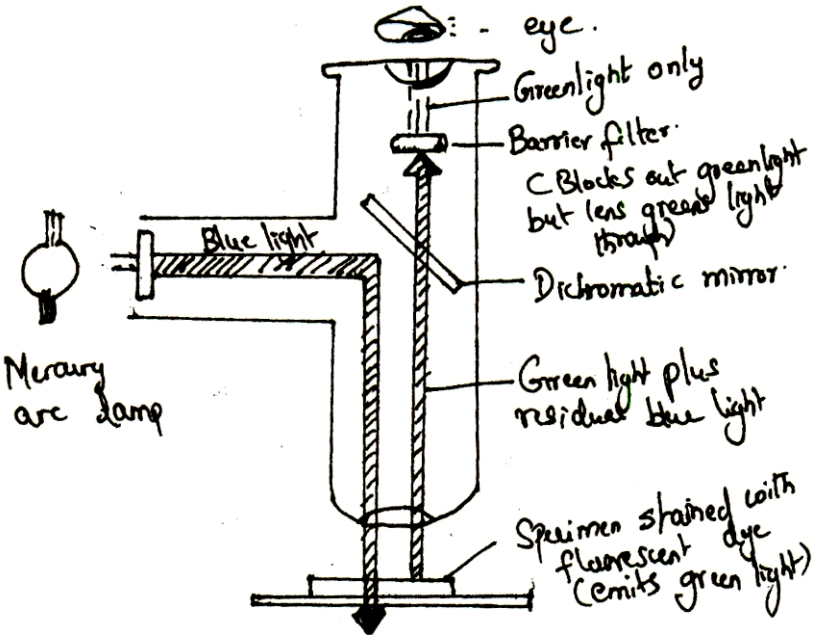
The narrow electron beam is rapidly moves over the surface of the specimen and he shower of secondary electrons and other types of radiation from the specimen surface. The secondary electrons are collected by a detector, which generates electronic signal. Then the signals are scanned in the manner television system to produce image.

6. Describe the fluorescent and phase contrast microscopy?

Fluorescent microscopy

The fluorescent microscopy functions based on the presence of chemical substances in the cell that are absorbs light and fluoresce. After absorbing the light of a particular wavelength and

energy, some substances will then emit light of a longer wavelength and a loses energy content. Such substances are called fluorescent and the phenomenon is termed fluorescence.



Schematic diagram of function of fluorescence microscope

Function: In practical function, microorganisms are stained with a fluorescent dye and then illuminated with blue light; the blue light is absorbed and green light emitted by the dye.

The function of the **Exciter filter** is to remove all but the blue light; the **barrier filter** blocks out blue light and allows green light (or other light emitted by the fluorescing specimen) to pas through and reach the eye. Barrier filters are selected on the basis of the dye used.

Application: It is used to identify specific proteins present in cell based on antibody and antigen techniques.

Phase contrast microscopy:

It is valuable for studying living unstained cells and is widely used in applied and theoretical biological studies. It uses a conventional light microscope fitted with a phase-contrast condenser. This special optical system makes it possible to distinguish unstrained structures with in a cell, which differ only slightly in their refractive indices or thicknesses.

Function:

The phase contrast microscope is based on the principle that rays of light move at different speeds through materials of different refractive index. So, the light ray that pass through the

specimen are **out of phase** (peaks of the waves occur at different times) to varying degrees with the rays that go around the specimen. When the out of phase rays are brought back together, they generate contrast by interference, meaning that peaks of light waves that arrive at a different time cancel each other out (interfere with one another). Those that arrive at or near the same time augment one another.

Application: The major advantage of phase – contrast microscopy in avoidance of the necessity of fixing and staining. So it can be used to study activities and properties of live cells, including cellular movement and internal structures that hence not been distorted. It is used to examine unstained specimens.

7. Write an essay on various staining procedures involve in examination of microorganism?

Stains are dyes that increase contrast by binding selectively to certain cells or to certain parts of them. As a result staining reveals structures in detail and help then to examine specimens in depth.

Stains are basically classified into 2 types as;

1. Simple stains and
2. Differential stains

to examine the structure and morphology of microorganism.

1. Simple strains

The aqueous or alcoholic solution of a single basic dye is called simple staining for example.

1. Methylene blue
2. crystal violet
3. carbol fuschin and
4. safaranin.

Some times a chemical helps to intensify the strain and such chemical is called mordant. The mordant is closely associated with strain and shows its affinity. In case, stain is applied to the fixed smear, then worked off, slide kept for drying and then it is to be examined under the microscope. Such procedure helps is studying the cellular shape and structures of microorganism.

2. Differential Staining

Differential stains are used to distinguish between types of microorganisms. Such procedures usually involve at least three steps as

1. Primary staining
2. De-staining and
3. Counter staining

The best example for differential staining is **Grams staining method.**

1. The **Primary staining** is same as the simple staining. The single dye (or) stain used.

2. **De-staining** in a treatment that removes stains from certain cells, in which the primary status stained in cells may (or) may not be removed which department on type of cells.

3. **Counterstaining** is the application of another dye to reveal the cells or parts off cells that have been destained.

There are 2 differential stains are used frequently in microbiology.

(i) Gram's stain
(ii) Acid fast stain

(i) Gram stain:

This technique has developed by Christian gram in 1884. This method is developed for grouping (or) classifying bacteria into 2 groups as the Gram positive and the Gram negative.

Step 1: The primary stain as Gentian violet (made from crystal violet, alcohol, ammonium oxalate and water) is applied to a fixed specimen of Bacteria. Both Gram positive and Gram negative bacteria becomes purple.

Step 2: Mordant: Iodine is applied, which sets the stain but does not change the general appearance of the cell.

Step 3: Decolonization:

A Decolorizing agent usually ethanol is added. Gram-negative bacteria lose their purple colour. Gram-positive bacteria retain it.

Step 4: Counter stain:

The red dye saffron is added, which turns the decoloured Gram-negative bacteria pink and the Gram-positive bacteria a slightly deeper violet colour.

Observation:

Based on the colour development of cells, the Bacteria is classified into either Gram-positive (violet coloured cells) and Gram-negative (red or) pink coloured cells).

(ii) The Acid-fast stain:

This stain colours only mycobacteria and some actinomycetes. Acids fast stain is usually to identify Mycobacterium tuberculosis and M. leprae. Mycobacteria are acid fast because they resist distaining with acid. This is due to the presence of wax like material in their cell wall.

Step 1: Primary stain;

Carbol fuschin is applied to the fixed specimen on a microscope slide. All cells stain red in colour.

Step 2 Mordant:

The slide is heated to steaming fro five minutes, which drives the stain into the cells,

Step 3: Decolorization:

A sulfuric acid is ethanol is added. This removes the red dye from all cells except the acid fast bacteria.

Step 4: Counter stain:

Methylene blue is added as a counter stain. It colours all decolorized cells and host tissue blue Acid fast bacteria remain red.

III Flagellar stain (the Leifsan flagellar stain)

Other special stains are (i) flagellar stain and (ii) Negative staining.

Flagellar staining renders them visible by adding a material that sticks to them and makes them thicker. Then the thickened flagella stained with a dye. All steps in flagella staining are designed to be gentle because flagella break easily.

Step 1: The suspension of bacteria is fixed chemically, with formalin and spread on the grass slide.

Step 2: It is allowed to air dry without heating .

Step 3: A freshly prepared mixer of tannic acid and rosaniline dye is then added to the slide. The tannic acids thickens the flagella. The rosaniline stain them.

Step 4: The excess stain is washed off by flooding the slide with water.

Step 5: It is again allowed to air dry before being examined under the microscope.

IV Negative Staining:

It is used for staining the capsule around the bacterial cell wall, which is protective to bacteria. This is revealed by Negative staining. In negative staining, the capsule is remain uncolored during staining.

Step1 1: A wet mount off specimen is prepared
Step 2: India ink is added. The carbon particles in the ink cannot penetrate the capsule, so only background is blackened. The capsule and cell wining are revealed ass clear zone.
Step 3: A simple stain may then be added to make the cell itself visible.

8. Write the principles and functions of phase contrast microscopy? Differentiate light with Electron microscope?

Phase contrast microscopy is extremely valuable for studying living cells and is widely used in applied and theoretical biological studies. In this microscope the contrast is achieved because various parts of the specimen absorb different amount of light. In a phase contrast microscope, contrast is generated because various parts of the specimen have different refractive indices. The special optical system available in the phase contrast microscope make possible to distinguish structures within all which differ only slightly in their refractive indices or thicknesses.

The principle that rays of light move at different speeds through material of different refractive index. So that the light rays that pan through the specimen are out of phase to varying degrees with the rays go around the specimen. When the out-of-phase rays are brought back together they generate contrast by interference. Interference means peaks of light waves that arrives at a different time cancel each other our. These differences, or wave front irregularities are translated into variations in brightness of the structures and hence are detectable by the etc.

The major advantage of phase –contrast microscopy is that is avoids the necessity of fixing and staining. So, it can be used to study cellular movement and internal cell structures in their native state.

The differences between Light microscope and electron microscope:

Description of parameter	Light microscope	Electron microscope
1. Magnification	Up to 1000x	up to 400,000 X
2. Cost	In expensive	Expensive
3. Sample preparation	Easy to prepare	Cumbersome & series of methods involve
4. lens system	Optical lens system used	Magnetic lens system used
5. Light source	Lamp is used	Electron gun is used.
6. Focusing of light	Light rays are used	Electron beam is used
7. Specimen used	Live specimen cells can be viewed	Dead cells (or) specimen only can be seen
8. Image	Light image is obtained	Image is through electron rays
9. Image viewing	Naked eye can be used to see image	Image is obtained (~) captured through Television screen & viewed.
10. Handling	Easy to handle by any person much training in not required	Handling is a problem + Trained persons (or) Technicians are required.
11. Structures	Simple morphological structures are able to see	Topographical & Textures are viewed.
12. Size	Small & potable	Very Big. Not potable

UNIT – II

PART – A

1. What are the different shapes of bacteria?

The shape of Bacteria is governed by its rigid cell wall, which determines it shape. These are;

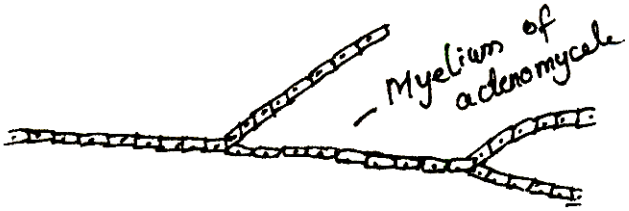
i) Cocci - Cells are in spherical shaped
ii) Bacilli shape - Cells are straight rods
iii) Spirilli shape - Cells are rod helical structure
iii) Pleomorphic - Bacterial cells exhibit a variety of shapes.

2. Write the different arrangements of Cocci bacteria.

1. Diplococci - Cells attached in pairs and spherical shape.
2. Streptococci - Cells attached in chain form and spherical shape.
3. Tetrads - Cells form in group of & cells and spherical shape.
&. Staphylococci - Cells divides and form as irregular bunches of cocci.
5. Sarcinae - Cells divide in tree plans and form as a cuboidal arrangement.

3. Describe the structure of Actinomycetes.

Actinomycetes are the group of Bacteria include the genus streptomyces sp. Actinomyces group of bacteria form long, branched, multinucleate filamentous vegetative form called Hyphae (singular hypha) which collectively called as mycelium. Reproductive structures are called as condiospores formed in a separate branch of mycelium. The spore bearing structure is called sporangium.



4. Write short notes on bacterial chromosome.

Bacterial chromosome appears as single naked highly condensed and coiled form directly exist (or) attach with cytoplasm. It is called as chromatin. Chromatin lacks distinct nuclear membrane. Bacterial chromosome lacks histone proteins.

5. What is Mesosome?

In most of the Gram-positive bacteria, the membrane invaginations in the form of systems of convoluted tubules and besides called mesosomes. These are classified as central mesosomes and peripheral mesosomes based on the existence or extra growth. This helps in photosynthesis to absorb more light and sometimes in help in DNA replication.

6. Describe flagella and its function of Bacteria.

It is hair like, helical appendages that protrude through the cell wall and are responsible for swimming motility. Flagella are occur in rod shaped Bacteria. Bacterial flagella are thinner than Eukaryotes. The location of the flagella varies with different bacterial species.

The main function of the flagella is it helping to Bacteria for locomotion.

7. What are flagellins?

The chemical component (or) protein present in the flagella as the hook and filament is called flagellin. These proteins are consist protein submits called monomors arranged in a helical fashion. These proteins gives strength to the bacterial flagella during locomotion or movement.

8. What are the different types of Bacterial movements?

1. Flagellar movement
2. Swimming motility
3. Gliding motility
4. Bacterial Chemotaxis.
5. Magnetotaxis
6. Phototaxis.

9. What is Chemotaxis?

The movement of bacterial based on the attraction chemical compounds present in the environment called Chemotaxis. Swimming toward chemical is positive chemotaxis and swimming away from the chemical substances is Negative chemotaxis. Chemoreceptors are the receptor molecules present in Bacteria which are responsible for chemotaxis.

10. Write short notes on Magnetosome and Magnetotaxis.

The direction of bacterial cells toward down of the aquatic environments i.e. oxygen deficient areas and more favour on its growth. Magnetosomes are a magnetic response components present in the Bacterial cells.

11. What is Phototaxis?

The response of light by bacteria and influence their movements are called phototaxis. The positive and negative movements of bacteria due to the presence of light in the environment like in chemotaxis. Photosomes are response components present in the bacteria.

12. What are Pili? Write its function.

Pili (Pilus singular) are hollow, non-helical, filamentous appendages that are thinner, shorter and more numerous than flagella.

The function of pili are i) Play a major role in human infection as help in attachment of bacterial cells on respiratory, intestine, genitourinary tract and (ii) some pili called F pili act as a sexual reproduction.

13. Write short notes on bacterial capsule,

Some bacterial cells surrounded by a viscous substance forming a covering layer or envelope around the cell wall is called capsule. In forming the layer of bactera is thick, it is called

as capsule and if it is thin which is called as microcapsule. The function of capsules is i) give protection against temporary drying ii) It may block attachment of bacteriophages and iii) may antiphagocyte.

14. What is the chemical composition of capsules?

Most bacterial capsules composed of polysaccharides. Single type of sugar called homopolysacharides (eg. Streptococcus mutans) and if more than one kinds of sugars present, then it is called as Heteropolysacharides. A few capsules are polypeptides (eg. Bacillus_anthrax)

15. What is the chemical composition of Bacterial cell wall?

The chemical composition of bacteria is mainly with peptidoglycon called murein on insoluble, porous, cross-linked polymer of enormous strength and rigidity. This further defined is N – acetylglucosomine, N – acetylmuramie acid L – alanine, D – alanine, D – glutamate.

16. Define Gram positive and Gram-negative bacteria.

Gram-positive bacteria usually have a much greater amount of peptidoglycan in their cell wall. It may account of 50 percent or more of the dry weight of the wall of some bacterial species.

Gram-negative bacteria contains thin peptidoglycan layer, which present in outer membrane. The cell wall of Gram-negative contains more lipids.

17. What are protoplasts and spheroplasts?

A protoplast is that portion of bacterial cell consisting of the cytoplasmic membrane and cell material bounded by it. Protoplasts can be prepared from Gram-positive bacteria by treating with enzyme lysozyme.

Spheroplasts are round; osmotically fragile forms of Gram-negative bacterial and prepared by a protoplast from Gram-positive bacteria.

18. Write short notes on Bacterial endospore.

The spores of certain bacteria produce within the cells (or) inside the cells are called endospore. They are thick walled, highly refractile, heat resistant and drought resistant. Endospores contain dipicolinic and (DPA) which is not found in vegetative cells and the DPA in combination with calcium ad the calcium-DPA complex may possibly play a role in the heat resistant.

19. Write short notes on bacterial conidiospores.

The reproductive spores or structures of actinomyctes are called bacterial condiospores. The actinomycetes group of bacteria forms a vegetative hyphae which is consist of chain of cells in continuous filamentous form. The spores (or) reproducing forms formed in the tip of hyphae called as a conidiosporangium and the spores produced are conidiospores.

20. Write about the vegetative form of fungi.

Fungi are Eukaryotic chemo-organotrophic organisms. The vegetative portion of fungi called as thallus (or) mycelium, which is multi cellular filament with more number of branches. Multicells of fungal mycelium or hyphae contains nucleus and cell contents with cytoplasm.

21. What are the methods of Asexual reproduction in Fungi?

Asexual reproduction called somatic or vegetative reproduction, in which the sex cells or sex organs do not involve. The types of asexual reproduction are;

1. Fission of somatic organs
2. Budding of somatic cells
3. Fragmentation
4. Spore formation

22. What are the different asexual spores produced by fungi?

The different asexual spores produced by Fungi are;

1. Sporangiospores
2. Conidiospores
3. Oidiaspores
4. Chylamydospores
5. Blastospores

23. What are the methods of sexual reproduction involved in Fungi?

Sexual reproduction is carried out by fusion of two compatible nuclei of two parent cells. The various methods of sexual reproduction involved in Fungi are;

1. Gametic copulation
2. Gamete – gametangial copulation
3. Gametangial copulation
4. Somatic copulation
5. Spermetization

24. What are the different sexual spores produced by fungi?

The different sexual spores produced fungi are;

1. Ascospores
2. Basidiospores
3. Zygospores
4. Oospores

25. Write any 4 differences between bacteria & fungi.

Description	Bacteria	Fungi
1. Cell type	Prokaryotic	Eukaryotic
2. Optimum P^H	6.5 to 7.5	3.8 to 5.6
3. O_2 requirement	Aerobic to Anaerobic	Strictly aerobic
4. Cell wall components	Peptidoglycan	Chitin, cellulose, or hemi cellulose

26. What are lichens?

Lichens are composite organisms composed of fungi and algae, each contributing to the benefit of both. The algae synthesize carbohydrates by photosynthesis and the fungi obtain other nutrients and water from the environment. The reproduction of lichens either by the involvement of algae or fungi.

27. Write short notes on Mycorrhizas.

A mycorihizae is composed of fungus, which occurred in infected root system of certain rootlets of seed plants. This association is beneficial to the higher plants and the mycorrhizal fungi live inside the root cells of plants. So, the fungi provide certain nutrients to plants and plants provide essential nutrients and space to live fungi.

28. Define algae.

Algae are living organisms ubiquotous in nature, single cells to multi-cellular organism which are mostly photosynthetic. Algae are live in aquatic (70%) and terrestrial environment. Algae are containing simple reproductive structure and reproductive processes. It is also economically important class of organism.

e.g Blue green algae

29. Write the economic importance of algae.

Many products derived from algae are economically important. The following are the few products for example.

1. Agar agar
2. Alginic acid
3. Carrageenan
4. Diatomaceous earth
5. Algae as food

30. What are the pigments present in algae?

The pigments present in algae are;

1. Chlorophylls a, b, c, d and e.
2. Carotenoides
   i) Carotenes
   ii) Xanthophylls
3. Phycobilins (or) Biloproteins.

31. What are the major classes of algae?

1. Rhodophyta
2. Xanthophyta
3. Chrysophyta
4. Phaeophycophyta
5. Bacillariophycophyta
6. Euglenophycophyta
7. Chlorophyta
8. Cryptophycophyta
9. Pyrrophycophyta

32. Write short notes on protozoa.

Protozoa are a group of microorganisms, which are Eukaryotic animal like (or) first animals. These are generally as single cells and specific life cycles they consist. Protozoa are lacking cell walls. Protozoa found (or) habitant in moist environment.

33. What are the Asexual reproduction methods found in protozoa?

Asexual reproduction in protozoa occurs by a simple cell division. The different methods are;

1. Binary fission
2. Multiple fission
3. Budding.

34. Write the major classes of protozoa studied.

The protozoa are mainly classified as,

1. Pytomastigophora (Plant like forms)
2. Zoomastigophora (Animal like forms)

Further it classified to following phylum.

Phylum 1 - Sarcomastigophora
" 2 – Labyrinthomorpha
" 3 – Apicomplexa
" 4– Microspora
" 5 – Acetospora
" 6 – Myxozoa
" 7 – Ciliophora

35. Define viruses.

Viruses are a group of microorganisms which is smaller in size i.e. less than bacterial size. They contain only one kind of nucleic acid as either DNA (or) RNA. The nucleic acid alone is necessary for their reproduction. They are facultative parasites and not live (or) reproduce outside of any host organisms. They are pathogenic to plants, animals, and bacteria.

36. Write short notes on Bacteriophage.

Viruses that are use bacterial cells as a host and infect on bacteria is called Bacteriophage. These produce plagues (or) lytic holes in a continuous bacterial lawn. The phages of E. coli (or) E. coli phage, α phage are the typical examples of Bacteriophage.

37. Define the structure of viruses.

The virus particles consist of Nucleic acid_ (NA) and protein coat. The protein coat is known as Capsid and the Nucleic acid and capsid together called as a Nucleocapcid. Individual proteins are called as Capsomears.

38. Write short notes on mycoplasma.

The mycoplasma is a group of microorganism belongs to or elevated from special class of bacteria. They are smallest, independently replicating prokaryotes. They are lack of cell walls and the protoplasts surrounded by cytoplasmic membrane. These are pathogenic to human & animals.

39. What is viriod? Why it is unique?

Viriods are in the form of small, naked i.e. not protein covered RNA molecules which are found in some plant diseases. Viriods are circular, closed, single-stranded RNA with a chain length of about 360 nucleotides. So, these are smallest aetiological agent of any disease.

40. What are prions?

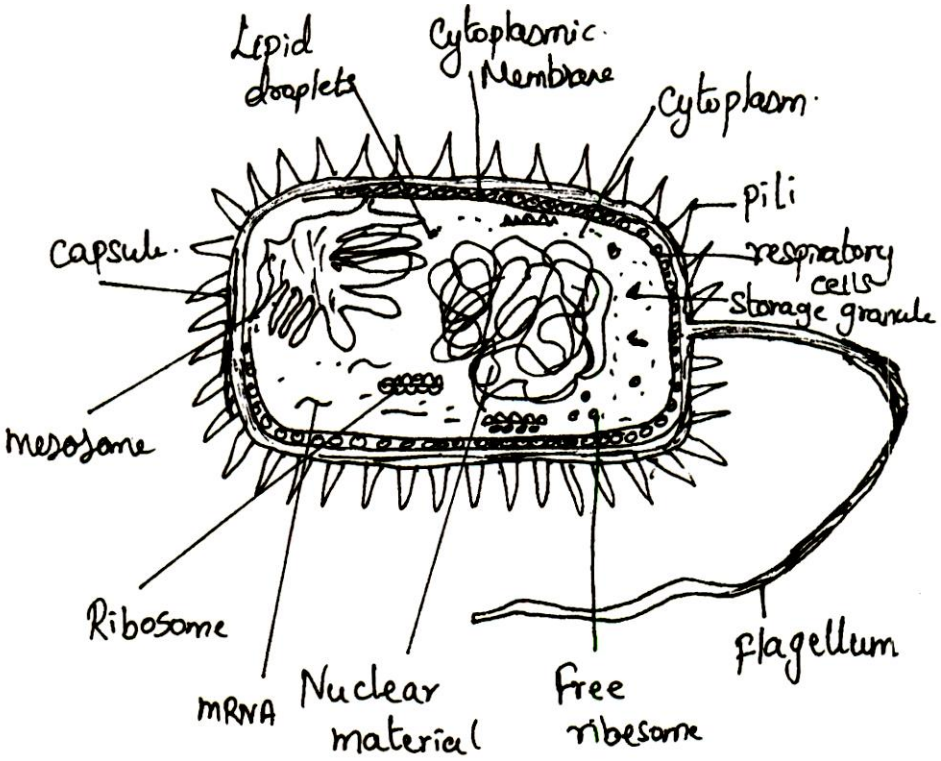
They are aetiological agents of diseases like Creutzfield –Jakob syndrome in man and scrapes in sheep are still largely known, except that they appear to be nucleic acid-free small proteins, of approximately 250 amino acids. It is thought possible those these activate a latent gene of the host, which codes for this protein, and that this causes the illness.

41. What are L-forms?

L-forms are a form of bacteria, which grows irregularly, and appear the change in length and diameter of antibiotic (penicillin) bacteria. This is much time increased in this structure and shape. These giant cells viable for a time on media. These come to normal spherical shape in the normal condition of environment.

PART – B

1. Draw a neat sketch of Bacterial cell and mention about the organelles.



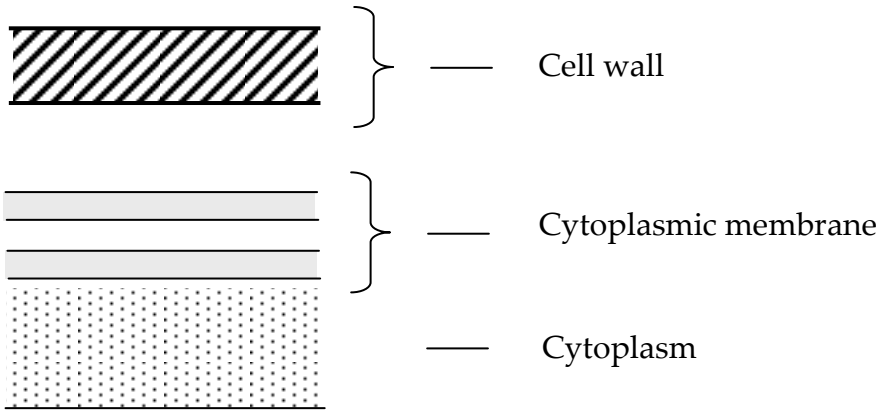
Typical Bacterial Cell

Description of Cell organelles

Bacterial cells are very small and most of them are rod shaped. The size is not more than 1 μm wide and 5 μm long. Some bacterial cells are appearing very big and up to 100μm long. These are giant bacterial cells.

Cell wall

Cell wall located beneath capsules, sheaths and flagella and external to the cytoplasmic membrane. Cell wall is a very rigid structure that gives shape to the cell. Its main function is to prevent the cell from expanding and eventually bursting because of uptake of water, since most bacteria live in hypotonic environment.



Structure of cell wall of Gram positive bacteria

Cell wall is made up of peptidoglycan (called as murein) an insoluble, porous, cross-linked polymer of enormous strength and rigidity. Peptidoglycan is basically with N-acetylglucosamine and N-acetylmuramic acid, L-alanine, D-alanine, D-glutamate and a diamino acid.

The Gram positive bacteria have a greater amount of peptidoglycan layer (about 50% or more) in their cell wall. Sometimes polysaccharides also present that are covalently linked to peptidoglycan.

The gram negative bacteria contain more complex cell wall structure. It contains outer membrane and inner membrane of cell which is with lesser amount of peptidoglycan and embedded with liquids layer. The outer membrane of cell wall of gram negative bacteria anchored by means of Braun's Lipoprotein. The membrane is bilayered.

Internal cell wall (Cytoplasmic membrane)

Immediately beneath the cell wall is the cytoplasmic membrane. This composed primarily of phospholipids and proteins.

The cytoplasmic membrane is a hydrophobic barrier to penetration by most water-soluble molecules. However, specific proteins in the membrane allow, indeed facilitate, the passage of small molecules (i.e. nutrients and waste products) across the membrane. The cytoplasmic membrane also contains enzymes for respiratory metabolism.

Membrane Intrusions (Mesosomes)

Bacteria may have specialized invaginations of the cytoplasmic membrane that can increase their surface area for certain functions. In Gram-positive Bacteria, the membrane invagination in the form of systems of convoluted tubules and vesicles termed mesosomes. The central mesosomes penetrate deeply into the cytoplasm and peripheral mesosomes shown only shallow penetration into the cytoplasm.

The Cytoplasm

The cytoplasm is surrounded by a membrane called Cytoplasmic membrane. Cytoplasm divided into 3 areas as; 1. The cytoplasmic area, granular in appearance and rich in the macro molecular RNA – protein bodies known as ribosomes on which proteins are synthesized. 2. The Chromatinic area, rich in DNA; and 3. The fluid portion with dissolved substances. No complex endomembrane organelles are present in Bacterial cytoplasm.

Cytoplasmic inclusion & Vacuoles

Concentrated deposits of certain substances are detectable in the cytoplasm of some Bacteria. These are called as volutin granules, also known as metachromatic granules, which are composed of polyphosphate molecules.

In some bacteria live in aquatic environment, have vacuoles that provide buoyancy.

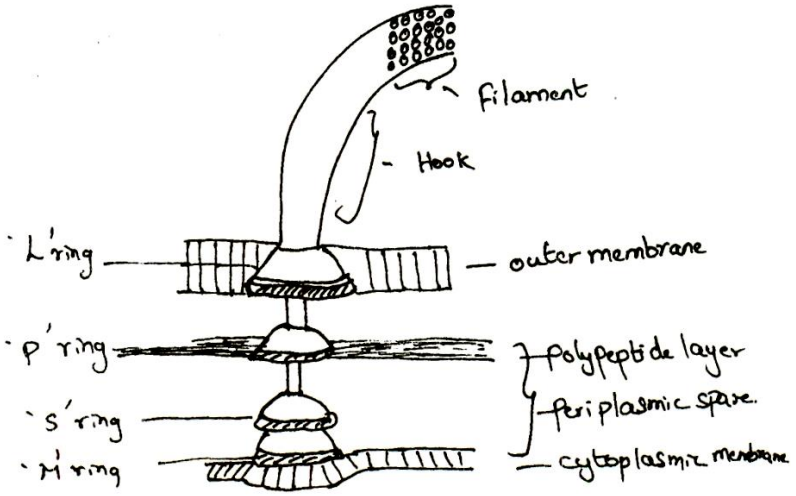
Nuclear material

Bacteria cells contain single naked chromosome dispersed near the centre of Bacteria is called chromatin. No nuclear envelope found in bacteria cell. DNA is circular.

Flagella

External to the cell wall of bacteria contains flagella are hair like, helical appendages which are responsible for locomotion or swimming of bacteria. Flagella is in 0.01 to 0.02 μm in diameter and much simpler in structure. The flagellum located on the cell wall is either polar or lateral side of the cell.

Structure and Attachment of Flagellum on Gram negative Bacteria



A flagellum is composed of 3 parts as;

1. Basal body – associated with cytoplasmic membrane and cell wall.
2. A short hook and
3. A helical filament which is several times longer than bacterial cell.

Flagella is useful for movement or motility of bacteria.

Pili (Fimbriae)

Pili are hallow, non-helical, filamentous appendages that are thinner, shorter and more numerous than flagella. The functions of the pili are: 1. Some pili known as "F" pili act as sex pilus which help in reproduction, and 2. Some pili play a role in infection process in which it attaches on host surfaces like intestine, respiratory and genitourinary tracts.

Capsules

Some bacterial cells surrounded by a viscous substance forming a covering layer or envelope around the cell wall is called capsule. This is in slimy nature.

1. Capsule provide a protection against bacteria from temporary drying.
2. They block attachment of bacteriophages.
3. They may be antiphagocytic.

Sheaths

In some marine or aquatic bacteria, the hollow tube encloses cells to form chain or trichomes are called sheath. Sheaths may impregnate with ferric and manganese hydroxides. The function of the sheath are which strengthen the cells.

2. Critically discuss about the cell wall of Gram positive and Gram-negative Bacteria.

The cell wall is located on the external to the cytoplasmic membrane. Cell wall is rigid structure and that gives shape to the cell. The main function of bacterial cell wall is preventing the cell expanding and eventually bursting because of uptake of water. The rigidity of cell wall can readily be demonstrated by subjecting bacteria to very high pressures or other severe physical conditions.

The cell wall portion gram positive bacteria is thicker than Gram negative bacteria.

The peptidoglycan is the main cell wall chemical components, which is insoluble in water and cross-linked polymer of enormous strength and rigidity. Peptidoglycan is basically a polymer

of N-acetylglucosamine, N-acetyl muramic acid, L – alanine, D – alanine, D – Glutamate and a diamino acid. The peptidoglycan helps in cell grow and new cell formation.

In archaeobacteria, the cell walls are composed of proteins, glycoproteins or polysacharides. A few genera as Methanobacterium have walls composed of pseudomurien.

Gram – positive bacterial cell wall

Gram-positive bacteria usually have a much greater amount of peptidoglycan in their cell walls than do Gram-negative bacteria species. It contains more than 50% of dry weight of the wall, but in gram negative only 10% other substances may occur in addition to peptidoglycan.

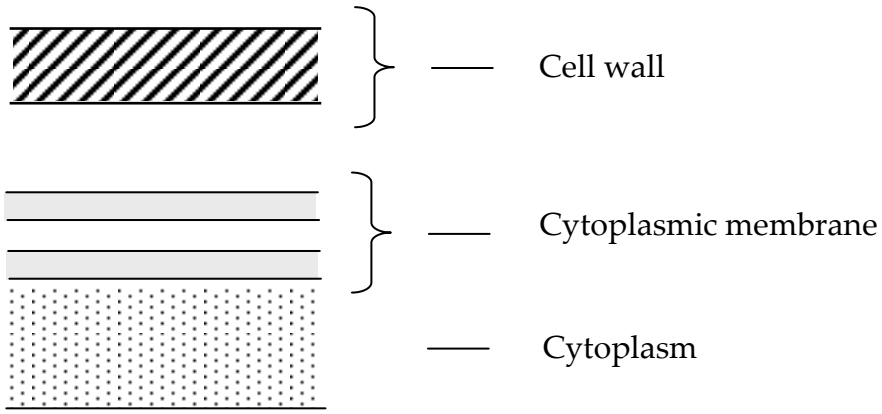


Fig. A gram positive bacteria contain thicker peptidoglycan layer of cell wall

In the walls of streptococcus pyrogens contain polysaccharides that are covalently linked to the peptidoglycan and which can be extracted. In staphylococcus arreus and S. feacalis contain techoic acids – acidic polymers of ribose phosphate or glycerol phosphate. Which are covalently linked to peptidoglycan, Techoic acids bind magnesium ions, and it believed to be give strength to the bacteria. In Mycobacterium the walls are correlated with the presence of mycolic acid.

Cell walls of Gram negative Bacteria

The cell walls of Gram-negative bacteria are more complex than those of Gram-positive bacteria. The important difference is the presence of outer membrane that surrounds a thin underlying layer of peptidoglycan. Due to presence of this membrane, the walls of Gram negative bacteria rich in lipids (11% to 22%) in contrast to those of Gram-positive bacteria.

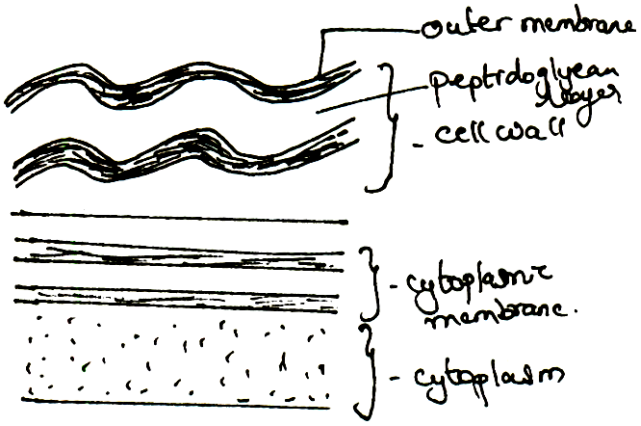


Fig. Structure of Gram-negative Cell Wall

The outer membrane server as a barrier which is impermeable to prevent the escape of enzymes such as those involved in cell wall growth and prevention of entry of various external chemicals and enzymes which could damage the cell.

The outer membrane is anchored to the underlying peptidoglycan by means of Braun's lipoprotein. The membrane is bilayered consisting of phospholipids, proteins and lipopolysacharides. The LPS has toxic properties and is known as endotoxin.

Although the outer membrane is impermeable for large molecules, it can allow smaller molecules such as nucleosides, oligosaccharides, monosaccharides, peptides and amino acids pass across. This is accomplished by means of channels in special proteins called Porins, which span the membrane.

The adhesions are the points which have direct contact between the two membranes which allows to export of LPS and Porins.

3. Describe in detail about the shape and arrangement of Bacterial cells.

Bacterial is very small, mostly from 0.5 to 1.0 μm in diameter. An important consequence of the small size of microorganisms is that the surface area volume ratio of bacteria is exceedingly high compared to the same ratio for larger organisms of similar shape.

Shape and Arrangement

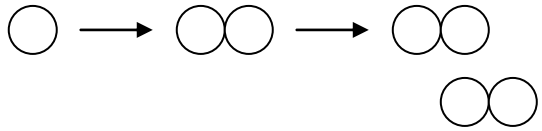
The shape of Bacterium is governed by its rigid cell wall; however exactly what attribute of this rigid material determines that a cell will have a particular shape is not yet understood. Typical bacterial cells are;

1. Spherical (Cocci (or) Coccus) – round
2. Straight rods (bacilli (or) bacillus) – rod shape
3. Rods that are helical – (Spirilla (or) spirillum)
4. Pleomorphic – In variety of shapes

Cocci

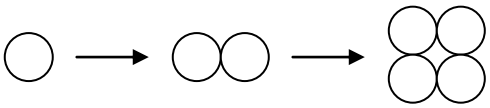
Cocci cells appear in several arrangements, depending on the plane of cellular division and whether the daughter cells stay together

1. Diplococci - Occur in pairs

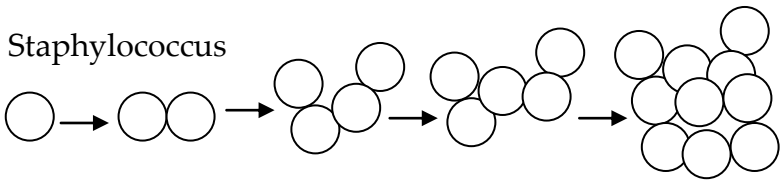


2. Streptococcus - appear in chain forms

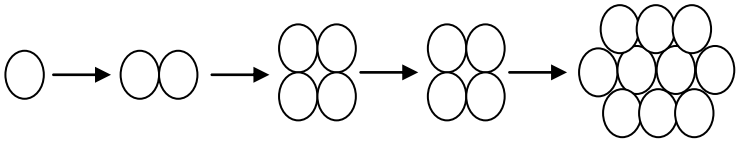
3. Tetrads

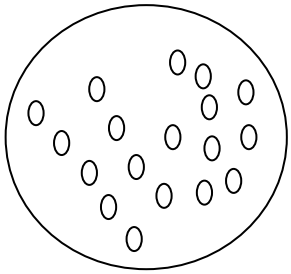


4. Staphylococcus

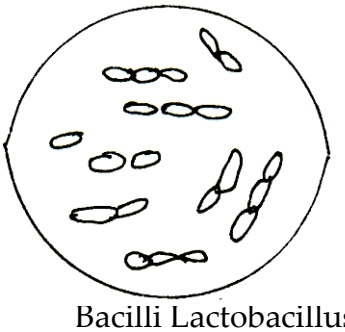


5. Sarcinae

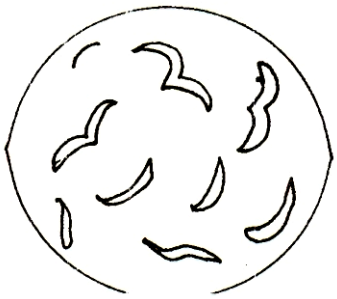


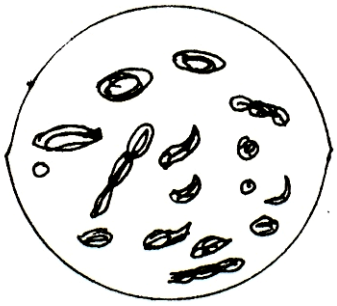


Cocei Strepto cocus



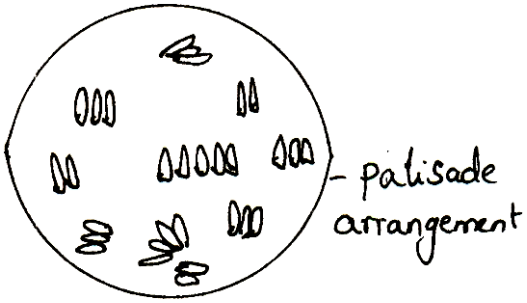
Bacilli Lactobacillus





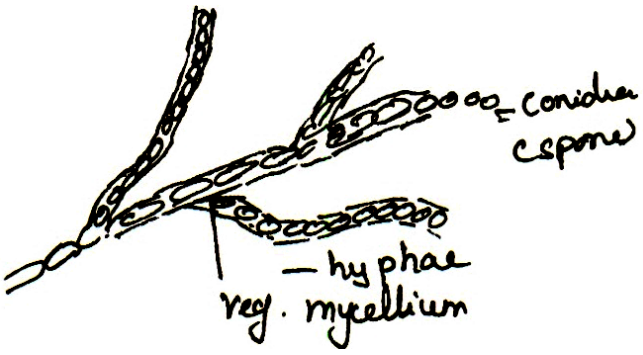
Vibrio (Coma shaped) Aquaspirillum Pleamorphic Arthobactor

Bacilli are not arranged in patterns as complex as those of cocci and most occur singly or in pairs (diplobacilli). But some species, such as Bacillus subutilis form chains (streptobacilli) others such as Beggiota and Saprospira species, form trichomes which are similar to chains but have much larger area of contact between the adjacent cells. In other bacillus species, such as corynebacterium diptheriae ; the cells are lined side by side like matchsticks (palisade arrangement) and at angles to one another.



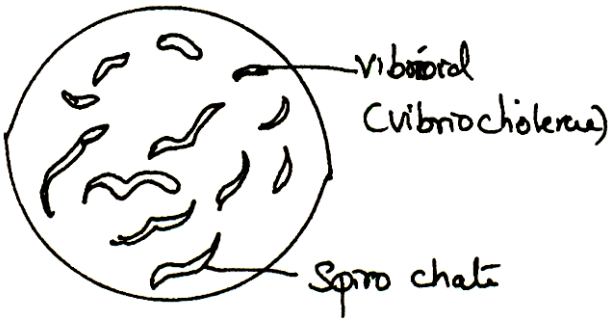
Corpnibacterium

Streptomyces species form long, branched, multinucleate filaments called hyphae (singular hypha) which collectively form a mycelium.



Streptomyces viridochromogenes

Some bacteria are in curved shape with twist. Bacteria have with less than one complete twist or turn have a vibrioid shape and more than one twist called spirochetes.



In addition most common shapes, others occurs in pear shaped, loped shaped, rods with squared, hemispherical ends (Bacillus anthracis), disk arrangement like stacks of coins, rods with helically sculptured surfaces (selibiria) and many others.

4. Explain the structure and functions of bacterial flagella. Write the method of movement of bacteria.

Bacterial flagella are like helical appendages that protrude through the cell wall and are responsible for swimming motility. They are much thinner than Eukaryotes. They are much simpler in structure and 0.01 to 0.02 μm in diameter.

The location of flagella on bacterial cell varies as polar (at one end) and lateral (along the sides of bacterium).

The arrangement of flagella are as follows:-

1. Monotrichous (Single flagellum) - Pseudeomonas auregenosa

2. Lophotrichous (cluster of flagella) - Pseudomonas fluoresces

3. Amphitrichous (at both ends of cell) - Aquaspirillum serpens

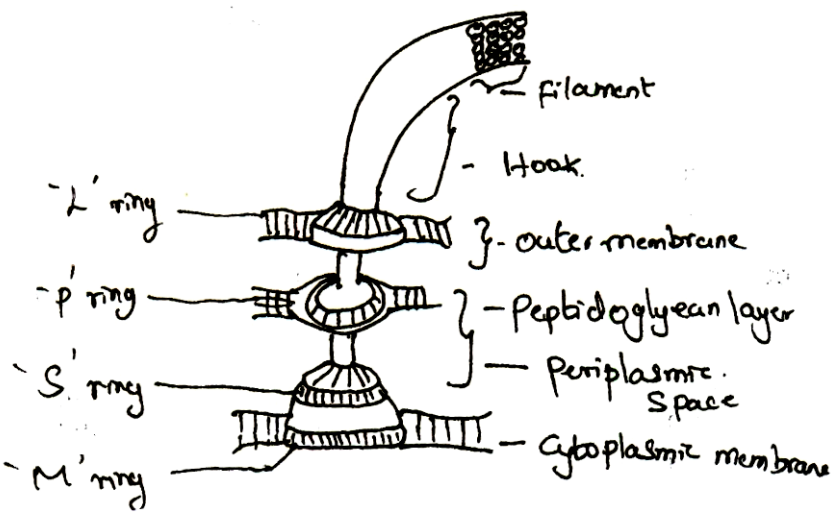
4. Peritrichous (Surrounded by lateral flagella) - Salmonella typhi

Structure (ultra structure)

Flagellum is composed of three parts.

1. A basal body – Associated with the cytoplasmic membrane and cell wall.
2. A short hook and
3. A helical filament – which is usually several times as long as the cell.

The chemical composition of hook & filament are composed of protein subunits arranged in a helical fashion. The protein of the filament is known as flagellin which has a relatively low molecular weight.



Structure of flagella

Motility (or) Movement

Bacteria propel themselves by rotating their helical flagella. The movement of flagella is controlled by basal body which derives electromotive force and the hydrogen-ion gradient across the cytoplasmic membrane.

Bacteria having polar flagella swim in a back – and forth; they reverse their direction of swimming by reversing the direction of flagellar rotation. Bacteria having lateral flagella swim in a more complicated manner. Their flagella separate in synchrony to form a bundle that extends behind the cell.

Motility without flagella

Certain helical bacteria exhibit swimming motility, particularly in highly viscous media, yet they lack flagella. However, they have flagellar like structures located within the cell, just beneath

the outer cell envelope. These are called periplasmic flagella. They have also been termed axis flagella (or) endoflagellae.

Gliding motility

Some bacteria, e.g. cytophaga species, are motile only when they are in contact with a solid surface. As they glide they exhibit a sinuous, flexing motion. This kind of movement is comparatively slow, only a few μm per second. The mechanism of gliding motility is unknown no organelles responsible for motility they have been observed.

Bacterial chemotaxis

Many motile bacteria are capable of directed swimming toward or away from various chemical compounds – a phenomenon called bacterial chemotaxis. Swimming toward chemical is called positive chemotaxis and swimming away is negative chemotaxis.

Bacterial Phototaxis

The responses of light by bacterial movement are called bacterial phototaxis. This show as increased intensities of light attracts and the reduced intensities repelled the bacteria.

Magnetotaxis

Bacteria attracted to the high-pressure atmosphere by the magnetic poles are called magnetotaxis. Magnetosomes are components present in the bacteria which response the magnetic force. E.g. marine bacteria live in the deepest area of sea.

5. Discuss the Asexual reproduction of fungi.

Fungi reproduce by means of various methods. Generally 2 types of reproduction observed.

1. Asexual reproduction
2. Sexual reproduction

I. Asexual reproduction

Asexual reproduction does not involve the union of nuclei, sex cells or sex organs. It may be accomplished by

1. Fission of somatic cells yielding two similar daughter cells.
2. Budding of somatic cells or spores, each laid a small outgrowth of the parent cell developing into a new individual.

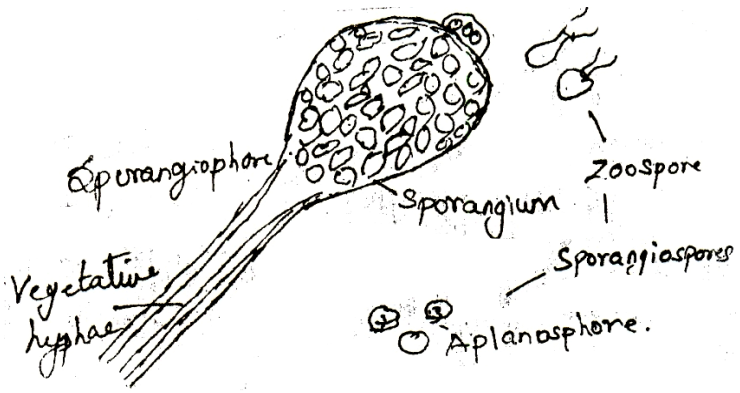
3. Fragmentation or disjointing of the hyphal cells, each fragment becoming a new organism or
4. Spore formation

A sexual spores are many kinds of asexual spores.

i) Sporangiospores

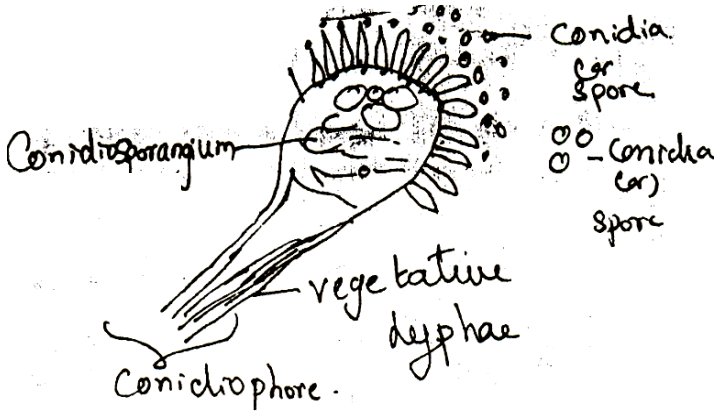
These single-celled spores are formed with in sacs called sporangia at the end of special hypae. i.e. sporangiophores.

Aplanospores are non motile sporangiospores Zoospores are motile sporangiospores, their motility being due to the presence of flagella.



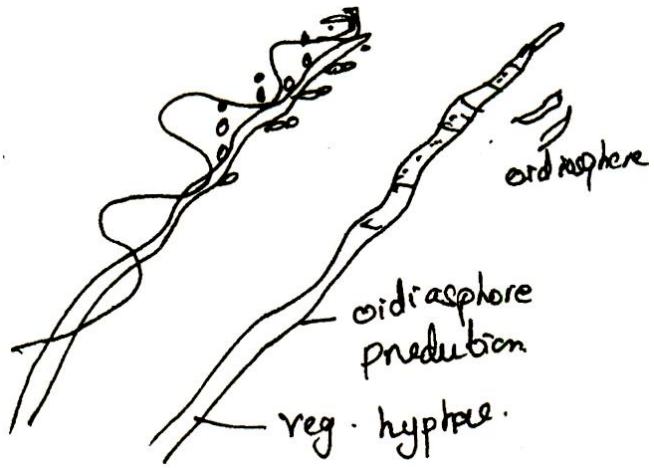
ii) Conidiospores or Conidia

Small singled conidia are called microconidia and large multicelled Conidia called macroconidia. Conidia are formed at the tip or side of a hypha.



iii) <u>Oidiospores (or) Oidea (or) Arthrospores</u>

Disjointing of hyphal cells forms these single celled spores in fungi.

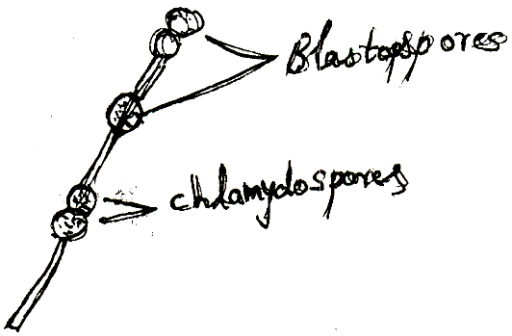


iv) <u>Chlamydospores</u>

These thick-walled, single-celled spores are highly resistant to adverse conditions. They are formed from cells of the vegetative hypae.

v) <u>Blastospore</u>

These are spores formed by budding from the tip cell of the mycelium or hyphae of fungi.



<u>Chlamydospore & Blastospores forming in Hypae</u>

The above asexual methods were found in fungi.

6. Discuss the sexual reproduction in fungi.

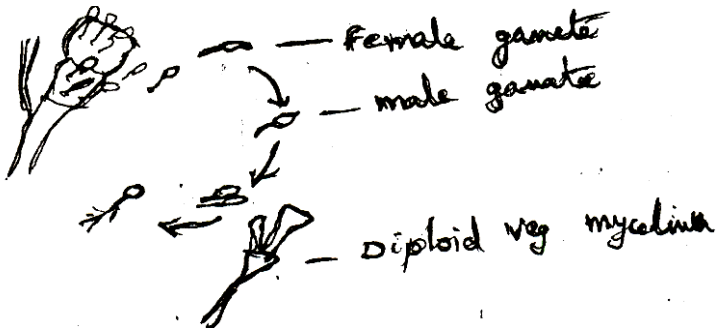
Sexual are reproduction is carried out by fusion of the compatible nuclei of two parent cells. The process of sexual reproduction begins with the joining of two cells and fusion of their protoplasts i.e. plasmogamy, thus enabling the two haploid nuclei of two mating types to fuse together (Karyogamy) to form a diploid nucleus. This is followed by meiosis, which again reduces the number of chromosomes to the haploid number.

The sex organelles of fungi are called gametangia. They may form differentiated sex cells (gametes) or may contain instead one or more gamete nuclei. If the male and female gametangia are morphologically different, the male gametangium is called the antheridium and the female gametangium is called the Oogonium.

The various methods of sexual reproduction may be described as follows:

1. Gametic copulation

Fusion of naked gametes, one or both of which are motile.

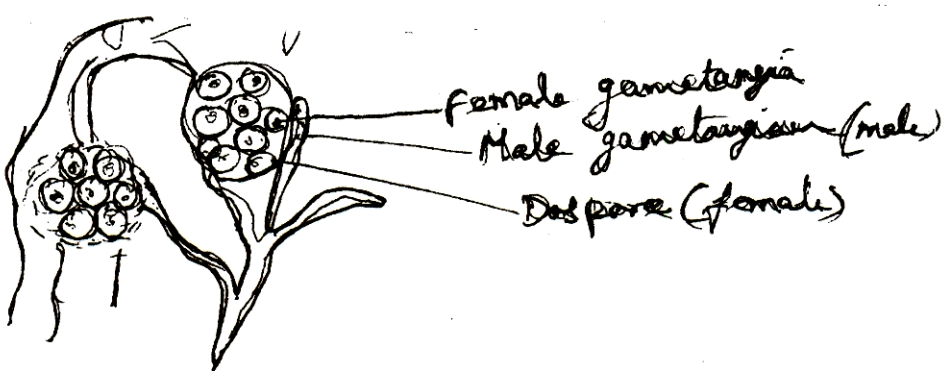


Gametic copulation

Both the gametics are fuse together and form a zygote. By meiosis haploid progemy is produced.

2. Gametic – Gametangial copulation

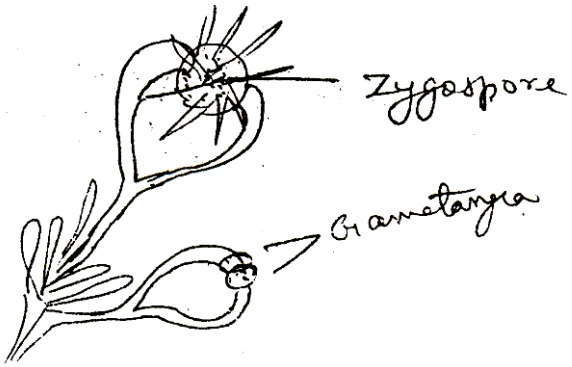
Two gametangia come into contact but do not fuse; the male nucleus migrates through a pore or fertilization tube into the female gametangium.



Gamete – gametangial copulation

3. Gametangial copulation

The gametangia or their protoplasts fuse and give rise to a zygote that develops into a resulting spore.



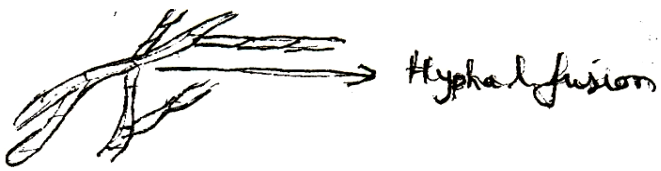
After fusion of gametes, zygospore is produced. Zygospore produces new progeny.

4. Somatic copulation

Fusion of somatic or vegetative cells and formation of spore.

5. Spermatization

Union of a special male structure called a spermatium with a female receptive structure. The spermatium empties its contents into the latter during plasmogamy.

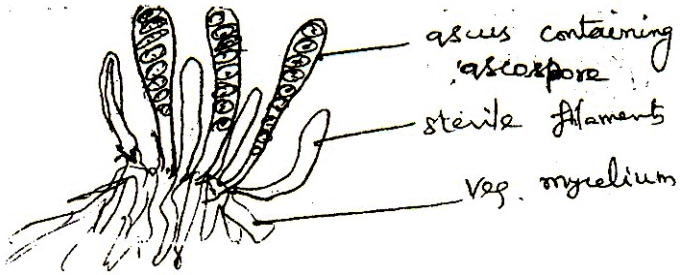


Somatic copulation

Sexual spores. Which are produced by the fusion of two nuclei, occur less frequently, later, and in smaller numbers than do asexual spores. There are several types of sexual spores.

1. Ascospores:

These single called spores are produced in a sac called an ascus (plural asci). These are usually eight ascospores in each ascus.

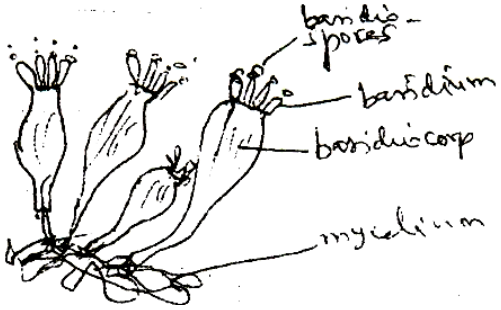


Ascospore formation

Nuclear fusion takes place in the ascus. The diploid zygote nucleus decides by meiosis and further mitosis to produce 8 ascospores.

2. Basidiospore

These single celled spores are born on a club shaped structure called a basidium (plural basidia). Their formation is illustrated in the following figure.



Basidiocorp

3. Zygospore

Zygospores are large thick-walled spores formed when the tips of two sexually compatible hyphae or gametangia of certain frungi fuse together.

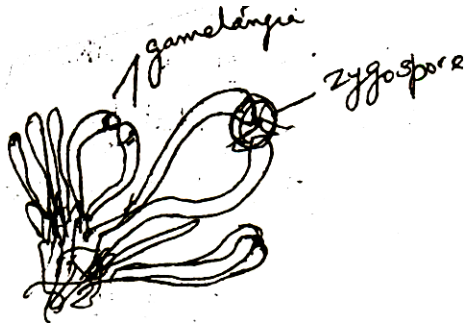


Fig. Zygosopore formation

4. <u>Oospores</u>

These are formed with in a special female structure, the fertilization of the eggs, or oospores, by male gamete formed in gives rise to oospore. There are one or more oospores in each organism.

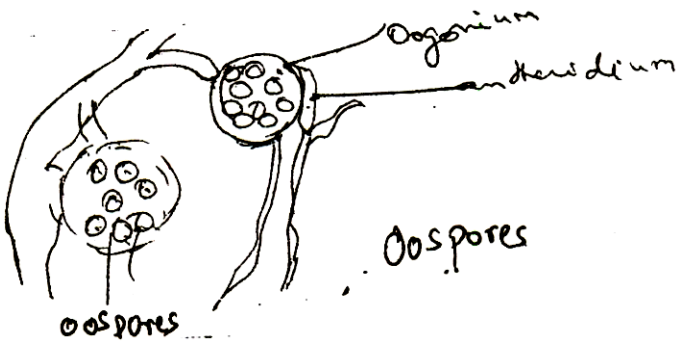


Fig. Oospores formation

Asexual and sexual spores may be surrounded by highly organised protective structures called fruiting bodies.

7. Discuss the classification of Algae.

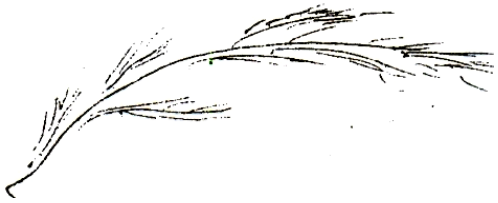
Algae are from unicellular to multicellular organisms, which belong to Eukaryotic group. They are habitants mostly in moist and aquatic environment. Few algae are live in terrestrial environment. Algae are classified on the basis of the following characteristics:

1. Nature and properties of pigments
2. Chemistry of reserve food products or assimilatory products of photosynthesis.
3. Type and Number, insertion and morphology of flagella.
4. Chemistry and physical features of cell walls.
5. Morphological characteristics of cells and thalli.
6. Life history, reproductive structures and methods of reproduction.

The major division of algae are discussed with few examples in the following text.

1. Rhodophycophyta: (The Red algae)

This group consist of Red algae and marine forms live in warmer seas and coasts, but some of them are grow in colder water as well as in fresh water. They grow in subtidal (submerged) areas and few are able to survive desiccation. It grows upto 2 or 4 feet long. Reproduction by asexual motile spores and sexually by heterogamous method. The union of well differentiates non-motile male cells called spermatia and female corpogonia. The pigments present are chlorophyll a, rarely d, β - carotene, zeaxanthine, phycoerythrin and phycocyamin. The economic importance is the agar made from Gelidium and carrageenan from chondrus curispus. The common is polysyphonia found in seawater.



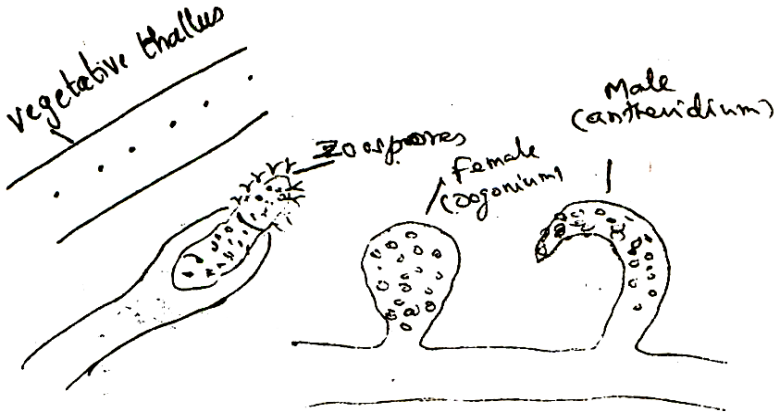
Polysyphonia lamosa

2. Xanthophycophyta (The yellow green algae)

They are mostly found in temperate regions in fresh water and marine habits, as well as and in soil. They have unique pigments chlorophyll a, c rarely chlorophyll e, β- carotene, diadinoxanthin, heteroxanthin and Vancheriaxanthin ester. Xanthophytes exist as single cells, colonies and both branched and unbranched filaments. Reproduction by asexual method through motile zoospore, which have unequal flagella.

Their cell walls are composed of cellulose and pectin. The cellulose storage product is an oil or chrysolaminarin. Voucheria is a well-known member of this group and habitant in moisty soil and flowing water. They have very long thallus with coenocytic filaments.

Asexual reproduction by Zoospores formation and sexual by differentiated antheridium and Oogonium.



Asexual reproduction and sexual reproduction Voucheria

3. Chrysophycophyta (The Golden Algae)

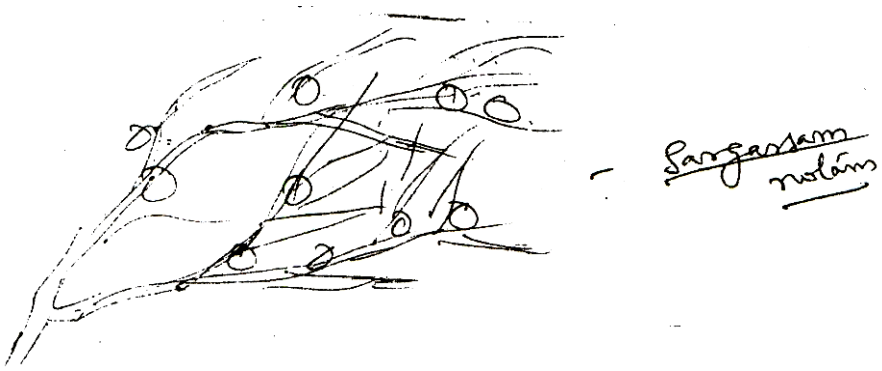
This group consists of characteristic pigment, which due to masking of chlorophyll by Brown pigments contains B-Carotene & Fucoxanthin. Hence it is Golden colour.

These are unicellular, amoeboidal with flagella with pseudopodial extension of the protoplasm. Non-motile coccid ad filamentous forms are available.

4. Phaeophyophyta (The Brown algae)

They are multi cellular and contain a Brown pigment which gives characteristic colour, hence brown algae. The pigments present are B-carotene, α-carotene, fucoxanthin and violaxanthin. They are almost occur in sea water (marine form) and grow in cold sea waters. They are complex and reach up to several hundred feet.

Reproduction by asexual method is by Zoospores and sexual methods by isogamy and Anisogamy. The economic value of this group is used as food and feed, medicinal properties and some are fertilizers. E.g. Sargassam notans.



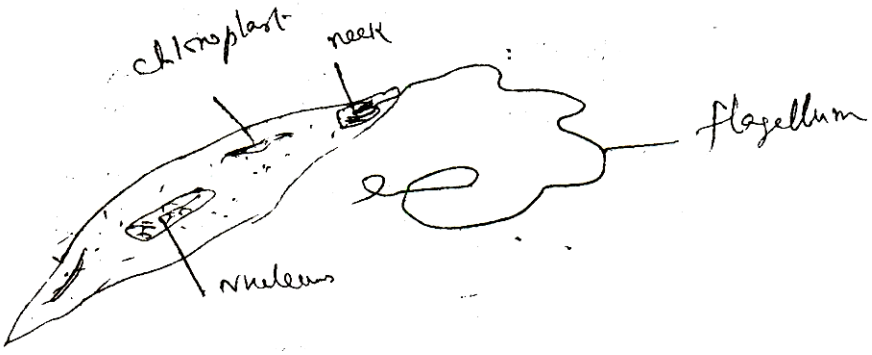
5. Bacillariophycophyta (The Diatoms)

They are called as Diatoms, which are found in both which are found in both fresh and salt water and in moist soil. Abundant in cold waters: They are in unicellular, colonial and filamentous forms and in wide variety of shape. They produce shell-containing silica. The deposits of these shells for long period called diatoms (or) diatomaceous growth. The economic importance of diatomaceous earth is used as a filter aid in filtering in industrial processes.

6. Euglenophycophyta (The Euglenoides)

These are unicellular organisms and actively motile by means of flagella. It is usually occur in soil as well as in water. The pigment present is B-carotene and diadinoxanthins sometimes α-

carotene. Reproduction by longitudinal Binary fission. Cyst (dormant cells) also formed sometimes for reproduction.



Euglena aces

7. Chlorophcophyta (The green algae)

They are green algae occur in fresh water. Occur from Unicellular motile cells to colonial forms and sometimes-filamentous forms. They have holdfasts, which helps in attaching (or) anchor the support. The pigments present are chlorophyll a and b, β- carotene, rarely α-carotene and lycopene and lutein. The storage granules contain starch and oil.

Asexual reproduction by vegetative cell undergoes longitudinal fission.

Sexual reproduction by isogamy, heterogamy and Oogamy. E.g. Chlamydamonas & Volvox (unicellular) Ulothrix, Ulva are filamentous.

8. Cryptophycophyta (The cryptomonads)

These are small group of biflagellate unicellular organisms, unequal 2 flagella. The pigments present are chlorophyll a, c, α-carotene, alloxanthin. They are live in fresh water.

Asexual reproduction by fission, cyst and Zoospores. Sexual reproduction by union of nuclei.

e.g. Cryptomonas

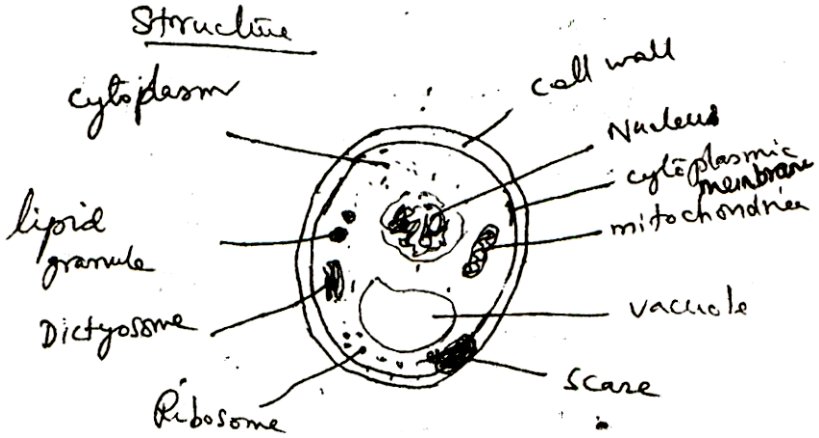
9. Pyrrophycophyta (The Dinaflagellates)

The diverse group with Biflagellate, unicellular organisms. They occur in marine, brackish water and fresh water environment. They move very fast. The pigments are chlorophyll a & c, β-carotene, peridinin, neoperidinin, dinoxanthin and neodinoxanthin present in this group. The formation of red tides in the ocean due to high concentration of organisms in more colour.

Asexual reproduction through cell division and sexual reproduction rarely observed as fusion of isogametes. E.g. Noctiluca.

8. Write an essay on yeast with special mention to its life cycle.

Yeast is unicellular, elliptical shaped Eukaryotic organisms. It belongs to class Ascomycetes. The size of the yeast is 6 to 8 μm long and 5 μm breath. It occurs in soil and on organic material. They are saprophytic in nature.



The yeast cell structure

Yeast cell contain cell organelles and vacuole. The nucleus contains definite nuclear membrane and consists of 16 linear chromosomes. Yeast has short doubling time as 90 mins. Mostly unicellular and few form as mycelial form.

The reproduction by asexual method as Budding of parent cell. The scares were observed on the cell wall of yeast, which is the indication of budding process. The yeast will give up to 23 scares by budding.

The budding is occurring due to division of single parent cell. Nucleus divides by constriction and portion of nucleus enters into the budding portion. Diploid and polyploid races are observed.

Sexual reproduction by mating of 2 yeast cells, which is called as Copulation. The result of fusion is zygote, which undergoes meiosis and produce Ascospores.

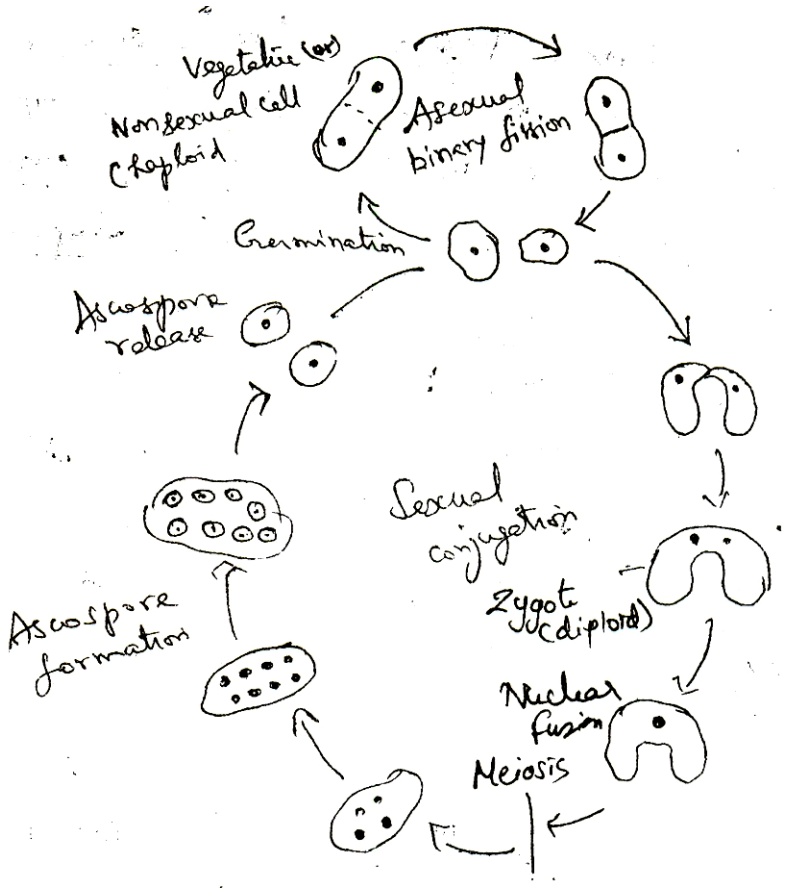
Heterothallic yeasts also were reported. Ascospores produced through 2 mating types as a, a+, Arthrospores also were found in Endomyces lactis. Endomycetous members form mycelium and in Endomycopsin, hyphae, buds and asci were found together.

Sacharomyces are true yeasts.

Chromosomes are 200 – 250 kb in size. Most of the yeast multiplying by budding.

E.g. Sacharomyces
Candida
Torulopsis
Cryptococcus
Rhodotorula
Pullularia
Endomyces
Yeast who having fermentable habits.

The life cycle of the typical yeast is explained in the following diagram.



Life cycle of common yeast (Shizo- sacharomyces)

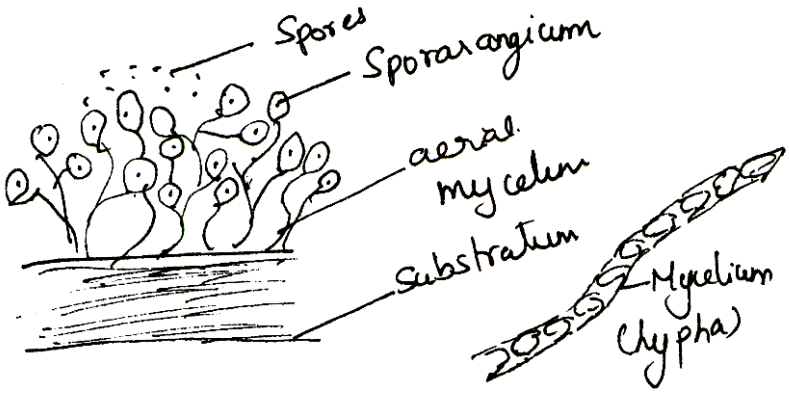
Asexual reproduction by binary fission and sexual reproduction by fusion of compatible nuclei or cells with formation of haploid Ascospores are the two type of reproductive cycles found in yeast cells.

9. Write an Essay on Actinomycetes.

Actinomycetes are a group of Bacteria, which forms vegetative mycelium with multinucleate mycelial structures. These are Gram positive Bacteria group conidiospores are formed in the tip of hyphae. These are Aerobic with few exceptions as anaerobic.

Actinomycetes are saprophytes and occur in soil and water. Some times it parasitize in animals and human.

It can be cultured in the laboratory in artificial culture media. It forms aerial mycelium and give spores from the tip of the mycelium and spore bearing bodies called sporangia. Sporangium are occur in two kinds as (1) Round, rod-shaped or irregular containing masses of spores and or (2) Finger like, club-shaped, or pear shaped, containing one or four spores arranged linearly with in the sporangium. The structure of mycelium and sporangial bodies is shown in the diagram.



The Actinomycetes cell wall contain peptidoglycan with meso-diaminopimelic acid & L-diaminopimelic acid which contain arabinose and galactose sugars.

Most of the organism from this group is economically important as they produce organic acids like citric acid, Succinic acid and Lactic acid. Many others produce antimicrobial compounds (antibiotics)

Streptomyces

Among the members present in this group. Streptomyces is the main member which posses many features and economically much valuable. They form as abundant aerial mycelium and chains of conidiospores. The substrate mycelium submerged in the substrate environment. About 340 species and 39 sub species were recognized from the single genus streptomyces.

The colour of the mycelium with pigments is characteristic feature. The flavour produced by the submerged mycelium also important character which is responsible for soil smell when ploughing the soil due to the presence of soil actinomycetes.

The actinomycetes group is classified based on the features of cultures as given below and examples for every family is given.

Class : Actinomycetes
Order : Actinomycetes

1. Family : Actinomycetaceae
   Genus : Actinomycetes – Pathogenic to human
   Bifidobacterium & animals

2. Family : Mycobacteriaceae
   Genus: Mycobacterium - pathogenic (leprosy)
   (Tuberculosis)

3. Family: Frankiaceae
   Genus : Frankia - Mycorrhiza fungus
   N_2 fixers

4. Family: Actinoplanaceae
   Genus : Actinoplanes
   Spirillospora

5. Family: Dermetophilaceae
   Genus: Dermetophilus - Dermetophytes
   Geodermetophilus

6. Family: Nocardiaceae
   Genus: Nocardia - Pathogenic
   Pseudonocardia

7. Family: Strepotomycetaceae
   Genus: Streptomyces - Antibiotics production
   Streptoverticillium

8. Family: Micromonosporaceae
   Genus: Micromonosporus
   Thermoactinomyces } Foil characteristic
   Actinofibidia

9. Antibiotic producing Actinomycetes

1. Amphotericin	- Streptomyces adosus
2. Chlortetracyclins	- S. amefaciens
3. Chloremphenicol	- S. Venezule
4. Cycloserine	- S, garyphabus
5. Erythromycin	- S. erythraceus
6. Kanamycin	- S. kanamycetous
7. Neomycin	- S. tradiae
8. Nystatin	- S. noursei
9. Oxytetracyclins	- S. rimosus
10. Streptomycin	- S. grieseus
11. Tetracyclin	- S. viridifacium

10. Describe viruses and its Reproduction methods.

Viruses are smaller particles categorized to microorganisms. These are smaller than Bacteria. The size of viruses is 20 nm to 300 nm.

Virus mean poison & virion means virulent or pathogenic particles.

Virus consist of 1. Nucleic acid and
2. Protein coat (capsid)
both Nucleic acid and capsid together called as Nucleocapsid.

Virus able to infect Plants
Animals &
Microorganisms (Bacteria)

Virus infecting Bacteria are called Bacteriophage virus can be demonstrated on host cells.

Characteristic features

1. One kind of Nucleic acid i.e. RNA or DNA present.
2. Nucleic acid alone is sufficient for replication or reproduction.
3. Unable to reproduce outside the host cell.
4. Not independently active
5. Depend host cells for synthesis and live, hence it is obligate intracellular parasites.

Viruses are classified mainly two types based on the presence of capsid or protein coat.

1. Enveloped (presence of capsid)
2. Non-enveloped (Naked virus)

Capsid is a protein coat. The sub units of capsids are called capsomeres.

e.g. Enveloped - Influenza virus
Herpes virus
Non-Enveloped - TMV, Papilloma
Adinovirus

The envelope consist of

1. Proteins - Polypeptides from host.
2. Carbohydrates – Polysacharides
   Galactose
   Mannose
   Glucose & Fructose
   Glucosamine & Galactosamine
3. Lipids - From Host
   Phosphotipids
   Cholesterol
   Fatty acids
   Glyco-lipids

Nucleic acids are
DNA or RNA
Single strand or double strand
Linear or circular
S_sDNA, d_s DNA, S_sRNA, d_sRNA

Classification

Holmer (1948) Proposed the classification of virus based on the Host specific infection

1. Phaginae - Infect Bacteria
2. Phytophaginae - Infect plants
3. Zoophaginae - Infect animals

Further classification of viruses based on the

Nature of organism
Site of infection
Structure of viruses.

Morphological structure based on

1. Enveloped
2. Non-enveloped
3. DNA virus
4. RNA virus
5. S_s DNA virus
6. d_s DNA virus
7. S_s RNA virus
8. d_s RNA virus
9. Shape of virus like
   - Helical virus
   - Polyhedron virus
   - Composite virus
   - Complex virus

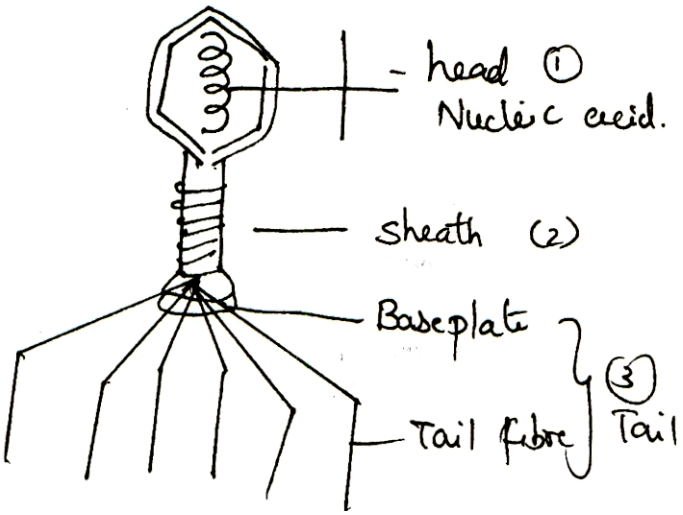
Are consider for the classification of viruses.

Bacteriophages are the group of viruses infects bacteria. They are mainly classified into two types based on the nature of multiplication in the host as;

1. Lytic virus (Virulent virus)
   - Infect host cell
   - Lysis occur in host cell
   - Multiplication of more viruses
   - Bursting (or) disintegration of cell wall of flesh
   - Release of new virus particles & infect other host.
   - Destruction of host.

2. Temperate virus (Avirulent virus)

   - Virus infect host cell
   - Replication of virus
   - Release of viruses
   - No lysis of host cells
   - Host cells are viable after release



Structure of Bacteriophage (T_2 Phage virion)

UNIT – III

PART – A

1. Write any 4 requirements for the growth of microorganisms?

1. Source of energy
2. Source of electron
3. Source of caution
4. Source of nitrogen
5. Source of oxygen
6. Trace elements
7. Vitamins and vitamins like compounds
8. Water

2. Define Phototrophs?

Bacteria species that use inorganic compounds as their source of electrons for oxidation by use of light is an energy source.

e.g. Chromatium okenii uses H_2S as its electron donar, oxidizing it to elemental sulphur.

$H_2S \rightarrow S + 2\ e^- + 2H^+$

3. What are chemotrophs?

Bacteria that use inorganic compounds as a source of electron in the absence of light and light energy.

e.g. Nitrosomonos use ammonia as their election source obtaining by oxidizing ammonia to nitrate.

$NH_4^+ + ½\ O_2 + H_2O \rightarrow NO_2^- + 2H_2O^+$

4. What are Autotrophs?

Bacteria that use CO_2 as their sole source of carbon for assimilation are termed as Autotrophs. **E.g. Nitrosomanos.**

Oxidize ammonia to nitrite their by obtaining sufficient energy to assimilate the carbon of CO_2 into cell components (CO_2 fixation):

$CO_2 + 4\ e^- + 4H^+ \rightarrow (CH_2O) + H_2O$

5. What is Heterotrophs?

Bacteria, which act as both Autotrophs as well as photographs are called as Heterotrophs. Heterotrophs are mostly cause diseases in human and animals. **e.g. Lactobacilli fastidious heterotrophs.**

6. Write short notes on obligate parasites?

Microorganisms, which are unable to cultivate in artificial media, are belong to this group. Those bacteria require a host permanently to grow is called obligate parasite. i.e. association with living host. e.g. **mycobacterium leprae, ricketsias & chlamydias.**

7. Define nutrient culture media?

Culture media are growth media or substrate that contains essentials nutrients for the growth of microorganisms in the laboratory. The essential nutrients include organic and inorganic substances including vitamins and growth hormones. This growth media designed in both **liquid & solid.**

8. Define simple medium or synthetic medium?

Synthetic medium or defined medium or simple medium is a nutrient culture medium, which contains known quantity of chemicals mixed in water, which support the growth, and reproduction of microorganism. In synthetic media inorganic salts or mixture of inorganic salts or compounds mixed in medium. E.g. Glucose salt broth.

9. What is complex media? Give suitable example?

The nutrient culture medium contains unlimited or undefined quantity of nutrients from natural substances. The natural substances give unlimited amount of nutrients to the growth of microorganisms.

E.g. Nutrient agar, Potato dextrose agar.

10. Write short notes on selective media?

The nutrient media, which provide nutrients that, enhance the growth and predominance of a particular type of bacterium and do not enhance other types of organism that may be present.

E.g. Mac Conkey agar.

11. Describe briefly on differential media?

Certain chemicals or nutrients present in the nutrient culture media, may allow differentiation of various kinds of Bacteria.

E.g. Blood agar – One can distinguish between hemolytic and Non-hemolytic bacteria.

12. Write the difference between the solid media and semi solid media?

The solid agar containing solidifying agent like agar powder about 1.5 to 2.0 percent.

Semi solid media prepared with agar about 0.5 or less agar have shoft custard like consistancy and are useful for the cultivation purposes silica gel is sometimes used as on inorganic solidifying agent or determination of bacterial motility.

13. What are psychrophiles?

Psychrophiles are the micro organisms which able to grow at the temperature of 0°C or lower, but they grow best at the optimum temperature of 15°C to 20°C the term **psychrotroph** or facultative psychrophiles is used those microorganisms able to grow at 0°C but which grow best at temperature in the range of 20°C to 30°C. **E.g. Vibrio psychroerythrus.**

14. What are thermophiles? Give suitable example?

Thermophiles are the microorganisms grow best at the temperature about 45°C i.e., higher temperature. The facultative thermophiles are grow best up to the mesophilic region or up to

30°C. True thermophiles, obligate thermorphiles or stenothermophiles are maintaining their growth at higher temperature as 45°C and above.

15. Compare the Aerobic and Anaerobic Bacteria?

Aerobic bacteria require free oxygen for their growth and can grow when incubated in an air atmosphere. They require 21% oxygen.

Anaerobic bacteria do not require free oxygen (or) atmospheric oxygen for their growth. Oxygen is toxic for them and O_2 inhibit the growth.

16. How oxygen toxic to the growth of microorganisms?

1. Oxygen inactivation of enzymes, when molecular oxygen can directly oxidize certain essential reduced groups, such as thiol (-SH) or enzymes resulting in inactivation. E.g. **Nitrogenase** inhibited by O_2 availability.
2. Damage due to toxic derivatives of oxygen. In certain reaction, addition of single electron to in oxygen molecule forms superoxide radical (OH), which is toxic.

17. How will you culture Anaerobic bacteria?

Providing the atmosphere, which is lack of atmospheric Oxygen, can culture anaerobic bacteria. There are 2 methods of culturing as;

1. **By Prepared media:** Medium is boiled for several minutes to drive out the free oxygen and reducing agents like Cysteine is added in the media to further lower the oxygen.

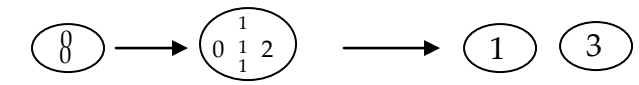
2. **By Anaerobic chamber:** This refers to a plastic anaerobic glove Box contain the atmosphere of H_2, CO_2 and N_2 and culture media is placed in the chamber which prevent the entry of free oxygen.

18. Write the method of reproduction in Bacteria?

1. **Binary fission:**

The most common method of bacterial reproduction and growth is by **transverse Binary fission.** In which single cell divides after developing a transverse septum (cross well) is asexual reproduction process.

Some Bacteria may undergo by the **budding** process and few others are asexually reproduced by **fragmentation** method.



Porent cell division New cell formed

Binary fission method

19. What is generation time of Bacteria? How will you calculate it?

During cell division or multiplications of bacteria the population doubles at regular intervals. The time interval requires to double the cell population is called **Generation time.**

Generation time can be calculated as:

$$g = \frac{t}{N} = \frac{t}{3.3(\log_{10} N - \log_{10} N_0}$$

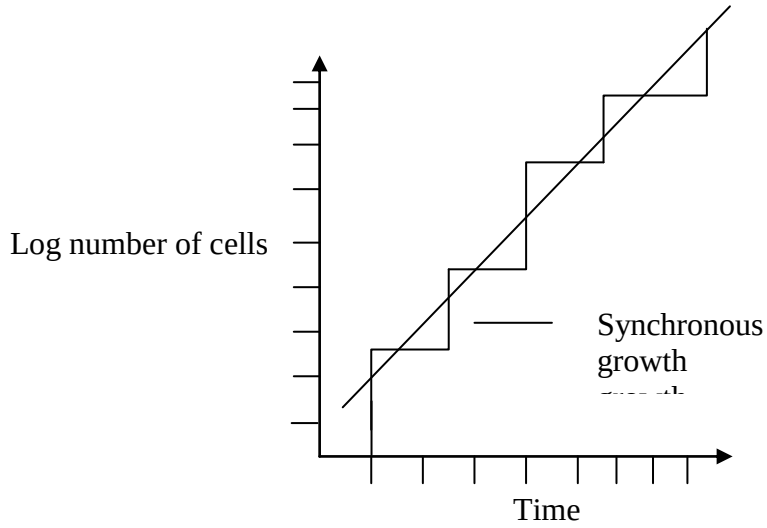
$t =$ time period

N = Number of cells during the time

N_0 = *Number* of cells at the initial stage.

20. Define Synchronous growth of Bacteria?

The occurrence of growth cycle of bacterial cells at the same stage by manipulating the culture in the laboratory is called Synchronous growth. Synchronous last only for few generation and it is required for research studies to relate the various aspects of growth, organization and differentiation at a particular stage.



Synchronous growth of Bacteria

21. What is a transitional period in the growth of bacteria?

A transitional period is a time catches up of bacteria from one growth phase to another stage of growth phase during the Growth cycle. The cultures proceed gradually from one phase of growth to the next stage.

E.g. from log phase to log phase or any other stage.

22. How will you calculate the growth rate of Bacteria?

The bacteria growth rate is calculated during exponential growth phase or log phase. The growth rate is reciprocal to the generation time. I.e. the number of generation per hour. It is obtained as a straight line of slop when the log number of cells is plotted against time.
Growth Rate (R) is calculated as

$$R = \frac{3.3(\log N - \log_{10} N_0}{t}$$

23. Describe chemostat?

Chemostat is the system provides continuous growth for microbial cells in the culture medium or environment. Here the medium is constantly maintained with **chemical nutrients** by fresh addition at the particular dilution rate. Hence, the growth of cells or population of microorganisms is maintained constantly. Using special instruments automatically sets this condition of environment.

24. What is Turbidostat? Write the application?

Turbidostat is the system or equipment set up which function based on the Turbidity occur in the culture medium due to the growth of bacterial or microbial cells. The cell population is maintaining constantly by use of sensor, which measure the turbidity.

This is used for provide continuous culture system of microbial cells during fermentation process or growth process.

25. Write any 2 methods used for quantitative estimation of bacteria by direct method?

1. Cell count:

The bacterial cell population is counted by using microscope with counting chamber.

2. Cell mass:

The cell mass is estimated by weighing the dry cell mass or by estimating the nitrogen content.

26. Define Cfu? Where it is used?

Cfu stands for colony forming units (Cfu). When the live cell population of bacteria is estimated by colony counting method by growing the cells in an agar plate as individual colony.

This method is otherwise called as **serial dilution plate method or viable count method.**

27. Brief any 2 indirect methods uses for quantitation determination of Bacterial growth?

1. **Turbidometry method:**

   Here the turbidity of the culture medium is determined by photocolorimeter and using standard values used the leadings for calculation of population.

2. **Measurement of cell activity by chemical change :**

The measurement of cell activity like acid production or enzyme production or any other product formation is used for calculating the population of Bacteria.

28. During log phase growth of a bacterial culture.

A sample is taken at 8 a.m. and found to contain 1,000 cells per ml. A second sample is taken at 5.54 p.m. and is found to contain 1,00,000 cells per ml. What is the generation time in hours?

29. During log phase of growth the numbers of bacteria remain constant? Does this mean the cells are dormant and inert? Explain?

Yes, during the log phase of growth the number of cells or population is remain constant because, during the initial period the bacterial cells require some time period to initiate their growth. That is adaptation in the new environment and increases of cell contents for cell division etc. are the factors for the cells remain constant.

30. Compare endergonic and exergonic reaction in cells?

During the course of chemical reaction is occurred the energy is utilized or released and it is referred to as free energy change (ΔG) of the reaction. When the energy (ΔG) in released during

the reaction in the cells it is called **exergonic reaction.** When the energy is consumed than the reaction requires energy (ΔG) is called **endergonic reaction.**

31. What is oxidative phosphorylation?

The flow of electrons during the chemical reaction (oxido-reductive) is occurring through various electron carrers and electron carrier enzymes. During the flow of electron through the chain of carriers, much of the free energy is conserved in the form of ATP, which process is called **Oxidative Phosphorylation.**

32. Compare Oxidation and reduction reaction?

Oxidation reaction refers loss of hydrogen atoms and Lon of electrons. Therefore the oxidation reaction occurs. **Reductant** is required in this reaction.

Reduction reaction will absorbs electron and gains electron therefore in the reaction the compounds reduced. **Oxidant** is required.

33. Write short notes on Oxidation-reduction potential or electromotive potential?

Electromotive potential to the expression of power or energy due to electron transfer, which is measured electrically under, standardized conditions and expressed as volt. The tendency to absorb electrons is **electromotive potential.**

34. Define fermentation?

Fermentation is the process of breakdown or decomposition of complex substances like polymeric compounds to simple substances like monomers and oligomers and subsequently to products by the action of **Microbial Organisms** is the presence of oxygen or absence of oxygen. The presence of oxygen is called **Aerobic Fermentation** and the absence of oxygen is called **Anaerobic fermentation**.

35. What are the products that are produced by fermentation of glucose by streptococcus lactis?

Streptococcus lactis is a lactic acid bacteriua, which is hetero fermentative. When ferment the glucose with streptococcus lactis following products will be formed.

$$\text{Glucose + water} \xrightarrow[\text{lactis}]{\text{Streptococcus}} \text{Pyruvic acid}$$

$$\text{Pyruvic acid} \xrightarrow{\text{Fermentation}} \text{lactic acid + } (CO_2)$$

36. What is amphibolic cycle/ Give an example?

During the cellular metabolism reaction, both the **catabolism** (breakdown of products) and **anabolism** (Synthesis of products) reaction occur, which is called **amphibolic cycle.**

E.g. Tricarboxylic cycle (TCA cycle).

37. Write brief note on Glycolysis?

This is most common pathway of glucose Catabolism is otherwise Embden-Meyerhof pathway of **Glycolysis** (splitting of sugar). This process occurs in microorganisms, plants and Animals. This does not require the presence of oxygen and therefore can occur in both aerobic and anaerobic cells. The overall reaction is as follows.

$$\text{Glucose} \xrightarrow{\text{Glycolysis}} \text{Pyruvic acid} + 2\text{ATP}$$

38. Briefly explain PPP (Pentose Phosphate Pathway)?

This is also a glucose catabolism pathway which occurs in both prokaryotic and Eukaryotic cells. This has been viewed as a "Shunt" of Glycolysis; hence it may also be called the hexose monophosphate shunt. Glucose is oxidized through the pentose phosphate pathway with the liberation of electron pairs, which may enter the respiratory chain. It is an alternative pathway for oxidation of glucose; it is also a mechanism for obtaining energy from 5- carbon sugars.

39. How many ATP, are produced during the glucose metabolism in microbial cells aerobic process?

1. Total number of ATP produced

(i). Total Number of ATP produced (8+6+24) (synthesised) } – 38*ATPs*

(ii). Total Number of ATP utilized - 2 ATP

Net gain of ATP during glucose metabolism in Aerobic process } - 36 ATPs

40. How alcohol is synthesized for glucose substrate?

Glucose $\xrightarrow{\text{Glylolysis}}$ Pyruvic acid +

Pyruvic acid $\xrightarrow{\text{Kreb's cycle}}$ Acetyl CoA +

Acetyl CoA $\xrightarrow[\text{dehydrogenase}]{\text{Alcoholic}}$ Ethyl alcohol + Acetic acid

PART – B

1. Write an essay on Nutritional requirements of Bacteria for their growth?

The bacterial growth dependant mainly on water, nutritional substances for cell synthesis and obtain energy. The Nutritional substance dissolved in water is called as a nutrient media. Different microorganisms have different media composition and environment. The nutritional media for microorganism must fulfill the following requirements.

1. **Elementary nutrients:**

a. Macro elements: Macro elements are the nutrients required in higher quantity for their growth and metabolism. The macro elements belongs to

Carbon (C)
Hydrogen (H)
Oxygen (O)
Nitrogen (N)
Sulphur (S)
Phosphorous (P)
Potassium (P)
Sodium (Na)
Calcium (Ca)
Magnesium (mg)
Furious (fe)

Which are considerably available in the culture media.

b. Micro elements:

Micro elements (or) trace elements are as group of Inorganic elements which require in a small quantity such as **nanogram, Milligram level.** The microelements are belongs to

Manganese (Mn)
Moleptenum (Mo)
Zink (or) copper (Cu)
Cobalt (Co)
Nickel (Ni)
Vanadium (Va)
Boron (Bo)
Chlorine (Cl)
Selenium (Se) and
Tin (T)

Almost all organisms require their macronutrients in a small quantity as nanogram to Milligram.

C. Heavy metals:

There are part of enzymes for metabolism of inorganic elements and compounds. i.e., O_2, H_2, S, P, K Most of heavy metals compounds are toxic at lower concentration example for heavy metals an Hg, Cu, Zn, Ni, Co, Cd, Ag, Cr, & Se. Most elements are provided in media as salt forms.

2. **Carbon and Energy sources:**

Microbes obtain energy is a **Photosynthesis** or **Oxidation** of inorganic compounds and use carbon dioxide as a main carbon sources. Autotrophs reduce CO_2 to carbon. Mostly from organic compounds. Microorganisms assimilate carbon to **cell material** and **energy.**

Carbon sources are Polysacharides as **starch** and **cellulose** & **Monomeric** form as **Glucose.**

3. **Acessary Nutrients**

Growth factors and **Vitamins** are certain accessory nutrients.

There are 3 types of accessory nutrients as;

1. Amino acids
2. Purine and pyrimidines bases from proteins and Nucleic acids.
3. Vitamins and co-enzymes or prosthetic groups that function as catalytic. They are requiring very less.

Autotrophs are dependant on the accessory nutrients.

Phototrophs are independent to accessory nutrients.

Sulphur and Nitrogen (S&N)

S & N are present in cells as sulfnitryles and amino groups.

Some can reduce atmospheric Nitrogen (N_2) Hydrogen sulfide and cysteine is sulphur compounds.

Nutritional types of Bacteria

Bacteria can be divided into 2 major groups passed on their nutritional requirement. They are given below.

1. Phototrophs

There are use in organic compounds are their source of electrons i.e., called as Photolithotrophs.
e.g. Chromatium okenii.

Some bacteria use organic compounds like fatty acids and alcohols are their source of electron and called as Photoorganogrphs.
e.g. Rhodospirillum rubrum.

2. Chemrotrophs:

Those bacteria use inorganic compounds as their source of electron i.e. called **chemolithotrophs. e.g**. **Nitrosomonas** use ammonia as their electron source.

Chemoorganotrophs are a group of bacteria which organic compounds like **sugars** and **amino acids** as their electron sources.

2. Describe various culture media used for culturing of micro organisms? How will you prepare the culture media?

For cultivation of bacteria basically 2 types of culture media are used as;

1. Simple media (or) chemically defined media:

Which contain chemical substances with known quantity dissolved in water and **agar** is **added** for **solid** media.

2. Complex media (or) undefined media:

Complex media or undefined media or Natural media contain complex raw materials like peptone, meat extract and yeast extract in addition to chemical substances. This supports variety of microorganism particularly **heterotrophic bacteria.**

Agar is generally added for **solidification** and gets solid media.

e.g. Nutrient Agar and Nutrient broth.

Types of Media

Based on the study purpose of bacteria like enumeration, isolation and identification, the culture media was classified in to numerous types on the basis of their application or function.

i. Selective media

These culture mediums provide nutrients that enhance the growth and predominance of a particular type of bacteria and do not enhance or may inhibit other types of organism.

E.g. cellulose contains media which enhance the growth of cellulose utilizing organisms and not others.

ii. Differential media

Certain chemicals and reagents present in the culture media may differentiate various kinds of bacteria from other organisms.

For example, blood-agar containing serum proteins distinguish the **hemolytic** organisms from **Non – hemolytic** microorganism on the same medium.

iii. Assay media

Certain media contain a prescribed compositions are used for the assay of **Vitamins amino acids and antibiotics** and testing of **disinfectants** by culturing of micro organism on the medium. This is called **assay media.**

iv. Enumeration media

Some kinds of media are used for determination of bacterial content of materials like milk, food materials and water. Their composition must adhere to prescribed specification.

E.g. **Potato dextrose agar** for **yeast & molds.**

v. Characterization media

A wide variety of media are conventionally used to determine the type of growth produced by bacteria and determine their ability to produce certain chemical changes.

E.g. **Triple sugar ion agar**
Indol agar.

vi. Maintenance media

Certain culture media require for the maintenance of the viability and physiological characteristics of a microbial culture over time may require a medium different from that, which is optimum for growth. Rapid growth also may be associated with rapid death of cells. So, show growth in a media is preferred for these purposes.

vii. Solid media

Solid media contain Agar power about 2% in the media with other nutrients and water. Agar provide solidification provide carbon to microorganisms, Solid media useful for isolation and characterization of bacteria.

viii. Semisolid media

It is appear as semisolid appear soft, custard like consistency, which contain agar concentration about 0.5 percent. This agar used for cultivation of **micro aerophilic bacteria** or for determination of motility of Bacteria. The above lyres of culture media are generally used for cultivation of bacteria in the laboratory.

Preparation of culture media

To prepare the microbial culture media either in the form of broth or solid media the following steps are followed.

i. The nutritional substances or natural substances are weighed in appropriate quantity required for media preparation.
ii. The preweighed quantity of nutritional compounds or chemicals are dissolved in a distilled water completely.
iii. It we need solid media, then 1.5 to 2.0% agar agar is added in the above solution.

iv. Check the P^H of the solution and adjust if necessary by using P^H meter.
v. Then the medium is boiled to dissolve agar and other contents.
vi. Now the medium is dispensed into glass tubes or culture flashes.
vii. Then the medium is sterilized by autoclave at 121°C for 20 minutes. The sterilization is done forheat labile ingredients as by the method of by Micro-filtration.
The above steps are necessary for culture media preparation.

3. Describe the methods involve in bacterial multiplication and their growth?

The growth and multiplication of bacteria is the occurrence of increase in **cell size** and **increase in cell number** or overall **population.** The complete cycle of bacteria for its growth and multiplication in a batch culture takes 24 hour of more. Nutrients are required for their growth continuously. The population or cell number of bacteria during it growth is determined by quantitative method.

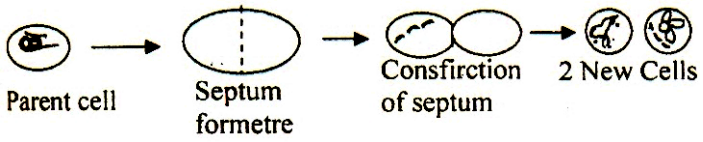
There are 4 types of reproduction or multiplication methods studied in Bacteria.

1. Binary fission or Division:

This is most commonly studied multiplication method in Bacteria. It is a mode of cell division or multiplication method. Which is called as **Asexual reproduction** method. In this process, one bacterial cell divides into 2 cells. i.e. Binary fission.

In this process the **transverse septum** is formed and the new cells are formed from the old cell steps involved in Binary fission are:

1. Each daughter cell drive complete genome
2. DNA precisely duplicate
3. No mitotic apparatus involved.
4. But, mesosome and cytoplasmic membrane play a role.
5. Formation of septum exactly middle portion of the cell.
6. Septum is formed after DNA synthesis.
7. Transverse Binary fission is formed.
8. Two new daughter cells formation.

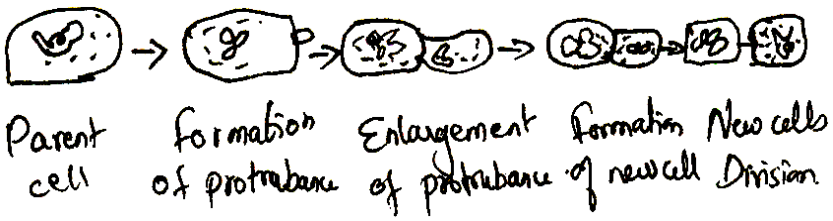


Stages of Binary fission

2. Budding:

Budding is an important method of cell multiplication method of Bacteria. In some species of bacteria Budding is occurred. During the process the following steps are occurred for new cell development.

1. In the parent cell, small protrubance is developed or formed at one end of cell.
2. The small protrubance enlarges and eventually develops as a new cell.
3. Then it separates from parent cell.
4. New cells grow and multiply.



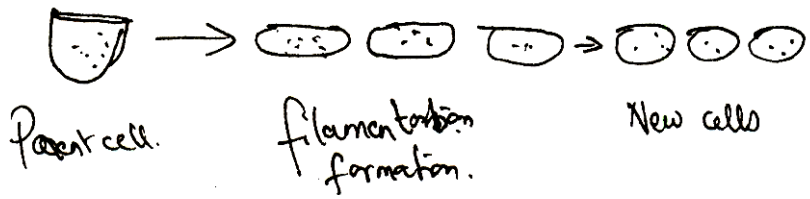
Budding type of multiplication

3. Fragmentation:

This is also a type of cell multiplication method in which the cells produce with extensive filamentous growth.

E.g. Nocardia spp.

The cells produce bacilli or cocci type of cells and each cell rise to new growth.

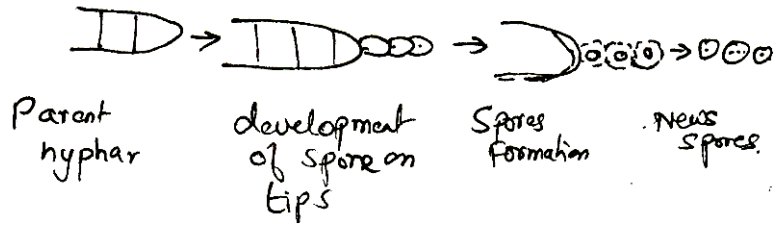


Fragmentation type

4. Formation of conidiospores:

Spores produced from the branch of **hyphal tip.** are develop from cross walls at the tips. Then each spore rise to as new organism.

e.g. Streptomyces



Conidiospore formation

spores are covered by death before release and produce **chain of conidiospores.**

During multiplication the nutrients are transferred from medium to cells by selective absorption process. Then the nutrients were converted by enzymes and Nuclear material formed & increased. Then cell elongation and increase of all contents. Then invagination of cell membrane takes place & formation of transverse cell well. Finally separation of daughter cells.

4. **Explain the bacterial growth and device equation for calculate the population, generation time, Number of generation?**

Bacterial growth and multiplication occurs by most common method as Binary fission. In this method 1 cell divides into 2 cells and whole population is derives.

i.e. From Single Bacterium to whole population.

$1 \rightarrow 2 \rightarrow 2^2 \rightarrow 2^3 \rightarrow 2^4$ …….. 2^n

1 -2 - 4 - 8 - 16 …….. X.

If no cell death, then the population will be double.

The population (N) at the end of the time or given period is

$N = 1 \times 2^n$

$$\boxed{N = 2^n} \quad \text{_____} \quad (1)$$

However, under practical condition the **initial population** (N_o) at the time of inoculation is not a single but several thousands.

So, the formula becomes,

$$N = No \times 2n \quad \text{_____} \quad (2)$$

N_o = Initial population
2 = Multiplication
n = Number of generation
N = Total population of cells.

Solving the equation of the above i.e. 2nd equation.

$$\log_{10} N = \log_{10} N_0 + n \log_{10} 2$$

$$n = \frac{\log_{10} N + \log_{10} N_0}{\log_{10} 2} ----(3)$$

Substitute $\log_{10} 2$, which is 0.301,
the equation becomes

$$n = \frac{\log_{10} N + \log_{10} N_0}{0.301}$$

$$n = 3.3\ (\log_{10} N + \log_{10} N_0) \text{_____}(4)$$

So, we can calculate the **number of generations (n)** with the above formula i.e. (4).

Generation time is the doubling time of the cells takes during the **Multiplication.**
i.e. double the population.

The doubling time may differ in different organisms during their growth process.

So, the generation time (g) can be calculated as:

$$g = \frac{t}{n}$$

$$g = \frac{t}{3.3\ (\log_{10} N + \log_{10} N_0} ----(5)$$

t = time period
g = generation time

n = number of generation

∴ Growth Rate (R) can be calculated as

$$R = \frac{n}{t}$$

$$R = \frac{3.3\ (\log_{10}N + \log_{10}N_0)}{t} \text{ ----(6)}$$

From the given populated and growth period. We can calculate the number of generation (n), generation time (g), time period (t) and growth rate (R) of the microbial cells.

5. Construct a growth curve and study the different phases of growth of bacteria in a given batch culture system?

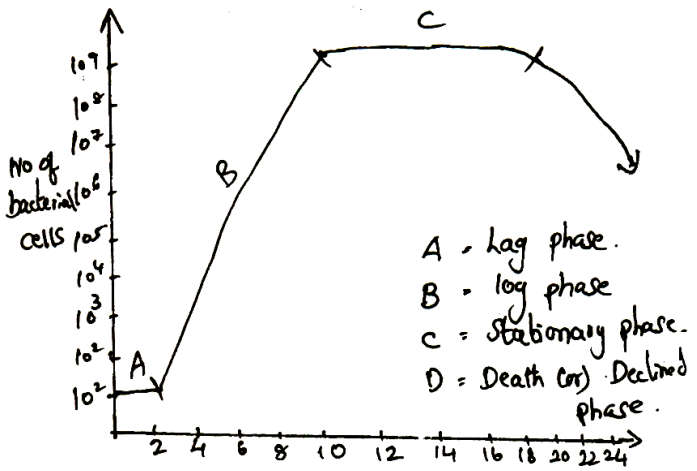
Normal bacterial growth cycle in a batch culture system means, the culture medium prepared in a flask or vessel is inoculated with predetermined quantity of bacterial cells. The inoculated medium is incubated at standard conditions like at a suitable temperature to allow the growth.

Then the growth of bacterial cells from the vessel containing culture medium is measured at different intervals period. Then the values are plotted in a graph against time periods.

From the above we can observe different phases of grown as;

1. Lag phase
2. The Log phase (or) exponential phase']
3. Stationary phase
4. Death phase (or) declined phase.

The following graph shows the different phases of bacterial growth in a batch system.



Different Growth phases of Bacteria

1. Lag phase

This is the initial phase of growth of Bacteria. During the phase, cell size increase but no change in the number of cells. Cell synthesis takes place for cell components. Cells absorb nutrients and take time to adjust with the environment due to change of medium and Age of cells. Younger cells takes less time and older cells take more time. Synthesis of cell components like ribosomes, protein, Nucleic acids, Enzymes, co-factors, ATP etc.

2. Log Phase (or) Exponential Phase

This is the second phase of growth cycle of Bacteria. During this phase, cells start to divide equally and grow steadily and constantly at maximum growth. The logarithmic stage of cells plotted in a graph as a straight line. Cells are uniform in biochemical composition and metabolic activity and physiological characters. In this stage, the population (N) of cells, Growth rate ® of cells, Number of generations (n) and generation time (t) etc., can be calculated. Growth rate depend on nutrient concentration. Balanced growth is occurred due constant environmental conditions. If environmental conditions change the unbalanced growth occurs. In this stage, specific nutrients are role play, which are called as growth limiting substrate. All the metabolic products are formed i.e. primary metabolites like alcohol, organic acids, amino acids etc., i.e., Growth associated products.

3. Stationary phase

Growth of cells ceases and there is no multiplication. It attains up to 10^9 cells/ml population. The steady growth lines stops and maintain Horizontal line population remain constant for certain period. This is due to depletion of nutrients, depletion of O_2 and accumulation of toxic waste. Sometimes starvation of cells also occurs. Secondary metabolites like antibiotics, Vitamin and co-factors etc. are formed. Cells get lyses and lysed product also support-the growth of other cells. Still this phase is maintained at the end, and the graph line starts to decline.

4. Death phase

During this phase the cells start to drying due to depletion of Nutrients environmental change, accumulation of inhibitory products like toxic inhibitors and various acids. Rate of Death vary depending on type of bacteria. Death rate mostly in logarithmic fashion. Death rate (R_D) also can be calculated based on the population dying with time period.

6. Discuss the different quantitative methods employed for the measurement of bacterial growth?

Measurement of the growth of population or magnitude of population of microbial cells is done by counting or estimating quantitatively. Otherwise it is counting the cell numbers or determining the cell mass (biomass).

There are many techniques available for estimation of bacteria quantitatively.

1. Cell count

Counting of cell numbers from the culture suspension by microscopic or Electronic counting device.

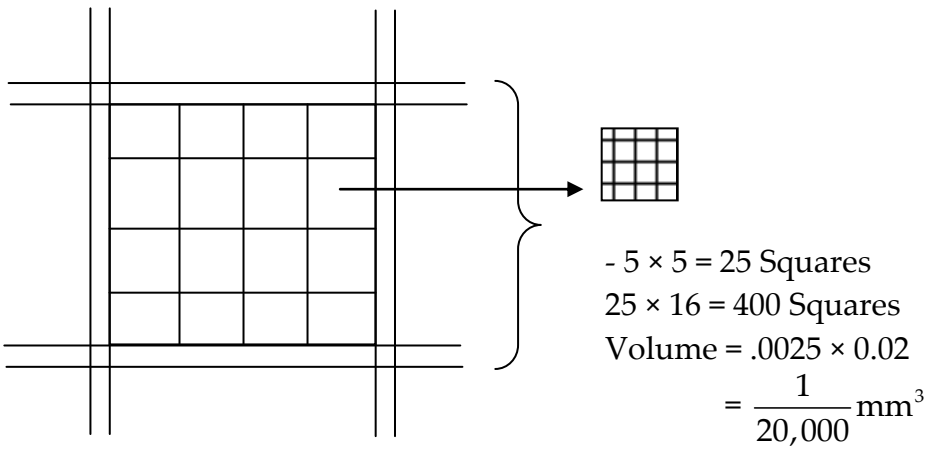
a. Microscopic count

Microscope is used to focus the counting chamber, which consists of chamber for keeping the cell suspension.

The counting chamber called Petroff-Houser counting chamber or Haemoeytometers are used counting chamber is a special slide ruled to squares and the square of counting area is $\frac{1}{400} mm^2$ *i.e.*$0.0025 mm^2$.

The height or depth is $\frac{1}{50} nm.\ or\ 0.02 nm$ the total number of squares about 400 squares

i.e. **1 mm².**



Counting chamber Haemocytometer

Bacterial suspension placed on **Slide grid** and **covered** with **cover slip.**

The cells occur in big square containing 25 squares are counted randomly. The average numbers of cells were calculated.

Calculation:

No. of cells per square (1 of 16 of 25) = N

N x 20,000/ ml

N x 2 x 10^4

if the N is 10 cells than 10 x 2 x 10^4

= 20 x 10^4 (or)

= 2 x 10^5 cells / ml.

[It cells are dense in culture, it can be diluted.]

2. Electronic counting of cells

There the electronic particle counter is present in the device.

Bacterial suspension placed inside and passes through the tiny **orifice** of 10 to 30 micron**.**

Orifice connects 2 compartments, which contain electrically conductive solution. Bacterial solution passes through orifice and electrical resistance increase in components. Which generate electrical signals and counting will be done automatically.

3. Plate count (or) Colony counting method

1. Bacterial cell suspension diluted to suitable dilution factor.

2. Then the diluted sample is inoculated in a suitable agar media and incubated at suitable atmosphere.

3. Then the colonies formed on the agar plates are counted by special counting device called Bacteria colony counter. The colony number to be counter should be in the range of 30-300 in a plate. It is to be illuminated background.

Magnifying glass (or) lens is used. The number of colony or count is called as colony forming units (Cu/ml or mg). Food samples like mike, water and food etc., can be analysed by this method.

4. Turbidometric method

Bacterial and yeast cells contain culture suspension can be analysed by this method. It the cell suspensions contain higher concentration then suitable dilution to be made before analysis.

The number of cells up to 10^7 & 10^8 cells / ml. Colorimeter (or) spectrophotometer is used. Live and Dead cells produce turbidity in the media is used for taking reading. The OD value of the sample is compared with standard values of cells (or) colony forming units and calculation is alone & values are expressed.

5. Determination of dry weight of cells

The dense population contain cells are suitable by this method.

Cell mass is harvested for its cell content by centrifugation method.

Then the cells are washed repeatedly and centrifuged.

After repeated centrifugation and washing the dried cells was weighed and the biomass is determined.

Here increased dry weight not related to growth.

This **method** is useful in **Research** other methods like.

N_2 content estimation are biochemical activity determination are indirect methods of measurement.

7. Describe the Embden-Meyerhof glycolytic Pathway in Microorganisms?

The most common pathway of glucose catabolism is the Embden-Mayerhof pathway (EMP) of glycolycis (splitting of sugar). This process occurs very widely and is found in microorganism as well as in animals and plants.

Glycolysis does not require the presence of oxygen and therefore can occur in both **aerobic and anaerobic cells.** Aerobic cells degrade glucose by glycolycis and this process contributes the preparatory stage for the aerobic phase of glucose oxidation. Thus, under anaerobic conditions this situation prevails;

Glucose $\xrightarrow{\text{Fermentation}}$ Fermentation products

Whereas under aerobic conditions, the following occurs:

Glucose $\xrightarrow{\text{Fermentation}}$ Intermediate (Pyruvate) $\xrightarrow[O_2]{\text{respiration}}$ $CO_2 + H_2O$

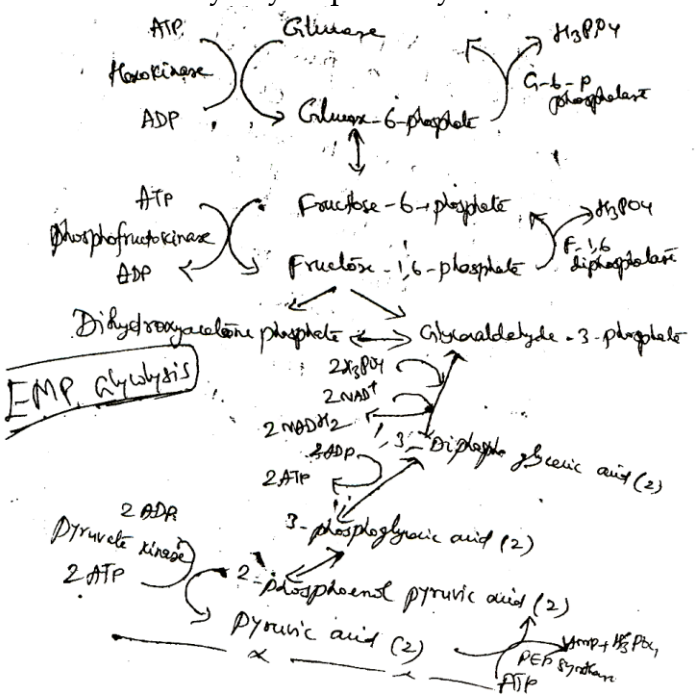
In Glycolysis, glucose converted to glucose -6- phosphate and from fructose-6- phase. Then the fructose -6- phosphate is split into 3-carbon units as dihydroxyacetone and glyceraldehyde -3- phosphate and they are subsequently oxidized into Pyruvic acid.

At this slip, where glyceroldehyde -3- phosphate is oxidized a pair of electrons is removed. In the absence of oxygen, this pair of electrons may be used may be used to reduce Pyruvic acid to lactic acid O_2 ethanol. In the presence of oxygen this pair of electron may enter the respiratory chain.

For each molecule of glucose metabolized two molecules of ATP are used up and 4 molecules of. ATP is formed. Therefore for each molecule of glucose metabolized by Glycolysis, there is a net yield of **two ATP** molecules. The overall reaction of Glycolysis can be summarized as follows:

$C_6H_{12}O_6 + 2NAD + 2ADP + 2\ P_I \rightarrow 2CH_3\,COCOOH + 2NADH_2 + 2ATP$

The following figure shows the EMP Glycolysis pathways.

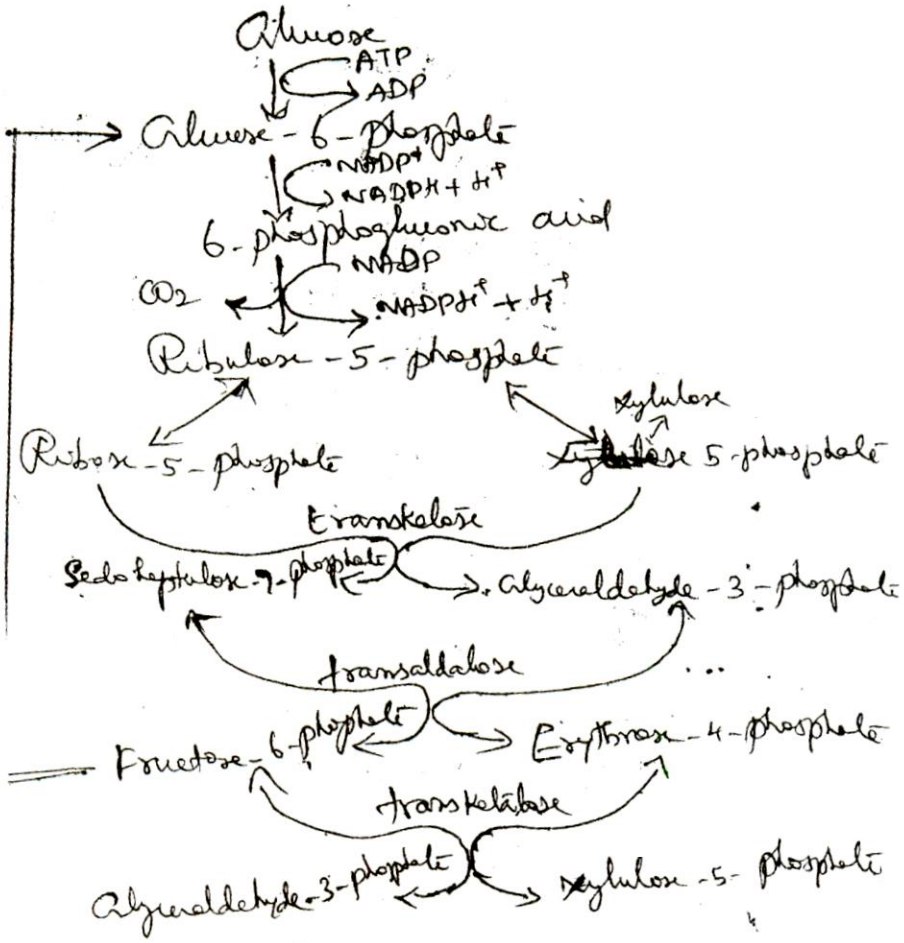


8. Describe the pentose phosphate pathway (PPP) glycolytic pathway?

The other glycolytic pathway for glycose metabolism is called pentose phosphate pathway (PPP). This occurs in both prokaryotic and Eukeryotic cells. Since it involves some reactions of the glycolytic pathway, it has been viewed as a "shunt" of Glycolysis, hence it may also may called the hexose monophosphate shunt. Its other synonym is the phosphor-gluconate pathway.

The pentose phosphate pathway with the liberation of electron pairs, which may enter the respiratory chain, can oxidize glucose. However, this cycle is not generally considered a major energy yielding pathway in most microorganisms. It provides reducing power in the form of NADPH + h + which is required in many biosynthetic reactions of the cell, and it provides pentose phosphates for are in nucleotide synthesis. Although it can produce energy for the cell as an alternate pathway for the oxidation of glucose, it is also a mechanism for obtaining energy from 5-carbon sugar.

The following diagram shows the PPP Glycolysis.



In PPP, phospherylation of glucose form glucose -6- phosphate; the later is oxidized to 6-phosphogluconic acid with the simultaneous production of NADPH. Decarboxylation of 6-

phosphor gluconic acid, together with a yield of NADPH, produces ribulose -6- phosphate. Epimerization reactions yield Xylulose -5- phosphate and ribose -5- phosphate. These two compounds are the starting point for a series of transketolase reactions and transaldolase reactions leading subsequently to the initial compound of the pathway, 6- phosphor gluconic acid, thus completing the cycle. Two intermediates of Glycolysis fructose -6- phosphate and glyceraldehyde -3- phosphate are formed.

The overall reaction is summarized as:

6 Glucose -6- Phosphate +12 $NADP^+ \rightarrow$ 5 glucose -6- phosphate + 6 CO_2 + 12 NADPH + 12 H^+ + P_i

Glucose -6- phosphate + 12 NADP $\rightarrow$ 6 CO_2 H_2 NADPH $^+$ + 12 H $^+$ P_i

The PPP feeds into the glycolytic pathway by means of fructose -6- phosphate and glyceraldehyde -3- phosphate.

9. Discuss the TCA cycle and its role in fermentation process?

TCA cycle is otherwise called as Tricarboxylic acid cycle or citric acid cycle or Kreb's cycle.

In this cycle the series of reactions that generate energy in the form of ATP and reduced co-enzyme molecules ($NADH_2$ and $FADH_2$). It also performs other functions. Many intermediate in this cycle are precursor in the biosynthesis of amino acids, urines, pyrimidines etc. for examples oxalo acetic acid and keto-glutoric acid are amino acid precursors as shown in the following reaction.

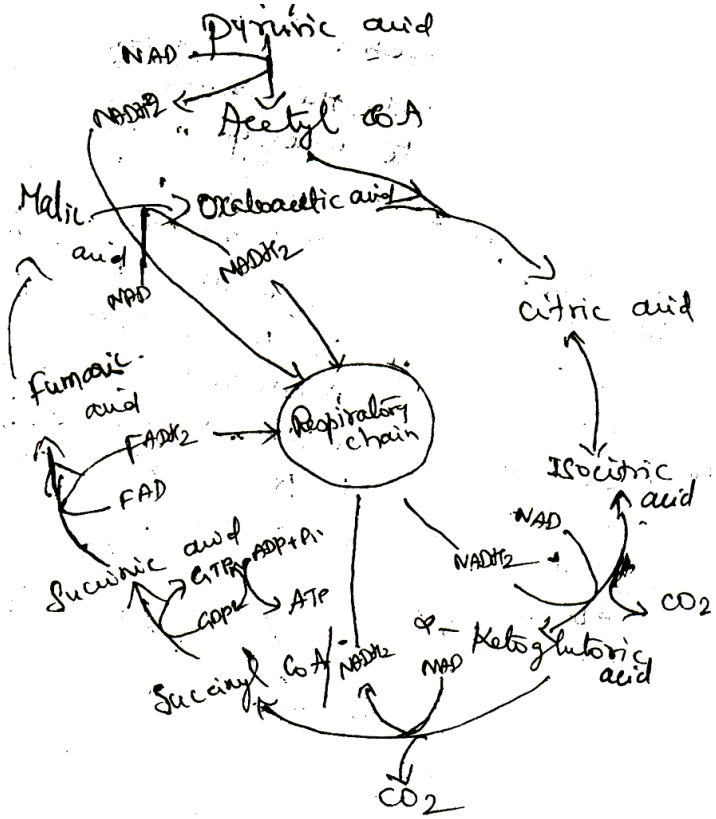
xaloacetate $\rightarrow$ Aspartate $\rightarrow$ Methioxine

Lysine threonine

2- ketoglutoric acid $\rightarrow$ Glutamate $\rightarrow$ Glutamine

Argixine Proline

Thus TCA cycle is an amphibolic cycle, which means that it functions not only in catabolic (breakdown) but also anabolic (synthesis) reaction. The cycle is shown in following figure.



The overall reaction of the TCA cycle can summarized as follows;

$$\text{Acelyl COA} + 3H_2O + 3NAD^+ + FAD + ADP + Pi \rightarrow 2CO_2 + COA + 3NADGH_2 + FADH_2 + ATP$$

On the TCA cycle 3 types of carbon compounds as 6 carbon compound, 5 carbon compound and & carbon cup up are formed.

Here, 2 Acetyl COA molecules are produced from 1 molecule of glucose. There twice the above reaction is takes place.

On the TCA cycle totally
24 HTP molecules are produced
18 ATP molecules from 6 $NADPH_2$
4 ATP molecules from 2 $FADH_2$
2 ATP molecules from 2GTP.

10. Discuss in detail about the lactic acid and mixed acid fermentation pathways occurring in Bacteria?

Anaerobic microorganisms also produce energy by reaction called fermentation, which use organic compounds as electron donor's and acceptors. Facultative anaerobic bacteria different kinds of fermentation to produce energy example are given below.

(1) lactic and fermentation

Streptococcus lactis, the bacterium is responsible for the normal souring of raw milk, dissimilates glucose to lactic acid, which accumulates in the medium as the sole fermentation product.

The fermentation process of lactic acid is by Glycolysis one molecule of glucose is converted to two molecules of pyruvic acid with concomitant production of two NADH $+H^+$. The Pyruvic acid is converted to lactic acid in the following reaction.

$$2CH_3COCOOH + 2NADH + 2H^+ \xrightarrow{\text{Reduction}} \underset{\text{Lactic acid}}{CH_3CHOHCOOH} + 2NAD^+$$

There are the types of lactic acid fermentation process by bacteria is studied.

(1) Homolactic Fermentation:

In Homolactic acid fermentation process Pyruvate is converted to lactic acid by reduction with Lactate dehydrogenase. No gas is produced e.g. Streptococcus lactis

$$\text{Pyruvic acid} \xrightarrow[\text{lactate dehydrogenase}]{\text{Reduction}} \text{Lactic acid}$$

(2) Heterolactic acid fermentation

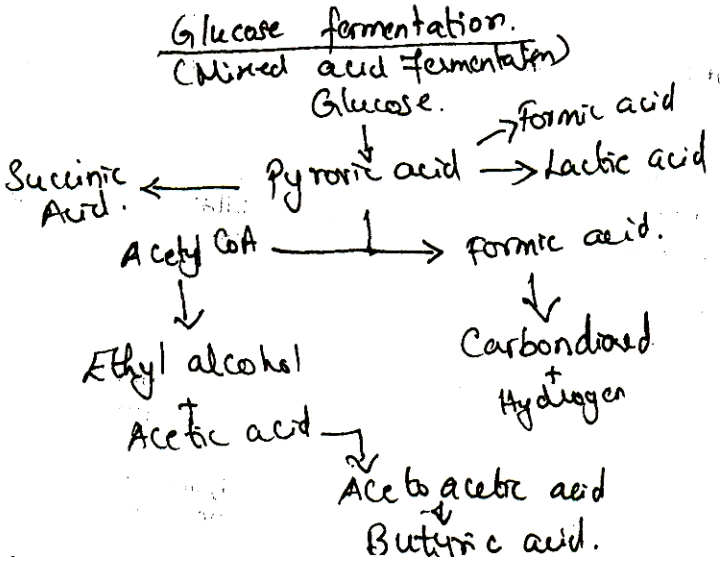
In heterolactic acid fermentation process, pyruvate is converted to Lactic acid and carbon dioxide. Here gas (CO_2) is produced. More amount of lactic acid and less amount of CO_2 are produced.

$$\text{Pyruvic acid} \xrightarrow[\text{lactate dehydrogenese}]{\text{Reduction}} \text{Lactic acid} + CO_2$$

II. Mixed Acid fermentation Process

In this fermentation process, glucose is converted to pyruvic acid and pyruvic acid is converted to many organic acids by different bacterial organism. This is called mixed acid fermentation.

In this anaerobic fermentation, process pyruvic acid is key compound and its is converted to various organic acids like propionic acid, Acetic acid, Formic acid, succinic acid, ethyl alcohol, Butyric acid, butyl alcohol, acetone, Isopropyl alcohol, gluconic acid and kojic acid by various species of bacteria. The following schematic diagram shows the process.



e.g. enterobacter- Acetic acid, Gluconic acid, kojic acid
Escherichia – formic acid, acetic acid, lactic acid
Closteridium- Butyric acid, acetic acid, formic acid
Acetobacter- Acetic acid, gluconic acid
Propionic bacterium- probionic and
Lactotobacillus – Lactic acid

Amino acids fermentation

This is otherwise called stick land reactions.
e.g. proteolytic bacterium

Clostridium

Fermentation of mixer of amino acids

Amino Acids

↓ -Oxidation

Other Amino acid (secondary amino acid

↓ -Reduction

Acetate, CO_2, NH=3

Amino acid fermentation are practically importance due to these process, in food product is give flavour (diacetyl flavour in milk) due to production of certain compounds which are responsible for flavours.

11. Briefly discuss the utilization energy by microorganisms and biosynthesis of important molecules?

Energy released in the glycolysis and fermentation process is utilized by the cells to synthesis of biological molecules which are require by cell for growth and multiplication process. The energy from ATP molecules is used to convert one chemical substance to another and to synthesize complex substances from simple molecules, which is otherwise called Biosynthesis.

(i) <u>Synthesis of smaller molecules like amino acids</u>

Amino acids about 20 different amino acids are available in cells which are the building blocks of proteins. The sequences and manner in which they are linked in determine the type of proteins.

Amino acids are formed form the intermediate products of Tricarbonxylic cycle or Kreb's cycle an oxaloacetic acid.

2-ketoglutanic acid, and
Succinic acid which are derive from pyruvic acids.

1.xaloacetate $\rightarrow$ Aspartate $\rightarrow$ Methioxine

Lysine threonine

2. α- ketoglutoric acid $\rightarrow$ Glutamic acid $\rightarrow$ Glutamine

Argixine Proline

Likewise all the 20 amine acids are synthesized.

II. <u>Synthesis of Macromolecules – (Cell wall –peptidoglycan)</u>

Peptidoglycans are composed of 1. Acetyl glucosamine, 2. Acetyl Muramic acid and 3. Peptide of 4 to 5 amino acids of limited variety.

The synthesis of peptidoglycan is as,

1. Amino acids are subsequently linked to acetyl muramic acid (AMA) requires energy (ATP)
2. The AMA-UDP precursor is coupled to membrane phospholipids called bactopremol.
3. the Acetylgluosamine (AGA) couples with AMA-UDP processor. This reaction requires the activated form of AGA, i.e. AGA-UGP derivative.
4. The precursor still linked to Bactopremol, is carried out of the cell through the cell membrane and is linked to a growing peptidoglycan chain in the cell wall.

III. Bio synthesis of Deoxyribo nucleic acids

Synthesis of DMA molecules requires a template of another DNA.

Bacteria can synthesize DNA, provided that the intracellular pool of nucleotides must be available. This reservoir of nucleotides can be synthesized from glucose, ammonium sulphate and some minerals. This process needs complex serially enzyme catalysed reaction and require energy (ATP)

$$\text{Nucleotide} + \text{ATP} \xrightarrow{\text{Kinase}} \text{nucleotide} - \text{phosphate} + \text{ADP}$$

$$\text{Nucleotide Phosphate} + \text{ATP} \xrightarrow{\text{Kinase}} \text{Nucleotide} - \text{di} - \text{phosphate} + \text{ADP}$$

As seen in the above equation energy in the form of ATP is utilized.

$$\left.\begin{matrix}\text{Nucleotide di-phosphates or} \\ \text{Nucleotide tri-phosphates}\end{matrix}\right\} \xrightarrow[\text{Mg}^{2+}\ \text{DNA polymerase}]{\text{preformed DMA}} \underset{\text{Inorganic Pyrophsophate)}}{\text{PPi}} + \text{New DNA strand}$$

The new DNA strand is complementary to the performed or template DNA strand

IV. Protein synthesis:

The first step in the protein syntheses is activation of amino acids. The Amino acids are activated by amino acids-activating enzymes called aminoacyl - tRNA synthesis. This activation reaction requires energy in the form of ATP.

$$\underset{\text{(enzyme)}}{\text{AminoAcidd} + \text{ATP} + \text{E}} \rightarrow \underset{\substack{\text{Aminoarcycl} \\ \text{adenylate -E}}}{\text{aminoacid.amp.E}} + \underset{\text{Pyrophosphate}}{\text{PPi}}$$

There is a specific activating enzyme for each kind of amino acid. The activated amino acid remains tightly bound to the enzyme after activation. Next the activated amino acids binds to an RNA molecule called transfer RNA ($_t$RNA). This reaction is catalyzed by the enzyme that was originally bound to the Amino acid.

$$\text{Amino acid .AMP-E} + {}_t\text{RNA} \rightarrow \text{amixoacid}_t\text{RNA} + \text{AMP} + \text{E}$$

The tRNA functions in protein synthesis to carry amino acids to and recognize codons in $_m$RNA. Then the subsequent process form derives of amino acids in chain and polypeptide chain make or synthesizes protein.

UNIT – IV

PART – A

1. Why we need to control of microorganisms?

The reason for control of microorganisms is;

i) Prevention of disease and it transmission

ii) Prevention of contamination or the growth of unwanted microorganisms

iii) Prevention of deterioration or spoilage of materials control of microorganisms means,

iv) Reduction in number of population

v) Reduction in activity of microorganisms.

2. What are different methods involved in control of microorganisms?

1) Control through physical agents such as heat, cold pressure, desiccation, incineration, Radiation and filtration.
2) Control through chemical opens, such as phenolic compounds, Alcohol halogen heavy metals, dyne related chemicals etc.
3) Biological methods like antibacterial, antifungal, anti-viral compounds available in nature or with other organisms

3. Write any 4 mode of action of antibacterial agents?

i) Damage to the cell wall by inhibiting the synthetic of the cytoplasmic membrane
ii) Change of physical of chemical state of protein and nucleic acids.
iii) Inhibition of enzyme action.

4. What is meant by thermal death time?

Thermal death time can be explained as the shortest period of time to kill bacterial cells at a prescribed temperature and at specific conditions. Thermal death time is calculated to understand the temperature regime for destruction.

5. Define decimal reduction time?

i) Decimal reduction time is the time taken to reduce bacterial population by 90% of stated it differently. That is the bacterial population less than 1 in 1000. It is the time in minutes for thermal death curve to pass through one log cycle.

6. Give any 2 physical agents, which prevent the growth of microorganisms?

1. High temperature:

High temperature treatment in the form of dry heat or wet (Moist) heat kills the microbes either spores or vegetative cells. High temperature coagulates the protein and inhibits the metabolism. Heat in the form of many ways used to kill bacterial.

2. Radiation:

Radiation is the type of physical factor, which controls the growth of bacterial. Energy transmission through space in a variety of forms in generally called radiation which all to kill the radiation by damage of cells. (e.g.) electromagnetic radiation.

7. What is mean by fractional sterilization?

It is a type of heat sterilization at 100°C at different intervals of period. When the nutrients present in the media are must suitable to heat above 100°C are sterilized by fractional sterilization. Here the media in heated at 100°C and cooled for incubation for three successive days. Here the resistant spores germinate in between and young vegetative cells die at 100°C heating.

8. Explain briefly about pasteurization?

Louis Pasteur adopted the method of heat sterilization of fermenting product called pasteurization. Pasteurization is a type sterilization method, which was established by Louis Pasteur.

Pasteurization is a type sterilization method, which was established by Louis Pasteur. This is applicable to milk and milk products, fruit juices and certain liquid beverages. In this process, the liquids are heated at 62.8°C (145°C) and held for 30 minutes period or 71.7°C (161°F) for 15 seconds.

9. How ultraviolet light is harmful to microorganisms?

Ultraviolet light is shorter wavelength rays emit high energy, which is absorbed by cellular nucleic acids when exposed. The DNA synthesis is inhibited or the abnormal synthesis of DNA occurs due to formation of pyrimidine dimer in nucleic acids ultimately cell death occurs.

10. What is mean by cold sterilization?

Sterilization of biological materials by use of ionizing radiation is called cold sterilization. Heat sensitive materials are sterilization by ionizing radiation (e.g.) food and pharmaceutical applications.

11. Write briefly on Gamma rays and sterilization?

Gamma rays have high-energy radiation. Energy emitted from radioactive isotopes and this is shorter than x rays.

It hit DNA indirectly and cause death of cells useful in thicker material like food and Pharmaceutical applications.

12. Write any 4 characteristic of chemical sterilizing agent?

Ideal chemical agent used for sterilization posses the following parameters:

i) Antimicrobial activity:

It should kills bacteria at lower concentration and it should be broad spectrum.

ii) Solubility:

Most soluble in water and other related solvents to extend the effectiveness.

iii) Stability:

Changes of activity low and the loss should be minimum during storage losses.

iv) Non-toxicity to animals and human:

The chemical find not give damage or accident to animals and human.

13. Write short notes on Disinfectant? Give suitable example?

It is a chemical agent that kills the growing forms of Bacteria but not the spores. It is used in inanimate objects.

Disinfection is the process of destroying infection agents. (e.g.) phenolic compounds.

14. Write short notes on Bactericides?

Bactericides are a chemical agents that kills bacteria and the compounds are said to be contain bactericidal activity, similarly fungicide, viricide, and sporicide refer the agents kill fungi, virus and spores respectively.

15. Write short notes on Antimicrobial agent?

Antimicrobial agents are compounds while may be chemicals (or) biological origin that interferes with the growth and metabolism of microorganisms. In common usage, the term denotes inhibition of growth and with reference to specie groups of organisms such terms as antibacterial or antifungal are frequently employed.

16. Write short notes on chemotherapy? Give one suitable example?

Chemotherapy is a method of using chemicals to treat infections or diseases caused by microorganisms in human. The chemicals used for treatment of chemotherapy on microbial diseases called chemotherapeutic agent.

(e.g.) phenolic compounds.

17. Write any four chemical agents used for chemotherapy?

i) Phonol and phenolic compounds.
ii) Alcohol
iii) Halogens
iv) Heavy metals and their compounds.

18. What are the criteria used for selection of chemical compounds?

1. Nature of the material to be treated
2. Types of microorganisms
3. Environmental conditions**.**

19. Write short notes on phenol co-efficient technique?

This is a technique used for testing or evaluating the antimicrobial agents, which is approved by AOAC and FDA. In this technique the suitable dilutions of antimicrobial substances were made in water and tested against microorganisms and compared with dilutions of phenol in water. The organisms are staphylococcus aurous and *salmonella typhii*.

20. Write the application and mode of action of halogen compounds used for control of microorganisms?

Halogen compounds are good bactericidal compounds and effective all kinds of bacteria. It is applied on skin and wounds to reduce population, disinfectant in water and sanitizer in food utensils.

Mode of action:

ii) Inactivate the protein with sulfhydryl group.
iii) Halogenation of tyrosine unit of enzymes.

21. Briefly explain MIC?

MIC means the Minimum Inhibitory Concentration. It is used for testing of antimicrobial compounds against microorganisms. The minimum concentration or lowest dilutions of concentration required to inhibit or kill more than 50% of bacterial population is called Minimum Inhibitory Concentration (MIC).

22. How Alcohol controls the growth of microorganisms?

It acts as disinfectant in clinical & research purposes.

It denatures the proteins of organisms. It penetrates in cell membrane and destroys lipid compounds in cell membrane.

Alcohols having cleansing action when cleaning of utensils and glass articles and remove all the debris with microorganisms.

E.g. Ethanol, Methanol

23. Write the mode of action of heavy metals and their compounds? Give example?

i) Heavy metals and their compounds combine with cellular proteins and inactivate them.

(e.g.) $Hgcl_2$ inhibit the enzymes contain sulfhydryl groups.

ii) Heavy metal salts coagulate the cytoplasmic proteins and damage (or) destroy cell.

Mg	- 0.1%
Ag	- 0.1%
Cu	- 2 ppm

24. Brief note on quaternary ammonium compounds (QAM) and their antimicrobial action?

QAM are cationic detergent chemicals, which are germicidal.

It acts on both gram positive and gram-negative bacteria. It denatures the protein, Interference of Glycolysis and membrane damage cytoplasmic membrane.

It uses as preservative in opthalmic solutions and used in cosmetic preparation. It acts as disinfectants and sanitizing agents.

25. What are the techniques available for evaluating antimicrobial agents?

There are three methods are used in evaluating antimicrobial compounds as,

i) Tube dilution and agar plate count method
ii) Agar plate count method
iii) Phenol co-efficient method.

26. Compare and contrast Antibiotics and Antitoxins?

Substances occur or produce naturally which cure diseases caused by microorganisms are called antibiotics (e.g.) penicillin by penicillium Antitoxins are compounds produced from the body of infected animals or human. This is act as disease resistant compounds.

27. Write any four characteristics required for chemotherapeutic agent?

i) Prevent / destroy the parasitic organisms without injury of host cells
ii) Able to penetrate the host cells and tissues deeper to have a contact.
iii) Leave the host natural mechanisms without charges.
iv) Work better in lowest concentration.

29. Write the Contribution of Ehrlich?

Paul Ehrlich invented the Salvarsan in 1910 which is an Arsenic compound cures syphilis. It has potential drug effect. It has less side effects very good active compounds against diseases and good chemical stability. He awarded Noble Prize sharing of with Elie Metchnikoff in 1910.

30. Define antibiotics?

Antibiotics are therapeutic compounds obtained from living organism like microorganisms. This is metabolic product of microbes. It is called as secondary metabolites. It acts in very small

concentration. It inhibits the growth disease causing microbes or other microbes.
(e.g.) penicillin produced by penicillium sp.

31. Write the contribution of Sale Wakesman?

Sale wakesman, 1945, discovered modern day antibiotics and its antimicrobial activity. He invented many antibiotics after the invention of penicillin. The antibiotics invented by him are as given below;

i. streptomycin,
ii. Tetracycline and
iii. many more antibiotics.

32. What are the characteristics of good antibiotics?

I. Ability to inhibit many pathogenic organisms. (i.e.) Broad-spectrum activity.
II. Ready resistant to organisms that are prevented by antibiotics.
III. No undesirable side effects.
IV. Antibiotics should not eliminate ordinary or normal microflora.

33. Write the mode of action of Antibiotics?

I. Antibiotics inhibit the cell wall synthesis.
II. Damage to cytoplasmic membrane.
III. Inhibition of nucleic acids and protein synthesis.
IV. Inhibition of specific enzyme system.

34. Write short notes on Nystatin?

Nystatin is a antifungal agent which is produced by streptomyces noursei.

Elizabeth Hagen, 1950 and Rachel Brown discover nystatin.

Nystatin is polyene compounds and its formula is $C_{46}H_{75}NO_{18}$

It is effect on Candida, Aspergillus, penicillium and Botrytis.

35. What are antiviral chemotherapeutic agents?

Some Chemotherapeutic agents effective control on viruses is antiviral chemotherapeutic agents.

(e.g.) Interferon

It is small glycoproteins, which inhibit the protein synthesis of viruses

36. Brief any one method for assay of Antibiotics?

Paper disc (plate) method. In this method, the paper disc is impregnated with antibiotics are placed on agar plates inoculated with microorganisms. After incubation, Zone of inhibition was observed and measured. Known concentration of antibiotics were compared with unknown concentration.

37. Write the contribution of Alexander Flemming?

Alexander Flemming 1929 invented the antibiotics penicillin and its effects on Bacterial cultures (staphylococcus). Later the organism responsible for the penicillin production was identified as a mycelial organism called penicillium notatum. He preserved these organisms in the laboratory.

38. Briefly write on antibiotics inhibit the cell wall synthesis?

The antibiotics as penicillin, ampicillin, Cephalosporin etc. are involve in inhibition of cell wall synthesis. It inhibit the synthesis of peptidoglycan in cell wall as

i) N-acetylglucosomine
ii) N-acetylmuramic acid.

39. What is chlorination of water? Write the significance of it?

Treatment of water with Sodium Hypochloride (NaOCl) where the free Chlorine is released. The free chl;orine available in the water kills the microorganisms at the minimum level of 1 ppm. It is used in water treatment food industry and domestic uses. For sanitation of public environment also can be done by using chlorination.

40. Briefly write on effect of osmotic pressure on microorganisms.

Release or removal of water from microbial cells to environment at higher ionic concentration. Due to release of water the osmotic potential is increased and shrinkage of cytoplasm occurs. This high osmotic potential causes the microbial cells to die.

PART – B

1. Write an essay on control of microorganisms by physical agents like temperature osmotic pressure surface tension and filtration?

The physical agents control prevents the growth of microorganisms, which are important in industry and related environment. Many physical agents control the growth and multiplication of microorganisms is given below:

I. Temperature: Temperature is a very important factor in control of microorganisms.

1. High Temperature:

a) Dry Heat and **b)** wet **heat** are the two forms of High temperature treatment for controlling the growth of microorganisms.

Microbes are susceptible to high temperature vegetative cells are more sensitive than spore forms. High temperature coagulates the protein and inhibits the metabolism of microorganisms. Moist heat is more effective than dry heat.

(e.g.) clostridium Solarium spores require moist heat at 121°C in 20 to 30 minutes. But in the dry heat it require 2 hour at 121°C. Vegetative cells sensitive than spores as vegetative cells require 5 to10 minutes at 70°C for bacteria yeast and fungi 5 to10 minutes at 60°C. Spores are 70°C - 80°C for 5 to 10 minutes.

a. Moist heat

Heat in the form of saturated steam under pressure. (e.g.) Autoclave.

Moist heat has advantage and rapid heating penetration abundance in moisture, which is help in coagulation of microbial proteins and kills microbes.

Autoclave in laboratory
Steam jacketed vassals - In industry.

Fractional Sterilization / Tyndalization

When the media is heat labile, higher temperature is not suitable. So, low temperature treatment with cooling intervals period pf incubation allows spores germinate to form vegetative cells and the vegetative cells are killed at low temperature.

During the intermittent cooling period of successive days allow killing all the microbial spores and cells present.

Boiling water:

Exposure of contaminated material on boiling water kills all the vegetative cells of microorganisms.

Pasteurization:

Pasteurization is a type of heating method of the temperature of 62.8°C (145°F) for 60 minutes or 71.7°C (161°F) for 15 mins called as HTST (High Temperature Short Time) method.

b. Dry Heat:

Heating the material without Moisture

1. Hot air sterilization:

Exposure of high temperature on materials kills microorganisms. Mostly this adopted to laboratory glassware and articles, oils, powders etc. for sterilization. This is normally 160°C for 2 hours or 120°C for 3 hours.

2. Incineration

Incineration is a burning of material (e.g.) Bunsen burner for inoculation needle in the laboratory kills the organisms.

Hospital waste, infected lab animals, Carcases are burnt for kill microbes. In this method care has to be taken for avoiding the fly off of infected material.

c) Low temperature

Temperature below the optimum condition depress metabolism. This is useful for preservation of culture in the laboratory refrigerator. Bacteria and fungi stored at 4 to 7°C for long period. Liquid nitrogen storage is at 196°C also used.

d) Desiccation

Keeping the culture (or) bacterial cells free of water (or) without water makes cessation of growth of microorganisms.

(e.g.) Lyophilization

Drying of culture with liquid at vaccum and sealed in ampoules.

ii. Osmotic pressure

Osmotic is a process of release of water from lower concentration to higher concentration i.e. from cell to atmosphere due to osmotic pressure and osmotic potential. Sine the plasma membrane is a semi permeable and selective membrane this osmotic potential occur in cell cytoplasm otherwise it is plasmoptysis. Plasmoptysis is a reverse process of the above and shrinkage occur in cytoplasm and bursting of cell wall and cell death occurs.

iii. Surface Tension:

The interface boundary between two phases of molecules i.e. surface of liquid and air i.e. unbalanced forces of attraction between molecular surfaces. In this process, the changes of surface tension of cells allow the permeability of cytoplasmic membrane. This results in damage to cell cytoplasum and death of cell.

e) <u>Filtration</u>

Filters made of membrane or molecular filters with smallest pore sizes like 0.01 to 10 μm diameter help in filtration or removal of microorganism.

E.g. Asbestos pad
Diatomaceous earth
Porcelain material
Sintered glass disc
Synthetic polyvinyl membrane – ultra filtration.

Uses in industry. Filtration of liquid through HEPA (High Efficiency Particulate Arrester) filters is a bacterial filter.

f) <u>Washing</u>

Washing is the method of cleaning by physical washing with detergents and cleansing solution. This type of physical washing with detergent solutions make surface tension on microorganisms and kill or remove microorganisms.

2. Discuss in details about the effect of radiation on control of microorganisms?

Radiation defined as the energy transmission through space that affect the growth of microorganisms and arrest the metabolism.

Radiation are studied as

1. Electromagnetic radiation as

   i) Light radiation
   ii) X – rays

2. Ionizing radiations as

   i) Gamma rays and
   ii) X – rays

These radiations pass through the cell and create free hydrogen radicals hydroxyl radicals and peroxides cause damage in intracellular level of cell.

Cold sterilization is a phenomenon of sterilization of biological materials by use of ionizing radiations. Ionizing radiation as with Gamma rays and X – rays.

UV – Light:

UV Light range from 150 to 3900 A° and the optional is 2650 A°C which is the highest bactericidal activity.

Sun light also composed of UV Light

Based on this, the UV lamp (or) Germicidal lamps are designed to emit high concentration of radiation in the effective region of 260 to 270nm. It is generally used in microbiology to kill (or) prevent the growth microorganism.

Mode of Action:

UV light absorbed by cellular material and the nucleic acids get damaged. Subsequently pyrimidines dimers are formed which lead to inhibition of DNA synthesis.

Application:

It is used in sterile room of Pharmaceutical Industry, Hospital Industry and the UV lamps are used in Food Industry, Dairy Industry. In the laboratory and research institutions, the UV lamp is used for avoid contamination.

X – rays

X rays are also highly lethal to microorganisms as well as in higher animal and man.

X rays emit very high energy form and has higher penetration power.

X rays are very expensive hence it is not economic.

Application

The X rays are generally used to produce microbial mutants for experimental purpose.

Gamma rays

Gamma rays are high – energy radiations emitted from radioactive isotopes like ^{60}Co.

Gamma rays are shorter wavelength than X-rays. So, Gamma rays are containing more energy and high penetrating power. Hence it is more effective on microorganisms.

Mode of Action

Gamma rays make a direct hit on DNA and causing ionizing radiation. Hence the death of cell occurs immediately.

Application

Gamma rays are applied on thicker volume of material like in packaged food, medical devices etc.

Electron beam radiation (cathode rays)

Cathode emits electrons when high voltage potential is generated between the anode and cathode. There are called cathode rays (or) electron beams. In this equipment the electron accelerated at high velocities (millions of volts). These high voltage electron beams cause deleterious effects on microorganisms as well as other living organisms. It has limited power of generation.

Application:

1.On surgical supplies
2. On drug and related products
3.Packaged medicines

3. What are the characteristic features of good antimicrobial compounds (or) chemical agents? How will you select the chemical agent?

A typical antimicrobial compounds should posses several selective characteristic features. Therefore no a single compound or chemical posses all the necessary characteristic features. So, combination of with others has been formulated for ecofriendly or desired use. Such combination of features is as given below:

1. Antimicrobial activity

The capacity of the substance to kill or inhibit microorganisms is the first requirement. The chemical, at a low concentration, should have a broad spectrum of antimicrobial activity.

2. Solubility

The substance must be soluble in water or other solvents to the extent necessary for effective use.

3. Stability

Changes in the substance up on standing should be minimal and should not result in significant loss of germicidal action.

4. Non toxicity to humans and other animals

Ideally, the compounds should be lethal to microorganisms and non-injurious to human and other animals.

5. Homogeneity

The preparation must be uniform in composition so that active ingredients are present in each application. Pure chemicals are uniform, but mixtures of material may lack uniformity.

6. Non-combination with extraneous organic material

Many disinfectants have an affinity for proteins or other organic material. When such disinfectants are used in situations where there is considerable organic material besides that of the microbial cells, little if any of the disinfectant will be available for action against the microorganisms.

7. Toxicity to microorganisms at room or body temperature

In using the compound, it should not be necessary to raise the temperature beyond that normally found in the environment where it is to be used.

8. Capacity of penetration

Unless the substance can penetrate through surfaces, its germicidal action is limited solely to the site of application. Sometimes, of course, surface action is all that is required.

9. Non-corroding and non-staining

It should not rust or otherwise disfigure metals nor stain or damage fabrics.

10. Deodorizing ability

Deodorizing while disinfecting is a desirable attribute. Ideally the disinfectant itself should either be odorless or have a pleasant smell.

11. Detergent capacities

A disinfectant which is also a detergent (cleansing agent) accomplishes two objectives, and the cleansing action improves the effectiveness of the disinfectant.

12. Availability

The compounds must be available in large quantities at a reasonable price.

Selection of a chemical agent for applications

The major factors that need to be assessed in the process selecting the most appropriate chemical agent for a specific practical application are as follows.

1. Nature of the material to be treated

Before selecting a chemical agent for treating the object to be studied thoroughly and to be selected if satisfactory. For example the chemical used for utensils might not be satisfactory for use in human skin. Consequently, the substance selected must be compatible with the material to which it is applied.

2. Type of microorganisms

Chemical agents are not all equally effective against bacteria, flung, virus and other microorganisms. Spores are resistant than vegetative cells. Differences exist between Gram negative and Gram-positive bacteria. Escherichia Coli is much more resistant to cationic disinfectants than staphylococcus aurous. Differences in action also exist between strains of the same species. Therefore the agent selected must be known to be effective against the type of organism to be destroyed.

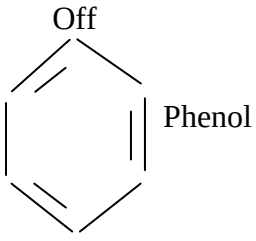
3. Environmental conditions

The temperature, P^H, time, concentration and presence of extraneous organic material may all have a bearing on the rate and efficiency on the antimicrobial action. The successful use of an understanding of the influence of these conditions on the particular agent, so it can be employed under the most favorable circumstances.

4. Discuss in detail about the control of microorganisms by chemical agents like phenolic compounds, alcohols and Halogen compounds?

Phenol is otherwise carbolic acid which was used as early as in 1880 by Josep Lister, surgeon to control the infection through surgical wounds, injuries and in cissions. Phenol at 5% solution kills microorganisms and control population and the range is 2% to 5% solution for effective control. Phenol solutions kill vegetative cells and not spores.

Hexylresorcinol a derivative of phenol, Phenol, cresol, Dimethyl phenol, 4-n Hexylphenol and 4-n Heptylphenol act as Bactericide, or Bacteriostatic and some are fungicidal. The structure of Phenol is as given below:



Phenol works less at alkaline pH and at low temperature

Presence of soap reduces the activity. Pure phenol is crystalline and colourless 2% to 5% solution is used as disinfectant.

Phenol- Mode of Action

- Disruption of cells and its activity.
- Precipitations of cells protein
- Inactivation of enzyme
- Leakage of Amino acids from cells

Application

- It is used as bactericidal or bacteriostatic.
- Some are highly fungicidal.
- It used as disinfectant on materials such as sputum, urine, feces and contaminated instruments or utensils
- Combination of phenolic compounds with detergents produces with good disinfectant as well as detergents.

II. Alcohols

Ethyl alcohol is a most widely used antimicrobial compounds at concentrations between 50% and 90% which is effective on vegetative and nonsporulating cells. Generally 70% is used for practical applications. Methanol is less effective and poisonous. Propel alcohol and Butyl alcohol, Amyl alcohol and others are more germicidal i.e. 40% to 80% more effective. High molecular weight alcohols are more effective than low molecular weight alcohols (Ethanol)

Alcohol - Mode of Action

Alcohols are protein denaturant Alcohols are destroy lipid compounds in cell membrane detergent action when cleaning with alcohols.

Alcohol Applications

Effective antimicrobial on skin used as disinfectant in clinical and Research laboratories.

It is also used for oral thermometers.

III. Halogen compounds

Iodine and Chlorine compounds are generally referred as halogen compounds. Iodine is used as a Tincture Iodine.

i.e. 1. 2% Iodine + 2% NaI in alcohol
2. 7% Iodine + 5% KI in 83% alcohol
3. 5% Iodine + 10% KI in aqueous solution

Iodophores are another class of halogen compounds which contain 5% Iodine + surface active agents.
Polvinylpirrolidone + Iodine (P+I complex)

Application

It is used as Bactericidal agents. It is effective m all kinds bacteria. It also acts as sporicidal activity. Bacteria on skin, wounds and injuries are controlled for its population disinfectants. It is used as disinfectant for water, Air, and sanitizer for food materials.

Mode of action

- It is a strong oxidizing agent.
- Inactivate proteins with .sulfhydroxyl groups.
- Halogenation of tyrosine unit of enzymes.
- Other cellular proteins require

Chlorine and chloride compounds

- In the forms of gas and chemical combination. (e.g.) Hypochlorides
- Hypochlorides is the form of gas

Mode of action

Hypochlorous acids release oxygen (nascent oxygen) more act as strong oxidizing agent. Sometime destroy microorganisms in combination of chloride with proteins of cell membrane and enzymes.

Chlorine and chlorine compounds

Chlorine in the form of gas and chemical combination is used for control of microorganisms.

1. e.g. Hypochlorides – i.e. Calcium Hypochloride
($CaoCl_2$)
and Sodium Hypochloride ($NaOCl_2$)

2. Chloramines also used as a disinfectant
(e.g.) Mono chloramine (NH_2Cl)
Chloramine -T and

Azo chloramine are also available with chlorine form for germicidal

Mode of action

When dissolved in water the hypochlorides forms Hypochlorides acid, which release oxygen (nascent oxygen) act as strong oxidizing agent and destroy microorganisms.

It works by combination of Chlorine with Proteins of cell membrane and Enzyme systems.

Applications used for water treatment with 1 ppm of Chlorine.

It is also used in Food Industry and Domestic uses and Medicine, Dairy Equipments Cleaning.

1% $NaoCl_2$ used for personal hygiene 5 to 12% used for household bleaches disinfectants. Residual Chlorine concentration of 1ppm of free chlorine is required.

5. Write short notes on the following for control of microorganisms.-

i) Heavy metals and their compounds

ii) Dyes and its chemicals

iv) Quaternary Ammonium compounds

v) Aldehydes

i) Heavy metals and their compounds

Heavy metals are available alone or in compounds which are effective on microbes.

E.g. Mercury (Hg)
Silver (Ag)
Copper (Au)

Mercuric Chloride ($HgCl_2$)
Mercuric Oxide
Silver nitrate
Copper Sulfate

Mode of Action

Heavy metals and their compounds are combine with cellular protein and inactivates them.

E.g. $HgCl_2$ inhibit enzyme systems which are contain sulfhydryl groups.

Heavy metal salts coagulate cytoplasmic proteins and damage due to death of cell.
Salts of Heavy metals also precipitate proteins and cause death of cell.

Application of Heavy metals

Heavy metals with salts are used for surface sterilization of various objects in industry as well as research laboratories.

$MgCl_2$ → 0.1%
$AgCl_2$ → 0.1%
$HgCl_2$ → 0.1%
Cu → 2 ppm

ii) Dyes and chemicals

Chemical dyes (or) synthetic Dyes are available as in 2 classes which are act as antimicrobial compounds.

1. Triphenyl methane dyes
2. Acridine Dyes

Triphenyl Methane dyes

E.g. Malachite green
Brilliant green
Crystal violet

These class of Dye effective at very low concentration as 1:1,00,000 dilution.
Gram-positive bacteria are more susceptible at low concentration.

Action: These dyes chemicals interface cellular Oxidation process and use in preparation of special medium.

2. Acridine Dyes

Acridine dyes also classified into two types.

i) Acriflavines
ii) Tryptoflavines

These are act against Strepotococci & Gonococci

These are active at very lower concentration useful in ophthalmic applications and used in burns and wounds.

iii) Quaternary Ammonium compounds

These are cationic detergent chemicals, which act as Germicides called Quaternary Ammonium Compounds (QAC) e.g. Quaternary Ammonium Chloride contain alkyl groups.

Several compounds of QAC are reported as antimicrobial compounds. Activity is high on Gram positive bacteria and low on Gram negative bacteria.
I.e. 1:200,000 to 1:300,000

Mode of Action

Denaturation of proteins
Interference with glycolysis
Cytoplasmic membrane damage and
Cause death of cell.

Application of QAC

QAC are used as Disinfectants and sanitizing agents used as preservative in ophthalmic solutions and cosmetic preparations.

iv) Aldehydes and its chemicals (RCHO)

Among the aldehyde groups low molecular compounds are effective.
E.g. Farmaldehyde &
Glutaraldehyde

Are used as microbicidal and sporicidal

Farmaldehyde

It produces gas in the form of fume which is stable in high concentration and elevated temperature.

Farmaldehyde available in aqueous form as 37% to 40% fumes are noxious and imitate eyes and tissues.

Mode of Action:-

Reacts with vital organic Nitrogen contain as proteins & Nucleic acids leading to death of cells.

Application

It is used for Sterilization and disinfection of various areas. Vegetative cells are more susceptible than spores.
Formalin and Parafarmaldehyde are available forms.

Glutaroldehyde

This is saturated dialdehyde.

About 2% of solution shows wide spectrum of Antimicrobial activity.

It acts on vegetative cells of Bacteria & Fungi and spores of Bacteria & Fungi and Viruses.

Application

The main application is sterilization of medical equipments.

6. Explain the methods used for evaluating the antimicrobial chemical compounds.

There are three techniques are available for evaluation of antimicrobial chemical agents in the laboratory, which are generally used. In each method, the chemical agent is tested against a specified microorganism referred as the test organism.
There are:

1. Tube Dilution and plate method
2. Agar plate method
3. Phenol co-efficient method

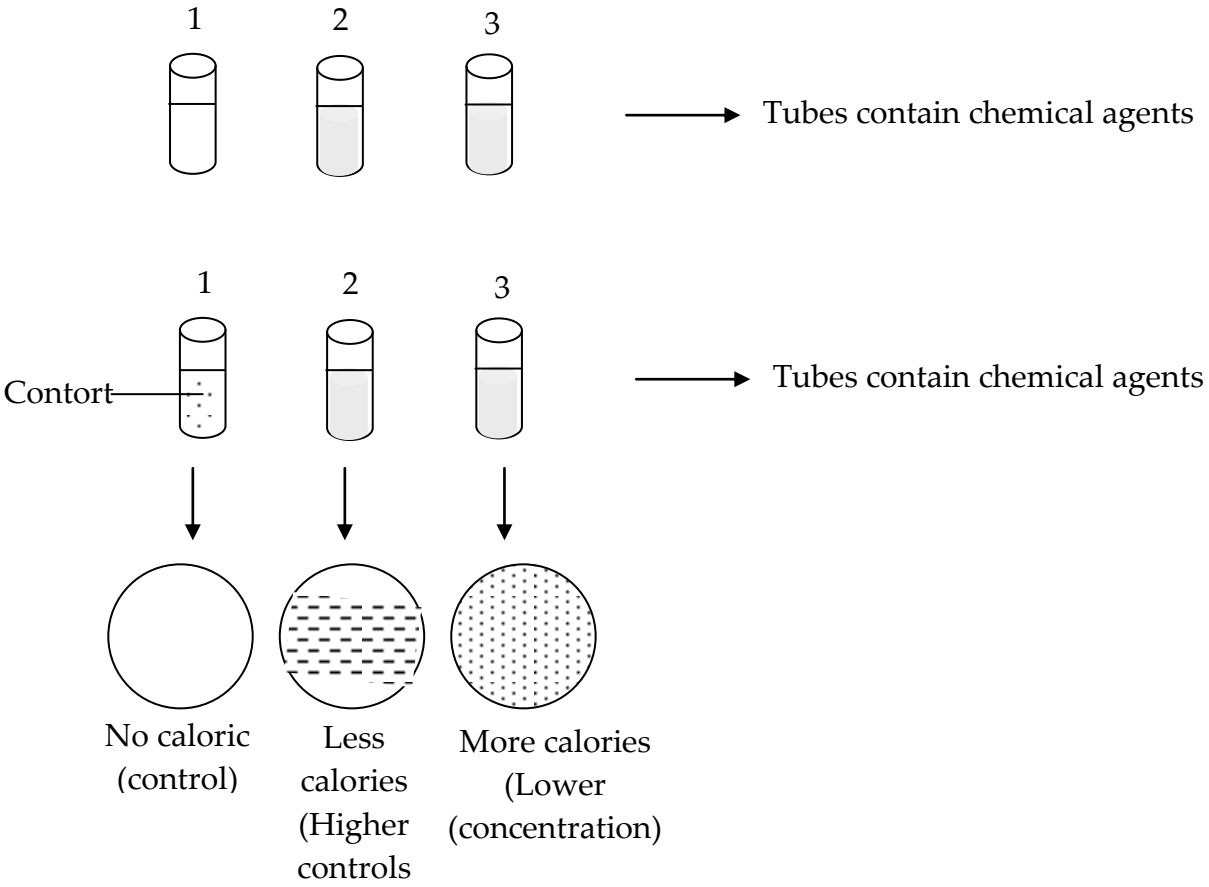
1. <u>Tube Dilution & Agar Plate Method</u>

In this method the chemical substances are diluted (or) dispensed with water in sterile test tubes.

The serially diluted solutions are dispensed in sterile tubes.
Addition of test organism in the above dilution contains test tubes.

The above culture tube (or) test organism is transfer (or) plated on sterile agar plate. The inoculated agar plates incubated for overnight at suitable temperature.

Then the plates were observed for the growth of Bacteria of interpretation done.



<u>In Test tubes</u>

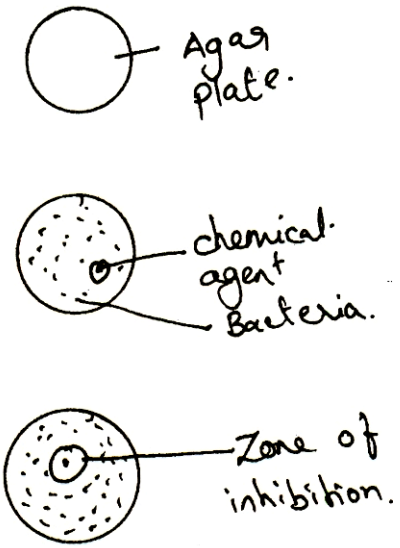
In the serially diluted sterile test tubes contains chemical agents are prepared. Then the test organism is added uniformly in above tubes contain chemicals.

Then the tubes were incubated for over right for the growth of organism.

Then the growth was observed by visible as well as by photometric method.

Record the values and interpretation.

2. Agar plate method



The sterile agar plate was prepared and the test organism was inoculated.

In the inoculated plates, Chemical agents were placed on the well made.

Then incubation of inoculated agar plates with chemical agents for overnight.

After overnight, the zone of inhibition of chemicals on culture plate was recorded and the interpretation was done.

3. Phenol co-efficient method

Phenol co-efficient methods are standardized internationally by AOAC method. This method is recommended and approved by FDA. (FDA – Food & Drug Administration)

This method is more suitable for testing disinfectants mixing (or) diluting in water. The antimicrobial activity of the chemical agents on test organisms was compared with phenol. Test organism is Salmonella typii and staphylococcus aureus

Procedure

1. The test infectant or chemical agent is diluted by series of dilution.
2. This is pound in test tube as 5 ml quantity.
3. In the above tube, 0.5 ml of 24 h broth culture is added.
4. Similar addition in same of series of phenol is done.
5. Then the inoculated culture tubes with chemical agents (set) and phenol (set) is incubated at 20°C for overnight.
    (i) Disinfectant (chemical) + organism – 1 set
    (ii) Phenol + organism – 1 set
6. Then subculture of above at 5, 10, 15 mins. intervals into sterile tube medium.
7. The above inoculated medium incubated for overnight.
8. Observation for growth is made.
9. The observation were recorded and the results Interpreted.

Interpretation

The greatest dilution (in disinfectant) killing the test organism at 10 min, which is divided by the dilution of phenol showing the same result.

The number obtained is called phenol coefficient ratio.

If the ratio is more than 1, then the compound (or) disinfectant is effective.

Results

Material Description	Dilution	Observation for growth		
		5 min	10 min	15 min
Disinfectant (K)	1:100	0	0	0
	1:125	+	0	0
	1:150	+	0	0
	1:175	+	+	0
	1:200	+	+	+
Phenol	1:90	+	0	0
	1:100	+	+	+
	1:125	+	+	+

From the results given in the table;

Phenol coefficient ratio (x)

$$= \frac{\text{the greatest dilution of disinfectant kill oranisms}}{\text{the dilution same results in phenol}}$$

$$= \frac{150}{90} = 1.6$$

Phenol coefficient ratio = 1.6

If the ratio is more than 1, then the disinfectant is effective.

7. What are the characteristic features of good antibiotics? Classify the antibiotics based on the mode of action?

A good antibiotic require desired characteristic features which are given below:

1. Antibiotics should have ability to inhibit many pathogens i.e. variety of pathogens (Broad spectrum).
2. It should prevent the growth of ready resistant pathogens.
3. It should not give undesirable side effects in host.
4. The antibiotics use for kill one organism should not eliminate normal mycoflora.
5. The antibiotics should work in the conditions of host like temperature, pH etc.,

The above are important characteristics of one antibiotics.

Classification of Antibiotics

Antibiotics can be classified based on the nature of function, mode of action and chemical nature in different ways as,

1) classified on
   - i) Bactericidal
   - ii) Bacteriostatic
2) Grouped based on chemical nature

3) Classified based on mode of action.

The classification based on mode of action is generally used.

1) Inhibition of cell wall synthesis
2) Damage to cytoplasm membrane
3) Inhibition of Nucleic acids & protein synthesis
4) Inhibition of specific enzyme system

1. Inhibition of cell wall synthesis

This group of antibiotics inhibits the synthesis of peptidoglycon present in the cell wall. Peptidoglycon composed of N - acetylglucosamine and N-acetylmuramic acid. Due to inhibition of cell wall material synthesis cell wall get weakened and burst which lead to cell death.

The following antibiotics were classified in this group as

i) **Penicillin** – It is produced by Penicillium chrysogenum and Penicillium notatum

ii) **Ampicillin** - Obtained from penicillin and called as semisynthetic penicillin.

iii) **Chepalosporin** – Cephalosporium acremonium effective on Gram Positive and Gram negative.

iv) **Cycloserine** – produced by chemical synthesis

v) **Bacitracin** - produced by Bacillus subutilis effective on Gram-positive bacteria.

vi) **Vancomychin** – Streptomyces orientalis inhibit peptidoglycon synthesis

II. **Damage to cytyoplasmic membrane**

This group of antibiotics is chemically consist of polypeptide antibiotics.

The antibiotics are,

1) Polymyxins
2) Germicidens
3) Tyrocidins

Which are produced by Bacillus species. These antibiotics adversely affect the permeability characteristics of cell membrane.

The polymixins are particularly effective against Gram-negative organisms.

The tyrosine and gramincidin are more effective against Gram-positive organisms. These are bactericidal and they cause leakage from the cytoplasmic membrane.

Another category referred as polyene antibiotics are large ring structures with many double ring. E.g. nystatin – streptomyces noursei amphoterecin – S- nodosus.

This group also cause damage to cytoplasmic membrane.

III. Inhibition of Nucleic acid and protein synthesis

Certain group of antibiotics involve in the inhibition of nucleic acid synthesis and protein synthesis require for cell growth and multiplication such antibiotics are;

Streptomycin produced by Streptomyces grieseus, which inhibit many microorganisms, which are resistant to Sulfonamides, and Penicillin include many Gram negative bacteria.

Streptomycin characterized by aminogluoside antibiotics. Kanamycin produced by S.kanamyceticus and neomycin by S. fradiae are other species of Streptomyces.

Streptomycin and other aminoglucoside antibiotics inhibit the protein synthesis by combining irreversibly with the 30s subunit m RNA.

Other antibiotics inhibit Nucleic acids and protein synthesis is Tetracyclins consist of chlortetracyclins, oxytetracyclins, tetracyclins, doxycyclins, and microcyclins are produced by streptomyces aeureofaciens were broad spectrum antibiotics.

Chloremphenicol is a broad spectrum antibiotics produced by S.venezule also inhibit the synthesis of Nucleic acids and proteins.

Erythromycin produced by S. Erythraeceus is active against Gram positive bacteria. Erythromycin belongs to macrolides antibiotics.

IV. Inhibition of specific enzyme system

The Sulfonamides and sulfonamide chemicals are antimicrobial compounds attack specific enzyme system. Those chemical similar to P – aminobenzoic acid is a precursor of Tetrahydrofolic acid which ender the cellular reaction enzyme and block the synthesis of cellular material THFA, is essential for synthesis of amino acid synthesis Thymidine synthesis etc. when basic of THFA cellular synthesis distrupted.

Antifungal antibiotics and Antiviral agents are other compounds inhibit the growth of fungi and virus respectively.

8. Write in detail about the methods involve in assay of antibiotics.

The potency of antibiotic content in samples can be determined by the following ways as,

1. Physical method
2. Chemical method
3. Biological method

Among these methods, biological assay are the most convenient method.

1. Chemical Assay

When the antibiotics is exist in pure chemical form, their concentration can be expressed in micrograms of the pure chemical per milligram of the specimen.

Such tests must give results that correlate well with those obtained in biological assays. Chemical assay methods are generally more accurate and require less time.

Less sensitive and less time required. Sometimes, the chemical assay method gives errors.

II. Biological assay method

Biological assay method is expressing in terms of either micrograms or units determined by comparing the amount of killing, or bacteriostasic, of a test organism caused by the substance under test with that caused by a standard preparation under rigidly controlled conditions.

This assay method is standardized by FDA (Food Drug Administration) regulations.

Using microbial organisms that are more sensitive to these antibiotics chemicals does these biological methods.

The techniques can be modified to make them more sensitivity for these specimens.

These are two types

1) Tube Dilution method
2) Disc assay method

1. Tube Dilution method

By tube dilution method, the smallest amount of antibiotic compound required to kill the microorganism is determined

i.e. 1) the Minimum Inhibitory Concentration (MIC)
2) the Minimum Lethal Concentrations (MLC)

The culture medium is taken into the series of test tubes. To the culture medium contain test tubes, the antibiotics at different concentration more added. Then the culture inoculum was added uniformly to all the tubes.

The different concentration of antibiotics added in the culture tubes were labelled and incubated for overnight.

After the incubation period, the concentration of drugs required to inhibit the growth of the organism is determined by observing the absence of growth.

This concentration is noted the results are interpreted. .

2. Disc plate method

The disc plate method is most commonly used technique for determining susceptibility of micro organisms to chemotherapeutic agents.

A small paper disc impregnated with antibiotics compound was placed on the agar plate containing inoculated Bacterial cultures.

Then the agar plates were incubated at suitable temperature for over the period of time.

After incubation period, the zone of inhibition of bacteria around the paper disc contain antibiotics were observed.

The zone of inhibition of bacterial growth was measured and compared with MIC (Minimal Inhibition Concentration) plates.
i.e. Known concentration of paper discs which is compared with unknown concentration of culture plate to determine the activity.

This method is FDA approved method, which was standardized, by Bayer, Kirby Sherries and Truck in 1966.

This is most widely used antibiotic assay method for find out the efficiency of antimicrobial activity.

9. Discuss any four clinically important microorganisms and it effects, symptoms and control methods?

Many microorganisms are clinically important due to their disease causing capability both in animals and human. The important pathogenic microorganisms and their disease symptoms, effects and their control methods are given below:

1. Pseudomonas - The Pseudomonads

This is Gram negative rod shaped, aerobic bacteria. This is opportunistic pathogen. Pseudomonas mallei, P. psuedomanallis are animal pathogen.

Pseudomonas auregenosa is a human pathogen produce lethal exotoxins and endotoxins.

This genes contain 2 species belong to Gram negative, coccobacilli are B. abortus (from cattle) and B.suis (from pigs).

Brucellosis occurs worldwide including India and Mexico.

It enters through damaged skin or mucus membranes of the digestive tract, conjunctiva and respiratory tract. Infected phagocytes releases bacteria in to the blood stream, creating focal lesions in the liver, spleen, bone marrow, and kidney. Brucellosis gives fever, accompanied by chills, profuse sweating, head ache, muscle pain and weakness and weight loss. The symptoms last for a few weeks to a year even with treatment.

The diagnosis is by serological tests of the patients blood and blood culture.

Control

A combination of tetracyclin and rifampin or streptomycin for 3-6 weeks is effective control.

2. Brucella and Brucellosis

Brucellosis otherwise Bang's diseases caused by Brucella species transmitted to human from infected animals.

This is enteric pathogen.

The disease complications include Pnuemonia urinary track infections, abcesses, otitis and coreneal diseases pseudomonas septicemia can give rise to diverse and gave conditions such as endocarditis, meningitis and bronchopneumonia. The colour of the area is blue to greenish yellow pigment due to bacterial growth.

Control

Drugs are found effective control on pseudomonas infections are third generation cephalosporins, aminoglycosides, Carbenicillin, Polymyxin, quinolones and monolactams.

3. Escherichia coli and its diseases

It is Enteropathogenic pathogens. These are Gram negative, rod shaped. This is strictly aerobic bacteria.. It lives in intestine of human and called as colon bacillus.

Enteropathogenic E-Coli cause Gasterointestinal diseases in human and produce endotoxins. The toxin producing E-Coli are otherwise called Enterotoxigenic and it produce Diarrheal diseases in adults and infants. The exotoxins are heat labile toxin (LT) and heat stable toxin (ST) stimulate diarrhea and fluid loss. Some are enteroinvasive and has fimbriae on that and produce dysentery similar to Shigella dysentery.

Traveller's syndrome is other diseases associated with travel associated gasterointestinal diseases. Travellers pick up with virulent E-coli strain through local food and water. Traveller's diarrhea associated with the syndrome as diarrhea, low-grade fever, nausea, and vomiting.

.

Other E-coli strain invades sites other than intestine, which cause 50% to 80% of urinary treat infections (VTI) in healthy people.

Other extra intestinal infection in which E-coli is involved are neonatal meningitis Pneumonia, septicemia, and wound infections.

Control

The control method is antibiotic drug with penicillin.

4. Vibrio - The Cholera

Different species of vibrio are human pathogenic, this is vibrio shaped (or) curve shaped enterobacteriacae.

Vibrio is ingested with food or water and reaches to stomach with high acidity. The size of bacteria increases drastically. Vibrio penetrates the mucosa of mucus barrier using their flagella adhere strongly.

The virulence strains of vibrio - Cholera produce enterotoxins called Cholera Toxin (CT) that disturb the intestinal cells. Due to this mechanism profuse loss of water and fluid and the disease is severe and violent.

The symptoms are heavy, 50% weight loss, loss of blood volume, acidosis and bicarbonate loss and potential depletion. The patient with severe thirst, flaccid skin, sunken eyes. Secondary consequences include hypotension, Tachycardia and collapse from shock.

Diagnosis

Vibrio can readily cultured in the lab and identified.

Therapy & Control

Heavy replacement of water and electrolytes and various rehydration techniques.

Oral antibiotics like tetracylin and trimethoprin- sulfa is best drug treatment for cholera.

UNIT – V

PART – A

1. What are primary metabolites? Give two examples?

Primary metabolites are metabolic products of microorganisms produced during the growth phases of growth cycle. The organisms themselves for their growth and metabolism utilize the Primary metabolic products.

e.g. 1. Organic acids –citric acid

2. Amino acids – glutamic acid

2. Write any two organic acids and the producer organism in industrial fermentation process?

1. Citric acid – Aspergillus niger
2.
3. Lactic acid- Lactobacillus lactis

3. What are the applications of lactic acid (LA)?

1. The technical grade LA is used for textile industry and ester manufacture.
2. Food grade LA is used as food additive, acidulant and production of sour flour and dough.
3. Pharmacopoeia grade used as Intestine treatment and metal ion lactates.
4. The plastic grade used as lacquers, varnishes and biodegradable polymers.

4. Name any 4 microorganism which produce lactic acid in Industrial process.

1. *Lactobacillus lactis*
2. *Lactobacillus bulgaricus*
3. *Streptococcus lactis*
4. *Leuconostac mesenteroides*

5. What are secondary metabolites? Give 2 examples?

Secondary metabolites are a metabolic products produced by microorganisms like bacteria and fungi during their stationary phase (or) Idiophase. Secondary metabolites are not utilized by organism themselves for their growth.

e.g. 1) antibiotics – Penicillin

2) Vitamins – vitamins B_{12}

6. Name any 4 Antibiotics and their producer's organism?

1. Penicillin – *Pencillium chrysogenum*
2. Streptomycin – *Streptomycin greiseus*
3. Erthyromycin – *Streptomyces erythreus*
4. Tetracyclins – *Streptomyes rimosus*

7. What is the mode of action of Antibiotics?

1. Inhibition of cell wall synthesis of microorganism
2. Damage to the cytoplasmic membrane (CM) of pathogenic microbes
3. Inhibition of nucleic acids and proteins synthesis.

8. What are the classes of antibiotics involved in the industrial production by fermentation process?

1. Beta lactum antibiotics - penicillins & cephalosporins
2. Amino glucosides - Tetralyclins, streptomycin
3. Macrolides - Erthromycin and Actinomycin
5. Other antibiotics - Include polyene compounds and nystatin etc.

9. Name any 2 Aminogluosides antibiotics and its application.

1. Streptomycin - *streptomyces grieseus*

It acts against both gram-negative and gram-positive bacteria. It controls the mycobacterium & tuberculosis disease.

2. Bacitracin - *streptomyces tenebrasius*

It is very important in medical use to control specific gram negative pathogen.

10. What is penicillin precursor? Write the importance of it?

Penicillin precursor are generally used in very low quantity as a starting material for the production of penicillin. 6-aminopenicillionic acid (6 APA) is known as penicillin precursor.

Presence of penicillin precursor in the penicillin production medium help in the formation of penicillin in the process.

11. What are the upstream and downstream processing in the fermentation process?

1. Upstream process is a process steps of fermentation from microbial strain development & propagation to fermentation process.

2. Downstream processing a part of the fermentation process steps which involve from completion of fermentation to product purification & recovery steps. This includes filtration, drying crystallization and packing.

12. What is meant by food yeast and Bakers yeast?

Food yeasts are single cell micro-organisms grow on solid media and rich in minerals, protein and vitamins etc. Which are used as a food.

E.g. *Kluveromyces, Torulopsis* etc

Bakers yeast is single called yeast organisms, which are used in baking industries like Bread & wheat dough etc. for leavening purposes.

E.g. *Sacharomyces cereviseae*.

13. What is a single cell protein? Give one example?

Single cell protein is the nutrients rich products like protein, minerals vitamin, hormones etc. Present in the single celled microorganisms which are growing on cheaper raw materials belong to food or agricultural waste products. High yield of cells are harvested and used as a food called single cell protein e.g. Spirulina sp., Torulopsis sp. etc..

14. What are the microorganisms that are found in the contaminated water?

In the contaminated water most of feacal coliforms are grow and cause diseases. Those organisms are;

1. *Escherichia coli*
2. *Pseudomonas sp.*
3. *Bacillus sp.*
4. *Salmonella sp.*
5. *Closteridium sp.*
6. *vibrio cholerae.*

15. Describe briefly on Bio-remediation?

Bio-remediation is the role of biological organisms include plants, microorganisms like bacteria, fungi, actinomycetes on degradation of environment as soil, water by special mechanism which have themselves. The waste includes toxic metals and harmful substances present in the environment are degraded or change into inactive form, which are harmless.

16. What are methanogens?

Methanogens are the group of bacteria that capable of produce methane gas is a major part or ratio in Biogas. The breakdown materials or simple solubilized materials converted from complex substances are utilized by methane producing bacterial e.g. *Methanococcus sp. Methanobactor sp.*

17. Differentiate food infection and food intoxications.

Food infection is the process of microbial infection through the contaminated or spoiled foods. The microorganism enters through the consumption of food.

E.g. *Salmonella sp.,*

Escherichia coli.

Food intoxication is the entering of toxic substances produced by pathogenic microorganism in the food which cause serious diseases on human and animals.

e.g. Shigella toxins, Vibrio cholera toxins.

18. What are the microorganisms present in the raw milk?

i. *Streptococcus lactis* *vi. Escherichia coli*

ii. S. cremoris *vii. Bacillus subutilis*

iii. Lactobacillus casei *viii. Pseudomonas sp.*

iv. L. fermentum *xi. Candida lypolytica*

v. L. lactis

19. Describe the role of Thiobacillus in the industry.

Thiobacillus ferroxidans and *Thiobacillus thioxidans* are acitotrophic, aerobic bacteria grow on the rocks with presence of copper ores produce acid with effect of oxidation and precipitate the metal (copper) or leaching of ores. This technique improves the recovery of metal from an ore and is non-polluting to the atmosphere.

20. What is meant by Biofertilizers? Give two examples.

Biofertilizers are the use of microorganism to supply of chemical nutrients to higher plants for its growth and yield. The microorganisms are

1. *Rhizobium spp*. – N_2 fixers
2. *Azotobacter* - N_2 suppliers
3. *Azospirillum* - N2 suplpiers
4. *Mycorhizae* - phosphate solublisers and supplier.

21. Write any 4 beneficially important organism, products and it uses.

1. Citric acid – *Aspergillus niger* – acidulans
2. Glutamic acid – *Corynebacterium glutamicum* – amino acids
3. Nisin – *Lactococcus lactis* – antimicrobial agents
4. α - Amylase – *Bacillus subutilis* – starch degrading enzymes.

22. Write short notes on Biopesticides. Give one example.

Biopesticides are the compounds produced by biological organisms or the microorganism, which act against insects and pests and kill the activity insects & pests. Those byproducts are ecofriendly and no side effects like chemical pesticides.

E.g. 1. *Bacillus thuringiensis*

3. Beaveria bassiana

3. Neem products.

23. Differentiate antibiotics and vaccines.

Antibiotics are the secondary metabolites that are produced by one microorganism and affect other microorganism, which leads to death of the organism.

Vaccines are proteins, which present in attenuated or killed pathogenic microorganism or the whole weakened cultures of pathogens, which react with antigens, or foreign proteins present in virulent pathogenic organisms inactivate the pathogen. Therefore the disease resistance occurred in host organisms.

24. How alcohol is produced from molases?

The molases containing sugar is converted into monosacharides like Glucose and fructose by the enzyme invertase produced by yeast. Then the reducing sugars are converted to pyruvic acid by glycolysis. The pyruvate is converted into alcohol by the yeast *Sacharomyces cereviseae*.

25. Write the application of Ethanol.

1. The pure ethanol is diluted and consumed by human.
2. Ethanol is used as a fuel in Automobile industry.
3. Ethanol is 92.4 % is used as solvent in the cosmetic and pharmaceutical industry.
4. Ethanol with 99.2% is used as motor fuel.

26. Mention the name of microorganism which produces vitamin B_{12} (or) cobalamine.

1. *Bacillus coagulans*
2. *Streptomyces grieseus*
3. *Bacillus megaterium*
4. *Probinobacterium sp.*

27. Describe Biogas.

Production of mixer of gases by microorganisms after degradation of organic substances at anaerobic environment is called Biogas.

Biogas contains;

1. Methane – 64%
1. CO_2 - 30%
2. N_2 - 4%
3. H_2S - 1%
4. races of H_2, O_2 & CO.

28. What is meant by Acitogenesis? Give suitable example.

Organisms called Acitogens are facultatively anaerobic and H_2 producing bacterial simple organism or material oxidized and produce Acitic acid + H_2 + CO_2

$$\text{Simple organic material} \xrightarrow{\text{oxidation}} \text{acetate} + H_2 + CO_2$$

e.g. 1. *Acitinobacter sp.*

2. Acinobactor sp

29. What is methenogenesis? Give examples.

Anaerobic organism involve in a production of methane gas by utilizing organic waste is called methenoenesis.

Acetate +H_2 +CO_2 → Methane +H_2+CO_2

e.g. 1) *Methanobacterium Methanospirillum*

2) Methanococcus Methanomicrobium

30. Write the application of biogas products.

1. Used as fuel for cooking
2. Used as a electricity for domestic lighting and heating
3. Used as a fuel for petrol and diesel engineers
4. Biogas production controls the pollution of environment.

31. Write the composition of biogas? What are the steps involved in biogas production?

Biogas composed of;

Methane (CH_4) – 63%
Carbondioxide (O_2) – 30%
Nitrogen (N_20 - 4%
Hydrogen sulphide (H_2S) -1%

Traces of H_2, O_2, O_2 & co

There are 3 stages are involve in Biogas production.

Stage I – Solubilization → Breakdown complex material

Stage II - Acitiogenesis → formation of acitic acid
from organic waste

Stage – III - Methanogeneis → Production of Mathane
from acetic acid.

32. Define bioleaching? Give an example?

Bioleaching or microbial leaching is biological processes in which the metals are dissolved are precipitated from ore bearing rocks and soil surface by the action of microorganisms. The microbial action is oxidation and reduction process.

E.g. *Thiobacillus thioxidans*
Thiobacillus peroxides
Solubilization of sulphur.

33. What is *in-situ* leaching process of microbial leaching?

Microbial leaching process occurs or performed in the original place of ores origin of rocks or soil surface. Rocks and soil surface are blasted on the surface and increase the permeability of water and bacterial culture suspension. The acidic water and bacteria seeps through the deposits and the solubilization of inorganic metals are carried out and purification process done.

34. What are the advantages of using bio-fertilizers?

1. Bio fertilizers are eco-friendly and reduce atmospheric pollution

2. Biofertilizers synthesis of growth promoting substance and enhance the plant growth and protection from diseases

3. Improve soil quality by crumps formation and water holding capacity

4. Biofertilizers can be manufacture in the farmer's place and less expensive.

35. What is nitrogen fixation? Give suitable example?

Fixation of atmospheric nitrogen in soil in the form of organic stage and make utilization to plants by microorganism like Bacteria and cyanobacteria is nitrogen fixation. There are two types of nitrogen fixation as;

1. Symbiotic nitrogen Fixation – Rhizobium sp
Nitrobacter sp.

2. Non-symbiotic nitrogen fixation –Blue green algae,
Azotobacter, Azospirillum, Anabena.

36. What is Bacterization?

Bacterization is a biological technique in which the symbiotic Nitrogen fixing bacteria are introduced (or) inoculated with the roots of higher plants for infection and further nitrogen fixation. Bacterial suspension prepared in a water or culture medium and it is available in commercial packs.

37. What is Mycorrhizae? Give the importance of it?

Mycorrhizae are the root infecting fungi, which are mutualistic, and living in the roots of higher plants. Mycorhizae supply mineral nutrients like phosphorus and Nitrogen. Instead mycorrhizal fungi derive space and nutrients from plant cells. There are 2 types of mycorhizae as;

1. Ectomycorrhizae

2. Endomycorrhizae

E.g. Acaricus bisporus

38. What is BT toxins?

BT toxins are otherwise called as *Bacillus thuringiensis* toxins. B_T toxins are endotoxins that are poisonous to insects and pests that infect crop plants like cotton. B_T toxins are works in the gut of bull worm, which infect cotton plants and kill the larvae by arresting the gut activity.

39. Write the advantages of using single cell proteins?

Single cell protein are otherwise called as microbial protein which are consist of high nutrients and has advantages over them food.

1. It has high protein content as 45% -85%
2. Microbes multiply and grow rapidly in short period. So, higher production.
3. Genetically modified organism used for synthesis of certain essential amino acids.
4. Quality of proteins is convenient to obtain in cultural condition.

5. Production requires small area cheaper raw materials.
6. The organism are efficient and ecofreindly.

40. Write brief note on Biosensors?

Biosensor is a analytical device or instrument which consisting of an immobilized layer of biological material like enzyme, antibody, organelle, hormones nucleic acids or whole cell sin the intimate contact with a transducer i.e. sensor which analyses. The biological signals and converts into an electric signal. The electrical signals are measured as a converted reading and estimation or testing is made.

E.G. Enzyme electrodes
(Glucose electrodes)

41. Write short note on preservation of foods?

Preservation of food mainly deals the elimination of spoiling microorganisms or controlling their growth by on food material by many ways like chemical methods, Biological, methods and physical methods. The common techniques are;

1. High temperature treatment
2. Low temperature storage
3. Storage with brine
4. probiotics culture (or) lactic and bacteria
5. Various organic acids
6. Radiation treatment

42. What are biopreservatives? Give suitable examples?

Preservation or storage of food materials with biological materials or biological preparations like lactic acid, Lactic acid bacterial culture (probiotics), and antimicrobial compounds from higher plants and microorganism (Nysin & Nystatin).

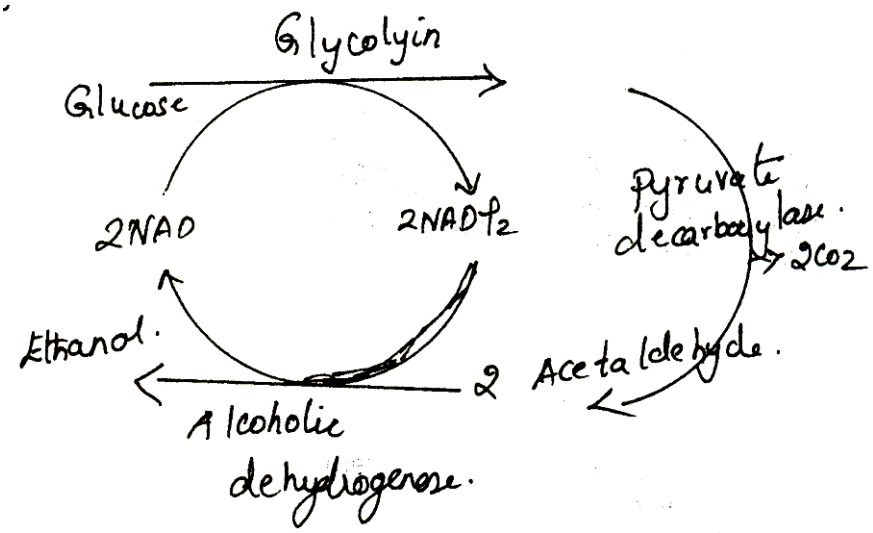
PART – B

1. Describe the Alcohol production process by industrial fermentation method?

Alcohol fermentation was first demonstrated by Louis Pasteur in 19th century. The conversion of Glucose to Ethanol was first discovered by Gay Lusssac in 1815

i.e. $C_6H_{12}O_6 \rightarrow 2C_2H_5O_H + 2CO_2$

The line conversion of glucose to ethanol through fermentation via fructorse bi-phosphate pathways as given below.



Pressure of oxygen inhibit the fermentation

Production Process;

Microorganism:

Yeast species are able to ferment sugar to ethanol. The organisms are

1. Sacharomyces ceevriseae
2. *Torulopsis sp.*
3. *Klebsiella sp*
4. *Candida sp.*

Among the above organisms, sacharomyces cerviseae is the most commonly used for ethanol production.

Large amount of yeast cells are prepared as an inoculum by laboratory cultivation method and size of the inoculum will be average of 4% v/v for fermentation process.

Raw material and fermentation medium:

Generally carbohydrates in the form of Molases contain sucrose (sugar) (48 to 55%) and Ammonium sulphate for N_2 source biotin also added in the medium.

pH of the medium is 4.8 to 5.0 and sulphuric acid is used for pH adjustment.

Fermentation;

The temperature of fermentation process is about 28 to 30°C and it is maintained by cooling coils. Anaerobic fermentation to be maintained.

Mild Agitation is required to maintain uniform temperature. The fermentation period is 30 to 72 hours. After this period the alcohol content will be 6% to 8% alcohol.

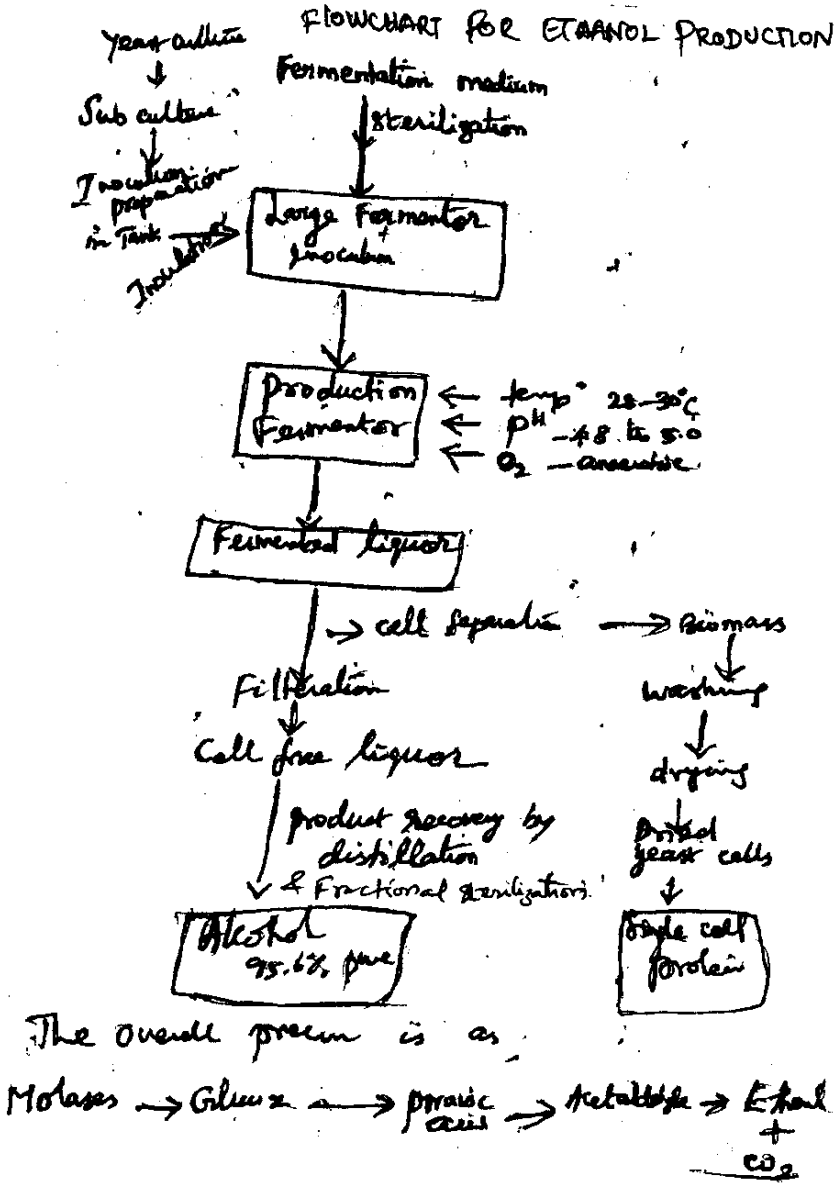
The yield of alcohol content about 90% of carbohydrate content.

Recovery:

Fermented mash contains medium alcohol and yeast cells are collected to settle for few hours.

Then the liquor is filter through filter press for removal of yeast cell biomass. Filtrate contained alcohol is distilled to obtained rectified spirit. Then the rectified sprit. then the rectified spirit is distilled by fractional distillation pure ethanol about 95.6% alcohol.

The process flow diagram of the ethanol production by industrial fermentation process is outlined below.



2. Explain the method or production of penicillin by the industrial scale?

Pencillins are β lactum group of antibiotics, which are mainly active on bacteria or bactericidal action. They inhibit the formation of peptide cross-language in the final stages of bacterial cell wall synthesis. Pencillin'G' and penicillin 'V' is fermented product from the fungus penicillium spp. which are effective on Gram-positive cocci.

Microorganism and inoculum preparation;

The microorganism involved in the production of penicillin is *penicillium chrysogenum* a spore forming fungi. The strain improvement for higher or overproduction is done by mutation techniques in time-to-time and high potent organisms are used for Industrial production.

The organism is cultured and the pure inoculum is produced to harvest spores for further inoculum development in seed tank. The spore development in the seed tank and germinated spores with young mycelium were taken as an inoculum for production tank of fermentation process.

Raw material and medium preparation:

The Raw materials are Agricultural waste material of starch and related polymers.

1. Carbohydrates are include starch, hydrolysed starch, Glucose, Dextrose syrup impure waste from Dextrose plant, Oligo sacharides.
2. Nitrogen sources in the form of Ammonium sulphate and cornsteep liquour (or) cornsteep powder.
3. minerals in the form of salts as sodium nitrate and pottasium chloride.

Addition of precursor molecules, like phenyl acetic acid or phenoxy acetic acid in the fermentation of penicillin is necessary for formation of penicillin.

The fermentation medium sterilized by in situ method and allocated to cool at ambient temperature.

Fermentation;

Pencillium chrysogenum in the form of spores or germinated spores are inoculated into the fermentor containing fermentation medium.

The fermentation process is Aerobic method; hence oxygen is to be supplied by aeration process.

Temperature of the fermentation to be maintains 30° to 32°C and the p^H to be maintained 5.0 to 6.0. the pH maintenance can be addition of Hydrochloric acid or sodium hydroxide. The time required is 2 to 3 days.

Recovery process:

Fermented liquour of the fermentor tank contained fungal biomass, penicillin in the liquour and other organic and in organic debris of the medium.

Filtration: The fermented liquour filtered through filtered press or rotary Drum filter and the mycelial mass in the form of cake is discarded. The spent liquour or filtrate is extracted by solvent extraction with the solvents choice off n-butyl acetate or methylisobutylketone.

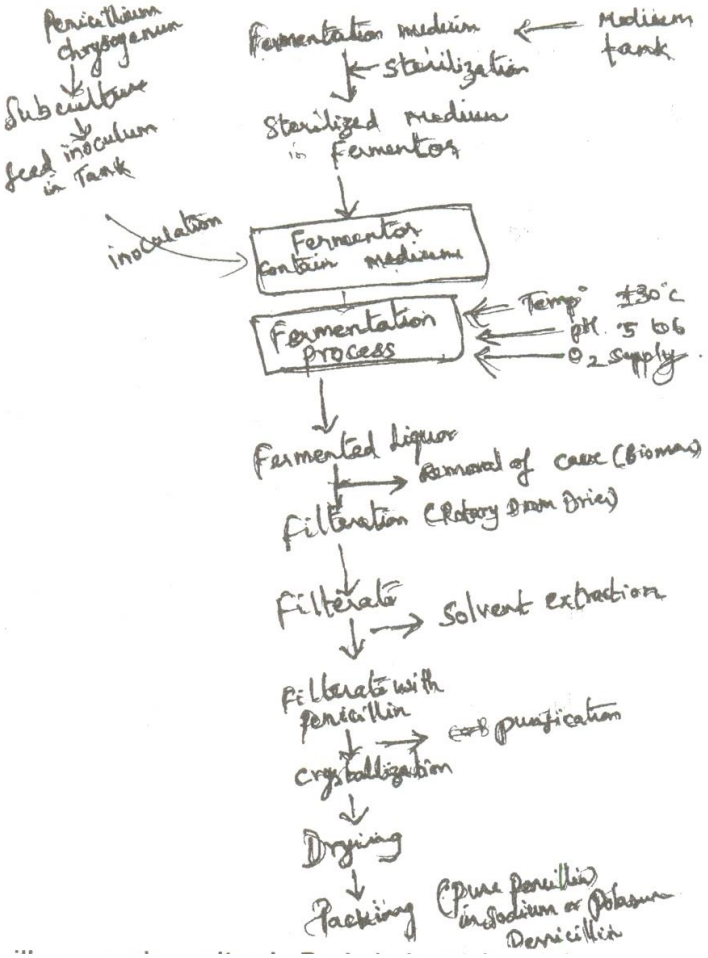
Then the pencillin is extracted with aquous solution contain phosphate buffer and again extracted with solvents.

The penicillin rich solvent is treated with activated carbon to remove pigments and coloring matter.

Then the pencillin is recovered as the sodium or potassium salt by adding sodium or potassium acetate.

The penicillin is then purified by crystallization and drying methods. The dried pencillin crystals are packed as sodium penicillin or Potassium penicillin in various quantities as preferred by the customers or marketing agency.

The complete process flow of industrial fermentation process of penicillin production is given below;



3. How will you produce vitamin B_{12} in industrial scale?

Vitamin B_{12} (or) cyanocobalamine is an important vitamin for human health. Deficiency of vitamin B_{12} causes Anaemia and improper functioning of central Nervous system (CNS). It is used as food and feed supplements. Required daily allowance is 0.2 to 1μg per day. Vitamin B_{12} is available as cyanocobalamine, which is produced in bulk by fermentation process to meet the requirements.

Microorganism & inoculum development:

The microorganisms used frequently to produce vitamin B_{12} are *Bacillus coagulans, strategies olivaceus, and Propinobacterium shermanii and Streptomyces sp.*

The improved strains of ATCC (American Type culture collection) were used for industrial production process.

Inoculum:

The bacterial spores or cells in the dry form as in Ampules or tube are available for subculturing. The above inoculum is subcultured in the laboratory in petriplate and scaled up done in flask. Feather scale up of inoculum for fermentation is done by seed fermentor (or) seed tank & kept as a bulk inoculum for fermentation.

Raw material & media preparation:

The growth medium and production medium are available for inoculum growth & fermentation respectively.

The growth media contain glucose, yeast extract, Beef extract, and N-Z- Amine A, (enzymatic rhydrolysate of cafeine) dissolved in distilled water and sterilized before inoculation.

The inoculum concentration is recommended as about 5% on medium.

The production medium composed of carbon sources as Distiller's soluble, Glucose and sacharides,

Nitrogen sources include distiller's soluble, yeast hydrolysate, Ammonium nitrate and Minerals & vitamins.
Cobalt is essential to formation of methylcobalamine.
Composition:

Distillers solubles - 5.0 g/lit
Glucose - 0.5 to 1.0 g/ lit
$Caco_3$ - 0.5 g /lit
$Cocl_2.\ 6H_2O$ - 1.5 to 10 pm
pH - 7.0 max

Media sterilization at 121°C for 20 mins. & Cooling of medium to ambient temperature. Inoculation of medium with 5% of inoculum after the temperature of the fermentation medium is cooled down to room temperature.

Fermentation /Incubation:

The inoculated medium incubated or fermented at 37°C for the period of 3 to 5 days (72 to 120 hrs) the PH of the medium to be maintained at 6.0 to 7.0 by addition of sulphuric acid.

Antifoaming agents as fatty acids are added to control foam.

The fermentation of vitamin B_{12} occur at 2 stage as

i) Anaerobic fermentation up to 72 hrs -during, which the cobinamide is produced and accumulated in medium.
ii) Aerobic fermentation stage Ii by another 50hrs.

5-6-demethyl benziminazol is added (0.1%) continuously and methylcobalamine is formed. Contamination to be prevented and sterility is maintained yield is maximum of 35 mg/lit

Recovery Process:

Fermented mash with liquour and mycelium is heated with pH 5.0 or below.

Then liberation of vitamin B_{12} achieved into the liquid medium.

The mycelial mass after heating treatment is removed by filtration (Rotary vacuum filter)

The filtrate-contained vitamin B_{12} is added with cyanamide and 2-6 dimethyl benziminazol to formation of vitamin B_{12}.

The vitamin B_{12} purified by adsorption in activated carbon and Ion exchange Resin, Bentonite etc.

Then the filter ate is discarded. The vitamin B_{12} is extracted with aqueous solvent as Hydrochloric acid water or water Acetone. Then Vitamin B_{12} is precipitated and further purified by column chromatography with Alumina. Then the aqueous form contain vitamin B_{12} is evaporated and concentrated. The concentrate is crystallized and dried by using vacuum drier. The dried powder of vitamin B_{12} is packed in suitable container.

The above methods are followed fro the prediction of vitamin B_{12} in the industrial fermentation process.

4. Explain the mechanism and production of Biogas by using Bio plant?

Production of mixture gases including methane as a major portion by microorganism through degradation of organic substances at anaerobic environment is called Biogas. Biogas composed of methane (63%) carbon dioxide (30%) Nitrogen (4%), Hydrogen sulfide (1%) and traces of H_2, O_2 & CO.

Biogas also referred as Biofuel, sewage gas, Clar gas, sludge gas, fools fine, gobar gas, bioenergy and fuel of the future.

The production of biogas by microorganism can be explain as;

$$\text{Organic matter} \xrightarrow{\text{Microbes}} \underset{\text{(gas)}}{\text{Biogas}} + \underset{\text{(sludge)}}{\text{organic fertilizers}}$$

$$\text{Organic matter} \xrightarrow[\text{microbes}]{\text{anaerobic}} CH_4 + CO_2 + H_2 + N_2 + O_2 + H_2S$$

Mechanism of production:

The production Biogas is the complicated process by microbial action. It involves several steps and reactions. Each step of reactions catalyzed by specific enzyme.

The process is mainly described by 3 stages.

i. Solublization/ Degradation
ii. Acitogenesis
iii. Methanogenesis.

Stage I - Solubilization:

The raw material or feedstock likes Agricultural biowastes and organic waste materials are get solubilized or degraded by a group of microbes called anaerobic organism. In this process, the organic polymeric substances are getting bread down into simple forms and to organic acids.

The sources of feedstock are

1. Plant source
2. Animal source

Cellulosic wastes, Lipocellulosic waste, many kinds of algae, Garbage, Kitchen wates, sugarcane waste and waste hyacinth etc are plant source. The animal source like cattle dung poultry manure, goats & sheep measure, slaughter house effluent, fishery waste and human exreta etc.

By enzyme action, the polymeric compounds are hydrolysed and converted and various organic acids which are the intermediates enter into the next stage.

Stage II - Acitogenesis:

The simple organic matter converted from the first stage after degradation utilized by various Autogenic bacteria like *Acitinobacter and Acinobactor*, which are facultative anaerobic organisms and Hydrogen producing bacteria and converted to Acetic acid, Hydrogen and carbon dioxide.

i.e.

$$\text{Simple organic matter} \xrightarrow{\text{Oxidation}} \text{Acetate} + H_2 + CO_2$$

These organic acids serve as a food for final stage and

$$\text{Fatty acids} \xrightarrow[\text{Obligate parasite}]{\text{dehydration}} \text{Acetate} + H_2 + CO_2$$

$$H_2 + CO_2 \xrightarrow[\text{Acetate producing Bacteria}]{\text{hydrogenation}} \text{Acetate} + \text{acids}$$

The above steps are carried out during the second stage called Acitogenesis by various acid-producing bacteria

Stage III - Methanogenesis:

In this process a special group of bacteria, which are anaerobic bacteria, involve in the production of Methane by utilizing the acetate and other acids. This group of organisms called "Methanogens'

$$\text{Acetate} + H_2 + CO_2 \xrightarrow[\text{Bacteria}]{\text{Methanogenic}} \text{methane} + CO_2 + H_2O$$

These organisms are primitive one and live in water lodged soil, gut of animals, sewage sludge. Ratting vegetation. Example for few methanogens is

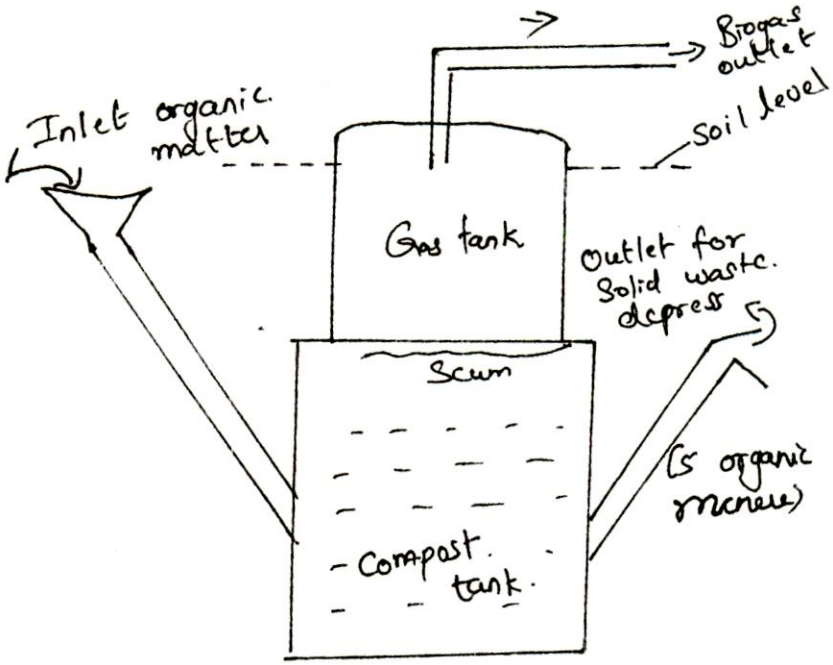
Methanobacterium
Methanococcus
Methano microbium
Methanospirillum
Methanosarcina

Production design
Anaerobic design

The airtight cyclindrical container made as a digester tank, concrete design or cement structure is designed as a digester. It has a side opening (charge pit) for charging organic material to digester.

The cylindrical outlet with container above the digester is for collection of biogas has valve outlet.

After digestion of organic matter, the gas is collected on the top container and gas is filled in small containers.



Schematic diagram for Biogas production

Uses:

1. It is used as cooking user and domestic lighting
2. used as heating purposes
3. Used as a motor fuels (petrol & diesel engines)

Advantages:

1. Indefinitely renewable: energy
2. Biodegradable organic wants are removed and environmental safety
3. Control the pollution of environmen

5. Write short notes on the following:

1. Biofertilizer production (N_2 fixing bacteria)
2. Biopesticides production
3. Biopreservatives and antibiotics
4. *in situ* ore leaching process.

1. Production of Biofertilizers (N_2 fixing bacteria):

The atmospheric free nitrogen is fixed in the organic as nitrate by bacteria (or) microorganism called biological Nitrogen fixation.

There are two types of N_2 fixers as

1.**Symbiotic Nitrogen fixation**:

As the bacteria live in the roots of plants and supply nitrogen and derive nutrients for them from plants.

E.g. Rhizobium – in Leguminous plants.

2. Nonsymbiotic Nitrogen fixation:

In this group the microorganism and free living on fix atmospheric nitrogen in their vegetative cells and they are available to plants through soil.

e.g. Azatobactor, Azospirillum, bluegreen algae } non leguminous plants

Production;

These bacteria and blue green algae are cultivated in mass level either in the artificial condition or natural condition.

The bacterial cells are multiplied from culture media in large scale.

Rhizobium cultivation:

The strains are isolated from injected root nodule and identified for further use.

They are grown in the laboratory with material rich medium and scaled up for fermentation process.

Fermentation method:

The media is prepared in large scale in the fermator and the bacteria is inoculated and kept for incubation for 3-4 days at 30-32° with pH of 7.0.

After a growth of bacteria in the fermentor the cell concentration was checked and the cell suspension taken for further blending with carrier material. The carrier material may be Talc powder, charcoal powder, paddy husk, shaw dust etc. The cell concentration in the powder is 1 $x10^9$ to 4 $x10^9$ cells/gram. This is dried to 5% moisture.

Biopesticides and their production:

Biopesticides are the biological materials include the plant and microbial products and microorganism, which are harmful to insects and pests. Many Bacteria, fungi and viruses are known to act against Insects and pests.

E.g. Bacteria-

Bacillus thurigiensis,

B. moritae

B. Propilliae is used as Biopesticides.

Biopepsticides:

Bacillus thuringiensis produce endotoxin otherwise called Delta toxin which is poisonous to insects and pests cause diseases of cotton plants. This insect called Bull worm eats cotton leaves. Other organisms are mainly fungi as:

Beaveria bassiana

Conidiobolus obscuses

Entomopthora grylli

Metarhizium anisopleae

are infect insects and kill them.

The production of biopesticides:

The bacteria or fungi, which are called as a Biopesticides, are first cultivated in the laboratory and the inoculum is prepared for bulk scale production.

The inoculum in the form of spores or cells is used for inoculation.

The culture media is prepared in bulk with respect to the organisms like either Bacteria or fungi in a big fermentor vessel and sterilized.

The inoculum is introduced into medium containing fermentor vessal and allows growing from few hours to few days. Then the biomass containing the culture booth is mixed with inert carrier and the biopesticides are packed in small packs for agriculture use.

The organism is available in viable (or) live form for field applications.

iii) Bio-preservatives and Lantibiotics:

Preservation of food products by natural products to avoid the spoilage of food during storage period is called Bio-preservation.

e.g. lactic acid bacteria (LAB) are capable of producing Bactereocidin which are antimicrobial compounds that inhibit the growth of food spoiling micro organisms.

Bactereocidin are proteinaceous compounds, which inhibit the food spoiling pathegon. It produced by

Lactobacillus fermentum

L. lactis

L. reuteri

Lactococcus sp.

Propioniobacterium sp

Leuconostoc mesenteroids

Pediococcus sp.

Streptococcus cremoris

These LAB are called Probiotics and very useful for food preservation.

The compounds Nysin produced by *streptococcus cremoris* and Nystatin produced by *streptomyces noursei* are antifungal compounds otherwise polyene antibiotics.

Lantibiotics:

Lantibiotics are otherwise the antimicrobial compounds produced by lactic acid bacteria. Lantibiotics contain dihydro residues and thioether amino acids in their polypeptide chain.

e.g. Nysin

Lanthionine rings found in the compounds (peptide chains). Prenysin is a nysin precursor.

IV. In situ ore leaching process:

This is a ore leaching process by the action of microbes in the ore bearing areas like rock & soil.

It is used to produce in Bulk scale. The leaching of ore occur in the place of ores availability as rocks & soil i.e. ores are remain in the original place of earth.

The ore deposit is identified for process surface blasting of rock and soil is done for free availability of ore. The blasted ore is more pronounced to permeability with water & bacterial inoculum.

The acidic water and Bacteria inoculum are introduced into drilled passage.

Then the growth of bacteria is occur and oxidation takes place. Seepage of acidic water with dissolved metals is seeping through the ores to the bottom areas.

Then the collection of water contain metals are done in big pool. Then the metals were extracted and separated by chemical processing.

The acidic water is again reused with bacteria or recycling in done

E.g. copper leaching is done by In situ method
Organism involved as *Thiobacillus ferroxidans.*

6. Describe the method of waste water treatment by Municipal water treatment plant?

Municipal wastewater treatment plants carry out a series of treatment process which are briefed as below.

1. Primary treatment:

To remove coarse solids and to accomplish removal of settlable solids. This is done by using screen in the collection tank to treatment tank.

2. Secondary (or) Biological treatment:

In this process the organic wastes were oxidized and absorbed by microorganisms, present in the effluent. The Biological oxygen Demand (BOD) of the wastewater is drastically reduce.

3. Advance treatment:

This process takes to remove additional objectionable substances to further reduce BOD. This includes removal of nutrients such as phosphorus and Nitrogen.

4. Final Treatment:

This process carries over to disinfect and dispose the liquid effluent water.

5. Solids Processing:

This process solubilizes solids to removed from liquid process to dewater solids and ultimately to dispose of solids (land application, land hill incineration). Detailed process:

I. Primary treatment:

Wastewater as it arrives at a wastewater disposal plant is treated to remove coarse solid materials by a variety of mechanical techniques including screening, grinding, and gril chambers, subsequent to this it is related to remove settlable solids.

Sedimentation:

Sedimentation tanks (units) provide the means for concentrating and collecting the particulate matter referred to as sludge. Following sedimentation, the sludge and the liquid effluent are processed separately.

II. Secondary (Biological) treatment:

Secondary treatment process accomplishes oxidation of the organic material in the liquid waste water by microbial activity. The oxidation methods employed are;

i) Filtration by intermittent sand filters, contact filters and trickling filters.

ii) Aeration by the activated –sludge process or by contact aerators.

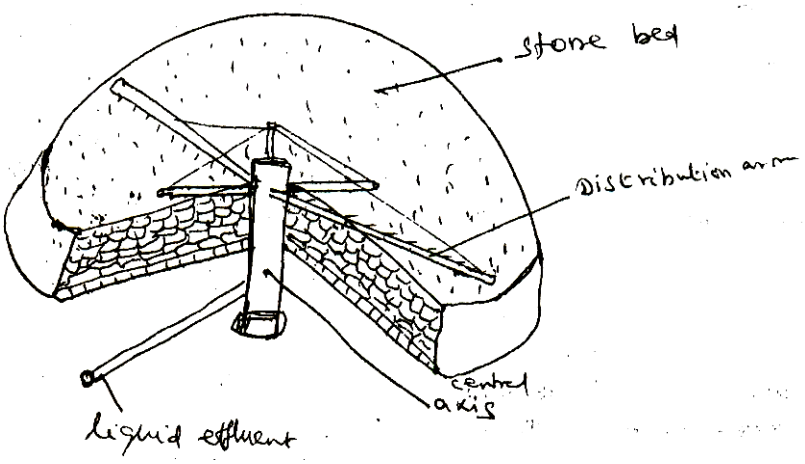
iii) Oxidation bonds.

Trickling filters:

The trickling filter consists of abed of crushed stone, gravel, slag or synthetic material with drains at the bottom of the tank. Trickling filters have been described as a pile of rocks over which sewage or organic wastes slowly trickle. The liquid waste is sprayed over the surface of the bed either by a rotating arm or through nozzles. The spraying saturates the liquid with oxygen. Intermittent application of the sewage permits maintenance of aerobic conditions in the bed. The filtering medium of the tank becomes coated with a microbial flora, the zoological film, which consists of bacteria, fungi, protozoa and algae.

As the sewage seeps over these surfaces the microorganism adsorb and metabolize the organic constituents to more stable end products. This operation may be regarded as a stationary microbial culture fed by a continuous supply of nutrients (organic contents off the sewage). A newly constructed bed must acquire the zoological film before it can functions efficiently. This requires operation over a period of few weeks. The organisms comprised of mixture of microbes.

The design of trickling filter is shown below.



The upper region of the trickling filter is favourable for the growth of algae and at times their growth may extensive that it impairs the operation of the filter. Many species off protozoa and fungi occur throughout the filter. Their growth is depending on availability of oxygen and nutrients.

The Activated sludge process:

Vigorous aeration of sewage results in the formation of floc, the finely suspended and colloidal matter of sewage forms aggregates designated floccules. If the floc is permitted to settle and then added to fresh sewage that is again vigorously aerated, flocculation occurs in a shorter time then before. By repetition of this process, i.e. addition of sedimentation, addition of sedimented floc to fresh sewage, aeration, sedimentation, addition of sediment to fresh sewage, aeration etc., a stage is reached where complete flocculation of fresh sewage occurs very quickly. i.e. a few hours. The particles of floc, i.e "activated sludge" contain large numbers off very actively metabolizing bacteria, together with yeasts, molds and protozoa. This combination of microbial growth is very effective in the oxidation of organic compounds. A poor settlement of activated sludge flocs adversely affects performance of a sewage treatment plant. The sludge becomes more voluminous and is difficult to control. The principal reason for poor settling of activated sludge is the growth of filamentous microorganisms. Many different species of microorganism have been isolated from sludge in this condition. The use of activated sludge is off great importance in wastewater treatment. This process usually employs an aeration period of 4 to 8 hours, after which the mixture is piped to a sedimentation tank. This tank contains effluents represents wastewater treated to secondary level and considerable level off suspended solids and the BOD is reduced. This effluent does not need higher dilution for disposal.

Oxidation Ponds (Lagoons)

These are shallow ponds designed to allow algal growth on wastewater effluent. Use of oxidation ponds should be preceded by primary treatment. Oxygen for biochemical oxidation of nutrients is supplied from the air, but the release of O_2. During photosynthesis of algae chlorella, pyrenoidse provide on important source off oxygen.

Advance treatment:

Advance treatment is required when removal of substance beyond the limits normally achieved by conventional primary and secondary processes are necessary. Unit processes have been developed to remove nutrients, simple organic substances, and complex synthetic organic compounds. Processes include biological treatment; however physical-chemical methods predominate common unit processes include biological nitrification, nitrification, filtration, reverse osmosis, carbon absorption, chemical addition and ion exchange. The major disadvantage of advanced treatment is high cost.

Final treatment:

The liquid treatment, upon completion of other treatment, is disinfected and usually discharged into body of water. This is necessary to protect public health when the receiving waters are used for purposes such a downstream water supply, recreation, irrigation or shellfish harvesting. Mostly chlorination is done for disinfection. Current use of sine and ultraviolet rays are also proved better than chlorination but costly.

Dissolved oxygen also to be added to the treated water prior to final discharge. This is called as post aeration process and is accomplished by mechanical means or a cascading slow technique.

The disposal of treated water on surface area in widely practiced and land application is a continuous feasible method.

V. Solids processing:

The solids processing consist of thickening, stabilization, dewatering and disposal of sludge. Solids are removed from the primary secondary and the advanced stages of the treatment process. Thickening is employed to further concentrate the solids or sludge prior to stabilization.

Many stabilization processes are employed including aerobic and anaerobic digestion, composting chemical addition, and heat treatment. The most common processes for modern municipal treatment facilities are the anaerobic digestion system.

Dewatering is accomplished by physical methods and is often enhanced by the addition of polymers or other chemical coagulant acids. Dewatering done by using vacuum filters, belt filters press, plate and frame presses and centrifuges.

(i) Anaerobic sludge digestion:

The solids, which accumulate during sedimentation, are pumped into a separate tank designed especially for the digestion of sludge under controlled conditions. Solids recovered from the aerobic treatment may also be returned to the sludge digestion. The microbial action on the constituents of sludge is termed sludge digestion.

In this process anaerobic and facultatively anaerobic bacteria are active and they digest the organic sludge and produce methane (70%) and CO_2 (20 to 30%) and smaller amounts of Hydrogen and Nitrogen. This process depends on the microbial inoculum, pH and temperature.

Composting:

Composting is a process where dewatered sludge undergoes decomposition, usually within the thermophilic temperature range. Dewatered sludge is mixed with a bulking agent such as wood chips. The bulking material is added to enhance circulation of air throughout the sludge to improve stabilization process. The mixer of sludge and bulking material is placed in aerated piles. Oxygen is furnished by forced aeration. The mixture is allowed to 'cure' or biologically decompose for a period of time as normally to be 21 days.

Then the bulking agent is separated from the sludge and the sludge is allowed to cure further for several weeks. Upon final curing the sludge has been transformed to a humus-type material and is suitable for use as a soil conditioner.

R 3117

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2007

Third Semester

Bio Technology

BT 1204 – MICROBIOLOGY

PART – A

1. What is the principle behind phase contrast microscopy?
2. What is meant by differential staining?
3. What is attenuation?
4. What is a palisade arrangement in bacillus?
5. What is competitive inhibition?
6. What is ubiquinone?
7. What is a peptidoglycan?
8. What is Transduction?
9. What are nuisance organisms?
10. What are exotoxins?

PART – B

11. (a) Describe with a neat diagram, the various components of a bacterial cell and its functions.

(or)

(b) Describe the basic mechanisms of bacterial metabolism.

12. (a) Discuss the bacterial growth curve and its applications.

(or)

(b) How will you obtain a pure culture of a bacterium and preserve it?

13. (a) Describe the classification of algae and its industrial applications.

(or)

(b) Describe the physical and chemical methods used for the control of micro organisms.

14. (a) Discuss in detail food microbiology.

(or)

(b) Discuss in detail soil microbiology.

15. (a) Write notes on:
    (i) Transmission electron microscopy.
    (ii) Bacteriophages.

(or)

(b) Write notes on:
    (i) Microorganisms and ore leaching.
    (ii) Quantification of bacterial growth.

T 8068

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2006

Third Semester

Bio Technology

BT 1204 – MICROBIOLOGY

PART – A

1. Define protista. Give 2 examples for higher protista.
2. What are the limits of resolution in light microscopy?
3. What is meant by bacterial chemotaxis?
4. Give an account on the structure of hypha of mycelium.
5. Give the significance of differential media with example.
6. What is meant by synchronous growth? How is it achieved?
7. List out the action of antibiotics on microorganisms.
8. Discuss the pathogenecity of haemophilus.
9. What are secondary metabolites? Give 2 examples with application.
10. Name two microorganisms used as biofertilizer and biopesticides.

PART – B

11. (a) Brief the history of microbiology and write an essay on the nomenclature of microorganisms.

(or)

(b) Write the principle and working hypothesis of light microscope. Give the principle of any two staining techniques.

12. (a) Explain the sexual and asexual mechanism of reproduction in fungi and write notes on Rhizopus.

(or)

(b) Describe the structural organization of any three algae and briefly discuss the sexual reproduction of protozoa.

13. (a) Discuss the different quantitative methods employed for the measurement of bacterial growth and comment on the selection of quantitative procedure.

(or)

(b) Explain the methods used for the maintenance and preservation of bacterial pure culture. Discuss the growth rate and generation time in bacterial growth.

14. (a) Describe the mode of action of any two physical and four chemical agents on the control of microorganism. Discuss the selection of chemical agent for practical applications.

(or)

(b) Write an essay on the host – microbe interaction in the process of infection and microbial virulence factors with suitable examples.

15. (a) Describe the industrial use of microbes in the production of penicillin, alcohol, vitamin B12 and biogas.

(or)

(b) Explain the applications of microbes in the food preservation, pollution control and bioremediation in detail.

C 127

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2005

Third Semester

Bio Technology

BT 1204 – MICROBIOLOGY

PART – A

1. Discuss the place of micro organisms in Whitakker's five kingdom classification scheme.
2. Compare the appearance of microorganisms as seen by dark field microscope and a phase contrast microscope.
3. During the log phase growth of a bacterial culture, a sample is taken at 8.00 a.m. and was found to have 1000 cells per ml. A second sample was taken at 5:54 p.m. and the population was found to be 1000000 cells per ml. What is the generation time in hours?
4. Give an example to illustrate the expenditure of energy in the biosynthesis of an amino acid.
5. What are the major differences between rickettsias and chylmydias?
6. What is multiple fission? Cite an example of its example in prokaryotes.
7. What is a viriod? Why is it unique?
8. How can potency of an unknown sample of pencillin be determined?
9. Why is Escherichia coli considered to be an indicator of pollution?
10. What is meant by food yeast and baker's yeast?

PART – B

11. (i) Explain the distribution and occurrence of natural flora.
    (ii) Describe the fractional sterilization (Tyndallization).

12. (a) Explain the history of microbiology.

    (or)

    (b) Explain the various classifications of living organism?

13. (a) Explain the various chemicals anti – microbial agents.

    (or)

    (b) What are the different physical agents used in control of micro organisms?

14. (a) Describe the significance, characteristics, morphology and structure and replication of bacteriophage.

(or)

(b) (i) Describe the life history of chlamydomonas.

(ii) What are diatomaceous earth? Why is it useful commercially?

15. (a) (i) Describe the sulfur cycle.

(ii) Describe the carbon cycle.

(or)

(b) (i) Under what circumstances will you add silica gel as a solidifying agent for cell culture?

(ii) Explain the bacterial growth curve.

K 1049

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2004

Third Semester

Industrial Bio Technology

IB 234 – MICROBIOLOGY

PART – A

1. Explain fluorescent microscopy with a suitable example.
2. Describe the organs of motility in a bacteria.
3. What are phototrophic bacteria?
4. Explain the method of preparation of protoplasts.
5. What is meant by a differential media in the culturing of microorganisms?
6. What are products that are produced by the fermentation of glucose using Streptococcus lactis?
7. Explain the difference between a gram positive and gram negative bacteria.
8. Explain Bacteriophages.
9. What are the microorganism that are found in the contaminated water?
10. What is a single cell protein?

PART – B

11. (i) Describe various types of bacteria based on their structure.
    (ii) Explain a normal bacterial growth curve

12. (a) Explain with suitable examples the various conditions that are adopted to obtain a pure culture of a microorganism.

    (or)

    (b) Describe the Embden – Meyerhof glycolytic pathway in micro organisms.

13. (a) Discuss in detail the classification of Algae.

    (or)

    (b) Describe the various classes of Fungi.

14. (a) Describe the chemical methods in the control of microorganisms.

    (or)

    (b) Describe the various uses of microorganism in the industry.

15. Write short notes on any TWO:
    (a) Pasteurization
    (b) Biological assay to determine the potency of an antibiotics
    (c) Electron microscopy and its use in microbiology.

E 290

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2003

Third Semester

Industrial Bio Technology

IB 234 – MICROBIOLOGY

PART – A

1. Write about contribution made by Joseph Lister in microbiology.
2. Is it possible to convert a bright field microscope to dark field microscope? If so explain.
3. What are L forms?
4. Differentiate Cyanobacteria from other photosynthetic bacteria.
5. What is chemoheterotroph?

6. What is bacterial endospore? Write about its composition.
7. Differentiate food infection from food intoxication.
8. Describe bio remediation.
9. What are methanogens?
10. What is endotoxin?

PART – B

11. (i) Explain the principle behind Electron Microscope. List out the difference between TEM & SEM.
    (ii) Discuss differential and special staining methods.

12. (a) (i) Explain the various types of culture media with examples.
    (ii) Discuss in detail chemical methods of sterilization.

    (or)

    (b) (i) Explain purification of municipal water supplies.
    (ii) How do you enumerate microbes in milk?

13. (a) Discuss lactic acid and mixed acid fermentation pathways occurring in bacteria.

    (or)

    (b) Discuss in detail the causative agents of:
    (i) Whooping count
    (ii) Pneumonia
    (iii) Dysentry
    (iv) Stomach ulcers.

14. (a) (i) Discuss the mechanism by which beta lactam antibiotic act on bacteria
    (ii) Note on Antifungal agents.

    (or)

    (b) Discuss
    (i) Growth curve
    (ii) Bioleaching
    (iii) Anoxygenic photosynthesis
    (iv) Biopesticides

15. Write notes on any TWO:
    (a) MIC
    (b) Cold sterilization
    (c) ED pathway
    (d) Nitrogen cycle

QUESTION BANK

MICROBIOLOGY

PART – A

1. Describe the difference between prokaryotes and sukaryotes with respect to
   a. Chemical composition
   b. Cytoplasm
2. Explain phase contrast microscopy.
3. Describe negative staining in EM.
4. Explain differential staining
5. What do you understand by bacterial crematories?
6. Describe endospours
7. What is a single all protein?
8. Describe the role of thiobacillus in the industry
9. What are the micro organisms present in the raw milk?
10. Describe the structure of fungi.
11. Write four important features of micro organisms
12. Write the contributions of Anton van leewonhock on microbiology.
13. Write shortly on known postulates.
14. Differentiate structurally the bacteria and fungi.
15. Write the economic importance of Actinomycetes
16. Differentiate the bacterial exospores with conidiospore.
17. Give two methods of numeration of micro organisms multiplication by indirect method
18. Write four important drugs and its role on controlling microbial diseases.
19. What is meant by bio – fertilizers? Give two examples of production of bio – fertilizers.
20. Write short notes on food presentation.
21. Differentiate the functions of bacterial flagella and pile?
22. Write short notes on structure of viruses?

23. Explain the common method of reproduction in bacteria. How will you calculate the generation time?
24. What is meant by selective media for culturing of microorganisms? Give one example with composition.
25. Differentiate the bacteriostatic and bactericidal activity of chemical agents. Give suitable examples
26. Write four beneficially important microorganisms products and its uses.
27. Define antibiotics and vaccines with examples.
28. Write the importance of thiobacillus traceries in mining industry.
29. Write short notes on Biopesticides
30. Give any four major difference between bacteria and yeast.
31. Explain the germ theory of fermentation
32. Write short note on magnetotacis
33. What is meant by flagella? Write the various arrangement of bacterial flagella.
34. Write the functions of mesosome
35. Differentiate the indispose with conidospore
36. Write about bacteriophage and its activity on host all.
37. What are the major nutrient requirements for the growth of microorganisms?
38. What is meant by doubling time? How will you calculate the bacterial generation time?
39. Define selective media and enrichment media with one example.
40. Write about the contributions made by Joseph leister in microbiology?
41. Is it possible to convert a bright field microscope to dark fluid microscope? It so explain.
42. What are L forms?
43. Differentiate gyanobacteria from other photosynthetic bacteria?
44. What is chemohetucateoth?
45. What is bacterial indispose? Write about its composition.
46. Differentiate food infection from food intoxication
47. Describe bioremediation

48. What are methanogens?
49. What is antitoxin?
50. Contributions of Alexander teeming?
51. Write any four applications of light microscope.
52. What are the various methods of domesticating the presence of flagella?
53. How does the T-even phages penetrate the bacterial cell?
54. How are bacterial growth measured?
55. Membrane filters
56. Deflective media.
57. Clostridium of bolulinum
58. Functions of Jimbriae
59. Explain fluorescent microscopy with suitable example.
60. Describe the organs of mobility in a bacteria.
61. What are phototrophic bacteria?
62. Explain the method of suppuration of protoplasts.
63. What is meant by a differential media in the culturing of microorganisms?
64. What are the products that are produced by the fermentation of glucose using streptococcus lactis?
65. Explain the difference between gram positive and gram negative bacteria.
66. Explain bacteriophages.
67. What are the microorganisms that are found in contaminated water?
68. What are different shape and arrangements of bacterial cells? Write one example in each.
69. Write about bacterial chemotaxis and phototacis.
70. Write the steps involved in the pupacation of nutrient culture media in the laboratory.
71. Explain the batch and fed batch cultures.
72. Reproductive method in actinamycetes.
73. Describe theory of spontaneous generation with one example.

74. Koch postulates.
75. Write short notes on i) Type culture and ii) Neotype strain.
76. What is meant by resolving power? How will you calculate the same.
77. Write about trichome an pynhae with an example.
78. Give four examples of bacterial cytoplasmic inclusions.
79. What are viruses? Give one example for each type of viruses which occur in nature.
80. Write the role of nucleic acids in classification of micro organisms.
81. What is numerical taconomy? Explain the role in taconomy of micro organisms.
82. Write a note on Anton Van Leewan hoeks invention.
83. Differentiate the electron microscope and light microscope.
84. Write the taxonomical concepts on classification of micro organisms.
85. Write short notes on
    i) Type culture
    ii) Neotype strain
    iii) Species & genes
    iv) Microscopy.
86. What is meant by thermal death time? How is it related to control of micro organisms?
87. Explain the phenomena of fractional sterilization.
88. Write briefly on oxidation and reduction reactions.
89. Give four important characteristic features of ideal antimicrobial companies.
90. Give 4 radiation energy and their effect on controlling growth of micro organisms.
91. What is free energy? How is it calculated?
92. Differentiate antibiotics and vaccines.
93. What is meant by phosphorylation? Give its importance.
94. Give four important Bacterial diseases occurring in man and their control.
95. Write briefly on catabolism of proteins.

PART – B

1. Describe the various conditions to obtain a pure culture of micro organism.
2. Explain a normal bacterial growth curve.
3. Describe in detail the various bacteria and their morphological characteristics giving suitable examples.
4. Describe various chemical agents and their action when used as a sterilizing agent.
5. Discuss soil microbiology.
6. Discuss algae.
7. Discuss fungi.
8. Write short notes on
   a) Methods for bacterial counting.
   b) Industrial uses of bacteria.
   c) Micro organisms in domestic and waste water.
9. Write an essay on anaerobic metabolic pathways of micro organisms.
10. Explain the principles and functions of phase contrast and fluorescent microscope.
11. Write an essay on principles and functions of electron microscope.
12. Write an essay on general structure on organization of bacterial cells.
13. Compare the structure and development of Actinomycetes and fungi.
14. Write an essay on different growth pattern on culture media microbes.
15. Essay on chemotherapy.
16. Explain the carbon and nitrogen cycle
17. Control of environmental pollution by micro organisms.
18. Sketch a typical bacterial cell and label the individual organelles. Discuss the structure and function of bacterial flagella with necessary diagram.
19. What are the major characters used for classification of micro organisms. Briefly explain the numerical taxonomy.
20. Explain the function of electron microscope with suitable sketch. Discuss various techniques used for electron microscope.

21. How will you construct the growth curve of bacteria? Explain the indirect method involved in the determination of growth of bacteria.

22. What are the different types of culture media used for isolation and culturing of micro organisms. Explain the method involved in isolation of fungi from soil and preservation in the laboratory.

23. Explain the metabolic pathways involved in aerobic micro organisms for utilization of glucose.

24. Discuss briefly on microbial toxins, antibiotics, food preservation, infection and immunity.

25. Discuss the role of microbes on carbon and nitrogen cycle.

26. Explain various steps involved in biogas production in large scale. How does it control environmental pollution?

27. Elaborate the bacterial cell wall (both oxygen positive and negative) with suitable examples. Explain the principle of gram staining.

28. Explain the mechanism and applications of light microscope with necessary sketches. Write the differences between light and electron microscope.

29. Write an essay on historical development of microbiology.

30. What are the criteria involved in classification of viruses? Write an account on classification of plant viruses.

31. Discuss various techniques involved in isolation, culture and preservation of various micro organisms.

32. How will you construct the bacterial growth curve? Explain the method commonly used for estimation of bacterial growth.

33. i) Bacterial estimation by colony counting method.
    ii) Bacterial biomass estimation.

34. i) Explain the principle behind electron microscope list out the difference between TEM and SEM
    ii) Discuss differential and special staining methods.

35. i) Explain the various types of culture media with examples.
    ii) Discuss in detail chemical methods of sterilization.

36. i) Explain purification of municipal water supplies.
    ii) How do you numerate microbes in milk?

37. Discuss lactic acid and mixed acid fermentation pathways occurring in bacteria.

38. Discuss in detail the causative agents of
    i) Whooping cough
    ii) Pneumonia
    iii) Dysentary
    iv) Stomach ulcers.

39. i) Discuss the mechanism by which beta lactam antibodies act on bacteria.
    ii) Note on Antifungal agents.

40. Discuss
    i) Growth curve
    ii) Bioleaching
    iii) Anoxygenic photosynthesis
    iv) Biopesticides

41. Write notes on any two
    a) MIC
    b) Gold sterilization
    c) ED pathway
    d) Nitrogen cycle

42. i) Describe various types of bacteria based on their structure.
    ii) Explain a normal bacterial growth curve.

43. Explain with suitable examples the various conditions that are adopted to obtain a pure culture of micro organism.

44. Describe the Embden – Meyerhoff fycolytic pathway in micro organisms.

45. Describe in detail the classification of algae.

46. Describe the various classes of fungi.

47. Describe the chemical methods in the control of micro organisms.

48. Describe the various uses of micro organisms in the industry.

49. Write short notes on any two:
    a) Pasteurization
    b) Biological essay to determine the pouncy of an antibiotics.
    c) Electron microscopy and its use in microbiology.

50. Sketch a typical bacterial cell and describe the components briefly.

51. Write in detail about bacterial cell wall.

52. Write the structure and importance of flagella.

53. Write the role of cytoplasmic membrane of bacteria.
54. Write an essay on yeast cells related to their structure and function.
55. Critically discuss the actinomycetes group of bacteria.
56. Write an essay on viruses.
57. Construct a bacterial growth curve for batch culture and explain individual phases of growth.
58. Sketch a chemostat for bacterial cultivation and explain the continuous culture system.
59. i) Synchronous growth.
    ii) Derive $n = 3.3 (\log_{10}N - \log_{10} N_0)$ from binary fission method of Bacterial multiplication.
60. Explain the methods of estimation of Bacterial all growth by counting method.
61. Explain the indirect method of estimation of bacterial growth.
62. Reproduction methods in bacteria and yeast.
63. Methods of reproduction in fungi.
64. Differentiate light microscope (compound microscope) with electron microscope with suitable schematic diagram.
65. Write in detail about
    i) Germ theory of fermentation.
    ii) Germ theory of diseases.
66. What are the tools used to classify the micro organisms? Write a brief account.
67. Write short notes on
    i) Numerical taxonomy
    ii) Taxonomic groups of classification.
68. What are techniques used for preparation of specimen for electron microscopic studies.
69. How are microbes classified?
70. Explain various methods of food preservation.
71. What are the properties that an antibiotic should fulfill? Classify them and explain with examples.
72. Explain the applications of various micro organisms in industrial use.
73. Write an essay on chemotherapy and its role in control of diseases caused in human and animals.

74. i) Virus diseases and their control in human.
    ii) Mycoses and their control.
    iii) Anaerobic growth process of micro organisms.

Department of Biotechnology

PART – A

1. What are the different shapes of bacteria?
2. What is mesosome? Mention its importance?
3. Write short notes on Chemotaxis?
4. Define Viruses?
5. What are L-forms?
6. Write the economic importance of algae?
7. Write short notes on Diatoms?
8. Write short notes on Mycoplasma?
9. Briefly write on bacterial condiospores?
10. What is meant by complex medium? Give one example?
11. Compare aerobic and anaeronic micro organism with one example in each?
12. Explain the synchronous growth and its importance?
13. Compare oxidation and reduction reactions?
14. What is amphibolic cycle? Give suitable examples?
15. Write any 4 differences between Batch culture system and Continuous culture system?
16. During the log phase growth of a bacterial culture, a sample is taken at 8 a.m. and found to contain 1,000 cells/ml. A second sample is taken at 5.54 p.m. and is found to certain 1,00,000 cells/ml. What is the generation time in hours?

PART – B

1. Discuss briefly on the cell walls of gram positive and gram negative bacteria?
2. Describe the structure and arrangement of flagella?

3. Discuss briefly on Actinomycetes group of bacteria?
4. Discuss the structural organization and economic importance of algae?
5. What is meant by nutrient media? What are the various nutrient media used for culturing of bacteria?
6. Describe the methods of reproduction in bacteria?
7. Explain the method of measurement of bacteria by direct cell count by microscopy?
8. Briefly explain the biosynthesis of micro molecules and macro molecules in microorganisms?

PART – C

1. Draw a neat sketch of bacterial cell and mention about various shapes and arrangements of bacterial cells?
2. Discuss various methods of reproduction involved in fungi?
3. What are the criteria involve in classification algae and mention various classes of algae?
4. Write an essay on yeast with special mention to its life cycle?
5. Explain the method of multiplication of bacteriophage?
6. Discuss various growth phases occur in bacteria with suitable diagram (growth curve)?
7. Mention any 4 methods involve in the measurement of Bacterial growth?
8. Describe in detail about the EMP pathway of glucose metabolism?

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MADHA
Expertise | Empathy | Excellence
ENGINEERING COLLEGE

DEPARTMENT OF BIOTECHNOLOGY

BT3392-BIOCHEMISTRY

LECTURE NOTES

REGULATION 2021

II YEAR & III SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

UNIT – I

PART - A

1. What is acid?

Any substance that dissociates to form H+ ions (protons) when dissolved in water.

2. Define Activation energy (ΔG+)?

Extra energy that must be possessed by atoms or Molecules in addition to their ground-state energy in order to undergo a particular chemical reaction. Activation energy can be applied externally as heat. But this is in appropriate for living organisms. They rely on biological catalysts (enzymes) which decrease the activation energy needed for the Reaction to take place.

3. What is Adenosine phosphate & its biological role?

A group of organic phosphate including adenosine Monophosphate (AMP),adenosine diphosphate (ADP)& adenosine triphosphate (ATP).They Function in phosphate transfer in the cell,particularlly in the transfer of the high –energy Phosphate bonds of ADP & ATP,ATP is the most directly utilizable source of energy of the cell.

4. Define Base.

Any substance that combines with H+ ions (protons) present in a solution and there by Increasing the number of hydroxyl ions.

5. Define Buffer.

A chemical substance which has the capacity to bond to H+ ions, removing them from solution when Their concentration begins to rise & releasing H+ ions when their concentrations begin to fall. In this way, buffers stabilize the pH of biological solutions & thus important in maintaining homeostasis.As an

Eg: hemoglobin maintains a stable pH in the erythrocyte.

6. Define Configuration.

The spatial arrangement of an organic molecule that is conferred by the presence of either (a) double bonds, around which there is no freedom of rotation ,or (b) chiral centres, around which substitutent groups are arranged in a specific sequence. Configurational isomers cannot be interconverted without breaking 1 or more covalent bonds.

7. Define Covalent bond.

A stable chemical bond formed by the sharing of 1 or more pairs of electrons among the atoms in a molecule.

8. What is Enantiomers?

Stereoisomers that are non superimposable mirror images of each other .The enantiomers of a compound rotate polarized light in opposite direction, hence also called as optical isomers.

9. What is Endergonic reaction?

A chemical reaction that requires an input of energy

Before it can proceed (that is, for which G is +ve), endergonic reactions never occur spontaneously; compare exergonic reaction.

10. Define Epimers.

Two stereoisomers differing in configuration at 1 asymmetric centre, in a compound having 2 or more asymmetric centres.

11. What is Hydrogen bond?

A weak electrostatic chemical bond that forms between 1.electronegative atom (such as oxygen or nitrogen) & a hydrogen atom covalently linked to a 2^{nd} electronegative atom. Hydrogen bonds hold the 2 complementary strands of DNA together Life would be impossible without this type of Linkage.

12. Define Hydrophilic compound.

Polar or charged, describing molecules or groups that form enough hydrogen bonds with water & hence dissolve freely in water; sugars are hydrophilic; compare hydrophobic.

13. Define hydrophobic compound.

Nonpolar or uncharged describing molecules or Groups, which do not form hydrogen bonds with Water &hence are not soluble in water.(but are Soluble in nonpolar solvents such as ether,Acetone), lipids & hydrocarbons are generally Hydrophobic; also called as lipophilic, compare Hydrophilic.

14. Define Ionic bond.

A noncovalent bond formed between 2 ions, one with a +ve charge & the other with a negative charge, as a Result of the attraction of opposite electrical charges.

15. Define Isomers.

Molecules that are formed from the same atoms in the Same chemical linkages but have different 3 dimensio-Nal configuration, alternatively, molecules with the molecules formulae but with different structural formulae.

16. What is macromolecule?

A large molecule having a Mol.wt in the range of a few Thousand to many millions; refers specifically to poly Saccharides, proteins, enzymes, lipids & nucleic acid.

17. Define molecule.

A group of atoms joined together by covalent bonds; The smallest unit of a compound that displays the properties of that compound & cannot be further subdivided without losing the quality of the compound.

18. Define mole.

A mole is the amount of a substance, in grams that equals the combined atomic mass of all the atoms in a molecule of that substance.

19. Define mole. Molecule.

The atomic weight of a substance, expressed in grams, 1 mole of any substance is defined as the mass of 6.0222 * 10^23 atoms.

20. Define Molecula weight.

Numerically the same as the relative molecular mass of a molecule expressed in Daltons, alternatively the Sum of the at. Wt of the atoms in a molecule.

21. Define Noncovalent bond.

A chemical bond which in contrast to a covalent Bond, no electrons are shared. Noncovalent bonds are relatively weak but they can sum together to produce strong, highly specific interactions between Molecules.

22. Define optical activity.

The capacity of a substance to rotate the plane of plane polarized light.

23. Define phagocytosis.

A form of endocytosis in which cells engulf organisms or fragments of organisms, prominent in carnivorous cells such as Amoeba proteus and in vertebrate Macrophages and Neutrophils.

24. Define trace element.

A chemical element required by an organisms in only trace amounts of less than 10-5 m. Absence can cause disease and death.

25. What is vander waals interactions?

The weak bonds formed between electrically neutral molecules or parts of molecules when they lie close together. Such interactions are common in the secondary and tertiary structure of protein.

26. Define water.

A colourless, odourless and tasteless liquid which is most abundant of all other substances on earth, mother liquor of all forms of life as there is no life without water. Life almost certainly originated in water, provides the medium for biological reactions to takes place; accounts for about 70% or more of the weight of most organisms. Unlike rest other substances, occurs in 3 states-solid, liquid and gaseous at the same time; has a melting point O C, boiling point 100 C, surface tension 72.8 and dielectric constant 80.

27. Define Zymogen.

An inactive precursor of an enzyme, particularly by those concerned with protein digestion. For example, pepsinogen the precursor of pepsin and trypsinogen the precursor pf trypsin.

28. Give example of biological buffer systems.

1. Phosphate buffer
2. Bicarbonate buffer
3. Protein buffer
4. Amino acid buffer
5. Hemoglobin buffer

PART - B

1. Describe the five general types of chemical transformations occurring in cells.

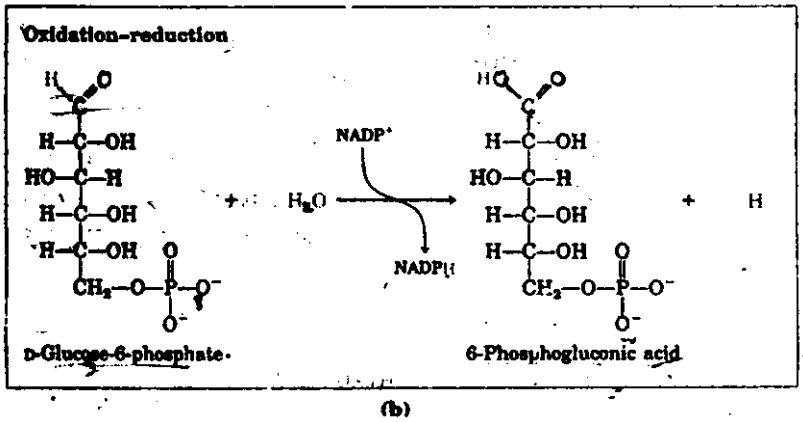
Most of the reactions in living cells fall into one of five general categories: (1) oxidation-reductions,(2)cleavage and formation of carbon-carbonbonds,(3) internal arrangements,(4)group transfers ,and (5)condensation reactions in which monomeric sub units are joined ,with the elimination of a molecule of water.Reactions within each general category usually occur by similar mechanisms.

Oxidation-Reduction Involve Electron Transfer

When the two atoms sharing electron in a covalent bond have the same electro negativity, as in the case of two carbon atoms, the bond is nonpolar. When the two elements that differ in the electronegativity form a covalent bond that is polarized.

In many biological oxidations, a compound loses two electrons and two hydrogen ions (i.e., two hydrogen atoms); these reactions are commonly called dehydogenations and the enzymes that catalyze them are called dehydrogenases. In some, but not all, biological oxidations, a carbon atom becomes covalently bonded to an oxygen atom

The enzymes that catalyze these oxidations are generally called oxidases or, if the oxygen atom is derived directly from molecular oxygen, oxygenases. Every reaction must be accompanied by reduction, in which an electron acceptor acquires the electrons removed by oxidation. Oxidation reactions generally release energy. Most living cells obtain the energy needed for cellular work by oxidizing metabolic fuels such as carbohydrate or fat.

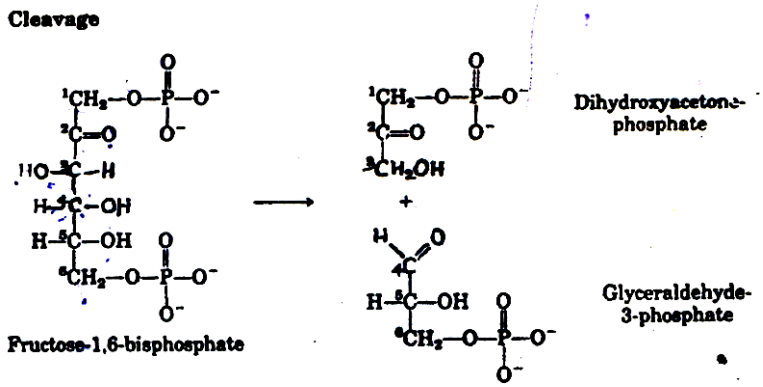


(b)

Carbon-Carbon Bonds Are Cleaved and Formed By Nucleophilic Substitution Reactions

A covalent bond can be broken in 2 general ways, hemolytic and heterolytic cleavage. In hemolytic cleavage, each atoms leaves the bond as a radical carrying one of the 2 electrons that held the bonded atoms together. This type of cleavage occurs rarely in living organisms. Heterolytic cleavages are more common, in which one atom keeps both bonding electrons, leaving the other atom one electron short. When a second electron rich group replaces the departing anion, a nucleophilic substitution occurs.

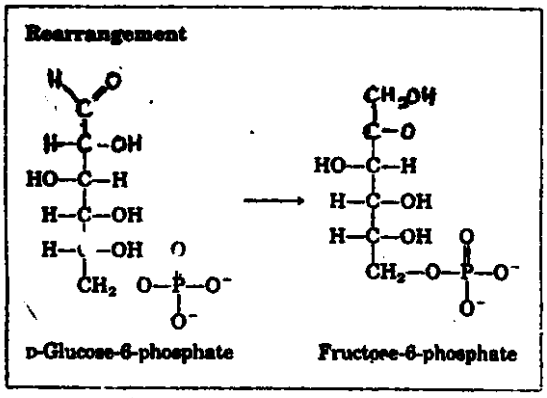
Many biological reactions involve interactions between nucleophile, functional groups that seek electrons and capable of donating them. There are 2 general mechanisms by which one nucleophile can replace another in the formation of carbon-carbon bonds. In the first the leaving group departs with its electrons, leaving the former partner as a relatively unstable cabocation, before the substituting group. This mechanism is known as **S_N 1 reaction.** In the second type of nucleophilic substitution reaction, an attacking nucleophile, arrives before the leaving group departs, and a pentacovalent intermediate forms transiently .This an **S_N2 reaction.**



Electron transfers within a molecule produce internal rearrangements.

Another common cellular reaction type is intra molecular rearrangement, in which redistribution of electrons results in isomerization, transposition of double bonds, and Cis-Trans rearrangements of double bonds.

e.g; of isomerization is the formation of fructose 6- phosphate from glucose 6-phosphate during sugar metabolism.

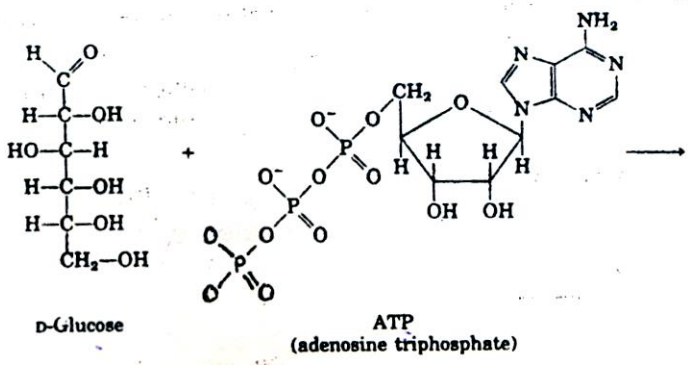


(c)

Group Transfer Reactions Activate Metabolic Intermediates.

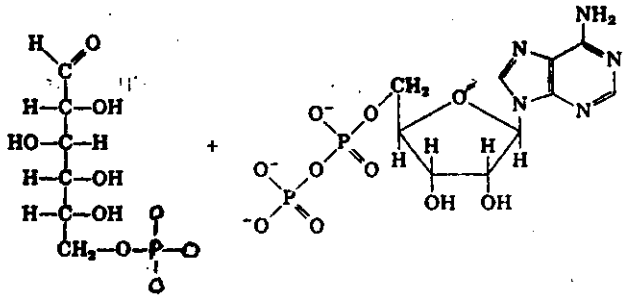
A general theme in metabolism is the attachment of a good leaving group to a metabolic intermediate to "activate" the intermediate for subsequent reaction. Among the better leaving groups in SN reactions are inorganic orthophosphate and inorganic pyrophosphate. Esters & anhydrides of phosphoric acid play central roles in cellular chemistry.SN reaction in which he phosphoryl group**($-PO_3^{2-}$)** serves as a leaving group occur in hundreds of metabolic reactions.

Group transfer



D-Glucose

ATP
(adenosine triphosphate)



D-Glucose-6-phosphate

ADP
(adenosine diphosphate)

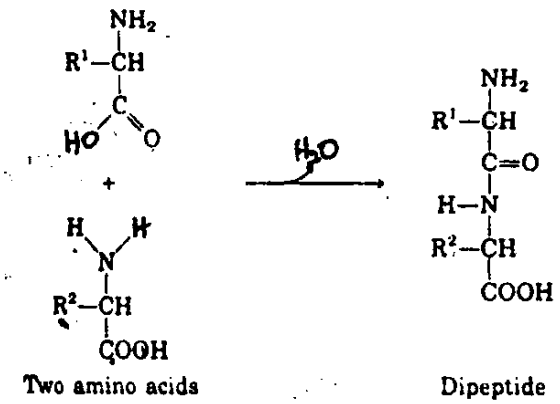
Bio polymers are formed by condensations

The monomeric sub units that make up proteins, nucleic acids, and polysacchrides are joined by nucleophilic displacement reactions that replace a good leaving group.

E.g, the joining of 2 aminoacids molecules to form a dipeptide could occur by the simple mechanism.

Macromolecules can be broken down by hydrolysis reactions, in which H_2O is the attacking nucleaphile, displacing the monomeric subunit. Enzymes that catalyze hydrolysis of biopolymers are essential in the digestive process and serve also tot regulate the level of such critical macromolecules as messenger RNA.

Condensation



Two amino acids

Dipeptide

2. Describe the Weak interactions in aqueous solutions of water molecule.

A. Hydrogen bonding between water molecules

The nearly tetrahedral arrangement of the oxygen electrons (bond angle 104.5°) allows each water molecule to form hydrogen bonds with four neighboring water molecules. At any moment in liquid water in room temperature, each water molecule forms hydrogen bonds with an average of 3.4 other water molecules. The water molecules are in continuous motion in the liquid state, have hydrogen bonds are constantly and swiftly being broken and formed.

In ice each water molecules is fixed in space and form hydrogen bonds with 4 other water molecules to produce a regular lattice structure. Much thermal energy is needed to break the large number of hydrogen energy is needed to break the large number of hydrogen bonds in such a lattice and this is the reason for a relatively high melting point of water.

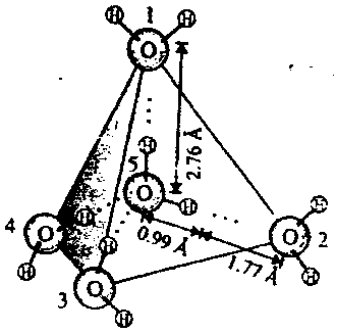


Figure: Tetrahedral hydrogen bonding of a water molecule, in ice

B. hydrogen bonding between water and solute molecules

Hydrogen bonding is not unique to water. They readily form between electronegative atoms in the same are another molecule. Hydrogen atoms covalently bonded to carbon atoms do not participate in hydrogen bonding. The distinction explains why butanol has a relatively high boiling point of 117°C in contrast to butane which has a boiling point of only -0.5°C. Uncharged but polar molecules such as sugar dissolve readily in water and form hydrogen bonds between the hydroxyl groups or the carboxyl oxygen of the sugar and the polar water molecules. Alcohols, aldehydes and ketones all form hydrogen bonds with water.

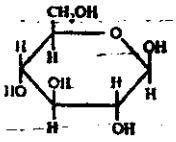
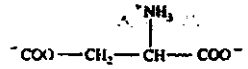
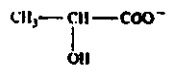
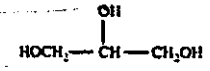
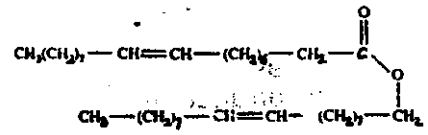
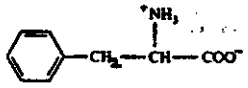
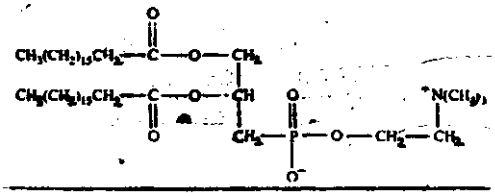
Hydrogen bonds are strongest when the bonded molecules are oriented to maximize electrostatic interaction. This happens when the hydrogen atom and the two atoms that share it are in the straight line. Hydrogen bonds are highly directional and are capable of holding two hydrogen bonded molecules or groups in a specific geometric arrangement.

C. Interaction between water and charged solutes.

Water is polar solvent. It readily dissolves most biomolecules, which are generally charged or polar compounds. Compounds that dissolve readily in water are hydrophilic. In contrast, nonpolar solvents (such as chloroform and benzene) are poor solvents for polar molecules, but readily dissolve nonpolar biomolecules such as lipids and waxes.

Water dissolves salts such as NaCl by hydrating and stabilizing the Na^+ and Cl^- ions, weakening their electrostatic interactions.

Some examples of polar, nonpolar and amphipathic biomolecules

Biomolecule	Ionic form at pH 7
Polar Glucose	
Glycine	
Aspartic acid	
Lactic acid	
Glycerol	
Nonpolar Typical wax	
Amphipathic Phenylalanine	
Phosphatidylcholine	

D. Interaction between water and nonpolar gases

The biologically important gases CO_2, O_2 and N_2 are nonpolar. The movement of these molecules from disordered gas phase into aqueous solutions constrains their motion and therefore represents a decrease in entropy. Some organisms have water soluble proteins that facilitate (hemoglobin and myoglobin) the transport of oxygen. Carbon dioxide gas forms carbonic acid (H_2CO_3) in aqueous solution, and is transported in that form.

E. Interaction between water and Non polar compounds:

When water is mined with a hydrocarbon such as benzene or hexane, two phases form: Neither liquid in soluble in the other. Non polar compounds such as benzene, hexane and ethane are hydrophobic as they are unable to undergo energetically favorable interactions with water molecules.

Amphipathitic compounds contain regions that are polar and regions that are non polar. When amphipathic compounds are mired with water the two regions of the solute molecule

experience conflicting tendencies. The Nonpolar of the molecules cluster together to present the smallest area to the solvent and the Polar Regions are arranged to minimize their interaction with the aqueous solvent. These stable structures of amphipathic compounds in water called miclles, may contain hundred or thousands of molecules.

Many biomolecules are amphipathic protein pigments, the sterols and phospholipids of membrane and certain vitamin all have polar and non polar surface region.

F. Vander waals Intraction:

Vander Waals interactions are weak non specific interatomic attractions and come into play when any two un charged atoms are 3 to 4 Aº depart. The basic of a van der waals bond is that the distribution of electronic charge around the atom changer with time.

All types of molecules exhibits vander waals force which arise from the attrcition of the bound electrons of one atom for the nucleus of another. When two atone are for aparts, these is a very weak attraction which becomes stronger as the atoms move closer together. However if the atom move close enough for their outer electron shells to over lap, then a force of repulsion occurs. At a certain distance, defined as the vander waals contact radices, there is a balance between, the force of attraction and those of repulsion.

The vender waals honding enough between two average atone is very small is, above, 1 kcal/mol, which is only slightly greater that the average thermal energy of molecules at room temperature. It is considerable weaker than a hydrogen or electrostatic bond, which is in the range of 3 to 7 kcal/mol.

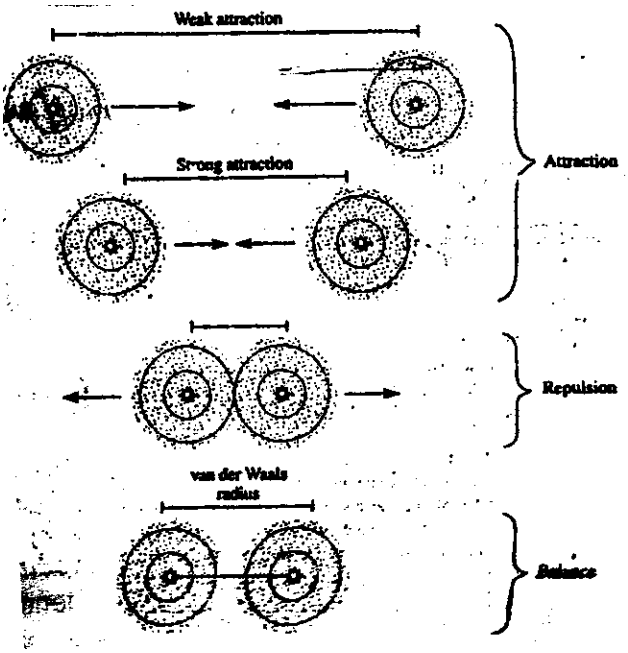


Figure: Schematic of van der weeks forces of attraction, repulsion and balance two atoms

3. Describe the Biological buffer systems and its types.

Almost every biological process is pH depended a small change in pH produces a large change in the rate of the process. Cells and organism maintain a specific and constant cystolic pH keeping biomolecules in their optimal ionic state, using near P^H 7 in multicelled organisms the pH of the extra cellular fluids in also tightly regulated constancy of pH is achieved primarily by biological buffers mixtures of weak acid and their conjugate bases. The importance buffering system of body fluids which maintaining pH are

Extra cellular fluids –bio carbonate buffer Protein buffer
Intercellular fluids – phosphate buffer protein
Erythrocytes- hemoglobin buffer

1. The phosphate buffer system

This system which acts in the cytoplasm of all cells consists of H_2PO4^{2-}as proton donor and $HPO4^{2-}$as proton acceptor:

H_2PO4^- H + + $HPO4^{2-}$

The phosphate buffer system works exactly like the acetate buffer system, except for the P^H range in which it functions. The phosphate buffer system is maximally effective at a P^H close to its PKa of 6.86 and thus tends to resist P^H changes in the range between 6.4 & 7.4

Since the concentration of phosphate buffer in the blood plasma is about 8% of that of the biocarbonate buffer its buffering capacity is much lower than bicarbonate in the plasma. The concentration of phosphate buffer is much higher in intercellular fluids than is extracellular fluids. The buffering capacity of the phosphate buffer is highly elevated inside the cells and the phosphate is also effective in urine inside the renal distal tubules and collecting duets.

2. The bicarbonate buffer system:

This is the main extra cellular buffer system which provides a means for the necessary removal of CO_2 produced by tissue metabolism. The bicarbonate buffer system is the main buffer in blood plasma and consists of carbonic acid as proton donor and bicarbonate as proton acceptor.

H_2CO_3 H^++HCO^{3-}
This system has equilibrium constant

$K_L = [H^+]\,[HCO^{3-}] / [H_2CO_3]$

And functions as a buffer in the same way as other conjugate acid base pair. with respect to the bicarbonate system, $[HCO^{3-}] / [H_2CO_3]$ ratio of 20 to 1 is required for the P^H of blood plasma to remain 7.4.

The P^H of blood is maintained at 7.4 when the buffer ration $[HCO^{3-}] / [H_2CO_3]$ becomes 20. If the bicarbonate neutralized any acid or base, there may be the change of buffer ratio and the

blood ph value. But the buffer ratio remains by the respiratory element of H_2CO_3 as CO_2 or the urinary eliminationation of HCO^{3-}.

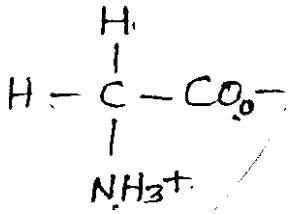
3. The protein buffer system:

The protein system is very important in the plasma and the intercellular fluids but their concentration is very low in cerebrospinal fluids lymph and interstitial fluids. The proteins exist as anions serving as conjugate base (Pr^-) as the blood P^H 7.4 and from conjugate acids (HPr) accepting H^+. They have the capacity to buffer some H_2CO_3 is the blood.

$H_2CO_3 + Pr^-$ $HCO^{3-} + HPr$

4. The amino acid buffer acid systems:

Amino acids contain in their molecule both an acidic and basic group. They can be visualized as existing in the form of a neutral zwitterion in which a hydrogen atom can pass between the carboxyl and amino groups. By the addition or substraction of a hydrogen ion to or form the zwitterions, either the cation or anion form will be produced.



Amino acids differ in degree to which they will produce the cation or anion form. In other words, a solution of an amino acid is not neutral but is either predominantly acidic or basic, depending on which form is present in greater quantity.

5. The Hemoglobin buffer system

These buffer systems are involved in buffering CO_2 inside erythrocytes. The buffering capacity of hemoglobin depends on its oxygenation and deoxygenation. Inside the erythrocytes, CO_2 combines with water to form carbonic acid under the action of carbonic anhydrase. At the blood P^H 7.4, H_2CO_3 dissociates into H^+ and HCO^{3-} and needs immediate buffering. Oxyhemoglobin (HbO_2^-). On the other side, loses O_2 to from deoxyhemoglobin (Hb^+) which remains undissociated (HHb) by accepting H^+ from the ionization of H_2CO_3.

Hbo_2 □ $Hb + O_2$

$Hb^- + H_2CO_3$ □ $HHb + HCO_3^-$

Some of the HCO_3^- diffuses out into the plasma to maintain the balance between intracellular and plasma bicarbonate. $HHbO_2$, produced in lungs by oxygenation hydrogen ions (H^+) are buffered by HCO_3^-, which is dissociated into H_2O and CO_2 by carbonic anhydrase. CO_2 diffuses out of erythrocytes and escapes in the alveolar air.

4. Explain Elemental composition of living matter and structure of atoms.

On earth, all cells, regardless of their origin (animal, plants or microbial), contain the same elements in approximately the same proportions. Thus of the 107 known elements, only about 20 are essential for terrestrial life. Six nonmetals (O, C, H, N, P, S), which contribute almost 98% of the total mass of cells, provide the structural elements of cytoplasm. From them, the functional components of cells (such as walls, membranes, genes, enzymes etc) are found. The relative abundance of these 6 lighter elements in the seas, crust and atmosphere of earth does not by itself explain their utilization for life. Aluminium, for example, is more abundant than carbon, but it performs no known function essential to life.

Carbohydrates and lipids contain C, H, and O while proteins and nucleic acids contain in addition N and S and N and P respectively. Na, K, Mg, Ca, Cl are usually found in ionic forms whose concentration must remain within narrow limits. The presence of BO, Si, V, Mn, Fe, Co, Cu, Zn and Mb, though in ultra trace amounts, is essential for functioning of enzymes. These elements are used as cofactors in the catalytic function of enzymes.

Small atoms form the tightest and most stable bonds – a distinct advantage for structural elements. H, O, N and C are the smallest atoms capable of forming 1-, 2-, 3- and 4- electron bonds respectively. Utilization of all possible types of electron bonds
Permits maximum versatility in molecular design. Advantages of carbon based versus silicon based life include the following:

1. Greater chemical stability of C-C bonds than the Si-Si bonds.
2. The ability of carbon, but not of silicon, to form multiple bonds; for example, the oxides of carbon are diffusible monomeric gases, whereas the oxides of silicon is a viscous polymer.
3. The stability of C-C bonds but not of Si-Si bonds, to rupture by nucleophilic reagents such as O_2, H_2O or NH_3.

Similar factors uniquely P and S for utilization in energy transfer reactions.

Table : The elemental composition of hiving cells

Element	Composition by weight (%)	Element	Composition by weight (%)
O	65		
C	18		
H	10	*Cu*, Zn, Se, Mo,	
N	3	F, Cl, I, Mn,	
Ca	1.5	Co, Fe	0.70
P	1.0		
K	0.35		
S	0.25	Li, Sr, Al, Si,	
Na	0.15	Pb, V, As, Br	Trace*
Mg	0.05		

- Variable occurrence in cells. No known function in most cases.

Six nonmetals, (O,C,H,N,P,S), which contribute almost 98% of the total mass of cells, provide the structural elements of protoplasm. From them, the functional components of cells, (such as walls, membranes, genes, enzymes etc) are formed. These 6 elements all occur in the first three period of the periodic table.

Table: The structural elements of protoplasm

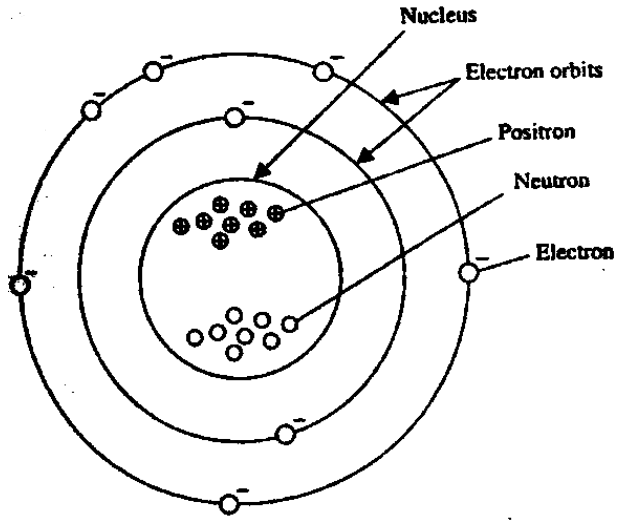
Period	Group I	II	III	IV	V	VI	VII	VIII
1	(H)							He
2	Li	Be	B	(C)	(N)	(O)	F	Ne
3	Na	Mg	Al	Si	(P)	(S)	Cl	Ar

* The six common nonmetals have been shown encircled.

Structure of an atom:

Each matter is composed of very small particles called atoms, which cannot be created, destroyed or subdivided. Atoms of the same element are similar to one another and equal to weight. Atoms of different elements have different properties and weight. The three fundamental subatomic particles are: proton, neutron and electron. Besides these fundamental particles, about 35 other atomic particles are also known to exist.

Regarding the arrangement of fundamental particles inside an atom, Ernest Rutherford proposed the most satisfactory model which is accepted even today with some modifications. Accordingly, an atom is made up of a central nucleus containing positively charged protons and neutral neurons, surrounded by negatively charged electrons which move around it in discrete, successive, concentric volumes in space known as orbits or shells. the electron shells are numbered as 1, 2, 3, 4, 5, 6 and 7 are designated by the letters K, L, M, N, O, P, Q respectively. Each shell has a certain number of electrons and maximum number of electrons for each shell is fixed. The maximum number is given by $2n^2$, where n is the serial number of the shell. Thus, the maximum number of electrons in K, L, M, N, O, P, Q shells will be 2, 8, 18, 32, 50, 72, and 98 respectively. The maximum number of electrons in the outermost shell is 8 and in the penultimate shell are 18.



Structure of an atom

The shells are subdivided into subshells. The number of subshells in a shell is equal to the number of the shell from witin. K shell has one subshell called s; second L has two subshells s and p; the third M shell has three subshells s, p and d and fourth N has four subshells s, p, d, and f. The subshells s, p, d, and f can have a maximum of 2, 6, 10 and 14 electrons respectively.

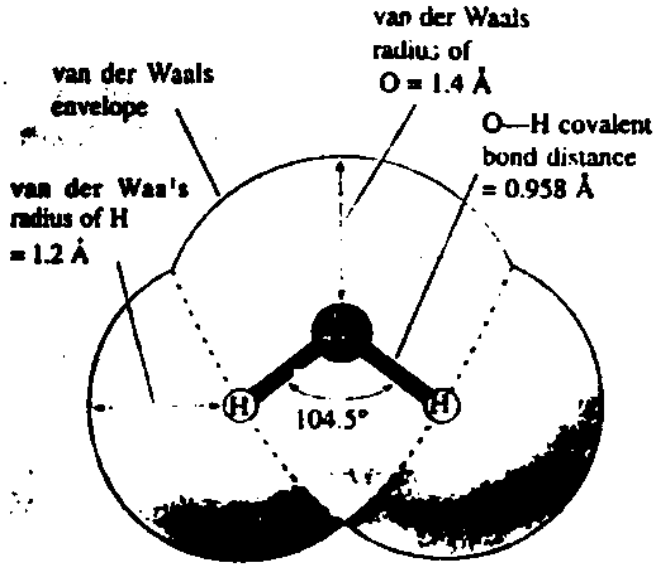
The position of electrons in the various shells and subshells are represented as follows. Major shells in which the electrons exist are indicated by the numbers 1, 2, 3 etc and the subshells designated by s, p, d, f etc. the superscript on s, p, d, and f gives the number of elrctrons in the subshell. Thus, $1s^2$ indicates the presence of two electrons in the s subshell of the first major shell (K). Similarly, $4f^8$ indicates the presence of 8 electrons in the f subshell of fourth major shell(N).

The mass of an atom depends entirely upon its nucleus. A neutron has nearly the same mass as a proton i.e. each proton or neutron weighs 1.66043×10^{-24}g. The mass of an electron is negligible, about 1/1823 of the mass of a proton or neutron. The mass of a proton or neutron is called the atomic mass unit.

5. Describe the Structure of water molecule.

In a water molecule each hydrogen atom shares an electron pair with the oxygen atom. The geometry of the water molecule is dictated by the shapes of the outer electron orbitals of the oxygen atom, which are similar to the bonding orbitals of carbon. These orbitals describe a rough tetrahedron, with a hydrogrn atom at each of the two corners and unshared electrons at the other two. The H-O-H bond angle is 104.5°, 5° less than the bond angle of a perfect tetrahedron which is 109.5°; the bonding orbitals of the oxygen atom slightly compress the orbitals shared by hydrogen.

The oxygen nucleus attracts electrons more strongly than does the hydrogen nucleus; oxygen is more electronegative. The sharing of electrons between H and O is therefore unequal. This unequal electron sharing creates two electric dipoles in the water molecule, one along each of thye H-O bonds. The oxygen atom bears a partial negative charge and each hydrogen a partial positive charge. The resulting electrostatic attraction between the oxygen atom of one water molecule constitutes a hydrogen bond.



The Structure of a water molecule

The outline represents the van der waals envelope of the molecule the skeletal model of the molecule indicates its covalent bonds. The H-O-H bonds bond angle is 104.5°. both hydrogen atoms carry a partial positive charge and the oxygen a partial negative charge, creating a dipole.

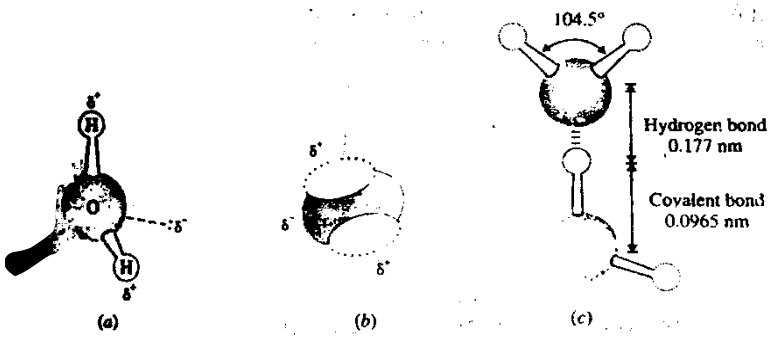


Figure: The dipolar nature of the water molecule

A. Ball and stick model. There is nearly tetrahedral arrangement of the outer shell electron pairs around the oxygen atom; the two hydrogen atoms have localized partial positive charges and the oxygen atom has two localized partial negative charges.
B. Space filling model.
C. Two water molecules joined by a hydrogen bond between the oxygen atom of the upper molecule and a hydrogen atom of the lower one. Hydrogen bonds are longer and weaker than covalent O-H bonds.

Polarity of water:

The larger the difference in electronegativities of 2 atoms, the more likely they are to form an ironic rather than a covalent bond. Sodium and chlorine, for example, have difference is electronegativities and hence form ionic bonds. Carbon and nitrogen on the contrary, have similar, moderate electronegativities and they usually form covalent bonds.

When atoms are differing in electronegativity, they do not share electrons equally. Instead, the diffuse clouds of shared electrons tilt toward the more electronegative atom. In a water molecule, for example, an oxygen atom shares electrons with 2 hydrogen atoms. But the shared electrons are more concentrated around the 2 hydrogen nuclei.

Molecules that have uneven distributions of electrical charge are said to be polar, since they have positive and negative poles in the same way that a magnet has 2 poles. Molecules with approximately uniform charge distributions are said to be nonpolar.

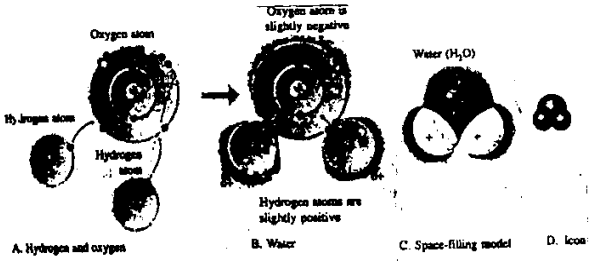


Figure: The Polarity of water

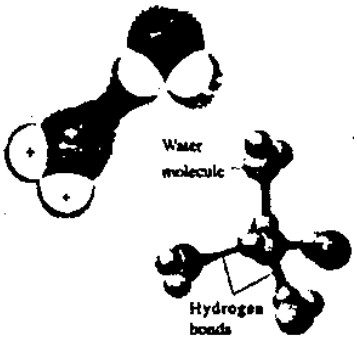


Figure: Hydrogen bonds between water molecules

The hydrogen atoms of one water molecule are attracted to the oxygen atoms of another water molecule.

The hydrogen bonds in liquid water have a bond energy of only about 20kJ/mol, as compared to 460 kJ/mol for the covalent O-H bond. When water is heated, the resulting temperature increase cause the faster motion of individual water molecules. Although at any given time, most of the molecules in liquid water are hydrogen bonded, the lifetime of each hydrogen bond is less than $1x10^{-9}$s. The hydrogen atoms in a few molecules are occasionally lost to neighbouring water molecules giving rise to hydrated proton, called a hydronium ion, H_3O^+ and a hydroxide ion, OH^-.

6. Explain the nature of chemical bonding and its types.

A bond is any force which holds two atoms together. The formation of bond between two atoms is due to some redistribution or regrouping of electrons to form a more stable configuration. The regrouping of electrons in the combining atoms may take place in either of three ways:

b. by a transfer of one or more electrons from one atom to another – electrovalent bonding
c. by a sharing of one or more pairs of electrons between the combing atoms – covalent bonding
d. by a combination of two processes of transfer and sharing – coordinate bonding

Electrovalent bonding:

Ionic bond formation takes place between atoms of strongly electropositive and strongly electronegative elements. An element preceding an inert gas in the periodic table is strongly electronegative and the element immediately following the inert gas is strongly electropositive. According to W.Kossel, a transfer of electrons takes place from the outer most shell of the electropositive atom to the outermost shell of the electronegative atom, resulting in the formation of stable positive and negative ions respectively which are held together by electrostatic forces of attraction to from a molecule or more precisely an ion pair.

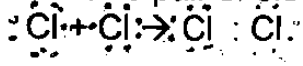
Covalent or nonpolar bond:

Covalent bond formation consists in sharing or holding a pair of electrons in partnership between two combing atoms, so that the pair counts towards the electronic grouping of both atoms. For each pair of electrons to be shared between two atoms, each of the constituent atom contributes one electron:

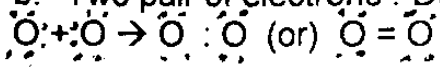
A ‘ + B’ A : B

This type of linkage which is the result of equal contribution and equal sharing of electrons is known as covalent bond. The covalent compounds always exist in molecular form, are nonelectrolytes or nonionizable, soluble in organic solvents (such as benzene, ether, pyridine) and have low melting and boiling points because of weaker nature of the bond. They are usually liquids or gases and are generally soft, easily fusible and volatile. The covalent bond is rigid and directional.

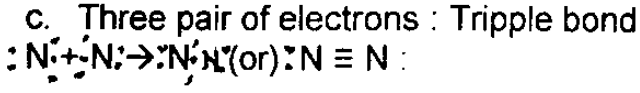
a. One pair of electrons : Single band



b. Two pair of electrons : Double band



c. Three pair of electrons : Tripple bond



The force for bond formation in covalent bond is the same as that of in ionic bonds. I.e., electrostatic attraction between the two atoms, although this force of attraction develops in a different manner.

Noncovalent bonds or interactions:

In addition to covalent bonding, there are weaker forces of interaction that profoundly influence conformation of biomolecules and their function.

A. Electrostatic or ionic bonds

Ionic bonds are formed due to the attraction between atoms or groups, of opposite charges (+ and -). A charged group on a substrate can attract an oppositively charged group on an enzyme. The force (F) of such an electrostatic attraction is given by Coulomb's law.

$$F = \frac{q_1 q_2}{r^2 D}$$

Where q_1, q_2 = charges of the two atoms,

r = distance between the two atoms

D = dielectric constant of the medium.

This kind of attraction is also called saline bond, salt linkage, salt bridge or ion pair. Ionic bonds occurs in crystals and salts that are ionized when dissolved in water. For example NaCl is a salt composed of Na^+ and Cl^- .

B. Hydrogen bonding:

Hydrogen bonds can be formed between uncharged molecules as well as charged ones. In a hydrogen bond, a hydrogen atom is shared by two other atoms. The atom to which the hydrogen is more tightly linked is called the hydrogen donor, where the other atom is called as the hydrogen acceptor. The acceptor has a partial negative charge that attacks the hydrogen atom. The donor in a hydrogen bond in biological systems is an oxygen or nitrogen atom that has a covalently attached hydrogen atom. The acceptor is either oxygen or nitrogen.

Hydrogen bonds in biomolecules are also more specific than other weak bonds because they require particular complementry groups that donate or accept hydrogen. An important feature of hydrogen bonds is that they are highly directional.

Hydrogen bondings are two types: intramolecular (within a molecule) and intermolecular (between two molecules).

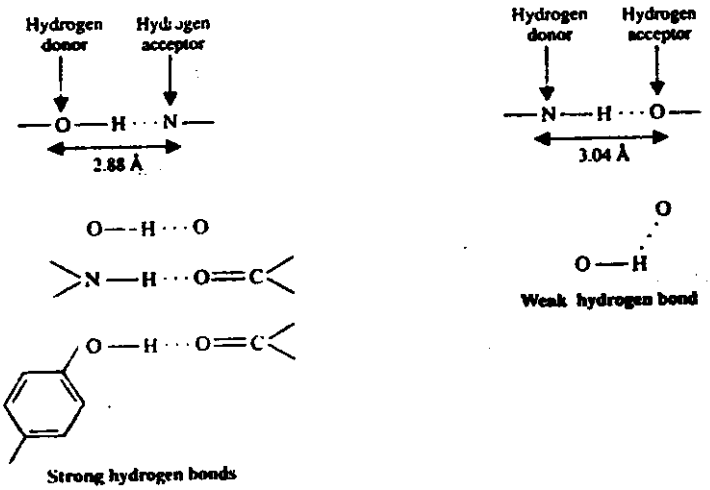


Figure: Strycture and types of hydrogen bonds

C.Hydrophobic or nonpolar interactions:

As in the formation of micelles, hydrophobic groups of macromolecules, if in proper spatial relation, will interact to the exclusion of solvent molecules and thereby reside in a hydrophobic environment. Hydrophobic interactions are a major driving force in the folding of macromolecules, the binding of substrate to enzymes and the formation of membranes that define the boundries of cells and their internal compartments.

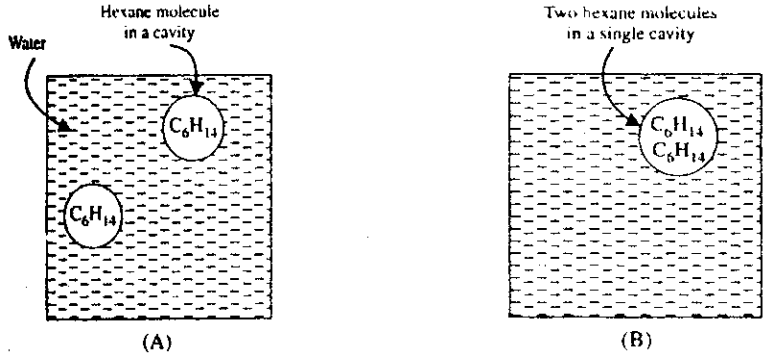


Figure: Schematic of two molecules of hexane in a small volume of water

The hexane molecules occupy different cavities in the water structure (A), or they occupy the same cavity, which is energetically more favoured (B).

D. **Vander waals interactions.**

Van der waals interactions are week non specific interactomic attractions and come into play when any two un charged atoms are 3 to 4 Aº Depart. The basic of a van der waals bond is that the distribution of electronic charge around the atom changer with time.

All types of molecules exhibits vander waals force which arise from the attrcition of the bound electrons of one atom for the nucleus of another. When two atone are for aparts, these is a

very weak attraction which becomes stronger as the atoms move closer together. However if the atom move close enough for their outer electron shells to over lap, then a force of repulsion occurs. At a certain distance, defined as the vander waals contact radices, there is a balance between, the force of attraction and those of repulsion.

The vender waals honding enough between two average atone is very small is, above, 1 kcal/mol, which is only slightly greater that the average thermal energy of molecules at room temperature. It is considerable weaker than a hydrogen or electrostatic bond, which is in the range of 3 to 7 kcal/mol.

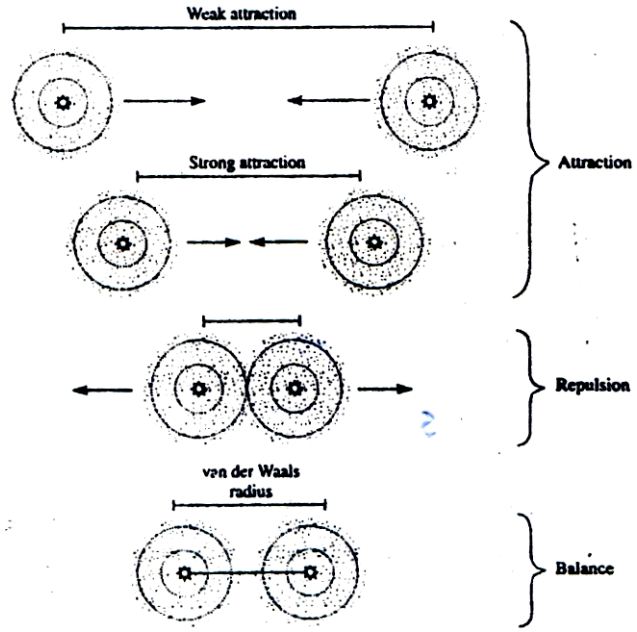


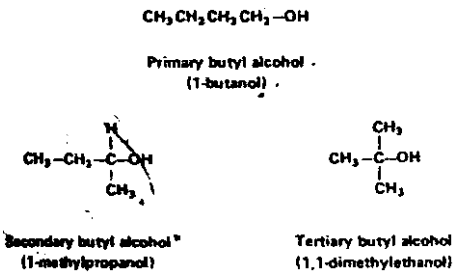
Figure: schematic of van der waals forces of attraction, repulsion and balance between two atoms

7. What are the functional groups important in biomolecules?

A functional group is a specific arrangement of elements (generally C, H, O, N, P or S) that has well defined chemical and physical properties. The properties of biochemical molecules are best understood in terms of the chemical and physical properties of the functional groups these molecules contain.

Alcohols

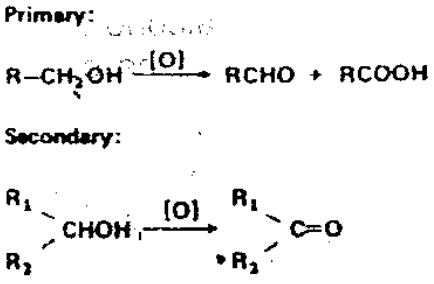
Many biological compounds (e.g, sugars, certain lipids, & aminoacids) are alcohols. These have both polar (hydroxyl, OH) and non polar (alkyl) character. They are the best regarded as both as hydoxylated hydrocarbons and alkyl derivatives of water. Although alcohols with up to 3 carbon atoms are infinitely soluble in water. water solubility decreases with increasing length of the carbon chain.i.e., with increasing nonpolar character. Primary. secondary,& tertiary alcohols have respectively 1,2,& 3 alkyl groups attached to the carbon atom bearing the –OH group.



Both monohydric (one-OH group) and polyhydric (more than one – OH group) alcohols are of physiologic significance. sugars are derivatives of polyhydric alcohols, as are cyclic or ring containing alcohols such as inositol. Their highly polar character makes plyhydric alcohols far more water soluble than corresponding monohydric alcohols with equivalent numbers of carbon atoms. thus even polyhydric alcohols with 6 or more carbon atoms(e.g., sugar) are highly water soluble.

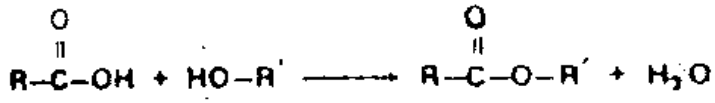
Chemical reactions of alcohols with biochemical analogies include are:

A. Oxidation: Primary alcohols are oxidized by strng oxidizing agents to aldehydes and acids, whereas secondary alcohols are oxidized to ketones.



Tertiary alcohols cannot be oxidized without rupture of a C-C bond.

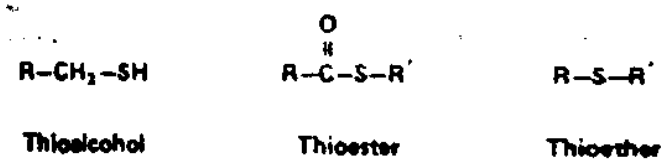
B. Esterification: An ester is formed when water is split out between an alcohol & an acid.



The acid may be organic or inorganic. Esters of H_3PO_4 and H_2SO_4 are of great significance in biochemistry. Many lipids contain carboxylic ester linkages

C. Ether formation: Ethers are derivatives of alcohols in which the hydrogen of the –OH group is replaced by an alkyl group (R-O-R') .the ether linkage is comparatively uncommon in living tissues.

Sulfur, which is in the same group of the periodic table as oxygen, forms similar compounds**.** Thioalcohols, thioesters & thioehters all occur in nature.



In addition, the disulfides (left) and peroxides (right)

R – S – S – R ‘ R – O – O – R’

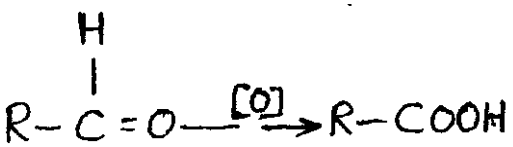
play an important role in protein structure & in prostaglandin biosynthesis.

Aldehydes and Ketones:

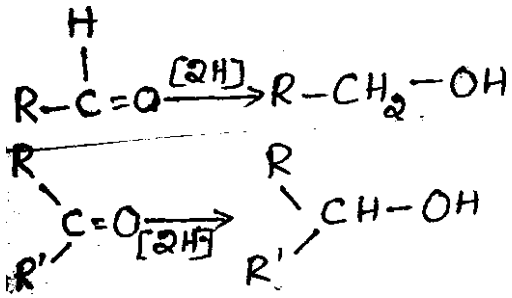
Aldehydes & ketones possess the strongly reducing carbonyl group > C=O.Aldehydes have one & ketones have 2 alkyl groups, attached to the carbon bearing the carbonyl group.

The sugars in addition to being polyhydric alcohols, are also either aldehydes or ketones.Reactions of aldehydes & ketones of biochemical interest include the following:

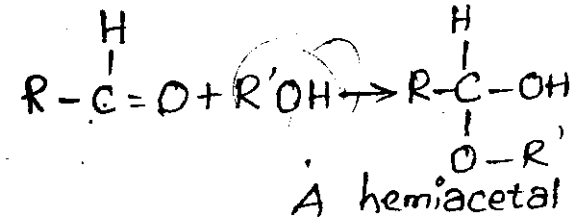
A.Oxidation: Oxidation of an aldehyde to the corresponding carboxylic acid. Ketones are not readily oxidized, since like tertiary alcohols, they cannot lose hydrogen without the rupture of a C – C bond.

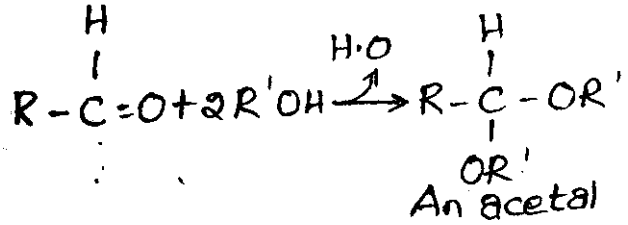


B.Reduction: Reduction of an aldehyde yields the corresponding primary alcohol & reduction of a ketone yields the corresponding secondary alcohol.



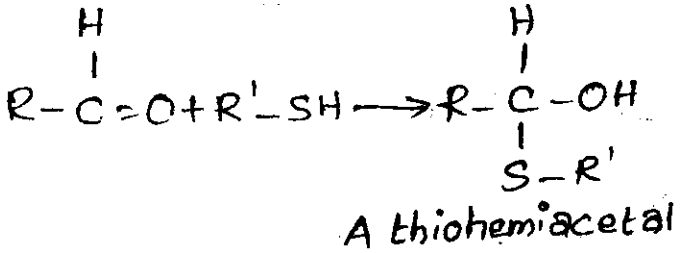
C.Hemiacetal and Acetal formation: Under acidic conditions aldehydes can combine with one or 2 of the hydroxyl groups of an alcohol, forming a hemiacetal or an acetal.



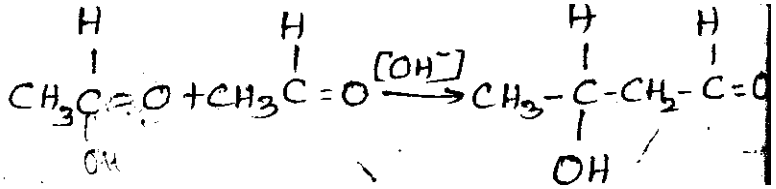


The carbonyl & alcohol functions may be part of the same molecule. For example the aldose sugars exist in solution primarily as the internal hemiacetal. Analogous structures are formed from alcohols and ketones.

Aldehydes may also form thioacetals and thiohemiacetals with thioalcohols.Thiohemiacetals function as an enzymic oxidation of aldehydes to acids.



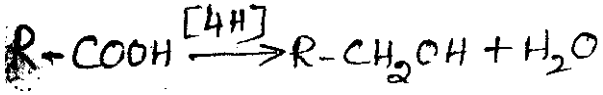
D.Aldol condensation: In alkali, aldehydes & to a lesser extent, ketones undergo condensation between their α-carbon atoms to form aldols or β –hydroxyl aldehydes or ketones. The β-hydroxy acids are derived fro these are important in fatty acid metabolism.



Carboxylic acids:

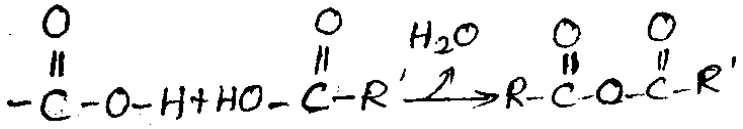
Carboxylic acids have both carbonyl(>C=O) & a hydroxyl group on the same carbon atom. They are typical weak acids and only partially dissociate in water to form a hydrogen ion (H^+) and a carboxylate anion ($R\text{-}COO^-$) with the negative charge shared equally by the 2 oxygen atoms. some reactions of carboxylic acids of biochemical interest include the following:

A. Reduction: Complete reduction yields the corresponding primary alcohol.

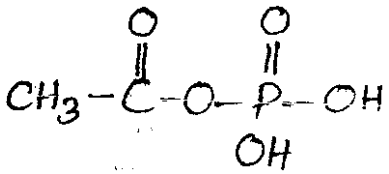


B. Ester & thioester formation: See alcohols.

C .Acid Anhydride formation: A molecule of water is split out between the carboxyl groups of 2 acid molecules.



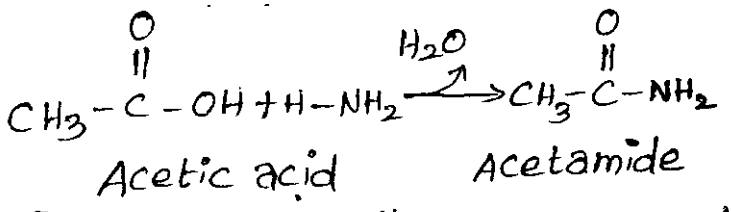
When both acid molecules are the same , a symmetric anhydride is produced. molecules of different acids yield mixed anhydrides. Anhydrides found in nature include those of phosphoric acid (in ATP) and the mixed anhydrides formed from phosphoric acid and a carboxylic acid, e.g:



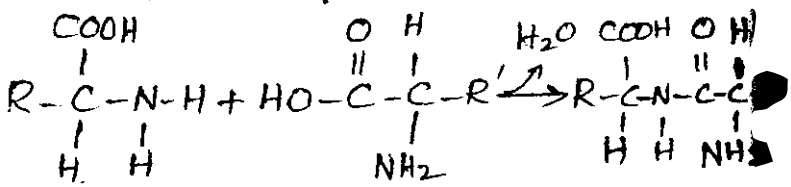
Acetyl Phosphate.

D. Salt formation: Carboxylic acids react stoichiometrically
With bases to form salts. Na+ and K+ salts are 100% dissociated in solution.

E. Amide formation: Splitting out a molecule of water between a carboxylic acid and ammonia or an amine forms an amide. Particularly important amides are peptides, formed from the amino group pf one amino acid and the carboxyl group of another.



Acetic acid Acetamide



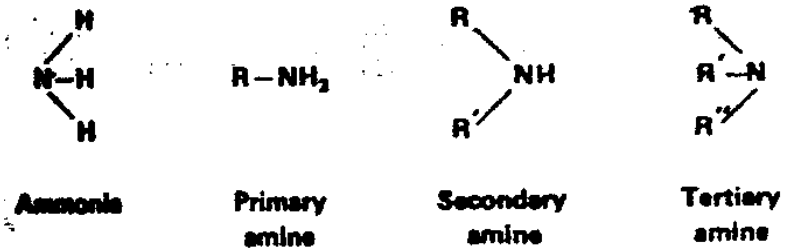
Peptide bond

Amines:

Amines alkyl derivatives of ammonia are usually gases or volatile liquids with odors resembeling ammonia but more, “fishlike.” Primary, secondary, & tertiary amines are formed by replacement of one, 2, 3 of th e hydrogens of ammonia.

Ammonia in solution exists in both charged & uncharged forms.

Amines behave in an entirely analogous way.



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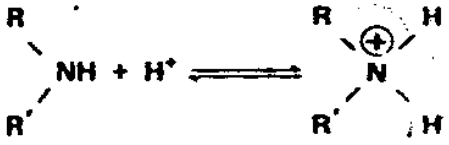
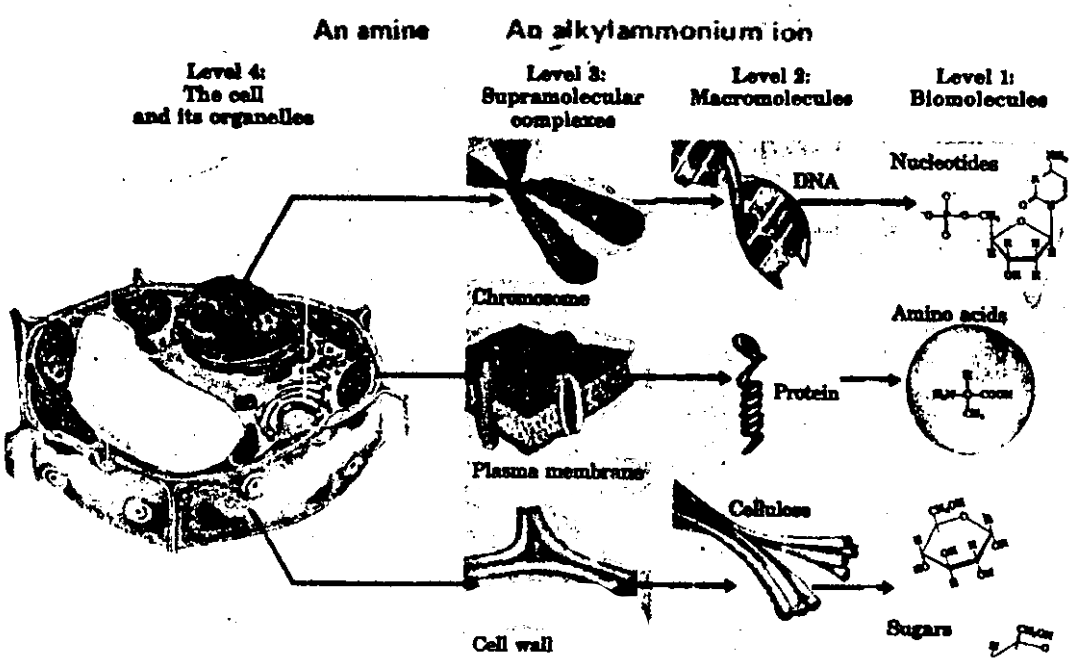




UNIT – II

PART – A

1. Define carbohydrates.

Carbohydrates are carbon compounds that contain hydrogen and oxygen in the ratio of 2 to 1. The term is also used for the derivatives of carbohydrates where the above definition may not be strictly true.

$C_x (H2O)_Y$

C_x – Carbon; $(H2O)_Y$ – Hydrate = Carbohydrate

These compounds are of fundamental importance in living organisms as a source of metabolic energy.

2. What is isomerism? Mention its types.

The term isomer (iso = equal, meros = part) to different compounds with the same molecular formula, and the phenomenon was called isomerism.

The isomers are of 2 types;

1. Structural isomers
2. Stereoisomers

3. What is ketose sugar? Give example.

The monosaccharides, often called simples are compounds which posses free ketone (=CO) group that type of sugar is called as ketose sugar.

E.g. Ribulose, fructose.

4. What is reducing sugar? Give example.

The carbohydrates molecules that contain free aldehyde (-CHO) or free ketone (=CO) are called reducing sugar.

E.g. Glucose, fructose.

5. Define non-reducing sugar.

The carbohydrates molecules that not contain free aldehyde (-CHO) or free ketone (=CO) group are called non-reducing sugars.

E.g. Sucrose

6. Define enantiomers.

Stereoisomers that are non superimposable mirror images of each other.

The two D and L forms of a compound constitute a pair of enantiomers or enantiomorphs.

e.g. D-glucose and L-glucose.

7. Define diastereoisomers.

Molecules with multiple chiral centers that exist in many forms with different physical and chemical properties.

The conversion of D-glyceraldehyde into an aldotetrose yields D-erythrose and D-Threose. These are called diastereoisomers i.e. isomers but not mirror images of each other.

8. Define optical activity.

The property of a molecule that causes rotation of plane polarized light. Chirality is an essential requirement for optical activity.

9. Define mutarotation.

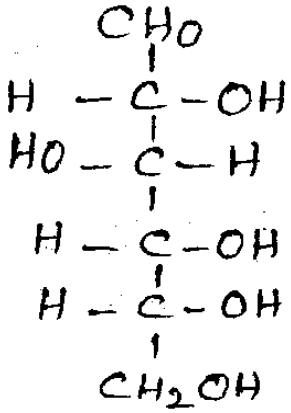
The change in specific rotation of a pyranose or furanose sugar or glycoside accompanying the equilibriation of its alpha and beta anomeric forms. The following equation explains mutarotation.

10. Draw the structure of pyranose and furanose.

Pyranose

Furanose

11. Give the structure of glucose.



12. Define dextrorotatory and levorotatory.

Dextrorotatory isomer: A stereoisomer that rotates the plane of ;plan – polarized light clockwise.

Levorotatory isomer: A stereoisomer that rotates the plane of plan-polarized light counter clockwise.

13. What is the use of Benedict's and Fehling's test?

These tests are used for confirmation of reducing sugars i.e. called as confirmatory test for reducing sugars.
In both the reagent, cupric oxide is formed as;

$Cu(OH)_2$ ------→ CuO + H2O

If a reducing substance is present, then the rust-brown cuprous oxide is ;precipitated. The reaction is as given below:

$2Cu(OH)_2$ -→ Cu2O + 2H2O +1/2O2

14. Define fermentation reaction.

Monosaccharides such as glucose, fructose and mannose are readily fermented by yeast. The process of yeast fermentation very complex. During this process, sugar phosphate and sugar acid ;phosphate are formed. Ordinarily, this process results in the formation alcohol and carbon dioxide.

Fermentation
C6H12O6 ----------→ 2C2H5OH + 2CO2
Monosaccharide Ethanol

15. What is disaccharides?

Disaccharides consist of two monosaccharides joined covalently by an O-glycosidic bond, which is formed when a hydroxyl group of one sugar reacts with anomeric carbon of the other.

e.g. Sucrose (Glucose and fructose)
Lactose (Galactose and Glucose)

16. Define polysaccharides.

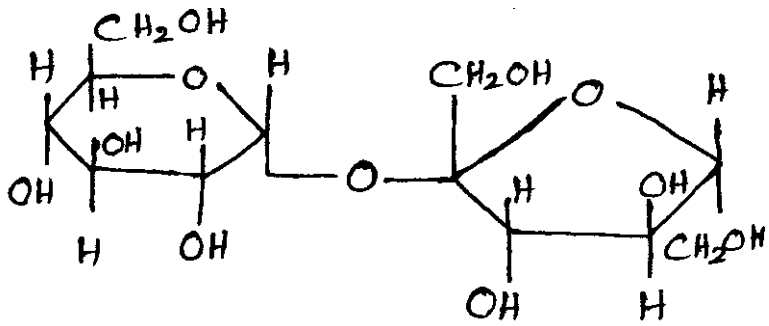
Polysaccharides are high molecular weight carbohydrates which, on hydrolysis yield mainly Monosaccharides.

e.g. Starch, glycogen, cellulose.

17. Give the structure of sucrose.

Sucrose is a disaccharide. The main source of sucrose is sugar cane. It is non reducing sugar.

It contain two monosaccharides as one glucose and one fructose which are linked together with 1,2 glucosidic linkage. The structure is given below;



18. Differentiate amylase with amylopectin.

Amylose: The amylose consists of long, unbranched chain of D-glucose residues connected by alpha 1, 4 lilnkages. Amylose may have molecular weight from few thousand to over a million.

Amylopectin: This consists of long branched chain in which the glucosidic linkages are alpha 1, 4 (straight chain) and alpha 1, 6 (cross chain) linkages. It has the molecular weight higher than the amylase as upto 100 million.

19. Define homopolysaccharides and heteropolysaccharides with examples.

Homopolysaccharides:
A polysaccharide containing only one type of monosacchride unit.

e.g. Starch, glycogen, linuliln

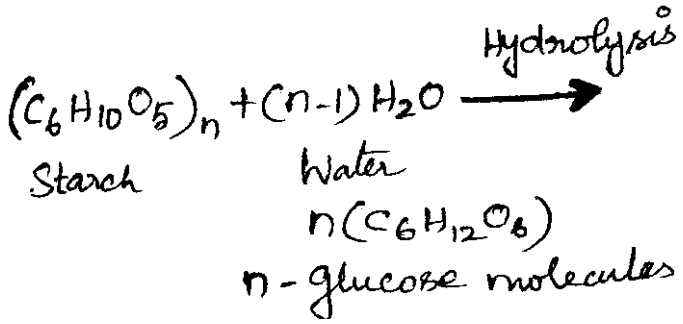
Heteropolysaccharides:
A polysaccharide that contains more than one type of sugar unit.

E.g. Hyaluronic acid.

20. Define hydrolysis reaction of starch.

The enzyme α-amylase fond in the digestive tract of animals (saliva and pancreatic juice) hydrolyse the linear amylase chain by cleaving α-1,4 linkages randomly throughout the chain and yield a mixture of glucose, maltose and oligosaccharides of glucose units.

The reaction is;



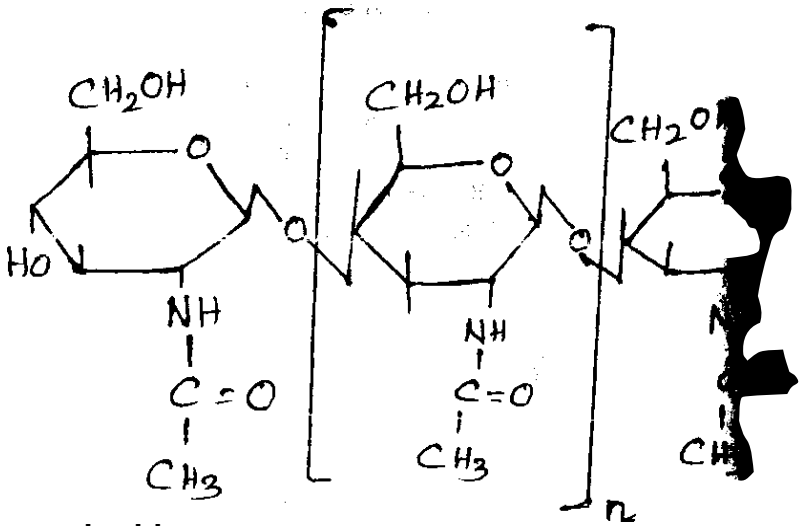
21. What is inuliln?

Inulin has a molecular weight of about 5,000 and consists of about 30-35 fructose units per molecule. It is formed in the plants by eliminating a molecule of water from the glycosidic OH group on carbon atom 2 of one 3-D-fructose unit and the alcoholic OH group on carbon atom 1 of the adjacent β-D-fructose unit.

22. What is chitin? Give its structure?

Chitin is closely related to cellulose. Here the alcoholic OH group on carbon atom 2 of the β-D-glucose units is replaced by an N-acetyamino group. It is, thus a linear polymer of N-acetyl D-glucosamine units joined together by β-1,4-glucosidic linkages.

Structure:

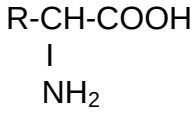


23. Define Mucopolysaccharide.

Mucopolysaccharides are polysaccharides that contain derivatives of sugars such as amino sugars and uronic sugars in addition to that of simple sugars. They are gelatinous substance of high molecular weight upto 5X10^6.

They consist of disaccharide units in which a uronic acid is bund by a glycosidic bond to the C3 of an acetylated amino acid (1→ linkage). These disaccharide residues are polymerized by 1 -->4 linkages to give a linear macromolecule.

24. **Define amino acid with its structure.**



Amino acid is a nitrogenous compound having both an acidic carboxyl (-COOH) and a basic amino ($-NH_2$) group. R stands for the side chains that are different for each amino acid. R can be as simple as a hydrogen atom (H) or a methyl group ($-CH_3$) or a more complex structure.

25. Name any 2 sulphur containing amino acids.

1. Cysteine
2. Methionine

26. **Give 2 example of hydroxyl aminoacids.**

1. Serinne (Ser)
2. Tyrosine (Tyr)

27. Give examples for aromatic amino acids.

1. Phenylalanine
2. Tyrosine
3. Tryptophan

28. Define protein.

A macromolecule composed of one or more polypeptide chains, each with a characteristic sequence of amino acids linked by peptide bonds.

e.g. Albumin, Globulin

29. Define isolectric point or isoelectric pH.

The pH at which a solute has no net electric charge and thus does not move in an electric field.

30. Define zwitter ion.

A dipolar ion, with spatially separated positive and negative charges.

Since the net charge is zero, the molecule does not move in an electric field. Amino acids are present as zwitterions and contain a positively charged amino group and a negatively charged carboxyl group.

31. Define conjugated protein with suitable example.

Conjucated proteins are proteins which are linked with a separable non protein portion called prosthetic troup. The prosthetic group may be either a metal or a compound.

E.g. Metalloproteins, glycoproteins and phosphoproteins.

32. Give any two biological importance of proteins.

1. Many proteins act a catalysts, thus usually enhancing the rate of chemical reactions to such extents as needed by the living cells.
2. Proteins perform transport functions.
3. Various proteins act as harmones.

33. Define disulphide bond.

A chemical bond that stabilizes the three dimensional structure of proteins. These bonds form between cysteine residues in the same or different peptide molecules which also termed Disulphide Bridge.

34. Define peptide bond.

The amino acid units are linked together through the carboxyl and amino group to produce the primary structure of the protein chain. The bond between two adjacent amino acids is a special type of amide bond, in which the hydrogen atom ;of amono (-NH2) group is replaced buy an R radical such a substituted amide is known as the peptide bond.

35. Give two differences between glycogen and starch.

Glycogen	**Starch**
It is animal storage carbohydrates molecule.	It is plant storage carbohydrates molecule.
It has one branch per 8 to 12 residues.	It has one branch per 24 to 30 residues in amylopecttin.

36. Write short note on proton motive force.

The electrochemical potential inherent in a transmembrane gradient of H^+ concentration. It is used in oxidative phosporylation and photophosphorylation to drive ATP synthesis.

37. What you mean by D- sugars?

Optical isomers differ from each other fin the disposition of the various atoms or groups of atoms in space around the symmetric carbon atom. They are infact, the mirror image of each other. These may also be likened to left and right handed gloves.
One form in which H atom at carbon 2 is projected to the left side and OH group to the right is designated aas D-form.

38. Write about the properties of starch:

Starch is a white soft amorphous powder and lacks seetness.
It is insoluble in water, alcohol a ether at ordinary temperature.
The specifc rotation starch is (α)D^20 is +196.

Starch breaks down into large fragments called dextrins.

39. Define lipids with example.

The lipids are heterogenous group of compounds related to fatty acids and include fat, oils, waxes and other related substances. These are oily or greasy organic substances and considerably soluble in organic solvents like ether, chloroform and benzene.

e.g. Phospholipids, glycolipids.

40. What is saturated fatty acids ? Give suitable example.

Fatty acids are long chain organic acids having usually from 4 to 30 carbon atoms. They have a single carboxyl group and a long, nonpolar hydrocarbon tail, which gives most lipids their hydrophobic and oily or greasy nature. If the fatty acid chain containing no double bonds, then it is called saturated fatty acids.

e.g., Butyric acid, lauric acid, Mysteric acid, Palmitic acid and steric acid.

41. Define unsaturated fatty acids with examples.

Fatty acids are long chain organic acids having usually from 4 to 30 carbon atoms. They have a single carboxyl group and a long, nonpolar hydrocarbon tail, which gives most lipids their hydrophobic and oily or greasy nature. If the fatty acid chain containing one or more double bonds, then it is called unsaturated fatty acids.

42. Write Biological role of fatty acids.

1. Lipids provide food, highly rich in calorific value. One gram lipid produces 9.3 kilocalorie ws of energy.
2. 2. Phospholipids play an important role in the absorption and transportation of fatty acids.
3. Lipids are an important constituent of the cell membrane.
4. Lipids involve in hormone synthesis like adrenocarticoids and cholic acids.

43. Define phospholipids and give examples.

Phospholipids is a calss of lipids and these are compounds containing glycerol, a phosphoric acid, nitrogen bases and other substituents, in addition to fatty acids.

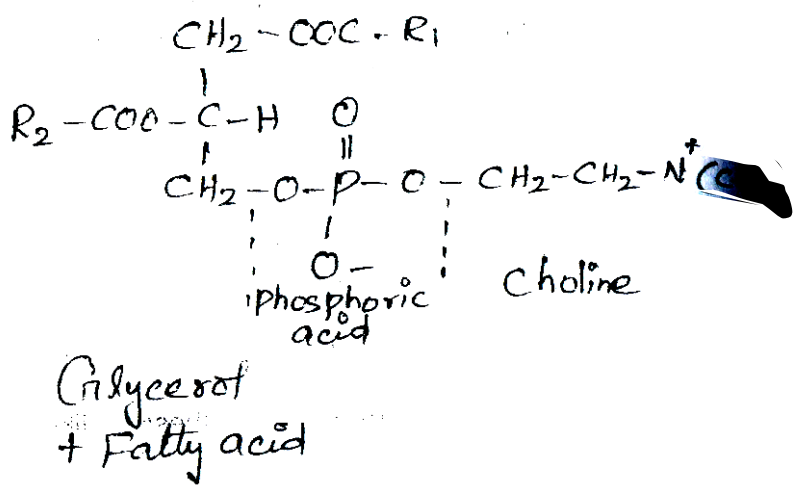
e.g., Lecithins, cebalins, plasmalogens.

44. Define derived lipids with example.

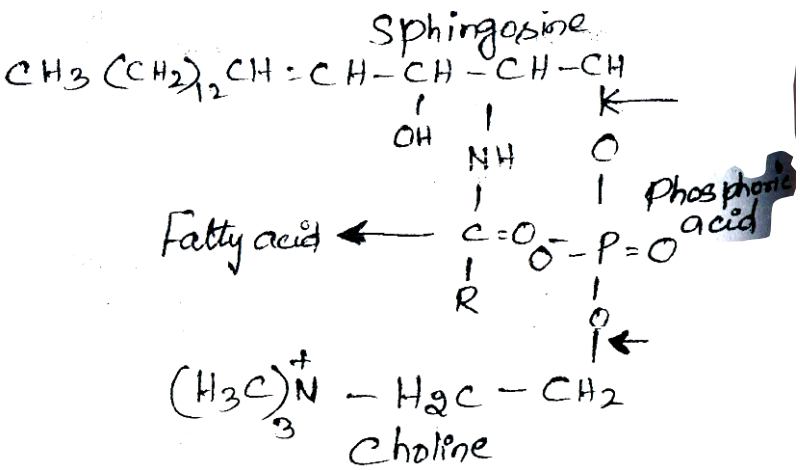
These are the substances derived from simple and compound lipids by hydrolysis. These include fatty acids, alcohols, mono and diglycerides, steroids, terpenes and carotenoids.

e.g., Cholesterol, Ergosterol, cholic acid and cortisol.

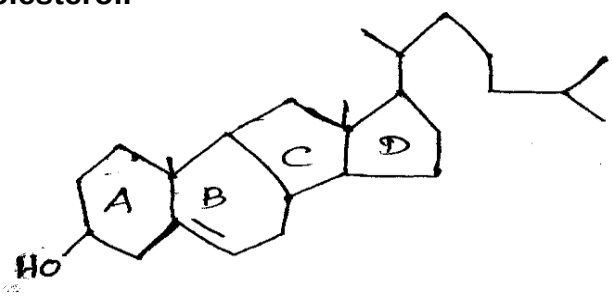
45. Give the structure of lecithins.



46. Give the structure of sphingomycelin.



47. Write the structure of cholesterol.



48. Define Acid Number.

It is a number of milligrams of KOH required to saponify 1 gm of fat. The acid number, thus, tell us of the quantity of free fatty acid present in a fat.

49. Define Saponification Number.

It is the number of milligrams of KOH required to saponify 1 gm of fat. The saponification number thus provides information of the average chain length of the fatty acids in the fat. It varies inversely with the chain length of the fatty acids.

50. Define Iodine Number.

It is the number of grams of iodine absorbed by 100g of fat. The iodine number is, thus a measure of the degree of unsaturation of the fatty acids in the fat. Oils like soybean, corn and cottonseed have higher iodine number (133, 127 and 109 respectively.

51. Differentiate nucleotide with nucleoside.

The nucleosides are compounds in which nitrogenous bases (Purine and pyrimidnes) and conjugated to the pentose sugars (ribose or dexoy ribose) bya beta-glucodic linkage.

Nucleotides are the phosphoric acid esters of nucleosides. These occur either in the free form or as subunits in nucleic acids.

52. Define nucleotide and give example.

Nucleotides are the phosphoric acid esters of nucleoside. These occur either in the free form or as subunits in nucleic acids. The components of nucleotides are phosphate group, sugars, purines and pyrimidnes.

e.g., Adenine,
Guanine,
Thymine,
Cytosine and Uracil.

53. Write different types of nucleic acids.

These are two types as;

1. Deoxyribonucleic acids (DNA) and
2. Ribonuncleic acid (RNA)

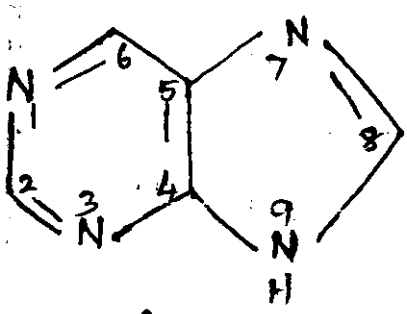
54. Give different types of RNA.

RNA are three types as;

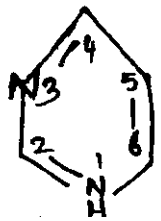
1. Transfer RNA (t RNA)
2. Messenger RNA (m RNA)
3. Ribosomal RNA (r RNA)

55. Structure of purine and pyrimidine.

Purines



Pyrimidines



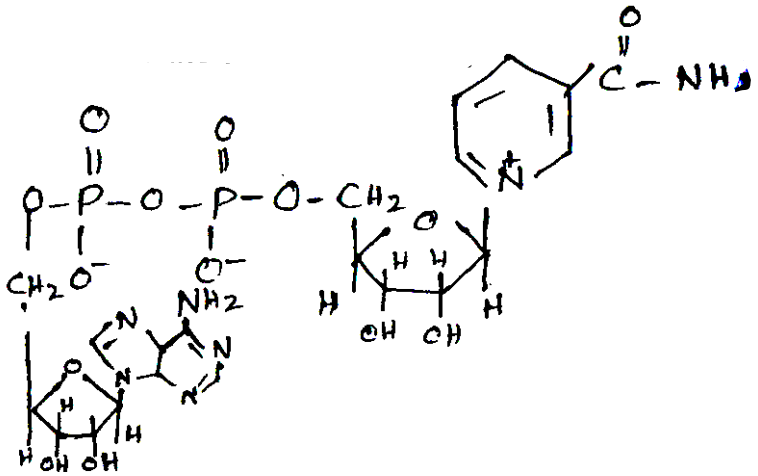
56. Write the difference between RNA and DNA.

DNA	**RNA**
Double stranded helix	Single stranded helix
It has deoxy ribose sugar	It has ribose sugar
It contain Adenine, quinine, cytosine and thymine basepairs	It contain Adenine, quinine, cytosine and Uracil basepairs
DNA is the genetic material	Some viruses only have RNA as genetic material.

57. Define biological importance of nucleotides.

Nucleotides are energy rich compounds that drive metabolic processes especially biosynthetic pathways in all cells. They also serve as chemical signals, key links in cellular systems that respond to hormones and other extracellular stimuli. They are structural components of a number of enzyme cofactors and metabolic intermediates.

58. Give the structure of NAD.



59. Define Oligonucleotide.

A short nucleic acid sequences is referred to as an oligonucleotide. The term oligonucleotide is often used for polymer containing 50 or fewer nucleotdides. A longer nucleic acid is called is called as polynucleotide.

60. State Chargaff's Rule.

i. In DNA, the sum of purines is equal to the sum of puyrimidines. i.e. pu/py = 1. In other words, A+G = T+C.

ii. The ratio of adenine to thymine is also one. Ie., A/T = 1.

iii. The ratio of guanine to cytosine is also one. i.e., G/C = 1.

61. Define denature in DNA.

Denaturation of DNA is a loss of biologic activity and is accompanied by cleavage of hydrogen bonds holding the complementary sequences of nucleotides together. This results in a separation of the double helix to the two constituent polynucleotide chains.

62. Define hyperchromocity.

All the nucleic acids are characterized by a maximum absorption UV light at wavelengths near 260 nm. When the native DNA is denatured, there occurs a marked increase in optical absorpbacny of UV light by pyrimidine and ;purine bases. This effect is called hyperchromocity effect.

63. Write different types of DNA.

i. B form DNA or B-DNA

ii. A form DNA or A-DNA
iii. Z form DNA or Z-DNA

64. Define codon and anticodon.

Anticodon: A specific sequence of three nucleotides in a tRNA, complementry to a codon for an amino acid in an mRNA.

Codon: A sequence of three adjacent nucleotides in a nucleic acid that codes for a specific amino acid.

65. Write the difference between coenzyme and cofactors.

Coenzyme: An organic cofactor required for the action of certain enzymes; often contains a nvitamin as a components.

Cofactor: An organic ion or coenzuyme require for enzuyme activity.

66. What is stop codon and give example?

The codon UAA, UAG and UGA which give signal for the termination of a polypeptide chain during the protein synthesis. Since the protein synthesis is stopped by the above codons, it is called as stop codons.

67. What is glycosidic bond in DNA?

The chemical bonds between a sugar and another molecule (typically an alcohol, purine, pyriminde or sugar) through an intervening oxygen is called glycosidic bond in DNA.

68. Define aminoacyl tRNA.

It is a tRNA carrying an amino acid during translation process or amino acid synthesis. The amino group of the amino acid is covalently linked to ether 2’ or 3’ OH group of the adenylate residue in the 3” CCA terminus of Trna.

PART – B

1. Define lipids and explain its classification & functions.

Lipids may be regarded as organic substances relatively insoluble in water, soluble in organic solvents (alcohol, ether etc.),potentially related to fatty acids and utilized by living cells.

Classification of lipids:

Lipids are broadly classified into simple & complex, derived & miscellaneous lipids, which are further, subdivided in different groups.

1. Simple Lipids: Esters of fatty acids with alcohols. These are mainly of 2 types.

(a) Fat and oils (triaceylglycerols): These are esters of fatty acids with glycerol. The difference between fat & oil is only physical. Thus oil is a liquid while fat is a solid at room temperature.

(b) Waxes: Esters of fatty acids with alcohol other than glycerol. These alcohols may be aliphatic or alicyclic. Cetyl alcohol is most commonly found in waxes.

2. Complex (or compound) lipids: These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc. These are further divided as follows.

(a) Phospholipids: They contain phosphoric acid frequently a nitrogenous base. This is in addition to alcohol and fatty acids.

(i)Glycerophospholipids: These phospholipids contain glycerol as the alcohol.e.g .lecithin, cephalin.

(ii)Shingophospholipids: Sphingosine is the alcohol in this group of phospholipids. E.g. sphingomyelin.

(b) Glycolipids: These lipids contain a fatty acids , Carbohydrate.and nitrogenous base.The alcohol is sphingosine, hence they are also called as glycosphingolipids.Glycerol and phosphates are absent. E.g. cerebosides, gangliosides.

(c) Lipoproteins: Macromolecular complexes of lipids with proteins.

(d) Other complex lipids: Sulfolipids, amino lipids and lipopolysacchrides are among other complex lipids.

2. Derived lipids: These are the derivatives obtained on the hydrolysis of group 1 & group 2 lipids which possess the characteristics of lipids. These include glycerol and other alcohols,fattyacids,mono-& diaceylglycerols,lipid(fat) soluble vitamins,steroid hormones,hydrocarbons and ketone bodies.

3. **Miscellaneous lipids:** These include a large number of compounds possessing the characteristics of lipids e.g, carotenoids, squalene, hydrocarbons such as pantacosane (in bees wax), terpenes etc.

4. **Neutral lipids:** The lipids which are uncharged are referred to as neutral lipids. These are mono-, di-, and triaceylglycerols, cholesterol and cholesteryl esters.

Function of lipids:

Lipids perform several functions,

1. They are the concentrated fuel reserve of the body.(triacylglycerols)
2. Lipids are the constituents of memberane structure and regulate the membrane permeability (phospholipids and cholesretol).
3. They serve as a source of fat soluble vitamins (A, D, E & K).
4. Lipids are important as cellular metabolic regulators.(steroid hormones and prostaglandins).
5. Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.

2. Describe the phospholipids and its class with biological important.

These are complex or compound lipids containing phosphoric acid, in addition to fatty acids, nitrogenous base and alcohol.

They are 2 classes of phospholipids.

1. **Glycerophospholipids** or (phosphoglycerides) that contain glycerol as the alcohol.
2. **Sphingophospholipids** or (sphingomyelins) that contain sphingosine as the alcohol.

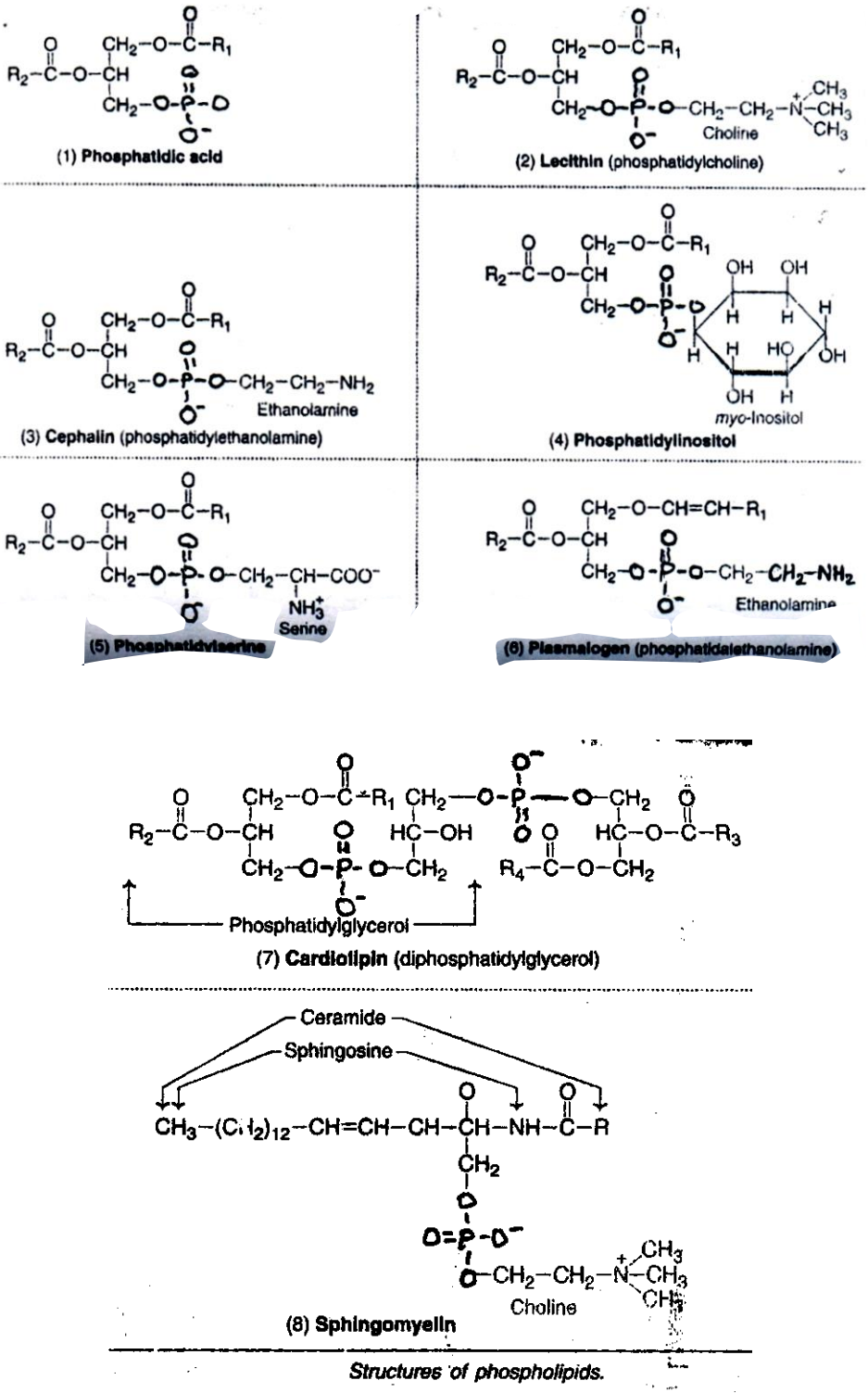
Glycerophospholipids

Glycerophospholipids are the major lipids that occur in biological membranes.They consist of glycerol 3-phosphate esterified at its C_1 and C_2 with fattyacids.Usually C_1 contains saturated fatty acids while C_2 contains an unsaturated fatty acid.

1. Phosphatidic acid: This is the simplest phospholipids. It does not occur in good concentration in the tissues. Basically, phosphatidic acid is an intermediate in the synthesis of triaceylglycerols and phospholipids.

The other glycerophospholipids containing different nitrogenous base or other groups may be regarded as the derivatives of phosphatidic acid.

2. Lecithins (phosphatidylcholine): These are the most abundant group of phospholipids in the cell membranes. Chemically lecithin is a phosphatidic acid with



Structures of phospholipids.

Choline as the base. Phosphatidylcholines represents the storage form of body's choline.

(a) Dipalmitoyl lecithin is an important phospa\hatidylcholine found in lungs. It is a surface active agent and prevents the adherence of inner surface of the lungs due to surface tension. Respiratory distress syndrome in infants is a disorder characterized by the absence of dipalmitoyl lecithin.

(b) Lysolecithin is formed by removal of the fatty acid either at C_1 or C_2 lecithin.

3. Cephalins: Ethanolamine is the nitrogenous base present in cephalins. Thus cephalin & lecithin differ with regard to base.

4. Phosphatidylinositol: The steroisomer myo-inositol is attached to phosphatidic acid to give phosphatidylinositol (PI).

This is an important component of cell membrane. The action of certain hormones (e, g. oxytocin, vasopressin) is mediated through PI.

5. Phosphatidylserine: The aminoacids serine is present in this group of glycerophospholipids. Phosphatidylthreonine is also found in certain tissues.

6. Plasmalogens: When a fatty acid is attached by an ether linkage at C_1 of glycerol in the glycerophospholipids, the resultant compoundis plasmogen. Phospahtidal-ethanolamine is the most important which is similar in structure to phosphatidylethanolamine but for the ether linkage. An unsaturated fatty acid occurs at C_1.Choline/inositol and serine may substitute ethanolamine to give other plasmogens.

7. Cardiolipin:It is so named as it was first isolated from heart muscle. structurally, a cardiolipin consists of 2 molecules of phosphatidi acid held by an additional glycerol through phosphate groups. It is an important component of inner mitochondrial membrane. Cardiolipin is the only phosphoglyceride that posses antigenic properties.

Sphingomyelins

Sphingosine is an amino acid present in sphingomyelins. They do not contain glycerol at all.Sphingosine is attached by an amide linkage to a fatty acid to produce ceramide. The alcohol group of sphingosine is bound to phosphorylcholine in sphingomyelin structure.sphingomyelins are important constituents of myelins and are found in good quantity in brain and nervous system.

Action of phospholipids

Phospholipases are a group of enzymes that hydrolyses phospholipids. There are 4 distinct phospholipases (A_1, A2, C &D) each one of them specifically acts on a particular bond.

Functions of phospholipids

1. In association with proteins, phospholipids from the structural components of membranes and regulate membrane permeability.

2. Phospholipids in the mitochondria are responsible for maintaining the conformation of electron

transport chain components, and thus cellular respiration.

3. Phospholipids participate in the absorbtion of fat from the intestine.
4. Phospholipids are essential for the synthesis of different lipoproteins and thus participate in the transport of lipids.

5. Accumulation of fat in liver can be prevented by phospholipids. Hence they are regarded as lipotropic factors.

6. Arachidonic acid an unsaturated fatty acid liberated from phospholipids serves as a precursor for the synthesis of eicosanoids.

7. Phospholipids participate in the reverse cholesterol transport and thus help in the removal of cholesterol from the body.

8. Phospholipids acts as surfactants .For instance dipalmitoyl phosphatidylcholine is an important lung surfactant. Respiratory distress syndrome in infants is associate with insufficient production of this surfactant.

9. Cephalins, an important group of phospholipids participate in blood clotting.

10. Phosphoipids are involved in signal transmission across membranes.

3. Describe the following

1. Sphingomyelins.

2. Glycolipids.

3. Triacylglycerides.

1. Sphingomyelins

Spingosine is the amino alcohol present in sphingomyelins (sphingophospholipids). They do not contain glycerol at all. Sphingosine is attached by an amide linkage to a fatty acid to produce ceramide. The alcohol group of sphingosine is bound to phosphorylcholine in sphingomyelin structure. Sphingomyelins are important constituents of myelin and are bound in good quantity in brain and nervous tissues.

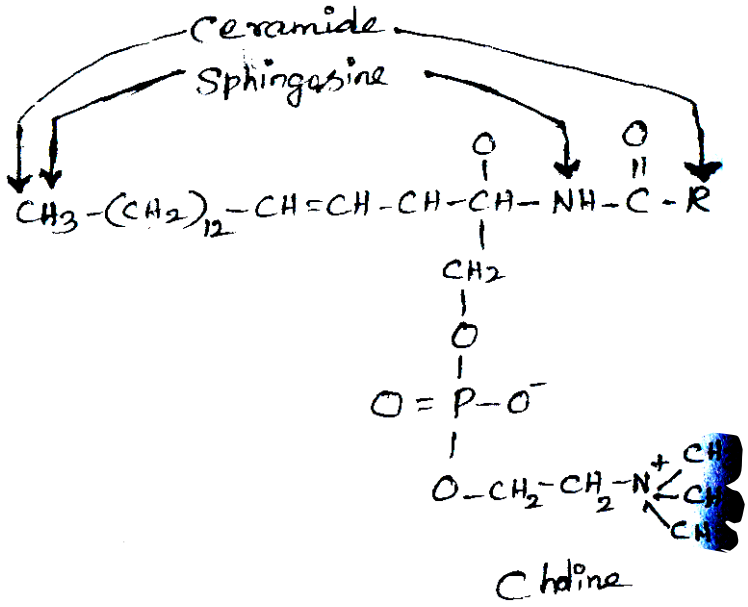
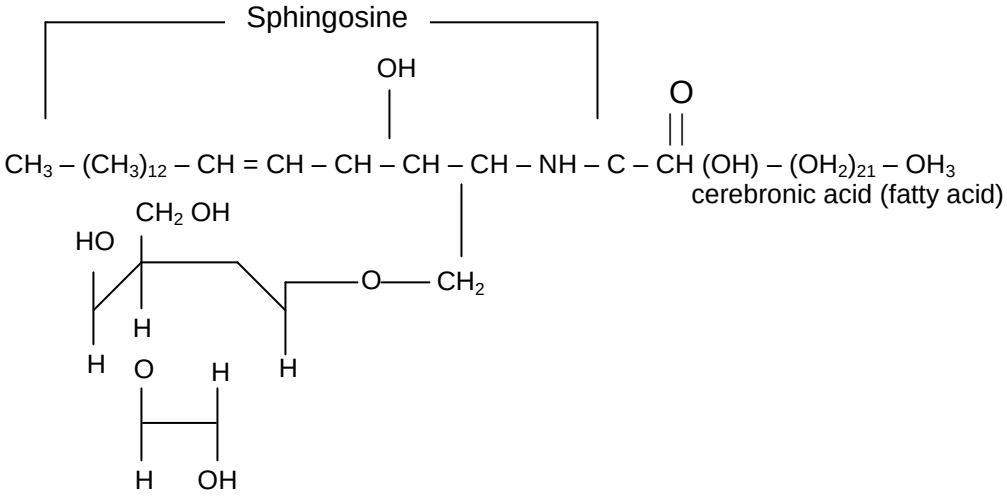


Figure: Sphingomyelin

2. Glycolipids

Glycolipids are important constituents of cell membrane and nervous tissues (particularly the brain). Cerebrosides are the simplest from of glycolipids. They contain a cetramide (sphingomine attached to fatty acid) and one or more sugars. Galactocerebroside (galactosylcereamide) ang glucocerebroside are the most important glycolipids. The structure of galactosylcereamide is given in fig. it contains the fatty acid cerebronic acid. Sulfagalactosylceramide is the sulfatide derived from galactosylceramide.



Structure of galactosylceramide (R = H) For sulfagalactosyl ceramide R is a sulfatide (R = SO_4^2)

Gangliosides: These are predominantly found in ganglions and are the most complex form of glycolipids. They are the derivatives of cerebrosides and contain one or more molecules of N-acetylneuraminic acid(NANA), the most important sialic acid. The structure of NANA is given in carbohydrate chemistry.

The most important gangliosides present in the brain are GM_1, GM_2, GD, and GT(G represents ganglioside while M,D and T indicate mono-, di- or tro- sialic acid residues, and the number denotes the carbohydrate sequence attached to the ceramide). The ganglioside, GM_2 that accumulates in Tay-Sachs disease is represented next.

3. Triacylglycerols: triacylglycerols(formally triglycerides) are the esters of glycerol with fatty acids. The fats and iols that are widely distributed in both in plants and animals are chemically triglycerols. They are insoluble in water and non-polar in character and commonly known as natural fats.

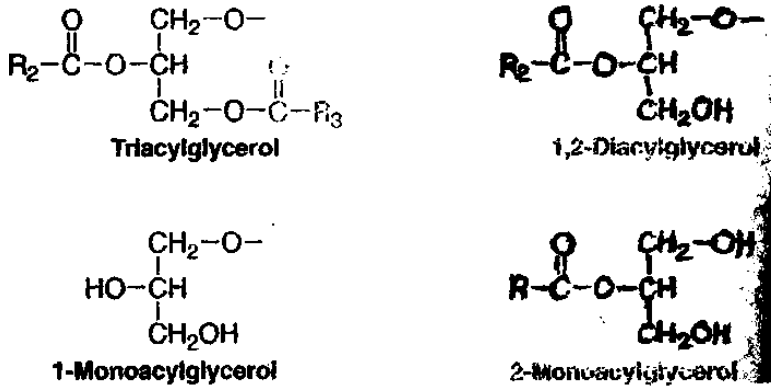


Figure: General structure of acylglycerols
(For palmitoyl R = $C_{15}H_{31}$; for stearoyl R = $C_{17}H_{35}$; For linoleoyl R = $C_{17}H_{31}$)

Fats as stored fuel: triacylglycerols are the most abundant group of lipids that primarily function as fuel reserves of animals. The fat reserve of normal humans (men 20%, women 25% by weight) is sufficient to meet the body's caloric requirement for 2-3 months.

Fats primarily occur in adipose tissue:

Adipocytes of adipose tissue-predominatly found in the shbcutaneous layer and in the abdominal cavity-are specialized for storage of triacylglycerols. The fat is stored in form of globules dispersed in the entire cytoplasm.

And surprisingly, triacylglycerols are not the structural components of biological membranes.

Structure of triacylglycerols:

Monoacylglycerols, diacylglycerols and triacylglycerols, respectively consisting of one, two, three molecules of fatty acids estrified to a molecule of glycerol, are known. Among these triacylglycerols are the most important biochemically.

Simple triglycerols contain the same type of fatty acid residue at all the three carbons e.g., tristearoyl glycerol or tristearin.

Mixed triglycerols are more common. They contain 2 or 3 different types of fatty acid residues. In general, fatty acid attached to C_1 is saturated, that attached saturated to C_2 is unsaturated while

that on C_3 can be either. Triacylglycerols are named according to placement of acyl radial on glycerol,e.g., 1,3-palmitoyl 2-linoleoyl glycerol.

Triacylglycerols of plants, in general, have higher content of unsaturated fatty acids

4. Describe briefly structure and biolical importance of steroids.

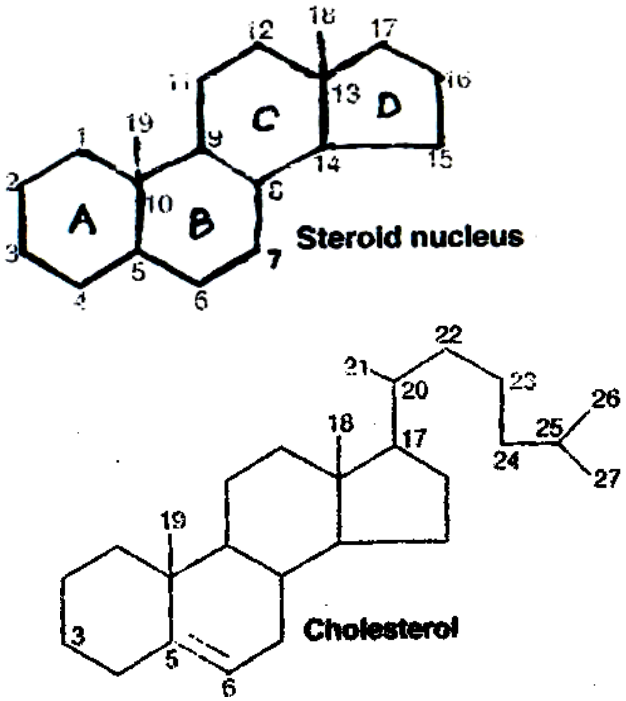
Steroids are the compounds containing a cyclic steroid nucleus namely cyclopentanoperhydrophenanthrene (cppp). It consists of a phenanthrene nucleus (rings A, B and C) to which a cyclopentane ring(D) is attached.

The structure and numbering of cppp are shown in fig. the steroid nucleus represents saturated carbons. The methyl side chains (19 and18) attached to carbons 10 and 13 are shown as single bonds. At carbon 17, steroids usually contain a side chain.

There are several steroids in the biological system. There include cholesterol, bile acids, vitamin D, sex hormones , adrenocortical hormones, sitosterols, cardiac glycosides and alkaoids. If the steroid contains one or more hydroxyl groups it is commonly know as sterol (means solid alcohol).

CHOLESTEROL

Cholesterol, exclusively found in animals, is the most abundant animal sterol. It is widely distributed in all cells and is a major component of cell membranes and lipoproteins. Cholesterol was first isolated from bile. Cholesterol literally means solid alcohol from bile.



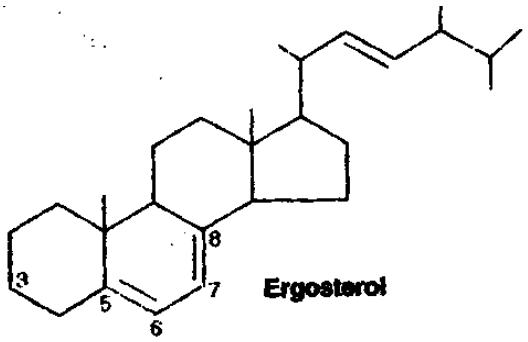


Figure: Structures of steroids (A, B, C, - Perhydro – phenanthrene; D – Cyclopentane)

Structure and occurrence

The structure of cholesterol (c 27H46 o) is depicted in fig. it has one hydroxyl groups at c3 and a double bond between c5 and c6. An 8 carbon aliphatic side chain is attached to c17. cholesterol contains a total of 5 methyl groups.

Due to the presence of an –OH group, cholesterol is weakly amphiphilic. As a structural componet of plasma membranes , cholesterol is an important determinant of membrane permeability properties. The occurrence of cholesterol is much higher in the membranes of sub-cellular organelles.

Cholesterol is found in association with fatty acids to from cholesteryl esters (esterification occurs at the OH groups of c3)

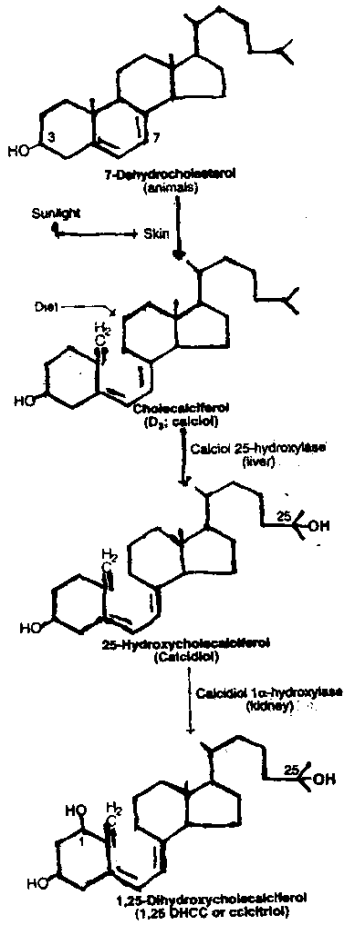
Properties and reactions:

Cholesterol is an yellowish crystalline solid. The crystals, under the microscope, show a notched appearance. Cholesterol is insoluble in water and soluble in organic solvents such as chloroform, benzene ether etc.

Several reactions given by cholesterol are useful for its qualitative identification and quantitative estimation. These include salkowsk’s test, Liebermann- Burchard reaction and Zak’s test.

Function of cholesterol:

Cholesterol is a poor conductor of heat and electricity, since it has a high dielectric constant. It is present in abundance in nervous tissues. It appears that cholesterol functions as an insulating cover for the transmission of electrical impulses in the nervous tissue. Cholesterol performs several other biochemical functions which include its role in membrane structure and function, in the synthesis of bile acids, hormones and vitamin D.



ERGOSTEROL

Ergosterol occurs in plants. it is also found as a structural constituent of membranes in yeast and fungi. Ergosterol is an important precursor for vitamin D. when exposed to light the ring B of ergosterol opens and it is converted to ergocalciferol, a compound containing vitaminD activity.

The other sterols present in plant cells include stigmasterol and p sitosterol compared to that of animals.

5. Describe the structure of DNA.

DNA is a polymer ***of deoxyribonucleotides*** *(*or simply deoxynucleotides*).* It is composed of monomeric units namely deoxyadenylate (damp), deoxyguanylate (dgmp), deoxycytidylate (dcmp) and deoxythymidylate (dtmp).

Schematic diagram of polynucleotides

The monomeric deoxynucleoides in DNA are held together by 3', 5'-phosphodiester bridges.DNA structure is often represented in short hand form. The horizontal line indicates the carbon chain of sugar with base attached to $C_{1'}$. Near the middle of the horizontal line is $C_{3'}$ phosphate linkage while at the other end of the line is $C_{5'}$ phosphate linkage.

Chargaff"s rule of DNA composistion

Erwin chargaff in late 1940s quantitatively analysed the DNA hydrolysates from different species. He observes that in all the species the studied, DNA had equal numbers of adenine and thymine residues (A=T) and equal numbers of guanine and cytosine residues (G=C). This is known as chargaff's rule of ***molar equivalence between the purines and prymidines in DNA*** structure. The significance of chargaff's rule was not immediately realized. The double helical structure of DNA derives its strength from chargaff's rule.

Single-stranded DNA and RNAs which are usually single stranded, do not obey chargaff's rule. However, double-stranded RNA which is the genetic material in certain viruses satisfies chargaff's rule.

DNA double helix

The double helical structure of DNA was proposed by ***James watsen*** and ***Francis crick*** in 1953 (noble prize, 1962). The elucidation of DNA structure is considered as a ***milestone in*** the era of ***modern biology***.DNA double helix is comparable to a twisted ladder. The salient features of Watson - Crick Model of DNA are described below.

1. The DNA is the right handed double helix. It consists of two poly deoxyribonucleotide chains (strands) twisted around each other on a common axis.
2. The two strands are ***antiparallel***, i.e., one strand runs in the 5' to 3' direction while the other in 3' to 5' direction. This is comparable to two parallel adjacent roads carrying traffic in opposite direction.
3. The width or diameter of a double helix is 20 Å (2 nm).
4. Each turn (picth) of the helix is 34 Å (3.4nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4Å
5. Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3'-5' phosphodiester bonds) on the outside (pherphery) of the molecule while the hydrophobic bases are stacked inside (core).
6. The two polynucleotide chains are not identical but complementry to each other due yo base pairing.
7. The two strands are held together by hydrogen bonds formed by complementry base pairs. The A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. The GΞC is stronger by about 50% than A=T.
8. The hydrogen bonds are formed between a purine and a ptrimidine only. If two purines face each other, they would not fit into the allowable space. And two prymidines would be too far to form hydrogen bonds. The only base arrangement possible in DNA structure, from spatial considerations is A-T, T-A, G-C and C-G.
9. The complementrybase pairing in DNA helix proves chagaff's rule. The content of adenine equals to that of thymine (A=T) and guanine equals to that of cytosine (G=C).
10. The ***genetic information resides on*** one of the two strands known as ***template strand*** or sense strand. The opposite strand is antisense strand. The double helix has major grooves and minor grooves along the phosphodiester backbone. Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

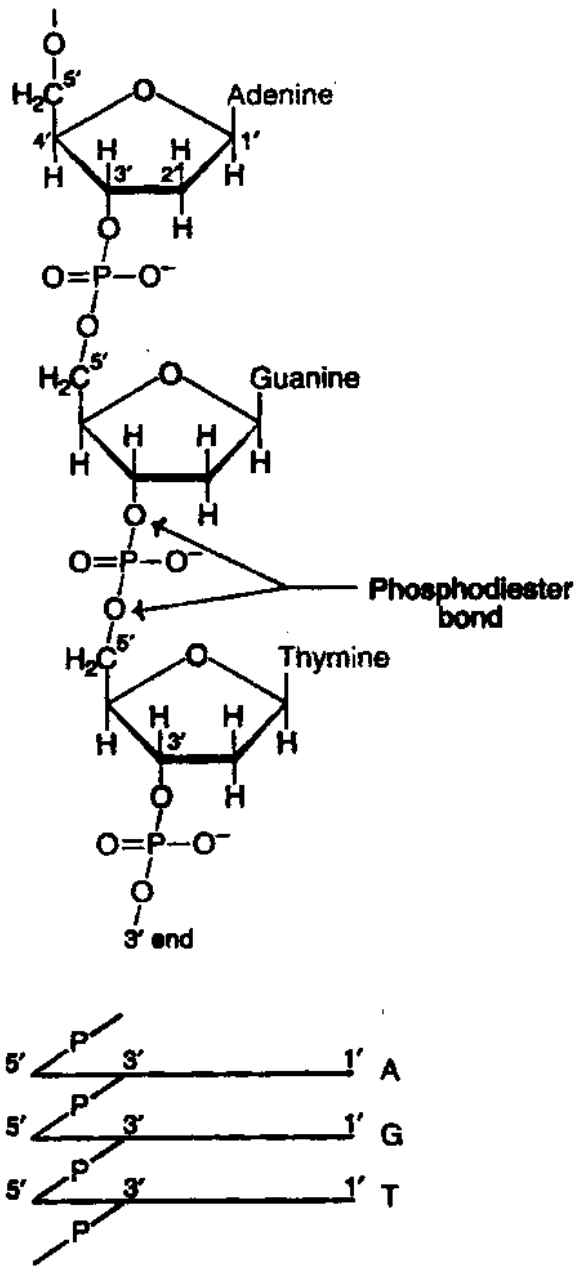


Figure: Structure of a polydeoxyribonucleotide segment held by phosphodiester bonds. On the lower part is the representation of short hand form of oligonucleotides.

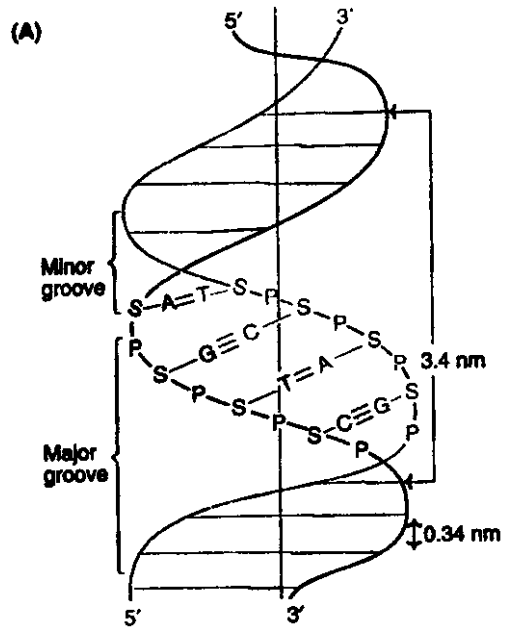


Figure: Watson – Crick model of DNA helix

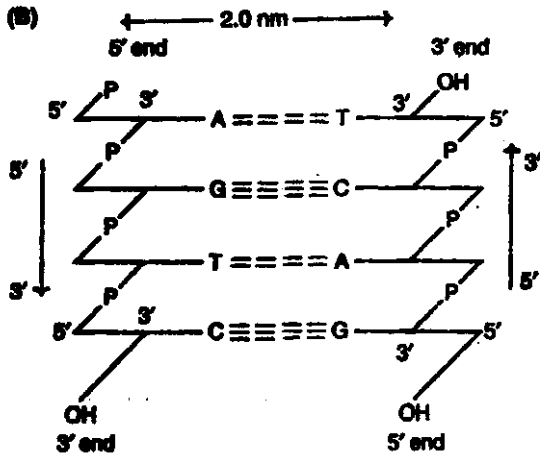


Figure: Complementary base pairing on DNA helix.

6. Describe the structure and types of RNA.

RNA is a polymer of ribonucleotides held together by 3', 5'-phosphodiester bridges. Although RNA has certain similarities with DNA structure, they have specific differences.

1. **Pentose:** The sugar in RNA is ribose in contrast to deoxyribiboise in DNA.
2. **Pyrimidine:** RNA contains the pyrimidine uracil in place of thymine (in DNA).
3. **Single strand:** RNA is usually a single-stranded polyunucleotide. However, this strand may fold at certain places to give a double stranded structure, if complementry base pairs are in close proximity.

4. **Chargaff's rule-not obeyed:** due to the single stranded nature, there is no specific relationbetween purine and pyrimidine content. Thus the guanine content is not equal to cytosine (as is the case in DNA).
5. **Susceptibility of alkali hydrolysis:** Alkali can hydrolysis RNA to 2',3' cyclic diesters. This is possible due to the presence of a hydroxyl group at 2' position. DNA cannot be subjected to alkali hydrolysis due to lack of this group.
6. **Orcinol colour reaction:** RNAs can be histologically identified by orcinol colour reaction due to the presence of ribose.

Types of RNA

The three major types of RNAs with their respective cellular composition are given below

1. **Messenger RNA (**mRNA): 5-10%
2. **Transfer RNA** (tRNA):10-20%
3. **Ribosomal RNA** (rRNA): 50-80%

Besides three RNAs reffered above, other RNAs are also present in the cells. These include heterogenous nuclear RNA, small nuclear RNA and small cytoplasmic RNA.

The RNAs are synthesized from DNA and are primarily involved in the process of protein biosynthesis. The RNAs vary in their structure and function. A brief description on the major RNAs is given.

Messenger RNA (m RNA)

The mRNA is synthesized in the nucleus as heterogeneous nuclear RNA on processing, liberates the functional mRNA which enters the cytoplasm to participate in the protein synthesis. mRNA has high molecular weight with a short half-life.

The eukayotic mRNA is capped at the 5'-terminal end by 7-methylguanosine triphosphate. It is believed that this cap helps to prevent the hydrolysis of mRNA by 5'-exonucleases. Further, the cap may be also involved in the recognition of mRNA for protein synthesis.

The 3'-terminal end of mRNA contains a polymer of adenylate residues (20-250 nucleotides) which is known as poly tail. This tail may provide stability to mRNA, besides preventing it from the attack of 3'-exonucleases.

mRNA molecules often contain certain modified bases such as 6-methyladenylates in the internal structure.

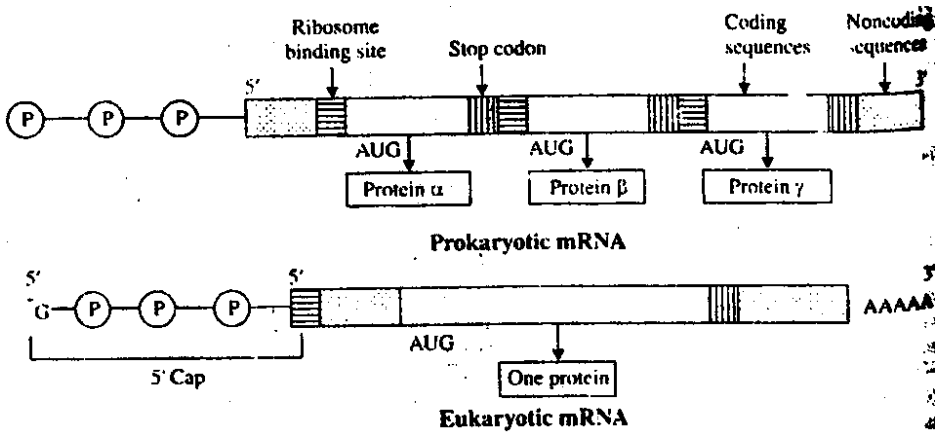
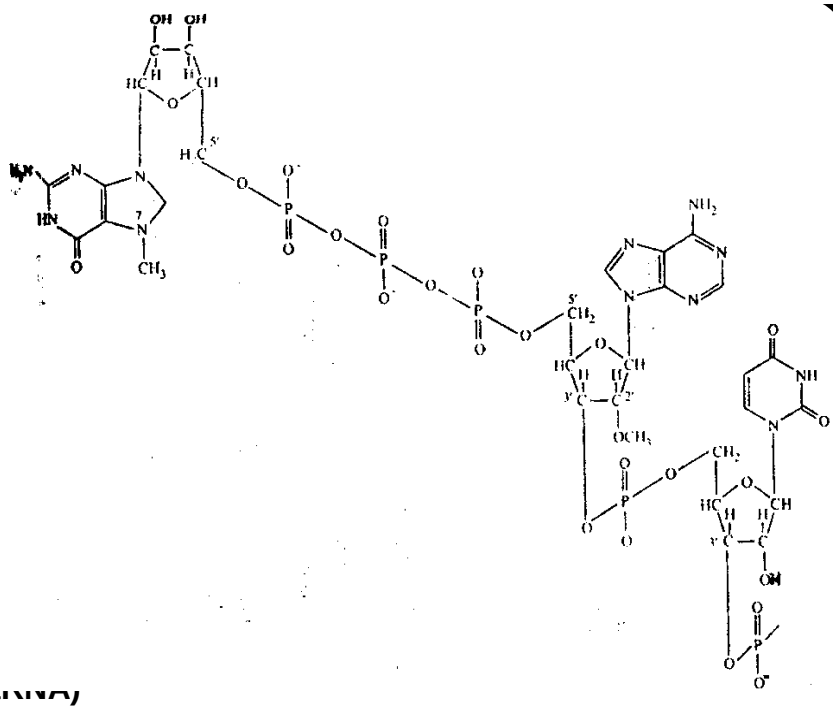


Figure: A comparison of the structures of prokaryotic and eukaryotic messenger RNA molecules



Transfer RNA (tRNA)

Transfer RNA (soluble RNA) molecule contains 71-80 nucleotides with a molecular weight of about 25000. There are atleast 20 species of tRNAs, corresponding to 20 aminoacids present in protein structure. The structure of tRNA was first elucidated by Holley.

The structure of tRNA, depicited in yhe diagram, resembles that of a clover leaf. tRNA contains mainkly four arms, each with a base paired stem.

1. **The acceptor arm:** this arm is caped with a sequence CCA (5' to3'). The aminoacid is attached to the acceptor arm.
2. **The anticodon arm:** this arm, with the three specific nucleotide bases, is responsible for the recognition of triplet codon of mRNA. The codon and anticodon are complementry to each other.
3. **The D-arm:** It is so named due to the presence of dihydrouridine.

4. **The TψC arm: this arm:** this arm contains a sequence of T, pseudouridine and C.
5. **The variable arm:** this arm is the most variable in tRNA. Based on this variability tRNAs are classified into two categories:

i. Class I tRNAs: the most predominant form with 3-5 base pairs length.

ii. Class II tRNAs: they contain 13-20 base pairs long

Base pairs in tRNA

The structure of tRNA is maintained due to the complementry base pairing in the arms. The four arms with their respective base pairs are given below;

The acceptor arm – 7bp
The TψC arm- 5bp
The anticodon arm-5bp
The D arm-4 bp

Ribosomal RNA (rRNA)

The ribosomes are the factors of protein synthesis. The eukaryotic ribosomes are composed of two major nucleoprotein complexes-60s subunits and 40s subunits. The 60s subunits contain 28s rRNA, 5s rRNA and 5.8s rRNA while the 40s subunit contains 18s rRNA. The function of rRNAs in ribosomes is not clearly known. It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.

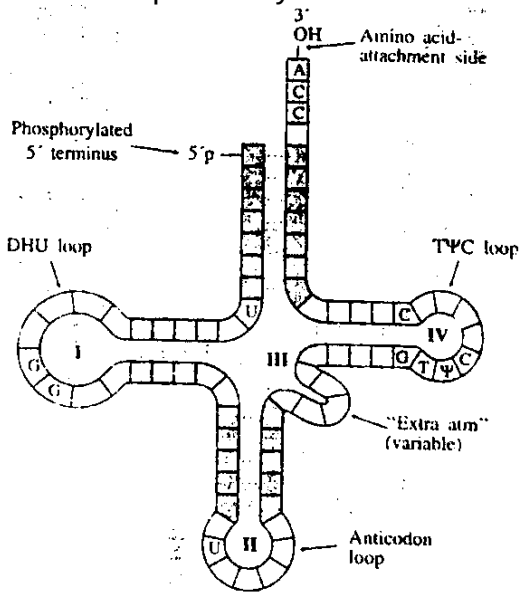


Figure: Common features of tRAN molecules

Comparison of the base sequences of many tRANs reveals a number of conserved features. Note the presence of an amino acid attachment site, the 4 loops and the 4 arms or stems.

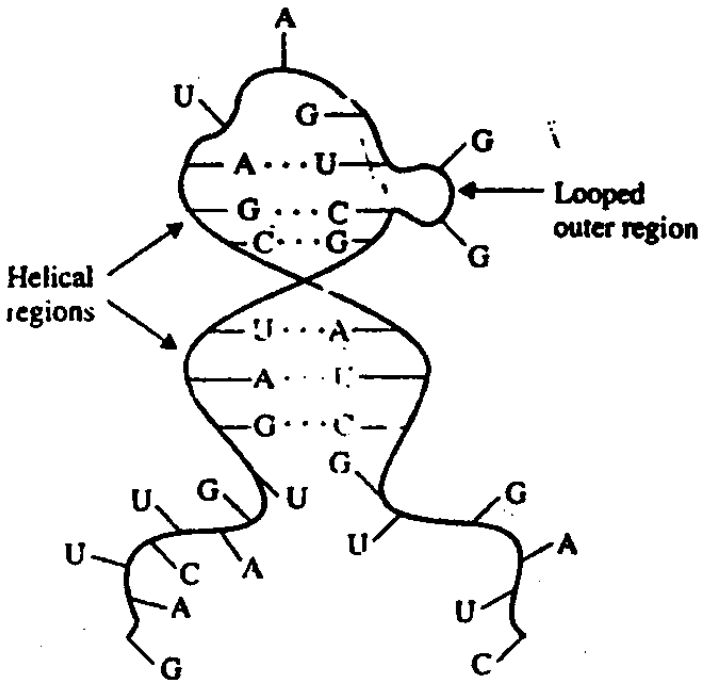


Figure: Molecular structure of rRAN Note the helical regions with complementary base pairs and a looped outer region.

7. Define nucleotides and its biological functions.

Nucleotides are the phosphoric acid esters of nucleotides. These occur either in the free form or as subunits in nucleic acids. As mentioned earlier, the phosphate is always esterified to the sugar moiety. The component units of nucleotides are shown in fig 15 – 10.

In the ribose moiety of a ribonucleotide, phosphorylation is possible only at three positions (C2', C3', C5') since C1' and C4' are involved in the furanose ring formation. In other words, the phosphate group could be esterified only at these three places. On the contrary, in the deoxyribose component of a 2' – deoxyribonucleoside, only two positions (C3', C5') are available for phosphorylation, since in this sugar C1' and C4' are involved in the furanose ring and C2' does not bear a hydroxyl group. Accordingly, hydrolysis of the two nucleic acids, RNA and DNA, by various methods and under different set of conditions gives rise to isomeric nucleotides of 3 types and 2 types respectively. Important ribonucleotides (also called ribotides) and deoxyribonucleotides (or deoxyribotides). While names of nucleosides and nucleotides are generally derived from the corresponding bases,there is one exception to this

Rule: the base corresponding to the nucleoside called inosine (and the derived nucleotides) is called

Hypoxanthine.

The following fig show the structure of nucleotides found in DNA and RNA;

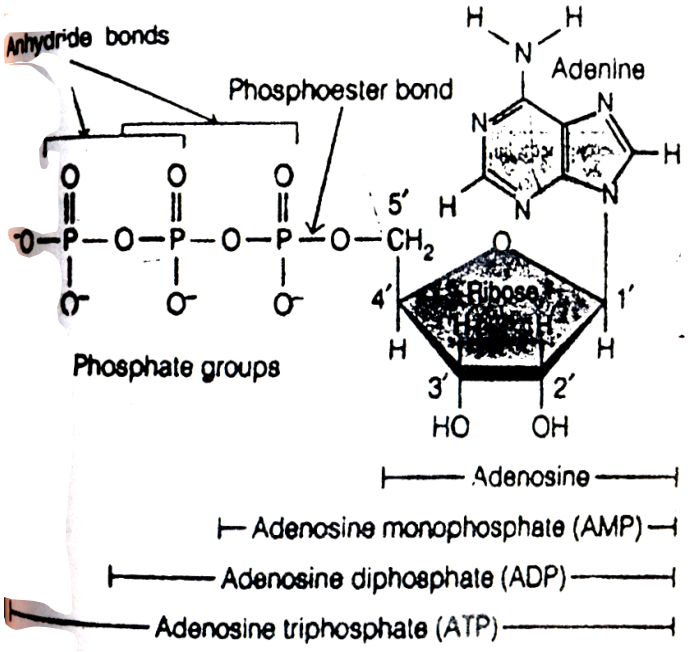
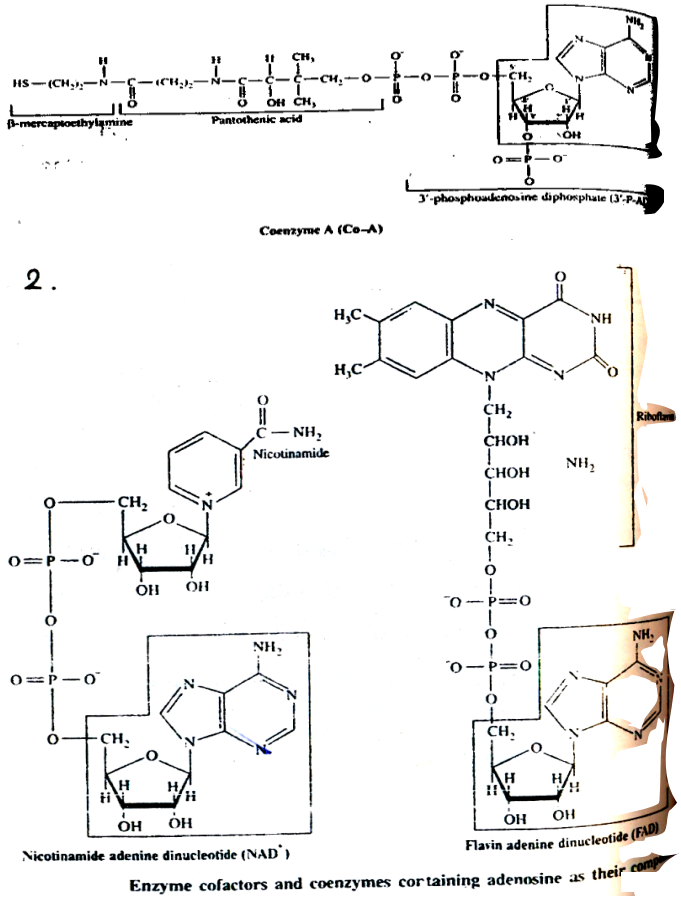
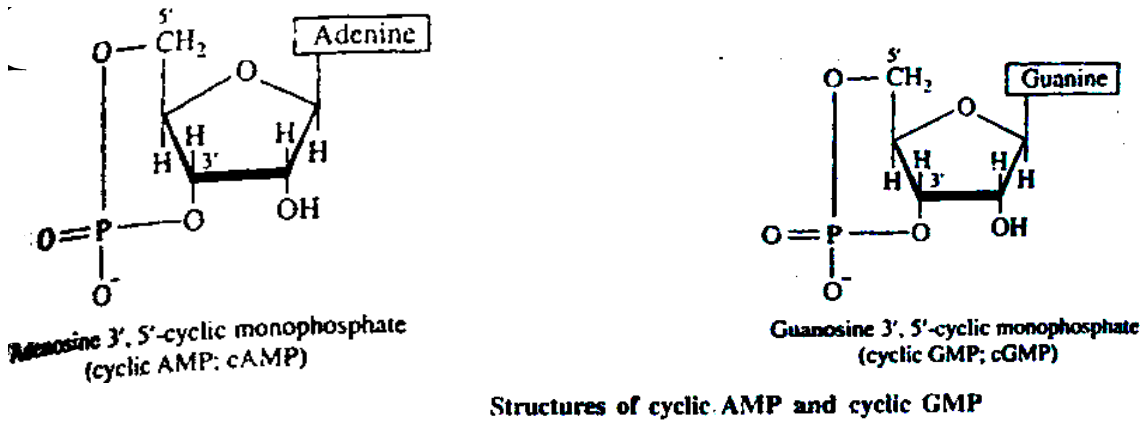


Figure: The Phosphate ester and phosphoric acid anhydride bonds of ATP

Hydrolysis of an anhydride bond yields more energy than hydrolysis of the ester.



Enzyme cofactors and coenzymes containing adenosine as their



Adenosine 3′, 5′-cyclic monophosphate (cyclic AMP; cAMP)

Guanosine 3′, 5′-cyclic monophosphate (cyclic GMP; cGMP)

Structures of cyclic AMP and cyclic GMP

Functions of Nucleotides

In addition to their roles as the subunits of nucleic acids, nucleotides perform some other functions. These are enumerated below :

1. **As carriers of chemical energy.** Nucleotides may have one, two or three phosphate groups covalently linked at 5' –OH of ribose. These are referred to as nucleoside mono-, di- and triphos-phates and abbreviated as NMPs, NDPs and NTPs, respectively. The 3 phosphate groups are generally labeled as α,β and γ, starting from the ribose. NTPs are used as a source of chemical energy to drive many biochemical reactions. Adenosine triphosphate (ATP) is, by far, the most widely used. Others such as uridine triphosphate (UTP), guanosine triphosphate (GTP) and cytidine triphosphate (CTP) are used in specific reactions.

The hydrolysis of ATP and other nucleoside

Triphosphates are an exergonic reaction. The bond between the ribose and the α-phosphate is an ester linkage. The α-β and β-γ linkages are Phosphoric acid anhydrides. Hydrolysis of the Ester linkage yields about 14 kJ/mol, whereas hydrolysis of each of the anhydride bond yields about 30 kJ/mol. In biosynthesis, ATP hydrolysis often drives less favourablr metabolic reactions (i.e., those with $\Delta G^{\circ\prime} > 0$). When coupled to a reaction with a positive free-energy change, ATP hydrolysis shifts the equilibrium of the overall process to favour product formation.

2. As components of enzyme factors. Many enzymes cofactors and coenzymes (such as coenzyme A,NAD+ and FAD) contain adenosine as part of their structure (Fig. 15-13). They differ from each other except for the presence of adenosine. In these cofactors, adenosine does not participate directly, but removal of adenosine from these cofactors usually results in drastic reduction of their activities. For instance, removal of adenosine nucleotide from acetoacetyl-CoA reduces its reactivity as a substrate for β-ketoacyl-CoA transferase, an enzyme of lipid metabolism, by a factor of 10^6 .

3. As chemical messangers. The cells respond to their environment by taking cures from hormones or other chemical signals in surrounding medium. The interaction of these

chemical signals(first messangers) with receptors on the cell surface often leads to the formation of second messangers (Fig.15-13) inside the cell, which in turn lead to adaptive changes inside the cell. often, the second messenger is a nucleotide.

4. One of the most common second messengers is the nucleotide adenosine 3',5' – cyclic onophosphate (cyclic AMP or cAMP), formed from ATP in arection catalysed by adenylate cyclase,associated with the inner face of the plasma membrane. Cyclic AMP serves regulatory functions in virtually every cell outside the plant world. Guanosine 3', 5' – cyclic monophosphate (cGMP) occurs in many cells and also has regulatory functions.

8. Describe the comparison between DNA and RNA.

DNA	RNA
Found mainly in the chromatin of the cell nucleus.	Most of RNA (90%) is present in the cell. Cytoplasm and a little (10%) in free state.
Never present in free state in cytoplasm	May be present in free state
Normally double stranded and rarely single stranded.	Normally single stranded and rarely double stranded.
DNA has both sense and antisense strands.	The sequence of an RNA molecules is the same as that of the antisense strand.
Sugars in DNA are in the C_2 endoform.	Sugars in RNA are in the C_3 endoform.
The common nitrogenous bases are adenine, guanine, cytosine and thyamine (but not uracil)	The common nitrogenous bases are adenine, guanine, cytosine and uracil (but not thymine)
Base pairing involves the entire length of DNA molecules.	Base pairs may takes place only in the helical regions of RNA.
DNA contains only few unusual bases.	RNA contain more un-usual bases.
DNA is alkali stable.	RNA is alkali labile.
DNA undergoes mutation.	It does not undergo.
DNA is the usual genetic material.	RNA I the genetic material of some viruses only.
DNA stains blue with azurepth thalate.	It stains red with azureph thalate.
DNA acts as a template for its synthesis.	It does not acts as template for its synthesis.
It is stained green with a dye, pyronin.	It is stained red with pyronin
DNA on replication forms DNA and on transcription forms RNA.	Usually RNA does not replicate or transcribe.

9. **Describe the components of nucleic acid and give the structure.**

The nucleic acids are the molecules mostly locate in the nuclei of cells and few of them are present in cytoplasm. The nucleic acids are the hereditary determinants of living organisms. They are the macromolecules present in most living cells either in the free state or bound to proteins as nucleoproteins. As regards their elemental composition, the nucleic acids contain carbon, hydrogen, oxygen, nitrogen and strangelyenough, phosphorus; percentage of the last two elements being about 15 and 10, respectively.

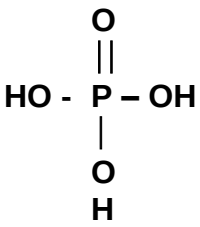
Types:

There are two kinds of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both types of nucleic acids are present in all plants and animals. Viruses also contain nucleic acids; however, unlike a plant or animal, a virus has either RNA or DNA , but not both.

The previously held view, that the DNA occurred only in animals and the RNA only in plants, is now known to be incorrect. DNA is found mainly in the chromatin of the cell nucleus whereas most of the RNA (90%) is present in the cell cytoplasm and a little (10%) in the nucleous. It may be added that extranuclear DNA also exists; it occurs, for example, in mitochondria and chloroplasts. Upon hydrolysis, under different set of conditions, the two nucleic acids yield 3 components; phosphoric acid, a pentose sugar and nitrogenous bases. Following table summarizes the structural components of the two types of nucleic acids, DNA and RNA.

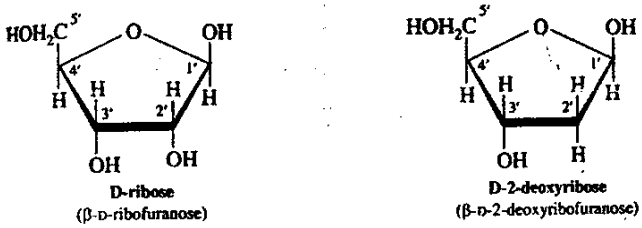
Phosphoric Acid

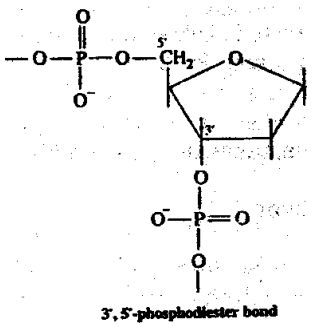
The molecular formula of phosphoric acid is H_3PO_4. It contains 3 monovalent hydroxyl groups and a divalent oxygen atom, all linked to the pentavalent phosphorus atom.



Pentose sugar

The two types of nucleic acids are distinguished primarily on the basis of the 5-carob keto sugar or ;pentose which they possess. One possesses D-2-deoxyribose, hence the name deoxyribose nucleic acid or deoxyribonucleic acid, while the other contains D-ribose, hence the name ribose nucleic acid or ribonucleic acid. Both these sugars in nucleic acids are present in the furanose form and are of β configuration.





A perusal of the structure of the two types of sugars reveals that D-ribose is the parent sugar while D-2-deoxyribose is a derivative in which OH group on C2 has been replaced by an H atom.

The two sugars may be differentiated by means of specific colour reactions. Ribose reacts with orcinol in hydrochloric acid solution containing ferric chloride. Deoxyribose reacts with diphenylamine in acid solution.

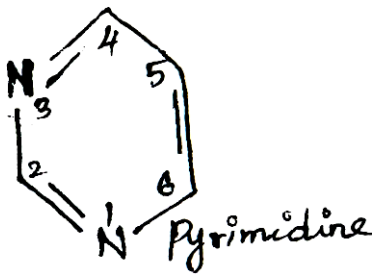
An important property of the pentoses is their capacity to form esters with phosphoric acid. In this reaction the OH groups of the pentose, especially those at C3 and C5 are involved forming a 3', 5'- phosphodiester bond between adjacent pentose residues. This bond, in fact, is an integral par of the structure of nucleic acids.

Nitrogen bases

Two types of nitrogenous bases are found in all nucleic acids. The base is linked to the sugar moity by the same carbon (C1) used in sugar-sugar bonds. The nitrogenous bases are derivatives of pyrimidine and purine. Owing to their π electron clouds, both the pyrimidine and purine bases are planar molecules.

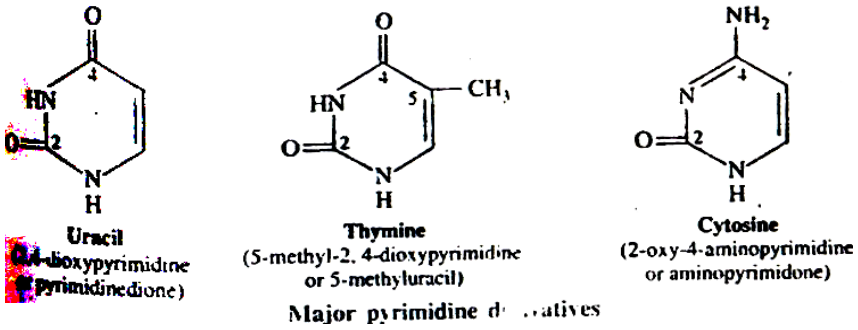
Pyrimidine Derivatives

These are derived from their parent hheterocyclic compound pyrimidine, which contains a six-membered ring with two-nitrogen atoms and three double bonds. It has a melting point of 22 ° C and a boiling point of 123.5 °C.



The common puyrimidne derivatives found in nucleic acids are uracil, thymine and cytosine.

Uracil ($C_4H_4O_2N_2$) found in RNA molecules only, is a white, crystalline pyrimidine base with MW = 112.10 daltons and m.p. 338 °C. Only rarely does uracil occur in DNA.



Uracil
(2, 4-dioxypyrimidine
or pyrimidinedione)

Thymine
(5-methyl-2, 4-dioxypyrimidine
or 5-methyluracil)

Cytosine
(2-oxy-4-aminopyrimidine
or aminopyrimidone)

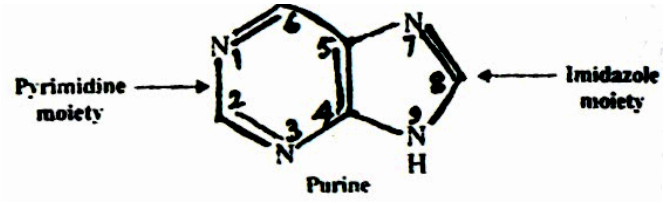
Major pyrimidine d· ··atives

Thymine ($C_5H_6O_2N_2$), found in DNA molecules only, has MW = 126.13 daltons. It was first isolated from thymus, hence so named. Only rarely does thymine occur in RNA.

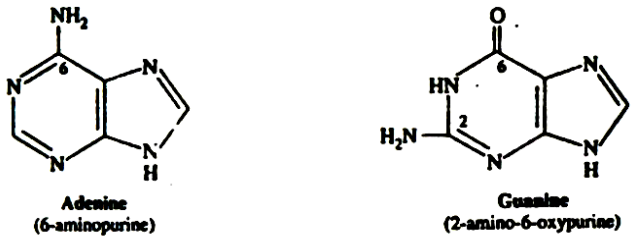
Cytosine ($C_4H_5ON_3$), found in both RNA and DNA, is a white crystalline substance, with MW = 111.12 daltons and a m.p. 320 – 325°C.

Purine Derivatives

These are all derived from their parent compound purine, which contains a six-membered pyrimidine ring fused to the five-membered imidazole ring and is related to uric acid. It has a melting point of 216 0°C.



The prevalent purine derivatives found in nucleic acids are adenine and guanine.



Major purine derivatives

Adenine ($C_5H_5N_5$'), found in both RNA and DNA**,** is a white crystalline purine basee, with MW = 135.15 daltons and a m.p. 360 – 365 °C.

Guanine ($C_5H_5ON_5$), also found in both RNA and DNA, is a colourless, insoluble crystalline substance, with MW = 151.15 daltons. It was first isolated from guano (bird maure), hence so named.

Other purine derivatives are also found in plants. For example, caffeine is present in coffee and tea and theobramine is found in tea and cocoa. Both caffeine and theobromine have important pharmological properties.

10. Describe briefly about saturated and unsaturated fatty acids.

Fatty acids are long-chain organic acids having usuallyfrom 4 to 30 carbon atoms; they have a single carboxyl group and a long, nonpolar hydrocarbon 'tail', which gives most lipids their hydrophobic and oily or greasy nature. Fatty acids do not occur in free or uncombined state in cells or tissues but are present in covalently bound form in different classes of lipids. Fatty acids which occur in natural fats are usually monocarboxylic and contain an even number of C atoms as these are synthesized from 2 carbon units. These are usually straight-chain derivatives. The chain may be saturated (containing only single bonds) or unsaturated (containing one or more double bonds). Some fatty acids may have hydroxyl group(s) in the chain (hydroxyl or oxygenated fatty acids) and still others may possess ring structure.

I. Saturated fatty acids

The general formula for these acids is CnH2n+1COOH. The following table shows some even-numbered straight chain saturated fatty acids, found distributed in both plant and animal worlds. In addition, lipids from all sources contain small amounts of saturated fatty acids with an odd number of carbon atoms (C 5 through C 17). Generally, these odd-carbon acids account for less than 1% of the total fatty acids.

In animal fats, palmitic and stearic acids (C16 and C18) are the most abundantly found saturated fatty acids, next in order are shorter chain fatty acids (C14 and C12) and longer chain fatty acids (C20, C22 and C24). Fatty acids of 10 carbon atoms or less are present in limited amounts in animal lipids except the milk fat which contains appreciable amounts of lower molecular weight fatty acids. The fpreponderance of these acids may thus, be shown in the descending order as below:

C16, C18 > C14, C12, C20, C22, C24 >C10 and less.

In addition to the straight chain fatty acids, a number of branched chain fatty acids having either an even or an odd number of carbon atoms have been denitrified as minor components of natural fats and oils.

Straight chain saturated fatty acids, commonly found in natural fats

Trivial Name	***Systematic name****	***Carbon skeleton***	***Structure†***	**Common source**
Butyric	n-Butanoic	4:0	$CH_3(CH_2)_2COOH$	Butter
Caproic	n-Hexanoic	6:0	$CH_3(CH_2)_4COOH$	Coconut and palm oils
Caprylic	n-Octanoic	8 : 0	$CH_3(CH_2)_6COOH$	Coconut and palm oils
Capric	n-Decanoic	10:0	$CH_3(CH_2)_8COOH$	Coconut and palm oils
Lauric (laurus^L = laurel plant)	n-Dedecanoic	12:0	$CH_3(CH_2)_{10}COOH$	Laurel oil, Spermaceti
Myristic (Myristica^L = nutmeg genus)	n-Tetradecanoic	14:0	$CH_3(CH_2)_{12}COOH$	Butter and wool fats
Palmitic (Palma^G = palm tree)	n-Hexadecanoic	16:0	$CH_3(CH_2)_{14}COOH$	Animal and plant fats
Stearic (Stear = hard fat)	n-Octadecanoic	18:0	$CH_3(CH_2)_{16}COOH$	Animal and plant fats
Arachidic (Arachis^L= legume genus)	n-Eicosanoic	20:0	$CH_3(CH_2)_{18}COOH$	Groundnut oil
Behenic	n-Docosanoic	22:0	$CH_3(CH_2)_{20}COOH$	Groundnut oil
Lignoceric	n-Tetracosanoic	24:0	$CH_3(CH_2)_{22}COOH$	Groundnut oil

(lignum^{L} = wood: cera^{L}= wax)				
Cerotic	n-Hexacosanoic	26:0	$CH_3(CH_2)_{24}COOH$	Wool fat
Montanic	n-Octacosanoic	28:0	$CH_3(CH_2)_{26}COOH$	–

II. Unsaturated fatty acids

These may be classified, based on the degree of unsaturation.

A. Monoethenoid acids – These contain one double bond and conform to the general formula,

CnH2n-1COOH. E.g. Oleic acid.

B. Diethenoid acids – The double bonds;

CnH2n-3COOH; E.G. Linoleic acid.

C. Triethenoid acids – Three double bonds;

CnH2n-5COOH; E.G. Linolenic acid.

D. Tetraethanoid acids – Four double bonds;

CnH2n-7COOH; E.G. Arachidonic acid

Monoethanoid acids are commonly called as monounsaturated fatty acids (MUFAs) and the remaining ones as polyunsaturated fatty acids (PUFAs). A few important unsaturated acids are listed in Table.

Trivial Name	Systematic name	Carbon skeleton	Structure	Commone source
Crotonie	2 – butenoie	4:1:2	$CH_3CH=CHCOOH$	Croton oil
Myrishto	9tetradecenoie	14:1:9	$CH_4(CH_2)_3CH=CH(CH_2)_7$ COOH	Pyenanthys
Palnitoleic	9 – hexadecenoic	16:1:9	$CH_3(CH_2)_5CH=CH(CH_2)_7$ COOH	Animal and plant fats
Oteic (olium^{G} = oil)	9 – octadecenoic	18:1:9	$CH_3(CH_2)_7CH=CH(CH_2)_7$ COOH	Animal and plant fats
Vaccenie	11-Octadecenoic	18:1:11	$CH_3(CH_2)_5CH=CH(CH_2)_9$ COOH	Bacterial fat
Linoteic (linon^{G} = flax)	9,12–octadecadienoic	18:2:9,12	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_3$ COOH	Linseed and cotton seed oils
Eleostearic	9,11,13–octadecadienoic	18:3:9,11,13	$CH_3(CH_2)_3CH=CH-CH=CH-CH=CH(CH_2)_7$ COOH	Tung oil
Linolenic	9,12,15–octadecadienoic	18:3:9,12,15	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7$ COOH	Animal fat
Nervonic	15 – tetracosenoic	24:1;15	$CH_3(CH_2)_7CH = CH(CH_2)_{13}COOH$	

Human body can convert stearic acid to oleic acid by inserting a double bond but is incapable of inserting further double bonds so that the oleic acid cannot be converted to either linoleic, linolenic or arachidonic acid. For normal cell functioning esp., of skin tissues any one of these acids is needed. Since they cannot be synthesized by the cells, they must be obtained from diet. On account of the important physiological rle, these 3 acids are collectively called as essential fatty acids (EFA), a term introduced by Burr and Burr in 1930.

Vegetable oils contain two types of ;polyunsaturated fatty acids (PUFAs)- linoleic acid and α-linolenic acid. In chemical jargon, line and longer chain fatty acids derived from it are referred to as n-6 fatty acids. Since the first double bond in their nikecyke iccyrs ib carvib bi. 3.Len and fatty acids derived from it are ref erred to as n-3 fatty acids since the first double bond in their molecule occurs on carbon no.3.

A most unusual unsaturated fatty acid, nemotinic acid, is excreted in the growth medium by a citrivorium mould. This fatty acid is unque in that it contains the single, double and triple C-C linkages. Nemotinic acid is one of the few naturally – occurring compound containing the allene.

The acetylene group has been detected in a number of unsaturated fatty acids, found in khigher plants and microorganisms. For instance, santalbic acid, a mahor component of the seed oil of sandlewood contains 1 acetylene group as against 2 of nemotinic acid.

Geometric isomerism: On account of the fpresence of double bond(s), the unsaturated fatty acids exhibit geometric (or cis-trans) isomerism. Most unsaturated fatty acids are found as the unstable cis isomer rather than as the more stable trans isomer.

The hydrocarbon chain of saturated fatty acids (stearic acid, for example) has a zigzag configuration with C-C bond forming a bond angle of 109°.

For the sake of simplicity, the long chains of CH_2 groups are represented by zigzag lines where each corner represents a C atom and the fhydrogen atoms are left out. The zigzag lline represents the most stable configuration of such carbon chains. The free end of this line, if not indicated otherwise, obviously represents the presence of methyl group. The simplified formula for stearic acid would, thus, be as represented in the following figure.

The double bond in the zigzag line is indicated by drawing an extra line in between the carbon atoms involved in double bond formation.

When ac is double lbond is inserted, the molecule bends assuming the shape, shown the following figure. This double lbond is rigid and creates a kink in the chain. The rest of the chain is free to rotate about the other C-C bonds.

Non conjugated double-bond system; Another structural peculiarity of naturally occurring polyunsaturated fatty acids is the fpresence of a nonconjugated double-bond system. It has a methylene group (-CH2-) flanked by double bonds on both the sides as in linoleic, linoenic and arachidonic acids. The conjugated double-bonded system is, however, rarely present. In it the methylene group is not found in between the double lbonds which, henceforth, occur one after the fother as in eleostearic acid. This acid has valuable properties as a drying oil since it polymerizes

readily. These two double-bond systems have different chemical reactivities which is shown in the following figure.

11. Explain Carbohydrate and its classification.

Carbohydrates are a class of compounds mostly were hydrates of carbon or represented by a general formula as $Cx(H_2O)y$. Some kinds of sugars like deoxyribose and rhamnose do not have the required ratio of hydrogen to oxygen. Certain other carbohydrates are now known to posses nitrogen, phosphorus or sulfur also obviously do not coincide with the above general formula.

Furthermore, formaldehyde, acetic acid and lactic acid which have C, H and O and the ratio of H: O is also the same as in water, but are not a carbohydrates.

To accommodate a wide variety of compounds, the carbohydrates are nowadays broadly defined as polyhdroxy aldehydes or ketones and their derivatives or as substances that yield one of these compounds on hydrolysis.

Classification:

Carbohydrates are usually classified into 3 groups which are discussed below in detail.

Manosacharides or Monosacharoses:

The monosacharides may be subdivided into troses, tetroses, pentoses, hexoses, heptoses etc., depending upon the number of carbon atoms they posses; and as aldoses or ketoses, depending upon whether they contain aldehyde or ketone group. Some important examples are;

Name	**Formula**	**Aldoses (aldo sugar)**	**Ketoses (Keto sugar)**
Trioses	$C_3H_6O_3$	Glycerose	Dihydroxyacetone
Tetroses	$C_4H_8O_4$	Erythrose	Erythrulose
Pentoses	$C_5H_{10}O_5$	Ribose	Ribulose
Hexoses	$C_6H_{12}O_6$	Glucose	Fructose
Heptoses	$C_7H_{14}O_7$	Glucoheptose	Sodoheptulose

Both these characters may also be combined ito one, Thus, for example, glycerose is an aldotriose,; ribulose, a ketopentose and glucose, an aldohexose. It is noteworthy that, except fructose, ketoses are not as common as aldoses. The most abundant monosaccharide in nature is the 6-carbon sugar, D-glucose.

Sometimes, a distinction in naming between aldoses and ketoses is also maintained. The suffix –oses is kept reserved for the aldoses and suffix –uloses is used for ketoses. Thus glucose is a hexoses and fructose, a hexulose. However, a few ketoses are named otherwise, such as fructose as fruits are a good suource of this sugar.

Oligosaccharides or Oligosaccharoses:

These are compound sugars that yield 2 to 10 molecules of the same or different monosaccharides on hydrolysis. Accordingly, an oligosaccharide yielding 3 molecules of monsacchrides on hydrolysis is designated as a disaccharide, and the one yielding 3 molecules of monosaccharide as a trosaccharide and so on. The general formula of disaccharides is $C_n(H_2O)n-1$ and that of trisacchrides is $Cn(H_2O)n-2$ and so on. A few examples are;

Disaccharides – Sucrose, Lactose, Maltose, Cellobiose, Trefhalose, Gentiobiose, Melibiose
Trisacchrides – Rhamnoses, Gemtoampse. Raffompse. Rabinose, Melezitose
Tetrasacharides – Stachyose, Scorodose
Pentasaccharide – Verbascose

The molecular compostition of the 3 legume oligosaccharides is shown below;

α-Galactose (1-6) α-Glucose (1-2) β-Fructose (Raffinose)
α-Galactose (1-6) α-Galactose (1-6) α-Glucose (1-2) β-Fructose (Stachyose)
α-Galactose (1-6) α- Galactose (1-6) α- Galactose (1-6) α-Glucose (1-2) β-Fructose (Verbascose)

Polysaccharides or Polysaccharoses

These are also compound sugars and yield more than 10 molecules of monosaccharides on hydrolysis. These may be further classified depending on whether the monosaccharide molecules prodluced as a result of the hydrolysis of polysaccharides are of the same type (homopolysacchrides) or different types (heteropolysacchrides). Their general formula is $(C_6H_{10}O_5)_x$. Some common examples are:

Homopolysaccharides – Starch, Glycogen, Inulin, Ceullulose, Pectin, Chitin.

Heteropolysaccharides – Specific soluble sugar of Pneumococcus type III, Hyaluronic acid, Chondrotin.

12. Define polysaccharide. Explain any two polysaccharides in detail.

Poysaccharides (variously called as glycanes or polyhonosides or polyosides) are high molecular weight carbohydrates which, on hydrolysis, yield mainly monsaccharides or products related to monsaccharides. They may also be regarded as polymeric anhydrides of simple sugars. D-glucose is is the commonest component of polysaccharides.

The great majority of carbohydrares of nature foccur as polysaccharies. Chemically, the polysacchrides may be distinguished into homopolysaccharides (or homohlycans) which yield, on hydrolyisis, a single monosaccharide and heteropolysaccharides (or heteroglycans), which produce a mixture of monosaccharides on hydrolysis.

Homopoysaccharides

These yield, on hydrolysis, a single monosaccharide. They serve as both storage (Starch, glycogen) and structural (Cellulose, pectin, chitin) polysaccharides.

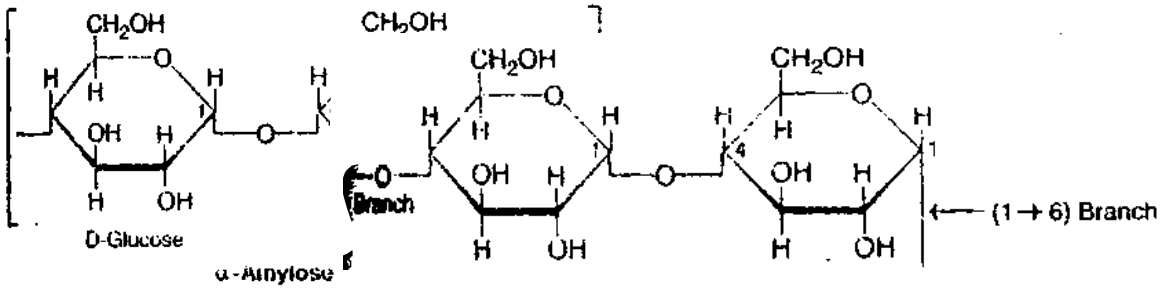
1. Starch

Occurrence: Starch is a homopolysaccharide. It is the most important reserve food material of the higher plants and is found in cereals, legumes, potatoes, Tapioca and other vegetables. More than half the carbohydrate ingested by humans is starch. It is usually present inside the plant cells as compact insoluble granules which may be spherical, lens-shaped or ovoid and which have distinctly layered structure.

Chemistry: Natural starches consist of two components as amylase (15 – 20%) a long unbranched straight-chain component and amylopectin (80 -85%), a branched chain polysaccharide. Starch from waxy corn is notable as it consists practically of amylopectin component, there being no amylose. Alpha amylase or simply amylase has a molecular weight range of 10,000 to 50,000. It may be formed in plant cells by elimination of a molecule of water ffrom glycosidic OH group of one α-D-glucose molecule and alcoholic OH group on carbon 4 of the adjacent α-D-glucose molecule. The linkage in amylase is, thus an α-2,4-glucoside, like that in maltose. Enzymatic hydrolysis of amylase with amylase, henceforth, yields maltose units mainly. Amylase may considered as an anhydride of α-D-glucose units.

B-amylose or isoamylose or amylopectin:

It has a high molecular weight range of 50,000 to 1,000,000, thus indicating the fpresence of 300-5,500 glucose units per molecule. This possesses the same basic chain of α-1,4-gllucoside linkage like that of amylase but has, in addition, many side chains attached to the basic chain by α-1.6-glucoside linkages, similar to those in isomaltose. It may, thus, be seen that the glusoe unit, present at each point of branching, has substituents not only on carob atoms 1 and 4 but also on carob atom 6. Inotherwords, these glucose units have 3 points of attachment to serve as branching points. The average chain length is about 24 gllucose units. Amylopectin, upon incomplete hydrlysis, yields the disaccharide isomaltose.



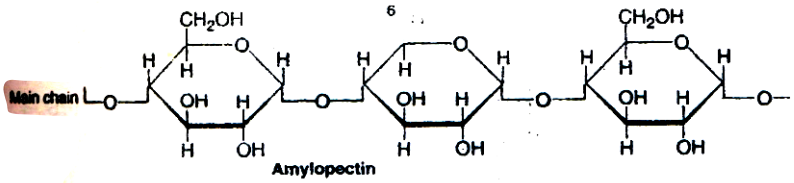


Figure: Structure of starch (α - amylase and amylopectin)

Properties:

Starch is a white soft amorphous powder and lacks sweetness. It is indouble in water, alcohol and ether at ordinary temperature. The specific rotation of Starch is +196o. The microscopic form of the starch grains characteristic of the source of starch. Starch, on partial hydrolysis by boiling with waater under pressure at about 250o C, breaks down into large fragments called dextrins. The resulting dextrins then confer stiffness to clothes that have been starched and ironed. Starch molecule is highly hydrated since it contains many exposed hydroxyl groups. With the result, starch, when extracted from granules with hot water, form turbid colloidal solutions. In common with other polysaccharides, starch is a nonreducing carabohydrate since carbonyl groups of all units participate in the glycosidic linkages.

Hydrolysis

The enzyme α-amylase, found in the digestive tract of animals (in saliva and pancreatic juice) hydrolyses the linear amylase chain by attacking α (1-> 4) linkages at random throughout the chain to produce a mixture of maltose and glucose. B-amylase, an enzyme found in plants, attacks the nonreducing end of amylase to yield successive units of maltose. Amylospectin can also be attacked by α- and β- amylases, but a separate enzymes called α (1->6) gllucosidae can hydrolyze the bod at the branch point.

2. Glycogen

Glycogen is also a homopolysacchride. It is the mahor reserve food in animals and is often called animal starch. Glycogen is stored in the lilver and muscles of animals. It is especially abundant in the liver where it may attain up to 7% of the wet weight. It occurs in animal cells as particles much smaller than the starch grains.

Chemistry

Glycogen is a branched chain polysaccharide and resembles amylopectin very much in structure, rather than amylase, but has somewhat more glucose residues per molecule and about one and a half times as many branching points. Also the chains are shorter (10-20 glucose units) and hence the molecule is even more highly branched and more compact. These differences, however, do not alter the functional beaviour of the fmolecule to any significant extent. The molecular weight is 1-2 X $10^{7.}$

Properties

It is a white powder and is more soluble in water than amylopectin. Hence, it readily forms suspension even in cold water. Glycogen is precipitated from aqueous solutions b7 addition of ethyl alcohol and is fairly stable in hot alkali. It is a nonreducing sugar and gives red colur with iodine. The red colur, however, disappears on boiling and reappers on cooling.

Hydrolysis

On incomplete hydrolysis with α-1, 4-gluconmaltohydrolase, glycogen yields maltose. However, the acids completely hydrolyze it into glucose.

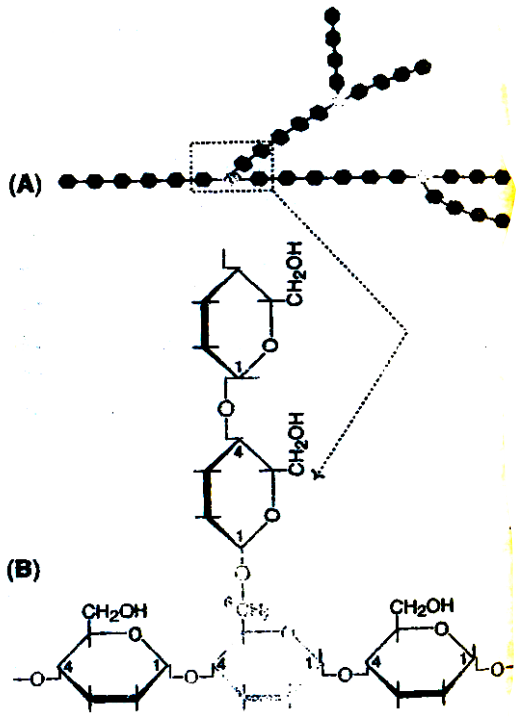


Figure: Structure of glycogen (A) General structure (B) Enlarged at a branch point

13. What is hetero poysacharide? Describe with suitable examples.

These yieid, on hydrolysis, a mixture of monosaccharides. They are numerous in both plants & animals.

The specific soluble sugar of pneumococcus type III is one of the simplest heteropolysaccharide. It contains repeating units of a mixed disaccharide consisting of glucose & glucuronic acid. Apparently, on hydrolysis, it yields equi molar amount of glucose & glucuronic acid.

Mucopolysaccharide

Polysaccharide that are composed not only of a mixture of simple sugars but also of derivatives of sugars such as amino acid & uronic sugars are called Mucopolysaccharide. They are gelatinous substance of high molecular wt. Most of these act as structural support material for connective tissues or mucous substance of a body. They serve both as a lubricant & a cementing substance.

Structurally, they have a common feature. They consist of disaccharideunits in which a uronic acid is bound by a glycosidic bond to the C3 of an acetylated aminoacid. These disaccharide residues are polymerized by 1→4 linkage to give a linear macro molecule. The uronic & sulphuric acid residues impart a strong acidic character to these substances.

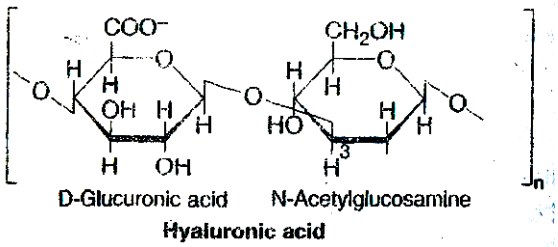
Hyaluronic acid

Occurrence

It is the most abundant member of mucopolysaccharides & is found in higher animals as a component of various tissues such as the vitreous body of the eye, the umbilical cord & the synovial fluid of joints. The high viscosity of the synovial fluid & its role as biological lubricant is largely due to the presence of its hyaluronic acid content.

Chemistry

Hyaluronic acid has the least complicated structure among mucopolysaccharide. It is a straight chain polymer of D-glucuronic acid & N-acetyl D-glucosamine alternating in the chain. Its mol. Wt. approaches approximately 5000000. Here apparently 2 linkages are involved, β-1→3& β-1→4. Hyaluronic acid is an acidic substance, because the carboxyl groups are largely ionized at celluloid pH.



Hyaluronic acid

Hydrolysis

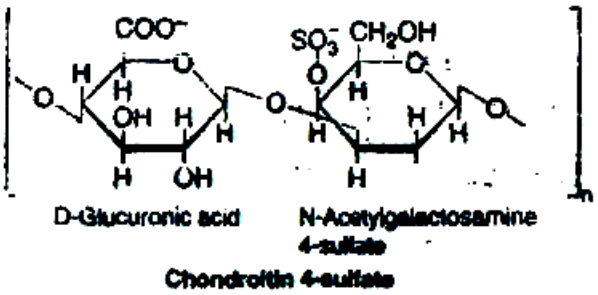
Hyaluronic acid, upon hydrolysis, yields an equimolar mixture of D-glucuronic acid, d-glucosamine &acetic acid.

It is noteworthy that hyaluronic acid swiftly by the enzyme hyaluranidase. The enzyme brings about depolymerization of hyaluronic acid & cleavage to smaller fragments. Hyaluranidase is the spreading factor of skin & connective tissue. The depolymerization effect allows any foreign bodies to penetrate tissue, since the cementing substance is being dissolved. The enzyme also has a physiologic role in fertilization. The sperm is rich in hyaluranidase & hence can advanced better in the cervical canal & finally fertilise the ovum.

Chondroitin

Occurrence

Chondroitin is of limited distribution. it is found in cartilage and is also a component of cell codes. it is parent substance for two more widely distributed muco polysacchrides chondroitin sulphates n chondrotin sulphates B.chondrotin is similar in structure to hyaralunic acid except it contains galactose amine .on hydrolysis chondrotin produces equal mixture of D glucoronic acid , D galactose amine and acetic acid



14. Define amino acids? Write the classification of amino acids.

Amino acids are referred as building blocks of proteins, because amino acids are monomers which are join together to produce polymers which are proteins.

The general formula of amino acid is as given below;

R-CH-COOH
|
NH_2

Each amino acid is a nitrogenous compound having both an acidic carboxyl (-COOH) and a basic amino (-NH_2) group. R stands for the side chains that are different for each amino acid. R can be as simple as a hydrogen atom (H) or a methyl group (-CH_3) or a more complex structure.

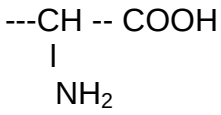
Classification

Generally three kinds of classification systems of amino acids are in use. The following text will deal the details of above 3 systems of classification.

On the basis of the composition of the side chain or R group

There are 20 different amino acids which regularly appear in proteins. These posses a side chain which is the only variable feature present in their molecules. The other features such as α-carbon, carboxyl group and amono group are common to all the amino acids. The common component of an amino acid appears as the following;

α



Based on the compostition of the side chain, the twenty amino acids, whose structure is shown in the above figure which may be grouped into the following 8 categories (Fairley and Kilgour, 1966).

i. Simple amino acids: These have no functional group in the side chain e.g. glycine, alanine, valine, leucine and isoleucine.

ii. Hydroxy amino acids: These contain a hydroxyl group in their side chain, e.g. serine, and threonine.

iii. Sulfur-containing amino acids: These possess a sulfur atom in the side chain, e.g. Cystein and methionine.

iv. Acidic amino acids: These have a carboxyl group in the side chain, e.g. aspartic acid and glutamic acid.

v. Amino acid amides: These are derivatives of acidic amino acids in which one of the carboxyly group has been transformed into an amide group ((-$CO.NH_2$) e.g. asparagines and glutamine.

vi. Basic amino acids: These possess an amino group in the side chain, e.g. lysine and arginine.

vii. Heterocyclic amino acids: These amino acids have in their side chain a ring which possesses at least one atom other than the carbon, e.g., tryptophan, histidine and praline.

viii. Aromatic amino acids: These have a benzene ring in the side chain, e.g., phenylalanine and tyrosine.

The classification given above is only a practical one and can conveniently be followed. It does not, however, strictly delimit the various categrories. For example, tryptophan may also be included under aromatic amino acids and similarly, lhistidine under basic amino acids.

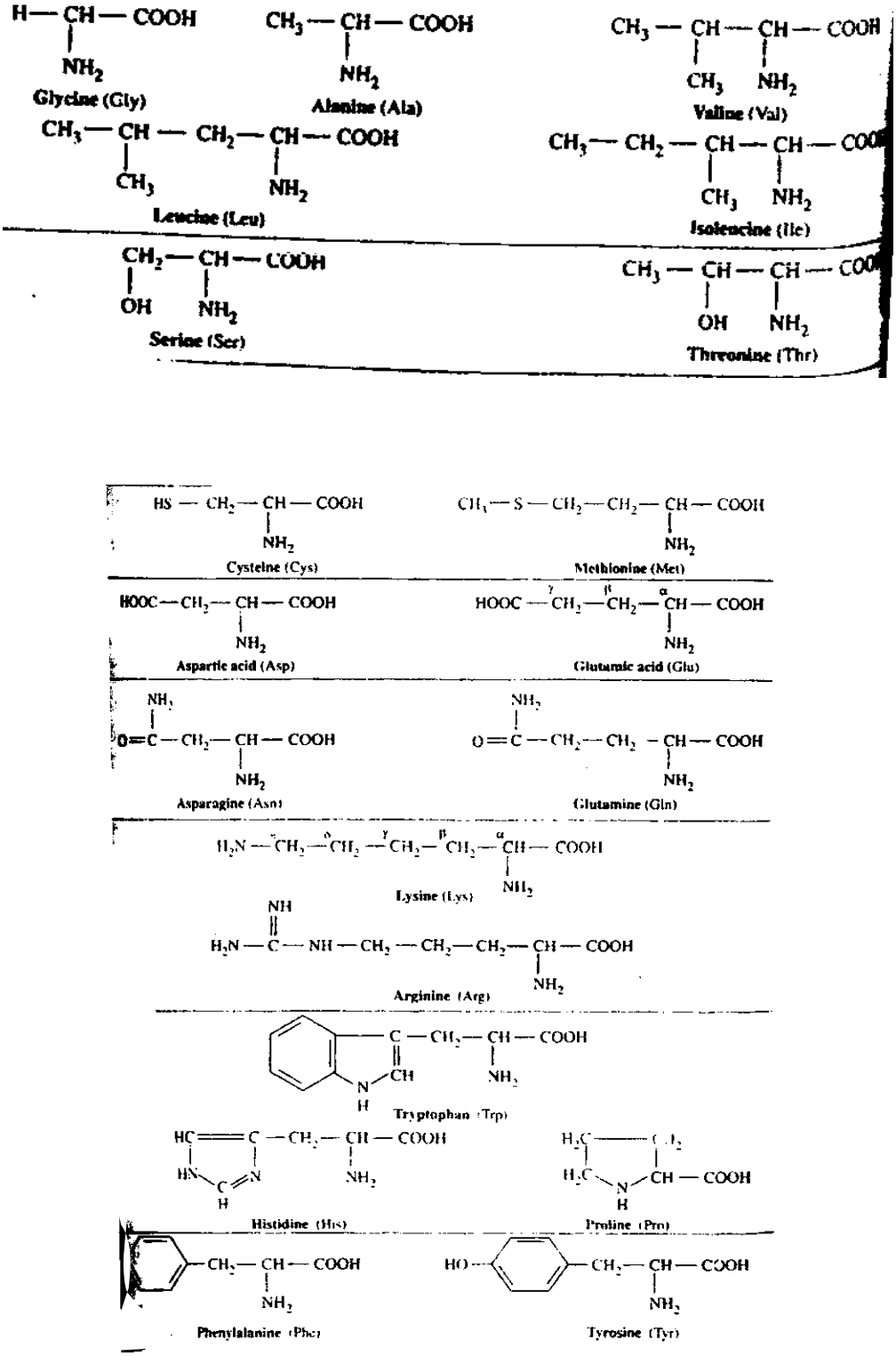


Figure: The twenty amino acids (“Magic 20”) found in proteins

The standard three – letter abbreviation for each amino acid is written in bracket. The common component is shown in a blue enclosure.

On the Basis of the number of amino and carboxylic groups: McGilvery and Goldstein (1979) have classified various amino acids as follows:

I. Monoamino-monocarboxylic amino acids:

1. Unsubstituted – Glycine, Alanine, Valine, Leucine, Isoleucine
2. Heteerocyclic – Proline
3. Aromatic – Phenylalanine, Tyrosine, Tryptophan
4. Thioether – Methionine
5. Hydroxy – Serine, Threonine
6. Mercapto – Cysteine
7. Carboxamide – Asparagine, Glutamine

II. Monoamino-dicarboxylic amino acids – Aspartic acid, Glutamic acid

III. Diamino-monocarboxylilc amino acids: lysine, Arginine, Histidine

On the basis of polarity of the side chain or R group:

A more meaningful classification of amino acids is , however, based on the polarity of the fR groups present in their molecules. i.e., their tendency to interact with water at biological pH (near pH 7.0). The R groups of the amino acids vary widely with respect to their polarity from totally nonpolar or hydrophobic (water-hating) R groups to highly polar or hydrophilic (water-loving) R groups. This classification amino acids emphasizes the fpossible functional roles which they perform in proteins.

15. Explain amino acid classification based on polarity of the side chain.

A more meaningful classification of amino acids is However based on the polarity of R groups present in their molecules i. e., their tendency to interact with water at biological pH. The R groups of aminoacids vary widely with respect to polarity from totally non polar or hydrophobic R groups to highly polar or hydrophilic R groups.
The 4 categories of amino acids are,

Aminoacids with non polar R groups

They are hydrocarbon and hydrophobic in nature. This group includes 5 amino acids with aliphatic R groups(alanine, valine, leucine, isoleucine, proline), Two with aromatic rings (phenyl alanine,tryptophan)and one containing sulphur (methionine).

Alanine

It is the parent substance of all aminoacids except glycine. The various amino acids may be derived from alanine by replacement of one or two H atoms of the methyl group present on α carbon atom. Alanine is the least hydrophobic of the 8 polar amino acids because of its small methyl side chain.

Valine

It is the branched amino acid and can be derived from alanine by the introduction of two methyl groups in the place of two H atoms of the methyl group present on α carbon atom.

Leucine

It is also branched chain amino acids and is the next higher homologue of valine. It is sparingly soluble in water.

Isoleucine

It is isomer of leucine and is also a branched chain aminoacid. It has two asymmetric carbon atoms and thus occurs in four stereometric forms.

Valine, leucine and isoleucine are chemically alike and posses the branched carbon chain. They are essential amino acids as they cant be synthesized inside the body.

Proline

It is a cyclised derivative of glutamic acid. Its α amino group is not free but is substituted by a portion of its R group to yield a cyclic structure. Unlike other amino acids , proline is very soluble in ethanol.

Phenyl alanine

It's a benzoid amino acid. It resembles tyrosine in structure. It cant be synthesised in animals, because of its aromatic ring.

Tryptophan

It is a complex amino acid found in proteins. Although wide spread, it is usually limited in quantity. Tryptophan is the only aminoacid of proteins which is nearly, completely destroyed upon acid hydrolysis.

Methionine

It is a common amino acid possessing ether linkage. Cereals have sufficient quantity to methionine where pulse is lack in it. It is a donor of active methyl groups.

I. Amino acida with polar but uncharged R groups:

The R groups of these amino acids are more soluble in water i.e., more hydrophilic than those of the non polar amino acids because they contain functional groups that form hydrogen bonds with water. This category includes 7 amino acids viz ., glycine , serine, threonine , tyrosine, cysteine, asparagines, glutamine. The polarity of these amino acids may be due to either a hydroxyl group (serine, threonine, tyrosine) or a sulfhydryl group (cysteine) or an amide group (asparagine, glutamine).

Glycine or glycocol:

It is a simple amino acid lacking asymmetric α carbon atom. It is optically inactive. Gycine is abundant in sclero proteins.

Serine

It is found in all proteins. It contains hydroxyl group which participates in ester formation. The hydroxyl group on serine makes it much more hydrophilic and reactive than alanine and valine.

Theonine

It has two asymmetric carbon atoms and thus occurs in four stereomertic forms, namely D and L threonine and D and L allothreonine. The unnatural form of threoninie is allothreonine.

Tyrosine

It is an aromatic amino acid normally found as component of proteins. The phenolic group of tyrosine is weakly acidic and loses its proton above pH 9. This is sparingly soluble in water. It is destroyed during acid hydrolysis of proteins.

Cysteine

It contains SH groups which is reactive and esp., easily dehydrogenated. When it is dehydrogenated (oxidized), two molecules join to form the amino acid cysteine. Fibropus proteins such as keratin from hair are especially rich in cysteine.

Asparagine

This is the β amide of aspartic acid and has been isolated from proteins after enzymic hydrolysis. it is a constituent of plant tissues.

Glutamine

It is a homologous of asparagine. This is the γ-amide of glutamic acid and has been isolated from proteins after enzymic hydrolysis.

Amino acids of categories I and II are jointly referred to as neutral amino acids because each one of them contain ne acidic and one basic group.

III. Amino acids with negatively charged (= acidic) R groups:

These are monoamino dicarboxylic acids. In other words, their side chain contains an extra carboxyl group with a dissociable proton. The resulting additional negative charge accounts for the electrochemical behaviour of proteins. The two amino acids which belong to this category are aspartic and glutamic acid.

IV. Amino acids with negatively charged (= acidic) R groups:

These are diamino-monocarboxylic acids. In other words, their side chain contains an extra amino group which imparts basic properties to them. Lysine, arginine and histidine belong to this category.

1. Discuss in detail on classification on proteins.

The modern day system of classification is based on the fproposals made by the committees of British physiological Society (1907) and the American Physiological Society (1908). This is widely accepted and used system of classification of proteins. This system divides the proteins into 3 major groups, based on their composition viz., simple, conjucated and derived.

I. Simple Proeins or Holoproteins

These are of globular type except for scleroproteins which are fibrous in nature. This group includes proteins containing only amono acids, as structural components. On decomposition with acids, these liberate the constituent amino acids.

These are further classified mainly on their solubility basis as follows:

Protamines and histones: These are basic proteins and occur almost entirely in animals, mainly in sperm cells; possess simplest structure and lowest molecular weight (approximately 5,000); soluble in water; unlike most other proteins, not coagulated by heat.; strongly basic in character owing to high content of basic amino acids (lysine, arginine); form salts with mineral acids and nucleic proteins. Protamines are virtually devoid of sulfur and aromatic amino acids. Histones are somewhat weaker bases and are, therefore, insoluble in NH_4OH solution, whereas protamines are soluble.

Albumins: These are widely distributed in nature but more abundant in seeds; soluble in water and dilute solutions of acids, bases and salts; precipitated with a saturated solution of an acid salt like $(NH_4)_2.SO_4$ or a neutral salt like $Na_2.SO_4$; coagulated by heat/. E.g., leucine in cereals, legumeline in legumes, ovalbumin from white of egg, serum albumin from blood plasma, myosin of muscles and lactalumin of milk whey.

Globulilns: These are two types – pseudoglobulins and euglobulins. Euglobulins are more widely distributed in nature than the fpseudoglobulins; either soluble or insoluble in water; precipitated with half saturated solution of $(NH_4)_2.SO_4$; coagulated by heat. E.g., pseudoglobulins – pseudoglobulin of milk whey. Euglobulins – serum globulin from blood plasma.

Glutelins: These have been isolated only from plant seeds; insoluble in water, dilute salt solutions and alcohol solutions but soluble in dilute acids and alkalies; coagulated by heat.

Prolamines: These have also been isolated only from plant seedsl insoluble in water and dilute salt solutions but soluble in dilute acids and alkalies and also in 60-80% alcohol solutions; not coagulated by heat. E.g., gliadin from wheat zein from corn, hordein from oat, etc..,

Scleroproteins or Albuminoids: These occur almost entirely in animals and are, therefore commonly known as the animal akeleton proteins; insoluble in water, dilute solution of acids, bases and salts and also in 60 – 80% alcohol solutions not attacked by enzymes. E.g., collagen of bones, elastin in ligaments, keratin in hair and horry tissues and fibroin of silk.

II. Conjugated or Complex Proteins or Heteroproteins

These are the fproteins linked with a separable nonprotein portion called prosthetic group. He prosthetic group may be either a metal or a compound. On decomposition with acids, these liberate the constituent amino acids as well as the fprothetic group.

The various inds of complex proteins are metallo proteins, chromoproteins, glycoproteins, phosphoproteins, lipoproteins and nucleoproteins.

Metalloproteins: These are the fproteins linked with various metals. These may be of stable nature or may be more or less labile. Based on their reactivity with metal ions, the metalloproteins may be classified into 3 groups;

i. Metals strongly bound by proteins
ii. Metals bound weakly by proteins
iii.Metals which do not couple with proteins

Chromoproteins: These are proteins coupled with a coloured pigment. Such pigments have also lbeen found among the enzymes like catalase, peroxidase and flavoenzymes. Similarly, chlorophyll is present in leaf cells in the form of ;a protein, the chlororplastin. The chloroplastin dissolves in water as a colloid and is readily denatured. E.g., myoglobin, hemoglobin, cytochromes, flavoproteins etc.,

Glycoproteins and Mucoproteins:

These are the proteins containing carbohydrate as prosthetic group. Glycoproeins contain small amounts of carbohydrates (less than 4%), whereas mucoproteins contain comparatively higher amounts (more than 4%).

e.g., glycoproeins – egg albumin, elastase, certain serum globulins.
e.g., mucoproeins – ovomucoid from egg white, mucin from saliva and Dioscorea tubers.

Phosphoproteins:

These are proteins linked with phosphoric acid; mainly acidic. E.g., casein from milk and ovovitellin from egg yolk.

Lipoproteins:

Proteins forming complexs with lipids (cephalin, lecithin, cholersterol) are called lipoproteins; soluble in water byt insouluble in organic solvents. E.g., lipovitellin, and lipovitellenin from egg yolk; lipoproteins of blood.

There are 4 group of lipoproteins as;

1. Very high density lipoproteins (VHDLs)
2. High density lipoproteins (HDLs)
3. Low density lipoproteins (LDLs) and
4. Very low density lipoproteins 9VDLs)

Nucleoproteins:

These are compounds containing nucleic acid and protein, especially protamines and histones. These are usual the salt-like compounds of proteins since the two components have opposite charges and are bound to each other by electrostatic forces They are present in nuclear substances as well as in the cytoplasm. These may be considered s the sites for the synthesis of proteins and enzymes.

e.g., nucleoproteins from yeast and thymus and also viruses which may be regarded as large molecules of nucleoproteins; nucleohistones from nuclei –rich material like glandular tissues nuclein.

C. Derived Proteins

These are derivatives of proteins resulting from the action of heat, enzymes or chemical reagents. These group also includes the artificially –produced polypeptides.

I. Primary derived proteins:

These are derivatives of proteins in which the size of protein molecule is not altered materially.

Proteans – Insoluble in water; appear as first product produced by the action of acids, enzymes or water on proteins.

e.g., edestan derived from destin and myosin derived from myosin.

Metaproteins or Infraproteins

Insoluble in waterbut soluble in dilute acids or alkalies. E.g., acid and alkali metaproteins.

Coagulated Proteins

Insoluble in water; produced by the action of heat or alcohol on proteins. E.g., coagulated eggwhite.

II. Secondary derived proteins

These are derivatives of proteins in which the hydrolysis has certainly occurred. The molecules are, as a rule, smaller than the original proteins.

Proteoses:

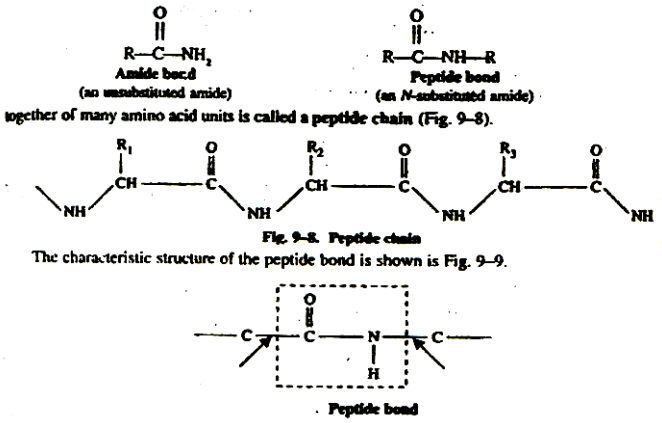
Insoluble in water; coagulate by heat; produced when hydrolysis proceeds beyond the level of metaproteins; primary proteoses are salted out by half saturation with $(NH_4)_2.SO_4$ and precipitated HNO_3 and picric acid. Secondary proteoses are salted out only by complete saturation with $(NH_4)_2.SO_4$, but are not precipitated by HNO_3 or picric acid.

e.g., albumose from albumin; globnulose from globulin.

Peptones: Soluble in water; nocoagulable by heat; produced by the action of dilutic acids or enzymes when hydrolysis proceeds beyond proteoses; neither salted out by $(NH_4)2SO_4$ nor ;precipitated by HNO_3 or picric acid.

Polypeptieds:

These are combination of two or more amino acid units. In fact, the proteins are essentially long chain polypeptides.



together of many amino acid units is called a peptide chain (Fig. 9–8).

Fig. 9–8. Peptide chain

The characteristic structure of the peptide bond is shown is Fig. 9–9.

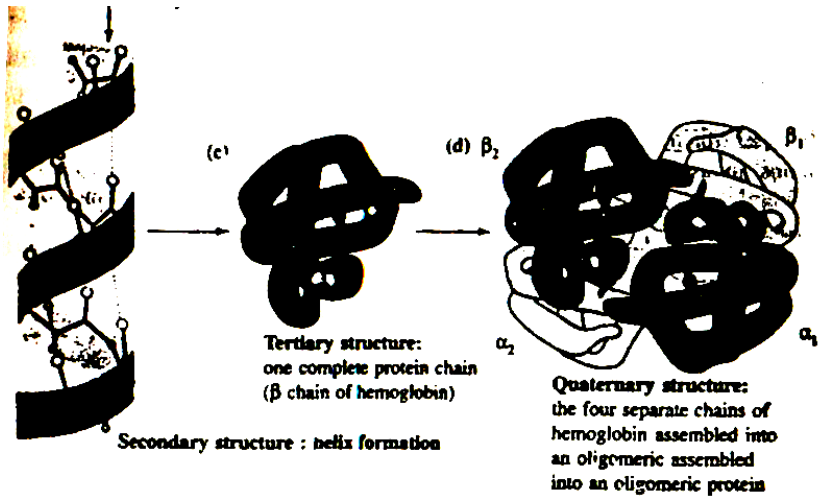
Peptide bond

16. Explain the structure of proteins.

I. Primary structure

The primary structure of protein refers to the number and sequence of amino acid, the constituent units of polypeptide chain. The main mode of linkage of the amino acids in protein is the peptide bond which links the α –carboxly group of one amino acid residue

To the α- amino group of the other. the proteins may contain one or more peptide chains.
(a) Lys – Ala – His - Gly – Lys – Val – Leu – Gly – Ala –
Primary structure: amino acid sequence in a polypeptide chain.



The structural hierarchy in proteins

Rigid and Planar peptide bonds:

Linus Pauling & Robert Corey, in the late 1930's demonstrated that α-carbons of adjacent amino acids are separated by three covalent bonds, arranged Cα-C-N-Cα.They also demonstrated that the amide C-N bond in the peptide is some what shorter.

Ramachandran Plot:

The conformation of the main polypeptide chain can be completely determined if the values Φ & Ψ for each amino acid in the chain are known.

In a fully stretched polypeptide chains Ψ=Φ=180º.G.N Ramachandran recognized that the an amino acid residue in polypeptide chain cannot have just any values of Ψ & Φ .By assuming that atoms behave as hard spheres, allowed ranges of Φ &Ψ can be predicted and visualized in steric contour diagram called **Ramachandran plots.**

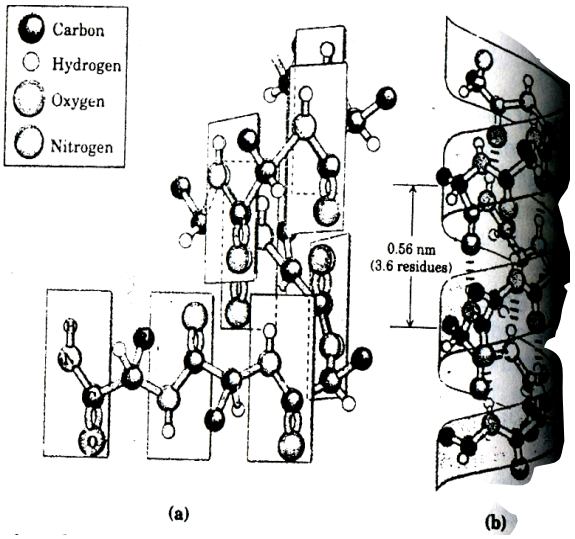
ii. Secondary structure: Helix formation

If the peptide is of only one type of linkage present in proteins behave as irregularly coiled polypeptide chains. this folding of peptide is due to the hydrogen bonds. folding between neighbouring amino acids results in a formation called helix.

Based on the nature of hydrogen bonding the secondary structure is classified into α – helix and β plated sheets.

α-helix

The polypeptide chain with planar peptide bonds would form a right handed helical structure by simple twists about α-carbon to nitrogen and the α –carbon to carboxyl carbon bonds. This is called α-helix .The α=helix is rod like structure. The α helix is stabilized by hydrogen bonds between the NH group and CO group of the main chain.
α –helix occurs in the protein α-keratin, found in the skin and its appendages such as hair, nails.

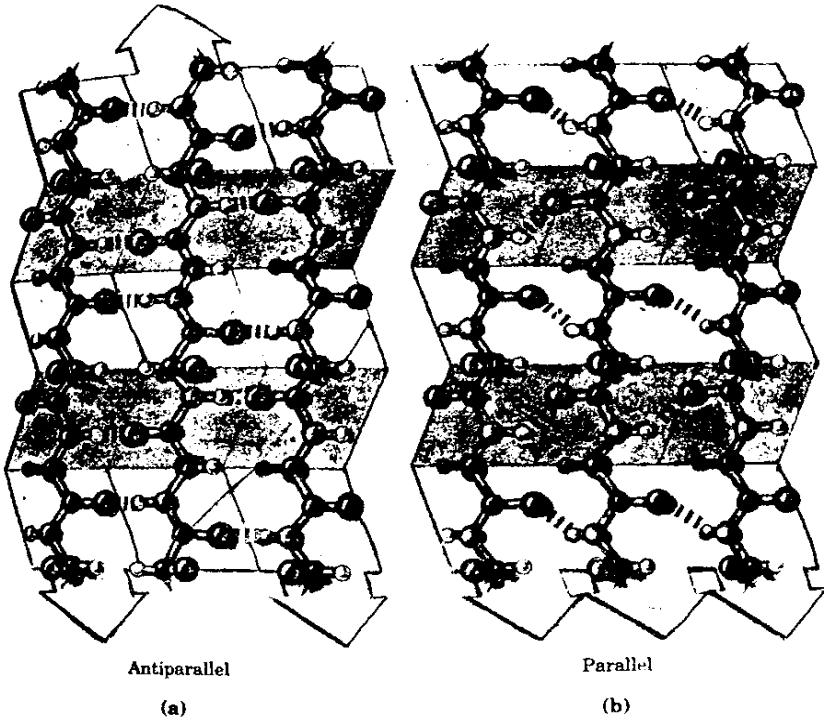


β-plated sheet:

The formation of β –plated sheet depends upon the intermolecular hydrogen bonding. The structure is formed by the parallel arrangement of a number of polypeptidechains. There are 2 types of β plated sheets..

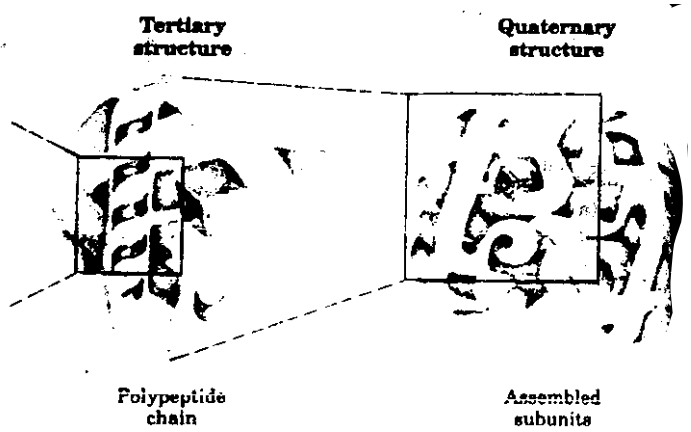
If the N=terminal ends of the polypeptide chains lie on the same edge of the sheet, with all the C =terminal ends on the opposite edge is known as parallel β-plated sheet.

If the direction of the chain alternates so that the alternating chains have their N-terminal ends on the same side of the sheet, while their C-terminal end lies on the opposite edge is known as anti parallel β-plated sheet.



II. Tertiary structure: Folding of the Chain

If the globular proteins consisted only of a small helix, these molecules would have been elongated structures with considerable length and a small cross-sectional area. But as we now know about the existence of globular proteins, the helix must, therefore, possess many other types of bonds placed at regular intervals. These additional bonds include disulfide, hydrogen, hydrophobic and ionic. In such globular proteins, polar groups because of their hydrophobicity are most often located on the molecule's exterior and nonpolar R groups in the interior, where their interactions create a hydrophobic environment. The teritary structure, thus, involves the folding of the fbelices of globular proteins. It refers to the spatial arrangement of amino acids that are far apart in lilnear sequence and to the pattern of disulfide bonds. The dividing line between secondary and tertiary structure is hence, a matter of state. X-ray crystallographic studies have revealed the detailed 3-D structures of more than 300 proteins.



17. Describe briefly about Haemoglobin and myoglobin.

Myoglobin (myo = muscle; globin = a type of protein) is relatively small, oxygen-binding heme protein, found in muscle cells. It has the distinction of being the first globular protein to have its 3-D structure elucidated by x-ray diffraction studies. This was accomplished by John C.Kendrew at a resolution of 6 A in 1957, 2 A in 1959 and 1.4 A in 1962. Myoglobin molecule contains a single polypeptide chain of 153 amino acid residues and a single prosthetic iron-porphyrin group, identical with that of hemoglobnin. The heme group is responsible for the deep red-brown colour of myoglobin. Myoglobin is especially abundant in the muscles of diving mammals such as the whale, seal and porpoise, whose muscles are so rich in this protein that they are brown. Storage of oxygen by muscle myoglobinn permits these animals to remain submerged for long periods. The function of myoglobin is to bind oxygen in the muscles and to enhance its transport to the mitochondria, which consume oxygen during respiration. Myoglobin (MW = 16,700) is an extremely compact macromolecule with oblate, spheroid shape and leaves little empty space in its interior. Its overall molecule dimensions are 45 X 35 X 25 A. The sausage like outline of territory structure of myoglobin chain is folded into an odd, irregular form. The backbone of the molecule is made up of 8 almost-straight alpha-helical segments, designated from the N-terminus as A through H. The first residue in helix

A is designated A1, the second A2 and so forth. Intersersed among the fhelical segments are 5 nonhelical regions , each identified by the two helical segments it joins, e.g, AB is located between helical sections A and B. There are also two other nonhelical regions: two residues at the

fN-terrminus and five residues at the C-terminus. The longest helical segment has 23 amino acid residues and the shortes only 7. All the helical segments are of α-type and right handed; there being no β-structure.

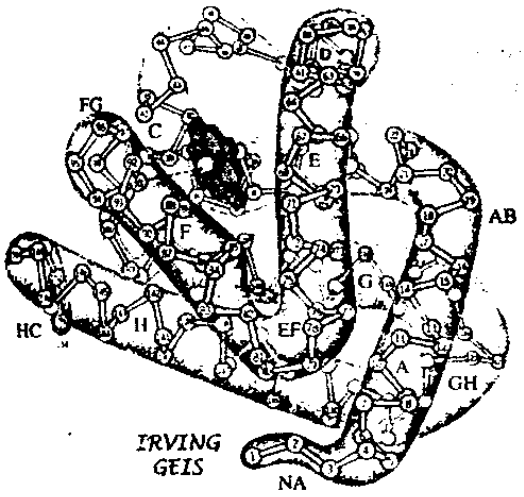
Of the 153 amino acid residues, 121 (i.e. 79%) are present on the helical regions and the remaining 32 amino acid residues are distributed over the nonhelical areas. The nonhelical areas possess various types of bojding such as hdrogen and nonpolar linkages. The flat heme group is tightly but nonvovelently bound to the polypeptide chain. A notable feature of whale myoglobin is the absence of a disulfide bridge, since both cysteine and cystine residues are lacking.

Other important features of myoglobinn are as follows;
The molecule is very compacrt and leaes so little space in its interior as to accomadate only 4 water molecules.

2. All the polar R groups of the molecule, except two, are located on its outer surface and all of them are hydrated.

3. Most of the hydrophobic R groups are located in the interior of the molecule . Hydrophobic R groups of helices E and F form the sides of a pocket into which the hydrophobic heme group fits. The porphyrin ring of the heme is largely hydrophobic, except for the 2 propionic acid side chains which stick out of the fpocket and into the swuoud rnbitonmrny.

4. Each of the 4 proline residues occurs at a turn. Other turns of bends contain serine, thronine and asparagines.



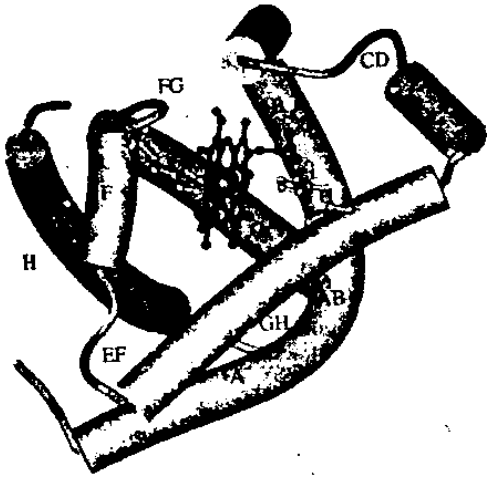


Figure: The eight α helical segments of myoglobin molecule

5. All the peptide bonds of the polypeptide chain are plannar, with the carbonyl and amide groups being trans to each other.

6. The heme group is flat and rest in a crevice in the molecule. The iron atom in the center of the fheme group has two coordination bonds that are perpendicular to the plane of the heme group. One bond is attached to the R group of histidine, whereas the other bond is the site to which an O2 molecule is bound.

7. The inside and outside are well defined. There is little empty space inside. The interior consists almost ent\irely of nonpolar residues such as Lue, Val, Met, and Phe. On the contrary, Glu, Asp, Gln, Asn, Lys and Arg are absent from the interior of the protein.

8. Myoglobin without its heme prosthetic group is designated apomyoglobin. The main functions of apomuyoglobin molecule is to ;provide a hydrophobic enggironment for the heme group and a properly oriented imidazole group to occupy the 5^{th} coordination posititon of the iron.

Hemoglobin (Hb)

Hemoglobin (hemo = blood; globin = a protein, belonging to the myoglobin-hemoglobin family), the oxygen transporter in erythrocytes, lconstitutes about 90% of the fprotein of red blood cells. It is a tetrameric protein. i.e., it contains 4 polypeptide chains/ The four chains are held together by noncovalent interactions. Each chain contains a heme group and single oxygen-binding site. Hemoglobin A, the principal hemoglobin in adults , consists of two alpha chains and two beta chains. Adults also have a minor hemoglobin called hemoglobin A2, which contains two delta chains in place of two beta chains of hemoglobin A. Thus the subunit composition of hemoglobin A is α2β2, and that of hemoglobin A2 is α2δ2.

Hemoglobin (mW = 64,500) provides an example of the interaction of unidentical protein subunits. Since hemoglobin is 4 times as large as myoglobin , much more time and effort were required to solve its 3-D structure, finally achieved by Max perutz, John C.Kendrew and their

colleagues in 1959. They determined the 3-D structure of adult hemoglobin of horse, which is very similar to that of human hemoglobin. The X – ray analysis has revealed that the hemoglobin molecule is nearly spherical with a diameter of 55 A (5.5 nm).

Human hemoglobin protein consists of 4 polypeptide chains of two types, two α-chains and two β-chains. The polypeptide portion is collectively called as globin. The α-chain has valine at the N-terminal and arginine at the C-terminal whereas in the β-chain, valine is situated at the N-terminal and histidine at the C-terminal. Each a chain is in contact with both β chains. In contrast. There are few interactions between the two α chains or between the two B-chains. Each chain has a heme prosthetic group in a crevice near the exterior of the molecule. The heme groups are involved in the binding of oxygen. The A-chain has 141 residues and the B-chain , which is more acidic, has 146residues. The protein, lthus, lhas a total 574 amino acid residues. Each of the 4 chains has a characteristic tertiary structure, in which the chain is folded. Like muyoglobin, the α- and β- chains are held together as a pair by ionic and hydrogen bonds. The 2 pairs are then joined to each other by additional iionic bonds, hydrogen bojnds and the fhydrophobic forces. Thus, the 4 polypeptide chains fit together almost tetrahedrally to produce the characteristic quaternary structure. The hemes are 2.5 nm apart from each other and titled at different angles. Each heme is partly buried in a ;pocket lilned with hydrophobic R groups. It is bound to its polypeptide chain through a coordination bond of the firon atom to the R group of a histidine residue. The sixth corordination bond of the oron atom of each heme is available to bind a molecule of oxygen. Myoglobinn and the α and β chains of hemoglobin have nearly the same tertiary structure. Both have well over 70% α-helical nature, both have similar lengths of α-helical segments and bends have about the same angles.

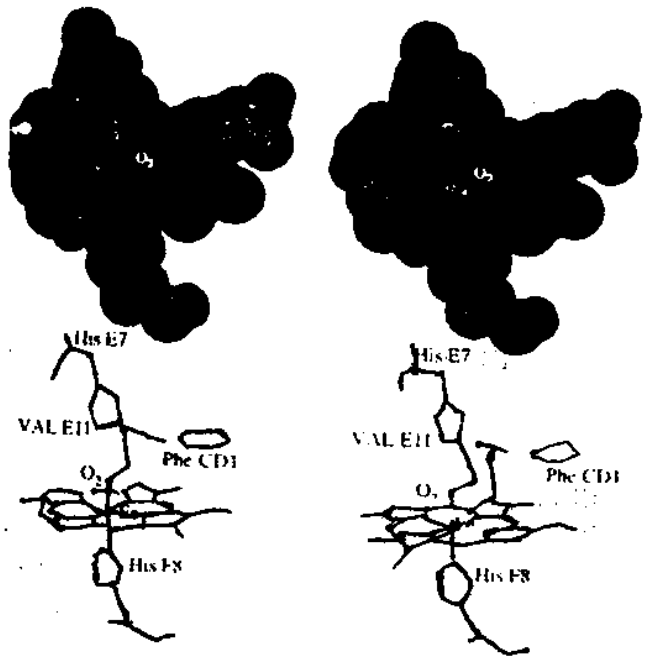


Figure: Stereo drawing of the heme complex in oxymyoglobin

UNIT – III

PART – A

1. Define enzyme and give examples.

Enzymes are bio-molecules, either protein or RNA that catalyzes a specific biochemical reaction. It does not affect the equilibrium of the catalyzed reaction. It enhances the rate of a reaction by providing a reaction path with a lower activation energy.

2. Write the classification of enzymes.

Enzymes are classified into six major classes, based on the type of reaction they catalyse;

i. Oxidoreductases
ii. Transferases
iii. Hydrolases
iv. Lyases
v. Isomerses
vi. Ligases

3. Define Holoenzyme.

Catalytically active enzyme including all necessary subunits, prosthetic groups and cofactors.

4. Define Apoenzyme.

The protein portion of an enzyme, exclusive of any organic or inorganic cofactors or prosthetic groups that might be required for catalytic activity.

5. Classify the enzyme specificity.

The specificity of enzymes are classified as given below;

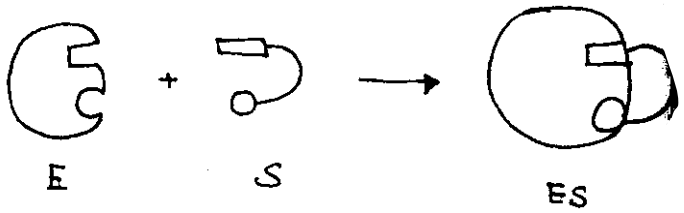
i. Optical specificity
ii. Group specificity

6. Define active site.

The substrate molecules are comparatively much smaller than the enzyme molecules, there should be some specific regions or sites on the enzyme for binding with the substrate. Such sites of attachment are variously called as ‘active sites’ or catalytic sites or substrate sites.

7. Write on Fischer’s Lock and key model of enzyme action.

According to this model, the union between the substrate and the enzyme takes place at the active site more or less in a manner in which a key fits a lock and result in the formation of an enzyme substrate complex.



8. Define metabolism.

The biochemical processes by which nutrients are built up into living matter, or by which complex substances and food are broken down into simple substances. The sum total of physical and chemical changes that occur in a tissue; includes anabolism and catabolism.

9. Define anabolism.

The phases of intermediary metabolism concerned with the energy requiring biosynthesis of cell components from smaller precursor molecules are called anabolism. During anabolism, energy is utilized and molecules are synthesized.

10. Define catabolism.

The phase of intermediary metabolism concerned with the energy yielding or degradation of complex molecules into simple molecules. During this reaction, energy is released through breakdown of complex organic molecules into simple molecules.

11. What is enzyme? Give examples for enzyme catalysis?

Enzyme are homogeneous biological catalysts. They are high molecular weight biological compounds having > 10000 mol wt. They may be proteins (or) nudeic acids that contain active sites in their molecular structure which is responsible for bindings the substrate namely the reactant and processing it into products.

Examples:-

1. Urea hydrolysis by Urease enzyme

12. What are the characteristics of enzyme catalysis?

It is a homogeneous catalysis

1. It is taking place in solution (aqueous medium).
2. Enzyme Catalysis is very specific. An enzyme which catalyses one reaction, will not catalyse another reaction. This is because of lock and key mechanism. The active sites will have definite orientation that will be suitable for a particular reaction.
3. Reaction that may not occur ordinarily takes places so easily by the presence of an enzyme.
4. Enzyme reactions may be inhibited by the presence of certain substances called inhibitors.

PART – B

1. Explain the active site and specificity of enzyme.

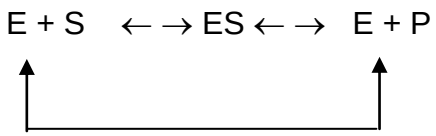
Enzymes are big in size compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis.

Active site

The active site (or active centre) of an enzyme represents as the small region at which the substrate(s) binds and participates in the catalysis.

Salient features of active site

1. The existence of active site is due to the tertiary structure of ;protein resulting three dimensional native conformation.
2. The active site is made up of amino acids (known as catluytic residues) which are far from each other fin the linear sequence of amino acids(primary structure of ;protein). For instance, the enzyme lysozyme has 129 amino acids. The active site is formed by the contribution of amino acid residues numbered 35, 52, 62, 63 and 101.
3. Active sites are regarded as clefts or crevices or pockets occupying a small region in a big enzyme molecule.
4. The active site is not rigid in structure and shape. It is rather flexible to promote the specific substrate binding.
5. Generally, the active site possesses a substrate binding site and a catalytic site. The latter is for the catalysis of the specific reaction.
6. The coenzymes or cofactors on which some enzymes depend are fpresent as a part of the catalytic site.
7. The substrate(s) binds ast the active site by weak noncovalent bonds.
8. Enzymes are specific in their function due to the existence of active sites.
9. The commonly found amino acids at the active sites are serine, as part at, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, serine is the most frequently found.
10. The substrate(s) binds the enzyme(E) att the active site to form enzyme-substrate complex (ES). The product (P) is released after the catalysis and the enzyme is available for reuse.



Enzyme specificity

Enzymes are highly specific in their action when compared with the chemical catalysts. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes. Three types of enzyme specificity are well-recognised.

1. Stereo specificity
2. Reaction specificity and
3. Substrate specificity.

Specificity of a characteristic property of the active site.

1. Stereo specificity or optical specificity

Stereoisomers are the compounds which have the same molecular formula, but differ in their structural configuration.

The enzymes act only on one isomer and therefore, exhibit stereospecificity.

e.g. L-amino acid oxidase and D-amino acid oxides act on L- and D- amino acids respectively.
Hexokinase acts on D-hexooses;
Glucokinasse on D-glucosel
Amylase acts on alpha-glycosidic linkages;
Cellulase cleaves beta-glycosidic bonds.

1. Reaction specificity

The same substrate can undergo different types of reactions, each catalysed by a separate enzyme and this is referred to as reaction specificity. An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc. The enzymes however, are different for each of these reactions.

2. Substrate specificity

The substrate specificity varies from enzyme to enzyme. It may be absolute, relative or broad.

Absolute substrate specificity: Certain enzymes act only on one substrate e.g. glucokinase acts on glucose to give glucose 6-phosphate, urease cleaces urea to ammonia and carbon dioxide.

Relative substrate specificity: Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin is a good example for group specificity. Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino acids (Phenylalanine, tyrosine and tryptophan). Examples of bond specificity glycosidases acting on glycosidic bonds of carbohydrates, lipases cleaving ester binds of lipids etc.

Broad specificity: Some enzymes act on closely related substrates which are commonly known as broad substrate specificity, e.g. hexokinase acts on glucose, fructose, mannose and glucosamine and not on galactose. It is possible that some atructural similarity among the first four compounds makes them a common substrate for the enzyme hexokinase.

UNIT - IV

PART - A

1. What are the sub compound present in the pyruvate dehydrogenase complex?

1 .Pyruvate dehydrogenase (E1)
2. Dihydrolipoyl transacetylase (E2)
3. Dihydrolipoyl dehydrogenase (E3)

2. Define amphibolic pathway.

The citric acid cycle has a dual or amphibolic (amphi = both) nature. The cycle functions not in the oxidative catabolism of carbohydrates , fatty acids and amino acids but also as the first stage in many biosynthetic (= anabolic) pathways for which it provides precursors.

3. Define Glycolysis.

Glycolysis (glycos = Sugar ; lysis = dissolution) is the sequence of 10 enzyme catalysed reactions that converts glucose into pyruvate with the simultaneous production of ATP. Moreover, glycolysis also includes the formation of lactate from pyruvate.

4. Give name of two phases of Glycolysis.

The glycolysis, 6 carbon glucose is broken down into two moles of 3 carbon pyruvate via 10 enzyme catalyzed Sequential reactions. These reactions are grouped under 2 phases , Phase 1 (preparatory phase) Phase 2 (pay off phase)

5. Define Glyoxylate Cycle.

A variant of the citric acid cycle, for the net conversion of acetate into Succinate and eventually, new carbohydrate, present in bacteria and some higher plant cells.

8. Define Gluconeogenesis.

The biosynthesis of a carbohydrate from simpler, non carbohydrate precursors such as oxyloacetate or pyruvate.

9. What is Calvin Cycle?

A major enzymatically catalyzed metabolic pathway in which CO2 is reduced to 3-phosphoglyceraldehyde (a C-3 compound) and the CO2 acceptor (ribulose 1, 5 bisphosphate) is regenerated.

10. Define Citric acid cycle or TCA cycle.

A central metabolic pathway found in all aerobic organisms and which acetyl Co A , derived from food molecules , is oxidized in mitochondria to CO2 ; each of the citric acid cycle also forms one ATP by substrate – level phosphorylation, reduces one NAD to NADH , and reduces one ubiquinone to ubiquinol; also known as Krebs Cycle or tricarboxylic acid (TCA) Cycle.

11. Define Coenzyme & Give the example.

A diaclyzable , non protein prosthetic group of an enzyme ; like enzymes , coenzymes are not altered or used up in the reaction and can be used many times ; most of them are derived metabolically from vitamins; NAD and Coenzyme A are examples of Coenzyme .

12. Define Deamination.

The enzymatic removal of amino group from a biomolecule, such as aminoacids or nucleotides. Deamination of adenine or cytosine causes base – pairing changes.

13. Define Decarboxylation.

The removal of carbondioxide from a biomolecule as in the conversion of oxalosuccinic acid (C6) to alpha – keto glutaric acid (C5) in the third step of the krebs cycle.

14. Define pentose phosphate pathway.

A pathway that serves to interconven hexoses and pentoses and is a source of reducing equivalents and pentoses for biosynthetic processes , present in most organisms , during this pathway . NADP is reduced to NADPH , but no ATP is produced also called the phosphogluconate pathway.

15. Define Urea Cycle.

A metabolic pathway pathway in vertebrates , for the synthesis of urea from amino groups and carbondioxide ; occurs in the liver . The reaction is essentially;

$2\ NH_3 + Co_2 \rightarrow NH_2\ CNH_2 + H_2o$

PART – B

1. Describe the citric acid cycle and its energetics.

The citric acid cycle (Krebs cycle, tricarboxylic acid cycle) is a series of reactions in Mitochondria that bring about the catabolism of acetyl residues, liberating hydrogen equivalents, which upon oxidation, lead to the release of most of the free energy of tissue fuels. The acetyl residues are in the form of acetyl –CoA (CH_3 -CO~S-CoA, active acetate) an ester of coenzyme A which with other acyl thioesters of CoA is classified as a High energy compound.

Significance of the citric acid cycle

Essentially, the cycle comprises the combination of a molecule of acetyl-CoA with the 4-carbon dicarboxylic acid oxalo acetate, resulting in the formation of a **6-carbon tricarboxylic acid, citric.** There follows a seris of reactions in the course of which 2 molecules of CO_2 are lost and oxaloacetate is regenerated. Since only small quantity of oxalo acetate is able to facilitate the conversion of large quantity of acetyl units toCO_2, oxalo acetate maybe considered to play a **catalytic role.**

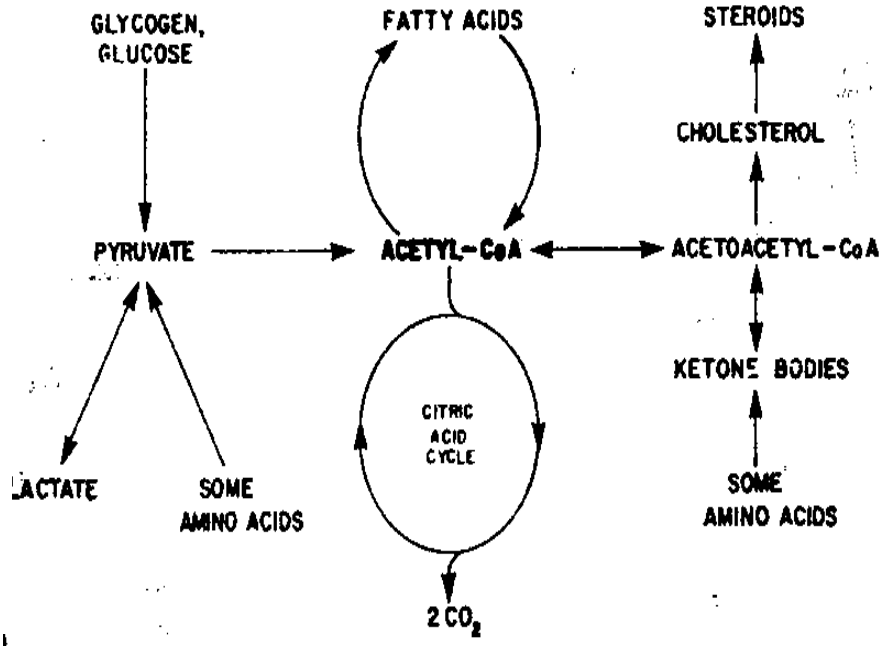
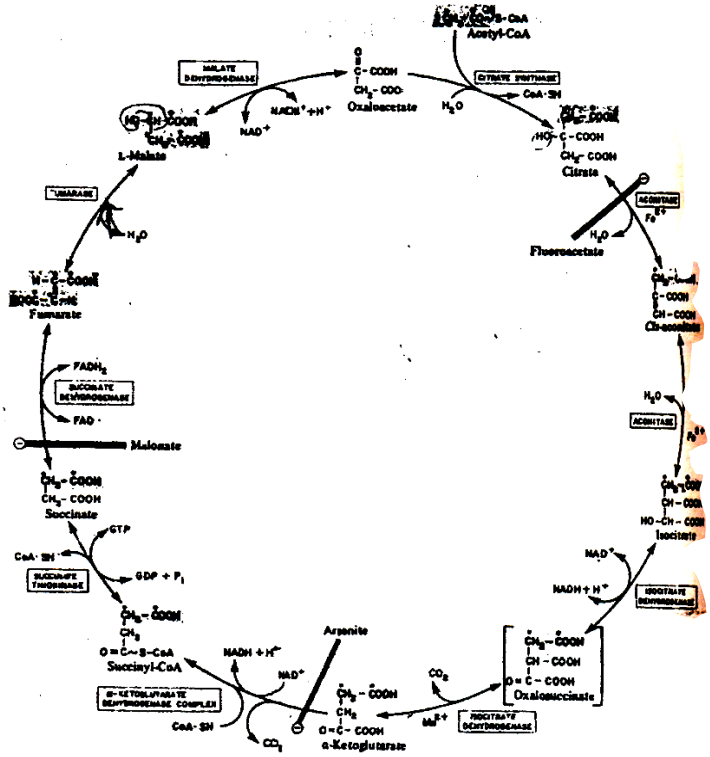


Figure: Overview of acetyl – CoA metabolism



AcetylCoa +Oxaloacetate +H_2O --> Citrate + CoA.SH

The initial condensation of acetylCoA with oxaloacetate to form citrate is catalysed by a condensing enzyme, citrate synthase, which effects a carbon to carbon bond between the methyl carbon of oxaloaceteate. The condensation oreation is followed by the hydrolysis of the thioester bond of CoA, which is accompanied by considerable loss of free energy as heat, ensuring that the reaction goes to completion.

Citrate is converted into isocitrate by the enzyme **aconitase** which contains iron in the Fe^{2+} state. This conversion takes place in two steps, dehydration to *cis*-aconitase, some of which remains bound to the enzyme and rehydration to iso citrate.

Citrate← → *cis*-aconitase ← →Isocitrate
H_2O

The reaction is inhibited by the presence of **fluofoacetate**, which in the form of fluoroacetyl-CoA, condenses with oxaloacetate to form fluorocitrate. The latter inhibits aconitase, causing citrate to accumulate.

Experiments using ^{14}C-labeled intermediates indicates that citrate reacts with aconitase in an asymmetric manner with the result that aconitase always acts on that part of the citrate molecule that this was due to a 3-point attachment of the enzyme to the substrate. The3-point

attachment would enable aconitase to differentiate the two $-CH_2$ COOH grp in citrate, thus conferring asymmetry on a apparently symmetric molecule. However the 3-point attachment hyposis is necessary to explain the asymmetric action of aconitase. It is now realized that the two $-CH_2$ COOH grps are not identical in space with respect to the –OH and –COOH groups. The consequences of the asymmetric action of aconitase may be appreciated by the reference to the fate of labeled acetylCoA in the citric acid cycle. It is possible that *cis*-aconitase may not be an obligatory intermediate between citrate and isocitrate but may in fact be a side branch from the main pathway.

Isocitrate undergoes dehydrogenation in the presence of **isocitrate dehydrogenase** to form oxalosuccinate. Three different enzymes have been described. One which is NAD-specific is found only in mitochondria . The other two enzymes are NADP-specific and are found in the mitochondria and the cytosol respectively. Respiratory chain oxidation of isocitrate proceeds almostcompletely through the NAD-dependent enzyme.

Isocitrate + NAD^+ ←→ Oxalosuccinate ←→ α-ketoglutarate + CO_2 + NADH + H^+

There follows a decarboxylation to α-ketoglutarate also catalysed by the isocatrate dehudrogenase. Mn^{2+} IS AN IMPORTANT COMPONENT OF THE DECARBOXYLATION REACTION. It would appear that oxalosuccinate remains bound to the enzyme as an inter mediate in the overall reaction.

Next α-ketoglutarate undergoes **oxidative decarboxylation** in the manner that is analogous to the oxidative decarboxylation of pyruvate both substrates being α-ketoacids.

α-ketoglutarate + NAD^+ CoA.SH -→ Succinyl-CoA + CO_2 + NADH + H^+

The reaction catallysed by an **ketoglutarate dehydrogenase** complex also requires identical cofactor and results in the formation of succinyl CoA, a thioester containing a high energy bond. The equilibrium of this reaction is so much in favour of succinyl CoA formation that the reaction must be considered as physiologically unidirectional. As in the case of pyruvate oxidation,arsenic inhibits the reaction, causing the substrate,α-**ketoglutatrate,**to accumulate.

To continue the cycle, succinyl-CoA is coverted to succinate by the enzyme **succinate thiokinase(succinly-CoA synthetase).**

Succinyl-CoA+p_i+GDP←→ succinate+GTP+CoA.SH

This reaction requires GDP or IDP, which is converted in the presence of inorganic phosphate to either GTP or ITP, This is the only example in the citric acid cycle of the **generation of a high-energy phosphate at he substrate level** and arises because the release of free energy from the oxidative decarboxilation of α-ketoglutarate is sufficient to generate a high-energy bond in addition to the formation of NADH(equivalent to 3~P). By means of a phosphokinase, ATP may be formed from either GTP or ITP,

Eg. GTP+ADP←→GDP+ATP

An alternative reaction inextrapatic tissues, which is catalyzed by **succinyl-CoA-acetoacetate CoA transferase(thiophorase),** is the conversion of succinyl-CoA to succinate coupled with the conversion of acetoacetate to acetoacetyl-CoA. In liver there is alsodeacylase activity, causing some hydrolysis of succinyl-CoA to succinate plus CoA.
Succinate is metabokized further by undergoing a dehydrogenation followed by the dehydrogenation which regenerates oxaloacetate.

Succinate+FAD←→ Fumarate+$FADH_2$

The first dehydrogenation reaction is catalyzed by **succinate dehydrogenase,** which is bound to the inner surface of the inner mitochondrial membrane, It is the only dehydrogenation in the citric acid cycle that involves the **direct transfer of hydrogen from the substrate to a flavoprotein without the participation of NAD.** The enzyme contains FAD and Fe:S Protein. Fumarate is formed as a result of the dehydrogenation. Isotopic experiments have shown that the enzyme is stereospecific for the *trans* hydrogen atoms of the methylene carbons of succinate. Addition of malonate or oxaloacetate inhibits succinate dehydrogenase competitively, resulting in succinate accumulation,

Under the influence of **fumarase(fumarate hydratase),** water is added to fumarate to give malate.

Fumarate+H_2o←→ L-Malate

In addition to br\eing specific for the L-isomer of malate, fumarase catalyzes the addition of the elements of water to the double bond of fumarate in the *trans* confugration. Malate is converted to oxaloacetate by **malate dehydrogenase,** a reactionrequiring NAD^+.

L-Malate+NAD^+ ←→ Oxaloacetate+NADH+H^+

Although the equilibrium of this reaction is much in favour of malate, the net flux is towards the direction of oxaloacetate because this compound together with the other productof the reaction (NADH) is removed continuously in further reaction

The enzymes participating in the citric acid cycle are also found outside the mitochondria expect for the α-ketoglutarate and succinate dehydrogenases. May not in fact be the same proteins as the mitochondrial enzymes of the same name.

Energitics of the Citric Acid Cycle

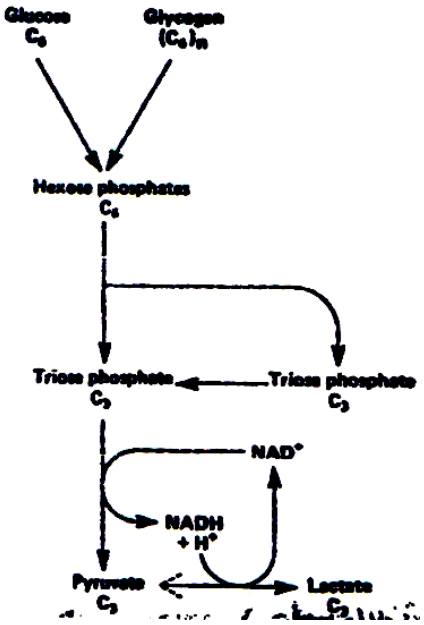
As a result of oxidation catalyzed by dehydrogenase enzymes of the citric acid cycle, **3 molecules of NADH** and **one of $FADH_2$** Are produced for each molecule of acetyl-CoA catabolized in one revolution of the cycle. These reducing equivalents are transferred to respiratory chain in the inner mitochondrial membrane. During passage along the chain, reducing equivkents from NADH will generate 3high-energyphosphate bonds by the esterification of ADP to ATP in the process of oxidative phosphorylation. However, $FADH_2$ produces only 2 high-energy phosphate bonds because it transfer its reducing power to Q, thus by passing the first site for oxidative phosphorylation in the respiratory chain. Thus, 12 new high-energy phosphate bonds are generated for each turn of the cycle.

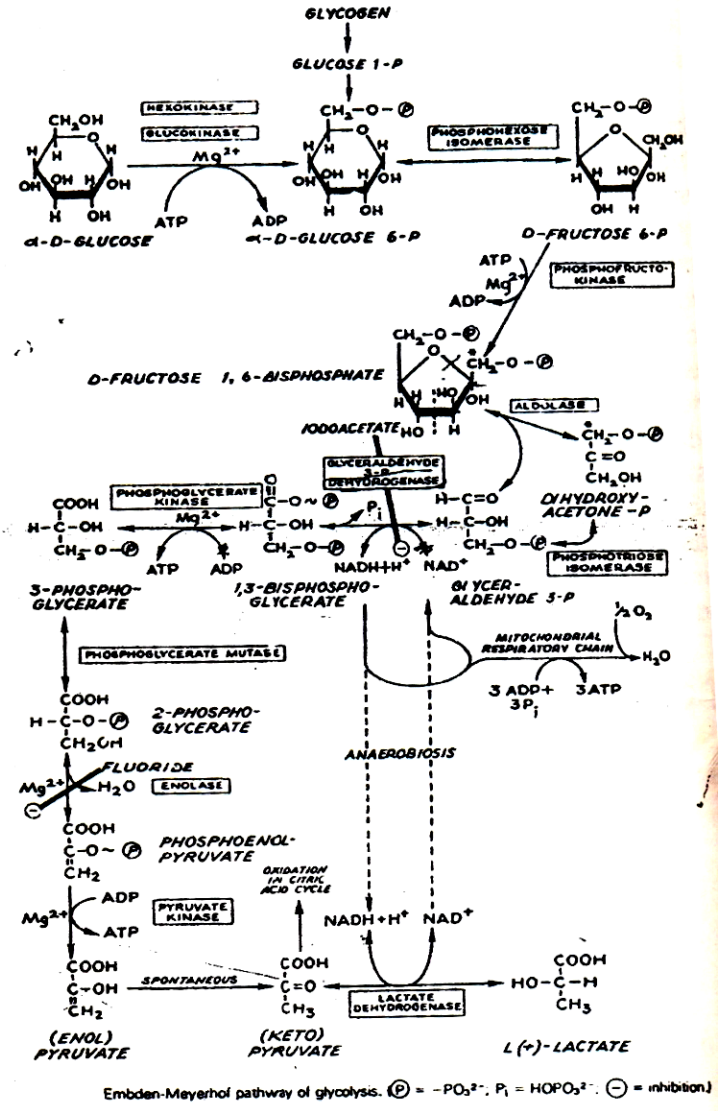
Reaction catalyzed by	Method of Prodution	Number of P formed
Isocitrate dehydrogenase	Respiratory chain oxidation of NADH	3
A-ketoglutarate dehydrogenase	Respiratory chain oxidation of NADH	3
Succinate thiokinase	Oxidation at substrate	1
Succinate dehydrogenase	Respiratory chain oxidation of $FADH_2$	2
Malate dehydrogenase	Respiratory chain oxidation of NADH	3
		Net 12

2. Explain glycolysis in all reaction.

At an early period in the course of investigations on carbohydrate metabolism it was realized that the process of fermentation in yeast was similar to the breakdown of glycogen in muscle. Although many of the early investigations of the glycolytic pathway were carried out on those 2 systems the process is now known to occur in virtually all tissues.

In many of the first studies on the biochemical changes that occur during muscular contraction it was noted when a muscle contracts in an aerobic medium ie one form which oxygen is excluded glycogen disappears and pyruvate and lactate appear as the principal end products. When oxygen is admitted aerobic recovery takes place and glycogen reappears while pyruvate and lactate disappear. However if contraction takes place under aerobic conditions, lactate does not accumulate and pyrvate is oxidation further to CO_2 and water. As a result of these observations it has been customary to separate carbohydrate metabolism into anaerobic and aerobic phases. This distinction is arbitrary since reactions are same in presence and absence except in the extent and end products.





Embden-Meyerhof pathway of glycolysis. (P) = $-PO_3^{2-}$; $P_i = HOPO_3^{2-}$; (−) = inhibition.)

When oxygen is short supplied reoxidation of NADH formed during glycolysis is impaired. Under these circumstances NADH is reoxidised by being coupled to the reduction of pyruvate to lactate the NAD so formed being used to allow further glycolysis to proceed.

The overall reaction for glucose to lactate is

Glucose + 2ADP + 2P_i → 2 L-Lactate + 2 ATP + 2 H_2O

Sequence of Reaction in Glycolysis

All of the enzymes of the EMBDEN- MEYERHOF pathway are found in the extramitochondrial soluble fraction of the cell the cytosol.

Glucose enters the pathway by phosphorylation to glucose-6-phosphate. This is done by the enzyme **hexokinase** and by an additional enzyme in the liver **glucokinase** whose activity is

inducible and affected by the changes in the nutritional state. The reaction takes place along with loss of engery as heat. ATP is required as phosphate donor, it reacts as Mg-ATP complex. One ATPis utilized and ADP is produced.

α-D-Glucose + ATP → α-D-Glucose-6-phosphate + ADP

Hexokinase has a affinity for its substrate glucose. Glucose-6-phosphate is an important compound being at the junction of several metabolic pathways. In glycoytsis it is converted into fructose-6-phosphate by phosphohexose isomerase

α-D-Glucose-6-phosphate ↔ α-D-Fructose-6-phosphate

Phosphofructose is another inducible enzyme whose activity is considered to play a major role in the regulation of the rate of glycolysis.

α-D-Glucose-6-phosphate + ATP → → α-D-Fructose1,-6-bisphosphate

The hexose phosphate is split by **aldose** into 2 triose phosphates, glyceraldehydes3-phosphate and dihydroxyacetone phosphate.

D-Fructose1,6-bisphosphate↔D-glyceraldehyde3-phosphate +Dihydroxyacetone phosphate

Glycolysis proceeds by the oxidation of glyceraldehyde3-phosphate to 1,3-bisphosphoglycerate and oxidized to 1,3 diphosphoglycerate via glyceraldehyde3-phosphate.

D-glyceraldehyde3-phosphate + NAD + Pi ↔1,3-bisphosphoglycerate + NADH +H^+

The enzyme responsible for the oxidation is NAD dependent. One of the SH grps is found at the active site of the enzyme. It is believed that the SH group participates in the reaction in which glyceraldehydes 3-phosphate is oxidized. Energy released during the oxidation is retained by the formation of te higher energy sulphur bond that becomes after phosphate bond in the position 1 of 1,3-bisphosphoglycerate

1,2-bisphosphoglycerate + ADP ↔ 3-Phosphoglycerate + ATP

3-phosphoglycerate arising from reaction is converted to 2-phosphoglycerate by the action of **phosphoglycerate mutase.** It is an intermediate in this reaction.

3-phosphoglycerate ↔ 2-phosphoglycerate.

It is then catalysed by **enolase** forming phosphoenol pyruvate. Enol is inhibited by **Fluoride.**

2-phosphoglycerate ↔ phosphoenolpyruvate + H_2O

Enolpyruvate formed is spontaneously changed into its keto form by **pyruvatekinase**

Phosphoenolpyruvate +ADP → Pyruvate + ATP.

The redox state of the tissue now determines which of the two pathways is followed. If **anaerobic** condition prevails the reoxidation is prevented. Pyruvate is reduced to lactate by **lactate dehydrogenase.**

Pyruvate + NADH + H^+ ↔ L-lactate + NAD^+

3. Describe the oxidation of pyruvate.

Pyruvate before entering into the citric acid cycle, it must be transported into the mitochondrion via a special pyruvate transporter that aids its passage across the inner mitochondrial membrane. Within the mitochondrion it is oxidatively decarboxylated into acetylCoA. This reaction is catalysed by the several different enzymes working sequentially in a multienzyme complex. They are collectively designated as the **pyruvate dehydrogenase** complex and are analogous to the α-ketoglutarate dehydrogenase complex of the citric acid cycle. Pyruvate is decarboxylated in the presence of thiamine diphosphate to a hydroxyethyl derivative of the thiazole ring of enzyme-bound thiamin diphosphate which in turn reacts with oxidized lipoate to form S-acetyl lipoate all catalysed by pyruvatedehydogenae. In the presence of **dihydrolipoyl transacetylase.** S-acetyl lipoate reacts with coenzyme A to form acetylcoA and reduced lipoate. The cycle of reaction is completed and latter is reoxidised in the presence of **dihydrolipoyl dehydrogenase.**

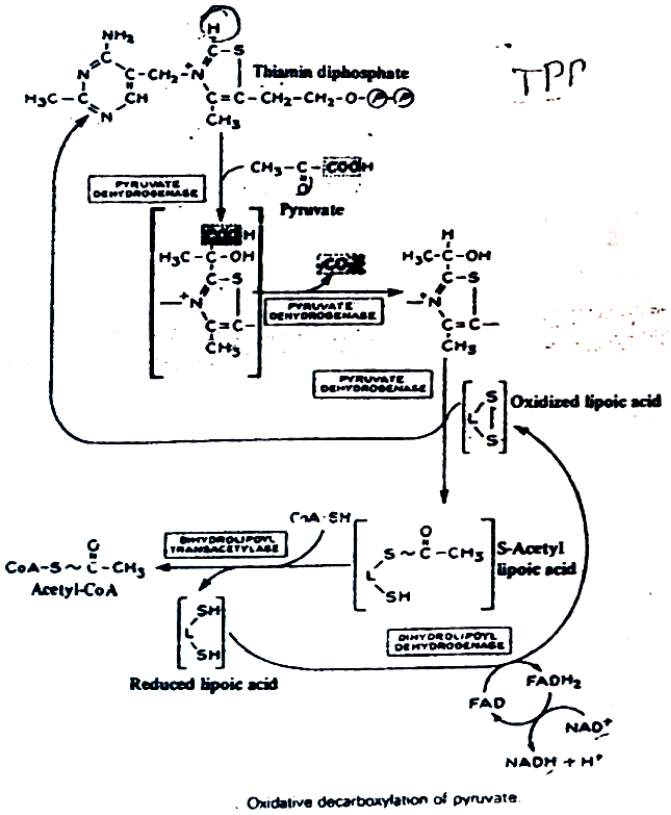
Pyruvate + NAD^+ + CoA → AcetylCoA + NADH + H^+ + CO_2

The pyruvate dehydrogenase complex consists of 29mol of pyruvate dehydrogenase and about 8mol of flavoprotein distributed around 1mol of transacetylase. Movement of the individual enzymes appear to restricted and the metabolic intermediates remain to the enzymes.

Table 15–1. Generation of high-energy bonds in the catabolism of glucose.

Pathway	Reaction Catalyzed By	Method of ~Ⓟ Production	Number of ~Ⓟ Formed per Mole Glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	6*
	Phosphoglycerate kinase	Oxidation at substrate level	2
	Pyruvate kinase	Oxidation at substrate level	2
			10
	Allow for consumption of ATP by reactions catalyzed by hexokinase and phosphofructokinase		−2
			Net 8
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Succinate thiokinase	Oxidation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $FADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
			Net 30
Total per mole of glucose under aerobic conditions			38
Total per mole of glucose under anaerobic conditions			2

*It is assumed that NADH formed in glycolysis is transported into mitochondria via the malate shuttle (p. 138). If the glycerophosphate shuttle is used, only 2~Ⓟ would be formed per mol of NADH, the total net production being 36 instead of 38.



Oxidative decarboxylation of pyruvate.

It is to be noted that the pyruvate dehydrogenase is sufficiently electronegative with respect to the respiratory chain that in addition to the generating a reduced coenzyme it also generates a high energy thro ester bond in the acetyl-CoA.

Arsenite or mercuric ions complex the –Sh grps of lipoic acid and inhibit pyruvate dehydrognase as does a dietry deficiency of thuamin allowing pyruvat to accumulate. Nutritionaly deprived alcohols are particularly susceptible to thiamin deficiency and if administered glucose exihibit rapid accumulation of pyruvate and lactic acidosis which can be life threatening. Mutations have been reported for virtually all of the enzymes of carbohydrate metabolim each associated with human disease.

4. Describe the pentose phosphate shunt.

A major function of the hexose monophosphate shunt is the provision of reduced NADP required by anabolism process outside the mitochondria, such as the synthesis of fatty acids and steroids

Sequence of the Reactions

This pathway for the oxidation of glucose occurs in certain tissues, notably liver, lactating mammary gland and adipose tissues in addition to the Embden-Meyerhof pathway of glycolysis. It is in the effect a multicyclic pathway molecules of glucose6-phosphate give rise to 3 molecules of CO_2 and three 5 carbon residues. The latter are rearranged to regenerate 2 molecules of glucose 6-phosphate and 1molecule of glyceraldehyde 3-phosphate can regenerate a molecule of

glucose6-phospate by reactions which are essentially a reversal of glycolysis the pathway. As in the Embden-Meyerof glycolysis pathway, NADP and not the shunt pathway are found in the extramitochondrial soluble portion of the cell.

A summary of the reaction of the hexose monophosphate shunt is shown below..

3-glucose6-P + 6 NADP+ $\rightarrow$3 CO_2 + 2glucose6-P + glyceraldehydes 3-P + 6 NADPH + 6 H^+

The sequence of reaction of the shunt pathway may be divided into 2 phases. In the first phase glucose6-P undergose dehydration and decarboxylation to give the pentose ribulose 5-P. In the second ribulose5-P is converted into glucose6-P involving **transketolase and transaldolase.**

Dehydrogenation of glucose 6-P to 6-phosphogluconate occurs via the formation of 6-phosphogluconolactone catalysed by **glucose6-P dehydrogenase.** The hydrolysis of 6-phosphogluconolactone is done by **gluconolactone hydrolase.** A second oxidative step is catalysed by **6-phosphogluconate dehydrogenase.**

Ribulose5-P now serves as substrate for 2 different enzymes **Ribulose5-P epimerase** alters the configuration about carbon 3 forming the epimer xylulose 5-phosphate. **Ribose 5-P ketoisomerase** converts ribulose 5-phosphate into ribose 5-phosphate. This reaction is analogous to the interconversion of fructose 6- phosphate in the Embden-Meyer pathway.

Transketolase transfers the 2carbon unit comprising of an aldose sugar. It therefore effects the conversion of a ketose sugar into an aldose with 2carbon less and simultaneously converts an aldose sugar into a ketose with 2carbon more. In addition to the enzyme transketolase the reaction requires thiamin diphosphate as coenzyme and Mg^{2+} ions. The 2-carbon moiety transferred is probably glycolaldehyde bound to thyiamin diphosphate ie “active glycoaldehyde” . In the hexose monophosphate shunt transeketolase catalyses the transfer of of the 2-carbon unit from xylulose 5-phosphate to ribose5-P producing the 7-carbon ketose sedoheptulose 7-phosphate and the aldose glyceraldehyde3-P. These 2products then enter another reaction known as transaldolation. Transaldolase allows the transfer of a 3-carbon moiety, “active dihydroxyacetone” from the ketose sedoheptulose 7-P to the aldose glyceraldehyde 3-P to form the ketose fructose 6-P and the 4-carbon aldose erythose 4-Phosphate.

In order to oxidize glucose to CO_2 via the shunt pathway it is necessary that the enzymes are present in the tissue to convert glyceraldehyde 3-P to glucose 6-. This involves the enzymes of the Embden-Meyerhof pathway working in a reverse direction and in addition **fructose-1,6-diphosphatase**.

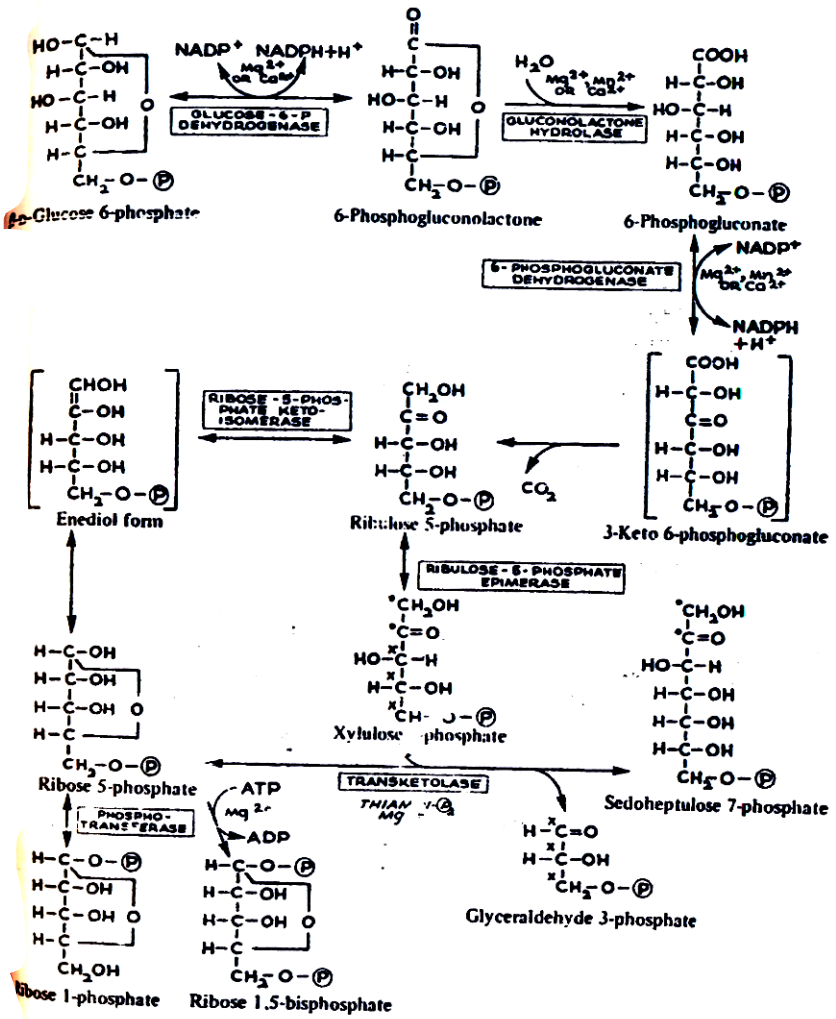


Figure The hexose monophosphate shunt (pentose phosphate pathway). (Ⓟ = $-PO_3^{2-}$)

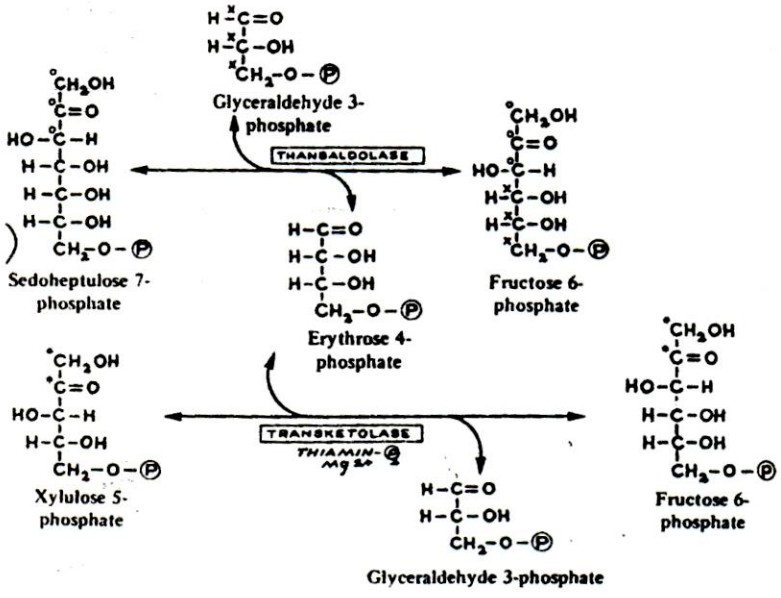


Figure (cont'd). The hexose monophosphate shunt.

5. Explain gluconeogenesis and three bypass enzyme.

Gluconeogenesis

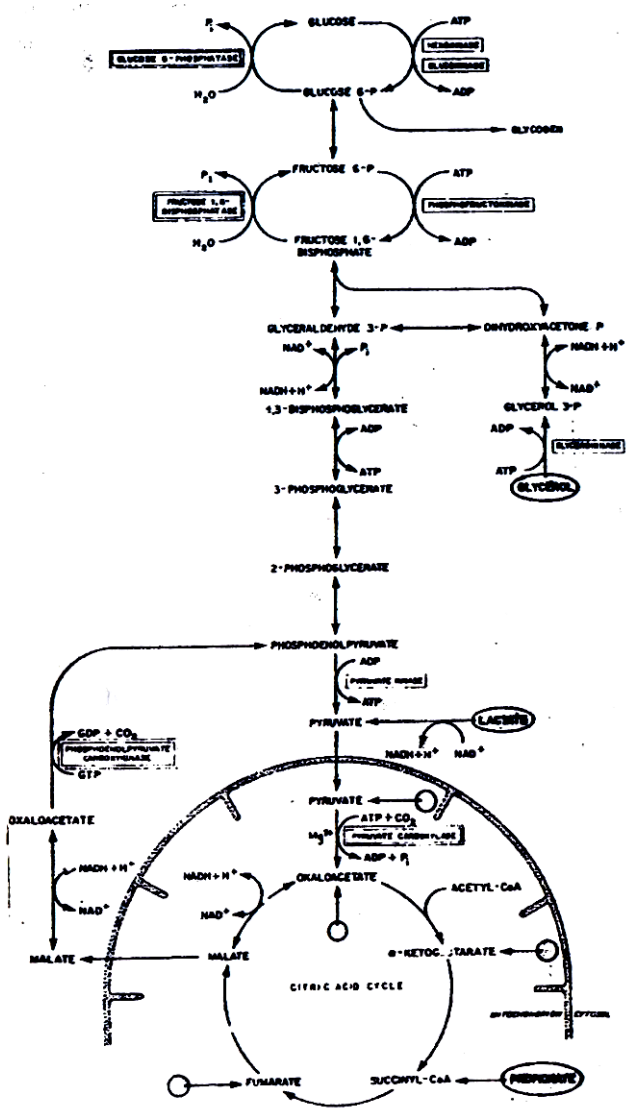
Meets the needs of the body for glucose when carbohydrates is not available in sufficient amounts from the diet. A continual supply of glucose is necessary as a source of energy, especially for the nervous system and the erythrocytes. Glucose is also required in adipose tissue as a source of **glyceride-glycerol,** and it plays a role in maintaining the level of intermediates of the citric acid cycle in many tissues. It is clear that even under conditions where fat may be supplying most of the calorific requirement for glucose. In addition glucose is the only fuel that will supply energy to skeletal muscle under anaerobic conditions. It is the precursor of milk sugar in mammary gland and it is taken up actively by the fetus. In addition these gluconeogenetic mechanism are used to clear the products of the metabolism of other tissues from the blood, eg: lactate produced by muscle, erythrocytes and glycerol continuously produced by adipose tissue. Propionate the principal glucogeonic fatty acid produced in the digestion of carbohydrates by ruminants is a major substrate for gluconeogenesis in the species.

In mammals the liver and the kidney are the principal organs responsible for the gluconeogenesis. As the main pathways for glycolysis is the reversal of gluconeogenesis.

Metabolic pathways involved in the gluconeogenesis.

These pathways are modifications and adaptations of the Embden-Meyerhof pathway and the citric acid cycle. They are concerned with the conversion of glucogenic aminoacid, lactate, glycerol, and in ruminants , propionate to glucose or glycogen. It has been pointed out by Kreb that energy barriers obstruct a simple reversal of glucolysis (1) between pyruvate and phosphoenolpyruvate (2) between fructose 1,6-bisphosphate and fructose 6-P (3) between glucose 1-P and glucose. These barriers are circumventedby special reaction described below.
(1)Present in mitochondria is an enzyme **pyruvate carboxylase** which in the presence of ATP biotin and CO_2 converts pyruvate to oxaloacetate. The function of the biotin is to bind CO_2 from bicarbonate onto the enzyme prior to the addition of the CO_2 to pyruvate. In the extramitochondrial part of the cell is found a second enzyme **phosphoenolpyruvate carboxykinase,** which catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. High energy phosphate in the form of GTP or ITP is required in this reaction and CO_2 is liberated. Thus with the help of these 2 enzymes and lactate dehydrogenase lactate can be converted to phosphoenolpyruvate.

However oxaloacetate does not diffuse readily from mitochondria. Alternative means are available to achieve the same end by coverting oxaloacetate into compounds that can diffuse from the mitochondria followed by their reconversion to oxaloacetate in the extramitochondrial portion of the cell. Such a compound is malate but conversion via aspirate α-ketoglutarate glutamate and citrate has also been proposed. There are species diff with regard to the distribution of phosphoenolpyruvate carboxykinase. The extramitochondrial location is true for the rat and mouse but in rabbit and chicken the enzyme is located in the mitochondrial.



(2) The conversion of fructose 1,6 -bisphosphate to fructose 6-P necessary to achieve a reversal of glycolysis is catalysed by the specific enzyme **fuctose1,6-bisphosphatase.** It is present in liver and kidney has been demonstrated in striated muscle and smooth muscle.

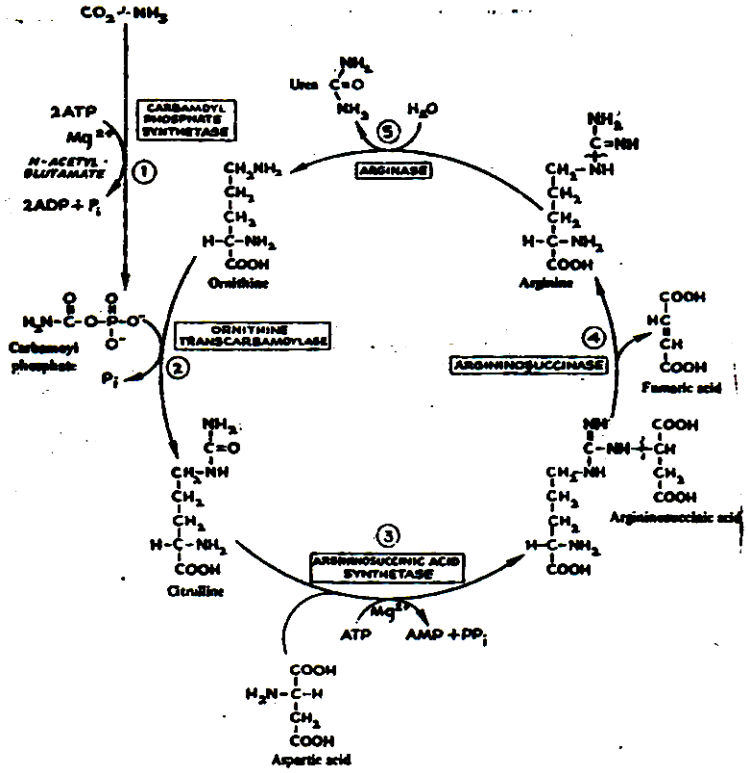
(3) The conversion of glucose 6-P to **glucose** is catalysed by **glucose6-phosphatase.** It is present in intestine platelet, liver and kidney where it allows these particular tissues to add glucose to the blood.

6. Describe the urea cycle.

A moderately active man consuming about 300g of carbohydrate 100g of fat and 100g of protein daily must excrete about 16.5g of nitrogen daily. The **major pathway of nitrogen excretion in humans is as urea** synthesized in the liver, released into the blood and clear by the kidney. In humans eating an occidental diet urea constitutes 80-90% of the nitrogen.

Reaction of the Urea Cycle.

The reaction s and intermediates in biosynthesis of 1mol of urea fom 1mol each of ammonia carbon dioxide and of the α-aminonitrogen of aspirate. Th e overall process requires 3mols of ATP and the successive participation of 5 enzymes catalyzing the numbered reactions. Of the 6 aminoacids involved in urea synthesis ,one(N-acetyl-glutamate) functions as an enzyme activator rather than as an intermediate. The remaining 5- aspirate arginine, ornithine, citruline and arginosuccinate- all functions as carriers of atoms which ultimately become urea. Two occur in proteins remaining three do not. The major metabolic role of these latter 3 in mammals is urea synthesis . note that urea formation is in part a **cyclical process.** The oornithine used in the reaction 2 is generated in reaction 5. There is no netloss or gain of ornithine, citruline, arginosuccinate or arginine during urea synthesis. However ammonia, CO_2 , ATP and aspirate are consumed.



Reaction 1: Synthesis of carbamoyl phosphate.

Condensation of 1mol each of ammonia, carbondioxide and phosphate to form carbamoyl phosphate is catalysed by **carbamoyl phosphate synthatase** a enzyme liver mitochondria. Of all ureotelic organisms. 2mols of ATP is hydrolysed during the reaction provide the driving force for the synthesis of two covalent bods - -the amide bond and the mixed carboxyluic acid phosphoric acid anhydride bond of carbamoyl phosphate.

In bacteria glutamine rather than ammonia serves as a substrate for carbamoyl phosphate synthesis.

Reaction 2: Synthesis of citruline.

Transfer of a carbamoyl moiety from carbamoylphosphate to ornithine forming citruline is catalysed by **ornithine transcarboxylase** of mitochondria.

Reaction 3: Synthesis of argininosuccinate.

In the **argininosuccinate synthetase reaction** aspirate and citruline are linked together via the amino grp of aspirate. The reaction requires ATP and the equilibrium strongly favours argininosuccinate.

Reaction 4: Cleavage of argininosuccinate to arginine and fumarate.

Reversisble cleavage of argininosuccinateto arginine plus fumarate is catalysed by **argininosuccinase,** a cold =liable enzyme of mammalian liver and kidney tissues. Loss of activity in the cold associated with dissociation into 2 protein components is prebented by Pi arginine and argininosuccinate or by p- hydroxymercuribenzoate which has no adverse effect on activity. The reaction proceeds via a *trans* elimination mechanism. The fumarate formed may be converted to oxaloacetate via the fumarase andmalate dehydrogenase reactions and then transaminated to regenerate aspirate.

Reaction 5: Cleavage of arginine to ornithine and urea.

This reaction completes the urea cycle and regenerates ornithine a substrate for reaction-2.Hydrolytic cleavage of the guanidine grp of arginineis catalysed by **arginase,** present in the livers of all ureotic organism. Smaller quantities of arginase also occur in renal tissues, brain, mammary gland, testiculartissues and skin. Mammalian liver arginase is activated by Co^{2+} or Mn^{2+}. Ornithine and lysine are potent inhibitors competitive with arginine the cell as is evident from the ATP-ADP cycle.

7. Describe the biosynthesis of glycogen ..

Gylcogenesis:

Glucose is phosphorylated to glucose 6- phosphate,a reaction that is common to the first reaction in the pathway of glycosis from glucose. Glucose 6- phosphate is then converted to glucose 1- phosphate in a reaction catalyzed by the enzyme phosphoglucomutase.The enzyme itself is phosphorylated & the phosphor group takes part in a reversible reaction in which glucose 1,6-bisphosphate is an intermediate.

Enz-P+ G-6-P ←--→Enz+Glucose 1,6 bis phosphate ←→Enz-P+ Glucose 1 phosphate.

Glucose 1 phosphate react with uridine triphosphate (UTP) to form the nucleotide uridine diphosphate glucose(UDPG).
The reaction between Glucose 1 phosphate and uridine triphosphate (UTP) is catalyzed by the enzyme UDPG pyrophosphorylase.

UTP+ Glucose 1 phosphate←--→ UDPG+ PPi

The subsequent hydrolysis of inorganic pyrophosphate by inorganic pyrophosphatase pulls the reaction to the right of the equation.

By the action of the enzyme glycogen synthetase, the C_1 of the activated glucose of UDPG forms a glycosidic bond with the C_4 of a terminal glucose residue of glycogen ,liberating UDP. The glycogen primer may in turn form a protein backbone, which may be a process similar to the synthesis of glycoproteins.

UPDG + $(C_6)n$ --→ UDP+ $(C_6)n+1$
Glycogen glycogen

The addition of a glucose residue to a preexisting glycogen chain occur at the non reducting outer end of the molecule so that the branches of the glycogen tree become elongated as a successive -1,4- linkages occur. When the chain has been lengthened to between 6 & 11 glucose residues a second enzyme, the branching enzyme acts on the glycogen. This enzyme transfers a part of the -1,4- chain to a neighbouring chain to form a -1,6 linkage, thus estabilishing a branch point in the molecule.

The action of the branching enzyme has been studied in the living animal by feeding C-labeled glucose and examining the liver glycogen at intervals thereafter. At first only the outer branches of the chain are labeled, indicating that the new glucose residues are added at this point. Later some of these outside chains are transferred to the inner portion of the molecule ,appearing as labeled- 1,6 linked branches.Thus under the combined action of glycogen synthetase & branching enzyme ,the glycogen molecule is assembled.

It will seen to be a branched polysaccharide composed entirely of α-D –glucose units. These glucose units are connected to one another by glucosidic linkages between the first and fourth carbon atoms except at branch points, where the linkages are between carbon atoms of 1 & 6. The molecular weight of glycogen may vary from 1 million to 4 million or more. A maximum molecular weight of 10-20 $x10^6$ would be possible because of the fact that the molecule would becomes more dense toward the periphery.

Glycogen synthetase activation & in activation :

In muscle, glycogen synthetase is present in 2 interconvertible forms:synthetase D(dependant), which is totally dependant for its activity on the presence of glucose 6- phosphate. And synthetase I (independent), whose Km for UDPG decreases in the presence of glucose 6-phosphate. Only the latter effect occurs with physiologic concentrations of glucose 6 –phosphate, implying that the synthetase I is the active form of the enzyme. Synthetase D is converted to synthetase I by synthetase phosphatase, a reaction involving dephosphorylation of a serine residue within the enzyme protein.Synthetase I is phosphorylated to form synthetase D ,with ATP acting as a phosphorylated donor, by an enzyme of rather wide specificity, cAMP-dependant protein kinase, which is active only in the presence of 3' , 5' –cyclic adenylic acid or cyclic AMP

cAMP is the intra cellular intermediate compound through which many hormones appear to act.It is formed from ATP by an enzyme adenylate cyclase, occurring in cell membranes. Adenylate cyclase is activated by hormones such as epinephrine, nonepinephrine, & glucagons, all of which lead to an increase in cAMP. cAMP is destroyed by phosphodiesterase, & it is the activity of this enzyme that maintains the level of cAMP at its normally low level. Insulin has been reported to increase its activity in liver. Thyroid hormones may increase the synthesis of adenylate cyclase, thus potentiating the effects of epinephrine in stimulating the formation of cAMP.

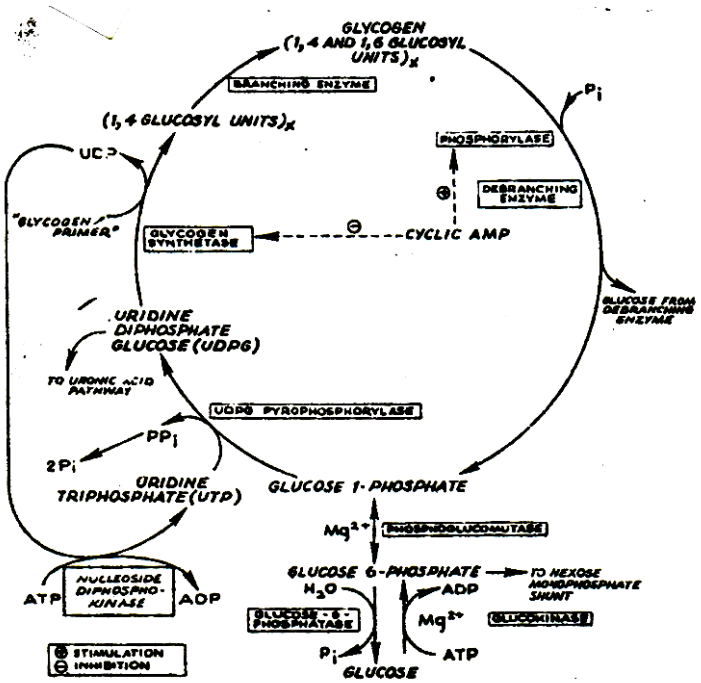


Figure: Pathway of glycogenesis and of glycogenolysis in the liver. Two high – energy phosphate bonds are used in the poration of 1 mol of glucose into glycogen.

In liver ,glycogen synthetase also exists in an active **a** form as well as an inactive **b** form ; **b** formed from **a** by phosphorylation of the enzyme protein in a reaction catalyzed by cAMP-dependant protein kinase,and **a** is formed from **b** by the action of synthetase phosphotase.

8. Explain the details about the biosynthesis of lipids.

The dietary carbohydrates and amino acids when consumed in excess can be convertad to fatty acids and stored as triacylglycerols. ***Denovo*** synthesis of fatty acids occurs predominantly in liver, kidney adipose tissues and lactating mammary glands. The enzyme machinery for fatty acid production is located in the Cytosomal fraction of the cell. Acetyl CoA is the source of carbon atoms while NADPH provides the reducing equivalents and ATP supplies energy for fatty acids formation. The fatty acid synthesis may be learnt in three stages.

I. Production of acetyl CoA and NADPH
II. Conversion of acetyl CoA to malonyl CoA
III. Reaction of fatty acid synthase complex.

I. Production of acetyl CoA and NADPH

Acetyl CoA and NADPH are the prerequisites for fatty acid synthesis. Acetyl CoA is produced in the mitochondria by the oxidation of pyruvate and fatty acids degradation of carbon skeleton of certain amino acids and from ketone bodies. Mitochondria however are not permeable to acetyl CoA. An alternate or a bypass arrangement is made for the transfer of acetyl CoA to cytosol. Acetyl CoA condenses with oxaloacetate in mitochondria to form citrate. Citrate is freely transported to cytosol where it is cleaved by **citrate lyase** to liberate acetyl CoA and oxaloacetate. Oxaloacetate in the cytosol is converted to malate.

II. Formation of malonyl CoA.

Acetyl CoA is carboxylated to malonyl CoA by the enzyme **acetyl CoA carboxylase.** This is an ATP independent reaction and requires ***biotin*** for the CO_2 fixation. Acetyl CoA is a regulatory enzyme in fatty acid synthesis.

III. Reactions of fatty acid synthase complex.

The remaining reactions of fatty acids synthesis are catalysed by a multifunctional enzyme known as ***fatty acid synthase(FAS)*** complex. In eukaryotic cells including man the fatty acid aynthase exists as a dimmer with 2 identical units. Each monomer possess the activities of seven diff enzymes and an ***acyl carrier protein*** bound to 4-phosphopantetheine. Fatty acids synthase functions as a single unit catalyzing all the seven reactions. Dissociation of the synthase complex results in loss of the enzyme activities. In the lower organisms the fatty acid synthesis is carried out by a multienzyme complex in association with a separate acyl carrier protein. This is in contrast to eukaryotes where ACP is a part of fatty acid synthase.

1. The 2carbon fragment of acetyl CoA is transferred to ACP of the fattyacid synthase catalysed by the enzyme **acetylCoA-ACP transacylase.** The acetyl units is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme **malonyl CoA-ACP transacylase** transfers malonate from malonyl CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transfered to malonyl grp. The malonyl moiety loses CO_2 which was added by acetyl CoA carboxylase. Thus CO_2 is never incorporated into fatty acid carbon chain. The decarboxylation is accompanied by the loss of free energy which allows the reaction to proceed forward. This reaction is catalysed by β-**ketoacyl ACP synthase.**

4. β-**ketoacyl ACP** reductase reduces ketoacyl grp to hydroxyacyl grp. The reducing equivalents are supplied by NADPH.

5. β-Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and double bond is introduced between α and β carbons.

6. A second NADPH dependent reduction catalysed by **enoyl-ACP reductase** occurs to produce acyl ACP. The four-carbon unit attached to ACP is butyryl group.

The carbon chain attached to the ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Ach time the fatty acid chain is lengthened by a two-carbon unit. At the end of 7cycles the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid – namely palmite –palmitoyl- bound to ACP is produced.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

Summary of palmitate synthesis.

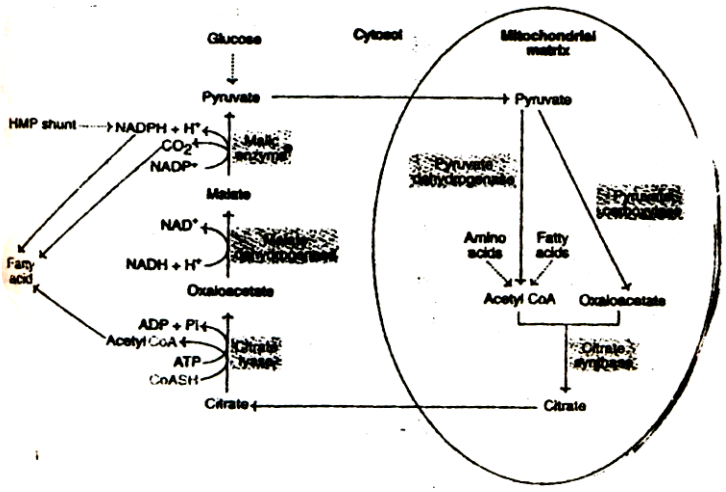
Of the 16carbon atoms present in palmitate only 2 come from acetyl CoA. The remaining 14 are from malonyl CoA which in turn is produced by acetyl CoA. The overall reaction of palmitate synthesis is summarized

8 Acetyl CoA + 7 ATP + 14 NADPH + H^+ → palmitate + 8 CoA + 7 ADP + 7 Pi + 6 H_2O.

Fatty acid synthase complex

Fatty acid synthase is a **dimer** composed of 2 identical subunits, each with a molecular weight of 240,000. Each subunit contains the activities of the 7enzymes of FAS and ACP with 4-phosphopantetheine –SH grp.

Each monomer of FAS contains all the enzyme activities of fatty acids synthesis. But only the dimmer is functionally active. This is because the functional unit consists of half of each subunit interacting with the complementary half of the other. The 2 functional units of FAS independently operate and synthesize 2 fatty acids simultaneously.



Functional significance of FAS complex

The organization of diff enzymes of a metabolic pathway into a single multienzyme functional unit has a distinct advantages for cellular function

1. The FAS complex offers *great efficiency* that is free from interference of other cellular reactions for the synthesis of fatty acids.

2. Since the entire process of the metabolic pathway is confined to the complex there are no permeability barriers for the various intermediates.

3. The multienzyme polypeptide complex is coded by a single gene. Thus there is a good coordination in he synthesis of all enzymes of FAS complex.

UNIT – V

PART – A

1. Give the three stages of respiration in cells.

1. First Stage : Oxidation decarboxylation of pyruvate to acetyl co A and CO2 .
2. Second stage : Citric acid cycle or acetyl coA catabolism.
3. Third stage : Electron transport and oxidative phosphorylation.

2. Define amphibolic pathway.

The citric acid cycle has a dual or amphibolic (amphi = both) nature. The cycle functions not in the oxidative catabolism of carbohydrates , fatty acids and amino acids but also as the first stage in many biosynthetic (= anabolic) pathways for which it provides precursors.

3. Give the energy yield of glycolysis.

S.No	Reaction	ATP Level	No.of ATPs
1	**ATP Production**		
	a. Glyceraldehyde 3–phospo dehydrogenase	Oxidation of 2NADH	6
	b. Phospho glycerate kinase	Oxidation at Substrate level	2
	c. Pyruvate kinase	Oxidation at substrate level	2
2.	**Consumption** Hexokinase & phosphor		10
			-2
		Net	**8**

4. Define Matrix.

The aqueous contents of a cell or organelle (the mitochondrion , for example) with dissolved solutes.

5. Define ATP Synthetase or F0 F1 ATPase.

The mitochondrial inner membrane contains the ATP synthesizing enzyme complex called ATP synthetase or F0 F1 ATPase .

This enzyme complex has 2 major components F0 and F1 (F for factor)

6. Define Electron transport system (ETS).

A collective term describing a sequence of membrane – associated electron carriers , generated by the citric acid cycle , that use the energy from electron flow to transport protons against a concentration gradient across the inner mitochondrial membrane .

7. Define Electron Carior Protien.

A protein, such as a Flavoprotien or a cytochrome , that can reversely gain an lose electrons; functions in the transfer of an electron from a donor molecule to an acceptor molecule
.

8. What is ATP phosphohydrolase (ATPase)?

One of a large class of enzymes that catalyze a process involving the hydrolysis of ATP. The energy so released is used to actively transport ions or other solutes against their concentration gradient.

9. Define ATP synthetase.

Enzyme Complex in the inner membrane of the mitochondrion and the thylakoid membrane of a chloroplast that catalyzes the formation of ATP from ADP and inorganic phosphate during oxidative phosphorylation and photosynthesis, respectively. Also present in plasma membrane of bacteria.

10. What is cytochrome oxidase?

An enzyme acting as the last hydrogen electron carrier in the electron transport system , receiving an electron from cytochrome and passing it on to oxygen , with the formation of water.

11. Define Cytochromes.

The coloured heme- containing metalloproteins that serve as electron carriers during cellular respiration , photosynthesis and other oxidation – reduction reaction; usually designated as cytochromes a, b , and c.

12. Define High – energy Compound.

A Compound that on hydrolysis undergoes a large decrease in free energy under standard conditions.

13. Define High – energy bond.

A covalent bond that has a low activation energy and is broken easily and which on hydrolysis releases an unusually large amount of free energy under the conditions existing in a cell. A group linked to a molecule by such a bond is readily transferred from one molecule to another ; common examples are the phosphodiester bonds in ATP and the thioester linkage in acetyle Co A

14. What is Iron – Sulfur Centre (Fe – S Centre)?

A prosthetic group of certain redox proteins involved in electron transfers; Fe2+ and Fe3+ is bound to inorganic sulfur and cystiene group in the protein.

15. Define Oxidative phosphorylation.

Phosphorylation of ADP to ATP that uses energy from a proton pump fueled by the electron transport system. The process is the major means by which aerobic organisms obtain their energy from food stuffs.

16. Define Proton – Motive Force.

The electro chemical potential inherent in a transmembrane gradient of H+ concentration , used in oxidative phosphorylation and photo phosphorylation to drive ATP synthesis.

17. Define Redox potential.

A quantitative measurement of the willingness of an electron carrier to act as a reducing or oxidizing agent. Redox potential is measured in volts . The more –ve the value the better the carrier will act as a reducing agent . Thus in an electron transport system the carriers are arranged in order of increasing redox potentials
(negative to positive).

18. Define Respiratory chain.

The electron transport chain, a sequence of electron – carrying proteins that transfer electrons from substrates to molecular oxygen in aerobic cells.

19. Define Substrate – level phosphorylation.

Phosphorylation of ADP (or some other nucleoside 5 – diphosphate) coupled to the dehydrogenation of an organic substrate , results in ATP production , independent of the electron transport system used in oxidative phosphorylation .

20. Define Ubiquinone.

A liquid – soluble quinone; ubiquinone accepts electrons from electron donors like NADH and from the oxidation of fatty acids; also called Coenzyme Q.

21. Define Ubiquitin.

A small, highly conserved protein present in all eukaryotic cells that becomes covalently attached to lysines of other proteins. Attachment of a chain of ubiquitones tags a protein for intracellular proteolytic destruction in a proteasone.

PART – B

1. Describe the citric acid cycle and its energetics.

The citric acid cycle (Krebs cycle, tricarboxylic acid cycle) is a series of reactions in Mitochondria that bring about the catabolism of acetyl residues, liberating hydrogen equivalents, which upon oxidation, lead to the release of most of the free energy of tissue fuels. The acetyl residues are in the form of acetyl –CoA (CH_3 -CO~S-CoA, active acetate) an ester of coenzyme A which with other acyl thioesters of CoA is classified as a High energy compound.

Significance of the citric acid cycle

Essentially, the cycle comprises the combination of a molecule of acetyl-CoA with the 4-carbon dicarboxylic acid oxalo acetate, resulting in the formation of a **6-carbon tricarboxylic acid, citric.** There follows a seris of reactions in the course of which 2 molecules of CO_2 are lost and oxaloacetate is regenerated. Since only small quantity of oxalo acetate is able to facilitate the conversion of large quantity of acetyl units toCO_2, oxalo acetate maybe considered to play a **catalytic role.**

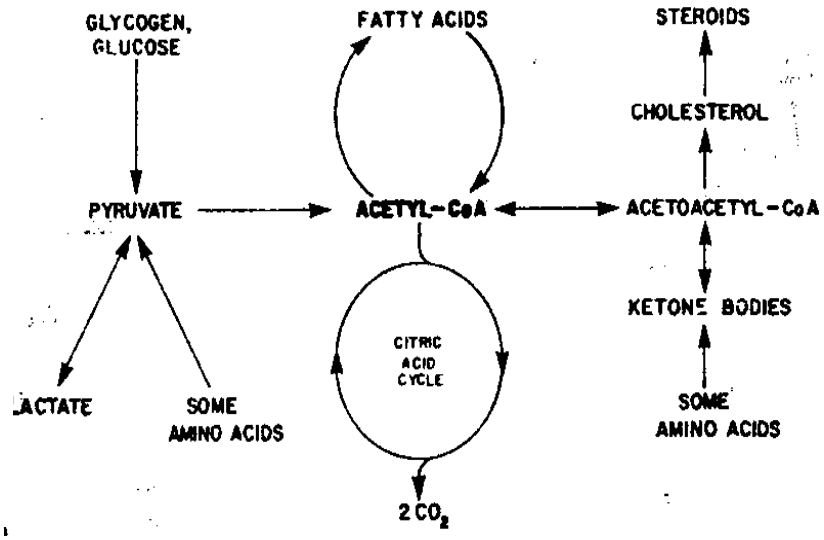
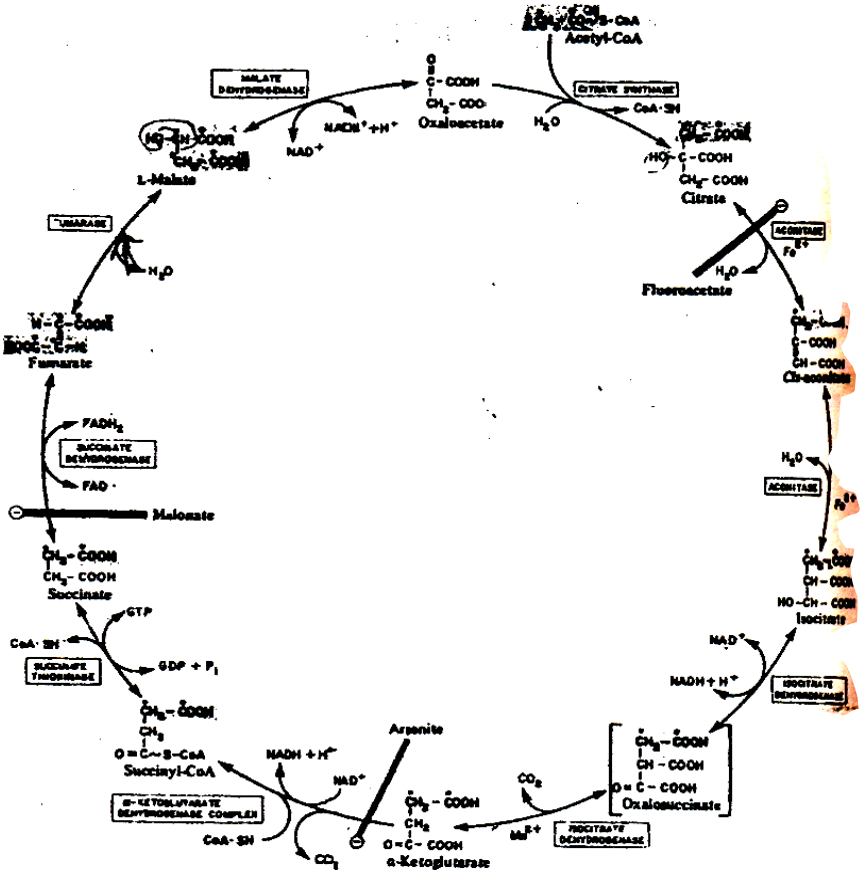


Figure: Overview of acetyl – CoA metabolism



Reactions of the citric acid cycle.

AcetylCoa +Oxaloacetate +H_2O --> Citrate + CoA.SH

The initial condensation of acetylCoA with oxaloacetate to form citrate is catalysed by a condensing enzyme, citrate synthase, which effects a carbon to carbon bond between the methyl carbon of oxaloaceteate. The condensation oreaction is followed by the hydrolysis of the thioester bond of CoA, which is accompanied by considerable loss of free engery as heat, ensuring that the reaction goes to completion.

Citrate is converted into isocitrate by the enzyme **aconitase** which contains iron in the Fe^{2+} state. This conversion takes place in two steps, dehydration to *cis*-aconitase, some of which remains bound to the enzyme and rehydration to iso citrate.

Citrate← → *cis*-aconitase ← →Isocitrate
H_2O

The reaction is inhibited by the presence of **fluofoacetate**, which in the form of fluoroacetyl-CoA, condenses with oxaloacetate to form fluorocitrate. The latter inhibits aconitase, causing citrate to accumulate.

Experiments using ^{14}C-labeled intermediates indicates that citrate reacts with aconitase in an asymmetric manner with the result that aconitase always acts on that part of the citrate molecule that this was due to a 3-point attachment of the enzyme to the substrate. The3-point attachment would enable aconitase to differentiate the two $-CH_2$ COOH grp in citrate, thus conferring asymmetry on a apparently symmetric molecule. However the 3-point attachment hyposis is noy necessary to explain the asymmetric action of aconitase. It is now realized that the two $-CH_2$ COOH grps are not identical in space with respect to the –OH and –COOH grps. The consequences of the asymmetric action of aconitase may be appreciated by the reference to the fate of labeled acetylCoA in the citric acid cycle. It is possible that *cis*-aconitase may not be an obligatory intermediate between citrate and isocitrate but may in fact be a side branch from the main pathway.

Isocitrate undergoes dehydrogenation in the presence of **isocitrate dehydrogenase** to form oxalosuccinate. Three different enzymes have been described. One which is NAD-specific is found only in mitochondria . The other two enzymes are NADP-specific and are found in the mitochondria and the cytosol respectively. Respiratory chain oxidation of isocitrate proceeds almostcompletely through the NAD-dependent enzyme.

Isocitrate + NAD^+ ←→ Oxalosuccinate ←→ α-ketoglutarate + CO_2 + NADH + H^+

There follows a decarboxylation to α-ketoglutarate also catalysed by the isocatrate dehudrogenase. Mn^{2+} IS AN IMPORTANT COMPONENT OF THE DECARBOXYLATION REACTION. It would appear that oxalosuccinate remains bound to the enzyme as an inter mediate in the overall reaction.

Next α-ketoglutarate undergoes **oxidative decarboxylation** in the manner that is analogous to the oxidative decarboxylation of pyruvate both substrates being α-ketoacids.

α-ketoglutarate + NAD^+ CoA.SH -→ Succinyl-CoA + CO_2 + NADH + H^+

The reaction catallysed by an **ketoglutarate dehydrogenase** complex also requires identical cofactor and results in the formation of succinyl CoA, a thioester containing a high energy bond. The equilibrium of this reaction is so much in favour of succinyl CoA formation that the reaction must be considered as physiologically unidirectional. As in the case of pyruvate oxidation,arsenic inhibits the reaction, causing the substrate,α-**ketoglutatrate,**to accumulate.

To continue the cycle, succinyl-CoA is coverted to succinate by the enzyme **succinate thiokinase(succinly-CoA synthetase).**

Succinyl-CoA+p_i+GDP←→ succinate+GTP+CoA.SH

This reaction requires GDP or IDP, which is converted in the presence of inorganic phosphate to either GTP or ITP, This is the only example in the citric acid cycle of the **generation of a high-energy phosphate at he substrate level** and arises because the releaseof free energy from the oxidative decarboxilation of α-ketoglutarate is sufficient to genetrate a high-energy bond in addition to the formation of NADH(equivalent to 3~P). By means of a phosphokinase, ATP may be formed from either GTP or ITP,

Eg. GTP+ADP←→GDP+ATP

An alternative reaction inextrapatic tissues, which is catalyzed by **succinyl-CoA-acetoacetate CoA transferase(thiophorase),** is the conversion of succinyl-CoA to succinate coupled with the conversion of acetoacetate to acetoacetyl-CoA. In liver there is alsodeacylase activity, causing some hydrolysis of succinyl-CoA to succinate plus CoA.
Succinate is metabokized further by undergoing a dehydrogenation followed by the dehydrogenation which regenerates oxaloacetate.

Succinate+FAD←→ Fumarate+$FADH_2$

The first dehydrogenation reaction is catalyzed by **succinate dehydrogenase,** which is bound to the inner surface of the inner mitochondrial membrane, It is the only dehydrogenation in the citric acid cycle that involves the **direct transfer of hydrogen from the substrate to a flavoprotein without the participation of NAD.** The enzyme contains FAD and Fe:S Protein. Fumarate is formed as a result of the dehydrogenation. Isotopic experiments have shown that the enzyme is stereospecific for the *trans* hydrogen atoms of the methylene carbons of succinate. Addition of malonate or oxaloacetate inhibits succinate dehydrogenase competitively, resulting in succinate accumulation,

Under the influence of **fumarase(fumarate hydratase),** water is addedto fumarate to give malate.

Fumarate+H_2o←→ L-Malate

In addition to bring specific for the L-isomer of malate, fumarase catalyzes the addition of the elements of water to the double bond of fumarate in the *trans* confugration. Malate is converted to oxaloacetate by **malate dehydrogenase,** a reactionrequiring NAD^+.

L-Malate+NAD^+ ←→ Oxaloacetate+NADH+H^+

Aithough the equilibriumof this reaction is much in favour of malate, the net flux is towards the direction of oxaloacetate because this compound together with the other product of the reaction (NADH) is removed continuously in further reaction

The enzymes participating in the citric acid cycle are also found outside the mitochondria expect for the α-ketoglutarate and succinate dehydrogenases. May not in fact be the same proteins as the mitochondrial enzymes of the same name.

Energitics of the Citric Aicd Cycle

As a result of oxidation catalyzed by dehydrogenase enzymes of the citric acid cycle, **3 molecules of NADH** and **one of $FADH_2$** Are produced for each molecule of acetyl-CoA catabolized in one revolution of the cycle. These reducing equivalents are transferred to respiratory chain in the inner mitochondrial membrane. During passage along the chain, reducing equivkents from NADH will generate 3high-energyphosphate bonds by the esterification of ADP to ATP in the process of oxidative phosphorylation. However, $FADH_2$ produces only 2 high-energy phosphate bonds because it transfer its reducing power to Q, thus by passing the first site for oxidative phosphorylation in the respiratory chain. Thus, 12 new high-energy phosphate bonds are generated for each turn of the cycle.

Reaction catalyzed by	Method of Prodution	Number of P formed
Isocitrate dehydrogenase	Respiratory chain oxidation of NADH	3
A-ketoglutarate dehydrogenase	Respiratory chain oxidation of NADH	3
Succinate thiokinase	Oxidation at substrate	1
Succinate dehydrogenase	Respiratory chain oxidation of $FADH_2$	2
Malate dehydrogenase	Respiratory chain oxidation of NADH	3
		Net 12

2. Describe the oxidation of pyruvate.

Pyruvate before entering into the citric acid cycle, it must be transported into the mitochondrion via a special pyruvate transporter that aids its passage across the inner mitochondrial membrane. Within the mitochondrion it is oxidatively decarboxylated into acetylCoA. This reaction is catalysed by the several different enzymes working sequentially in a multienzyme complex. They are collectively designated as the **pyruvate dehydrogenase** complex and are analogous to the α-ketoglutarate dehydrogenase complex of the citric acid cycle. Pyruvate is decarboxylated in the presence of thiamine diphosphate to a hydroxyethyl derivativeof the thiazole ring ofenzyme-bound thiamin diphosphate which in turn reacts with oxidized lipoate to form S-acetyl lipoate all catalysed by pyruvatedehydogenae. In the presence of **dihydrolipoyl transacetylase.** S-acetyl lipoate reacts with coenzyme A to form acetylcoA and reduced lipoate. The cycle of reaction is completed and latter is reoxidised in the presence of **dihydrolipoyl dehydrogenase.**

Pyruvate + NAD^+ + CoA → AcetylCoA + NADH + H^+ + CO_2

The pyruvate dehydrogenase complex consists of 29mol of pyruvate dehydrogenase and about 8mol of flavoprotein distributed around 1mol of transacetylase. Movement of the individual enzymes appear to restricted and the metabolic intermediates remain to the enzymes.

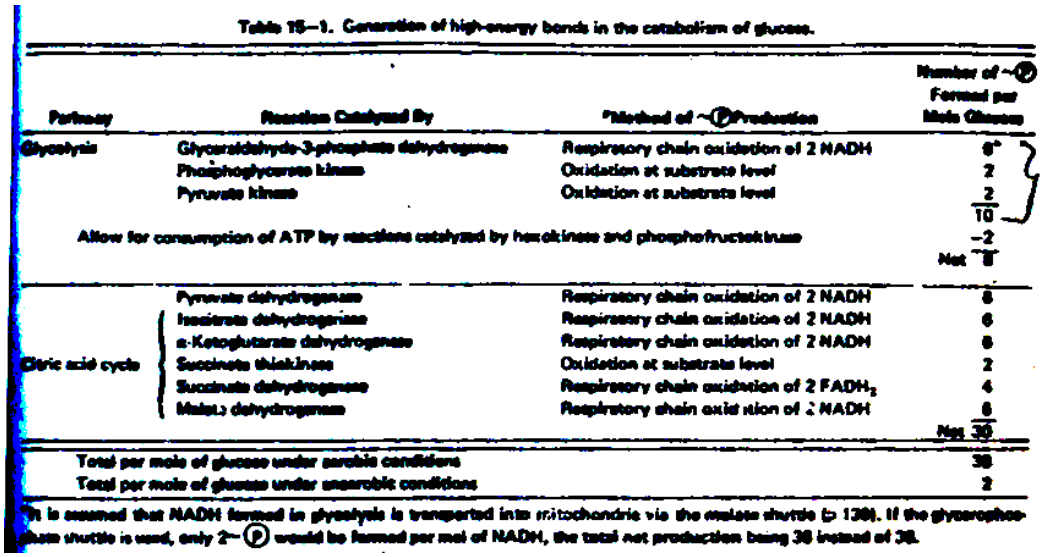
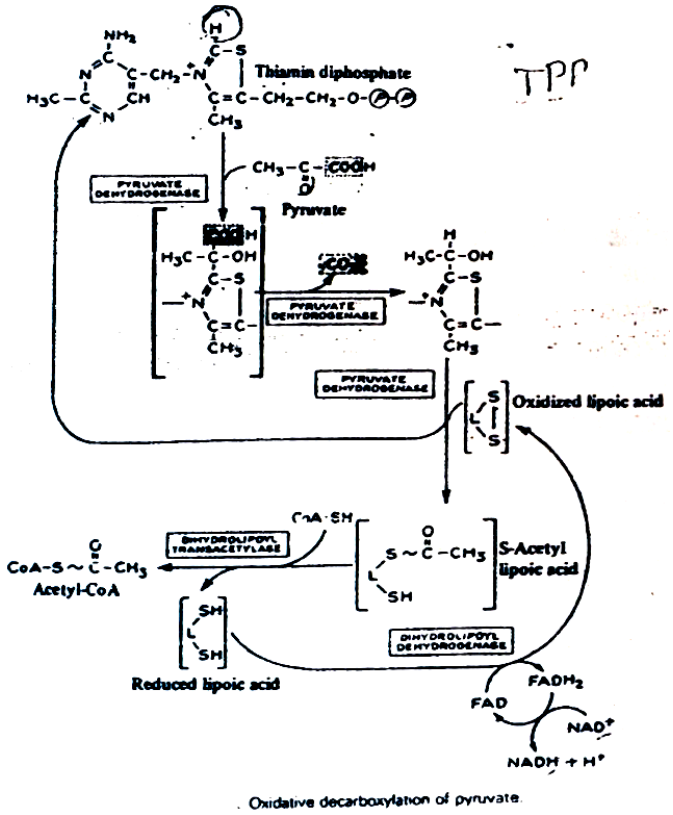


Table 15—1. Generation of high-energy bonds in the catabolism of glucose.

Pathway	Reaction Catalyzed By	Method of ~Ⓟ Production	Number of ~Ⓟ Formed per Mole Glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	6*
	Phosphoglycerate kinase	Oxidation at substrate level	2
	Pyruvate kinase	Oxidation at substrate level	2
			10
	Allow for consumption of ATP by reactions catalyzed by hexokinase and phosphofructokinase		−2
			Net 8
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
	Succinate thiokinase	Oxidation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $FADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH	6
			Net 30
Total per mole of glucose under aerobic conditions			38
Total per mole of glucose under anaerobic conditions			2

*It is assumed that NADH formed in glycolysis is transported into mitochondria via the malate shuttle (p. 138). If the glycerophosphate shuttle is used, only 2~Ⓟ would be formed per mol of NADH, the total net production being 36 instead of 38.



Oxidative decarboxylation of pyruvate.

It is to be noted that the pyruvate dehydrogenase is sufficiently electronegative with respect to the respiratory chain that in addition to the generating a reduced coenzyme it also generates a high energy thioester bond in the acetyl-CoA.

Arsenite or mercuric ions complex the –Sh grps of lipoic acid and inhibit pyruvate dehydrognase as does a dietry deficiency of thuamin allowing pyruvat to accumulate. Nutritionaly deprived alcohols are particularly susceptible to thiamin deficiency and if administered glucose exihibit rapid accumulation of pyruvate and lactic acidosis which can be life threatening. Mutations have been reported for virtually all of the enzymes of carbohydrate metabolim each associated with human disease.

3. Describe the high energy compounds and give examples?.

Certain compounds are encountred in the biological system which, on hydolysis, yield energy. The term high-energy compounds or energy rich compounds is usually applied to substances which posses sufficient free energy to liberate at least 7 Cal/mol at pH 7.0. Certain other compounds which liberate less than 7.0 Cal/mol(lower than ATP hydrolysis to ADP+ Pi) are reffered to as low-energy compounds.

All the high-energy compounds-when hydrolysed-liberate more energy than that of ATP. These include phosphoenol pyruvate, 1,3-bisphosphoglycerate, phosphocreatine etc. Most of the high-energy compounds contain phosphate group(exception acetyl CoA) hence they are called high-energy phosphate compounds.

Classification of high-energy compounds

There are at least 5 groups of high-energy compounds.

1. Pyrophosphates e.g. ATP.
2. Acyl phosphates e.g. 1,3-bisphosphoglycerate
3. Enol phosphates e.g. phosphoenolpyruvate.
4. Thioesters e.g acetyl CoA.
5. Phosphagens e.g. phosphocreatine.

High-energy bonds: The high-energy compounds posses acid anhydride bonds(mostly phosphoanhydride bonds) which are formed by the condensation of two acidic groups or related compound. These bonds are referred to s high-energy bons, since the free energy is liberated when these bonds are hydrolysed. Lipmann suggested use of the symbol ~ to represent high-energy bond. For instance, ATP is written as AMP~P~P.

ATP-the most important high-energy compound Adenosine triphosphate(ATP) is a unique and the most important high-energy molecule in the living cells. It consists of an adenine, a ribose and a triphosphate moiety. ATP is a high-energy compound due to the presence of two phosphoanhyride bonds in the triphosphate unit. ATP serves as the energy currency of the cell as is evident from the ATP-ADP cycle.

ATP-ADP Cycle

The hydrolysis of ATP is associated with the release of large amount of energy.

$ATP + H_2O \rightarrow ADP + Pi + 7.3$ Cal.

The energy liberated is utilized for various process like muscle contraction, active transport etc. ATP can also act as a donor of high-energy phosphate to low-energy compounds, to make them energy rich. On the other hand, ADP can accept high-energy phosphate from the compounds possessing higher free energy content to form ATP.

ATP serves as an immediately available energy currency of the cell which is constantly being utilized and regenerated. This is represented by ATP-ADP cycle, the fundamental basis of energy exchange reactions in living system. The turnover of ATP is very high.

ATP acts as an energy link between the catabolism (degradation of molecules) and anabolism (synthesis) in the biological system.

Synthesis of ATP

ATP can be synthesized in two ways

1. Oxidative phosphorylation: This is the major source of ATP in aerobic organisms. It is linked with the mitochondrial electron transport chain (details described later).

2. Substrate level phosphorylation : ATP may be directly synthesized during substrate oxidation in the metabolism. The high-energy compounds such as phosphoenolpyruvate and 1,3-bisphosphoglycerate(intermediates of glycolysis) and succinyl CoA(of citric acid cycle) can transfer high-energy phosphare to ultimately produce ATP.

Storage forms of high-energy phosphates

Phosphocreatine(certain phosphate) stored in vertebrate muscle and brain is an energy-rich compound. In invertebrates, phosphoarginine(arginine phosphate) replaces phosphocreatine.

4. Describe Electron Transport Chain.

The energy-rich carbohydrates(particularly glucose), fatty acids and amino acids undergo a series of metabolic reactions and finally, get oxidized to CO_2 and H_2O. The reducing equivalents from various metabolic intermediates are transferred to coenzymes NAD^+ and FAD to produce, respectively, NADH and $NADH_2$. The latter two reduced coenzymes pass through the electron transport chain(ETC)or respiratory chain and finally, reduce oxygen to water. The passage of electrons through the ETC is associated with the loss of free energy. A part of this free energy is utilized to generate ATP from ADP and Pi.

Mitochondria-the power houses of cell

The mitochodria are the centers for metabolic oxidative reactions to generate reduced coenzymes(NADH and $FADH_2$) which, in turn, are utilized in ETC to liberate energy in the form of ATP. For this reason, mitochondrion is appropriately regarded as the power house of the cell.

Mitochondrial organization

The mitochondrion consists of five distinct parts. These are the outer membrane, the inner membrane, the intermembrane space, the cristate and the matrix.

Inner mitochondrial membrane: The electron transport chain and ATP synthesizing system are located on the inner mitochondrial membrane which is a specialized structure, rich in proteins. It is impermeable to ions(H^+,K^+,Na^+) and small molecules (ADP, ATP). This membrane is highlyfolded to form cristae. The surface area of inner mitochondrial membrane possesses specialized particles(that look like lollipops), the phophorylating subunits which are the centres for ATP production.

Mitochodrial matrix: The interior ground substance forms the matrix of mitochondria. It is rich in the enzymes responsible for the citric acid cycle, β-oxidation of fatty acids and oxidation of amino acids.

Structural organization of respiratory chain

The inner mitochondrial membrane can be disrupted into five distinct respiratory of enzyme complexes, denoted as complex I, II, III, IV and V. The complexes I-IV are carries of electron while complex V is responsible for ATP synthesis. Besides these enzyme complexes, there are certain mobile electron carries in the respiratory chain. These include NADH, coenzyme Q, cytochrome C

and oxygen.

The enzyme complexes(I-IV) and the mobile carriers are collectively involved in the transport of electrons which, ultimately, combine with oxygen supplied to the body is utilized by the mitochondria for the operation of electron transport chain.

Components and reactions of the electron transport chain

There are five distinct carriers that participate in the electron transport chain (ETC). These carriers are sequentially arranged and are responsible for the transfer of electrons from a given substrate to ultimately combine with proton and oxygen to form water.

1. Nicotinamide nucleotides

Of the two coenzymes NAD^+ and $NADP^+$ derived from the vitamin niacin, NAD^+ is reduced to NADH + H^+ by dehydrogenases with the removal of two hydrogen atoms from the substrate(AH_2).The substrates include glyceraldehydes-3 phosphate, pyruvate, isocitrate, α-ketoglutarate and malate.

$AH_2 + NAD^+ \leftrightarrow A + NADH + H^+$

NADPH+ H^+ produced by $NADP^+$- dependent dehydrogenase is not usually a substrate for ETC. NADPH is more effectively utilized for anabolic reactions (e.g. fatty acid synthesis, cholesterol synthesis).

II. Flavoproteins

The enzyme NADH dehydrogenese (NADH- coenzyme Q reductase) is a flavoprotien with FMN as the prosthetic group. The coenzyme FMN accepts two electrons and a proton to form $FMNH_2$. NADH dehydrogenase is a complex enzyme closely associated with non-heme iron proteins (NHI) or iron-sulfur proteins (FeS).

$NADH + H^+ + FMN \rightarrow NAD^+ + FMNH_2$

Succinate dehydrogenase(succinate-coenzyme Q reductase) is an enzyme found in th inner mitochondrial membrane. It is also a flavoprotein with FAD as the coenzyme. This can accept two hydrogen atoms ($2H^+ + 2e^-$) from succinate.

Succinate + FAD → Fumarate + $FADH_2$

III. Iron-Sulfur Proteins

The iron-sulfur (FeS) proteins exist in the oxidized (Fe^{3+}) or reduced (Fe^{2+}) state. About half a dozen FeS protein connected with respiratory chain have been identified. However the mechanism of action of iron-sulfur proteins in the ETC is not clearly understood.

One FeS participates in the transfer of electrons from FMN to coenzyme Q. Other FeS proteins associated with cytochrome b and cytochrome c_1 participate in the transport of electron.

IV. Coenzyme Q

Coenzyme Q is also known as ubiquinonw since it is ubiquitous in living system. It is a quinine derivative with a variable isoprenoid side chain. The mammaliam tissues possess a quinine with 10 isoprenoid units whichis known as coenzyme Q_{10} (CoQ_{10}).

Coenzyme Q is a lipophilic electron carrier. It can accept electrons from $FMNH_2$ produced in the ETC by NADH dehydrogenase or $FADH_2$ produced outside ETC(e.g. succinate dehydrogenase, acyl CoA dehydrogenase).

Coenzyme Q is not found in mycobacteris. Vitamin K perform similar function as coenzyme Q in these organisms. Coenzyme Q has no known vitamin precursor in animals. It is directly synthesized in the body.

V. Cytochrome

The cytochrome are conjugated proteins containing heme group. The latter consists of a porphyrin ring with iron atom, The heme group of cytochromes differ from that found in the structure of hemoglobin and myoglobin. The iron of heme in cytochromes is alternately oxidized (Fe^{3+}) and reduce (Fe^{2+}), which is essential for the transport of electrons in the ETC. This is in contrast to the heme iron of hemoglobin and myoglobin which remains in the ferrous(Fe^{2+}) state.

Three cytocrome were initially discovered from the mammalian mitochondris. They were designated as cytochrome a, b and c depending on the type of heme present and the respective absoption spectrum. Additional cytochromes such as $c_{1,}b_{1,}b_{2,}a_3$ etc. were discovered later.

The electron are transported from coenzyme Q to cytochrome (in the order) b,c_1, c,a and $a_{3.}$ The property of reversible oxidation-reduction of heme iron $Fe^{2+} \leftrightarrow Fe^{3+}$ present in cytochromes allows them to function as effective carriers of electron in ETC.

Cytochrome c (mol.wt. 13,000) is a small protein containing 104 amino acids and a heme group. It is a central member of ETC with an intermediate redox potential. It is rather than loosely bound to inner mitochondrial membrane and can be easily extracted.

Cytochrome a and a_3 : The term cytochrome oxidase is frequently used to collectively represent cytochrome a and a_3 which is the terminal component of ETC. Cytochrome oxidase is the only electron carrier, the heme iron of which can directly react with molecular oxygen.Besides heme (with iron), this oxidase also contains copper that undergoes oxidation reduction ($Cu^{2+} \leftrightarrow Cu^{+}$) during the transport of electrons.

In the final stage of ETC, the transport electrons, the free protons and the moleculas oxygen combine to produce water.

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LECTURE NOTES

REGULATION 2021

II YEAR & III SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

UNIT – I

PART - A

1. Define fermentation:

The anaerobic breakdown by micro organisms of complex organic substance, especially carbohydrates like glucose. The process is energies yielding.

Eg: Alcohd fermentation

$$\text{Glucose} \xrightarrow[\text{(Sacharomyces cervisiae)}]{\text{Yeast}} \text{Ethyl alcohol} + 2Co2$$

2. Define sterilization.

Sterilization refers to any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses) from a surface, equipment, food, medication or biological medium sterilization can be achieved through application of heat, chemicals, irradiation or filtration.

3. What are the basic steps involved in the operation of a bioreactor?

The basic steps involved in the bioreactor operation are,

(i) Sterilization
(ii) Inoculum and sampling
(iii) Aeration
(iv) Control system
(v) Cleaning

4. What is meant by bioreactor / fermentor?

A container, such as a large fermentation chamber, for growing living organisms that are used in the industrial production of substances such as pharmaceuticals, antibodies or vaccines.

OR

The biotechnological process is carried out in a specially designed container where the correct environment for optimization of growth and metabolic activity can be possible to produce the commercial product. That container is called fermenter.

5. What is downstream processing? Give an example?

The process used for actual recovery of the desired product after the completion of fermentation is called downstream processing.

e.g. filtration, centrifugation, extraction, crystalization, drying, etc.

6. What are the unit operations involve in the fermentation processes?

The major unit operations involved in the fermentation process are,

(1) Filtration
(2) Centrifugation
(3) Coagulation & Flocculation
(4) Liquid-Liquid extraction
(5) Aqueous two phase extraction
(6) Adsorption
(7) Dialysis
(8) Ultrafiltration and micro filtration
(9) Chromatography
(10) Crystallization

7. Name major steps involved in the separation of fermentation products.

The major categories of separation of fermentation product are

(i) Separation of biomass or other insoluble product
(ii) Concentration or primary isolation of product and
(iii) Purification

8. Write short notes on filter sterilization.

In addition to steam sterilization, process fluids can be filter sterilized. Filter sterilization is necessary when the medicine contains heat sensitive materials such as vitamins, antibiotics. An important example of filter sterilization in bioprocesses is the sterilization of media to support the growth of animal cells. Micro process filters (pre sizes <0.2mm) are typically used. Filter sterilization is also used to sterilize process air.

9. Define industrial biotechnology.

Industrial biotechnology is the application of biotechnology for industrial purposes, including manufacturing alternative energies (or "bioenergy"), and biomaterials. It includes the practice of using cells or components of cells like enzymes to generate industrially useful products.

10. What is meant by traditional Biotechnology?

Traditional biotechnology refers to early forms of using living organisms to produce new commodities or modify existing ones. The development and modification were achieved at the organism, not cellular level. It includes such techniques as selective breeding, fermentation and hybridization.

11. What is modern biotechnology?

Modern biotechnology refers to techniques that are used to intentionally manipulate genes cells or living tissue in a predictable and controlled manner to produce new tissue or to generate change in the genetic make-up of an organism. Some examples include recombinant DNA technology and tissue culture.

12. What is meant by recombinant DNA technology?

A set of techniques which enable one to manipulate the DNA. One of the main techniques is DNA cloning. It produces an unlimited number of copies of a particular DNA segment, and the result is called DNA clone. An organism manipulated using recombinant DNA techniques is called a genetically modified organisms (GMO). Among other things, recombinant DNA technology involves identifying genes, cloning genes, studying the expression of cloned genes and producing large quantities of the desired gene product.

13. Define mutant strain.

An organism or an allele that difference from the wild type because it carries one or more genetic changes in its DNA or its DNA sequences. A mutant organism may carry mutated genes (s); mutated chromosomes); or mutated genome (s).

14. What are the major types of organisms used in the fermentation process?

A wide range of organisms can be used in fermentation for the production of various chemicals and antibiotics. It includes Bacteria, yeast, fungi, plant cells, mammalian cell culture and Algae.

15. Give any three examples for anaerobic fermentation products.

Anaerobic fermentation products:

1) Ethanol
2) Lactic acid
3) Acetone and butanol

16. Name two aerobic fermentation processes.

Aerobic fermentation processes:

1) Citric acid production
2) Production of Baker's yeast
3) Production of penicillin
4) Production of High fructose corn syrup

17. What is Tissue Engineering?

Tissue engineering has a primary focus on developing invitro bioartifical tissues, typically based on cells derived from donor tissue. These tissues can be used as transplants to improve biological function in the recipient. Commercial examples include living skin tissue and chondrocytes implanted in a damaged knee for production of hyaline like cartilage.

18. List commercially important fermentation products.

Antibiotics, organic solvents, Beverage, food products, flouring agent, organic acids, Amino acids, vitamins and other growth stimulants, enzyme, fats, Bacterial insecticides and other chemical intermediates.

19. Mention the different ranges of fermentation process.

The range of fermentation process can be classified into,

(i) Microbial cells (or Bimodal)
eq: Baker's yeast, SCP (single cell protein).

(ii) Microbial enzymes
eq: Protease, amylase, cellulose

(iii) Microbial metabolites
eq: primary metabolites, secondary metabolites

(iv) Transformation products
eq: Steroids, Antibiotics:

20. How microbial strains are selected in a given fermentation process?

The most important factor for the success of any fermentation industry is of a production strains. The production strain must possess the following four characteristics:

(i) It should be a high yielding strain
(ii) It should have stable biochemical characteristics
(iii) It should not produce undesirable substances
(iv) It should be easily cultivated on a large scale.

21. Define batch fermentation.

Batch fermentation is a closed culture system in which all the nutrients are initially added to the vessel and inoculated to proceed for the harvest and recovery of the product. This ends the batch fermentation vessel is cleaned and desterilized for the subsequent batches.

22. Define continuous fermentation process.

It is a process in which cells or micro organisms are maintained in the culture, in the exponential growth phase by the continuous addition of fresh medium that is exactly balanced by the removal of cell suspension from the bioreactor.

23. What is meant by fed batch fermentation?

In fed batch fermentation process, substrate is added periodically to the culture cells or micro organisms as the fermentation progresses. The formation of many secondary metabolites is subject to catabolite repression by high concentration of glucose, other carbohydrates, or nitrogen compounds. In such situation, in the fed batch method, the critical elements of the nutrient solution are added in small concentration in the beginning of the fermentation and these substrates continue to be added in small doses during the production phase.

24. Define gene therapy.

Gene therapy is a technique in which the defective gene can be replaced with a functional gene copy & it expressed correctly. So the disease caused by the defect gene can be prevented. There are two possible approaches to gene therapy.

(i) Introduction of the gene into the reproductive (germ line) cells or
(ii) Introduction of transgene into the somatic cells of the affected tissue.

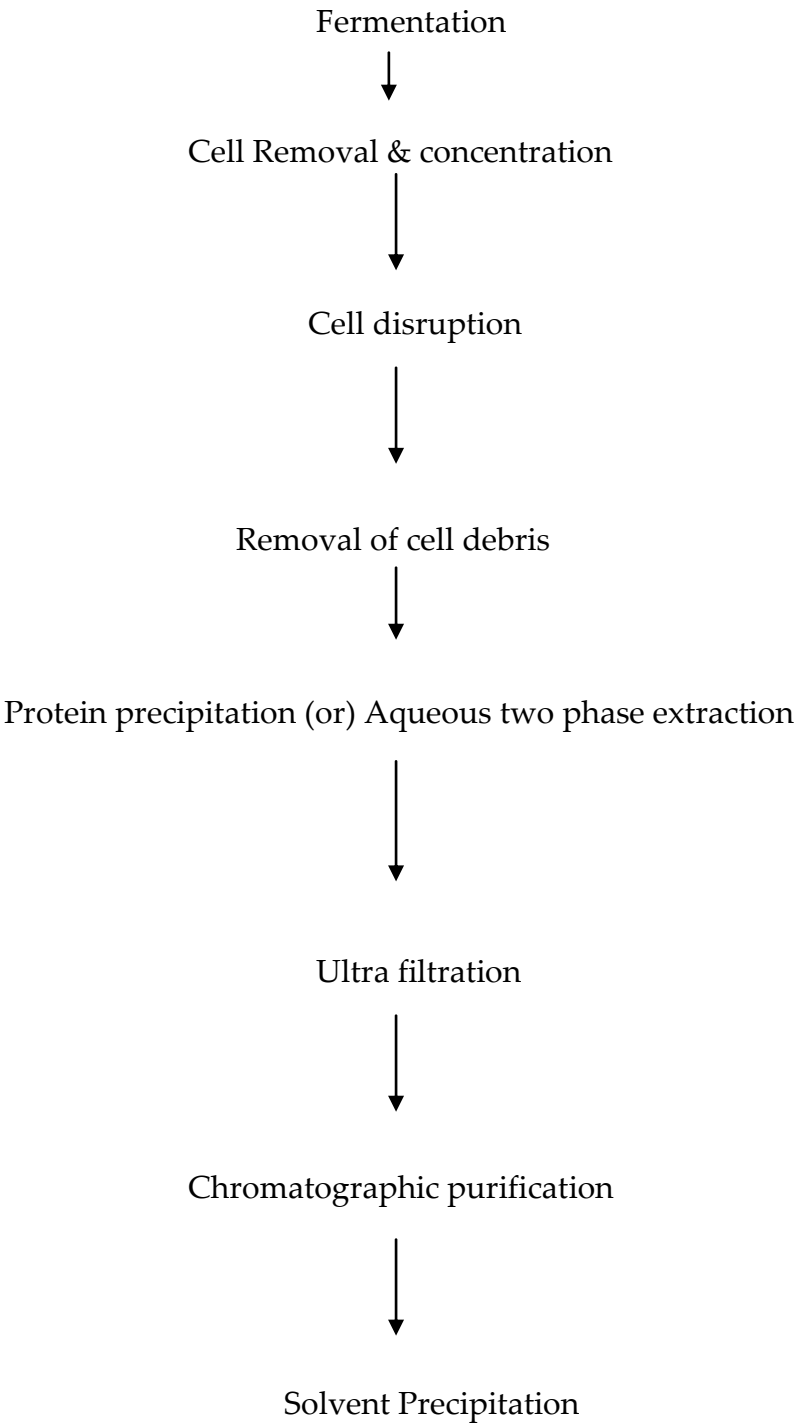
25. Write the contribution of Louis Pasteur.

Pasteur pursued schwann's idea further and became a pioneer in experimental studies on fermentation. He confirmed the observation made by schwann and showed that a living organism, a yeast, is required for the chemical change that transforms sugar into alcohol and carbonic acid. In 1851 Pasteur discovered that a different kind of organism was associated with process by which sugars were broken down to lactic acid.

26. Given examples for modern biotechnology production.

Monoclonal antibodies, recombinant insulin, transgenic plants, transgenic animals, Biopesticides, recombinant proteins, artificial skin, therapeutic proteins and vaccines.

27. Draw a flow chart for a general protein separation process in a fermentation processes.



Dialysis

Lyophilization

28. What is meant by aqueous two phase extraction?

Aqueous two phase extraction is an approach under active development for the extraction of soluble proteins such as enzymes between two aqueous phases containing incompatible polymers, such a polyethylene glycol (PEG) and dextran.

The phase containing PEG and dextran are more than 75% water and are immiscible. Typical aqueous phase used for this purpose are PRG-water/dextran water and PEG-water/K-phasphate-water PEG/dextran and PEG/K phosphate are reasonably immiscible.

29. List some important industrial fermentation microbial products.

(1) Antibiotics: Streptomycin, penicillin,
(2) Organic solvent: acetone, butanol, ethanol
(3) Beverages: wine, beer
(4) Foods: cheese, fermented milks, yeast
(5) Organic acids: lactic, acetic, citric acid.
(6) Enzymes: amylases, proteases, pectinase.

PART – B

1. Define Fermentation and explain.

The term fermentation covers any process by which microbes are grown in large quantities to produce any type of product or service of economic value. The word bio processing, fermentation and industrial microbiology are virtually similar in their scope, objectives and activities. Fermentation involves a multitude of complex enzyme catalyzed reactions using specific micro organisms under critical physical and chemical conditions. Traditionally, fermentation processes were employed for production of wines, bread, etc. Still these practices are in use and with the advent of technology new commercially significant bio-products are increasingly being derived from microbial fermentation and include

(i) Primary metabolites, e.g. acetic acids, lactic acids, glycerol, acetone, butyl alcohol, amino acids, vitamins and polysaccharides.

(ii) Secondary metabolites (that are produced as by products of cellular metabolism by microbes and are not essential for the survival and growth of organism) namely, antibiotics, growth hormones, inhibitions, tannins, resins, alkaloids, steroids, etc.

(iii) Industrially useful enzymes, e.g. amylases, pectinases, proteases, invertases, asparginase, restriction enzymes etc.

(iv) Products obtained by fermentation of various substrates using yeast, algae, filamentous fungi and bacteria that are collectively called biomass (e.g. single cell protein) and that are employed as food, feed and fodder.

All these microbial products have commercial importance and are essential to modern society. Recently, bioprocess technology is increasingly using cells derived from plants and animals to produce several significant bio products such as follows:

(i) Plant cell and tissue culture involving vegetative propagation, embryogenesis and genetic improvement is aimed largely at secondary products formation such as flavours, perfumes and drugs.

(ii) Mammalian cell culture has been concerned with production of vaccine, antibodies and pharmaceutical proteins such as interferon, interleukins, etc.

Fermentation technology essentially involves the use of suitable microorganisms, nutrient medium, optimum temperature and suitable PH and above all a physical or technical containment system called fermenter or bioreactor.

2. Explain the steps involved in the fermentation process.

Steps involved in fermentation Process

The fermentation process is usually developed in three stages. In the initial stage basic screening procedures are carried out by relatively simple microbiological techniques, using petri dishes, Erlenmeyer flasks etc. This is followed by a pilot plant investigation to determine the optimal operating condition in a volume of 5 to 200 litres. The third stage is the transfer of technology to plant or production scale and final economical exploitation of the desired product.

The general steps required to optimize fermentation as shown in figure and are as follows:-

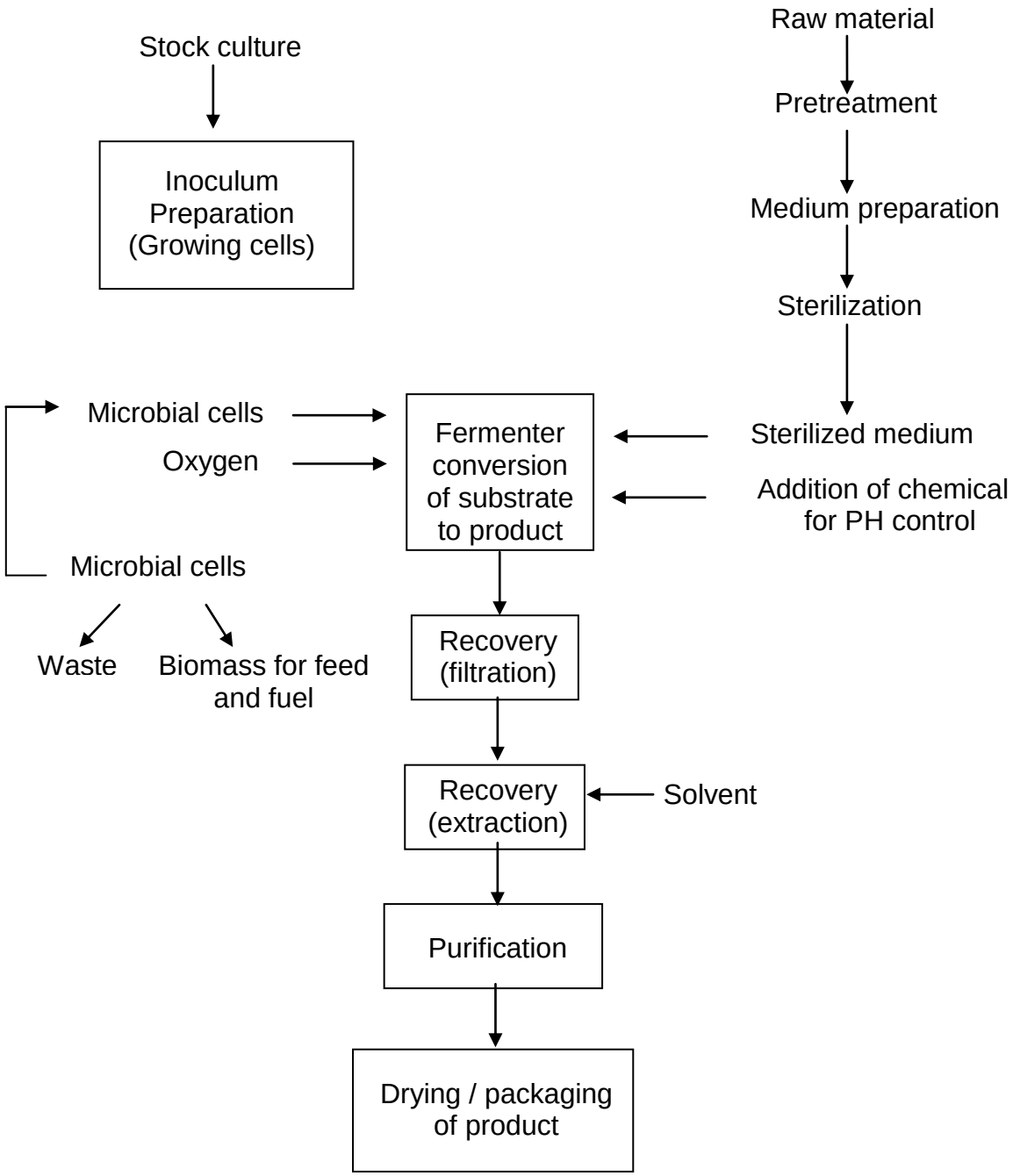


Figure: Steps involved in the Process of fermentation

1. Isolation and screening of Microorganism

The success of fermentation mainly depends on the micro organism strain used. The strain that is genetically pure, easy to manipulate by genetic engineering, and having minimum risk of contamination should be selected. Industrially important microbes like bacteria, fungi, algae etc. are isolated from soil, lakes and river mud. The desired microbes are then screened by studying their products or biosynthetic pathways and a pure culture of selected microbes is developed.

2. Selection of nutrient medium

The fermentation medium includes nutrients essential to achieve optimum growth of microbes and formation of desired end products. The choice of the nutrient is based on nutritive value and economic aspects. Usually locally available and cheap raw material like corn sugar, molasses, starch, vegetable oils, soybean meal, corn steep liquor, etc. is selected for preparation of nutrient medium.

3. Pretreatment

The selected raw material is treated and made suitable for fermentation. It may involve hydrolysis, dilution, acidification, etc. for example molasses are treated for removal of iron salts in it. Similarly, starch in corn syrup is hydrolyzed to sugar before yeast can convert it to ethanol.

4. Sterilization

The fermenter and nutrient media are sterilized to inactivate or to remove the living organisms and can be done by heating, irradiation, filtration and treatment with chemicals like formaldehyde, H_2O_2 etc. This avoids contamination of fermenter and nutrient media by unwanted micro organisms.

5. Transfer of Nutrient medium

The pretreated and sterilized nutrient medium is transferred to the fermenter for the process.

6. Inoculation:

The medium is inoculated with pure culture of selected micro organisms to achieve fermentation.

7. Adjustment of p^H and temperature

The nutrient medium is adjusted with optimum p^H and temperature so that the activities of microorganism can produce desired products in sufficient quantity. At the same time medium requirement, aeration, agitation and duration should also be adjusted as per predetermined values.

8. Recovery of Products

The process used for actual recovery of the desired product after completion of fermentation process is called down stream processing. Since the desired product may be present in very small quantities. It is generally mined with other molecules from which it has to be separated. The down stream processing involves filtration, centrifugation, flocculation and

floatation to separate the cells from liquid medium various methods like extraction, concentration and purification are employed to disintegrate the cells and finally the product is dried, making it suitable for handling and storage. In many cases, the fermentation products are inhibitory to the product formation. In such situation, in sites recovery of the products is employed to increase the yield of the desired product. This can be achieved by use of vacuum, adsorbant, anion exchange resin or dialysis.

Once the fermentation process is completed and the product is recovered, it is necessary to clean the bioreactor.

3. How does modern biotechnology differ from traditional biotechnology?

Some of the main ways that modern biotechnology differs from traditional biotechnology include modern knowledge, more varied applications and more advanced techniques. With modern biotechnology, it also takes less time to achieve the desirable changes with greater precision during development of Novel Products.

Modern knowledge

Before the discovery of genes and DNA, genetic changes in organisms (including plants) were carried out at the organism level. For example, a plant with the desirable trait was cross-breed with other plants in the hope that through cross-pollination, the desirable traits would be transferred to the offspring of the parent plants.

In modern biotechnology, achieving desired traits in an organism is done mostly at the gene level. Hence, the gene responsible for the desired trait is identified, transferred and inserted into the organism at the cellular level, to produce genetic changes. Also, in other modern techniques of biotechnology such as mutagenesis, past knowledge of causes of mutation known as mutagenisis, (such as exposure to radiation or temperature extreme) has been harnessed to generate intentional changes in the genetic make-up.

Most of the techniques used in modern biotechnology have been developed as a result of the increased knowledge of genetics and micro organisms. For example, in the traditional biotechnology technique of selective breeding, only plants that were similar (of the same species) were cross-breed. However, through recombinant DNA technology in modern biotechnology, genes can be transferred between unrelated species, for example between bacteria and humans.

Also, modern techniques such as recombinant DNA techniques and mutagenesis have made it possible to develop novel products in larger amounts than was possible in traditional biotechnology. For instance, in rDNA technology, genes with useful (desirable) traits from plants

and animals are transferred into micro organisms, such as yeasts and bacteria, which are easy to grow in large quantities. This technique is used in producing genetically modified bacteria to produce humulin, (human insulin) used to treat diabetics.

Time and Precision

The discovery of the structure of the DNA molecule and the way genetic information is passed from generation to generation has made the transfer of genes and therefore desired traits between organisms using modern biotechnology. More precise and less time consuming than traditional biotechnology. Although it is not guaranteed that every cell used in the process incorporates the transferred gene, the probability of cells incorporating the desired gene is much higher using modern biotechnology techniques than with traditional biotechnology of a cell or plant tissue. For example, mutation breeding is a biotechnology technique commonly used to develop plants with novel traits. In mutation, breeding, plant tissues are exposed to powerful mutagens in hopes of causing beneficial changes in the genetic make-up of the plant cells and then exposed to the conditions under which the plants would have to grow. Those plants with experienced beneficial mutations survive the exposure to the conditions and are breed and developed into plant lines.

Applications of Biotechnology

Modern biotechnology has many more applications than traditional biotechnology. Traditional biotechnology focused mainly on food and agriculture through techniques such as selective breeding and fermentation. Science and technology advances have broadened the scope in which biotechnology can be applied to include the environment (bio-remediation and bio-filtration), human and animal heath (development of vaccines and drugs, gene therapy, diagnostics), energy, (Production of bio-fuels), foresting (production of bio-pesticides and genetically modified tree seedlings) and other areas.

Techniques of Biotechnology

An advance is science and technologies have led to the development of new types of biotechnology techniques that did not exist in traditional biotechnology. For instance, modern biotechnology includes such techniques as recombinant DNA techniques (also known as genetic engineering), mutagenesis breeding, hybridoma technology and tissue culture.

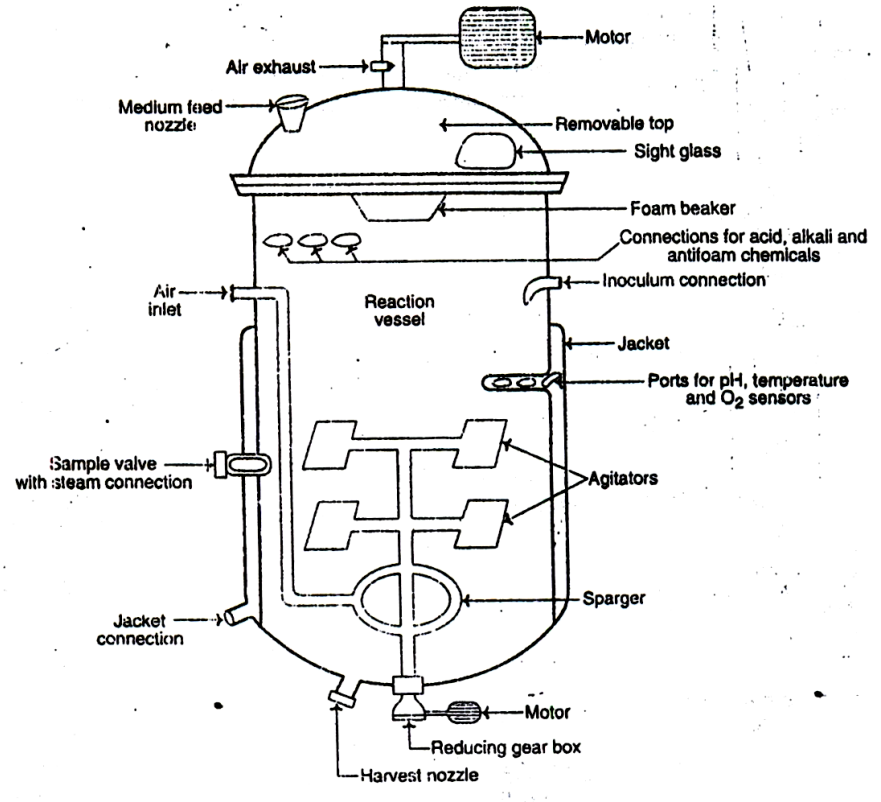
In addition, using traditional techniques it took many generations and was a lot more time consuming to produce plant, and animals with desired traits, since those techniques were mostly based on trial and error. Knowledge of the actual genes being transferred, coupled with modern technology, has significantly reduced the time it takes to obtain the same results in traditional biotechnology.

4. Define Fermenter, and write short notes about fermenter with clear diagram.

The biological process is carried out in a specially designed container where the correct environment for optimization of growth and metabolic activity can be possible to produce the commercial product. That container is called fermenter. In simple words, the fermenter is a container where fermentation is carried out; it may be a large vessel constructed as an upright cylinder. It is usually made up of stainless steel or copper of suitable grade to provide resistance to steam sterilization. Majority of fermenters are fitted with stirring gear for agitation that helps in breaking clumps of organisms. Bioreactors range from simple stirred or non-stirred open containers to complex aseptic, integrated systems with varying levels of advanced computer inputs.

Fermenters are of two distinct types. The first is open that allows continuous processing with substrate entering at one end and products leaving at another. In the second closed type the processing is done in batches and aseptic conditions are essential for successful product formation. e.g. antibiotics, vitamins, etc.

The microbes are grown on nutrients placed in a typical fermentation vessel at the start of the fermentation. The vessel is cooled by a water jacket, filtered air is pumped into the bottom of the liquid, and acid or alkali added as necessary. A stirrer keeps the contents well mixed. Steam lines are provided so that the container can be sterilized after each fermentation batch.



Diagrammatic representation of a typical bioreactor.

For designing the fermenter and to achieve optimization of the bioreactor system, the following points should be considered.

(i) The fermenter should provide an environment suitable for the growth of a pure culture and or a defined mixed culture, which can run free from contamination by unwanted microbes.

(ii) The culture volume inside the fermenter should remain constant i.e. it is essential to avoid undue release of culture organism into environment. Indeed, it is very essential to retain the organisms within the bioreactor particularly when genetically engineered microbes as a part of culture.

(iii) All the nutrients, including dissolved oxygen must be provided to diffuse into each cell, and waste products like heat, CO_2 and waste metabolites should be removed.

(iv) During fermentation, it is necessary to regulate many factors within predetermined values: Oxygen, CO_2, P^A, temperature, nutrient media concentration, etc.

(v) The standard of materials used in the designing of bioreactors is also important i.e. the materials must be corrosion-resistant, nontoxic, be able to withstand repeated sterilization with high pressure steam and should not be deformed or broken under mechanical stress. If possible the material should be transparent since visual inspection of the medium and culture is advantageous.

5. Write short notes on historical review of fermentation process.

Fermentation: An ancient tradition.

Fermentation has been known and practiced by mankind since prehistoric times, long before the underlying scientific principles were understood. That such a useful technology should arise by accident will come as no surprise to those people who live in tropical and subtropical regions, where as majority Stephenson put it, "every sandstorm is followed by a spate of fermentation in the cooking pot". For example, the products of bread, beer, vinegar, yogurt, cheese and wine were well-established technologies in ancient Egypt. It is an interesting fact that archaeological studies have revealed that breed and beers were the two most abudant components in the diet of ancient Egyptians. Archaeological evidence has also revealed that ancient Egyptians were fully aware not only of the need of malt barley or the emmer wheat but also of the need for starter cultures, which at the time may have contained lactic acid bacteria in addition to yeast.

In fact, the scientific understanding of fermentation microbiology and, in turn, biotechnology only began in the 1850s, after Louis Pasteur had succeeded in isolating two different forms of a myl alcohol of which one was optically active while the other was not. In 1857, Pasteur published their results of his studies and concluded that fermentation is associated with the life and structural integrity of the yeast cells rather than with their death and decay. He reiterated the view that the yeast cell is a living organism and that the fermentation process is essential for the reproduction and survival of the cell.

During the course of his further studies, Pasteur was also able to establish not only that alcohol was produced by yeast through fermentation but also that so uring was consequence of contamination with bacteria that were capable of converting alcohol to acetic acid souring could be avoided by heat treatment at a certain temperature for a given length of time. This eliminated the bacteria without adversely affecting the organoleptic qualities of the beer or the wine a process we now know as pasteurization.

A Second stage in the development of fermentation microbiology and biotechnology, more collectively known as fermentation biotechnology, began in 1877, when Moritz trouble proposed the theory that fermentation and other chemical reactions are catalyzed by protein like substances at the end of the reactions. Further more; he described fermentation as a sequence of events in which oxygen is transferred from one part of the sugar molecule to another, culminating in the formation of a highly oxidized product, i.e., CO_2, and a highly reduced product, i.e., alcohol.

In 1897, two years after Pasteur died; Edward Buchner was successful in preparing a cell free extract that fermented sugar. This discovery was received with a great deal of interest, not only because it was the first evidence of fermentation without a living organism but also because it was in sharp contrast to the theory proposed by Pasteur. In the early 1903, the views of Pasteur were modified and extended to stress the idea that fermentation is a function of a living, but not necessarily multiplying, cell and that it is not a single step but rather a chain of different reactions, each of which is probably catalyzed by a different enzyme.

The next stage in the development of fermentation biotechnology was dominated by the success in the use of regulatory control mechanisms for the production of amino acids. The first breakthrough was the discovery of glutamic acid over production by Cornebacterium glutaminum in the late 1950s and early 1960s.

6. What is mean by sterilization and explain industrial sterilization processes in detail?

Sterilization refers to any process that effectively kills or elimination transmissible agents (such as fungi, bacteria, viruses) from a surface, equipment, food, medication, or biological medium. Sterilization can be achieved through application of heat, chemicals, irradiation or filteration.

In all industrially important fermentation processes it is essential to use contamination – free cultures at all stages, from the preliminary culture to the production fermenter. The fermentor can be sterilized either by destroying the organisms with some lethal agent, such as heat, radiation, or a chemical, or by removing the viable organisms by a physical procedure such as filteration. During fermentation the following points must be observed to ensure sterility.

- Sterility of the culture media
- Sterility of the incoming and outgoing air
- Appropriate construction of the fermentor for sterilization and for prevention of contamination during fermentation.

Pure culture fermentations, tissue culture and some food products require stringent measures. Essentially all containing microbial life must be excluded from the system

Media Sterilization:

Initially prepared nutrient media contain a variety of vegetative cells and spores, derived from the constituents of the culture medium, water and the vessel. These must be eliminated by a suitable means before inoculation. A number of methods are available for media sterilization but in practice for large scale installations, heat is the main mechanism used.

Liquids, usually aqueous, can be sterilized by several means, including radiations such as ultra violets and X-rays. These methods include sonication, filtration, heating and chemical addition.

Heat sterilization

This is the most useful method for the sterilization of nutrients media. A number of factors influence the success of heat sterilization. These are

a) Number of type of micro organism Present
b) Composition of culture medium
c) P^H
d) Size of the suspended particles

Spores of Bacillus st ear other mop hilus are the most heat – resistances. Therefore they are used as assay organism for testing the various procedures used to sterilize equipment.

Drawbacks of Heat Sterilization Using Steam:

In industrial fermenters, if takes 2-3 hours to reach the sterilization temperature of 121^{o}C depending on the steam condition and fermentor size. Then the proper temperature is maintained for 20 to 60 minutes for the actual killing of the contaminant micro organisms. Then the fermentor should be cooled. The energy required for all these operations is very high. Another important disadvantage is that the heating, sterilization and coding phases not only kill micro organisms but also severely alter nutrient solutions. Discoloration and change is the P^H values are the common effects. Vitamins are destroyed and the quality of the culture medium deteriorates.

Radiation;

This includes UV, X-ray, and γ-radiations. They are used in the food industry but not in industrial fermentation.

Chemical methods:

A number of chemicals act as disinfectants. But they cannot be used to sterilize the nutrient media because there is a risk that inhibition of the fermentation organism could occur from the residual chemical, or it may destroy the inoculums used also.

Mechanical Removal of Organisms:

Centrifugations, adsorption to ion exchangers, adsorption to an activation carbon or filtration are possible. Amongst these, filter sterilization is widely used.

Filter sterilization is often used for all components of nutrient solutions, which are heat sensitive and would thus be denatured through the steam sterilization process normally used. Vitamins, antibiotics and blood components are some of such heat liable compounds, which must be sterilized by filtration.

The disadvantages of filtration are certain components of the nutrient solutions may be adsorbed on the filtration material and high pressures must be used.

Air sterilization:

Heat sterilization of gases is not economical then, filters may be used to remove undesirable viable cells and viruses from the appropriate process stream.

Filters may be made form sintered porcelac asbestos fibermats or synthetic micro porous polymer membrane. Nearly all the filtration today is associated with sterilization relies on the use of polymeric membrances. These filters are now routinely used to render sterile a gas flow or a dilute liquid suspension entering a fermenter.

Batch Sterilization:

Batch sterilizer is a well mixed closed vessel containing the liquid to be sterilized. The liquid is sterilized by heating and then cooled to a suitable temperature for subsequent processing. Common batch sterilization designs include one or more of the following heat sources. Steam sparging, electrical heating, and heating or cooling with a two fluid heat exchanger.

Synthetic media require relatively shorter sterilization times. The more crude media requires considerably longer sterilization times because the greater viscosity of these media impedes heat penetration and because relatively heat resistance bacterial spores may be present in some of the crude medium components. Prolonged heating of a medium will caramelize the sugars present in the medium most nutrient media are present by sterilized in batch volumes in the fermentor at 121^{o}C.

Continuous sterilization:

The medium constituent at double or triple the strength are mixed with water in a separate mixing tank, and the resulting medium is then pumped though retention tubes and heat exchangers before passing into the fermenter which previously sterilized by live steam. The retention tubes contain steam jet heater that inject high – Pressure steam into the medium to sterilize it as it passes through the pipes – The rate of passage is adjusted to provide complete media sterilization without over cooking it. The retention time is two to ten minutes. After eating into the fermenter, the medium is diluted is the required volume by sterile water.

7. What is mean by down stream processing and explain different types of down stream process involved in the fermentation process?

The extraction and purification of a biotechnological product from fermentation is referred to as down stream processing (DSP) or product recovery.

Down stream processing can be divided into series of distinct unit processes linked together to achieve product purification. Generally the number of step is kept minimum. This is not only because of cost, but because even through individual steps may obtain high yield, the overall losses of multistage purification may be prohibition.

Cell Harvesting:

In the down stream processing, the first step of suspended culture is a solid- liquid separation to remove the cells from the spent medium. Further, each fraction can undergo processing, depending on whether the product is intracellularly located, or has been secreted into the periplasmic space to the medium size and morphology of micro organism, specific gravity, viscosity and Rheology of the fermentation medium greatly influence the choice of solid – liquid separation method.

Broth Conditioning

These techniques are generally used for the separation of cells from liquid media in association with sedimentation and centrifugation. They alter or exploit some property of a micro

organism, or other suspended material, such that it flocculates and usually precipitates. In certain cases however it may be used to promote floatation this uses the ability some cells to absorb to the gas liquid interfaces of gas bubbles and float to the surface for collection, which occurs naturally in traditional are and Baker's yeast fermentation. Their low cost and ability to separate microbial cells from large volumes of medium are the major advantages of this technique.

Sedimentation:

This technique is extensively used for primary yeast separation in the production of alcoholic beverages, and in waste water treatment. This technology is low cost and is relatively slow. It is only suitable for large flows. The rate of particle sedimentation is a function of both size and density. Hence, the larger the particles and the greater its density the faster the rate of sedimentation.

Centrifugation:

A centrifugal field is applied to separate suspended particles instead of simply using gravitational force, the rate of solid liquid separation is significantly increased and many particles can be separated. Centrifugation may also be used to separate particles as small as 0.1mm diameter and is also suitable for some liquid – liquid separations. Its effectivenesses also depend on the size of the particle, density difference between the cells and the medium and viscosity.

Centrifugation is very useful, as it provides fully continuous systems which can process large volumes in small volume centrifugal. Centrifugal are steam sterializable, allowing aseptic processing and for membranes, chemicals or filter aids, there are no consumable costs. However the disadvantages of centrifugation include high initial capital costs, the noise generated during open operation and the cost of electricity.

Filtration:

Filtration is the most cost effective method for the separation of large solid particles and cells from fermentation broth. Fermentation broth is passed through a filter medium, and a filter cake is formed as a result of deposition of solids on the filter surface. Continuous rotary filters or rotary vacuum precoat filter are the most widely used types in the fermentation industry.

Filtration is commonly used for separating mycelium from fermentation broth in anti biotic industries. It is also commonly used in waste water treatment facilities.

Coagulation and Flocculation:

Coagulation and flocculation are usually used to form cell aggregates before centrifugation, gravity setting or filtration to improve the performance of this separation process. Coagulation is

the formation of small flows from dispersed colloids using coagulating agents, which are usually simple electrolytes. Flocculation is the agglomeration of these small flows into larger settlable particles using flocculating agents, which are usually poly electrolytes or certain salts, such as $CaCl_2$.

Simple electrolytes used in coagulation are acids, bases, salts and multivalent ions, which are relatively inexpensive, but least effective than poly electrolytes. Poly electrolytes used in flocculation are high molecular weight, water soluble organic compounds, which can be anionic, cationic, or nonionic. The important parameters to be considered in flocculation are flocculant – coagulant concentration, concentration of colloidal cells, kinetics of the binding, and settling phenomena.

Liquid – Liquid Extraction:

Liquid extraction is commonly used to separate inhibitory fermentation products such as ethanol and aceto, butanal from a fermentation broth. Antibiotics are also recovered by liquid extraction (using amyl acetate or croamylacetate). The liquid extractant should be nontoxic, selective, inexpensive and immiscible with fermentation broth and should have a high distribution coefficient for the product. The extraction of a compound from one phase to the other is based on solubility differences of the compound is one phase relative to the other.

Adsorption:

Adsorption of solutes from liquid media on the solids is a common practice in separating soluble materials from fermentation broth. Various mechanisms may be involved in solute is transferred from liquid to solid phase, and an equilibrium is reached after a while in a batch operation. the type of adsorbent of used. Depends on the particulars application. The most widely used adsorbent for waste water treatment applications is activated carbon, since it has large internal surface area per unit weight. Ion exchange resins and other polymeric adsorbents can be used for protein separation of small organics for example; a carboxylic acid cation exchange is used to recover streptomycin.

Chromatography:

Chromatography separates mixtures into components by passing a fluid mixture through a bed of adsorbent material – typically a column is packed with adsorbent particles, which may be solid, a porous solid, a gel, or a liquid phase immobilized in or on a solid. A mobile phase or fluid phase with a mixture of solutes is injected. This pulse is followed by a solvent or event. This pulse enters as a narrow concentrated peak, but exits dispersed and diluted by additional solvent. Different solutes in the mixture interact differently with the adsorbent material; some interact weakly and some interact strongly. Some important types of chromatographic methods are

(i) Adsorption chromatography
(ii) Ion exchange chromatography
(iii) Gel filtration chromatography
(iv) Affinity chromatography
(v) High pressure liquid chromatography.

Crystallization:

Crystallization is the last step in producing highly purified products such as antibiotics. Crystallization operates at low temperatures, which minimize thermal degradation of heat sensitive materials. High purity crystals are recovered using batch Nutsche- type filters or centrifugal filters.

UNIT – II

PART - A

1. What are metabolites?

Metabolites are low molecular weight biological compound that is usually synthesized by an enzyme. It is generally classified into primary metabolites and secondary metabolites.

2. Define Primary metabolites.

Primary metabolites are microbial products made during the exponential phase of growth whose synthesis is an integral part of the normal growth process. They include intermediates and end products of anabolic metabolism, which are used by the cell as building blocks for essential macromolecules (e.g. vitamins).

3. Name industrially important primary metabolite products.

Industrially important primary metabolites are amino aids, nucleotides, vitamins, solvents, and organic aids.

4. Give example for some important commercially produced organic aids.

Citric acid, lactic acid, acetic acid, Fumaric acid, itaconic acid, kojic and, propionic acid, pyruvic acid, succinic acid, Fumaric acid & Malic acid.

5. Give, some example for organic acid producing micro organisms.

Citric acid - Aspergillus niger
Lactic acid - Lactobacillus delbrueckii
Acetic acid - Aspergillus terreus
Gluconic acid - Aspergillus Niger
Malic acid - lactobacillus brevis.

6. Write the citric acid applications in the industry.

Citric acid is used in many fields.

(1) As an acidulant in food (e.g. jams, preserved fruits, fruit drinks, etc) and pharmaceutical industries.
(2) As a chelating and sequestering agent (e.g. in the tanning of animal skins).
(3) In the Production of carbonated beverages.
(4) Citrate and citrate esters as plasticizers.

7. Write the application of lactic acid in industry.

The major use of L-lactic acid is in foods (more than 50 V.) as an acidulant and preservative. Lactic acid is also used as a chemical intermediate to produce other chemicals and in the pharmaceutical industry. It is also used in some plastic industries.

8. Name lactic acid producing microbial strains.

There are numeric microbial specifies capable of producing large quantities of lactic acid. These include lacto bacillus bulgaricus, L. delbrueckii, L. Leichmannii, L.casei, strepto coccus, lactis and phizopue oryzae. The selection of a micro organism largely depends on the nature of the carbon source being used in the fermentation process.

9. Short Note on commercial use of amino acids.

Amino acids are used for a variety of purpose. The food industry requires L – glutamate as a flavour enhancer and glycine as a sweetener in juices. The chemical industry requires amino acids as building blocks for a diversity of compounds. The pharmaceutical industry requires the amino acids themselves in infusions in particular the essential amino acids – or in special dietary food.

10. Lift some commercially important producing amino acids.

(1) L. Lysine - Coryne bacterium. glutamicum
(2) L – threonine - E. coli
(3) L – argineni - Bacillus sub tiles.
(4) L – Glutamate - C. glutamicum
(5) Proline - C. glutamicum
(6) Phenylalanine - C. glutamicum.

11. How Glutamic acid produced in the industry?

L – glutamic acid may be produced in many ways

(i) By the hydrolysis of wheat gluten, soyabean cake, or other proteinaceous material,
(ii) By the cleavage of the pyrrolidone carboxylic acid found in Steffen's molasses;
(iii) By a one stage fermentation process involving a single micro organism;
(iv) By a two stage fermentation process where $\propto$ - ketoglutaric acid, is converted to L glutamic acid by another microbe.

12. Name the glutamic acid producing micro organisms:

Carbon source		Micro organism
(1) Glucose	-	(i) Corynebacterium glutamicum (ii) Brevibacterium flavum
(2) Acetic acid	-	(i) Brevibacterium flavum (ii) Brevibacterium thiogentalis
(3) Ethanol	-	Brevibacterium sp.
(4) Benzoic acid	-	Brevibacterium sp.

13. Write the importance of L – Lysine.

L – Lysine is an essential amino acid for the nutrition of humans. It is used for supplementing ceral proteins lacking amino acids. Thus, protein quality of certain foods (e.g. wheat based foods) is improved resulting in an improved growth and tissue synthesis. This amino acid has also been used medically as a nutrient.

14. Name L – Lysine producing micro organisms.

Micro organisms	Fermentable subtractes
(i) Brevibacterium flavum	- Glucose
(ii) Corynebacterium glutamicum	- Glucose
(iii) Brevibacterium flavum	- Acetic acid
(iv) Brevibacterium lacto fermentum	– Ethanol.

15. Define feed back inhibition.

A mechanism in which the end products of a given pathways acts as an inhibitor of an earlier reaction in the pathway. Generally, the end product acts as a non competitive inhibitor of the first committed step in the pathway.

16. Name phenylalanine producing microbial strain.

E. Coli and corynebacterium glutamicum are the two major strains used for the products in of L – phenylalanine.

17. Write the uses of aspartic acid in industry.

L – Aspartic acid is widely used as a food additive and in pharmaceuticals. Demand increased rapidly with the introduction of aspartame as an artificial sweetner. This is a diopeptide consisting of L – aspartate and L – phenylalanine which is about 200 – fold sweetner than sugar.

18. Name the organic solvents mainly used in fermentation industries.

Ethanol, glycerol, acetone, butanol and 2,3 – butane dio are widely used in chemical as well as fermentation industries. For example, ethanol is used as a solvent and a precursor of many synthetic chemicals.

19. Name the microbars which are used for the production of industrial alcohol.

(i) Alcohol production from starch and sugar raw materials utilizes specially selected strains of saccharomyces cerevisiae and, to lesser extent, of saccharomyces ellipsoidus.

(ii) Production from the lactose of whey, after protein removal, is accomplished with Candida pseudotropicals.

(iii) The sulfite waste, liquor fermentation employs Candida utilize, because of the ability of this organism to ferment pentoses.

20. Define anaerobic fermentation process, Whether it beneficial to fermentation process.

Anaerobic fermentation are those fermentations that are carried out in the absence of oxygen by strictly anaerobic or facultatively anaerobic bacteria or yeasts.

Micro organisms growing anaerobically recover less energy per unit of carbon substrate utilized than do aerobes. Also, there is a tendency in this fermentation for carbon substrate to undergo only partical decomposition. so that various organic acids, organic amine, and 30 forth accumulate in the growth medium; these products can present problems in p^H maintenance of the fermentation. Thus, the incomplete utilization of substrate and low cellular yields for these fermentations often requires that much carbon substrate be decomposed for the growth and maintenance of the microbial cells. This phenomenon, however, can be beneficial to fermentation yields, it relatively greater passage of substrate carbon through the metabolic sequences of the organism causes a resultant conversion of large amount of substrate carbon to fermentation product.

PART – B

1. Explain the production of citric acid formation in the industry.

The fermentation processes are carried out either in surface culture or in fermenters up to $220m^3$ in volume. Citric acid is marketed as citric acid hydrate or as anhydrous critic acid. Most Citric acid is used in the food and beverage industry. The flavour of fruit juices, fruit juice extracts, candy ice cream and mar malade is enhanced or preserved by the addition of citric acid. The pharmaceutical industry uses citric acid as a preservative for shored blood bottles, oinments and in cosmetic preparations. In chemical industry it is used as an antifoam agent, as a softener and for the treatment of textiles stains for citric acid production.

Many strains excrete traces of citric acid as a metabolite of primary metabolism. Examples are aspergillus Niger, A.Wenti, pencillium luteum, P.citrinum, citromyces pfeferianus, arthobacter paraffineus. But only mutants of aspergillus Niger are used for commercial production. Compared to penicilliam strains, the aspergilli produce more citric acid per unit time.

Biosynthesis:

Citric acid (2 – hydroxyl propane – 1, 2, 3 – tricarboxylic acid) is a primary metabolic product and is formed in the tricarboxylic acid cycle. Glucose is the main carbon source used for citric acid production. In many organisms, 80% of the glucose used for citric acid biosynthesis is break down by reactions of the Embdden – Meyerhof – parnas (EMP) pathway and 20% by reactions of the pentose phosphate cycle. During the growth phase, the relationship between these two pathways is 2:1.

When pyruvate is decarboxylated with the formation of actual – CoA, the actuate residue is channeled into the tricarboxylic acid cycle during the idiophase, all enzymes of the kreb cycle are expressed except x – ketsglutarate dehydrogenase. The citrate synthesis activity is increased by a factor of 10 during the production of citric acid, while the activities of the enzymes which catabolism citric acid, surbar aconites and isocitrate dehydrogenate are sharply reduced as compared to their activity in the prophase.

In the triphosphate, part of the added glucose is used for the production of mycelium and in the idiophase; the rest of the glucose is converted into the organic acids. The theoretical yield is 123 of citric acid 1 – hydrate of 112 g anhydrous citric acid per 100g surface

Nutrient Media:

Carbohydrate source:

A variety of materials can be used as a carbohydrate sources. Starch from potatoes, glucose syrup from sac charified starch, sugar cane syrup, sugar cane molasses, and sugar batmolasses.

Trace elements:

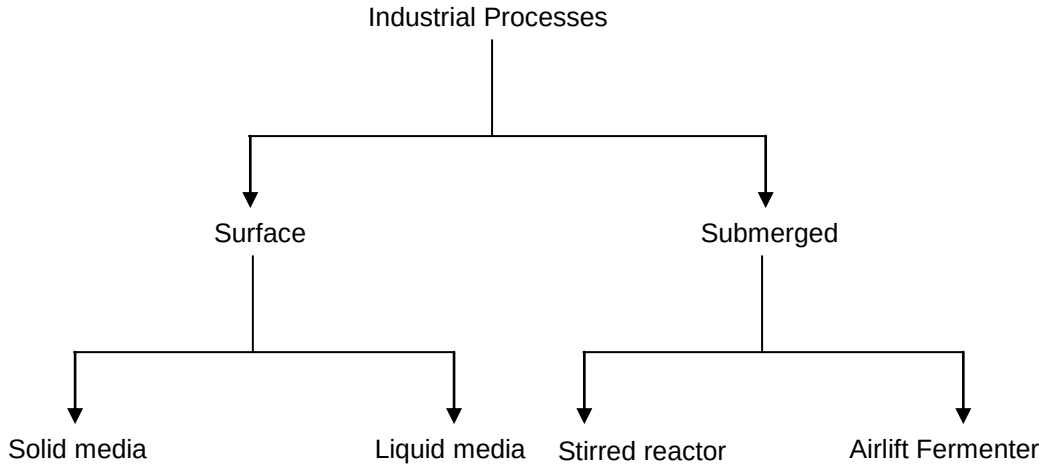
Copper, manganese, magnesium, iron, zinc and moly bolenum in the ppm range for optimal growth. Besides the composition of the nutrient solution the p^H of the medium influences the yield. During the idiophase, the pH must be below 3 in order to suppress oxalic and gluconic acid formation, but during the brophophase the pH is generally started at 5.

Production Processes:

Citric acid is produced by both surface and submerged processes. Surface processes can be further subdivided according to the state of the culture medium used: solid or liquid. Two types of submerged processes are used, stirred fermenters or airlift fermenters.

Production of inoculums:

The material used as inoculums for citric acid production is a spore suspension. Sports are produced in glass bottles on solid subtrate at 25°C with incubation times of 10 – 14 days. The spores germinate at 32°C and form pellets 0.2 – 0.5 mm in diameter within 24 hours. These pellets are used as the inoculum for production fermenters.



Surface Processes:

In surface processes wheat bran or pulp from sweet potato starch production as a culture medium. After sterilization the material is inoculated with spores, spread on trays in layers 3 – 5 cm thick and incubated at 28°C. Growth accelerated by adding α - amylases, although the fungus can hydrolyze starch with its own amylase. The solid surface process takes 90 hours, at the end of which the entire solution is extracted with hot water to isolate the citric acid.

Surface processes using liquid nutrient solution are the oldest production methods. These processes are still in use because of the low investment the low energy cost for the cooling system and the simple technology. The following figure shows the layout of a typical fermentation system. The sterilized nutrient solution automatically flows over a distribution system onto the brays. Within 24 hours after inoculation, the germinating spores form a thin cover of mycelium on the surface of the nutrient solution. The fermentation is stopped after 8 – 14 days.

Yields from the surface process using liquid nutrient solution amount to 1.2 to 1.5 kg citric acid monohydrate |m2 of fermentation surface per hour.

Submerged Processes:

Eighty percent of the world's supply of citric acid is produced by submerged processes. There factors are especially important for production in submerged processes: a) Quality of the material used to construct the fermenter b) mycelium structure c) oxygen supply

Fermenters for a citric acid production must be either protected from acids or constructed to stainless steel. At p^H values between 1 – 2 the heavy metals leached from normal steel fermenter walls can inhibit the formation of citric acid.

The structure of the mycilium that forms in the submerged culture during the trophophase is vital to a successful production processes. If mycelium is loose and filamentous, with limited branches and no chalmydospores, little citric acid is produced in the idiophase. Mycelium for optimal production rates consists of very small solid pellets. The ratio of iron to copper in the medium determines the mycelium structure.

Although aspergillus Niger requires relatively little oxygen, it is sensitive in oxygen deficiency. Through hour the entire fermentation period, there must be a minimum oxygen concentration of 20 – 25% of the saturation value.

Foaming is a problem in submerged fermentation. Anti foam agents, such as lard oils can be added at frequent intervals and mechanical anti foam devices can also be used.

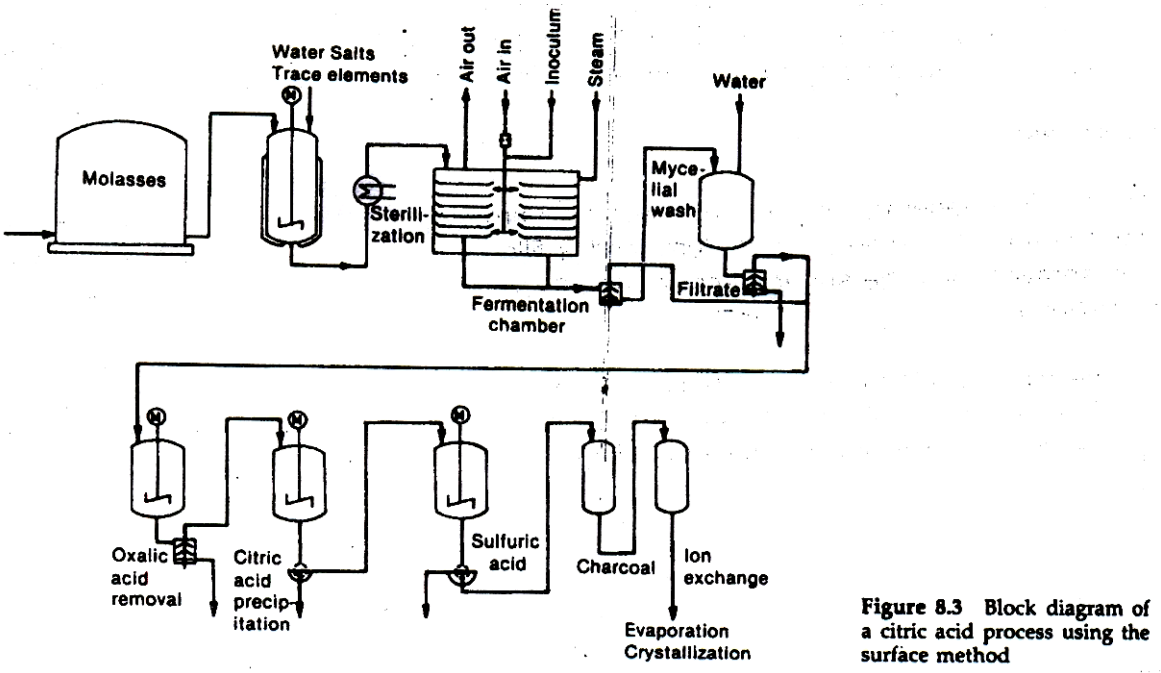


Figure 8.3 Block diagram of a citric acid process using the surface method

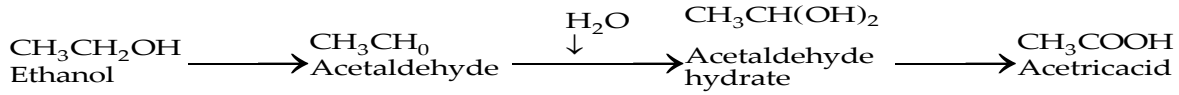
2. Explain the production process of Acetic acid in industry.

Acetic acid is produced by many fermentative bacteria, only members of a special group, the acetic acid bacteria, are used in commercial production. The acetic acid bacteria can be divided into two genera, Gluconobacter and Aceto bacter the first group oxidizing ethanol solely to acetic acid and the second group able to oxidize ethanol first to acetic acid and then further to CO_2 and H_2O.

Strains used commercially belong to the species Aceto bacter acetic, A.Pasteurianus or A.Peroxidans

Biosynthesis:

Acetic acid production is an incomplete oxidation rather than a true fermentation because the reducing power which is produced is transferred to oxygen. The first oxidation step from ethanol leads acetaldehyde with a NAD or NADP special alcohol dehydrogenase. Then there is a hydration to acetaldehyde hydride and a second oxidation with acetaldehyde dehydrogenase to acetic acid.



During the oxidation, 1 mole of acetic acid is produced from 1 mole ethanol. From 1 liter of 12% (v/v) ethanol, 1 liter of 12.4% (w/v) acetic acid is produced.

Production:

The starting materials with low alcohol content such as wine, whey, moles or cider, do not require any additional components to the nutrient solution. But, if potato or grain spirits is used, nutrients must be added in many cases to obtain optimal growth and acetic acid production.

Surface Process:

The trickling generator is used for acetic acid production by surface process. The wooden bioreactor has a total volume of up to 60m^3 and is filled with beach wood shavings. The starting material is sprayed over the surface and trickles through the shavings containing bacterial into a basis in the bottom.

Of the alcohol added, 88 – 90% is concentrated to acetic acid in the trickling generator process. The rest of the alcohol is used for primary metabolism. The time needed to produce 12% acetic acid in this process is about three days.

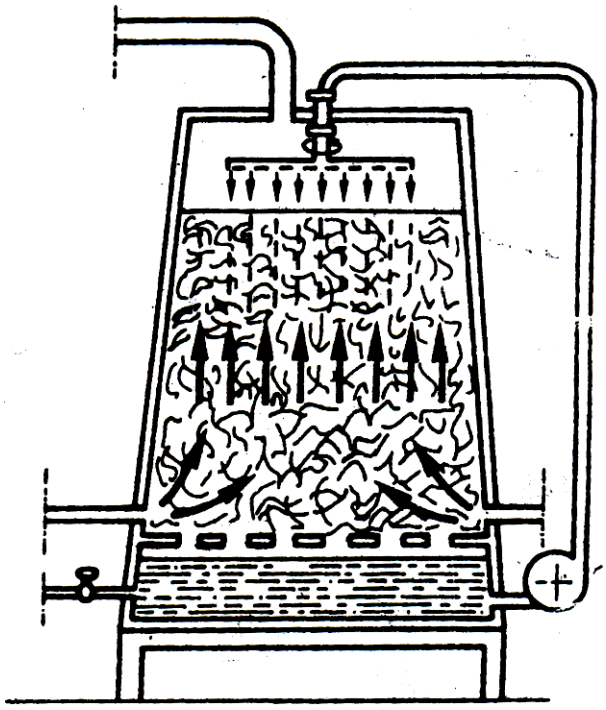


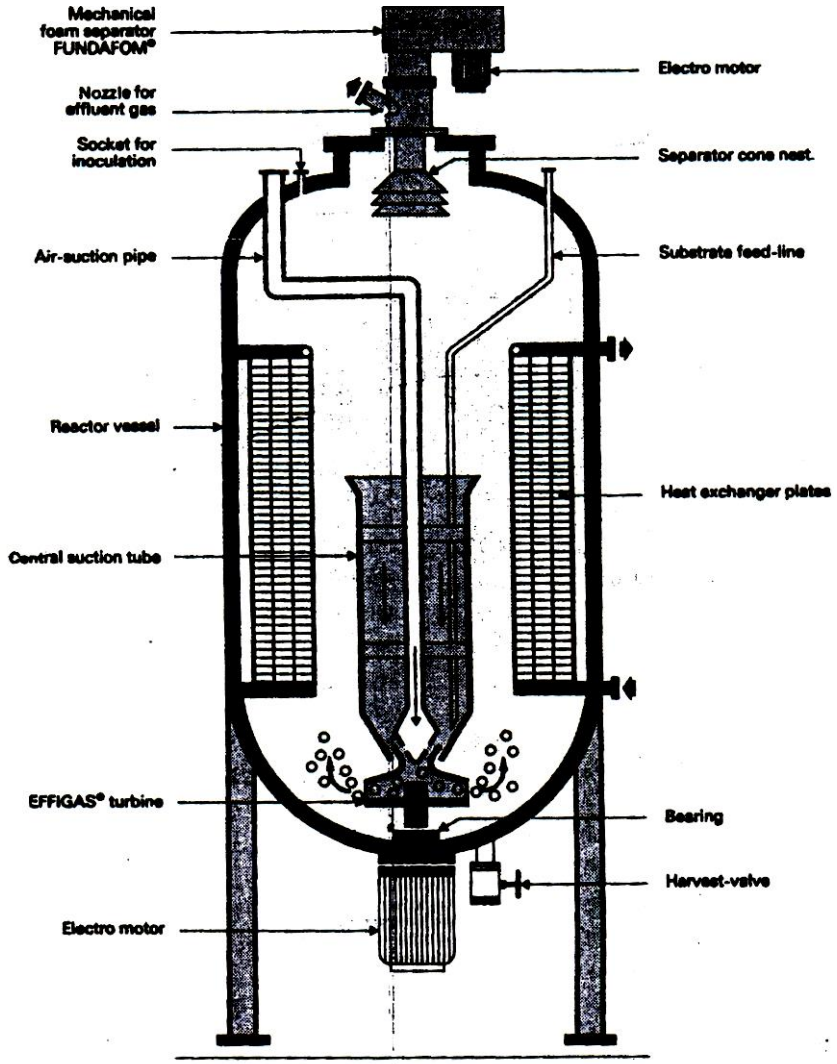
Diagram of a trickling generator for vinegar

Submerged Processes:

Fruit wines and special mashes with low total concentrations of alcohol were first used in submerged processes. The banks of the bioreactor, are constructed of stainless steel and are stirred from the bottom. The typical reactor shown in figure. The acceleration apparatus consists of a section rotor with the incoming air coming down through a pipe from the top of the fermented.

Heat exchangers control the temperature and mechanical foam elimators must usually be installed. House hold vinegar (13% acetic acid) is produced in a semi continuous, fully automatic process. With the submerged process, the production rate per m^3 is 10 times higher than with the surface fermentation and about 5% higher than with the trickling generator process. Other advantages are: lower capital investment per production amount, only 20% of the plant area required for the installation, capability of conversion to other mashes in a shorter time and low personel cost due to fully automatic control.

The acetic acid obtained in the submerged process is turbid due to the present of bacteria and the product must be clarified by filtration.



Submerged fermenter for acetic acid production (Chemap)

3. Explain the production process of Lactic acid in the industry.

The first microbial production of an organic acid was the production of lactic acid, carried out in 1880. Today chemical methods for the production of lactic acid are very competition at the same cost as biological processes. Two kinds of lactic acid bacteria are used for the production of lactic acid, heterofermentative and homofermentative.

The heterofermentative organisms produce a great number of by products and are net suitable for commercial purposes. With homofermentative organisms as little substrate as possible is converted into all material plus by products and as much as possible is metabolized into lactic acid.

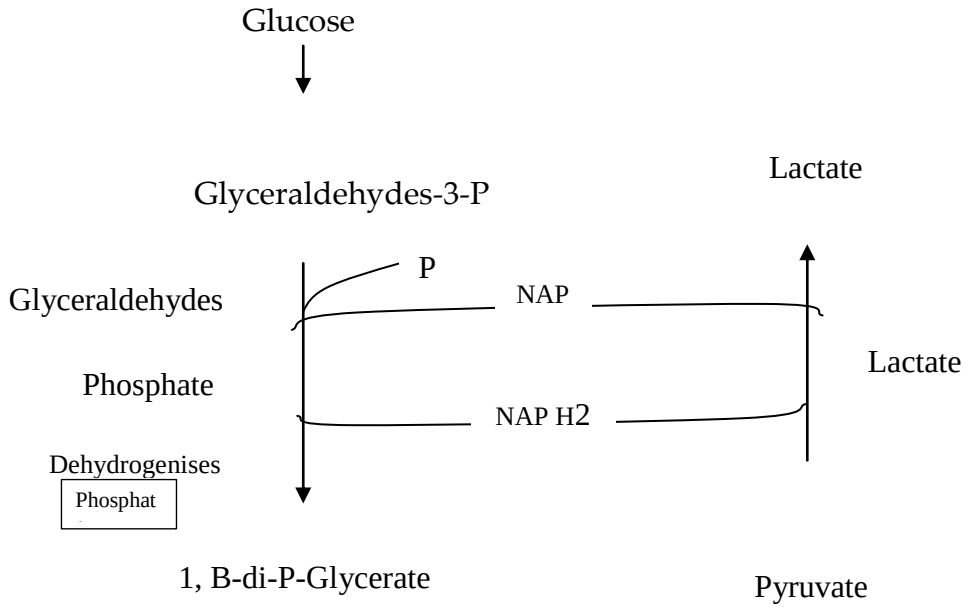
The biosynthesis of lactic acid from glucose proceeds via glyceraldehydes 3 – P, 1, 3 – di – P glycorate and pyruvate. The reducing power produced during the oxidation of glyceraldehyde

phosphate is transferred with a dependent lactate dehydrogenase pirate, which is reduced sterospecifically to L(t) or (D-1) lactic acid.

Theoretically R moles of lactate are produced from 1 mole of glues. Over 90% of this theoretical yield is actually attained in practice. The organisms used are Laetobacillus delbruedckii and L.leichamanii when a glass is used as a substrate, lactobacillus burglarious with whey, and lactobacillus pentose with sulfite waste liquor.

In addition to 12-13% glucose and (N+14) 2HPO4 (0.25%), the fermentation medium must contain B vitamins. Production is carried out on 25-120m^3 fermenters at 45-50°C with an excess of $CaCo_3$ added to maintain the P^H between 5.5 and 6.5. The fermentation time is about 72 hours. Since free lactic and is Bowie to organisms so continuously removed during production.

Only L+ lactic and is produced commercially when the fermentation has completed the broth is heated to dissolve the calcium lactate. The heated broth is filtered and the calcium precipitated by addition of surphuric acid. After concentration of the lactic acid, it is further purified.

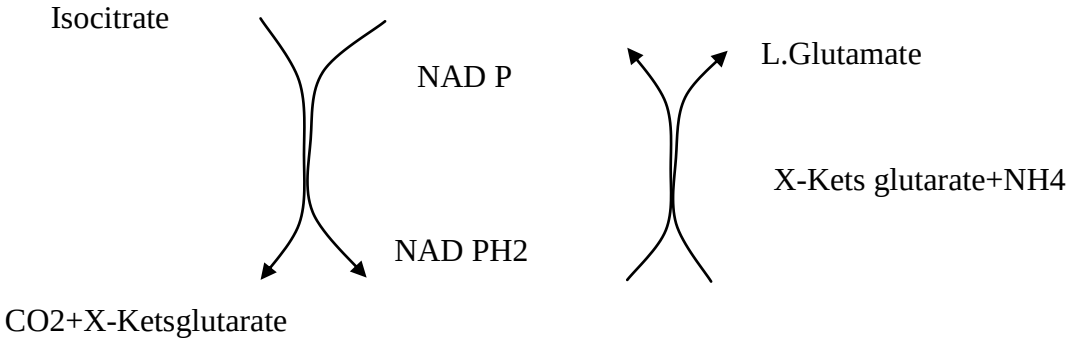


4. Explain Glutamic acid production in detail.

L.glutamic acid is manufactured predominantly by microbial means although it is also manufactured chemically. L.glutamic acid production was fecund to occur in a wide variety of bacteria, streptomyces, yeasts and fungi. The industrially important strains are corynebacterium glutamicum, Brevibacterium, Microbacterium or Arthrobacter.

Biosynthesis of glutemic acid:

The glucose carbon source is broken down into c3 and C2 fragments by glutamic acid producing microorganisms through the Embden Meyer of parnas (EMP) pathway and the pentose phosphate cycle, and the fragments are channeled into tricarboxylic acid (TCA) cycle. The key precursor of glutamic acid is α-ketoglutamatic, which is formed in the TCA cycle via citrate and isocitate and then converted into L.glutamic acid through reductive amination with free NH4+ ions. This reaction is catalyzed by the NADP dependent glutamate dehydrogenase.



Carbon Source:

A wide variety of carbohydrate can be used as carbon sources in the fermentation process. Among the monosacentarides, glucose and sucrose are frequently used, and fructose, maltose, ribose and xylose are find some role of the unrefined carbohydrate source, sugar cane and sugar beat molasses are more important.

Nibrogen Sources:

In addition to ammonium salts, ammonium can be used as a nitrogen source. Ammonia feeding permits p^H control and abvitates the problem of ammonia toxicity. Must glutamic acid producing bacteria process urease activity, so that urea is also frequently used as a nitrogen source.

Growth factors:

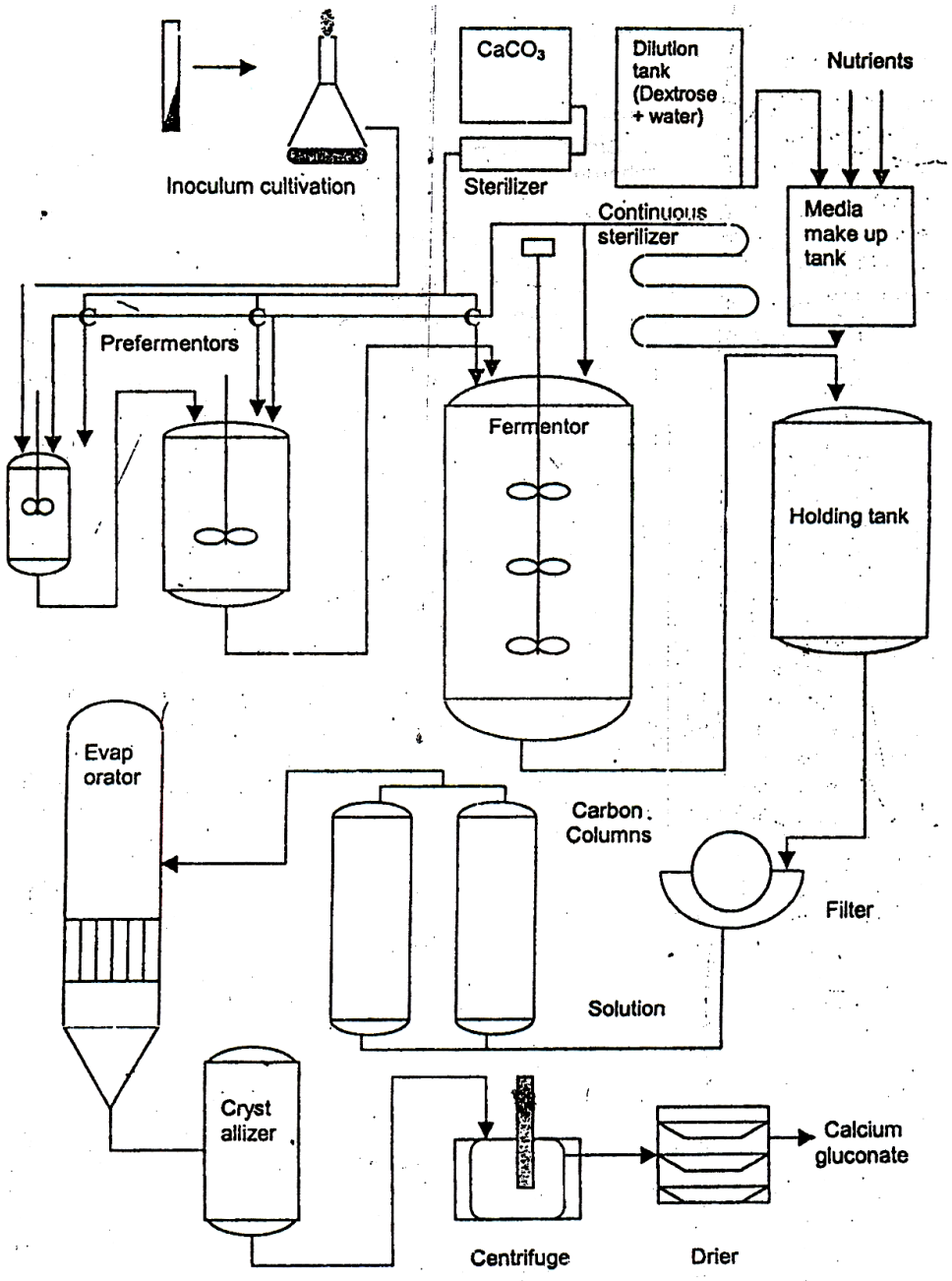
Mainly biotin is added to the strains of growth factor. The optimal biotin concentration is dependent on the carbon source used. Some strains require L.cystine as an additional growth factor; for media based on an n-alkane, supplementation with thiamin may be necessary.

Production Processes:

A typical fermentation from glucose with brevibacterium divaricatum rane a follows.

- Seeds culture: glucose, $Mgso_4$. $7H_2O$, yeast extract, urea, tap water &16h incubation at 35° C.
- Main culture: glucose, ammonium acetate, molasses from starch saccharification, KH_2PO_4, K_2SO_4, $MgSO_4$, H_2 O, antifoam agent , tap water and inoculation volume: 6%

At the beginning of the fermentation 0.65 ml/l of oleic acid is added. The p^H is set as 8.5 with ammonia and is automatically maintained at 7.8 during the course of the fermentation. After the beginning growth of the glucose down to a level of 0.52%, glucose feeding is done until the fermentation is completed, 16g/L are fed on the average. Aeration is controlled so that the CO2 content in the exhaust gas does not exceed 4.5 vol%. The glutamic acid constent is analyzed hourly. As a rule, the fermentation is stopped after 30-35 hours with a glutamic acid yield of about 100/l. If molasses from starch sacharrification is substituted for glucose, the glutamic acid yield is 94g/l after 36 hours.



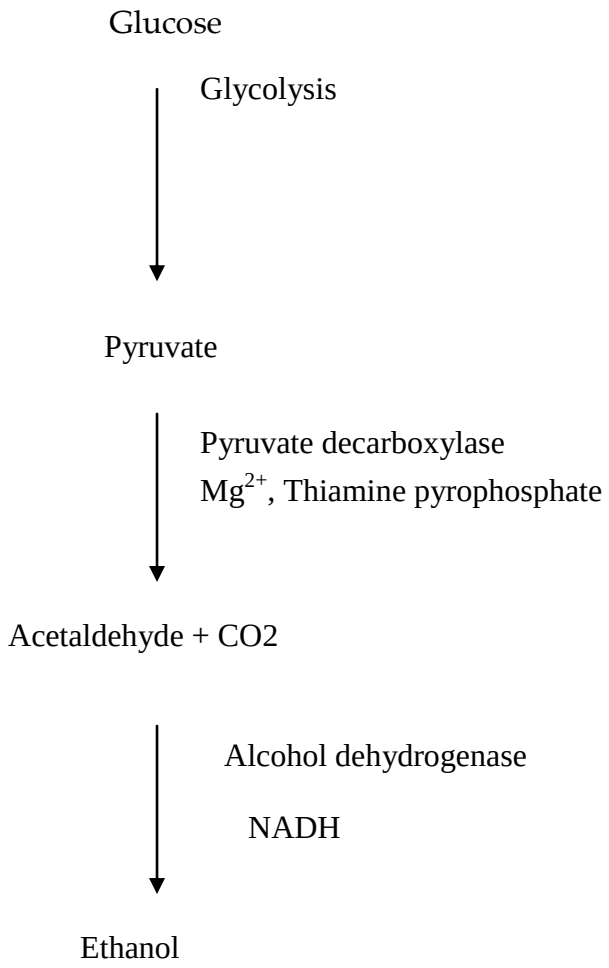
PRODUCTION OF CALCIUM GLUCONATE

5. Explain Ethanol production in detail.

Ethanol:

Both yeasts and bacteria have been used for the production of ethanol. Among the bacteria, the most widely used organism is zymomonas mobilis saccharomyces cerevisiae is the most commonly used yeast but Saccharomyces fragilis is also been employed.

Under aerobic conditions and in the presence of high glucose concentrations, sacharomyces cerevisiae grows well, but produces no alcohol. Under anaerobic conditions, however growth slows and pyruvate from the glycolytic pathway is split with pyruvate decar -boxylase into acetaldehyde and CO2. Ethanol is then produced from the acetaldehyde by reduction with alcohol dehydrogenase.



Theoretically from 1gram of glucose 10.511 gram of ethanol can be obtained. When pure subtrate are fermented, the yield is 95% and reduces to 91% when industrial grade starting materials are used. Ethanol is inhibitory at high concentrations and the alcohol tolerance of the yeast is critical for high yields. As the concentration of ethanol increases the growth rate is first reduced, where as at higher concentrations the biosynthesis of ethanol itself uninhibited. Growth generally ceases at 5% ethanol and the production rate is reduced. **Zen** at 6-10% (v /v).

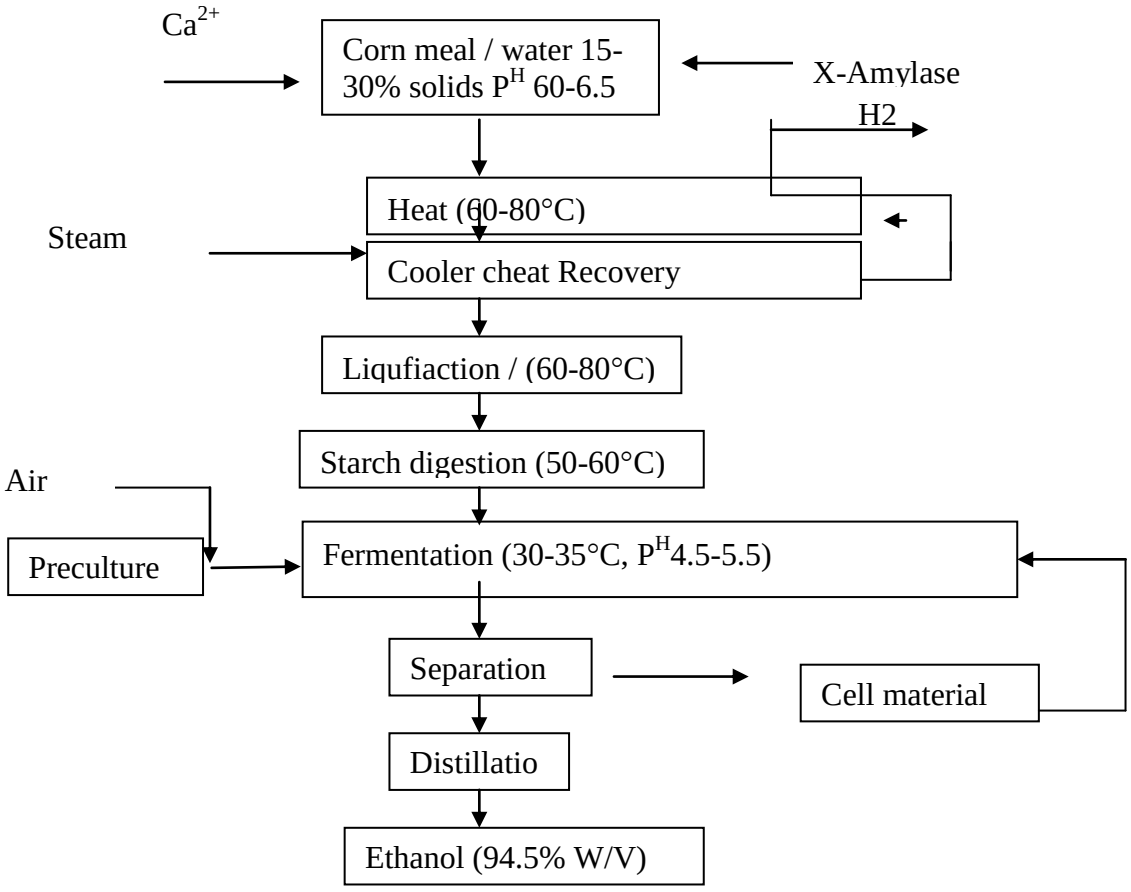
The P^H optimum of the bacterial ethanol fermentation is considerably broader (P^H5-7) and the temperature optimum is higher (30°C). In order to increase the productivity, both the bacteria and yeast processes are being carried out in large scale by continuous fermentation . Maximal productivity with glucose as carbon source that has been reached is about 82g/l-h for yeast and 120g/l-h for zymomonas.

Ethanol Production Process:

Ethanol is produced in three steps, each of which must be optimized.

(1) Preparation of the nutrient solution
(2) Fermentation
(3) Distillation of ethanol.

The following fig. shows the stages in the production of ethanol from corn meal.



Preparation of the nutrient solution:

Three types of substrates are used in the ethanol fermentation.
(1) Starch containing roots, tubers, **organs**,
(2) Molasses or juice from sugar cane or sugar
(3) Wood or waste products from processed wood.

The most important root starch is derived from the tropical plant **manihet esculenta**.

Examination:

Continuous fermentation are brought into large scale operation the P^A value was adjusted to 5 with H_2SO_4 and pasteurization was then carried out. The fermentation temperature was 35° and the yeast production was 10g/l. After the fermentation, the cells were separated by centrifugation and channeled back into the first fermenter.

Batch fermentation is more commenly employed for ethanol products. Production is carried out in batch processes with either starch hydralysate or molasses, using a 3% inoculum. Within 12 hours, sacharomyces produces 10% (v/v) ethanol with 10-20g cell dry weight/l. When the process is carried out at 35-38°C; P^H 4.0-45; the maximal productivity was 1.9g/l-h. The short fermentation time accomplished by use of cell **realing** before dilation, the mass is separated by centrifugation or sedimentation.

Ethanol with a purity of 92.4% is used as a solvent in the cosmetic, pharmaceutical and chemical industry and at 99.2% purity as a motor fuel.

6. Explain Butane fermentation process in detail.

Butanel fermentation:

Butanol is required for the synthesis of butadiene to produce synthetic rubber. Butanol is extensively used in break fluids, for antibiotic recovery, as amine for gasoline additives and as ester in protective coating industry.

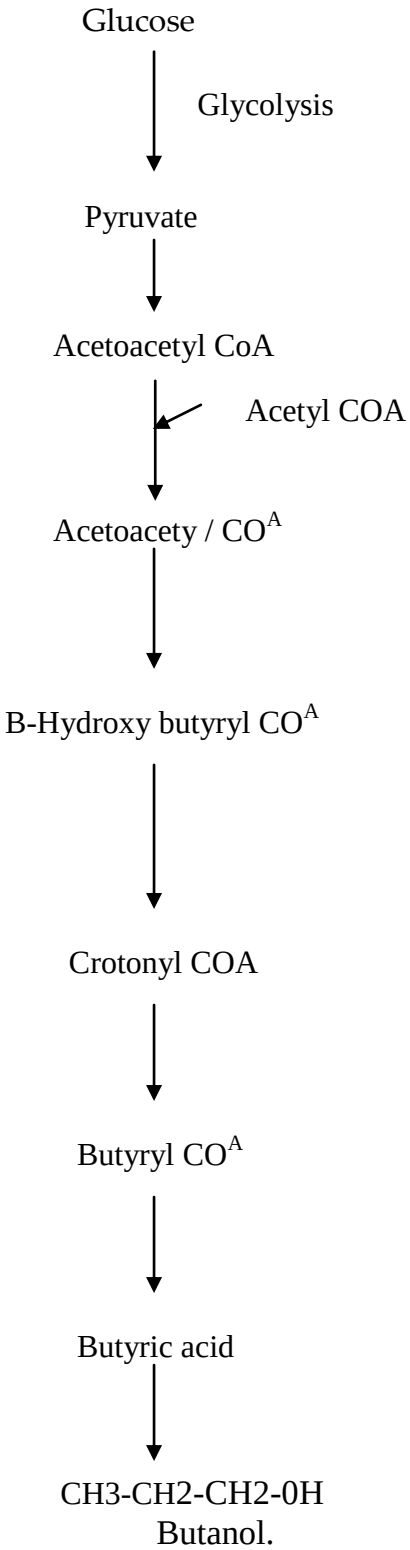
Production of butanol:

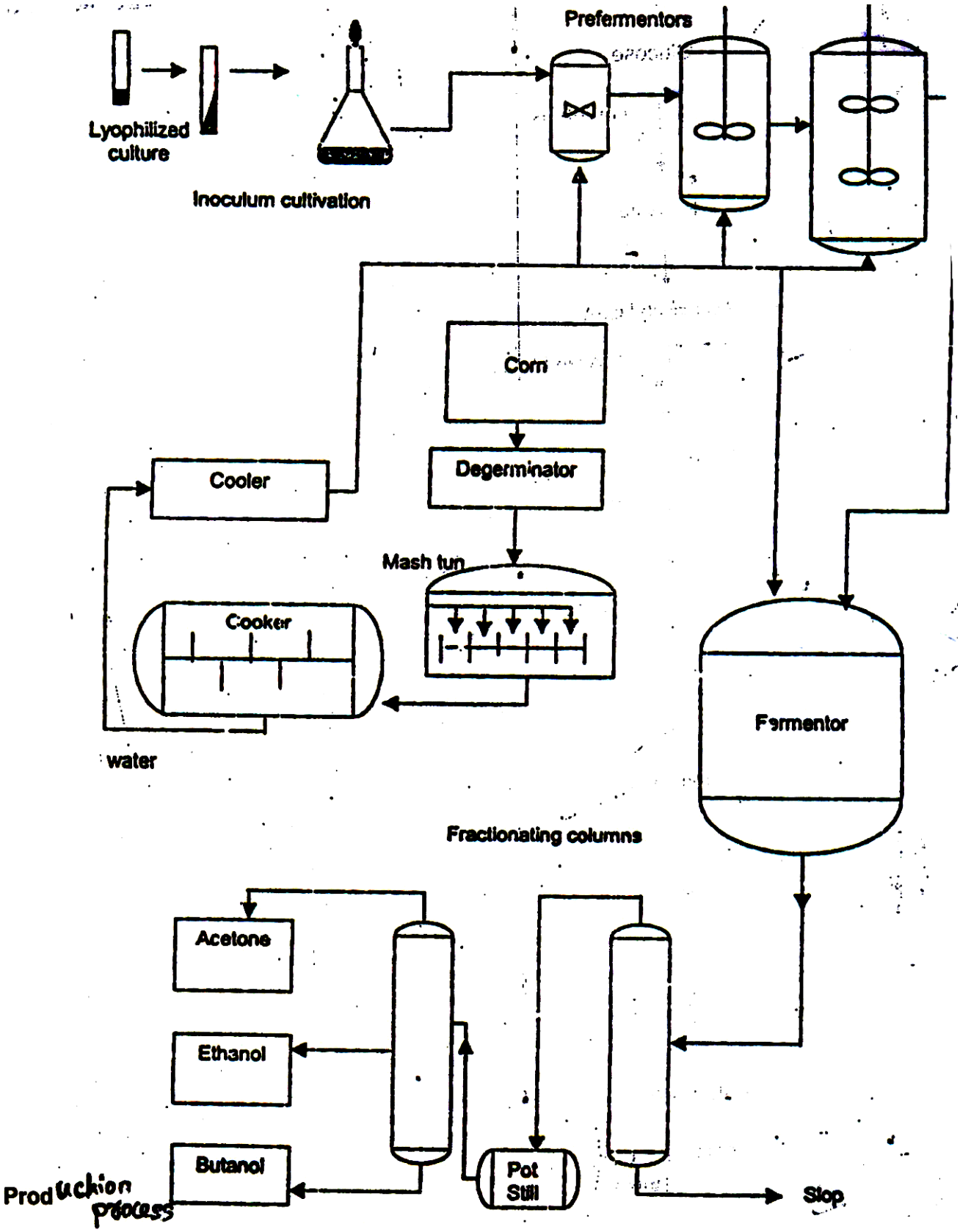
Certain bacteria of clostridium species are capable of producing acetone, butanol, butyric acid and isopropanol through fermentation of molasses, wood hydrolysates, the relative production of the four compounds varies. For clostridium acetobutylium, the fermentation products are actone and butanol.

Biosynthesis:

The sequence of reactions leading to the formation of butanol.

The following flow charts represent the biosynthesis pathway.




Prefermentors
Lyophilized culture
Inoculum cultivation
Corn
Degerminator
Cooler
Mash tun
Cooker
water
Fermentor
Fractionating columns
Acetone
Ethanol
Butanol
Pot Still
Slop
Production process

Production Process:

Butanol & acetone producing bacterium clostridium acetobutylium can be stored in the form of spores for 25-30 years. The inoculum is building up in several stages & it efficiently ferments sugars to give a high yield. These organisms are also resistant to contamination.

The fermentation is usually carried out is corn or molasses based medium. The molasses medium contains molasses, ammonium sulphate, calcium carbonate and corn steep liquor. The fermenters after sterilization is gased with CO_2. After the addition of the medium and inoculum the fermenter contents are again stirred with CO_2. The fermentation is carried out with a starting P^H 5.8-6.0 and temperature 34°C. The actual process of fermentation, spread over 36hours, & it occur in three phases.

Absolute sterile conditions are required for good production of butanol & acetone contamination due to bacterophage and lactobacilli is a major problem that hinder the yield. Approxiamately, 30% of the carbohydrate gets converted to neutral solvent in the fermentation. The actual production of butanol is influenced by its toxicity to organism. Butanol is toxic at a concentration higher than 13.5% in the medium.

Butanol is recovered through continuous distillation and **fraqtinaltion** process. The left over residue after distillation can be dried and used as animal feed.

UNIT – III

PART - A

1. Define Antibiotic give example.

An antibiotic is the complex organic chemical substance which is produced as the secondary metabolite by one Micro organism and acts as a toxin against other micro-organisms; either inhibiting their growth or killing them.

Eq: Streptomycin, Erythromycin, penicillin, Actinomycin.

2. Write four applications of antibiotics.

(i) Antibiotics are particularly important as antimicrobial agents for chemotherapy.

(ii) Few selected antibiotics are used to control the cancer growth, although with a limited success eq: actinomycin D, mitomycin C.

(iii) Some antibiotics are used as a food preservative in canning industry. (eq: chlortetracyline) and for preservation of fish, meal and polutry.

(iv) Antibiotics are used to control the plant diseases: eq: blasticiden, **tetranadin**, polyoxin.

3. Define β - Lactam antibiotics.

β - Lactam is specific inhibitors of bacterial cell wall (peptidoglycan) synthesis. They combine specifically with penicillin binding protein (PBP) of the bacterial cell and, by inhibiting the enzyme activity of this protein, bring about cell death.

eq: Penicillin, cephalosporin, **Nocardins**.

4. Name the penicillin producing micro organisms.

Penicillium notatum & penicillium chrysogenum are mainly used in large scale production of penicillin.

5. What are micro organisms are used for antibiotics production?

(i) Antibiotics produced by fungi. Like genus of Aspergillaceae and **Moniliables.**
eq: penicillin, cephalosporin antibiotics.

(ii) Antibiotics produced by actinomyceses, particularly, of the genus streptomyces
eq: tetracyclines, actinomycin D.
(iii) Antibiotics produced by certain bacteria also.
eq: bacitracin, polymyxins.

6. Give example for Beta lactam antibiotics.

(i) Penicillin
(ii) Cephalosporins
(iii) Clavulanic acid
(iv) Thienamycins
(v) **Nocardins**
(vi) Monobactame.

7. Define Amino glycoside antibiotics.

Amino glycosides are oligosaccharide antibiotics and consist of an aminocyclohex and moiety, which is glycosidically linked to other amino sugars. They are primarily used against gram – negative bacteria in a wide range of applications.

8. Give example for aminoglycoside antibiotics.

Streptomycin, Neomycin B and C, kanamycin A and C gentamicin and sisomicin.

9. What are Macrolide antibiotics?

Macrolides are hydrophobic compounds. They consist of 12 -, 14 - , 16- or 17 – membered lactione rings with 1 – 3 sugars glycosidically lined with the aglycone (tactone ring) and with each other. The sugars are amino sugars and / or 6 – deoxy – hexoses. Macrolides are very effective against Gram positive bacteria, particularly staphylococci, and mycoplasmas and over frequently used against penicillin resistant organisms.

10. Give example for Macrolide antibiotics.

Carbomycin A, Erythromycin, Leucomycins, **Maridomycins**, Oleandomycin, Tylosin and spiramycins I , II, III.

11. Define vitamins.

Vitamins are organic compounds that perform specific biological functions for normal maintenance and optimal growth of an organism. These vitamins cannot be synthesized by the higher organisms, including man, and there fore they have to be supplied in small amounts in the diet.

12. Name the vitamins which are produced by micro organisms in commercial process.

Thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin, vitamins B_{12} & β carotenoid.

13. Name the sources of vitamin A.

Vitamin A, a fat soluble vitamin, occurs in two principal forms in nature: retinal and certain carotenoids. Retinal is found only in animal sources, in foods such as fish, meat, eggs and full fat milk. In plant foods, vitamin A can be obtained from a family of substances called carotenoids which are found in brightly colored fruit and vegetables and green leafy vegetables.

14. Define secondary metabolites.

Secondary metabolites are compounds that are not necessary for growth or maintenance of cellular functions but are synthesized, generally for the production of a cell or microorganism, during the stationary phase of the growth cycle.

15. Give examples for secondary metabolite products.

(i) Antibiotics eq: Penicillin, cephalosporin
(ii) Vitamin eq: vitamin B_2, vitamin B_{12}
(iii) Steroids.

16. Define Bio transformation.

The conversion of one chemical or material into another using a biological catalyst: a near synonym is biocatalysis, and hence the catalyst used is called a biocatalyst usually the catalyst is an enzyme, or a whole, dead micro organism that contains an enzyme or several enzymes.

17. Define Bio conversion.

Bio conversion is defined as, conversion of one chemical into another by living organisms, as opposed to their conversion by enzymes (which is bio transformation) or by chemical processes. The usefulness of bio conversions is much the same as that of bio transformation – in particular its extreme specificity and ability to work in moderate conditions. A major commercial application is in the manufacture of steroids.

18. Give example for important industrially producing antibiotics.

(i) Natural penicillins - Penicillium chrysogenum

penicillin V - penicillium chrysogenum
Penicillin V - penicillium chrysogenum
Ampicillin - penicillium chrysogenum
(2) Cephalosporins - cephalosporium acremonium
(3) Erythromycin - **Saccharaploy oporaery thraea**.
(4) Gentamicin - Micromonspora purpurea.
(5) Streptomycin - Streptomyces griseus.

19. Name the different types of bio conversion reactions.

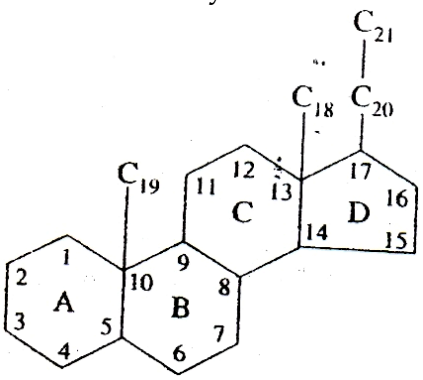
(i) Hydroxylation
(ii) Hydrolysis
(iii) Condensation
(iv) Decarboylation
(v) Oxidation
(vi) N-and – O – Demethylation
(vii) Hydration and Amintion
(viii) Dehydration
(ix) Deamination
(x) Reduction
(xi) Isomerization.

20. Name tetracycline producing microbial strain.

There are three important actinomycetes namely, streptomyces aureofaciens, S. ramosus and Nocardia sulphurea which form tetracyclines.

21. What are steroids?

Steroids are complex organic compounds. Since the several chemical reactions involved in transformation of basic 4 – membered ring into various other products. For example 32 reactions or steps were required to get cortisone from deoxycholic acid.



(a) Structure of steroid.

22. Name the microbes which are involved in the steroid bio transformation.

Several fungi, such as rhizopnsnigricans, Curvularia lunata, Aspergillus sp., penicillium sp., Gliocladium sp., and yeasts are some of the important organisms of steroid bio transformation. Besides, bacteria namely corynebacterium simplex and actinomycet Nocardia restrictus are also known for bio transformation.

23. Give example for steroid bio transformation by micro organisms.

(i) Corynebacterium simplex -- Hydrocortisone to predisolone
(ii) rhizopnsnigricans -- Progesterone to 11-∝ - hydroxy – progesterone.

24. Explain the Role of vitamin B_{12}.

Vitamin B_{12} is produced by intestinal micro organisms. For scoine and poultry feeds,10-15ing vitamins B_{12} is added per ten of feed, since animal protein can be replaced with less expensive vegetable protein if the vegetable protein is fortified with vitamin B_{12}. It also play a major role in biological nitrogen fixation.

25. Name the micro organisms which are employed in the industrial production of vitamin B_{12}.

Streptomyces griseus, S. olivaceus, Bacillus mega Eerium, B. Coagulans, Pseudomonas denitrificans, Propionibacterium freudenreichii, P. Shermanii, and a mixed fermentation of a proteus spp. and a pseudomonas sp.

26. Name the microbes used for the production of Riboflavin.

Micro organisms include bacteria, yeasts or fungi. the outstanding two closely related species of ascomycetes are: (i) Eromothecium ashbyii and (ii) Ashbya gossypii.

27. Explain the rate of vitamin B_{12}.

It is essential for the growth and reproduction of both humans and animals, and, thus, it often is recommended as a feed additive for the animal nutrition. The riboflavin deficiency in rats causes stunted growth, dermatitis and eye damage. Ariboflavinosis is a disease in humans caused by riboflavin deficiency.

28. Name some Narrow spectrum and Broad spectrum Antibiotics.

Narrow Spectrum: Active Against limited types of Organisms include
Pecnicillin, Streptomycin, Bacitracin, polymyxin, Neomycin.
Broad Spectrum: Wide range of action: Tetracycline, Chlor tetracycline,
Chloramphenicol, ampicillin

29. Who discovered Penicillin? Its Source and Classification.

Alexander Fleming (1928)
Source: Moulds eg. Penicillium notatum
Classification:
Naturally Occuring Penicillins: Destroyed by penicillinase-Penicillin G
Semi synthetic Penicillin:
Prepared by combining 6 APA with various side chains

(a) Resistant to acid Gastric juices: Oral Penicillin (Phenoxy methyl Penicillin)
(b) penicillinase resistant: methicillin, Cloxacillin
(c) Broad Spectrum Penicillin: Ampicillin, Carbenicillin

30. What is the mode of action of Penicillin?

Penicillin is a bactericidal drug. It interferes with bacterial cell wall synthesis. So water enters through branches of cell wall leading to lysis of bacteria.

31. What is Ampicillin? What are its Uses?

It is a broad spectrum penicillin, orally effective in both gram Negative and Positive Organisms. Effective against Salmonella, Shigella, E.Coli, H. influenzae, Klebsiella.

Uses: Typoid fever, Urinary Tract infection, bacillary dysentery, entercoccal Endocarditis.

32. What are Cephalosporins? Cite their use.

The mould cephalosporins elaborates cephalosporium cephalosporidine is useful in streptococci, staphylococci, Pneumococci infection. They inhibit cell wall synthesis.

PART - B

1. Define Antibiotics and explain its products in detail.

Antibiotics:

" An antibiotic is a complex Organic chemical substance which is Produced as the secondary metabolite by one micro organism and acts as a toxin against another micro organisms; either inhibiting their growth or killing them".

Antibiotics are products of secondary metabolism. Antibiotics are chemical substance excreted by some micro organisms, which inhibit the growth and development of other organisms. In recent years several antibiotics have been obtained semi synthetically.

One of the large number of known antibiotics of microbial origin, only few are currently produced by fermentation. In addition, more than 50 antibiotics are produced as semi-synthetic compounds and three antibiotics, chloramphenicol, phospomycin and **pyrrolinitrin** are produced completely synthetically.

Chemotherapeutic antibiotics can be either broad spectrum antibiotics, active against many organisms or narrow spectrum antibiotics, active against only a restricted range of organisms. Antibiotics are also used as animal growth Promoters and in veterinary medicine.

The microbial groups Producing Antibiotics:-

Antibiotics are produced by bacteria, actinomycetes and fungi. In the fungi, only the antibiotics produced by Aspergillaceae and moniliates are of practical importance. Only 10 of the known fungal antibiotics are produced commercialy by and only the penicillins, cephalosporin C, griseofulvin, and fludic acid are clinically important.

In the bacteria, there are many taxonomic group which produce antibiotics such as gramicidin and polymyxins. The greatest variety in structure and number of antibiotics is found in the actinomycetes, especially in the genes streptomycin. eg: streptomycin, chloramphenical, tetracycline, erythromycin, kanamycin.

Classification of Antibiotics:-

Antibiotics can be classified according to their antimicrobial spectrum, mechanism of action, producer strain, and manner of biosynthesis or chemical structure.

1. Carbohydrate – containing antibiotics: Streptomycin, **strapsthricin, Everniomycin,** vancomycin
2. Macrocyclic lactones -Erythromycin, Candicidin, Rifamycin, tetranactin.
3. Quinones and related antibiotics- Tetracyclin, Mitomycin, **adxiamycin,** Actinorhodin.
4. Amino acid and peptide antibiotics- Penicillin, Bacitracin, Actinomycin, cycloserine, valinomycin, Bleomycin
5. Heterocyclic antibiotics containing nitrogen. polyoxins.
6. Heterocyclic antibiotics containing oxygen Monensin
7. Alicyclic derivatives- cycloheximide & Fusidic acid.
8. Aromatic antibiotics- chloramphenical, Griseofulvin, Novobiocin.
9. Aliphatic antibiotics -Fosfomycins.

Application of antibiotics:-

Most antibiotics are manufactured as antimicrobial agents for chemotherapy, but some have other applications, as out lined below.

Antitumor antibiotics:-

Such antibiotics are clinically used as cytostatic agents. It is generally toxic, with careful control of the dose certain of these antibiotics are effective in the treatment of certain kinds of tumors.

eq: Aclacinomycin, Mithramycin, Adriamycin.

Antibiotics for plant Pathology:-

Antibiotics may be more useful than synthetic chemicals in the control of plant diseases for the following reasons: they may be applied selectively in low concentrations, they are only slightly tonic to warm blooded animals and beneficial soil micro organisms.

ea: Blasticidin S, pol yoxin, cycloheximide**, tetranactin**.

Antibiotics as food Preservatives:-

Government regulations in each country directly control the use of antibiotics as food preservatives. Pimaricin, a fungicide applied to food surface; tylosin (effective against clostridia), both used in the canning industry; chlortetracycline, used to maintain freshness in fish, meat and poultry by in corporation into ice. Antibiotics used as animal growth promoters and in veterinary medicine:

Animal feed is more efficiently processed in the animals digestive system of an antibiotic additive is used in subtherapeutic concentrations weight gain may also be accelerated. Example

for the therapeutically useful antibiotics are penicillins, tetracyclins, erythromycins, streptomycin are added to feed.

Antibiotics as tools in biochemistry and Molecular biology:-

The use of antibiotics as selective inhibiter & has made a vital contribution to the understanding of certain cell functions, such as DNA replication, transcription, translation and cell wall synthesis.

2. What are β -Lactam Antibiotics and give one example with their production?

β -Lactam Antibiotics:

The β -Lactam antibiotics can be divided into five distinct classes. The penicillin and the cephalosporins belong to the most effective of all therapeutic agents and for the control of infectious diseases.

Penicillins:-

Penicillin was the first antibiotic to be produced industrially. The credit for the discovery of penicillin goes to sir Alexander fleming (1929). Penicillins are produced by many fungi, particularly penicillium and Aspergillus species. Natural penicillins are effective against numerous gram positive bacteria. They are liable in acid and may be inactivated by spliting the B – Lactam ring with penicillin β lactamases. Because of its low toxicity, large doses of penicillin can be used; only a small percentage of patients develops allergies (0.5 – 2%).

β - Lactam antibiotics are specific inhibitors of bacterial cell wall (peptidoglycan) synthesis. They combine specifically with the so called penicillin – binding Protein (PBP) of the bacterial cell and, by inhibiting enzyme activity of their protein, bring about cell death.

The basic structure of the penicillins is 6 – aminopenicillanic acid (6 – APA), which consists of a thiozolidine ring with a condensed β - Lactam ring. The 6 – APA carries a variable acyl moiety in position 6. the penicillin fermentation is carried out without addition of side chain precursors, the natural penicillins are produced.

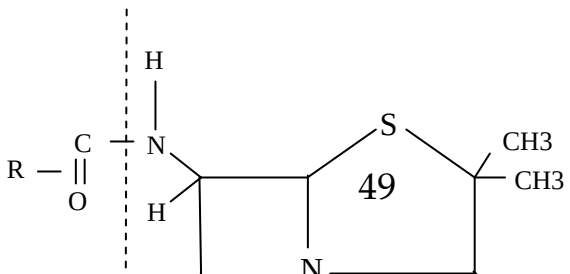


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Figure:

Over 100 biosynthetic penicillins have been produced. In commercial processes only penicillin G, penicillin V and penicillin O have been produced. About 38% of the penicillins, 12% in veterinary medicine and 43% are used as starting material for the production of semi synthetic penicillins.

Production of Penicillin:

Penicillin G and V are produced using submerged processes in 40,000 – 200,000 liter fermentors. Due to difficulties with O_2 supply, large tanks cannot be employed. Penicillin fermentation is an aerobic process. Various turbine impellers are used in the fermenters. Depending on the production strain used, the optimal temperature ranges is between 25 – 27^oC.

The inoculum is started using lyophilized sporces. After several stages of growth the production culture is ready. The micro organism used is penicillium crysogenum. The production medium consists of carbon source (such as lactose, molasses or glucose), Nitrogen source (corn steep liquor, Soya meal, yeast extract or whey) and phenylacetic acid or phenoxyacetic acid (as precursor).

The P^H is maintained at 6.5. The precursor is added to suppress other types of penicillins. In the typical penicillin fermentation there is a growth phase of 40 hours duration, with a doubling time of 6 hours during which time the greatest part of the cell mass is formed. After the growth phases, the culture proceeds the actual penicillin production phases. By feeding with various culture medium components, production phase can be extended to 120 to 180 hours.

Penicillin is excreted in the medium and less than 1% remains in the mycelium. The completed penicillin fermentation culture is filtered on a rotary vacuum filter is remove the mycelium and other solids, phosphoric or sulphuric acid are added to lower the P^H to 2 to 2.5 in

order to convert the penicillin to a anionic form. The broth is then immediately extracted in a two stage counter current extractor with the organic solvent such as amyl acetate; **methyl is duty xelone** or butyl acetate at 0 – 3°C. The yield is around 90%.

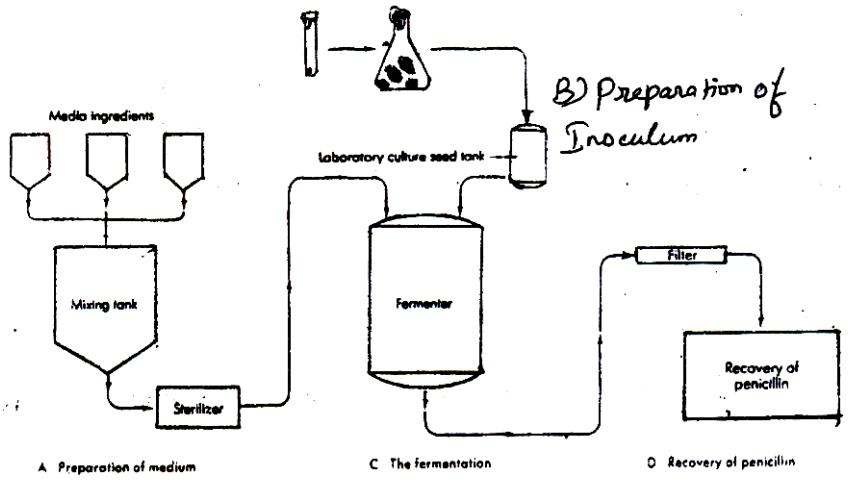


Figure: Flow chart of Penicillin fermentation Nutrient.

3. What are cephalosporins and given detailed account about its Production?

Cephalosporins are β– lactum antibiotics containing a dihydrothiazine ring with D-X aminoadipic acid as acyl moiety.

The pharmaceutical uses of penicillins are associated with allergic reactions in some individuals. To overcome these allergic problems, cephalosporins were developed. They have improved stability against β - lactamases, and are more active against gram-negative bacteria.

Cephalosporin C was first discovered in the cultures of fungus cephalosporium acremonium and this organism to be used still. The other organisms employed for cephalosporin production are Emericellopsis sp. paecilomyces sp and streptomyces sp. several mutant of C. acremonices have been developed for improved production of cephalosporin.

Cephalosporins are valued not only because of their low toxicity but also because they are broad spectrum antibiotics, comparable in action with ampicillin. With about 29% of the antibiotic market, cephalosporins are the single most important group of antibiotics. Therapeutically only semi synthetic derivatives of cephalosporin and **cephamycin** are used. Cephalosporins are economically significant as penicillins.

<u>Production process</u>:

The production process is similar to that of penicillin. The culture media consists of corn steep liquor and soy a flour based media in a continuous feeding system. The other ingredients of the medium include sucrose, glucose and ammonium salts. Methionine is added as a source of sulfur.

The fermentation is carried cut in temperature 25^0 – 28^o C and PH 6 – 7. The growth of micro organisms substantially increases with good O2 supply, although during production phase, O2 consumption decline. Cephalosporin C from the culture broth can be recovered by ion-exchange resins and by using column chromatography cephaloporin C can be precipitated as zinc, sodium or potassium salt and is dated.

In recent years, by penicillin v as the starting material, chemical synthesis of cephalosporin has become possible. This is being done due to low cost of production of penicillin.

4. What are Amino glycosides and explain its production in detail?

Amino glycosides:

Aminogly cosides are oligosaccharide (carbohydrate) antibiotics. They contain an amino cycle hexane moiety which is bound to other amino sugars by glycosidic linkages. More than 100 aminogly cosides are known. eq: streptomycin, neomycin, kanamycin, gentamycin, **hygromycin**, sisomicin.

Aminoglycosides are very potent antibiotics an act against Gram negative and Gram positive bacteria, besides mycobacteria. Prolonged use of aminglycosides causes damage to kidneys and hearing impairment. For the treatment of severe and chronic infections, aminoglycosides are the antibiotics of choice.

Aminoglycoside antibiotics are produced by Actinomyces sp. Recombinant DNA techniques have been used to produce hybrid aminoglycosides, and for increasing the fermentation yield.

Eq: Aminoglycoside	**Producing Organism**
Streptomycin	Streptomycin griseus
Neomycin B CC	S. fradiae
Kanamycin A,B,C,C	S. Kanamyceticus
Hygromycin B	Hygroscopicus
Gentamycin	Micromonospora purpurea.

Streptomycin:

Streptomycin is produced by streptomycin griseus. If was the first aminoglycoside that was successfully used to treat tuberculosis (i.e., against Mycobacterium tuberculosis).

It has been used therapeutically in the treatment of infections caused by organisms resistant to penicillin. In, man prolonged streptomycin treatment at high dosage can produce neurotoxic reactions and partial hearing loss. Some micro organisms gain resistance relatively easily to streptomycin, and hence streptomycin therapy is often carried out in conjunction with para – amino salicylic acid or isoniazid.

Production Process:-

Most of the media for the commercial production of streptomycin are some what similar to that of penicillin. The medium consists of soya meal or corn syrup that can supply glucose at a slow rate. The initial supply of nitrogen (NH3) and phosphate is also obtained from soya meal. This is required since glucose, ammonia and phosphate in high quantities inhibit streptomycin synthesis.

The fermentation conditions for optimal production of streptomycin are temperature 27^0 – $30^{\circ}C$, P^H 6.5 – 7.5, aeration rate 0.5 – 1.0 vvm. The duration of fermentation process depends on the strain used, and is between 6 to 8 days. Spores of the strains of streptomyces griseus are maintained as soil stocks or are lyophilized in a carrier such as sterile skim milk. The spores from this culture are then transferred to a sporulation medium to provide enough sporulated growth to initiate liquid culture buildup of mycelial inoculum in flasks or inoculum in tanks.

During the first phase lasting 24 hours, there is rapid growth of mycelium. In phase 2 streptomycin is produced at a rapid rate. Approximately no growth occurs during this period. In phase 3, sugar is depleted and the production of streptomycin ceases. The cell lyses. However before this phase the fermentation is stopped.

The mycelium is separated from the broth by filtration, and the antibiotic is recovered by one of the several procedures. In one procedure, the streptomycin is adsorbed from the broth to activated carbon and the eluted from the carbon with dilute acid. The eluted streptomycin is then precipitated by solvents, filtered and dried before further purification.

In an alternated procedure, the fermentation broth is acidified, filtered and neutralized. It is then passed through a column containing ion exchange resins to adsorb the streptomycin from the broth, the column is then washed and the **biotic** eluted with hydrochloric acid and concentrated in vacuum to almost dryness. The streptomycin is dissolved in methanol and filtered, and acetone is added to the filtrate to precipitate the antibiotic. Finally the precipitate is

washed with acetone and dried in vacuum.

5. What are Macrolides and explain its industrial production in detail?

Macrolides:-

Macrolides are a group of antibiotics with large lactone rings. They consist of 12, - 14 or 16 – membered lactone rings with 1 – 3 sugars linked by glycosidic bonds. The sugars may be 6 – deoxy hexoses or amino sugars. Erythromycin and oleandomycin and tylosin are example of 16 – membered macrolides.

Erythromycin and its derivative clarithromycin are the most commonly prescribed macrolides. They are effective against Grame positive bacteria, and are frequently used to penicillin – resistant organisms. Clarithromycin is currently used to combat stomach ulcers caused by H. Pylori. The macrolides inhibit the protein synthesis by binding to 50s ribosome.

Production of Macrolides:-

Macrolides are produced by actinomycetes. The major macrolide antibiotics and the corresponding organisms synthesizing them as follows.

Macrolid antibiotic	Producing organism
Erythromycin	Streptomycin erythroces
Oleandomycin	S. antibiotics
Pikromycin	S. felleus
Tylosin	S. fradiae
Leucomysins	Streptsverticillium kitas atoensis.

All macrolide antibiotics are inhibitors of protein synthesis, binding is the 50 S submit of bacterial ribosome. Erythromycin is known to inhibit the elongation. Factor G – dependent release of deacylated t – RNA from the P site of the ribosome.

Macrolide antibiotics are produced in aerobic submerged fermentation process. Yields in large scale industrial processes are around 20 g/l. Industrial production of erythromycin is carried out by aerobic submerged fermentation the culture medium mainly consists of soya meal or corn steep liquor, glucose, yeast extract and ammonium sulphate. Fermentation is carried out at 30 – 34°C for about 3 – 7 days.

6. Define vitamins, and explain various vitamin productions in detail.

Vitamins:

Vitamins are chemical substances essential to the physiological processes in humans as well as animals. They are catalytic in activity and effective in small doses. As a rule they cannot be synthesized by the organisms. They must be provided by exogenous sources which may be natural food, synthetic preparation. They are produced by bacterial fermentation or by treatment of natural vitamin precursors called provitamins. Such as thiamine, **vitoflvin**, folic and panrtothenic and pyridoxal, vitamin B12 and biotin.

(i) Vitamin A:

Vitamin A (Retinol, axerophthol) is usually referred to as vitamin A1. It is diterpenoids. It influences growth in animals, and also apparently increases resistance to diseases. Night blindness is due to vitamin A1 deficiency in the human diet. Vitamin A, a fat soluble vitamin, occurs in two principal forms in nature: retinal and cestain carotenoids.

β-Carotelne is produced by various organisms, but particularly by members of the choane shoraceae family of the phycomycetes. Increased yield of B carotene are obtained if both the plus and minus mating types of choanephor a cucubitarcem are grown together.

The fermentation medium includes among other ingredients, B-ionone, nonionic detergent, and vegetable oils. Inoculum of each mating type is grown separately and approximately 5 % of each is added to the fermentation medium. Although it is not necessary both types be added simultaneously. The β-ionone is asceptically added to the fermentation approximately 48 hours inoculations.

(2) Vitamin B12 (cobalamin)

The term vitamin B12 refers to a group of molecules called cobalamins which all contain cobalt. These include hydroxocobalamin, adenosyl cobalamin active coenzyme form), Methylcobalamin (active coenzyme form) and cyanocobalamin (synthentic form of vitamin B12). The main diatary sources of vitamin B12 are animal Products for example, organ meats, fish, eggs & dairy Products. It is involved in the cellular metabolism of carbohydrate, protein and fat and in the production of wood cells in. bone marrow, Nerve sheaths and proteins.

Vitamin B12 is synthesized by bacteria is the gut and exists in all foods from animal source. People with gastrointestinal disease that cause absorption problems, including **corhn's** disease, ulcers and **sprue**, may have a vitamin B12 deficiency. The recommended daily allowance for vitamin B12 is 2.0 micrograms per day.

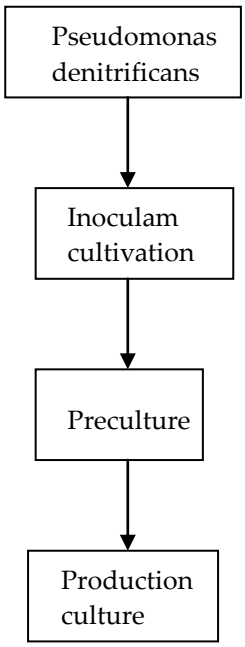
Synthesis:

The industrial production of vitamin B12 involves bacterial fermentation. Most of the B12 fermentation processes use glucose as a carbon source. The strains with high yield are: Bacillus megaterium (0.45 mg/l), streptomyces olivacers (3.3 mg/l), micro monospoa (11.5 mg/l), propionibactericum freudenreichi (19mg/l), propionitruterium shermanii (30-40 mg/l) and pseudomonas denitrificans (60 mg/l).

Pseudomonas denitrificans is found to be the most productive special among the different vitamin B12 prouduing organisms.

Process with Pseudomonas denitrificants:

It is a one stage process, the process is shown below



Inoculam stage: lyophilized with dry milk

Agar slant with medium A.
Incubation: 96h, 280°C.

1 litre Erlemeyer flask/with 150ml Medium B. Incubation: 72h, 280°C, shaker

5 litre fermenter with 3.3 litre medium C, Sterilization 75 min and 1200°C.
Inoculum: 150 ml Preculture, Incubation: 90 hours, 290C
Stirring 40 rpm Aeration 1 vvm
Medium A (p/l) : Sugar beet molasses 60; yeast Extract 1; N-Z-Amine 1; (NH_4) HPO_4 2;

Mg $SO_4.7H_2O$ 0.02; $Na_2MnO_4.2H_2O$ 0.005; Agar 25; Tap water; **p^H7.4.**

Medium B(g/l) : Medium A, without Agar.

Medium C (g/l): Sugar beet molasses 100; yeast extract 2; $(NH_4)2\ HPO_4$ 5;
$MgSO_4.7H_2O3$; $MnSO_4$, H_2O 0.2; $Co(NO_3)2.6H_2O$. 0.188;
5,6-Dimethyl benzimidzol 0.025; $ZnSO_4$. $7H_2O$ 0.02; Na_2 Mo O_4 $.2H_2O$

$.2H_2O$ 0.005; Tap water; p^H 7.4.

Pseudomonas denitrificans has been found to be the most productive species among the different vitamin B12 is produced during the entire fermentation process. Cobalt and 5,6, dimethyl benzimidazole must be added as supplements. If has also been found that additions of the compound betaine result increased yield; *sugar* beet molasses is used as a low cost betaine source.

(3) Vitamin B2 (Riboflavin)

Vitamin B2 or riboflavin is an intermediary the transfer of electrons in the cellular oxidation reduction reactions which generate energy from protein, carbohydrate and fat. The principal rich sources of riboflavin in the dietary sources are milk and milk products, Egg white, meats, Leafy green vegetables and Egg yolks.

There are two methods in the manufacture of riboflavin

- Fermentation
- Synthesis from xylene, D ribose and alloxan (usually the processes used are refinements of the method developed try Kuhn and Karrer in 1934).

Commercial Production Vitamin B2

(a) Chemical synthesis:

The procedure involves a reductive condensation of 6-nitro-3, 4-xylidine with D-ribose to give the nitro compound, which is catalytically, reduced to phenylenediamine. This when treated with alloxan in acid medium gives riboflavine in 16 % yield, based on D-ribose.

(b) Microbial synthesis:

Riboflavin can be obtained by fermentation process using different micro organisms and appropriate media. Most of the riboflavin produced by fermentation is consumed in the form of crude concentrates for animal feed supplements.

Three group of micro organisms have been found to synthesize riboflavin in significant

amounts

- Bacteria of the butanol, acetone group of which the chief representative is clostridium acetobutylicum certain candida yeasts

- Two closely related yeast like fungi, eremothecium ashbyli and ashbya gossypii; yields are usually expressed in micrograms of riboflavin per millitre of brok.

The general process involves the preparation and sterilization of suitable carbohydrates containing mash cereals like corn, rice, whey and semisynthetic starch materials. All have been successfully used when it is adjusted to pH 6-7, buffered with calcium carbonate.

As a result of the discovery that iron in concentrations exceeding about 5 ppm exerts a is carried out in non ferrous or glass lined vessels or else iron-sequestering agents like 2,2 bipyridine are added to the mash. Optimum iron concentration is reported to be between 1 and 3 ppm. Following inoculation with C. Acetobutylicium, the mash is incubated at 37-40°C for 2-3 days.

Several techniques are employed for recovering riboflavine from the liquors; butanol extraction followed by precepitation from the extract by means of petroleum eshen yeilds upto 70g/ml have been reported for the c.aut obutylicum method.

Another organism used industrially for the fermentation of riboflavin is the yeast like fungus Eremothecium ashbyii, which is grown in a medium of protein materials, carbohydrate and vegetable oil lipids the inoculated mixture is aerated and agitated for 50-90 hours at a temperature between 200°C to 340°C, after which the final beer is heated for an hour at 60-120°C to free the riboflavin from the mycelium. By this method, yields of 500 g/ml have been obtained.

Processes employing several candida yeasts also have been potented. The substrate consists of a sugar, phosphate, ammonia, glycine, biotin, mineral salts and trace elements. Fermentation is allowed to proced for a week in the dark at temperatures around 300°C yields are of the order of 250-300 mg/ml

7. What are steroids and explain their production in the industry?

Steroids are complex organic compounds because several chemical reactions involved in transformation of basic 4 membered ring various other products, hence biological transformation is important due is the reason that only few steps are required getting products.

For example 32 reactions or steps are required to get cortisone from deoxycholic acid, by sasset.

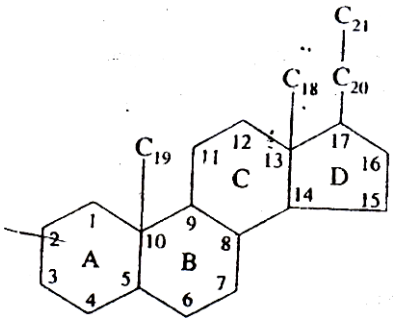


Figure: Structure of steroid nucleus showing numbering system.

Microbial Preparations of many steroids by their enzyme action at the specific site let is the synthesis of Novel varieties of steroids. Such processes are more viable and specific. Steroid transformation is different with that of micro biological process due to the fact that in later that organic acids, solvents, antibiotic are synthesized from the ingredient in the medium which also serve as substrate for growth and reproduction of micro organisms.

(i) Micro organisms:

Several fungi, such as Rhizopus nigricans, curvularia lunata, Aspergillus sp., Penicillium sp., Gliocladium sp., Fusarium solani and yeasts are some of the important organisms of steroid biotransformation. Besides bacteria namely corynebacterium simplex and actionmycete Nocardia restrictus are known for bio transformation.

(ii) Production:

The production of steroids can be carried out by the following steps.

(a) Cultivation of microbe:

The appropriate microbe is grown in a way to get maximum growth in short period of time. Generally, glucose or sucrose are recommended as carbon source and corn steep liquor as nitrogen source.

(b) Incorporation of steroids and inhibitors into the medium:

A suitable amount of steroid (0.25 to 1.0g lit -1) to be transformed is first dissolved with the desired solvent (solvent should not inhibit with the microbe) and then is to add in the medium after the growth of the organism. The desired inhibitors are added to inhibit the undesired enzyme activities.

(iii) Transformation of steroids:

Microbes transform the steroid in a few hours to several days.

(iv) Separation and Purification:

For this, regular sample is to be analyzed chromatographically using TLC. The spots are eluted and their quantity can be measured spectrophotometrically. The transformation product is extracted with a suitable solvent and then purified by using column chromatgraphy or crystallization.

The structure is determined by using classical organic chemistry methods. Some importent bio transformations as shown in below.

Microbe involved	Substrate into Product	Reaction
(i) Corynebacterium simplex	Cortisol →Prednisolone Cortisone → Prednisone	Nuclear double bounds in ring A
(ii) Rhizopus nigricans	Progesterone → 11 ∝ Hydroxy progesterone →	Hydroxylation at 11 ∝
(iii) yeast	Androstenedione Testrosterone	Hydroxylation at 17β

Important Biotransformations:

Steroids are organic molecules which have common as per hydrocyclopentaphenantherne nucleus. Steroids are named because they are related to sterols which are abundant in nature. e.g. cholesterol (found in brain and nerve tissue), stigma sterol (in vegetable oils), ergosterols (in yeast), sapogenins (diosgenin and hecogein in plant)etc. The sapogenins are extremly useful starting material for sex hormone sysheing and later on for corticoides and contraceptive drugs. It is observed that an agar plate exposed on the window yielded a. culture of the genus rhizopus. When this culture was grown using Progesterone as a substrate, they found that is unexpectedly converts Progesterone to 11 ∝-hydroxy Progesterone 80 higher yield.

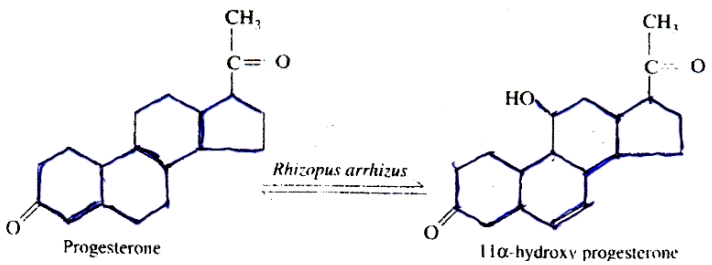


Figure: Steroid transformation by chemical means 1: Progesterone ints 11∝ - hydroxy Progesterone.

Steroid biotransformation by certain micro organisms as follows.

Micro organism	Reaction	Substrate to product
(i) corynebacterium simplex	Introduction of 1, 2 position	Hydrocortisone to prednisolone
(ii) Rhizopins nigricans	Hydroxylation at 11 ∝ position	Progesterone to 11 ∝ hydroxy progesterone

These days steroid bio transformation is carried out in submerged cultures in large fermenters. These transformations differ from the conventional process in that the product are not synthesized from the medium ingredients. Steroid precursors are added into the culture towards the end of growth phase. In recent years, purified enzymes have also been tried in places of organisms in steroid transformation.

UNIT – IV

PART - A

1. What are enzymes?

Enzymes are biocatalysts. They catalyze a particular reaction or a group of closely related reactions with or without the aid of cofactors. All the functions of the body such as digestion, synthesis and breakdown of carbohydrates, Proteins, fats and nucleic acids are catalyzed by specific enzymes. They are Protein in nature. Their physical and chemical properties are similar to proteins.

2. Give some therapeutic uses of enzymes.

S.No	Enzymes	Therapeutic uses
1	∝ chymotrypsin, trypsin and papain	Treatment of abscess and infected burns
2	Fibrinolysin, pancreatic dornuse and streptodornase	Infected skin ulcers, cervitis and vaginitis
3	Streptskinase plus streptodornase	Dissolves thrombi and fibrinous adhesions
4	Pepsin, trypsin, peptidase and lipase	Enzyme deficiency, gasterointestinal disorders
5	Amylase, nuclease, elastase, cellulase	Chronic Panoratitis And Puncreatectsmy.

3. What are the advantages and disadvantages of enzymes as commercial agents?

Advantages:

1. Enzymes have high catalytic activities
2. They catalyze a great variety of reactions
3. They are useful for stereo specific reactions
4. They work under mild reaction conditions

5. Unnecessary reactions and unwanted products do not occur.

Disadvantages:

1. Enzymes are fragile and unstable molecules
2. They are available generally is small quantities only
3. They are expensive from a commercial point of view

4. Explain the industrial use of enzymes present in the whole microbe.

Brewing is the Process in which the enzymes of years (saceharomyces cerevisiae, a single cell micro organism) degrade the sugars present in fruit as matted barlely to ethanol and carbon dioxide. This process is also known as fermentation, takes several days or weeks and results in accumulation of maximum alcohol. Above which the yeast is itself killed.

5. Why are microbes used as common source of industrial enzymes?

Micro organisms are capable of producing enzymes both inside their cells and outside the cell. Selected micro organisms are usually cultured in large fermentation chambers, known as fermenter, under controlled conditions to maximize enzyme production. Some specific genes of mammalian origin can be introduced very easily into the era of micro organisms through genetic engineering so that they produce enzymes which are highly difficult to be produced from their parent sources. Therefore microbes are commonly used as a source of industrial enzymes.

6. What are the advantages and disadvantages of using enzymes in industries?

Advantages:

1. They are specific in their action and therefore less likely to produced unwanted by products
2. Since they biodegradable, they cause less environmental pollution.
3. They are energy saving because they work in mild conditions such as low temperature and neutral p^H

Disadvantages:

1. They are highly sensitive to change in their environment conditions in which they work must be scrupulously controlled.
2. They are highly susceptible to various types of inhibitors.
3. The enzyme and other components of the reaction mixture must be in highly purified state.

7. What are the limitations in using enzymes as therapecetic agents?

Since enzymes are specific biological catalyts, application of enzymes as therapeutic agents for the treatment of various metabolic diseases should produce the expected desirable results.

Unfortunately, the following number of factor, severely reduce their activity and hence their utility.

1. Since applied enzymes are generally foreign proteins to the body, they are antigenic and elicit immune response which may cause severe and life threatening allergic reactions.
2. The effective lifetime of applied enzymes within the circulation is only a matter of minuets, continuous addition enzymes or enzymes with long lifetime is needed for effective therapeutic results. But the cost of the purified enzyme is very high. Such costly medical treatments are not reachable to common people.

8. Give example for some industrially important producing enzymes.

∝ Amylase, Glucoamylose, Protease, Lipase, Cellulase, Pectinase, Glucose, Gudase**,** Invertace, Aspartase, Amino acylose, Glucose Isomerase and Catalase.

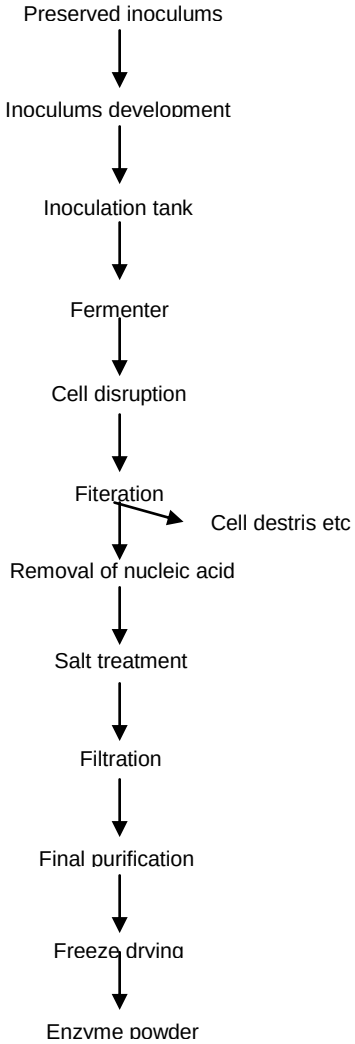
9. List some industrially produced enzymes and their applications.

1. Hyaluronidase	- Medical use
2. Lipase	- Digestive aid, flavoring of milk products
3. Cyto chrome C	- Medical use
4. Catalase	- Sterilization of milk
5. Microbial rennet	- Cheese manufacture
6. Glucose isomerase	- Glucose → Fructose
7. Lactase	- Drying of lacquer
8. Cellulose	- Digestive aid

10. Name the microbes which are used for the commercial production of enzymes.

Commercial microbial enzyme productions utilize mainly various fungi, bacteria and yeast and in fact, bacteria and fungi often produced similar enzymes.

11. Draw flow chart for the production of enzymes by micro organisms.



12. Write ∝ amylases producing beateria.

Bacillus subtotes, B.cereus, B.amloliquefaciens, B.coagulans, B.aciducaldarius, B.licheniformis, Lactobacillus, Micrococcus, Pseuedomonae, Arthrobacter & Escherichia.

13. Write ∝ amylase producing fungi strains.

Some ∝ amylase producing fungi are from the genera Aspergillus, Penicillium, cephalous porium, Mucor, candida, Newrospora and Rhizopus.

14. Mention the application of a ∝ amylases in industry.

1. Starch industry: Liquefaction of starch for production of glucose, fructose, maltose,
2. Alcohol: Liquefaction of starch before the addition of malt for saccarification.
3. Backed goods: Increase in the proportion of fermentable carbohydrates.

4. Brewing: Barley Preparation, liquefaction of additives.
5. Textiles: Continuous desizing at high temperatures.
6. Sugar: Improvement of filterability of cane sugar juice via breakdown of starch in juice.
7. Laundary and detergent: Increase in cleaning power for laundary soiled with starch, additive in dish washer detergents.

15. Name β-amylase Producing micro organisms.

Bacillus prelumyxa, B.cereus, B.megaterium, streptomyces sp., pseudomonas sp., and Rhizopus fapanicus.

16. Name the microbes which are used for the production of Glucoamylases.

Micro organisms used to produce gluco-amylases are Aspergillus niger, A. oryaze, A.awamori, Rhizopus niveus, R.determar and R.favanicus.

17. Name Glucose isomerase producing micro organisms.

The most important glucose isomerase producers are bacillus coagulane, streptomoyces rubigincosue, Actinoplane, Actinoplanes missouriensis, Flavobuserium arborescens.

18. Write the industrial applications of proteases.

Proteases are used primarily in the detergent industry and in the dairy industry. The other areas in which proteases are used include the pharmaceutical industry, the lather industry, the manufacture of the protein hydrolysates, the food industry, the film industry, and waste processing.

19. Name the micro organisms which are produced alkaline proteases.

Many bacteria and fungi exerete alkaline proteases. The most important producers are Bacillus strains such as B.licheniformis, B.amyloliquefaciens, B.firmus, B.megaterium, and B.pumilis; Streptsmyces strains such as s.fradiae, s.griseus, and s.rectus; and the fungi Aspergillus niger, A.sojal, A.oryzal and A.flaves.

20. Name neutral Proteases producing micro organisms.

Producing organisms include: Bacillus subtiles, B.cereus, B.megaterium, Pseudomonas actuginosa, strepto myces griseus, Aspergillus orgzae. A.sojae and pericularia oryzal.

21. Name pectinases Producing micro organisms and their applications in industry.

Aspergillus niger or A.wentic fungal strecin are mostly used for pectinase enzyme production. It also produced by bacteria, protozoa, insects and higher plants.

Pectinases are used primarily to clarify fruit juices and grape must for the maceration of vegetables and fruits and for the extraction of olive oil.

22. List lipases Producing Organisms.

Fungi:

Aspergillus mucor, Rizopus, Penicillium, Geotrichum, and the yeasts torulopsis and condida.

Bacteria:

Pseudomonas, Achromobacter and staphylococcus

23. Define SCP.

Single cell protein (SCP) refers to the microbial cells or total protein extracted from pure microbial cellculler, which can be used as protein supplement for humans or animals. Besides high protein content, SCP also contains for carbohydrates, nucleic aids, vitamins and minerals.

24. What are the advantages of using micro organisms for SCP production?

1. Micro organisms grow and a very rapid rate under optimal culture conditions. Some microbes double their mass in less than 30 minutes
2. The quality and quantity of protein content in micro organisms is better compared to higher plants and animals.
3. A wide range of raw materials can be fruit fully used for SCP production.
4. The culture conditions and the fermentation processes are very simple
5. Micro organisms can be easily handled, and subjected to genetic manipulations.

25. Name SCP producing micro organisms.

Bacteria:

Methylophilus methylotrphys, pseudomonades sp., Brevibacterium sp.

Yeasts:

Saccharomy copis lipolytica, saccharomyces cerevisiae, lactobacillus bulgaricus, tosulopsis sp.,

Fungi:

Chaetsmium cellulolytiuem, paeutomyces varioti, aspergillus niger.

Algae:

Spirulina maxima, Chlorella pyrenoidosa, Scenedesmus acutus.

26. Name important microbial polysaceharide and their producing micro organisms.

1. xanthan gum – xanthomonas canoestris
2. Dextran – Leuconostoe mesenterides autsbacter sp., streptscoccus mutans
3. Alginate – Pseudomonas aeraginosa Azobacter vinelandic
4. Gellon – Pseudomonas elodea
5. Polluan –Aureobasidum pellulans
6. Emulsan – Acinetobacter calcoaceticus

27. Write the applications of biopoly saccharh aides.

1. Xanthan → As a food additive for stabilization, gelling and viscosity control, i.e. for the preparation of soft foods e.g. ice cream, cheese.
2. Dextran → Blood plasma expander, used in the prevention of thrombosis, and in wound dressisng (as adsorbent). In the laboratory for chromatgraphic and other techniques involved in purification, used as a food stuff.
3. Alyginate → In food industry as thickening and gelling agent. Alginate beeds are employed in immobilization of cells and enzymes. Used as non-exchange agent.
4. Polluan → Being a bio degradable podysauccaride, it is used in food coating and packaging
5. Einrelsan → In oil industry for enhanced recovery. For cleaning of oil spils.

28. What are biofertilizers?

Biofertilizers is used to refer to the nutrient inputs of biological origin to support plant growth. This can be achieved by the addition of microbial inoculants as a source of biofertilizers. Biofertilizers broadly includes the following categories.

- Symbiotic nitrogen fixers
- Asymptotic nitrogen fixers
- Phosphate solubilising bacteria
- Organic fertilizers

29. Name the microbes which are used as biofertilizers.

Micro organism	Useful for corps
1. Symbiotic nitrogen fixers Rhizobium leguminosarcem , R. meliloti, R.ciceri, Bradyrhizobium japonicum	Legumes (pulses oil seeds)
2. Asymbiutis Nitrogen fixers Azobacter, Azospirillum, Blue green algae	Wheat, rice, sugar, cane, joker, vegetables, rice,
3. Phosphate Solubilizing bacteria Thiobacillus, Baullss Mycorrhiza	Pulses Pulses

30. Write the benefits of using biofertilizers.

1. low cost and easy to produce small farmers are immensely benefited
2. Fertility of the soil is increased year after year
3. Free from environmental pollution
4. Besides nutrient supply, some other components which promote plant growth, are also produced e.g. plant growth hormones, antibiotic.
5. Biofertilizers increase physico – chemical properties of the soil, soil texture and water holding capacity.
6. Biofertilizers improve the tolerance of plants against toxic heavy metals
7. Plants can better withstand biotic and a biotic stresses and improve in product yield.

PART - B

1. Write short notes about enzyme production in industry.

Enzyme Production:-

Enzyme are organic substances, which are produced by living cells numbers of enzymes have been isolated in a pure form and even crystalline state and are found to be proteins. They have the ability to catalyze the numerous reactions. Enzymes can be divided into two categories depending on the in vivo location where they normally carry the biological function. They are intracellular and extra cellular enzymes.

The extra cellular enzymes or ext-enzymes are synthesized with in the cell and then secreted into the extra cellular space. Majority of the commercially utilized enzymes are extra cellular type, the intra cellular enzymes are both synthesized and utilized within the cell they are bound or scrapped within intra cellular membranes. Hence isolation of intra cellular enzymes are much more difficult.

Over 2000 enzymes have been isolated and characterized, the majority of them potentially available from any biological organism, enzymes can be extracted from a plant, animal or a microbial cell. The only major animal enzymes in use at present are trypsin, a variety of lipases

and rennets. Because of the long time for growth and the difficulty in isolation, animals serve as a source of enzyme only under special conditions, such as no other source or unavailability of a large supply of animal organs locally.

Plant enzymes are most prevalent and include the proteases, papain, bromelain, and ficin, cereal amylases, soyabeans lipoxygenase and some specialized enzymes from citrus fruits. These enzymes are mostly used in the food processing industries.

Microbial enzymes account for the rest. This source is by far the largest interms of volume of production.

Advantages of microbial enzymes:

Micro organisms have two major types of advantages: economical and technical. The first major economic advantage is that of bulk production very large quantities of product can be produced in a short time and in a small area when compared to animal and plant enzymes. The second advantage is that of case of extraction. A large proportion of industrial enzymes for example most hydorlases, from microbial sources are produced extracellularly into the growth medium. Two other economic advantages are the predictability of enzyme yield and the absence of seasonal variation.

The following enzymes are currently produced commercially.

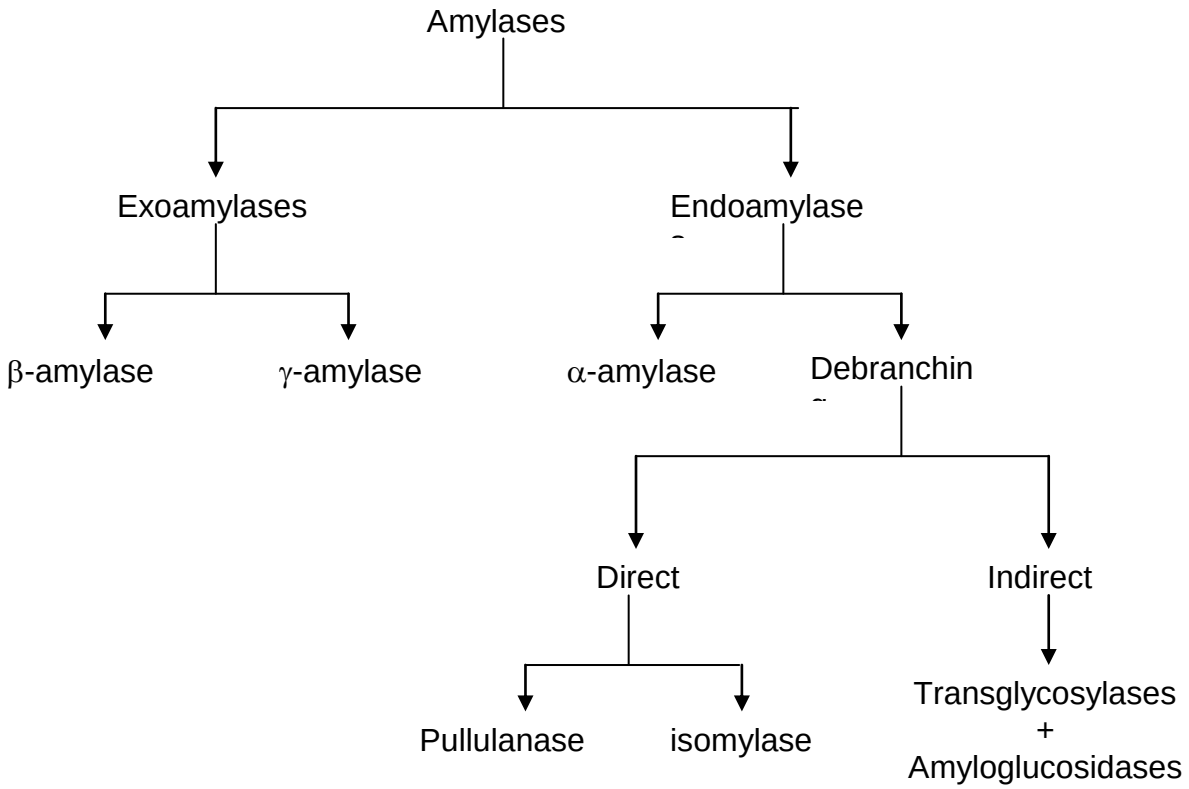
- Enzymes used in industry, such as amylases, proteases catalases, isomerases and penicillin acylases,
- Enzymes used for analytical purposes, such as glucose oxidase, alcohol dehydrogenase, muramidase and cholestral oxidase.
- Enzymes aused in medicine, such as asparaginase, proteases, lipses and streptskinase.

2. Explain amylase enzyme production in detail.

Amylases:

Amylases are enzymes which hydrolyze starch. One of the main uses of amylases is in the production of sweeteners for the food industry. The hydrolysis of starch with amylases results first in the production of short chain polymers called dextrains , then the disaccharide mallose and finally glucose. The most important enzymes in the starch – saccharification process are α-amylases P-amylases, gluco amylases – glucose isomerase, pullutanases and isomylases.

Amylases can be classified as endo amylases and exoamylases. Further classification is are given below



α- Amylases:

α- amylases are extra cellular enzymes which hydrolyze α-1, 4-glycosidic bonds. These enzymes are endoenzymes, splitting the substrate in the interior of the molecule. α amylases are formed by many bacterial and fungi. They are classified according to their starch liquiefying and / or saccarogenic effects, PH optimum, temperature range and stability. Saccharogenic amylases produce free slegars, where as starch- liquefying amylases break down the starch polymer but do not produce free sugars.

Bacteria which produce α-amylases are Bacillus subtilis, β-cereus, β- amyloliquefacines, B.coagulans, B.polymyra, B.caldolylicus, amylosaccharatius, lactobacillus, micrococess, iseudomonas, arthrobacter, Escherichia, Thermomonospora and serratia.

α-Amylase producing fungi are from the general aspergillus, penicillium, cephalosporium, mucor, candida, Neurospora and Rhizopus.

Production of bacterial –amylases

In industry α-amylases are produced either in batch or in fed batch fermentation. The enzyme formation rate is very low during exponential growth in many productions, but just before the growth rate decreases and spore formation begins, amylase production increases.

The production of α-amylases is regulated by several genes, which have been only partially

characterized single step mutations increase yields by a factor of 2-7, mutants have been selected after 5 steps which produce yields 250 times greater than the wild strain.

A medium for the production of α-amylases in a 100 m3 fermenter with B-subtilis consists of 5% starch, 0.56% NH_4NO_3, 0.28%. Sodium citrate, 0.13% KH_2 PO_4, 0.05% MgSO4. $7H_2O$, 0.01% Cacl2 $7H_2O$, 0.5% peptone, 0.2% yeast extract; P^H6.8. At 45°C, the maximal amount of enzyme produced (upto. 3000 units /ml) is obtained at considerably lower temperature (27° to 30°C). Thermophilic strains are used in newer processes. Thermomonospora, isolated from compost, has a temperature optimum for growth and amylase production at 53°C.

α-amylase Production from fungi:

The production of fungal amylases is constitutive, but as with other enzymes, it is repressed by regulators. For amylase production using aspergillus oryzae, the following nutrient solution can be used: 8% starch, 1.R% $NaNO_3$, 0.1% mg$(NO_3)2$, 0.05%mg $(H_2PO_4)2$,2.0% malt extract. The optimal temperature lies in a narrow range between 28-30°C, and the duration of the fermentation process is 3-4 days.

3. What are the process and explain how it is produced?

Proteases:

Proteases hydrolyse proteins into smaller peptide units. They constitute a large and industrially important group of enzymes. They cover 60% of the total enzyme market. Proteases are primarily used in the detergent industry and in the dairy industry other areases which include proteases are used include the pharmaceutical industry, the leasster industry, the manufacture of proteins hydrolysates, the food industry, the film industry and waste processing.

Proteases are produced from bacteria (Bacillus), molds (Aspergillus, Rzizopur, mucor), animal pancrease and plants. The proteases on the market include alkaline, neutral and acid proteases.

Alkaline Proteases:

Most bacteria and fungi excrete alkaline proteases. The most important producers are Bacillus strains such as B. licheniformis, B. amyloliquefaciens, B.firmus, B.megateum; streptomyces strains such as S.Fradiae, S. Griseue and S. rectus; and fungi Aspergillus niger, A. Sojae, A. oryzae and A. flavus the best known proteases are subtilisin.

Fermentation Process:

Serility is mandatory for protease production, as with other enzyme fermentations. Cultures are stored in the lyophilized state or under liquid nitrogen. Initial growth is carried out in shaken flask and small fermentors at 30 to 37°C. for production, 40 to 100 m^3 fermentors are used. Production of extra cellular protease is chefly regulated by the medium composition. Fermentation period is 48 is 72 hours. High aeration rate is maintained.

Neutral proteases:

Neutral proteases are excreted by both bacteria and fungi producing organisms include. Bacillus subtilis, B. cereus, B. Megaterium, Pseudomonas aeruginosa, streptomyces griseus, Aspergillus oryzae, A. sojae.

Neutral proteases are relatively unstable and calcium, sodium and chloride must be added for maximal stability. The P^H range of activity is fairly narrow and these enzymes are not very stable to increased temperature (as shown in following figure).

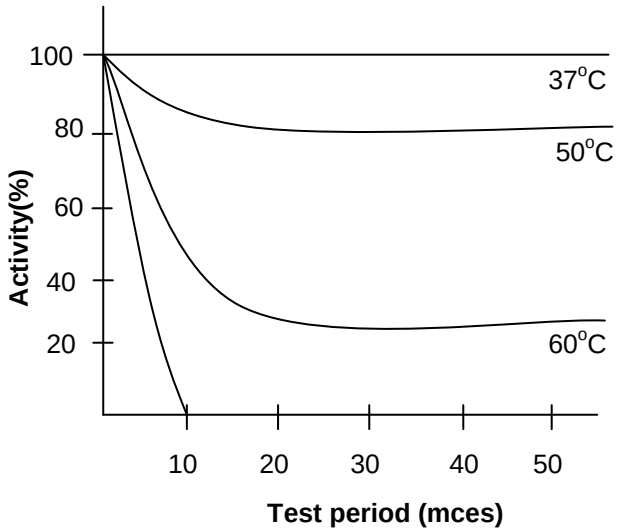


Fig. Temperature stability of a neutral protease

The neutral proteases are also quickly inactivated by alkaline proteases. Because of these limitations, they have restricted industrial application. But they find their use in the leather industry and in the food industry for the manufacture of crackers, bread and rolls.

Acid proteases:

In this category are rennin like proteases from fungi which are chiefly used in cheese production. Acid proteases are used in medicine in the digestion of say protein for say source production and to break down wheat gluten in the backing industry.

4. Write short notes on the following enzyme production.

(i) Pectinases

Pectinases are used primarily to clarify fruit juices and grape must these enzymes eliminate pecting and pectinike protective colloids in fruit juices and facilitate clarification of the juice. They are also used for the maceration of vegetables and fruits, and for the extraction of olive oil.

A number of commercial firms produce fungal pectinases using Aspergillus niger or A. Wentii or Rhizopus. Both surface and submerged processes are used.

The fermentation with aspergillus niger runs for 60 to 80 hours in fed batch cullulars at pH 3-4 and 37°C sugar and 2% pectin. The purification of enzyme is simple the biomass is removed by filtration or centrifungation stabilizing agents are added. The enzyme is precipitated with organic solvents and the crude protein dried.

Since the pectinase is retained in parts in cells and excreted to the medium is parts, the enzyme must be recovered from both. The mycelium is dried, ground, and the pectinase is extracted with water. It is then precipitated as before.

(ii) Invertase:

It is also known as sucrase or saccharase. It is an enzyme that hrdrolyzes sucrose is yield glucose and fructose although the enzyme acts on some tri and tetra saccharides. It is widely distributed in naturing, occurring in animal and plant tissues. It is prevalent among various bacteria, yeasts and fungi.

Invertase is employed commercially to prepare invert sugar from sugar. The inversion of sucrose increases its sugar solubility so that sugar crystallization is diminished. Use of this enzyme prevents sugar crystallization during preparation of food products, such as candies and ice creams. Invertase, in addition is employed to enzymatically yield invert sugars for use as plasticizing agents in paper industry.

Invertase is produced commercially from baker's yeast or brewer's yeast. Invertase is an endoenzyme, liberated to the fermentation medium only on autolysis of the cell. There fore, the enzyme is recovered from the yeast either by mechanical disruption of the cell wall or by autolysis induced by chloroform, ethyl acetate or toluene. The enzyme is then precipitated from aqueous solution by alcohol addition.

(iii) Glucose Isomerase:

Glucose isomerase causes the isomerization of glucose to fructose. The reaction is reversible fructose is the sweetest monosaccharide and has twice the sweeteinigl strength of suoars starch can be hydrolyzed enzymatically to glucose and this glucose can be converted to fluctose by glucose isomerase

Starch
(Wheat,60m, Potato

↓ hydrolysis (encymatic or acid)

glucose

↓ glucose isomerase

glucose fructose (syrup)

(60:40)

The glucose isomerase used must fulfill the following criteria low pH optimum to avoid side reactions, high specific activity, high temperature optimum. The most important glucose isomerase producers are : Baccilus coagulans, streptomyces rubiginosus, Actin oplances missouriensis and Flavobacterices arborescens

Glucose isomerases from streptomyces strains are produced only in batch processes. Production is begun with a spore inoculum of the working strain, which is cultured for 24-48 hours at 30°C in shaken culture. The culture medium used contains soya meal, yeast extract, glucose, starch, phosphate and deionized water. A number of such shake flasks are used is inoculate (5% inoculum) the production fermentor which is so to 150 m^3 volume. Streptomyces olivaceus, produces glucose isomerase at pH 8.5 and between 60-70°C, the enzyme is extracted from the broth and purified.

5. Define (SCP) and explain its production and its importance.

Single cell protein (SCP)

Single cell protein is refer to microbial biomass used as food and feed additives. Either the isolated cell protein or the total cell material may be called SCP. Single cell protein is of high protein, vitamin and lipid content and the general presence of a complete carry of all essential amino acids the following substrates are presently being studied for SCP production.

(1) alkanes
(2) methane
(3) Methanol
(4) Cellulose
(5) Carbohydrates
(6) Waste materials

Production of SCP from alkanes:

Alkanes are classified as short chain alkanes and long chain alkanes. Alkanes can be catabolized by many yeasts and by some bacteria (partially through cooxidation). The following yeast species have been intensively studied for SCP production. Candida tropicalis, candida oleophila and saccharomycopsis lipolylica.

The disadvantage of the use of alkanes is that, they are not easily soluble during growth in bioreactors with impellers or in airlift fermenters, large alkane drops are formed which are 1-100μm in six and which remain suspended. considering the low water soluability of alkanes the observed high growth rates of micro organism on alkanes cannot be explained merely by transport of a kane dissolved in water. Alkane molecules can then reach the cytoplasmic membrane through the cell wall via passive diffusion cells growing on alkanes are enriched in lipids and its seems likely that these lipids play a role in the transfer of alkanes through the cell membrane.

Catabolism of longer – chained alkanes:

The first step in the utilization of alkanes is the introduction of molecular oxygen into the molecule. There are two pathways, for oxygen introduction terminal oxidation and subterminal oxidation.

In terminal oxidation, the corresponding monocarboxylic acid is produced via the inter mediate stages of the primary alcohol and a dehyde. After this terminal oxidation, break down generally proceeds to acetyl – COA units by means of β- oxidation. Terminal oxidation is the chief pathway of metabolism for bacteria and yeast.

In subtermina, the approximate ketone is first produced via a secondary alcohol. This can either happen in the C_2 position or in the interior of the molecule, eg. At C_3, C_4, C_5 or C_6. Further catabolism of the secondary alcohol is variable. α-oxidation with subsequent decarbocylation and P- oxidation has been found in candida.

Large scale processes using Yeast:

Two petroleum products have been used as starting material.

- Gas oil, also knows as fuel oil or diesel oil, is a fraction derived from crude oil and contains 10-25% $C_{15} – C_{30}$ alkanes.
- C_{10}-C_{13} alkanes or C_{13}-C_{17} alkanes one separated from gas oil with molecular sieves.

The gas oil fermentation was run in an air lift bioreactor with an increased aeration rate. Since alkanes make up only a small proportion of gas oil, insoluble gas oil and is be fed repeatedly to growth medium. This resulted in poor mixing, poor oxygen transfer and low yields.

Methanol Fermentations:

Methanol was the most important substrate for single cell protein production and extensive research on methanol utilizing organisms. Methanol may be obtained from synthesis gas, natural gas, methanol oil or coal. Bacteria, yeasts and fungi may all be considered for the production of SCP from methanol besides the obligate methylotrophic bacteria which only grow on C1 compounds, facultative methylotrophic bacteria, yeasts and fungi which metabolize longer chained hydrocarbons – Example for this

1. Obligate methylotrophic bacteria:

Methylobacter, methylococcus, methycomonas, methylocystis

2. Faurlative methylotrophic organisms.

(a) Bacteria:

Arthrobacter, bacillus, micrococcus, pseudomonar, strreptmyces, vibrio

(b) Yeast:

Candida boidinic, candida parapsilosis, Hansenula casulatae, Hansenula minuta, pichia pastoris, torulopsic glabrata.

In methanol fermentation for SCP production, bacteria rather than yeasts are employed in essentially all existing production processes for the following reasons. Rapid growth, higher protein content, better yields and simpler culture requirements.

Methanol oxidation:

Methanol is oxidized to CO_2 by bacteria via the following intermediates steps

$$CH_3OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2$$

The first step to formaldehyde requires an inducible nonspecific methanol dehydrogenase.

Yeasts oxidize methol by means of a non specific, FAD- containing, inducible methanol oxidase. Methanol oxidation to formaldehyde results into energy gain for the yeast.

The next step is formaldehyde oxidation, which in bacteria can be carried out by several enzymes.

- ☺ Conversion of formaldehyde to formats with reduced glutathione (GSH) by means of an NAD- dependent formaldehyde dehydrogenase, a reaction which also occurs in yeast.

$$HCHO + NAD + H_2O \xrightarrow{GSH} HCOOH + NADH_2$$

- ☺ A dichlorophenol – indophenol (DCPIP) – dependent formaldehyde dehydrogenase.
- ☺ An unspecific methanol dehydrogenase

Two is three ATP per mole of substrate are obtained at this oxidation level.

The last step, the oxidation of formaldehyde, is common to all the methanol utilizing micro organisms formaldehyde oxidation involves a NAD – dependent format dehdrogenase and yields 3 ATP per mole of substrate

$HCOOH+NAD \rightarrow CO_2 + NADH_2$

Production Processes:-

Imperial chemical industries (ICI) was the first company to develop a continuous methanol fermentation for the commercial production of SCP they studied the effect of O2, CO2 and methanol concentration on productivity and the effect of the pressure differential between the bottom and the surface of the bio reactors. The ICI pressure cycle Fermenter", a combination of air lift and loop reactor.

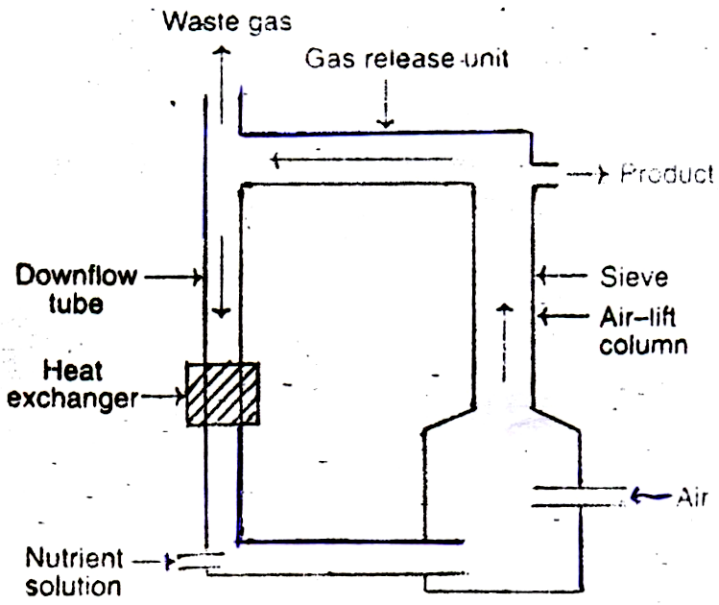


Fig. ICI Pressure cycle fermenter.

This 37m3, 30m high pilot fermenter consists of 3 unit's air lift column (I), down flow tube with heat removal (II) and gas release space (III). The pilot plant system, which had a capacity of 1000 tons / year, operated at PH 6.5-6.9 and 34 - .37°C. The organism used for industrial SCP production is the methanol oxidizer pseudomonas methylotrophus, which was isolated by ICI and then significantly improved through generator and physiological development.

In the product recovery process, partial cell lyses is first achieved via heat and acid treatment and the nutrient solution is then clarified by decanting. The water is then recycled bank into the fermenter and the cells are spray – dried.

6. Explain cheese production in detail.

CHEESE PRODUCTION:

Cheese production is the largest dairy industry in the world. They are broadly of two types, unripened cheeses (cottage cheese with low fat, cream cheese with high fat) and ripened cheeses. (hard cheese e.g. cheddes, blue cheese; soft cheese e.g. limburger, (amembert). All of them are made form the casein of milk, that is produced after separating the whey (liquid portion of milk). Milk from different animals can be used. E.g. sheep, cow, goat, buffalo.

Production Process:

Cheese is produced from milk by a process of dehydration where in casein (milk portion) and fats are concentrated 5-15 fold. Cheese production is kenjcomplicated, and broadly involves

four stages, acidification of milk, coagulum formation, and separation of curd from whey and ripening of cheese.

(1) Acidification of Milk:

By employing lactic acid bacteria (streptococceis lactis, Lactobacillus lactis) the sugar of milk (lactose) can be converted to lactic acid. This lowers the PH around 4.6, and thus acidifies milk.

(2) Coagulum formation:

When the acidified milk is treated with rennet (i.e. the enzyme chymosin of animal of fungal origin), casein gets coagulated. Casein mainly consists of three components – insoluble αand β caseins and k- casein that keeps them in soluble state. By the action of chymosin casein is degraded. Consequently, αand β casein and the degraded products of αcasein combine to form a coagulum (curd). This process of coagulation is dependent on calcium ions.

(3) Separation of curd from whey:

When the temperature of the coagulum is raised to around 40˚C, the coagulum and whey get separated the separated curd is cut into blocks, drained and pressed into different shapes.

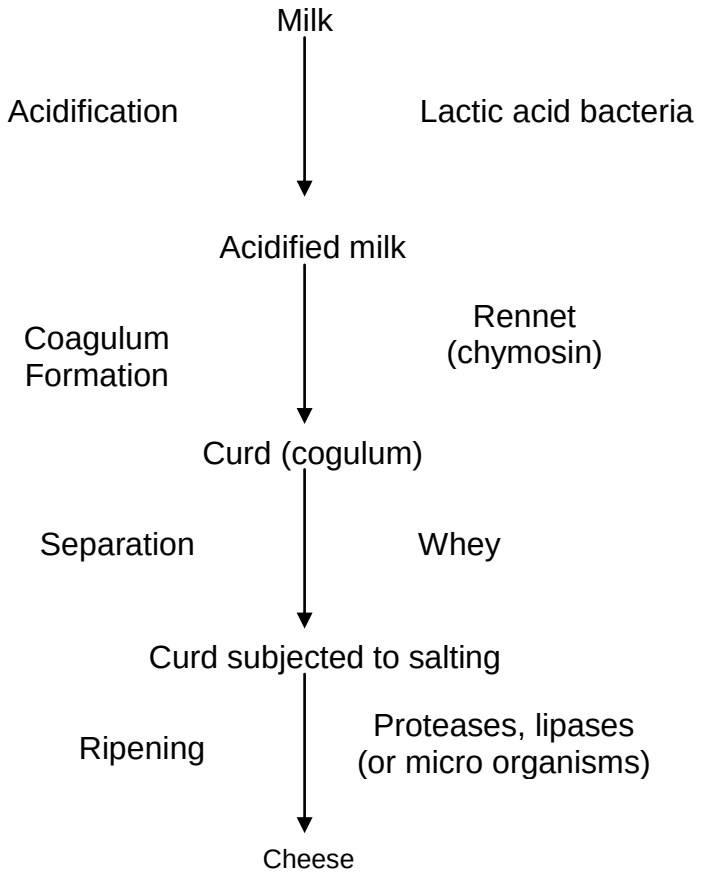
(4) Ripening of cheese:

The flavour of raw cheese (with rubber texture) such as cheddar is bland. Ripening in parts flavors, besides making changes in its texture. The blocks of curd separated are subjected to the action of processes and or lipases. Alternatively, they may be inoculated with certain fungi (e.g. penicillium roqueofortii) the hydrolysis of proteins and fats results in certain compounds which imparts flavour to the cheese. Mild hydrolysis of fats usually carried out by lipases or Aspergillus niger or mucor maihai results in butyric acid formation with characteristic flavour.

Sources of chymosin:

There are several sources of rennet (chymosin enzyme) for cheese production. These include calves, adult cows, pigs and fungal sources.

A diagrammatic representation of cheese production is shown below.



7. Briefly explain the production of Xanthan gum.

Xanthan gum:

Xanthan or more frequently referred to as xanthan gum was the first poly saccharide available commercially.

Xanthan has a molecular weight in the range of 2-15 × 10^4 daltons. Xanthan is a poly saccharide containing glucose (GlC), mannose (Man) and glucuronic acid (G/CA) with acetate (AC) and pyruvate (Pyr) as depicted below.

Basically, Xanthan is a branched polymer with β (1-4) linked glucon (glucose poly back bone bound to a tri saccharide (Man, Glen, Man) side chain on alternate glucose residues the mannose has either acetate on Pyruvate groups.

Biosynthesis:

For the bio synthesis of xanthan, the monomers are bound to a carrier lipid molecule and then transferred to a growing polymer chain the activated monosccharide nucleotides supply energy for the formation of glycosidic bonds between adjacent units.

Production:

Xanthan is commercially produced by the gram negative bacterium, xanthomonas campestris. The culture medium usually consists of 4-5% carbohydrate (glucose, sucrose, corn starch hydrolysate), 0.05-0.1% nitrogen cammonicem nitrate, urea, yeast extract) and salts. The PH is maintained around 7.0, and the fermentation is carried out by batch culture broth is precipitated by isopropano or methanol. These agents also kill the micro organisms. the precipitated xanthan can be dried and used for commercial purpose.

The wild type X campestris can efficiently utilize glucose, sucrose or starch as a carbon source, but unable to use lactose as a carbon source. Genetically engineered X. campestris have been developed that can utilize lactose (from whey) for the production of xanthan.

Applications:

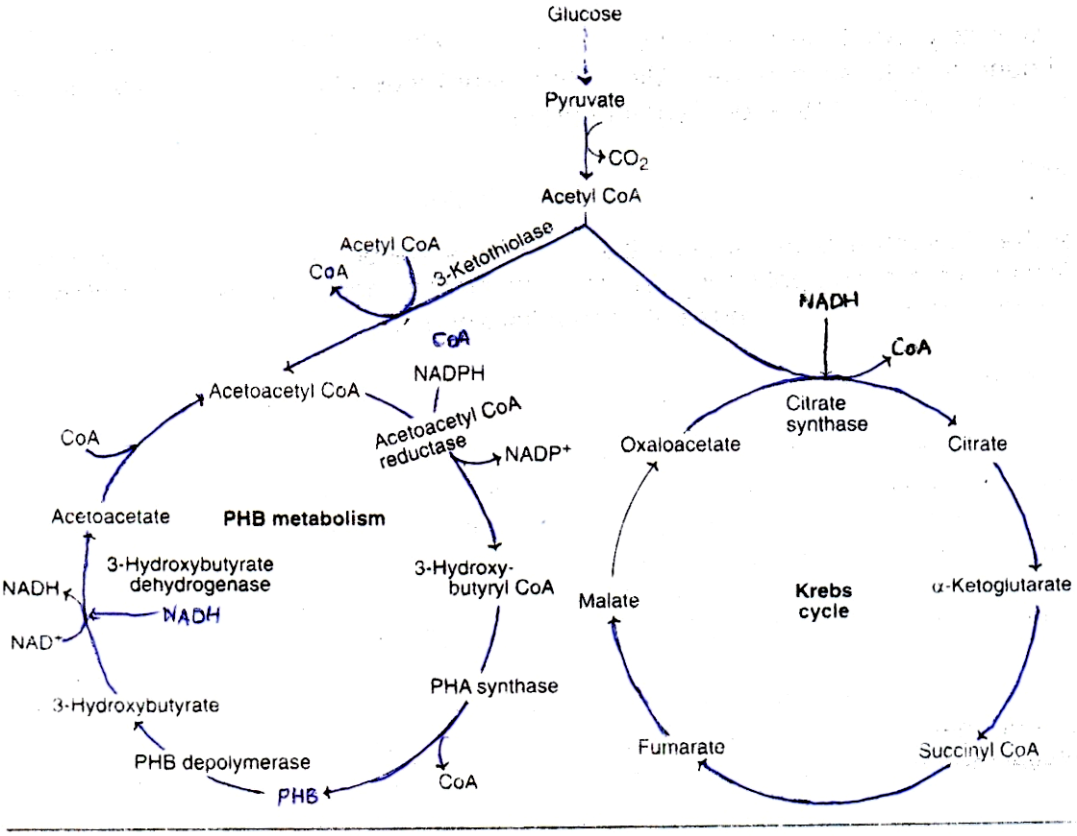
Xanthan gum is used as a food additive for the preparation of soft food, (ice cream, cheese). It is also used in oil industry for enhancing oil recovery. Further, xanthan is useful for the preparation of tooth pastes and water based paints.

8. Write short note on PHB production in detail.

Polyhydroxy butyrate (PHB):

PHB is synthesized in three reaction steps. Acetyl COA is converted to acetoacetyl COA by the enzyme 3-hetotholase which is then reduced to 3-hydroxybutyryl COA by acetoacetyl COA reductase. The reducing equivalents are supplied by NADPH. The enzyme PHA synthase is responsible for the addition of hydroxyl butyrate residues to the growing PHB chain.

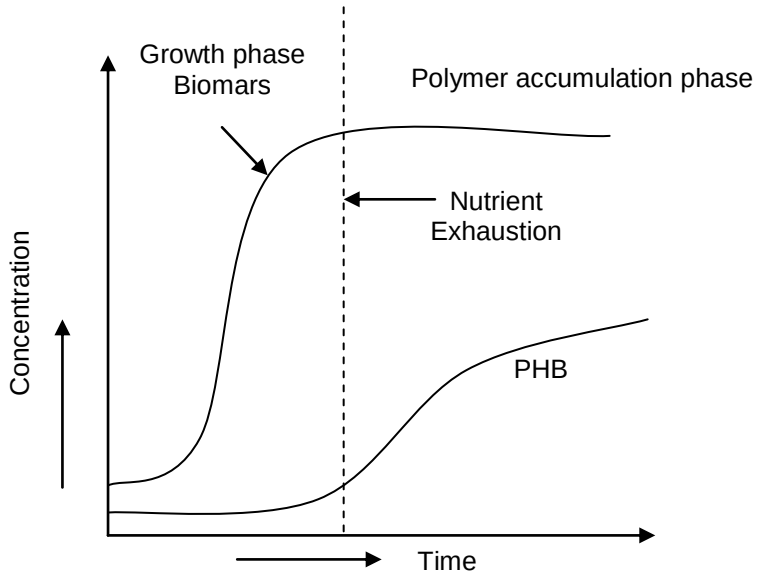
PHB, the storage energy reserve compound, can be degraded to acetyl COA and metabolized via Krebs cycle. This occurs when the organism is deprived of energy supplying carbon sources. The biosynthesis and break down of PHB form a cyclic process as depicted as follows.



Regulation of polyhydroxybutyrate (PHB) metabolism.

Production of PHB:

Poly hydroxyl butyrate is mostly manufactured by batch culture. PHB production occurs when there is an excess supply of carbon source and limitation of some other essential nutrient such as nitrogen, phosphorus or sulfur source. The production / accumulation of PHB is shown in the figure there are two distinct phases a growth phase and a polymer accumulation.



As a growth phase cease, due to nutrient exhaustion, synthesis of polymer (PHB) commences. It is also possible to produce PHB by restricting the oxygen supply to aerobic bacteria.

Applications of PHB

PHB can be implanted in the human body without rejection. This is because PHB does not produce any immune response and thus it is biocompatible. PHB has several medical applications e.g. as durable bone implants for wound dressings.

9.Write short notes about the production of biopolymers detran, alginate and scleroglucon.

Write short notes on dextran polymer production?

Chemically, dextrans are glucans (polymers of glucose) containing 1-6 glycosidic linkages. Dextrans are used as blood plasma expanders for the prevention thrombosis and in wound dressing. In addition dextrans are useful in the laboratory analytical techniques for purification of biomolecules.

Production:

Dextrans can be produced by a wide range of gram positive and gram negative bacteria. E.g. leuconostoc mesenteroides and streptococcus mutans. Dextrans are produced by extracellular enzyme in the medium. The enzyme is dextran sucrose which acts on sucrose and brings about polymerization of glucose residues, and simultaneously liberates free fructose into the medium.

The commercial production is carried out by using lactic acid bactericum L. Mesenteriodes by a batch fermentation process. Besides sucrose, the culture medium contains organic nitrogen source and inorganic phosphate the crude dextran produced is precipitated by alcohol and then subjected to acid hydrolysis. The resultant dextrans can be fractionated and dried.

It is also possible to use a cell free system for the production of dextran. The extracellular enzyme dextran, sucrose can tansform sucrose into dextran in a cell free nutrient solution. This reaction is optimum at PH 5.0-5.5 and temperature 25-30˚C.

10. Write short notes on the following biopolymer production.

(i) Alginate:

Alginate is a linear polymer composed of mannuronic acid and glucuronic acid in a proportion ranging from 4:1 to 20:1. Alginate is commercially produced by gram negative bacteria, pseudomonas aeruginosa and Azobacter vinelandii.

The type of organism used and the culture conditions determine the relative proportion of mannuronic acid and glucuronic acid residues and the degree of acetylation in alginate. For commercial purposes, seaweed alginates are more commonly used than bacterial alginate. This is mainly because bacterial alginates are relatively unstable and get easily degraded. Alginates are useful as thickening agents in food industry and for un mobilization of cells and enzymes.

(ii) Scleroglucan :

Scleroglucan is a glucose polymer. It is a neutral polysaccharide with P1→ 3 glucon backbone and single glucose (G/c) residue branches (β 1→6 linkage). The branching occurs at a regular sequence at every third glucose unit in the polymer backbone chain.

Scleroglucan is a fungal heoxpolysaccharide. It is commercially produced by sclerotuem glucanicum, s. rolfsii and s. delphinii sclero glucan is useful for stabilizing later paints pringing ink and drilling muds.

11. What are bio fertilizers? Explain is production in the industry.

Bio fertilizers:

They are active products (or) microbial inoculants of bacteria, algae and fungi which may help biological nitrogen fixation for the benefit of plants. The use of chemical / synthetic fertilizers is the common practice to increase crop yields. Besides the cost factor, the use of fertilizer is associated with environmental pollution.

The term biofertilizers is used to refer to the nutrient input of biological origin to support plant growth. This can be achieved by the addition of microbial inoculants as a source of biofertilizers.

Biofertilizers broadly includes the following categories.

- ⌘ Symbiotic nitrogen fixers
- ⌘ Asymbiotic nitrogen fixers
- ⌘ Phosphate solubilising bacteria
- ⌘ Organic fertilizers.

The most important microorganisms used as bio fertilizers are listed below.

S.No.	Category/micro organism.	Useful for corps.
1	Symbiotic Nitrogen fixers Rhizobium, leguminoscercem R. melitoti, R. ciceri, Bradyrhizobium japonicum	Legumes
2	Asymbiotic Nitrogen fixers Azobacter, Azospirillum Blue green algae (Anabaena Noslit)	Wheat , rice, sugarcane, **jouier**, Rice
3	Phosphate solubilizing bacteria Thiobacillus, Bacillus Mycorrhizer	Pulses

Symbiotic Nitrogen fixers:

The diazotrophic micro organisms are the symbiotic nitrogen fixers that serve as biofertilizers. Eq: Rhizobium sp and Bradrhi zobium sp.

Green manuring:

It is a farming practice where in the leguminous plants which are benefited by the symbiotic nitrogen fixing bacteria are floughed into the soil and a non – leguminous crop is growth to take benefits from the already fixed nitrogen. Green manuring has been is practice in India for several centuries. It is a natural way of enriching the soil with nitrogen, and minimizing the use of chemical fertilizers rhizobicem sp can fix about 50-150kg nitrogen/hectare / annum.

Asymbiotic Nitrogen fixers:

The asymbiotic nitrogen fixing bacteria can directly convert the gaseous nitrogen to nitrogen rich compounds when these asymbiotic nitrogen fixers due, they enrich the soil with nitrogenous compounds, and thus serve as biofertilizers eig Azobacter sp, Azospirillum sp.

Blue green Algae (cynobactericem)

Blue green algae multiply in water logging conditions. They can fix nitrogen in the form of organic compounds (proteins, amito acids). The term algalization is used in the process of cultivation of blue green algae in the field as a source of biofertilizer.

Blue green algae, besides fixing nitrogen, accumulate biomass, which improves the physical properties of the soil. This is useful for reclamation of alkaline soils besides providing partial tolerance to pesticides. Cynobacteria are particularly useful for paddy fields phosphate solubilizing bacteria.

Certain bacteria (e.g. Thiobacillus, Bacillus) are capable of converting non available inorganic phosphorous present in the soil to utilizable organic or inorganic) form of phosphate. These bacteria can also produce siderophores , which chelates with iron, and make it unavailable to pashopgenic bacteria. Thus the plant are protected from disease – causing micro organisms.

My corrhizas:

My corrhizas are the fungus roots (eg: Glomus sp) with distinct morphological structure. They are developed as a result of mutual symbiosis between certain root inhabiting fungi and plant roots. My corrhizas are formed in plants, which are limited with nutrient supply. These plants may be herbs, shrubs & trees.

For the development of mycorrhizas, the fungal may be located on the root surface or inside the root. Mycorrhizas also produce plant growth-promoting substances.

12. Define Bio preservatives give on example and explain its production in detail.

Bio-Preservatives:

Bio preservation is defined as specific kind of organism used to preserve specific substance from spoilage organism.

The lactic acid bacterial (LAA) or lacto coccus lactil are there microbial products add to the feed particle to improve the safety and quality of the products chemical preservative and chemical processed feed even though provide unparallel safety and diversity of feed quality.

Nisin:

Nisin is a peptide that is made by the bacterium lacto coccus lactis. It is a small molecule that kills gram positive bacteria by binding to their membrance and by disrupting the protsn motive force. It appears to be active against the endospores of the food –borne, pathogenic gram positive bacteria, clostridium botulinum and bacillus nisin obtained a GRAS (generally recognized as safe) status for use as a biopreservative in the food industry.

When food is processed it normally goes through a heat treatment. This kills bacteria that are found in food. While vegetative cells of because are killed by heat, the endospores are most resistant. The endospores that survive tarn into vegetative cells and start producing toxins. These toxins cause food poisoning. Adding nisin to the food, provides a second barrier for the growth of the bacteria.

Structure of Nisin:

Nisin containing unusual aminoavids. Dehydroalanine, Dehyadrobutyrine, lanthionine and B-Methyl lanthionine. The nisin structure contains 5 internal ring structure of disulfide bridges linkage (37) is lanthionine and other 4 ring called β-methy lanthionine linking residuces. Each nisin contains (1) Lanthionine and (4) β-methy lanthionine.

General Characteristics:

Nisin for food preservation was first permitted in Britain in 1959. Nisin intibit the "Spoilage and pathogenic bacteria" without changing their physical-chemical nature of food.

Nisin are bacterial poly deptider or proteins containing group of 34 amino acid residues. It kills gram positive bacteria by binding to their membrane and by disrupting the organism.

Activity of Nisin against organism:

Nisin(Bacterician) has a narrow antimicrobial spectrum. Nisin kills only the specific type of organism. The main step in nisin action is adsorplion to the microber. The addition of nisin to the vegetative cells result in a rapid and Non specific efflur of preacaumulated ione, Amino acids and ATP molecules. This increase flun of compounds across the membrane rapidly dissipates chemical and electrical gradiente across the membrane. Bacterio treated cells have decreased infracelluar ATP level. The proton motive force (PMF), an electrochemical grandient which serve as the major driving force of many vital energy-dependent processes, is dissipated within minutes of nisin addition.

Commercial Production of Nisin:

Nisin is commercially produced from lactococus lactie bactericum. It is grown for shire in a culture media containing BHI + 3% yease extract (BHI-Brain heart Infusion). The temperature is maintained at 30°C.

The obtained product is filter sterilized and it contains 1000Ng/ml. The filtered solution is extracted with 50% NH4SO4 solution to precipitate nisin collect the precipitate and dialyzed against with distilled water to remove salts, then we can get purified nisin.

Nisin is added to milk, cheese and dairy products, canned food baby food atc as food biopreservatives.

UNIT – V

PART – A

1. Give example for recombinant proteins.

Hemoglobin, erythropoietin, tissue plasminogen activator, epidermal growth factor, interferons colony stimulating factor, inter laukinns, tumor necresis factor and insuten.

2. Name rDNA derived therapeutic agent and their application.

rDNA product	**Applications**
1. Insulin	Diabetes
2. Growth form one	Pituitary dwarfism
3. ∝ - Interferon	Hairy cell leukemia
4. Hepatitis B vaccine	Hepatitis B
5. Tissue plaminogen activator	Myocardial infarction
6. Factor VIII	Hemophilia
7. DNase	Cystic fibrosis
8. Erythropoietin	Severe anemia with kidney damage

3. What is tissue plasminogen activator?

Tissue plasminogen activator (tPA) is a naturally occurring protease enzyme that helps to dissolve blood clote. tPA is a boon for patient suffering from thrombosis

4. What are interferon?

Interferon is an antiviral substance and is the first line of defense against viral attacks. All the interferon are protein in nature and many of them are glycol proteins. They are broadly categorized into three groups bused on their structure and function.

5. What is mean by vaccination?

Vaccination is the phenomenon of preventive immunization. In the modern concept, vaccination involves the administration of an antigen to elicit an antibody response that will protect the organism against future infections.

6. Define vaccine.

A preparation introduced into the body to stimulate immunity against a pathogen. A vaccine triggers the body's immune system to produce antibo dies against a specific disease causing organisms. (Virus, bacteria or other parasite)

7. Mention different types of vaccines.

Vaccines are mainly of three types

1. Dead banters a or inactivated virus
2. Live non-virulent or weakened (attenuated) bacteria / or viruses
3. Viral fragment or bacterial molecules (subunit vaccines)

8. What are the drawbacks in the traditional production of vaccines?

1. it is not possible to develop vaccines for the organisms not grown in culture
2. The yield of vaccines is very low
3. Cell cultures are costly to maintain
4. There is a danger of non-virulent organisms getting converted to virulent ones. Vaccinations by such organisms may causative disease itself
5. It is not possible to prevent all the disease by use of traditional vaccines. e.g. AIDS.

9. Define recombinants vaccines.

Recombinant vaccines are the new generation of vaccines produced by employing recombinant DNA technology. Recombinant Vaccines may be broadly categorized into three groups

1. subunit recombinant vaccines
2. Attenuated recombinant vaccines
3. Vector recombinant vaccines

10. Define subunit vaccine's give example.

Subunit vaccines are the components of pathogenic organisms – subunit vaccines include peptides, protein and DNA. The advantages of these vaccines include their purity in preparation, stability and safe use. The disadvantages are high cost factor and possible alteration in native conformation.

e.g. Hepatitis B

11. What is meant by Attenuated recombinant vaccines?

These are the genetically modified pathogenic organisms (bacteria or viruses) theta are made non-pathogenic and used as vaccines.

12. Define vector recombinant vaccines.

These are the genetically modified viral vectors that can be used as vaccines against certain pathogens.

13. What are DNA vaccines?

The immune response of the body is stimulated by a DNA molecule is called DNA vaccines. A DNA vaccine consists of a gene encoding an antigenic protein inserted ants a plasmid, and then incorporated it's the cells in a target animal.

14. What are the advantages of DNA vaccines?

There are several advantages of using DNA vaccines in immunization.

1. The tedious and costly procedures of purifying antigens or grating recombinant vaccines are not necessary.
2. DNA vaccines are very specific in producing the target proteins. Thus they trigger immune response only against the specific antigen.
3. In general, DNA vaccines elicit much higher immune response compared is other winds of vaccines.
4. DNA vaccines are more stable for temperature variations than the conventional vaccines, thus the storage and transport problems associated with vaccines are minimal.

15. What are the disadvantages of DNA vaccine?

1. The fate of the DNA vaccine in the host cells is not yet clear. There is a possibility of their DNA getting integrated into the host genome and this interrupt the normal function.
2. There also exists a danger of cancer due to DNA vaccines
3. The post translational modification of the gene (DNA vaccine) product in host cells may not be the same as that found in the native antigen

16. What are edible subunit vaccines?

Transgenic plants have been developed for expressing antigens derived from animal viruses (rabies virus, herpes virus). The edible vaccines can be easily ingested by easting plants their climates the processing and purification produces.

17. Give some example for plant edible vaccine.

Antigen	Host plants
1. Rebio glycoprotein	Tomato
2. Foot and virus	Arabidopsis
3. Herpes virus mouth B surface antigen	Tobacco
4. Cholera torn B subunit	Potato
5. Human cytomegalovirus glycoprotein B	Tobacco

18. Define monoclonal antibody (MAB).

Monoclonal antibody is a single type of antibody that is produced by hybridoma cells. The MAB is directed against a specific antigenic determinant (epitope)

19. What is meant by hybridoma cells?

A clone of hybrid cells produced dry function of myeloma cell with an antibody producing cell. Each hybridoma produces only one type of monoclonal antibody.

20. Define myeloma cells.

A tumax line derived from a lymphocyte which usually produces a single type of immunoglobulin.

21. What is meant by recombinant protein?

A protein that is produced by the expression of cloned gone of a recombinant cell.

22. Write the applications of monoclonal antibodies.

Monoclonal antibodies with specificity and high purity have a wide range of applications which can be broadly categorized as follows.

1. Diagnostic application
2. Therapeutic uses
3. Protein purification
4. Miscellaneous applications

23. Give examples for animal cell culture products.

Immunoregulators monoclonal antibodies, enzymes, hormones virus vaccines, tumor necroses factor, factor VIII, factor IX, erythropoietin Granulocyte macrophage colony stimulating factor.

PART – B

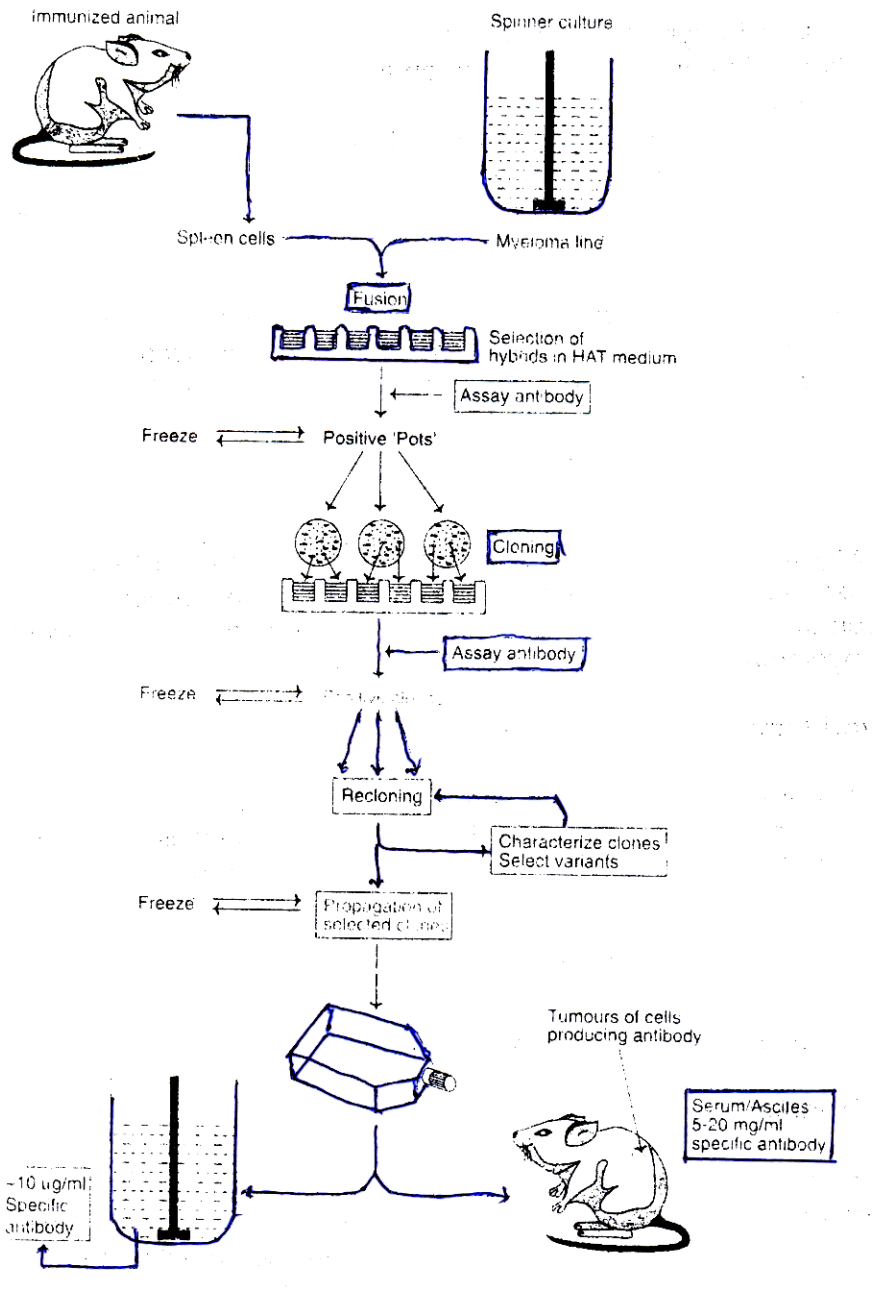
1. How Monoclonal Antibodies are produced explain in detail?

Monoclonal antibody (MAb) is a single type of antibody that is directed against a specific antigenic determinant (epitope). The production of monoclonal antibodies by the hybrid cells is referred to as hybridoma technology.

Production:

The establishment of hybridomas and production of MAbs involves the following steps:

1. Immunization
2. Cell fusion
3. Selection of hybridomas
4. Screening the products
5. Cloning and Propagation
6. Characterization and storage



Basic protocol for the derivation of monoclonal antibodies from hybrid myelomas.

1. Immunization:-

The very first step in hybridoma technology is to immunize an animal (usually a mouse), with appropriate antigen. The antigen, along with an adjuvant like Freund's complete or incomplete adjuvant is injected subcutaneously by several times. This enables increased stimulation of β-lymphocytes which are responding to the antigen. Three days prior to killing of the animal, a final dose of antigen is intravenously administered. The immune-stimulated cells for synthesis of antibodies have grown maximally by this approach. The concentration of the desired

antibodies is assayed in the serum of the animal at frequent intervals during the course of immunization.

When the serum concentration of the antibodies is optimal, the animal is sacrificed. The spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells. The lymphocytes of the spleen are separated from the rest of the cells by density gradient centrifugation.

2. Cell fusion

The thoroughly washed lymphocytes are mixed with HGPRT defective myeloma cells. The mixture of cells is exposed to polyethylene glycol (PEG) for a short period (a few minutes), since it is toxic. PEG is removed by washing and the cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.

3. Selection of hybridomas

When the cells are cultured in HAT medium, only the hybridoma cells grow, while the rest will slowly disappear. This happens in 7-10 days of culture. Because, when the cells deficient (mutated cells) in HGPRT are grown in a medium containing hypoxanthine aminopterin and thymidine (HAT medium), they cannot survive due to inhibition of de novo synthesis of purine nucleotides. Thus cells lacking HGPRT, grown in HAT medium die.

Selection of a single antibody producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually.

4. Screening the Products

The hybridomas must be screened for the secretion of the antibody of desired specificity. The culture medium from each hybridomas culture is periodically tested for the desired antibody specificity. The two techniques namely ELISA and RIA are commonly used for this purpose. In both the assays, the antibody binds to specific antigen and the unbound antibody and other components of the medium can be washed off. Thus, the hybridoma cells producing desired antibody can be identified by screening. The antibody secreted by the hybrid cells is referred to as monoclonal antibody.

5. Cloning and Propagation

The single hybrid cells producing the desired antibody are isolated and cloned. Two techniques are commonly employed for cloning hybrid cells – limiting dilution method and soft agar method.

6. Characterization and storage

The monoclonal antibody has to be subjected to biochemical and biophysical characterization for the desired specificity. It is also important to elucidate the MAb for the immunoglobulin class or sub-class, the epitope for which it is specific and the number of binding sites it possesses.

The production of MAb's in the culture bottles is rather low (5-10μg/ml). so in case of large scale production, the yield can be increased by growing the hybrid cells as ascites in the peritoneal cavity of mice. The ascetic fluid contains about 5-20 mg of MAb/ml.

2. Write the applications of Monoclonal antibodies.

(i) The utility of monoclonal antibodies has been envisaged for the labelling and precise identification of specialized cells such as neurons.

(ii) Monoclonal antibodies have been useful in determining the structure of cell membranes.

(iii) Currently monoclonal antibodies are being used directly in serotherapy as well as in the preparation of very specific vaccines active against viral strains.

(iv) Monoclonal antibodies are now being seriously considered to neutralize the action of lymphocytes responsible for the rejection of grafts and destroy the auto antibodies produced in auto-immune diseases.

(v) Recently monoclonal antibodies are now finding application in turnover and cancer therapy. The medical literature is full of several recent trials and results in this connection and the detailed description is beyond the scope of the text.

(vi) Monoclonal antibodies are useful in identification and classification of major histocompatibility complex products for tissue typing in transplantation and population genetic studies.

(vii) For serogenetic classification of infectious micro organism and protozoans and metazoan parasites.

(viii) It is envisaged that application of a monoclonal antibody based assay will replace conventional tissue section immuno florescence assays. It is likely that such assay will be very useful in forensic sciences.

(ix) The potential of monoclonal antibodies as tools in enzyme genetics is developing fast as a discipline of immunogenetics.

(x) Monoclonal antibodies are further useful in enzyme purification which cannot be purified as much by biochemical methods.

(xi) Some other applications include assignment of genes encoding particular enzymes to specific chromosomes or regions of chromosomes in somatic cell hybrid studies; studies on the cellular or intracellular locations of different enzymes by immunocytochemistry.

3. Explain in detail about the Production of recombinant Proteins having therapeutic and diagnostic applications.

In the past, mammalian therapeutic proteins and peptides were available in very limited quantities. They could only be prepared from animal or human tissues and body fluids. These preparations were extremely expensive to produce, some had unwanted side effects, and in certain cases there were unfortunate problems with virus and prior contamination. The demand and market size of therapeutic proteins is now growing. With the help of recombinant DNA technology, many therapeutic proteins from various sources can be produced.

These products are not synthetically produced but are produced by microbial fermentation or mammalian cell culture or by transgenic animals. Four times of today's capacity of production is required to meet the demand of therapeutic proteins. There are 13 major classes of therapeutic proteins in the market. As a class "Protein Therapeutics" include (1) naturally occurring human proteins; primarily plasma proteins. (2) Recombinant copies of naturally occurring proteins. (3) Mutated or modified versions of naturally occurring proteins having higher efficiency, lower toxicity or higher functionality and (4) Monoclonal antibodies.

Important groups of therapeutic proteins manufactured today are

1. Interferons
2. Interleukins
3. Colony Stimulating factors
4. Blood Products such as Factor VIII and Factor IX
5. Structural proteins such as serum albumin, collagen, fibrinogen.
6. Insulins
7. Human Growth hormones
8. Erthropoietins

9. Enzymes like Alfa Glucosidase, Cerazyme / Ceredase
10. Monoclonal antibodies
11. Recombinant Protein vaccines

Therapeutic proteins can be used in a variety of applications including surgery, trauma, and cancer therapy, urinary and fecal in continence, cosmetic reconstruction and chronic diseases. However, current production methods are limited by the inability to produce high value complex proteins, high cost of production and long lead times before production facilities are operational producing therapeutic proteins in the milk of cloned transgenic animals can be increase efficiencies in the industry. Transgenic cows have the potential to produce enormous quantities of therapeutic proteins in their milk. For example, transgenic cows have produced human collagen at a concentration of 8g/L and human Fibrinogen at 2.4g/L. Both molecules have been purified and shown to be bioactive.

DNase

There is a fatal genetic disorder called cystic fibrosis which includes malfunctioning in epithelial tissue. A characteristic feature of these diseases involves the emergence of thick mucus which is produced in a number of organs mainly the lungs, where it impairs breathing and increases the risk of microbial infection. Following infection part of the immune response includes phagocytes attacking the micro organisms. As a result of this free DNA are released into the lungs from both bacteria and phagocytes. The DNA is very viscous and helps in further thickening of the mucus.

Genetically engineered DNase preparations are now available that can help clear these secretions by breaking up the long DNA strands into smaller sections to reduce the viscosity of the mucus.

Erythropoietins

It is a glycoprotein hormone, and is produced in kidney. It is a haemopoietic growth factor i.e., a regulating factor involved in the control of mammalian erythrocyte production in the bone marrow. The numbers of red blood cells dramatically reduce in the body without sufficient amounts of erythropoietin and results in anaemia.

Human growth hormone

Human growth hormone (hGH) is a proteinaceous hormone secreted by pituitary gland present at the base of the brain. This hormone controls both growth and stature. hGH are prepared and used to cure children with 'hypopituitary dwarfism'. It is a congenital disease in

which the pituitary fails to secrete sufficient hGH for normal growth. This hormone cannot be administered orally, but must be injected. In addition hGH has a therapeutic value in the treatment of a range of other diseases, particularly those which are associated with ageing and wound healing.

hGH is a single protein chain and is manufactured in the body as a precursor / pre hormone composed of 217 amino acids. The pre hormone contains a signal sequence of 26 amino acids that is enzymatically cleaved to give the biologically active protein.

hGH was first of all clinically used to treet dwarfism in 1958. It was done by using hormone extracted from the pituitary glands of human cadavers.

Insulin

It is a polypeptide hormone and is synthesized in the pancreas by the islet of langerhans. This hormone plays important role in the regulation of blood glucose levels, and in the metabolism of carbohydrate, fat and starch. It contains two peptide chains – an 'A' chain contains 21 amino acids and a 'B' chain constitutes 30 amino acids.

In the past, insulin extracted from animal pancreas, particularly bovine and preine has been used to fulfill the requirements of diabetic patient who suffer from insulin – dependent diabetes mellitus. But now this has been partly replaced by recombinant human insulin.

Genetech was the first one to develop the recombinant product but it was marketed by Eli Lilly as Humulin. It was the first recombinant product which got a marketing license by the FDA in 1981 and was approved for the treatment of human disease. Insulin chain A and B were cloned separately and each host is cultivated independently. Each chain is purified from its respective fermentation and is then combined in a chemical step to form the complete insulin molecule. Now recombinant insulin is available from either E. Coli or Saccharomyces cerevisiae.

Interferon:

It is a member of cytokines, a large family of small signalling proteins involved in regulation of cell mediated immunity which also includes interleukins, tumor necrosis factor, colony – stimulating factor, erythropoietin and thrombopoietin. Almost all vertebrates produce a variety of interferons, and mammals, including humans produce three types – a, b, and g.

Interferon a forms are primarily produced by leucocytes. They consist of a single polypeptide chain of 165-166 amino acid residues. Some are glycosylated with varying amounts of carbohydrate moietics. The carbohydrate portion does not appear to confer any functionality on

interferon μ and may be removed without affecting their activity. Due to this property, recombinant interferon a is allowed to be produced in prokaryotic systems like E.Coli, which are not capable of post-translational modifications essential to form glycosylated polypeptides.

Interferon a is now been approved for the treatment of hairy cell laukeamia, chronic myeloid leukeamia, renal cancer, melanoma, multiple myelomas and genital warts. Interferon – b is synthesized naturally by mammalian fibroblast cells and may be produced recombinantly from E.Coli. These products like Betaseron and Rebif can be used in curing relapsing multiple sclerosis.

Interferon g is also called immune interferon. It is naturally produced by activated T. Lymphocytes. It is the most important interferon involved in the immune system as it activates T-cells, natural killer cells, cytotoxic T-lymphocytes and macrophages. Preparations of recombinant interferon g from E.Coli are used in the treatment of chronic myeloid leukeamia and renal cancer eg. Genetech's Act immune.

Collagen:

It is the most abundantly found protein in the human body. It is generally used by surgeons for suturing and repairing. It is currently obtained for this purpose from cattle or human cadavers. The yeast pichiaaugusta and pichia pastoris have now been genetically engineered to produce human type I and III collagen fragments respectively. They may be safe future sources of this protein for surgical and medical use.

4. Define Animal cell culture and explain their products in commercial application.

Culturing animal cells invtiro condition by providing specific techniques or methods is called as animal cell culture.Hormones, metabolites and regulatory molecules are the products naturally expressed in animal cells. Other cell products use the cell purely as a manufacturing unit. E.g. viral vaccines. Sometimes cell itself may be a product. A whole range of therapeutic proteins of potential value to the pharmaceutical industry and even vaccines are now produced in cultured recombinant mammalian cells.

1. For study of biochemistry and biophysics of cell growth and division.
2. For research on animal viruses.
3. For production of wide range of biological products of commercial interest –
    (A) Immuno regulators
    (B) Monoclonal antibodies.

Largest system currently in operation is Hybridoma cells being grown in 2000 litre airlift fermentor. The yields are 100 mg / litre. Alternatively hollow fibre reactors, ceramic cartridges,

membranes, porous microcarriers, and encapsulation are used by other monoclonal antibody producers. To meet the increasing demand of monoclonal antibodies cell technologist plans to scale up the production to 5000 litres cultures in Airlift fermentor.

(C) Enzymes
(D) Hormones
(E) Viral Vaccines

Viruses are obligate intracellular parasites and earlier viral vaccines were made in intact animals. Today except for influenza and yellow fever vaccines which are made in developing chick embryo, all other viral vaccines are made by growth of virus in cell culture.

List of some vaccines prepared by cell cultures are

a. Measles – Chick embryo fibroblast
b. Polia (inactivated) – Monkey kidney cells
c. Polio (active live) – Monkey kidney cells, human diploid cells, verocells
d. Rabies – Human diploid cells, verocells
e. Rubella - Rabbit kidney cells, duck embryo cells, human diploid cells.
f. Hepatitis β - rCHO (Chinese Hamster Overy cells)

Most of the common vaccines are still produced by multiple culture processes using roust flasks and roller bottles since these methods were used at the time of development and case in obtaining of licence for manufacture for these vaccines.

There are also some viral genes which have been successfully expressed in vaccinia recombinants. These can be useful to produce novel vaccines. Some examples are –

(a) Hepatitis B virus surface antigen
(b) Herps simplex virus glycoprotein D
(c) Rabies virus glycoprotein G.
(d) Influenza virus haemoglutinin
(e) Malaria sporozoite antigen
(f) Therapeutic proteins.

List of Therapeutics produced in Recombinant animal cells.

(a) Tissue plasminogen activator (tPA) – It is useful for thrombosis.
(b) Interleukin – 2 – for cancer therapy.

(c) Tumor necrosis factor – for cancer therapy
(d) Factor VIII – for haemophilia A
(e) Factor ICX – for haemophilia B
(f) Erythropoetein for anaeamics
(g) Granulocyte – macrophage colony stimulating factor for stimulating WBC production after cancer therapy.
(h) Interferon – P – for cancer therapy
(i) Interferon - α
(j) Tumor specific antigens (for inclusion in diagnostic test kits)
(k) Fibroblast culture – This is being considered for burns patients. It can be used as a reconstitute skin to facilitate wound healing.
(l) To evaluate new drugs and toxic chemicals. It can be used in concert with animal models. It will reduce time, cost, efforts and will save animals. For example genotoxicity can be assessed by using incorporation of radio active nucleotides as a measure of DNA repair. Such testing is important for before use.

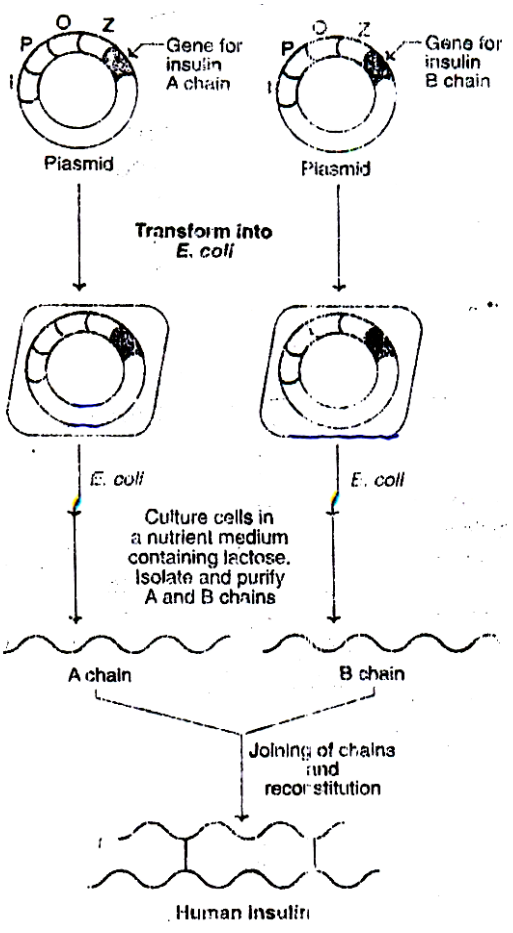
5. Write short notes on the following therapeutic proteins production.

(a) Insulin

The hormone insulin is produced by the β-cells of islets of langerhans of pancreas. Human insulin contains 51 amino acids, arranged in two polypeptide chains. The chain A has 21 amino acids while B has 30 amino acids. Both are held together by disulfide bonds.

Production of recombinant insulin:-

Attempts to produce insulin by recombinant DNA technology started in late 1970s. The basic technique consisted of inserting human insulin gene and the promoter gene of lac operon on to the plasmids of E.Coli. By this method human insulin was produced. The procedure employed for the synthesis of two insulin chains A and B is illustrated in the following figure:



: The production of recombinant insulin in E. coli (I-Inducer gene, P-Promoter gene, O-Operator gene, Z-β-Galactosidase gene; all these genes are of lac operon system).

The genes for insulin A chain and B chain are separately inserted to the plasmids of two different E.Coli cultures. The lac operon system (consisting of inducer gene, promoter gene, operator gene and structural gene Z for βgalactosidase) is used for expression of both the genes. The presence of lactose in the culture medium induces the synthesis of insulin A and B chains in separate cultures. The so formed insulin chains can be isolated, purified and joined together to give full-pledged human insulin.

(b) Human Growth Hormone:

Growth hormone is produced by the pituitary gland. It regulates the growth and development. Growth hormone stimulates over all body growth by increasing the cellular uptake of amino acids, and protein synthesis and promoting the use of fat as body fuel.

In sufficient human growth hormone in young children results in retarded growth, clinically referred to as pituitary dwarfism.

Production of recombinant hGH:

The hGH is a protein composed of 191 amino acids. During the course of its natural synthesis in the body, hGH is tagged with a single peptide (with 26 amino acids). The signal peptide is removed during secretion of release the active hGH for biological functions. The entire process of hGH synthesis goes on in an orderly fashion in the body. However, signal peptide interrupts hGH production by recombinant technology. The complementary DNA (CDNA) synthesized from the mRNA encoding hGH is inserted into the plasmid. The plasmid containing E.Coli when cultured, produces full length hGH along with signal peptide. But E-Coli cannot remove the signal peptide. Theoretically, CDNA encoding signal peptide can be cut to solve these problems. Unfortunately, there is no restriction endonuclease to do this job.

Biotechnologists have resolved the problem of signal peptide interruption by a noval approach. The base sequence in CDNA encoding. Signal peptide plus the neighbouring 24 amino acids (i.e. a total 50 amino acids) is cut by restriction endonucleases ECoRI. Now a gene (CDNA) for 24 amino acid sequence of hGH (that has been deleted) is freshly synthesized and ligated to the remaining hGH CDNA. The 30 constituted CDNA, attached to a vector, is inserted into a bacterium such as E.Coli for culture and production of hGH. In this manner, the biologically functional hGH can be produced by DNA technology.

Recombinant hGH was approved for human use in 1985. It is marketed as protropin by Genetech Company and Humatrope by Eri Lilly company.

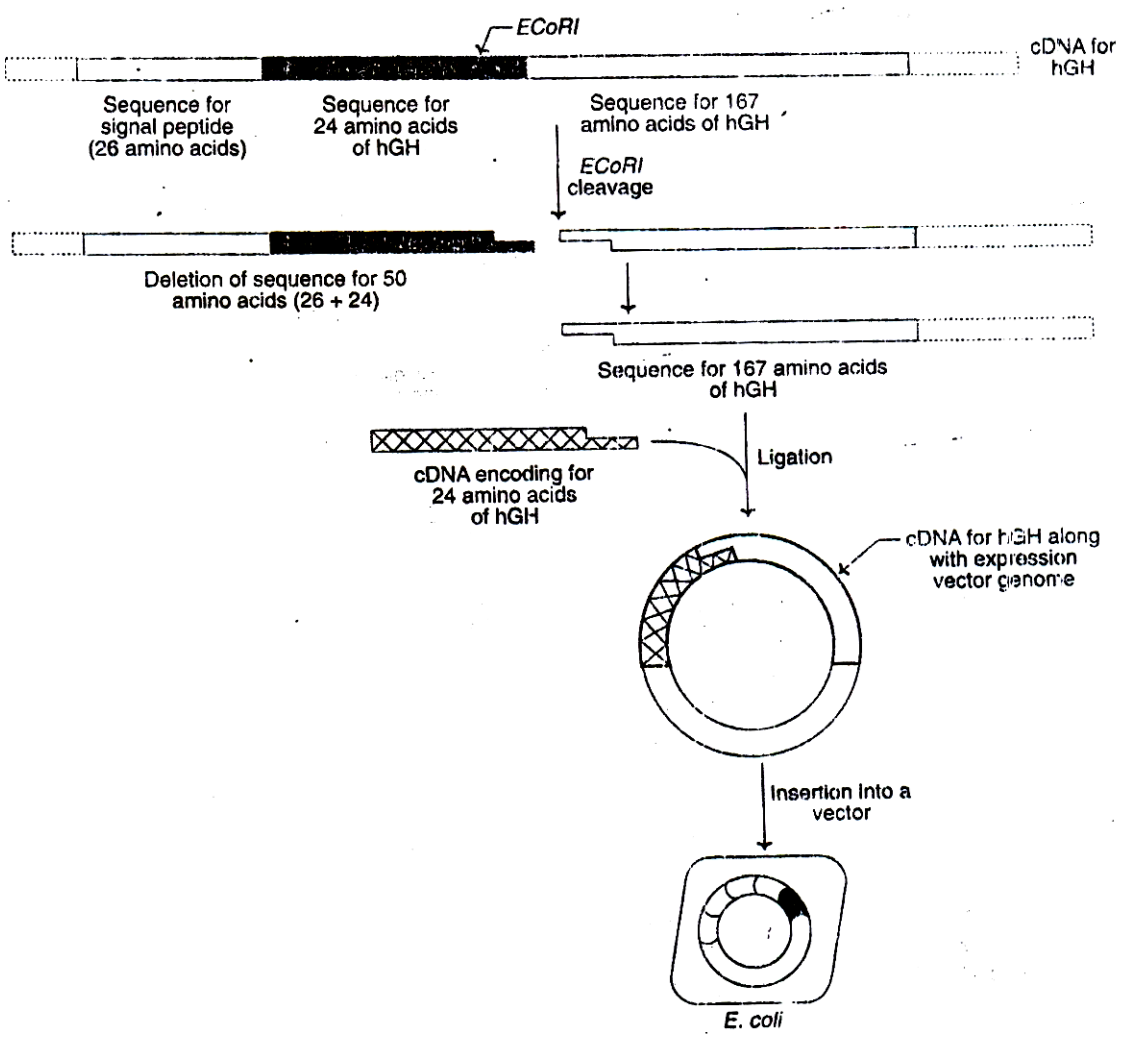


Fig. The production of recombinant human growth hormone

6. Write short notes on the following therapeutic agents.

(a) Tissue plasminogen Activator:- (tPA)

Tissue plasmingen activator (tPA) is a naturally occuring protease enzyme that helps to dissolve blood clots. tPA is a boon for patients suffering from thrombosis.

Production of recombinat tPA:

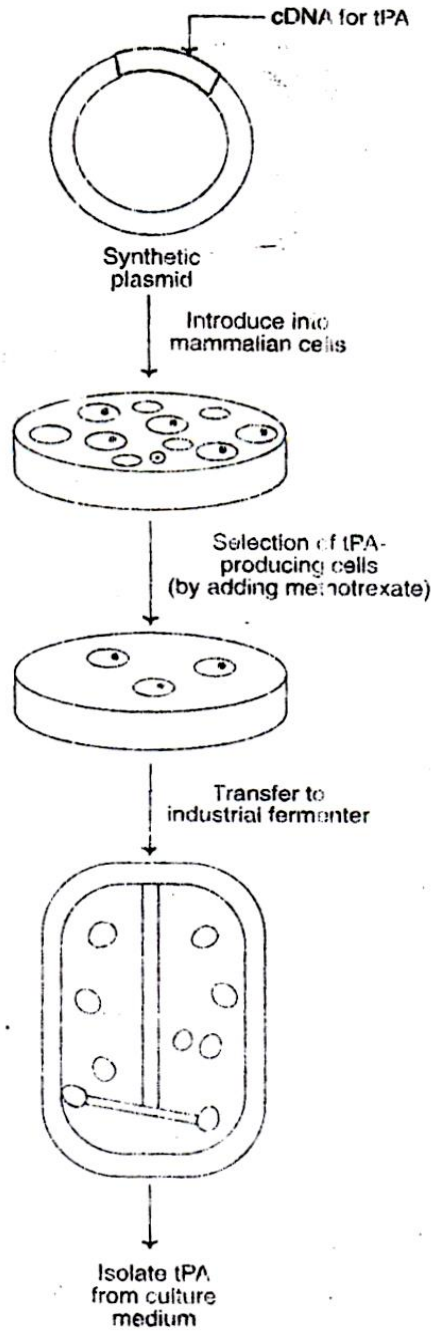


Fig. The production of recombninant tissue plasmingen activator (tPA)

DNA technologies synthesized the complementary DNA (c-DNA) molecule for tissue plasminogen activator. This cDNA was then attached to a synthetic plasmid and introduced into mammalian cells. They were cultured and tPA producing cells were selected by using methotreate to the medium. tPA producing cells were transferred to an industrial tank (fermenter). tPA,

secreted into the medium, is isolated for theraepeutic purpose. If may be noted that tPA was the first pharmaceutical product to be produced by mammalian cell culture.

(b) Interferons:

Interferon is an antiviral substance, and is the first line of defense against viral attacks. It is now known that interferon actually consists of a group of more than twenty substances with molecular weights between 20,000-30,000 daltons. All the interference are proteins in nature and many of them are glycoproteins. They are broadly categorized into three groups based on their structure and function.

(1) interferon - α(IFN-α)
(2)Interferon - β(IFN-β)
(3)interferon - γ(IFN-γ)

Production of recombinant interferons:

The complementary DNA was synthesized from the m-RNA of a specific interferon. This is inserted to a vector which is introduced into E. coli or other cells. The interferon can be isolated form the culture medium. This is the basic mechanism of producing recombinant interferons.

The production of interferons is relatively less in bacterial hosts, although E. coli was the first to be used. This is mainly because most interferons are glycoproteins in nature and bacteria do not passes the machinary of glycosylation of proteins

Production Interferon's fry yeasts:

The Yeast saccharomyces cervicae is more suitable for the production of recombinant interferons. This is mainly because the yeast possess the mechanism to carry out glycosylation of proteins, similar to that occurs in mammalian cells. The DNA sequence coding for specific human interferon can be attached to the yeast alcohol dehydrogenase gene in a plasmid and introduced into be yeast cells. The yield of interferons is several fold higher compared to E.coli.

7. Define vaccine and write short note on different types of vaccines.

Vaccine was first developed by Edward Jenner (1796) against for small box. Vaccines are antigenic preparations from bacteria (or) virus either by killing them (or) keep attenuated them, to produced antibodies and protect body from disease.

Vaccination is the phenomenon of preventive immunization. Vaccination involves the administration (injection or oral) of oxygen to elicit an antibody response that will protect the organism against future infections.

Vaccines are mainly of three types

(i) Dead bacteria or inactivated viruses
(ii) Live non – virulent or weakened (attenuated bacteria / or viruses)
(iii) Viral fragments or bacterial molecules (sub unit vaccines)

A Vaccine triggers the body's immune system to produce anti bodies against a specific disease causing organism (virus, bacterium or other paradise). Their provides surveillance against future exposure to such an organism and thus protects the body.

Recombinant DNA technology has become a boon to produce new generation vaccines. The recombinant vaccines may be broadly categorized into three groups.

(i) Sub unit recombinant vaccines:

These are the components of the pathogenic organisms. Sub unit vaccines include proteins, peptides and DNA

(ii) Attenuated Recombinant Vaccines:

These are the genetically modified pathogenic organisms (bacteria or viruses) that are made non – pathogenic and used as vaccines.

(iii) Vector recobinant vaccines:

These are the genetically modified viral vectors that can be used as vaccines against certain pathogens.

The list of diseases for which recombinant vaccines are developed or being developed is given in the following table.

Disease viral disease	Pathogenic organism
(i) Accuse in fantile gastroenteritis	Rotaviruses
(ii) Accuse respiratory diseases	Influenza A & B viruses
(iii) AHOS	Human immunodeficiency viruses
(iv) Chicken box	Vairicella – zoster viruses
(v) Liver damage	Hepatitis is A virus Hepatitis is B virus

Bacterial diseases:

(1) Cholera	Vibro choleras
(2) Diarrhea	E – Coli
(3) Dysentery	Shigella strain
(4) Leprosy	Mycobacterium leprael
(5) Tetanus	Clostridium tetani
(6) Typhoid	Salmonella typhoid

Parasitic diseases:

(1) Filariasis	Wuchereria bancroftic
(2) Malaria	Plasmodium sp
(3) River blindness	Onchocerca volvulus
(4) Sleeping sickness	Trypanosome Sp

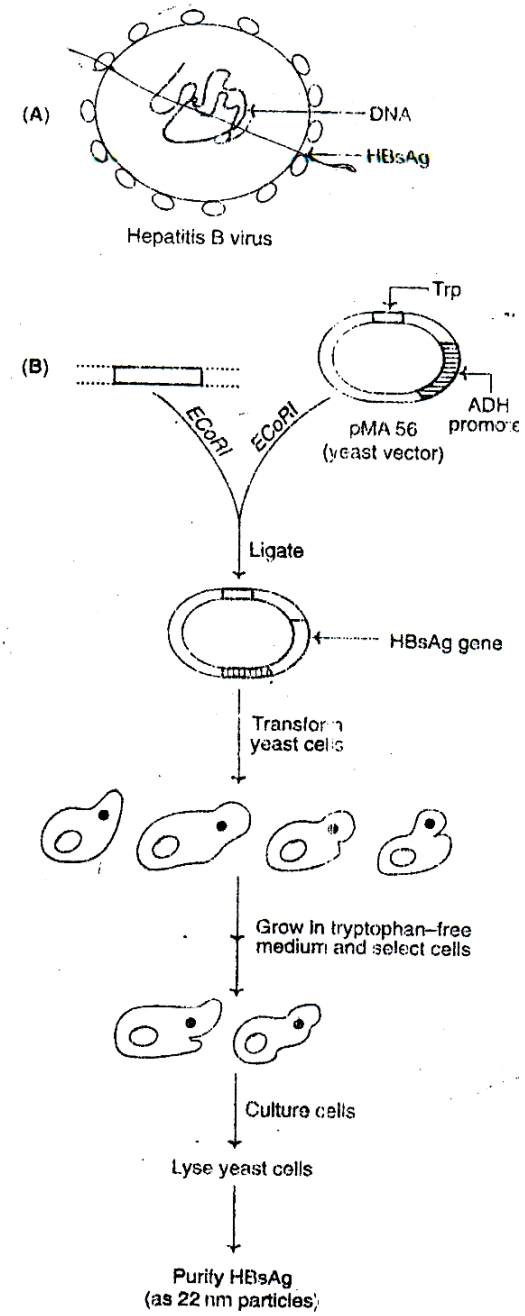
8. What are subunit vaccines and explain its production?

Subunit Vaccines

Subunit recombinant vaccines are the components (proteins, peptides, DNAs) of the pathogenic organisms. The advantages of these vaccines include their purity in preparation, stability and safe use. The disadvantages are high cost factor and possible alteration in native conformation. Example: Hepatitis

Hepatitis

It primarily affects liver causing chronic hepatitis, cirrhosis and liver cancer. Hepatitis virus is a 42 nm particle, called Dane particle. If consists of a core containing a viral genome surrounded by a phospholipids envelop carrying surface antigens.



(A) Hepatitis B virus–Dane particle (42 nm particle); (B) Production of hepatitis B surface antigen (HBsAg) in yeast cells (Trp–Tryptophan, ADH–Alcohol dehydrogenase)

Production

The gene encoding for hepatitis B surface antigen (HBsAg) has been identified. Recombinant hepatitis B vaccine as a subunit vaccine is produced by cloning HbsAg in yeast cells. Saccharomyces cerevisiaoe, a harmless backing and brewing yeast is used for this purpose. The gene for HBsAg is inserted which is linked to the alcohol dehydrogense promoter. These

plasmids are then transferred and cultured. The cells grown in tryptophan, free medium are selected and cloned. The yeast cells are culled.

The HBsAg gene is expressed to produce 2nm sized particles similar to these found in patients infected with hepatitis B. The subunit HBsAg at 22nm particles can be isolated and used to minimize individuals against hepatitis B. This is the first produced synthetic vaccine for public use.

9. Define DNA vaccines and explain its production.

DNA Vaccines:

Genetic immunization by using DNA vaccines is a novel approach that came into being in 1990. The immune response of the body is stimulate by a DNA molecule. A DNA vaccine consists of a gene encoding an antigenic protein, inserted into a plasmid, and then incorporated into the cells in a target animal. The plasmid carrying DNA vaccine normally contains a promoter site, cloning site for the DNA vaccine gene, origin of replication, a selectable marker sequence (example: a gene for amplicillin resistance) and a terminator sequence (a poly – A tail).

DNA vaccine – plasmids can be administrated to the animals by one of the following delivery methods

- Nasal Spray
- Intramuscular injection
- Intravenous injection
- Intradermal injection
- Gene gun or biolistic delivery

DNA Vaccine and immunity:

The plasmid vaccine carrying the DNA for antigenic protein enters the nucleus of the in occulted target cell of the host. As the antigen molecules bind to B – lymphocytes, they trigger the production of antibodies which can **entropy** the pathogens. Some of the B – Lymphocytes become memory cells that can protect the host against future infections.

10. What are edible vaccines & explain its production? How it differ from attenuated recombinant vaccines?

Plants as Edible subunit vaccines:

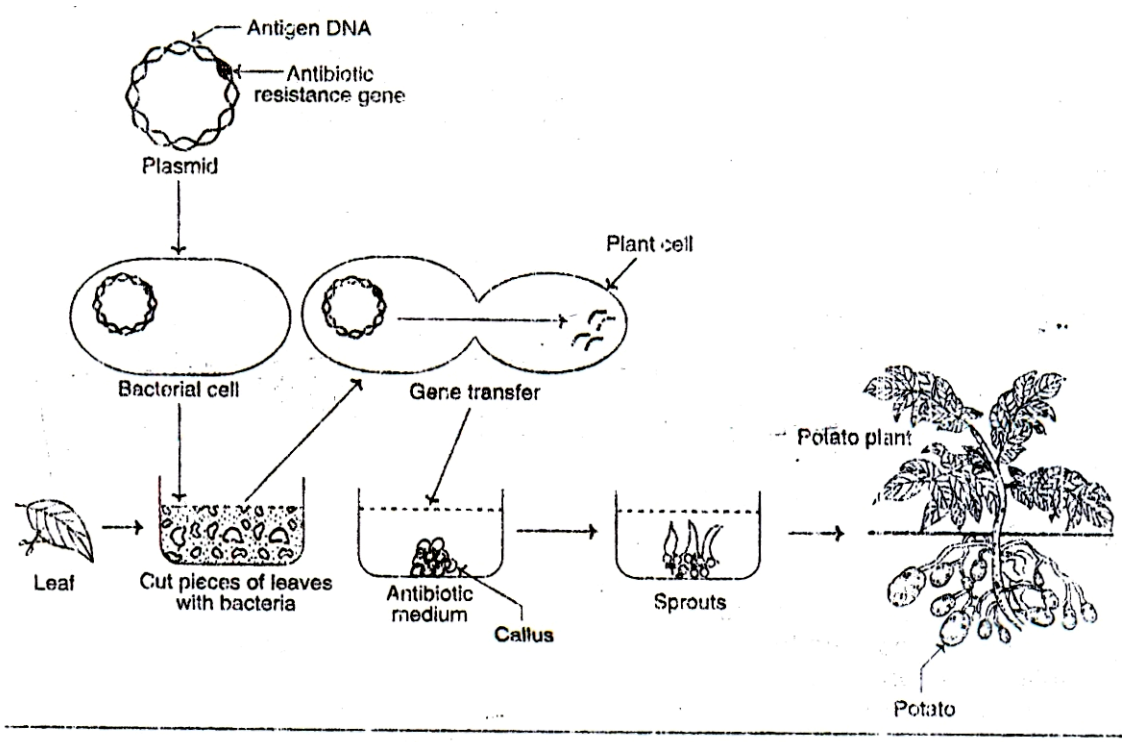
Plants serve as a cheap and safe production systems for subunit vaccines. The edible vaccines can be easily ingested by eating plants. This eliminates the processing and purification

procedures that are otherwise needed. Transgenic plants (tomato, potato) have been developed for expressing antigens derived from animal viruses produced in plants is given below.

Antigen	**Host Plant**
Rabies glycoprotein	Tomato
Food and mouth virus (VP)	Arabidopsis
Herpes virus B surface antigen	Tobacco
Cholera Toxin B subunit	Potato

Edible vaccine Production:

The production of vaccine potatos is illustrated in the following figure



An illustration for edible vaccine production.

The bacterium, Agro bacterium tumefacions is commonly used to deliver the DNA for bacterial for bacterial or viral antigens. A plasmid carrying the antigen gene and an antibiotic resistance gene are incorporated into the bacterial cells. The cut pieces of potato leaves are exposed to an antibiotic which can kill the cells that lack the new genes. The surviving cells (i.e., gene altered ones) can multiply and form a callus. Their callus is allowed to sprout shoots and roots, which are grown in soil to form plants. It about three weeks, the plants bear potatos with antigen vaccines.

Attenuated Recombinant Vaccines:

It is now possible to genetically engineer the organisms (bacteria or viruses) and use them as live vaccines, and such vaccines are referred to as attenuated recombinant vaccines. The genetic manipulations for the production of these vaccines are broadly of two type.

(1) Defection or modification of virulence genes of pathogenic organisms.
(2) Genetic manipulation of non pathogenic organisms to carry and express antigen determinants from pathogenic organisms.

The advantage with attenuated vaccine is that the native conformation of the immune determinants is preserved; hence the immune response is substantially high.

Example: Cholera.

T 8069

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2006.

FOURTH SEMESTER

BIOTECHNOLOGY

BT 1251 – BASIC INDUSTRIAL BIOTECHNOLOGY

PART A

1. Name some modern biotechnology products and their source.
2. What is meant by Bioconversion? Give examples.
3. What are the steps followed in a fermentation process?
4. What is the role of biotin in glutamic acid production?
5. How do the foams formed during fermentation and how to control it?
6. How do we sterilize heat labile components in a medium?
7. What are Precursors?
8. Write the applications of Proteases.
9. Define Submerged fermentation.
10. How can we recover the by-product formed in citric acid production?

PART – B

11. (a) (i) Compare: Traditional and Modern Biotechnology.
(ii) Explain the ranges of fermentation products.

Or

(b) (i) What does upstream and downstream processing mean?
(ii) Explain the construction and working principle of a fermenter with a neat diagram.

12. (a) (i) Describe with a metabolic pathway the production of aspartic acid and its applications.
(ii) Write the recovery process for Ethanol.

Or

(b) Describe the steps involved for the production of Acetic acid, both surface and submerged fermentation with neat diagrams.

13. (a) Discuss the steps involved in the production of aminoglycoside antibiotic and its recovery with a neat flow chart.

Or

(b) What are Steroids and discuss the bioconversion steps involved in the production of Steroids?

14. (a) Explain the commercial method of production of Proteases with a flow chart.

Or

(b) Write briefly about:
(i) SCP
(ii) Nisin
(iii) Xanthan Gum
(iv) Biopesticides.

15. (a) Discuss about the Vaccines and the production of any vaccine, using Modern Biotechnology.

Or

(b) Explain the method of production of animal cell culture products.

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S 169

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2006

THIRD SEMESTER

INDUSTRIAL BIO TECHNOLOGY

IB 237 – BASIC INDUSTRIAL BIOTECHNOLOGY

PART A

1. What are the applications of lipases?
2. What are the methods of preservation of industrially important micro organisms?
3. What are secondary metabolics?
4. How does C:N ratio of the medium influence microbial product formation?
5. What are recombinant antigens?
6. Define biofertilizers.
7. How do minerals help microbes in fermentative production of aminoacids?
8. What are chemoheterotrophs?
9. Write the importance of SCP.
10. Write the applications of recombinant proteins.

PART - B

11. With suitable examples explain how over production of metabolites by microbes be achieved.

12. (a) Write in detail the method of commercial production of (i) citric acid (ii) glutamic acid.

Or

(b) How is streptomycin produced commercially?

13. (a) What are biopolymers? How are they produced?

Or

(b) Write one method each for treatment of solid and liquid wastes.

14. (a) Write in detail the method of production of any two important aminoacids.

Or

(b) With suitable examples illustrate the commercial production of important enzymes involved in food and pharmaceutical industry.

15. (a) Animals/plants serve as the most efficient and cost effective factories for production of therapeutic drugs. Justify with suitable examples.

Or

(b) Write the principles and methods employed in the reduction of BOD and COD in industrial effluents.

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C 3084

B.E./B.Tech. DEGREE EXAMINATION, MAY/JUNE 2007.

FOURTH SEMESTER

BIOTECHNOLOGY

BT 1251 – BASIC INDUSTRIAL BIOTECHNOLOGY

PART A

1. Explain Quantitative Aspects of Cell Growth.
2. Comment on Continuous Culture.
3. Short note on different type of sterilization methods.
4. Applications of Aspartic acid and Acetic acid.
5. Comment of pH and Biomass production in fermentation technology.
6. Define the role genetic engineering in Bioprocess.
7. Give an example for Single Cell Protein and its applications.
8. What are aminoglycosides? Explain its applications.
9. Role of air-lift reactor in Plant cell culture.
10. Explain in brief "Animal cell culture".

PART – B

11. (a) (i) Overview of Downstream Processing; Centrifugation, Filtration and Ultrafiltration.
(ii) Explain in detail about Oxygen Transfer in Biological Reactors.

Or

(b) (i) Write a brief note on different fermentation products.
(ii) Explain the principle of a fermenter with clear diagram.

12. (a) (i) Comment on Optimal Feed Rate Control in Optimization of a Primary Metabolite Fermentation Process.
(ii) Describe in detail about acetic acid production with neat diagram.

Or

(b) Describe in detail about Ethanol production with neat diagram and explain about the purification process and its applications in various field.

13. (a) (i) Explain the step involved in the production and Purification of Penicillin from the microbial broth.
(ii) Comment on Interactions Between Fermentation and Recovery.

Or

(b) Explain the steps involved in the production of Vitamins and steroids with one example each.

14. (a) (i) Explain Industrial production of Cellulases and their applications.
(ii) Explain in brief about Biopesticides.

Or

(b) Describe in detail about Biopreservatives and Biopolymers with relevant examples.

15. (a) Discuss about the production of recombinant proteins with any commercially important protein as examples.

Or

(b) Discuss in detail about the production and various uses of monoclonal antibodies.

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BT3301-BIOCHEMICAL THERMODYNAMICS

LECTURE NOTES

REGULATION 2021

II YEAR & III SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

UNIT 1 NOTES

THERMODYNAMIC SYSTEMS: BASIC CONCEPTS

1.1 Introduction

The word “Thermodynamics” originates from its Greek roots (*therme*, heat; *dynamis*, force). As a subject it is concerned with quantification of *inter-relation* between energy and the *change ofstate* of any real world system. The extent of such change of state due to transfer of energy *to* or *from* the system is captured through the basic equations of thermodynamics which are derivedstarting from a set of fundamental observations known as “Laws of Thermodynamics”. The laws are essentially ‘postulates’ that govern the nature of interaction of real systems and energy. They are products of human experiential observations to which no exceptions have been found so far, and so are considered to be “laws”. The scope of application of the laws of thermodynamics ranges from the microscopic to the macroscopic order, and indeed to cosmological processes. Thus, all processes taking place in the universe, whether in non-living or living systems, are subject to the laws of thermodynamics.

Historically speaking, thermodynamics, is an extension of Newtonian mechanics which considered mechanical forces (or energy) as the agent of change of state of a body (anything possessing mass), the state being defined by its position and momentum with respect to a frame of reference. With the discovery steam power which propelled the so-called ‘Industrial Revolution’ of the 18^{th} century, it became evident that not only the direct application of mechanical energy can change the state of a system, but that fluids themselves can act as reservoir of energy, which can be harnessed to effect changes in the real world to human advantage. It was this observation that laid the foundations of thermodynamics, which now constitutes a generalized way of understanding and quantifying *all* changes that occur during processes taking place in the universe as a result of application of energy in any form.

1.2 Thermodynamic System: Select Definitions

It may be evident from the foregoing introduction, that for the purpose of any thermodynamic analysis it is necessary to define a ‘system’. A ***system***, in general, is any part of the universe which may be defined by a boundary which distinguishes it from the rest of the universe. Such a thermodynamic system is usually referred to as ***control volume*** as it would possess a volume and

would also contain a definite quantity of matter. The system boundary may be real or imaginary, and may change in shape as well as in size over time, i.e., increase or decrease.

A system can either be ***closed*** or ***open***. A closed system does not allow any transfer of mass (material) across its boundary, while an open system is one which does. In either case energy transfer can occur across the system boundary in any of its various forms; for example, heat, work, electrical / magnetic energy, etc. However, for most real world systems of interest to chemical engineers the primary forms of energy that may transfer across boundaries are heat and work. In contrast to closed or open systems, a system which is enclosed by a boundary that allows neither mass nor energy transfer is an ***isolated*** system.

All matter *external* to the system constitutes the ***surroundings***. The combination of the system and surroundings is called the ***universe***. For all practical purposes, in any thermodynamic analysis of a system it is necessary to include only the immediate surroundings in which the effects are felt.

A very common and simple example of a thermodynamic system is a gas contained in a piston-and-cylinder arrangement derived from the idea of steam engines, which may typically

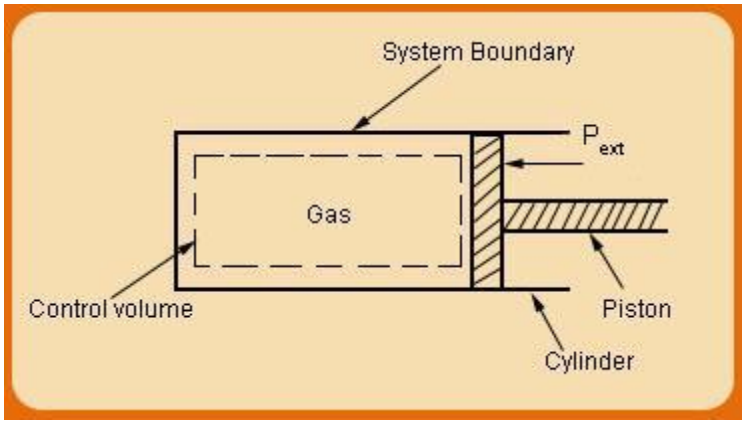


Fig. 1.1 Example of simple thermodynamic system

exchange heat or work with its surroundings. The dotted rectangle represents the 'control volume', which essentially encloses the mass of gas in the system, and walls (including that of the piston) form the boundary of the system. If the internal gas pressure and the external pressure (acting on the moveable piston) is the same, no net force operates on the system. If, however, there is a force imbalance, the piston would move until the internal and external pressures

equalize. In the process, some net work would be either delivered *to* or *by* the system, depending on whether the initial pressure of the gas is lower or higher than the externally applied pressure. In addition, if there is a temperature differential between the system and the surroundings the former may gain or lose energy through heat transfer across its boundary.

This brings us to a pertinent question: how does one characterize the changes that occur in the system during any thermodynamic process? Intuitively speaking, this may be most readily done if one could measure the change in terms of some properties of the system. A thermodynamic system is, thus, characterized by its ***properties***, which essentially are *descriptors* of the *state* of the system. Change of state of a system is synonymous with change in the magnitude of its characteristic properties. The aim of the laws of thermodynamics is to establish a *quantitative* relationship between the energy applied during a process and the resulting *change* inthe properties, and hence in the state of the system.

Thermodynamic properties are typically classified as ***extensive*** and ***intensive***. A property which depends on the size (i.e., mass) of a system is an extensive property. The total volume of a system is an example of an extensive property. On the other hand, the properties which are independent of the size of a system are called intensive properties. Examples of intensive properties are *pressure* and *temperature*. The ratio of an extensive property to the mass or the property per unit mass (or mole) is called *specific* property. The ratio of an extensive property to the number of moles of the substance in the system, or the property per mole of the substance, is called the molar property.

Specific volume (volume per mass or mole) $V=V^t/M$ ..(1.1)

Molar Volume (volume per mole) $V=V^t/N$

where,

V^t=total system volume (m^3)
M = total system mass (kg)
N = total moles in system (kg moles)

1.3 Types of Energies associated with Thermodynamic Processes:

We know from the fundamentals of *Mechanics*, that the energy possessed by a body by virtue of its position or configuration is termed potential energy (PE). The potential energy of a body of mass *m* which is at an elevation *z* from the earth's surface (or any particular datum) is given by:

$$PE = mgz \qquad ..(1.2)$$

Where, *g* is the acceleration due to gravity (= 9.81 m/s^2).

The energy possessed by a body by virtue of its motion is called the kinetic energy (KE). For a body of mass *m* moving with a velocity *u*, the kinetic energy of the body is given by:

$$KE = \frac{1}{2}mu^2 \qquad ..(1.3)$$

It follows that, like any mechanical body, a thermodynamic system containing a fluid, in principle may possess both *PE* and *KE*. It may be noted that both PE and KE are expressed in terms of macroscopic, directly measurable quantities; they, therefore, constitute macroscopic, mechanical forms of energy that a thermodynamic system may possess. As one may recall from the basic tenets of mechanics, PE and KE are inter-convertible in form.

It may also be noted that PE and KE are forms of energy possessed by a body as a whole by virtue of its *macroscopic* mass. However, matter is composed of atoms /molecules which have the capacity to translate, rotate and vibrate. Accordingly, one ascribes three forms intra-molecular energies: *translational, rotational* and *vibrational.* Further, energy is also associated with the motion of the electrons, spin of the electrons, intra-atomic (nucleus-electron, nucleus-nucleus) interactions, etc. Lastly, molecules are also subject to inter-molecular interactions which are electromagnetic in nature, especially at short intermolecular separation distances. All these forms of energy are *microscopic* in form and they cannot be readily estimated in terms of macroscopically measurable properties of matter. It needs to be emphasized that the microscopic form of energy is distinct from PE and KE of a body or a system, and are generally *independent* of the position or velocity of the body. Thus the energy possessed by matter due to the

microscopic modes of motion is referred to as the *internal energy* of the matter. The microscopic variety of energy forms the principal consideration in case of transformations that occur in a thermodynamic system. Indeed, as mentioned earlier, it is the realization that matter or fluids possessed useful form of microscopic energy (independent of macroscopic KE or PE) that formed the basis of the 18^{th} century Industrial Revolution.

As we will see later, the majority of practical thermodynamic systems of interest are the ones that do not undergo change of state that entails significant change in its macroscopic potential and kinetic energies. Thus, it may be intuitively obvious that in a very general sense, when a thermodynamic system undergoes change of state, the attendant change in the internal energy is responsible for the energy leaving or entering the system. Such exchange of energy between a thermodynamic system and its surroundings may occur across the system boundary as either ***heat*** or ***work*** or both.

Thermodynamic Work:

Work can be of various forms: electrical, magnetic, gravitational, mechanical, etc. In general work refers to a form of energy transfer which results due to changes in the external *macroscopic* physical constraints on a thermodynamic system. For example, electrical work results when a charge moves against an externally applied electrical field. As we will see later, it is mechanical work that is most commonly encountered form in real thermodynamic systems, for example a typical chemical plant. In its simplest form, such work results from the energy applied to expand the volume of a system against an external pressure, or by driving a piston-head out of a cylinder against an external force. In both the last examples, work transfer takes place due to the application of a differential (or finite) force applied on the system boundary; the boundary either contracts or expands due to the application of such a force. In effect this results in the applied force acting over a distance, which results in mechanical energy transfer.

Consider the system in fig.1.2, where a force F acts on the piston and is given by pressure x piston area. Work *W* is performed whenever this force translates through a distance.

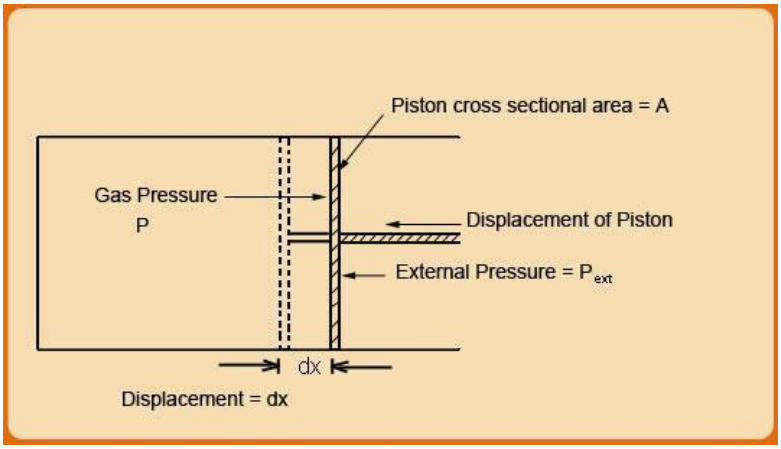


Fig. 1.2 Illustration of Thermodynamic Work

Thus for a differential displacement '*dx*' of the piston the quantity of work is given by the equation:

$$dW = Fdx \qquad ..(1.4)$$

Here *F* is the force acting along the line of the displacement *x*. If the movement takes place over a finite distance, the resulting work is obtained by integrating the above equation. By convention, work is regarded as *positive* when the displacement is in the same direction as the applied force and negative when they are in opposite directions. Thus, for the above example, the equation 1.4 may be rewritten as:

$$dW = -PAd\,(V^t/A) \qquad ..(1.5)$$

If the piston area 'A' is constant, then:

$$dW = -PdV^t \qquad ..(1.6)$$

As may be evident from eqn. 1.6, when work is done on a system (say through compression) the volume decreases and hence the work term is positive. The reverse is true when the system performs work on the surroundings (through expansion of its boundary).

Heat

We invoke here the common observation that when a hot and a cold object are contacted, the hot one becomes cooler while the cold one becomes warmer. It is logical to argue that this need be due to transfer of 'something' between the two objects. The transferred entity is called *heat*. Thus, heat is that form of energy that is exchanged between system and its surrounding owing to a temperature differential between the two. More generally, heat is a form of energy that is transferred due to temperature *gradient* across space. Thus heat always flows down the gradient of temperature; i.e., from a higher to a lower temperature regions in space. In absence of such temperature differential there is no flow of heat energy between two points. Heat flow is regarded to be *positive* for a thermodynamic system, if it enters the latter and *negative* if it leaves.

Like work, heat is a form of energy that exists only in transit between a system and its surrounding. Neither work nor heat may be regarded as being possessed by a thermodynamic system. In a fundamental sense, the ultimate repositories of energy in matter are the atoms and molecules that comprise it. So after transit both work and heat can only transform into the kinetic and potential energy of the constituent atoms and molecules.

1.4 Thermodynamic Equilibrium

In general change of state of a thermodynamic system results from existence of gradients of various types *within* or *across* its boundary. Thus a gradient of pressure results in momentum or convective transport of mass. Temperature gradients result in heat transfer, while a gradient of concentration (more exactly, of *chemical potential*, as we shall see later) promotes diffusive mass transfer. Thus, as long as *internal* or *cross-boundary* gradients of any form as above exist with respect to a thermodynamic system it will undergo change of state in time. The result of all such changes is to annul the gradient that in the first place causes the changes. This process will continue till all types of gradients are nullified. In the ultimate limit one may then conceive of a state where all gradients (external or internal) are non-existent and the system exhibits no further changes. Under such a limiting condition, the system is said to be in a state of thermodynamic *equilibrium*. For a system to be thermodynamic equilibrium, it thus needs to also satisfy thecriteria for mechanical, thermal and chemical equilibrium.

Types of Thermodynamic Equilibrium

A thermodynamic system may exist in various forms of equilibrium: *stable*, *unstable* and *metastable*. These diverse types of equilibrium states may be understood through analogy with asimple mechanical system as depicted in fig. 1.3 – a spherical body in a variety of gradients on a surface.

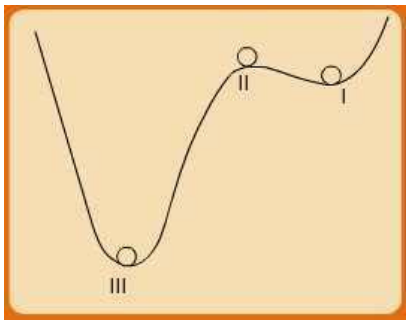


Fig. 1.3 Types of Mechanical Equilibrium

Consider the body to be initially in state 'I'. If disturbed by a mechanical force of a very small magnitude the body will return to its initial state. However, if the disturbance is of a large magnitude, the body is unlikely to return to its initial state. In this type of situation the body is said to be in *unstable* equilibrium. Consider next the state 'II'; even a very small disturbance will move the body to either positions 'I' or 'III'. This type of original equilibrium state is termed *metastable*. Lastly, if the body is initially in state 'III', it will tend to return to this state evenunder the influence of relatively larger disturbances. The body is then said to be in a *stable* equilibrium state. If 'E' is the potential energy of the body and 'x' is the effective displacement provided to the body in the vertical direction, the three equilibrium states may be described by the following equations:

Stable Equilibrium: $\frac{\partial E}{\partial x} = 0; \frac{\partial^2 E}{\partial x^2} > 0$ ..(1.7)

Unstable Equilibrium: $\frac{\partial E}{\partial x} = 0; \frac{\partial^2 E}{\partial x^2} < 0$ ..(1.8)

Metastable Equilibrium: $\frac{\partial E}{\partial x} = 0; \frac{\partial^2 E}{\partial x^2} = 0$ ..(1.9)

The above arguments may well be extended to understand equilibrium states of thermodynamic systems, which are relatively more complex in configuration. The disturbances in such cases could be mechanical, thermal or chemical in nature. As we shall see later (section 6.3), for thermodynamic systems, the *equivalent* of (mechanical) potential energy is *Gibbs free energy*. The considerations of change of Gibbs free energy are required to understand various complex behaviour that a thermodynamic system containing multiple phases and components (either reactive or non-reactive) may display under the influence of changes brought about by exchange of energy across its boundary.

1.5 The Phase Rule

Originally formulated by the American scientist Josiah Willard Gibbs in the 1870's, the phase rule determines the number of independent variables that must be specified to establish the *intensive* state of *any* system at equilibrium. The derivation of the general phase rule is shown inchapter 6, but here we state it without proof:

$F = 2 + N - \pi - r$ ..(1.10)

Here, F = degrees of freedom of the thermodynamic system in question; N = Number of components; π = number of co-existing phases, and r = number of independent reactions that may occur between the system components. For a non-reactive system, the phase rule simplifies to: $F = 2 + N - \pi$..(1.11)

In the most general sense a thermodynamic system may be multiphase and multi-component in nature. A phase is a form of matter that is homogeneous in chemical composition and physical state. Typical phases are solids, liquids and gases. For a multiphase system, interfaces typically demarcate the various phases, properties changing abruptly across such interfaces. Various phases can coexist, but they *must be in equilibrium* for the phase rule to apply. An example of a three-phase system at equilibrium is water at its triple point (~ 0^0C, and 0.0061 bar), with ice, water and steam co-existing. A system involving one pure substance is an example of a single-component system. On the other hand mixtures of water and acetone have two chemically independent components.

The intensive state of a system at equilibrium is established when its temperature, pressure, and the compositions of all phases are fixed. These are therefore, regarded as phase-rule variables; but they are not all independent. The degrees of freedom derivable from the phase rule gives the number of variables which must be specified to fix all other remaining phase-rule variables. Thus, *F* means the number of intensive properties (such as temperature or pressure), which are independent of other intensive variables. For example, for a pure component gaseous system, phase rule yields *two* degrees of freedom. This implies that if one specifies temperature and pressure, all other intensive properties are then uniquely determined these two variables. Similarly for a biphasic system of a pure component – say water and steam – there is only one degree of freedom, i.e., either temperature or pressure may be specified to fix all other intensive properties of the system. At the triple point the degrees of freedom is zero, i.e., any change from such a state causes at least one of the phases to disappear.

1.6 Zeroth Law of Thermodynamics and Absolute Temperature

Thermometers with liquid working fluids are usually used for measurement of temperature. When such a device is brought in contact with a body whose temperature is to be measured, the liquid column inside the thermometer expands due to heat conducted from the body. The expanded length can be said to represent the degree of hotness in a somewhat *quantitative* manner.

The *Zeroth Law of Thermodynamics states* that if two bodies are in thermal equilibrium with a third body, then the two given bodies will be in thermal equilibrium with each other. The zeroth law of thermodynamics is used for measurement of temperature. In the Celsius temperature scale, two fixed points – ice point and steam point – are used to devise the scale. Thus, the freezing point of water (at standard atmospheric pressure) is assigned a value of zero, while the boiling point of pure water (at standard atmospheric pressure) denoted as 100. However for introducing detail, the distance between the two end points of the liquid column marks is arbitrarily divided into 100 equal spaces called *degrees.* This exercise can be extended both below zero and above 100 to expand the range of the thermometer.

The entire exercise can be carried out with any other substance as the thermometric fluid. However, for any specific measured temperature the extent of expansion of the liquid column

will vary with the thermometric fluid as each fluid would expand to different extent under the influence of temperature. To overcome this problem, the *ideal gas* (see next section) has been arbitrarily chosen as the thermometric fluid. Accordingly, the temperature scale of the SI system is then described by the Kelvin unit (T^0K). Its relation to the Celsius (t^0C) scale is given by:

$T\,(^0K) = 273.15 + t\,(^0C)$

Thus the lower limit of temperature, called absolute zero on the Kelvin scale, occurs at -273.15^0C.

1.7 The Ideal Gas

In the foregoing discussions we have pointed out that a thermodynamic system typically encloses a fluid (pure gas, liquid or solid or a mixture) within its boundary. The simplest of the intensive variables that can be used to define its state are temperature, pressure and molar volume (or density), and composition (in case of mixtures). Let us consider for example a pure gas in a vessel. As mentioned above, by phase rule the system has two degrees of freedom. It is an experimentally observed phenomenon that in an equilibrium state the intensive variables such as pressure, temperature and volume obey a definitive inter-relationship, which in its simplest form is expressed mathematically by the Boyle's and Charles's laws. These laws are compositely expressed in the form of the following equation that is said to represent a behaviour termed as *Ideal Gas Law*:

$$PV = RT \qquad ..(1.12)$$

Where, P = system pressure (say, Pa = N/m^2), T = system temperature (in ^{0}K), V = gas molar volume (mol/m^3) and, R = universal gas constant ($=8.314\ J/mol^0K$). The above relation is said to represent an equation of state, and may alternately be written as:

$$PV^t = nRT \qquad ..(1.13)$$

Where, V^t = total system volume; n = total moles of gas in the system.

The equations (1.10) and (1.11) are also termed *Equations of State* (EOS) as they relate the variables that represent the thermodynamic state of a system in the simplest possible manner. It is

obvious that the EOS indicates that if one fixes temperature and pressure the molar volume is automatically fixed as well, i.e., the latter is not an independent property in such a case.

The ideal gas law is a limiting law in the sense that it is valid primarily for gaseous systems at low pressure, strictly speaking at pressure far below the atmospheric. However, for practical purposes it is observed to remain valid at atmospheric pressures as well. As we shall see later, the ideal gas law serves as a very useful approximation as well as a datum for estimation of both the volumetric (chapter 2) as well as all other real fluid thermodynamic properties of practical interest (chapter 5, for example).

1.8 State and Path Dependent Thermodynamic Variables

Consider a gas at a certain temperature and a pressure within a piston-cylinder assembly (for example, fig. 1.2), which for arguments' sake we may assume to be isolated. If the piston position is held fixed at this point the gas state is said to be characterized by the temperature and the pressure and its corresponding volume. In its simplest form the relationship between these intensive variables may be described by (say) eqn. 1.12. Consider next that the gas is compressed by application of an extra force on the piston so that it moves inwards into the cylinder. This motion will continue till it reaches a point when the internal gas pressure equals the externally applied pressure on the piston. If there is no further increase in the force applied to the piston, the gas will also attain a new equilibrium state wherein the pressure and temperature would attain a new set of values. If, on the other hand the extra applied pressure is removed and the gas reverts to the earlier state the original temperature and pressure (and, of course volume) is restored. Extending this argument, in general, if the gas is heated or cooled, compressed or expanded, and then returned to its initial temperature and pressure, its intensive properties are restored to their initial values. It is evident, therefore, that such properties do not depend on the past history of the fluid or on the path by which it reaches a given state. They depend only on present state, irrespective of how they are attained. Such quantities are thus defined as *state variables.* Mathematically, this idea may be expressed as follows:

$$\int_{T1}^{T_2} dT = T_2 - T_1 = \Delta T\ ; \quad \int_{P1}^{P_2} dP = P_2 - P_1 = \Delta P; \quad \int_{V1}^{V_2} dV = V_2 - V_1 = \Delta V \qquad ..(1.14)$$

The changes in the above intensive properties depend only on the initial and final states of the system. They constitute point functions and their differentials are exact.

Let us next consider the case of thermodynamic work as defined by eqn. 1.6. It may be readily evident that if one can depict the exact variation of pressure and volume during a change of state of a system on a two-dimensional P-V graph, the area under the curve between the initial and final volumes equal the work associated with process. This is illustrated in fig. 1.4.

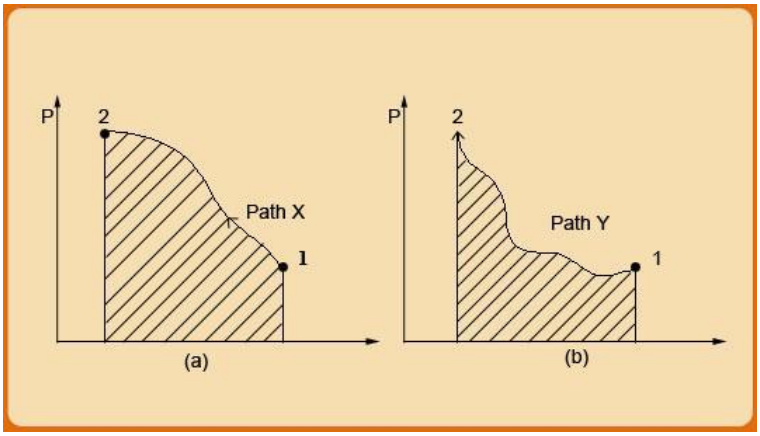


Fig. 1.4: Depiction of thermodynamic work on P-V plot

As shown in the above figure the work associated with a thermodynamic process clearly in dependent on the path followed in terms of P and V. It follows that if one were to go from state '1' to '2' by path X and then return to '1' by path Y the work in the two processes would differ and so one would not be giving and taking work out of the system in equal measure. An entity such as P-V work is, therefore, described as a *path variable*, and therefore is not directly dependent on the state of the system. This is obviously distinctive from the case of state variables such as P and V (and T). Thus, for quantifying work, one cannot write an equation of the same type as (1.12). The more appropriate relation for such variables may be written as:

$$\int_1^2 \delta W = W_{12} \qquad ..(1.15)$$

It may be pointed out that the notation δ is used to depict differential quantum of work in order to distinguish it from the differential quantity of a state variable as in eqn. 1.14. We demonstrate in chapter 3 that, like P-V work, heat transferred between a system and the surrounding is also a path variable and so one may also write:

$$\int_1^2 \delta Q = Q_{12} \qquad ..(1.16)$$

Heat and work are therefore quantities, and not properties; they account for the energy changes that occur in the system and surroundings and appear only when changes occur in a system. Although time is not a thermodynamic coordinate, the passage of time is inevitable whenever heat is transferred or work is accomplished.

1.9 Reversible and Irreversible Thermodynamic Processes

We have seen above that in absence of any gradients (or motive forces) a thermodynamic system continues to remain in a state of equilibrium. Obviously, if a disturbance (i.e., mechanical, thermal or chemical potential gradient) is impressed upon such a system it will transit from its initial state of equilibrium. However, as it moves away from its initial state the originally applied gradients will diminish progressively in time, and ultimately when they are reduced to infinitesimal levels the system will attain a new equilibrium state. A question arises here as to the nature of the process of change: if the initially impressed disturbances are reversed in direction (not magnitude) can the system return to its first equilibrium state back through the same intermediate states as it went through during the first phase of change? If that happens we depict the process as *reversible*, if not, then the process is termed *irreversible*.

It is necessary to understand the concept of *reversibility* of thermodynamic process more deeply as it is an *idealized* form of process of change and without that consideration it is not possible to represent or understand real thermodynamic processes, which are generally irreversible in nature.

What makes a thermodynamic process reversible? To answer the question let us again take the example of the simple gas-in-piston-and-cylinder system as shown in figure 1.5.

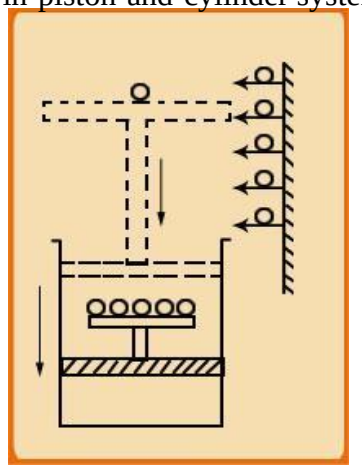


Fig. 1.5 Illustration of Reversibility of Thermodynamic Process

The system initially contains a pure gas whose pressure equals that exerted externally (due to piston weight), and its temperature is the same as that of the environment. Thus it is at equilibrium (say state 'A') as there are no mechanical, thermal or chemical concentration gradients in the system. Now a ball of a known weight is transferred on to the piston, whereupon the external pressure exceeds the gas pressure and the piston moves down to attain a new lower position at which point the gas has been compressed and its pressure once again equals that applied externally. At the same time if any differentials in temperature (within or across the system boundary) and internal concentration distribution of the gas molecules result due to the applied mechanical imbalance, heat and mass transfer will take place simultaneously until these gradients are also annulled and the system eventually comes to rest at a new equilibrium point (say, 'B'). We say that the system has undergone a process due to which its state has changed from A to B. Note that this process can be continued as long as desires by sequentially transferring more and more balls individually onto the piston and impelling the system to change in steps till say the end point state 'X'. The question that one may pose: is the process A-X reversible? That is, if one reversed all the initial steps of sequentially moving each ball off the piston so as to reach from state 'X' back to 'A' would all the interim states of the system as defined by temperature, pressure and volume at any point be identical to those obtained during the process of going from A to X?

To answer this question we need to understand the process occurring in the system a little more deeply. Consider first that a mass m_o is suddenly moved onto the piston from a shelf (at the same level). The piston assembly accelerates downwards, reaching its maximum velocity at the point where the downward force on the piston just balanced by the pressure exerted by the gas in the cylinder. However, the initial momentum of the plunging piston would carry it to a somewhat lower level, at which point it reverses direction. If the piston were held in this position of maximum depression brought about by transfer of the mass m_0, the decrease in its potential-energy would very nearly equal the work done on the gas during the downward movement. However, if unrestrained, the piston assembly would oscillate, with progressively decreasing amplitude, and would eventually come to rest at a new equilibrium position at a level below its initial position.

The oscillation of the piston assembly cease because it is opposed by the viscosity of the gas, leading to a gradual conversion of the work initially done by the piston into heat, which in turn is converted to internal energy of the gas.

All processes carried out in finite time with real substances are accompanied in some degree by dissipative effects of one kind or another. However, one may conceive of processes that are free of dissipative effects. For the compression process depicted in Fig. 1.4, such effects issue from sudden addition of a finite mass to the piston. The resulting imbalance of forces acting on the piston causes its acceleration, and leads to its subsequent oscillation. The sudden addition of smaller mass increments may reduce but does not eliminate this dissipative effect. Even the addition of an infinitesimal mass leads to piston oscillations of infinitesimal amplitude and a consequent dissipative effect. However, one may *conceive* of an ideal process in which small mass increments are added one after another at a rate such that the piston movement downwards is continuous, with minute oscillation only at the end of the entire process.

This *idealized* case derives if one imagines of the masses added to the piston as being infinitesimally small. In such a situation the piston moves down at a uniform but infinitesimally slow rate. Since the disturbance each time is infinitesimal, the system is always infinitesimally displaced from the equilibrium state both internally as well with respect to external surroundings. Such a process which occurs very slowly and with infinitesimal driving forces is called a *quasi-static* process. To freeze ideas let us assume that the gas in the system follows the ideal gas law.Thus the pressure, temperature and volume at any point during the process are related by eqn. 1.12 (or 1.13). Now imagine that the process of gradual compression is reversed by removing each infinitesimal mass from the piston just as they were added during the forward process. Since during the expansion process also the system will always be differentially removed from equilibrium state at each point, the pressure, temperature and volume will also be governed by the relation 1.12. Since the latter is an equilibrium relationship and hence a unique one, each interim state of the system would exactly converge during both forward and backward progress of system states. Under such a condition the process of compression is said to be thermodynamically reversible. Both the system and its surroundings are ultimately restored to their initial conditions. In summary, therefore, if both the system and its surroundings can be restored to their respective

initial states by reversing the direction of the process, then the process is said to be reversible. If a process does not fulfill this criterion it is called an *irreversible* process.

It need be emphasized that a reversible process need be a quasi-static process, and that the origin of irreversibility lie in the existence of dissipative forces in real systems, such as viscosity, mechanical friction. These forces degrade *useful* work irreversibly into heat which is not re-convertible by simply reversing the direction of the process, since during a reverse process a fraction of useful work will again be lost in the form heat in overcoming the dissipative forces. Thus, in the above example system if there were no viscous or frictional forces opposing the motion of the piston the processes of compression and expansion would be reversible, provided of course all changes occur under infinitesimal gradients of force. The argument in the last sentence may be extended to state that if changes are brought about by finite gradients (in this case finite difference in force across the piston, associated with addition of finite mass to the piston), the process would necessarily be irreversible. This is because finite gradients will force the system to traverse through non-equlibrium interim states, during which the pressure, temperature and volume will not be constrained by a unique relationship such as eqn. 1.12, which holds for equilibrium states. Indeed, it would not be possible to define the non-equilibrium states in terms of a single temperature or pressure, as there would be internal gradients of these variables during processes induced by finite force imbalances across the system boundary. These very same considerations would apply for the reverse process of expansion as well, if it occurs under finite mechanical gradients. So in general during such processes it would not be possible to ascribe unique intensive properties to interim states during a change, and hence the forward and reverse “paths” would not coincide as they would if the process occurs under quasi-static conditions.

An additional point that obtains from the above considerations is that only under reversible conditions can one calculate the thermodynamic work by integrating eqn. 1.6, since at all points during the process the variables P, V and T are always uniquely related by the eqn. 1.12. Clearly if the process were occurring under irreversible conditions no such relation would hold and hence the calculation of the thermodynamic work would not be possible through a simple integration of eqn. 1.6.

The foregoing discussion has used the example of a single-phase closed-system, where compression and expansion processes are induced by gradients of mechanical force across the system boundary. There are, however, many processes which are occur due to potential gradients other than mechanical forces. For example, heat flow is induced by temperature differences, electromotive force gradients lead to flow of electricity, and chemical reactions take place as there is a difference between the chemical potential of reactants and products. In general, it may be shown that all such processes brought about by potential gradients of various kinds would tend to reversibility if the gradients are themselves infinitesimal. For example, heat transfer across the boundary of a thermodynamic system would be reversible if the difference across it is of a differential amount *'dT'*, and so on.

1.10 Significance of Chemical Engineering Thermodynamics: Process Plant Schema

Before we conclude the present chapter it would be appropriate to obtain a brief preview of the scope and utility of the principles of thermodynamics insofar as application to real world processes is concerned. Although based on relatively *abstract* principles, the laws of thermodynamics provide the fundamental *constraints* under which all *real* world process take place. The ultimate application of the knowledge of the core principles of chemical engineering is in the design of a chemical process plant. Engineering thermodynamics constitutes one of the principal elements of such knowledge. Typically such a plant converts a set of raw materials to a desired product through a variety of steps that are schematically represented by Fig. 1.6.

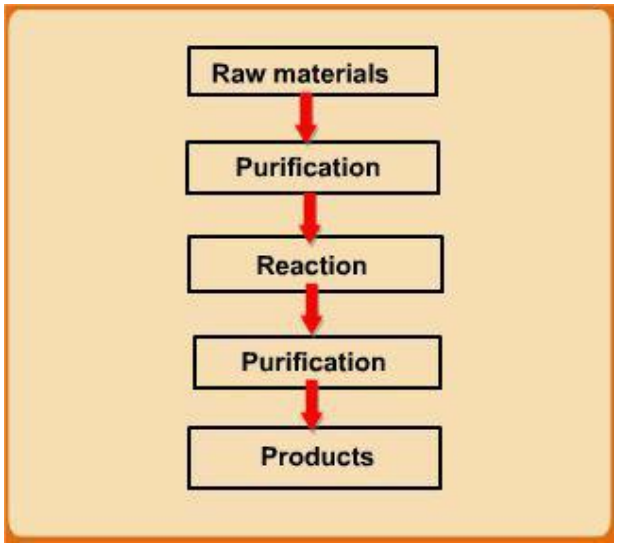


Fig. 1.6 Chemical Process Plant Schema

The raw materials most often are mixtures which need to be purified to obtain the right composition required for conversion to products A wide variety of separation processes are available for carrying out such purification; examples include distillation, liquid-liquid extraction, precipitation from solutions, crystallization, etc. Practically all such separation processes involve generation of two or more phases, in one of which the desired raw material components are *preferentially* concentrated, which is then used recover the substances in a relatively purer form.For a typical large scale chemical plant the separation process equipments may constitute more than half of the total capital investment.

The chemical reactor forms the “heart” of a chemical plant. It is here that once the feed materials are available in the right proportions (and compositions) they are reacted to yield the product. Obtaining the desired product requires an optimal choice of conditions under which the reactor may be operated. However, the product formed is very rarely obtained in a pure form. This is because typically the feed is never fully converted to product molecules and therefore the stream exiting the reactor is not a pure substance. In addition it is usually a common phenomenon that the intended chemical reaction is accompanied by often more than a single side reaction. The latter leads to the formation of side products, which results in “contamination” of the final product. Therefore, it is usually required to subject the reactor exit stream to another round of purification to obtain a product with the desired specifications of the product

With regards to all such processes of purification and reaction, the laws of thermodynamics play a very fundamental role: they allow the calculation of the *principal* entities that form the basis of design and operation of process plants:

1. The *maximum degree of purification* that is possible under a given set of processing conditions
2. The *maximum degree of conversion* possible under the reaction conditions
3. The *optimal operating conditions* for separation and reaction processes
4. The *total energy required* to achieve the intended degree of separation and reaction, and therefore the plant energy load

The calculation of the above parameters tends to constitute 50-70% of the computational load encountered during the stage of basic process plant design. Thus, the principles of chemical

engineering thermodynamics is one of the mainstays of knowledge needed to realize the goal of plant design and operation.

Volumetric Properties of Real Fluids

In the previous chapter we have introduced the concept of ideal gas and the corresponding equation of state (EOS). However, such a state obtains for a gas only at pressures around and below atmospheric (and at high temperatures), and, therefore, constitutes a limiting case. As we know, substances exist also in other forms: solids, liquids, etc. Also more often than not, in practice (as in process plants) gases (as well as other phases) may exist at substantially higher pressures (up to several thousands of atmospheres). This necessitates the development of other EOSs not only for gases, but also for relating *P-V-T* behavior of liquids and solids. Such mathematical relations, if expressed in suitably generalized forms, provide the added advantage of being able to quantify *P-V-T* behaviour for a large number of individual substances.

Such volumetric properties form a group of macroscopic thermodynamic state variables which are most easily measured. As we will see in later chapters, all other intensive thermodynamic properties can be represented in terms of mathematical expressions which denote functions of volumetric and a number of other directly measurable state variables. In the last chapter we have already introduced the ideas of thermodynamic work which clearly can be calculated if the relation between P and V is known. In chapters 3-5 we will demonstrate that heat transfer occurring under reversible conditions, as well as a host of other intensive, state variables (internal energy, enthalpy, entropy, Gibbs free energy, etc) can also be calculated using volumetric properties of a substance in question. Since work and heat are two principal modes of energy transfer in most thermodynamic systems of practical interest, it follows that the knowledge of volumetric properties is fundamental to all such calculations. Finally, as part of process plant design one needs volumetric properties for the purpose of sizing of process pipelines and all major process equipments such as reactors, heat exchangers, distillation columns, and so on. These considerations underscore the precise significance of *P-V-T* behavior of substances in all plant design activity.

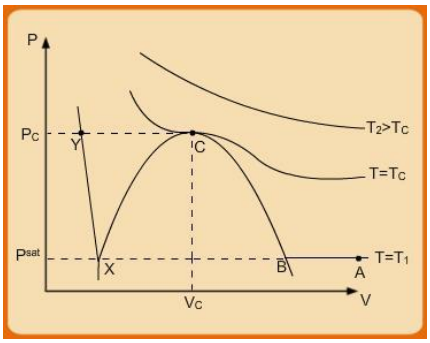
In the following sections we first describe the general nature of the *P-V-T* behavior of pure substances: in gaseous, liquid and solid forms. The various EOSs available to quantify such real fluid behaviour are then considered. Lastly, generalized correlations to relate gas and liquid behaviour are presented. Such analytical EOSs and generalized correlations allow prediction of

P-V-T values of real fluids and are, therefore, of great value as they can obviate the need for detailed experimental data.

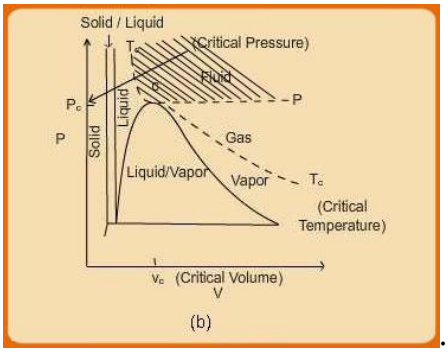
2.1 General P-V-T Behaviour of Real Fluids

P-V Diagrams

Fig. 2.1 represents the general pure component, real fluid phase behavior that typically obtains from experimental measurements. Consider first the fig. 2.1a. Let us take a substance at some temperature T_1 and certain pressure P_1 such that it is in a gaseous state A_1. Keeping the temperature fixed at T_1 if one pressurizes the gas (say in a piston-cylinder assembly as in fig. 1.2) its molar volume will decrease along the curve *A*-*B*. At point *B*, any further pressurization leads to commencement of condensation of the gas into a liquid from. Point '*B*' is thus said to correspond to a state where the substance is in a *saturated vapour state*. Once condensation begins any attempt at reducing the volume by further



(a)



(b)

Fig. 2.1 General P-V plots for real fluids

pressurization more of the saturated vapour present at *B* progressively liquefies until a point *X* is reached where all the original gas (or saturated vapour) is fully converted to liquid state. Point *X* is described as a *saturated liquid state*. It follows that at all point between *B* and *X* the substance exists partitioned into two phases, i.e., part vapour and part liquid. As one transits from *B* to *X*, pressure and temperature both remain constant; the only change that occurs is that the fraction of the original gas at point *A* (or *B*) that is liquefied increases, until it is 1.0 at point *X*. The line *B*-*X* connecting the saturated vapour and liquid phases is called the *tie-line*. For a given *T* and *P*, the relative amounts of the phases determine the effective molar (or specific) volume at any point within the two-phase region. Any further attempt to pressurize the saturated liquid results in relatively very little compression, and this is captured by the steep slope of the curve *X*-*Y*, which signifies that the liquid state is far less compressible, compared to the gas state (i.e., points over *A*-*B*). Essentially points between *X*-*Y* (including *Y* itself) represent *compressed liquid* states.

An important point to re-emphasize is that on the two-phase line *B*-*X*, the pressure of the system remains constant at a fixed value. This pressure is termed the *saturation pressure* (P^{sat}) corresponding to the temperature T_1. We recall your attention to the phase rule described in section 1.5, and eqn. 1.11. By this eqn. the degrees of freedom is *one*, which is borne by the fact that if one fixes temperature the system pressure also becomes fixed. However, in both regions *A*-*B* and *X*-*Y* the degrees of freedom is *two*, as pressure becomes fixed only if one defines bothtemperature and volume.

In general, the same behviour as detailed above may repeat at another temperature *T* ($>T_1$). One can on the one hand connect all the saturated vapour phase points at differenttemperatures and on the other connect all the points representing saturated liquid phase, the locus of such points give rise to the dome-shaped portion *X*-*C*-*B* of the *P*-*V* diagram which essentially signifies that at any pressure and volume combination within this dome, the state of the system is biphasic (part gas and part liquid). The region right of the dome *B*-*C* represents saturated gas phase while to the left (*X*-*C*) the state is saturated liquid. If one continues to conduct the pressurization at increasingly higher temperatures, one eventually arrives at a temperature for which the tie-line is reduced to a point and the *P*-*V* curve turns into an inflexion point to the two-phase dome. The temperature which such a behavior obtains is called the *critical temperature* (T_C), while the pressure at corresponding point of inflexion is termed the *critical pressure* (P_C).

The molar volume at the point is termed the *critical volume*, and the state itself the *critical point*. A fluid which is at a temperature and pressure above the critical point values is said to be in a supercritical state; this is indicated by the hatched region in fig. 2b. As has been shown for the *P-V* curves for a $T > T_C$, there exists no liquid phase as the curve passes beyond the two-phasedome region. Thus, the critical temperature is a temperature above which a gas cannot be liquefied by compressing, as can be below it. Compilation of values of critical properties and ω for a large number of substances are available readily from several sources **(see:http://srdata.nist.gov/gateway/gateway).**Values of these parameters for some select substancesare provided in *Appendix II.*

In fig. 2.1b the phase behavior depicted in fig. 2.1a is extended and more generalized to include solid phase as well. Accordingly, not only vapour-liquid region, other two phase regions, i.e., solid-vapour and solid-liquid regions are also displayed. The same arguments as made above for explaining the nature of co-existence of vapour and liquid phases apply to the other two biphasic regions.

P-T Diagrams

The phase behaviour described by fig. 2.1 can also be expressed in a more condensed manner by means of a pressure-temperature (*P-T*) diagram shown in fig 2.2. Just as P-V curves were depicted at constant temperature, the P-T diagram is obtained at a constant molar volume. The two phase regions which were areas in the P-V diagram are reduced to lines (or curves) in fig. 2.2. The P-T curves shown by lines *X-Y, Y-Z, and Y-C* result from measurements of the vapour

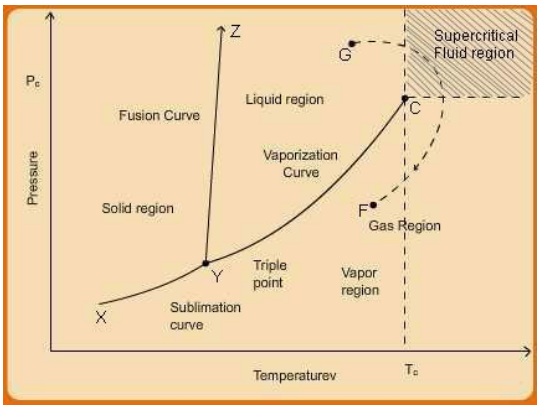


Fig. 2.2 Pressure-Temperature Diagram of a Pure Substance

pressure of a pure substance, both as a solid and as a liquid. *X-Y* corresponds to the solid-vapour (sublimation) line; *X-Y* represents the co-existence of solid and liquid phases or the fusion line, while the curve *Y-C* displays the vapour-liquid equilibrium region. The pressure at each temperature on the *Y-C* curve corresponds to the equilibrium vapour pressure. (Similar considerations apply for *P-T* relation on the sublimation curve, *X-Y*). The terminal point C represents the critical point, while the hatched region corresponds to the supercritical region. It is of interest to note that the above three curves meet at the triple-point where all three phases, solid, liquid and vapour co-exist in equilibrium. By the phase rule (eqn. 1.11) the degrees of freedom at this state is zero. It may be noted that the triple point converts to a line in fig. 2.1b. As already noted, the two phases become indistinguishable at the critical point. Paths such as *F to G* lead from the liquid region to the gas region without crossing a phase boundary. In contrast, paths which cross phase boundary *Z-Y* include a vaporization step, where a sudden change from liquid to gas occurs.

A substance in the compressed liquid state is also often termed as *sub-cooled*, while gas at a pressure lower than its saturation vapour pressure for a given temperature is said to be "superheated". These descriptions may be understood with reference to fig. 2.2. Let us consider a compressed liquid at some temperature (T) and pressure (P). The saturation temperature for the pressure P would be expected to be *above* the given T. Hence the liquid is said to be sub-cooled with respect to its saturation temperature. Consider next a pure vapour at some temperature (T) and pressure (P). Clearly for the given pressure P the saturation temperature for the pressure P would be expected to be *below* the given T. Hence with respect to the saturation temperature the vapour is *superheated*.

The considerations for *P-V* and *P-T* diagrams may be extended to describe the complete P-V-T phase behaviour in the form of three dimensional diagrams as shown in fig. 2.3. Instead of two-dimensional plots in figs. 2.1 and 2.2 we obtain a *P-V-T surface*. The *P-V* plots are recovered

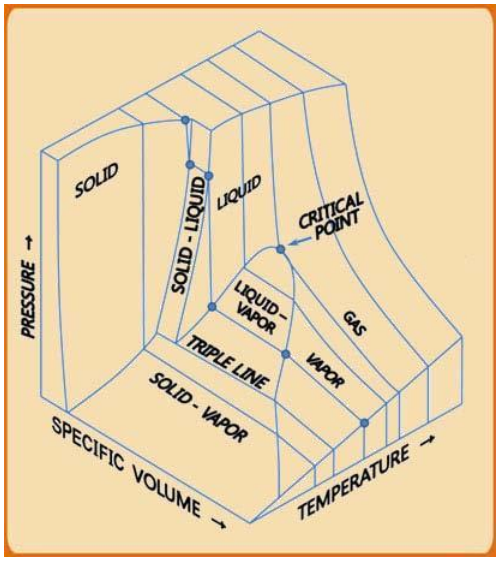


Fig. 2.3 Generalized Three-dimensional P-V-T Surface for a Pure Substance

if one takes a slice of the three dimensional surface for a given temperature, while the *P-T* curve obtains if one takes a cross-section at a fixed volume. As may be evident, depending on the volume at which the surface is cut the *P-T* diagram changes shape.

Fig. 2.4 illustrates the phase diagram for the specific case of water. The data that is pictorially depicted so, is also available in the form of tables popularly known as the "steam table". The steam table (seehttp://www.steamtablesonline.com/) provides values of the following thermodynamic properties of water and

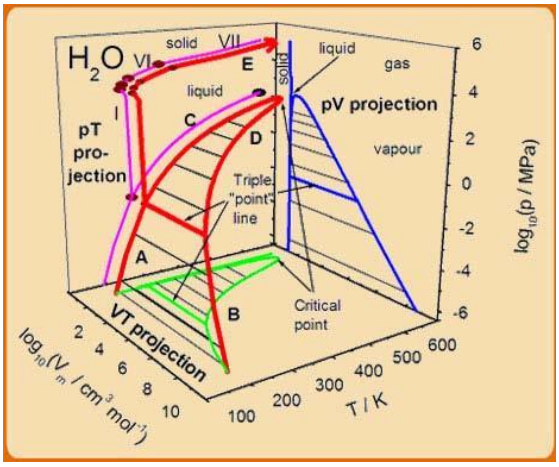


Fig. 2.4 Three-dimensional P-V-T Plot for Water

vapour as a function of temperature and pressure starting from its normal freezing point to the critical point: molar volume, internal energy, enthalpy and entropy (the last three properties are introduced and discussed in detail in chapters 3 and 4).

The steam tables are available for saturated (two-phase), the compressed liquid and superheated vapour state properties. The first table presents the properties of saturated gas and liquid as a function of temperature (and in addition provides the saturation pressure). For the other two states the property values are tabulated in individual tables in terms of temperature and pressure, as the degree of freedom is two for a pure component, single state. For fixing the values of internal energy, enthalpy and entropy at any temperature and pressure those for the saturated liquid state at the triple point are arbitrarily assigned zero value. The steam tables comprise the most comprehensive collection of properties for a pure substance.

2.2 Origins of Deviation from Ideal Gas Behaviour

The ideal gas EOS is given by eqn. 1.12. While this is a relationship between the macroscopic intensive properties there are two assumptions about the microscopic behaviour of molecules in an ideal gas state:

O The molecules have no extension in space (i.e., they posses zero volume)

P The molecules do not interact with each other

In particular, the second assumption is relatively more fundamental to explaining deviations from ideal gas behavior; and indeed for understanding thermodynamic behavior of real fluids (pure or mixtures) in general. For this, one needs to understand the interaction forces that exist between molecules of any substance, typically at very short intermolecular separation distances (~ 5 – 20 ^{0}A (where 1 ^{0}A = 10^{-8}m).

Uncharged molecules may either be polar or non-polar depending on both on their *geometry* as well as the *electro-negativity* of the constituent atoms. If the *centre* of total positiveand negative charges in a molecule *do not* coincide (for example, for water), it results in a *permanent* dipole, which imparts a polarity to the molecule. Conversely, molecules for which thecentres of positive and negative charge coincide (for example, methane) do not possess a permanent dipole and are termed non-polar. However, even a so-called non-polar molecule, may possess an *instantaneous* dipole for the following reason. At the atomic level as electrons

oscillate about the positively charged central nucleus, at any point of time a dipole is set up. However, averaged over time, the net dipole moment is zero.

When two polar molecules approach each other closely the electric fields of the dipoles overlap, resulting in their re-orientation in space such that there is a net *attractive* force between them. If on the other hand a polar molecule approaches a non-polar molecule, the former *induces* a dipole in the latter (due to displacement of the electrons from their normal position) resulting once again in a net attractive interaction between them. Lastly when two non-polar molecules are close enough their instantaneous dipoles interact resulting in an attractive force. Due to these three types of interactions (dipole-dipole, dipole-induced dipole, and induced dipole-induced dipole) molecules of any substance or a mixture are subjected to an attractive force as they approach each other to very short separation distances.

However, intermolecular interactions are not only *attractive*. When molecules approach to distances even less than ~ 5 $\overset{0}{A}$ or so, a *repulsive* interaction force comes into play due to overlap of the electron clouds of each molecule, which results in a repulsive force field between them. Thus if one combines both the attractive and repulsive intermolecular interactions the overall interaction potential *U* resembles the schematic shown in fig. 2.5.

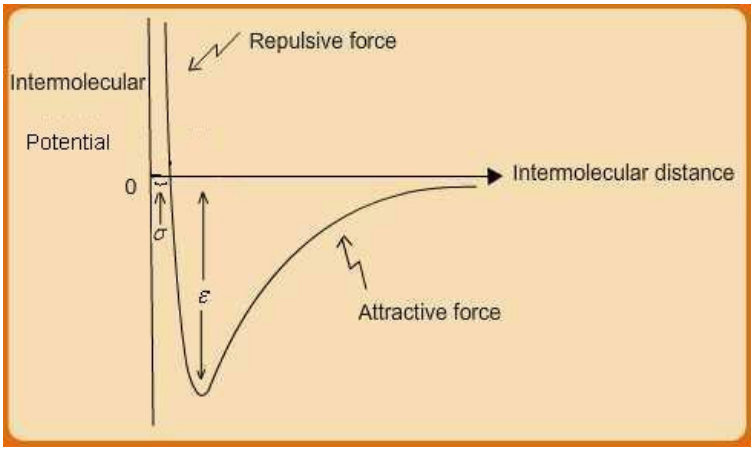


Fig. 2.5 Schematic of Intermolecular potential energy *U* for a pair of uncharged molecules

Many expressions have been proposed for the overall interaction potential *U* [see, J.M. Prausnitz, R.N Lichtenthaler and E.G. Azevedo, *Molecular Thermodynamics of Fluid PhaseEquilibria,* (3^{rd}ed.), 1999, Prentice Hall, NJ (USA)]. These are essentially empirical, althoughtheir functional forms often are based on fundamental molecular theory of matter. The most

widely used equation in this genre is the *Lennard-Jones* (LJ) *12/6 pair-potential function* which is given by eqn. 2.1:

$$U(LJ)=4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12}-\left(\frac{\sigma}{r}\right)^{6}\right] \quad ..(2.1)$$

Where, r = intermolecular separation distance; ε,σ = characteristic L-J parameters for a substance.

The r^{-12} term represents the repulsive interaction, whereas the r^{-6} term corresponds to the attractive interaction potential. As already indicated, the domain of intermolecular interactions is limited to relatively low range of separation distances. In principle they are expected to be operative over $r=0-\infty$; but for practical purposes they reduce to insignificant magnitudes for separations exceeding about 10 times the molecular diameter.

The L-J parameters ε,σ are representative of the molecular interaction and size respectively. Typical values of the L-J equation parameters for various substances may be found elsewhere (G. Maitland, M. Rigby and W. Wakeham, 1981, *Intermolecular Forces: Their Originand Determination*, Oxford, Oxford University Press.)

Since gases behave ideally at low pressures, intermolecular separation distances therein are typically much higher than the range over which intermolecular interactions are significant. This is the reason why such interactions are negligible in case of ideal gas, which essentially is one of the assumptions behind the definition of ideal gas state. Indeed while the ideal gas EOS is expressed in macroscopic terms in eqn. 1.12, the same equation may be derived from microscopic (thermodynamic) theory of matter.

The root of non-ideal gas behavior, which typically obtains at higher pressure, thus is due to the fact that at elevated pressures, the intermolecular separations tend to lie within the interactive range and hence the ideal gas assumption is no longer valid. Thus, the ideal gas EOS is insufficient to describe the phase behavior of gases under such conditions.

Intermolecular interactions also help explain the behavior of fluids in other states. Gases can condense when compressed, as molecules are then brought within the separations where the attractive forces constrain the molecules to remain within distances typical of liquid phase. It follows that a pure component liquid phase cannot be *ideal* in the same sense as a gas phase can be. Further, the fact that liquids are far less compressible also is due to the repulsive forces that

operate at close intermolecular distances. Obviously these phenomena would not be observed unless there were interactions between molecules. Thus, it follows that while properties of the ideal gas depend only on those of isolated, non-interacting moleclues, those of real fluids depends additionally on the intermolecular potential. Properties which are determined by the intermolecular interaction are known as *configurational properties*, an example of which is the energy required for vapourization; this is because during the process of vapourization energy has to be provided so as to overcome the intermolecular attractive force between molecules in the liquid phase and achieve the gas state where the seprations are relatively larger.

2.3 Equations of State for Real Fluids

The generic form of an equation of state (EOS) is: $f(P,V,T)=0$

However, as we have already seen by the phase rule, for a single phase pure component the degrees of freedom are two. This may be expressed in the form of an EOS equation as follows:

$V = V(T, P)$

It follows that:

$$dV = \left(\frac{\partial V}{\partial T}\right)_P dT + \left(\frac{\partial V}{\partial P}\right)_T dP \qquad ..(2.2)$$

Defining Volume Expansivity as $\beta \equiv \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_P$ ..(2.3)

Isothermal compressibility as $\kappa \equiv -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T$ ..(2.4)

The generic EOS (2.2) may be written as:

$$\frac{dV}{V} = \beta dT - kdP \qquad ..(2.5)$$

2.3.1 EOS for Liquids

For **liquids,** which are relatively incompressible, the factors β and κ are generally show an weak dependence on T and P and hence **averaged** values of these parameters may be used for

estimating the liquid volume at any temperature using the following integrated form of the equation (2.5):

$$\ln\frac{V_2}{V_1} = \beta(T_2 - T_1) - k(P_2 - P_1) \qquad ..(2.6)$$

For liquids the usual datum volume (i.e., V_1 in eqn.2.6) can be the saturated volume at a given temperature, which may be obtained from the Rackett equation (H. G. Rackett, J. *Chem. Eng.Data, 1970,* vol. 15, pp. 514-517); i.e.,:

$$V_{sat} = V_c Z_c^{(1-T_r)^{0.2857}} \; ; \; where \; T_r = T/T_C = reduced \; temperture. \qquad ..(2.7)$$

Where Z_C is the critical compressibility factor (see below).

Example 2.1

For a liquid 'A' at 350K and 1 bar, $\kappa = 50 \times 10^{-6}$ bar^{-1}. (i) To what pressure must water be compressed at 350 K to change its density by 0.5%? Assume that κ is independent of P.

2.3.2 EOS for Gases

In contrast to liquids, **gases** are relatively far more compressible, and so volume is strongly dependant on temperature and pressure. Consequently eqn. 2.6 cannot be used easily to estimate volume at a given T & P, as both β and κ are strong functions of T and P. Thus, various EOSs have been proposed to describe gas phase volumetric properties. The next section presents select EOSs that are typically used for the gas phase, ranging from those applicable to moderate pressure to others which are more accurate at high pressures.

Virial EOS:

Generally applicable to moderate deviations from ideal gas behavior, the virial EOS is given by two alternate forms:

$$\frac{PV}{RT} = 1 + \frac{B}{V} + \frac{C}{V^2} + \ldots \qquad ..(2.8)$$

Where B, C = second and third virial coefficients

$$\frac{PV}{RT} = 1 + B'P + C'P^2 + \ldots \quad ..(2..9)$$

Or:

It may be shown that the following relations hold further:

$$B = \frac{B'}{RT}; \text{ and } C = \frac{C' - B^2}{(RT)^2} \quad ..(2.10)$$

As may be evident, the second and third terms on the right side of eqns. 2.8 and 2.9 constitute corrections for the non-ideal behavior of a gas. The virial coefficients are essentially dependent of temperature. The more the number of virial coefficients used in the equation the better is the prediction of gas molar volume. While the estimation of the second virial coefficient is relatively straightforward, that of the third virial coefficient is generally far more complex, and there is scant data in the literature on its value for different substances. Because of this the virial EOS is most commonly used in the truncated form, and applies to moderate deviations from ideal gas behavior:

$$\frac{PV}{RT} = 1 + \frac{B}{V} \quad ..(2.11)$$

Alternately, $Z = 1 + \frac{BP}{RT} = 1 + \frac{BP_c}{RT_c}\frac{P_r}{T_r}$ ..(2.12)

Where, P_r and T_r are *reduced* pressure and temperature respectively, given by:

$P_r = P / P_C$; $T_r = T / T_C$

A set of generalized correlations have been proposed by Pitzer and co-workers (K. S. Pitzer, *Thermodynamics,* 3d ed., App. 3, McGraw-Hill, New York, 1995) for computing the secondvirial coefficients whereby:

$$\frac{BP_c}{RT_c} = B^0 + \omega B^1 \quad ..(2.13)$$

The terms B^0 and B^1 are given by:

$$B^0 = 0.083 - \frac{0.422}{T_r^{1.6}} \quad ..(2.14)$$

$$B^{1} = 0.139 - \frac{0.172}{T_r^{4.2}} \qquad ..(2.15)$$

The parameter ω, discussed in a later section, is the *acentric factor*, which is a fundamental thermodynamic property of a substance, and is a measure of *asphericity* of molecular shape The value of acentric factor may be obtained from the following expression from experimental vapour pressure data of a pure substance (K. S. Pitzer, *Thermodynamics*, 3d ed., App. 3, McGraw-Hill, New York, 1995):

$$\omega \equiv -1.0 - \log_{10}\left(P_{r}^{sat}\right)_{T_r=0.7} \qquad ..(2.16)$$

$\left(P_{r}^{sat}\right)_{T_r=0.7}$ = reduced saturation vapour pressure at a reduced temperature equalling 0.7.

Since the virial EOS applies to only moderate deviations from ideal behaviour several complex extensions of it have been proposed for increasing accuracy of prediction at higher pressures; an example of such an EOS is the so-called Benedict-Webb-Rubin (BWR) EOS (G.B.Benedict, G. B. Webb, and L. C. Rubin, *J. Chem.Phys.*, vol. 8, pp. 334-345, 1940) used in the petroleum and natural-gas industries:

$$P = \frac{RT}{V} + \frac{B_0 RT - A_0 - C_0/T^2}{V^2} + \frac{bRT - a}{V^3} + \frac{a\alpha}{V^6} + \frac{c}{V^3 T^2}\left(1 + \frac{\gamma}{V^2}\right)\exp\left(-\frac{\gamma}{V^2}\right) \qquad ..(2.17)$$

where A_0, B_0, C_o, a, b, c, and γ are all constant for a given fluid.

Example 2.2

Calculate the molar volume for butane at 2.5bar and 298 K using the truncated virial EOS using the following data: T_C=425.1K; P_C=37.96bar; ω=0.2.

2.3.3 Cubic EOS for Gases

While the truncated virial EOS applies to moderate pressures, it is often necessary to obtain volumetric properties of gases at relatively much higher pressures. It has generally proved to be computationally unwieldy to use extensions of the virial EOS at higher pressure. To obviate this problem an entire range of alternate EOSs, termed *cubic* EOS, have been proposed by various workers. The term “cubic equation of state” implies an equation which, if expanded, would

contain the volume terms raised to the first, second, or third power. Such an EOS provides two distinct advantages over many other more complex EOS found in the literature; they allow:

5. Prediction of both gas and liquid (saturated) phase molar volumes
6. Provide a trade-off between complexity and accuracy of prediction

The first of such cubic EOS was proposed by the Dutch physicist Johannes Diderik van der Waals in 1873, and has the following form:

$$P = \frac{RT}{V-b} - \frac{a}{V^2} \qquad ..(2.18)$$

Where, a and b are characteristic constants for a pure substance. For example, for nitrogen the values of a and b are $0.1368 Pa.m^6/mol^2$ and $3.864 \times 10^{-5} m^3/mol$ respectively. Values of these parameters for other substances may be found in the literature (see, for example: S.I. Sandler, *Chemical, Biochemical and Engineering Thermodynamics*, 4th edition, Wiley India, 2006). Once can take advantage of the phase P-V behaviour at the critical point (see fig. 2.1) to determine the expressions for a and b. Since the P-V curve offers a point of inflection at the critical point one may write:

$$(\partial P / \partial V)_{T_C} = 0 \qquad ..(2.19)$$

$$(\partial^2 P / \partial V^2)_{T_C} = 0 \qquad ..(2.20)$$

On applying eqns. (2.19) and (2.20) on 2.18 two independent equations obtain, which may solved simultaneously to show that for the vdW EOS:

$$a = (27/64) R^2 T_C^2 /$$

$$P_C b = (1/8) RT_C / P_C$$

$$V_C = (3/8) RT_C / P$$

It follows that the critical compressibility factor is then given by:

$$Z_C = P_C V_C / RT_C = 3/8 = 0.375$$

The vdW EOS suggests that for all pure substances the critical compressibility factor is a constant (= 0.375). This, however, is contrary to experience. Indeed, although similar in magnitude, the value of Z_C is specific to a pure substance. For example, for methane it is 0.286, while for helium 0.302. This indicates that although an improvement over ideal gas EOS, the

vdW EOS does not reflect the specificity needed to predict the behaviour of all substances at high pressures.

To bridge this gap several other cubic EOS of higher accuracy have been advanced, the three most widely used of which are:

- Redlich-Kwong (RK) EOS (Otto Redlich and J. N. S. Kwong, *Chem. Rev.*,vol. 44, pp. 233-244, 1949)
- Soave-Redlich-Kwong (SRK) EOS (*G.* Soave, *Chem. Eng.****Sci.,*** vol. 27, pp. 1197-1203, 1972.)
- Peng-Robinson (PR) EOS (D. **Y.** Peng and D. B. Robinson, *Ind. Eng. Chem. Fundam.,* vol. 15, pp. 59-64, 1976.)

All the above EOSs including that due to van der Waals may be expressed by a single equation of the following form:

$$P = \frac{RT}{V - b} - \frac{a}{V^2 + ubV + wb^2} \quad ..(2.21)$$

As part of a generalized approach to representing non-ideal gas behavior parameter *Z*, termed the *compressibility factor*, may be defined. This is discussed further in the following section.However, here we provide the expression for it:

$Z = PV/RT$ ..(2.22)Using eqn. 2.20, eqn. 2.19 may be rewritten as:

$$Z^3 + \alpha Z^2 + \beta Z + \gamma = 0 \quad ..(2.23)$$

Where:

$A = aP / (RT)^2$

$B = bP / RT$

$\alpha = -1 - B + uB$

$\beta = A + wB^2 - uB$

$-uB^2\gamma = -AB - wB^2 - wB^3$

Expressions for all the above parameters for each EOS are provided in Table 2.1 below.

Table 2.1 Values of $u, w, \alpha, \beta, \gamma$ for various cubic EOSs

EOS	u	w	α	β	γ	a	b
vdW	0	0	$-1-B$	A	$-AB$	$\frac{27}{64}\frac{R^2T_c^2}{P_C}$	$\frac{RT_C}{8P_C}$
RK	1	0	-1	$A-B-B^2$	$-AB$	$\frac{0.42748R^2T_c^2\alpha_{RK}}{P_c}$	$\frac{0.08664RT_C}{P_C}$
SRK	1	0	-1	$A-B-B^2$	$-AB$	$\frac{0.42748R^2T_C^2\alpha_{SRK}}{P_c}$	$\frac{0.08664RT_C}{P_C}$
PR	2	-1	$-1+B$	$A-2B-3B^2$	$-AB+B^2+B^3$	$\frac{0.45724R^2T_C^2\alpha_{PR}}{P_C}$	$\frac{0.07779RT_C}{P_C}$

The parameters $\alpha_{RK}, \alpha_{SRK}$ and α_{PR} given by:

$$\alpha_{RK} = T_r^{-1/2}$$

$$\alpha_{SRK} = [1 + (0.48 + 1.574\omega - 0.176\omega^2)(1 - T_r^{1/2})]^2$$

$$\alpha_{PR} = [1 + (0.37464 + 1.5422\omega - 0.62992\omega^2)(1 - T_r^{1/2})]^2$$

As may be evident from table 2.1 all the parameters are determinable from the critical properties, ω and the temperature at which the molar volume or pressure needs to be calculated. Once theseare computed the eqn. 2.21 may be solved to yield the value of Z, which in turn may be used to obtain the molar volume. Eqn. 2.21 may be solved using a suitable *iterative* algorithm or more readily by the general analytical solution for cubic algebraic equations, which is outlined below.

For solving eqn. 2.21, substitute, $Z=X-(\alpha/3)$ which gives:

$X^3 + pX + q$

$=0$where:

$p=\beta-(\alpha^2/3)$

$q=(2\alpha^2/27)-(\alpha\beta/3)+\gamma$Now, let

$D=(q^2/4)+(p^3/27)$

Then the roots of eqn. (2.21) are as follows:

Case 1:

If $D>0$, then only one real root exists:

$$Z=-\frac{q}{2}+\sqrt{D}^{1/3}+-\frac{q}{2}-\sqrt{D}^{1/3}-\frac{\alpha}{3}$$

Case 2 :

If $D=0$, then there are three real roots, of which two are equal:

$$Z_1=-2\frac{q}{2}^{1/3}-\frac{\alpha}{3};\ Z_2=Z_3=\frac{q}{2}^{1/3}-\frac{\alpha}{3}$$

Case 3:

If $D<0$, then there are three unequal real roots:

$$Z_1=2r^{1/3}\cos(\theta/3)-\frac{\alpha}{3}$$

$$Z_2=2r^{1/3}\cos\frac{2\pi+\theta}{3}-\frac{\alpha}{3}$$

$$Z_3=2r^{1/3}\cos\frac{4\pi+\theta}{3}-\frac{\alpha}{3}$$

where: $\cos\theta=-\frac{q}{2}\sqrt{-\frac{27}{p}^3}^{1/2}$

$$r=\sqrt{\frac{-p^3}{27}}$$

The true advantage of use of cubic EOS derives from the fact that not only can it provide gas phase molar volumes, but also that of saturated liquid at a given temperature (note that this is not feasible by use of virial EOS). To understand this point we refer to fig. 2.6, which is a simplified

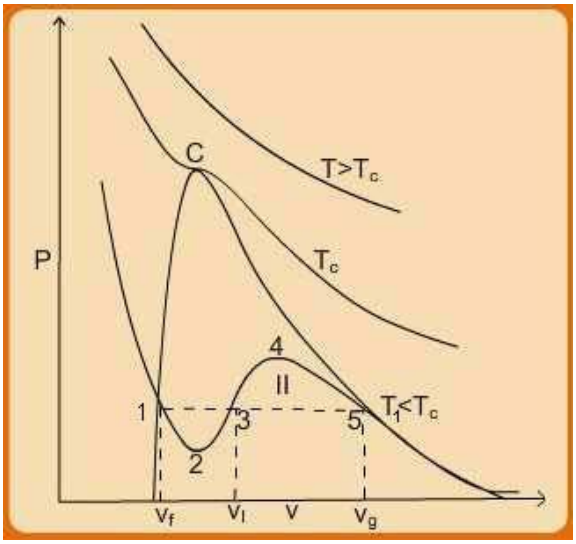


Figure 2.6 P-V plots at constant temperatures (as obtained from a cubic equation of state)

version of fig. 2.1.

For given values of V and T one can calculate P (using any cubic EOS) as a function of V for various values of T. Figure 2.4 is a schematic P-V curves for three different temperatures. The variations shown by $T = T_C$ and $T > T_C$ have already been discussed in section 2.1. We draw your attention here to the plot at $T < T_C$. In this case the pressure decreases rapidly in the subcooled liquid region with increasing V; after crossing the saturated-liquid line, it goes through a *minimum* (1-2-3), rises to a *maximum* (3-4-5), and then decreases, crossing the saturated-vapor line and continuing downward into the superheated-vapor region. However, experimentally obtained plots of this kind (at $T < T_C$) do not show this manner of transition from saturated liquid to saturated vapor. As already discussed in section 2.1, in the two phase region within the dome, the curve connecting the saturated vapor and liquid states constitutes a tie-line. Along this horizontal segment the two phases coexist in varying proportions at the saturation vapor pressure. Clearly then the true behavior of the substance cannot be exactly reproduced by an analytical cubic EOS in the two phase region.

Nevertheless, the P-V behavior that obtains in the two-phase region using the cubic EOS is not entirely unrealistic. Indeed the two *points of intersection* of the analytical P-V curve (at a given temperature) with the boundary of the dome occurs (approximately) at the points where the experimental saturated vapour and liquid state volumes obtain (i.e., points 1 and 5). Cubic equations of state have three volume roots, of which two may often be complex conjugates.

Physically relevant values of V are always real, positive, and greater than constant b *which* appears in the cubic EOSs. For $T > T_C$, at any pressure the solution for eqn. 2.21 yields one real root. This is also true at any pressure on the $T = T_C$ curve, except for $P = P_C$; at the latter condition three real roots obtain all equal to V_C. For $T < T_C$, in general there could be one or three real roots. If the pressure is different from the saturation vapour pressure (P^{sat}, at the given temperature) the roots are not realistic. If, however, the pressure is taken to be P^{sat}, three real roots are found. The highest amongst these correspond to the saturated vapour phase volume, while the lowest one approximates the saturated liquid phase volume; the third root, however, is not physically meaningful.

Example 2.3

For methane at 298K and 2 MPa compute the molar volume using SRK equation. For methane, T_c= 190.7 K, P_c= 46.41 bar, ω = 0.011.

2.3.4 Principle of Corresponding States

The compressibility factor Z has already been defined in eqn. 2.20. A typical plot of Z as a function of T & P for methane is shown in fig.2.7. Experimentally measured values of Z for different fluids display similar dependence on reduced temperature and pressures, i.e., $P_r$andT_r.

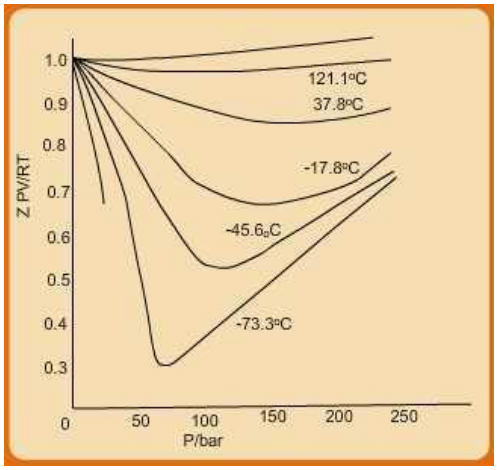


Fig. 2.7 Variation of Compressibility-factor with pressure and temperature (Source: J.M. Smith, H.C. Van Ness and M.M. Abbott, *Introduction to Chemical Engineering Thermodynamics*, 6th ed., McGraw-Hill, 2001)

This observation has been generalized to formulate the *two-parameter theorem of correspondingstates* which is stated as follows: *"All fluids at the same reduced temperature and reduced pressure have approximately the same compressibility factor, and all deviate from ideal-gas behavior to about the same degree."* Fig. 2.8 presents select experimental data which support thisobservation.

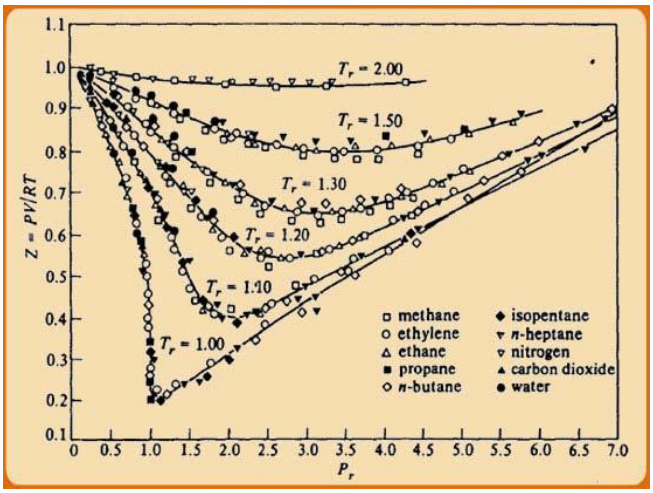


Fig. 2.8 Experimental compressibility factors for different fluids as a function of the reduced temperature and pressure. [Source: H. C.Van Ness and M.M Abbott (1982) based on data from G.-J. Su (1946). *Ind. Engr. Chem.* 38, p 803.]

While this theorem applies fairly reliably to the *simple fluids* (argon, krypton, and xenon), for more complex fluids the deviations are significant. To address this gap Pitzer and coworkers introduced a third corresponding-states parameter, characteristic of molecular structure, more particularly the "degree of sphericity" of the molecule; the most widely used one is *acentricfactor 'ω'*(already utilized in eqn. 2.13 and for computation for cubic EOS parameters in table2.1) (K. S. Pitzer, *Thermodynamics*, 3d ed., App. 3, McGraw-Hill, New York, 1995). The

expression for ω was provided in eqn. 2.16. As is evident, it can be computed for any substance using critical properties and a single vapor-pressure measurement made at $T_r = 0.7$.

By definition ω(see J.M. Smith, H.C. Van Ness and M.M. Abbott, *Introduction toChemical Engineering Thermodynamics*, 6th ed., McGraw-Hill, 2001) is zero for the *simple* fluids argon, krypton, and xenon, which are generally regarded as spherical molecules. For other substances, the greater the deviation molecular sphericity, the larger is the departure of its corresponding ω from zero. For example for methane it is 0.012, while for butane it is 0.2, and so on. Experimentally determined values of Z for the three simply fluids coincide if measured at identical reduced temperature and pressures. This observation forms the basis for extending the two-parameter theorem (stated above) to the *three-parameter theorem* of corresponding states:

"The compressibility factor for all fluids with the same value of ω, when compared at the same reduced temperature and pressure are approximately the same, and hence the deviation from ideal-gas behavior is nearly the same."

This theorem leads to vary convenient approach involving generalized correlations for computing not only the volumetric properties but for estimating a wide variety of other thermodynamic properties.

Generalized Compressibility factor Approach to EOS: Pitzer Correlations

For prediction of volumetric properties (using the compressibility factor Z or the second virial coefficient) the most commonly used correlations are those due to Pitzer and coworkers (op. cit.). According to this approach, compressibility factor is decomposed as follows:

$$Z = Z^0 + \omega Z^1 \qquad ..(2.24)$$

Where Z^0andZ^1 are both functions of $T_r$andP_r only. When $\omega = 0$, as for the simple fluids, the second term disappears. Thus the second term generally accounts for relatively small contribution to the overall Z due to the *asphericity* of a molecule. As noted earlier, the value of ω may be computed using the following equation:

$$\omega = -1.0 - \log(P_r^{sat})_{T_r=0.7} \qquad ..(2.16)$$

Fig. 2.9 shows a plot of reduced vapor pressures for select substances as a function of reduced temperature. At a value of about ~ 0.7 for the reduced temperature (T_r) of the typical simple fluids (argon, krypton, xenon) the logarithm of reduced pressure is – 1. For other molecules the

greater the departure from sphericity of the structure the lesser is the value of reduced pressure at T_r=0.7.This is indicative of lower volatility of the substance, which suggests relatively strongerintermolecular interactions in the condensed phase. Stronger interactions result from higher polarity of molecules, which is in turn originates from the asymmetry of the molecular structure. Equation 2.16 indicates that the difference between the reduced pressures at the common reduced temperature (T_r=0.7) is the measure for the acentric factor.

Based on the Pitzer-type correlations, Lee-Kesler (B.I. Lee and M. G. Kesler, *AIChE J.*, vol. 21, pp. 510-527,1975) has developed generalized correlations using a variant of the BWR-EOS (eqn. 2.17) for computing Z^0andZ^1 as function of $T_r$andP_r. The values of Z^0andZ^1 are

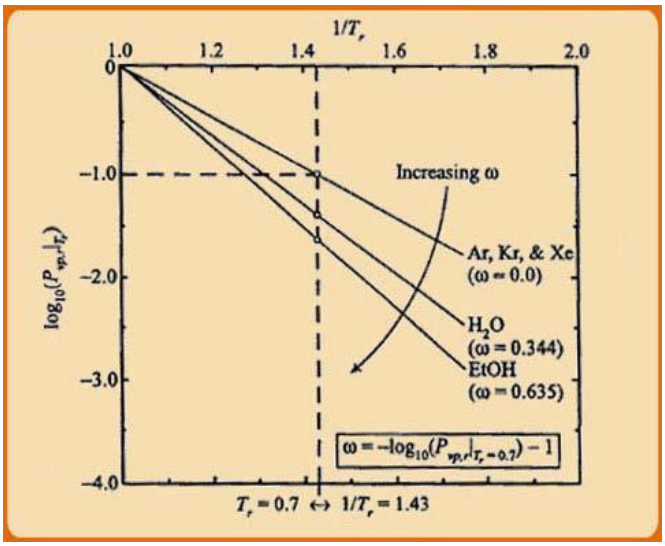
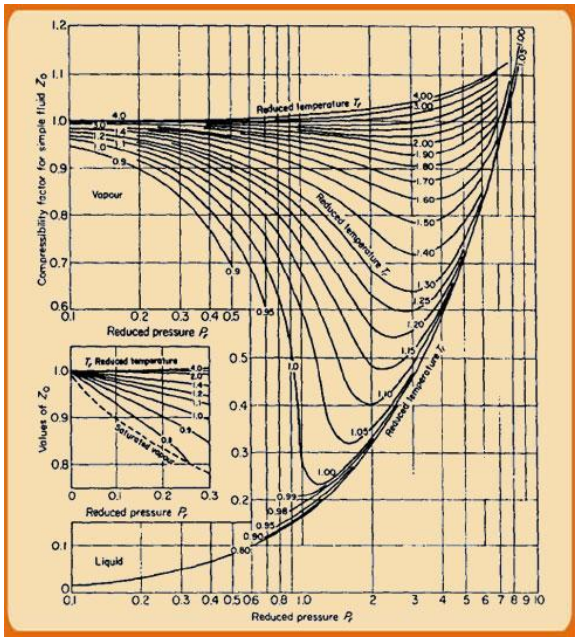
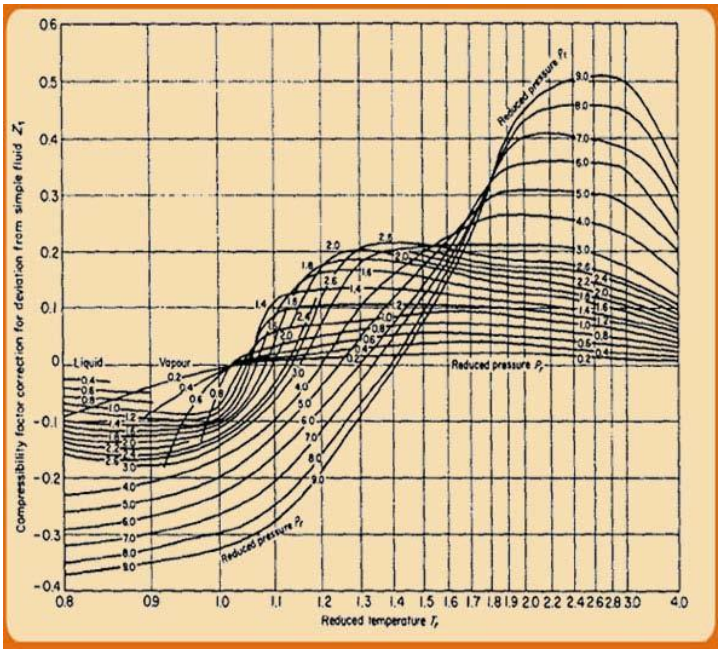


Fig. 2.9 Plot of P_r vs. T_r for select substances of varying polarity (Source: J.W. Tester and M. Modell, *Thermodynamics and its Applications*, 3rd ed., Prentice Hall, 1999).

available in the form of tables from where their values may be read off after due interpolation wherever necessary, or in the form of figures (see figs 2.10a and 2.10b). The method is presented in the detail in Appendix1.1 of this chapter.



(a)



(b)

Fig. 2.10a & 2.10b Z^0 and Z^1 contributions to generalized corresponding states correlation developed by Pitzer and coworkers (1955) [Source: *Petroleum Refiner,* April 1958, Gulf Publishing Co].

Example 2.4

A rigid 0.5-m^3 vessel at 25^oC and 2500kPa holds ethane; compute the number of moles of ethane in the vessel. For ethane: T_c = 305 K; P_c = 48.72 bar, ω = 0.1

Example 2.5

Compute the saturate liquid phase molar volume for methane at 150K. For methane T_c= 190.7 K, P_c= 46.41 bar, V_C = 98.6cm^3/mol, Z_C = 0.286, ω = 0.011.

2.4 Volumetric Properties of Mixtures

The EOSs discussed in the preceding sections may be applied to mixtures as well, through use of what are called "mixing rules". Such rules help re-define the fundamental parameters of each type of EOS in terms of those corresponding to pure species and overall composition. The principle behind these rules is that the mixture parameters are equivalent to those of a "hypothetical" pure species, which would display the same behaviour as the mixture. They are essentially semi-empirical in nature, in that they contain parameters which although grounded in molecular theory are difficult to predict fully. Nevertheless, such rules have proved reasonably reliable for prediction of mixture properties for most practical, engineering applications.

Virial EOS

For a gas mixture the virial EOS is exactly the same as for a pure species (eqn. 2.12):

$$Z = 1 + \frac{BP}{RT}$$

However, for the mixture the second virial coefficient 'B' is dependent not only on temperature but also on the mixture composition. Its exact composition dependence is derivable from the relations provided by statistical mechanics, and takes the following form:

$$B_m = \sum_i \sum_j y_i y_j B_{ij} \qquad \text{..(2.26)}$$

Where, y_i = mole fractions in a gas mixture. The indices i and j identify species, and both run over all species present in the mixture. The virial coefficient B_{ij} characterizes a bimolecular interaction between molecule i and molecule j, and therefore $B_{ij} = B_{ji}$. The summation in eqn. 2.26 accounts for all possible bimolecular interactions. For a binary mixture i = 2 and j = 2; the expansion of eqn.2.23 yields:

$$B = y_1^2 B_{11} + 2 y_1 y_2 B_{122} + y_2^2 B_{22} \quad ..(2.27)$$

Since they correspond to pure species, the calculation of the parameters B_{11} and B_{22} can be made directly employing eqns. 2.13 – 2.15. For any cross-coefficient B_{ij} (where i and j are not same) pseudo-critical" parameters need to be employed in general as shown below:

$\omega_{ij} = (\omega_i + \omega_j) / 2$

$T_{cij} = (T_{ci} T_{cj})^{1/2} (1 - k_{ij})$

$k_{ij} = 1 - 8(V_{ci} V_{cj})^{1/2} / (V_{ci}^{1/3} + V_{cj}^{1/3})^3$

Often k_{ij} is set to zero for simplicity

$P_{cij} = Z_{cij} RT_{cij} / V_{cij}$

$Z_{cij} = (Z_{ci} + Z_{cj}) / 2$

$V_{cij} = [(V_{ci}^{1/3} + V_{cj}^{1/3}) / 2]^3$

Now, $T_{rij} = T / T_{cij}$

$B_{ij}^0 = 0.083 - (0.422 / T_{rij}^{1.6})$; $\quad B_{ij}^1 = 0.139 - (0.172 / T_{rij}^{4.2})$

Finally: $B_{ij} = [(RT_{cij}) / P_{cij}][B_{ij}^0 + \omega_{ij} B_{ij}^1]$ ..(2.28)

Next B_m is computed by eqn. 2.26, which is used in eqn. 2.12 for computing the mixture molar volume.

Example 2.6

Estimate the second virial coefficient for an equimolar mixture of propane and n-pentane at 500K and 10 bar.

	T_c (K)	P_c (bar)	$V_c \times 10^3$ (m^3/mol)	Z_c	ω	y_i
Propane (1)	369.9	42.57	0.2	0.271	0.153	0.5

Pentane (2)	469.8	33.75	0.311	0.269	0.269	0.5

Cubic EOS:

The parameters in all cubic EOSs are principally*: a, b and ω.* It follows that for computing the molar volume of a mixture these parameters need to be re-defined using mixing rules. For a binary mixture they are:

For a binary mixture (*m*):

$$a_m = y_1^2 a_{11} + 2 y_1 y_2 (a_{11} a_{12})^{1/2} + y_2^2 a_{22} \quad ..(2.29)$$

$$b_m = y_1 b_1 + y_2 b_2 \quad ..(2.30)$$

$$\omega_m = y_1 \omega_1 + y_2 \omega_2 \quad ..(2.31)$$

The values of each parameters can be computed after those of the individual species are calculated using the expressions that apply to each type of cubic EOS (see table 2.1).

Generalized Correlations

Generalized correlations presented above for pure species may also be conveniently extended to prediction of volumetric properties of gas phase mixtures as well. For the mixture, the apparent critical properties are computed using the following set of linear relations:

$$T_{C,m} = \sum_i y_i T_{C,i}; \qquad P_{C,m} = \sum_i y_i P_{C,i} \qquad \omega_m = \sum_i y_i \omega_i \quad ..(2.32)$$

The subscript '*i*' runs over all the species present in the mixture '*m*'. The above properties are designated as "pseudo-critical" as they do not represent the true critical properties of a mixture; indeed the latter are most often difficult to obtain.

The *pseudo-reduced* temperature and pressure, are then determined by: $T_{r,m} = T/T_{C,m}$; $P_{r,m} = P / P_{C,m}$. As for pure components, the compressibility factor for the mixture is next obtained using standard functions of $Z^0(T_r, P_r)$ *and* $Z^1(T_r, P_r)$, which are then used in the following equation: $Z_m = Z_m^0 + \omega Z_m^1$ ..(2.33)

Example 2.7

Calculate the molar volume of an ethylene and propylene mixture comprising 70 mole percent ethylene and 30 mole percent propylene at 600 K and 60 bar. Assume that the mixture follows the Redlich-Kwong equation of state.

	T_c (K)	P_c (bar)
Ethylene (1)	283.1	51.17
Propylene (2)	365.1	46.0

2.5 Property Representation of 2-phase systems:

Consider a system consisting of liquid and vapor phases of a pure component coexisting in equilibrium. The total value of any extensive property of the two-phase system is the sum of the total properties of the phases. Let the total volume of the system be V^t; n^L = mass (or moles) of liq, n^V = mass (or moles of vapour.

Then:

$V^t = nV = n^L V^L + n^V V^V$

$n = n^L + n^V$

$x^L = n^L / n$

$x^V = n^V / n$

$x^L + x^V = 1$

$V = x^L V^L + x^V V^V = (1 - x^V) V^L + x^V V^V = V^L + x^V V^{LV}$

$V^{LV} = V^V - V^L$

Let M= Any Molar Thermodynamic Property

Thus, one may write: $M = M^L + x^V M^{LV}$ ..(2.34)

Where, $M^{LV} = M^V - M^L$ ..(2.35)

It may be noted that M is not a state variable, but an effective molar property of the system. The actual phase rule state variables are M^V and M^L. The parameter x^V – the mass fraction of vapour phase – in the system if called the *'quality'*.

Example 2.8

Find the molar volume and internal energy of a system containing water and steam at 50% quality at 200^0C.

2.6 Heat Effects of Phase Change

A change of phase is generally accompanied by heat release or absorption. The heat effect during the change of phase from liquid to gas, for example, is called the latent heat (or enthalpy of vapourization), while for a transition from soild to liquid the effect is termed heat of fusion. Such enthalpy changes typically quantify the amount of heat need to change the phase of one mole of a substance and are usually obtained experimentally. However, correlations have been developed for their prediction as well. The Reidel equation (L. Riedel, *Chem. Ing. Tech*., vol. 26, pp. 679-683, 1954) provides a suitable expression for the enthalpy of vapourization at the normal boling point (ΔH_n^{vap}) of a pure substance:

$$\frac{\Delta H_n^{vap}}{T_n} = \frac{1.092(\ln P_C - 1.013)}{(0.093 - T_{rn})} \qquad ..(2.36)$$

Where, n = normal boiling point, P_C (bar).

The Watson equation may be used (K. M. Watson, *Ind. Eng. Chem.,* vol. *35*, pp. 398-406, 1943) for computing the enthalpy of vaporization of a pure liquid at any temperature using its value at a chosen datum temperature:

$$\frac{\Delta H_{2vap}^{vap}}{\Delta H_1} = \frac{1 - T_{r2}}{1 - T_{r1}}^{0.38} \quad ; \Delta H_i^{vap} = \textit{Heat of vapourization at } T_i \qquad ..(2.37)$$

Example 9

Using Riedel's correlation, estimate the enthalpy of vaporization of water at its normal boiling point and compare the result with that given in steam tables.

Example 2.10

The enthalpy of vaporization of water at 100^{o}C is 2256.94 kJ/kg. Determine the value at 150^{o}C, and compare the value with that listed in the steam tables.

Appendix: Lee-Kesler and Pitzer Methods

Lee Kesler Method:

The method used a modified Bendict-Webb-Rubin equation using Pitzer's acentric factor (ω). The compressibility factor of a real fluid is related to the properties of a simple fluid ($\omega = 0$) and those of a *reference* fluid; for the latter *n-Octane* is used... The compressibility factor Z for the fluid is given by:

$$Z = Z^{0} + \frac{\omega}{\omega^{*}}\left(Z^{R} - Z^{0} \right) \qquad \text{(A.2.1)}$$

Where Z = compressibility factor for the fluid

Z^{0}= compressibility factor for the simple fluid

Z^{R}= compressibility factor for the reference

fluidω = acentric factor for the fluid

ω^{R}= acentric factor for the reference fluid (n-octane) = 0.3978.

According to the theory of corresponding states $\omega = 0$, for "simple fluids": i.e. whose molecules are considered spherical; examples include noble gases, such as argon, krypton and xenon. Sphericity of a molecule tends to reduce its polarity, as the centre of negative and positive

charges approach each other, leading to low dipole moments. This is also true for relatively linear but symmetric molecule. For example, carbon dioxide has virtually no dipole moment; however, it possesses a *quadrupole* moment. The acentric factor, therefore, is an indirect measure of the dipole moment of a molecule, and hence of the deviation of the intermolecular potential of real fluids from that of simple fluids.

The compressibility factors for the simple fluid (Z^0) and the reference fluid (Z^R) are generated from the following equation which is a variant for the BWR EOS (eqn. 2.17):

$$\frac{PV}{RT}^{0} = Z^{0} = 1 + \frac{B_0}{V_r} + \frac{C_0}{V_r^2} + \frac{D_0}{V_r^5} + \frac{C_4\gamma_0}{T_r^3 V_r^2}\left(\beta + \frac{\gamma_0}{V_r^2}\right)\exp\left(-\frac{\gamma_0}{V_r^2}\right)$$

$$\frac{PV}{RT}^{R} = Z^{R} = 1 + \frac{B_R}{V_r} + \frac{C_R}{V_r^2} + \frac{D_R}{V_r^5} + \frac{C_4\gamma_R}{T_r^3 V_r^2}\left(\beta + \frac{\gamma_R}{V_r^2}\right)\exp\left(-\frac{\gamma_R}{V_r^2}\right)$$

Where $B = b_1 - \frac{b_2}{T_r} - \frac{b_3}{T_r^2} - \frac{b_4}{T_r^3}$

$$C = c_1 - \frac{c_2}{T_r} + \frac{c_3}{T_r^3} \qquad ..(A.2.5)$$

$$D = d_1 + \frac{d_2}{T_r} \qquad ..(A.2.6)$$

..(A.2.7)

Where P_r *and* T_r *are the* reduced pressure and temperature, respectively, at which the volume of the real fluid is required.

Further, $V_r^0 = PV_c^0/RT_c$

Where, V^0 = molar volume of the simple fluid (at the given T & P). The Lee-Kesler constants for the simple fluid and the reference fluid to be used with Eqns. 2-6 are enumerated in Table A.1.1 below.

Table A.1.1 Lee-Kesler Constants for use with Eqns.A.2.2-A.2.6

	fluid	fluid		fluid	fluid
b_1	0.1181193	0.2026579	c_3	0.0	0.016901
b_2	0.265728	0.331511	C	0.042724	0.041577
b_3	0.154790	0.027655	$d_1 \times 10^4$	0.155488	048736
b_4	0.030323	0.203488	$d_2 \times 10^4$	0.623689	0.0740336
c_1	0.0236744	0.0313385	β	0.65392	1.226
c_2	0.0186984	0.0503618	γ	0.060167	0.03754

The method of calculating the volume at a given T_r and P_r is as follows:

- Step 1: using the critical properties of the real fluid find V_r^0 by eqn. A.2.7
- Step 2: using V_r^0 , calculate Z^0 by eqn. A.2.1
- Step 3: using the given values of T_r and P_r, Eqn.A.2.2 is solved again, but *with referencefluid constants* from above table. The solution obtained for V_r^0 is now called V_r^R
- Step 4: Z^R, the compressibility for the reference fluid, is next computed with: given by
  $Z^R = PV^R / T$ (A.2.8)
  $r\ rr$
- Step 5: With the above values of Z^0 and Z^R, the compressibility factor Z for the fluid in question can be calculated from Eqn.A.2.1.

The *Generalized* Lee-Kesler Charts essentially present the values of Z^0 and Z^1 obtained by the above procedure as function of T_r and P_r (as). A variety of other thermodynamic variables can also be computed in an identical manner. Examples include *residual* enthalpy and entropy, and gas fugacity. These properties are presented either in the form of figures or charts (see, for example, Appendix E of J.M. Smith, H.C. Van Ness and M.M. Abbott, Introduction to Chemical Engineering Thermodynamics, 6th ed., McGraw-Hill, 2001).

Pitzer Method:

The Pitzer method is nearly identical to that of Lee-Kesler; it assumes that the compressibility factor is linearly dependent on the acentric factor. Thus, eqn. A.2.1 is reformulated using the compressibility factors of *both* the simple (1) and reference fluid (2), whence:

$$Z = Z^{R1} + \frac{\omega - \omega^{R1}}{\omega^{R2} - \omega^{R1}} \left(Z^{R2} - Z^{R1} \right) \quad \text{..(A.2.9)}$$

Any two fluids may be used as the reference fluids. The method of computing the values of Z^{R2} and Z^{R1} and hence, Z for the fluid of interest follows the same procedure described abovefor Lee-Kesler.

First Law of Thermodynamics

3.1 Concept of Internal Energy

We have noted in chapter 1 that the two most common modes of energy exchanged by a thermodynamic system and its surroundings are work and heat. The interconvertibility between these two forms of energy was first demonstrated by the British scientist James P. Joule during 1840s by a series of carefully executed experiments. The experimental setup he used is shown as a schematic in fig. 3.1. Known quantities of a set of fluids (water, oil, and mercury) were placed in an

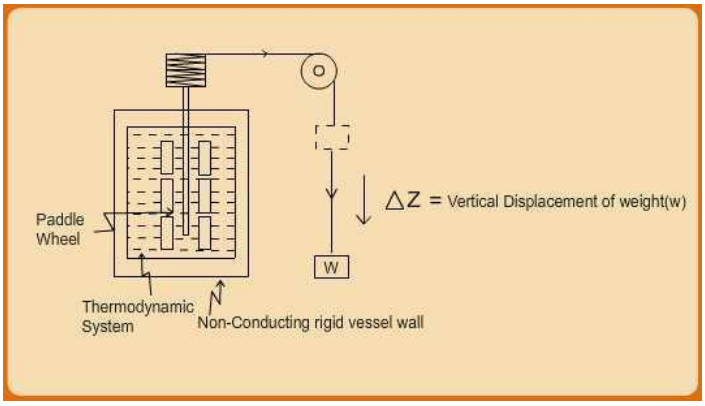


Fig. 3.1 Schematic of Joule's Experimental System

insulated, rigid vessel and stirred by means of rotating shaft provided with vanes. The amounts of work done on the fluid by the stirrer were measured in terms that needed to lower or raise a weight, and the resultant change in the temperature of the fluid was recorded. The key observation made by Joule was that for each fluid a *fixed* amount of work was required per unit mass for every degree of temperature rise caused by the rotating paddle wheel. Further, the experiments showed that the temperature of the fluid could be restored to its initial value by the transfer of heat by bringing it in contact with a cooler object. These experimental findings demonstrated for the first time that inter-convertibility exists between work and heat, and

therefore the latter was also a form of energy.

Joule's observation also provided the basis for postulation of the concept of *internalenergy* (introduced briefly in section 1.3). Since work are heat are distinctly different forms ofenergy how is it possible to convert one into another? The question can be answered if one assumes that although these two types of energies are distinct in *transit* across a thermodynamic system boundary, they must eventually be stored within a thermodynamic system in a commonform. That common form is the so-called internal energy. As we have already discussed in section 1.3, such a form of energy can only repose at the microscopic level of atoms and molecules, essentially in the form of translational, vibrational and rotational energies. To this may be added the potential energy of intermolecular interactions (as introduced in section 2.2). On a sub-molecular scale energy is associated with the electrons and nuclei of atoms, and with bond energy resulting from the forces holding atoms together as molecules. With these considerations one is in a position to rationalize the observation that while a system may receive energy in the form of work done on it, it may part with it also in the form of heat to another body or surroundings and be restored to its state prior to receipt of work. This is possible as in the interim between these two processes all energy may be stored in the form of internal energy.

As may be evident from the foregoing discussion, the addition of heat or work from an external source can lead to enhancement of the microscopic form of systemic (internal) energy. As also noted in chapter 1 the terminology "internal" is applied mainly to distinguish it from the mechanical potential and kinetic energies that a thermodynamic system may also possess by virtue of its position and velocity with respect to a datum. The latter two may then be thought of as "external" forms of energy.

It is important to note that like other intensive, macroscopic variables such as pressure, temperature, mass or volume, internal energy is a *state variable* as it is wholly dependent on the energy states that its atoms / molecules. Thus any change in the (say, specific) internal energy due to a process would only depend on the initial and final states, and not on the path followed during the change. Thus as for changes in P, V or T, one may write:

$$\int_{U}^{U_1^2} dU = \Delta U = U_2 - U_1$$

However, unlike P, V, T or mass, U is not a directly measurable property. Besides, in common with potential and kinetic energies, no absolute values of internal energy are possible. However, this is not of particular significance as in thermodynamic processes one is always interested in

changes in energies rather than their absolute values

3.2 The First Law of Thermodynamics

The empirical conclusion that heat and internal energy belong to the general category of energies, help extending the law of conservation of mechanical energy, which states that potential and kinetic energies are fully inter-convertible. As already discussed in chapter 1, a thermodynamic system may possess any other forms of energy such as surface energy, electrical energy, and magnetic energy, etc. Thus one may arrive at an extended postulate that all forms are energies are inter-convertible. This constitutes the basis of the *First Law of Thermodynamics*, which may be stated as follows:

Energy can neither be destroyed nor created, when it disappears in one form it must re-appear at the same time in other forms.

It must be said that there is no *formal* proof of the first law (or indeed of other laws of thermodynamics) is possible, but that no evidence have been found to date that violates the principle enunciated by it.

For any thermodynamic process, in general one needs to account for changes occurring both within a system as well as its surroundings. Since the two together forms the "universe" in thermodynamic terms, the application of the first law to a process leads to the following mathematical form:

$\Delta(\textit{Total energy of the universe}) = 0$

$\Delta(\textit{Total energy of the system}) + \Delta(\textit{Total energy of the surroundings}) = 0$

Where $\equiv$*finite change occurring during the thermodynamic process*

3.2 Application of the First Law to Closed Systems

In general, a thermodynamic system in its most complex form may be multi-component as well as multiphase in nature, and may contain species which react chemically with each other. Thermodynamic analysis tends to focus dominantly on the energy changes occurring within such a thermodynamic system due to change of state (or vice versa), and therefore it is often convenient to formulate the first law specifically for the system in question. Here we focus on closed systems, i.e., one that does not allow transfer of mass across its boundary. As

already pointed out work and heat may enter or leave such a system across its boundary (to and fro with respect to the surrounding) and also be stored in the common form of internal energy. Since in a system may also possess potential and kinetic energies, one may reframe the first law as follows. Using the notations U , E_K , E_P for specific internal, kinetic and potential energies, respectively:

$\Delta[U_{total} + E_{\substack{total \\ kinetic}} + E_{\substack{total \\ potential}}]_{system}$ = Total energy input to system in all forms

If the energy transfer across the system boundary takes place only the form of work and heat:

$\Delta[U_{total} + E_{\substack{total \\ kinetic}} + E_{\substack{total \\ potential}}]_{system} = Q^{total}(heat\ input) + W^{total}(work\ input)$

The above relation may be written per unit mass / mole of closed system, i.e.,:

$\Delta U + \Delta E_K + \Delta E_P = Q + W$

The above equation may also be written in a differential form:

$$dU + dE_K + dE_P = \delta Q + \delta W \qquad ..(3.1)$$

If there is no change in potential and kinetic energies for the system or it is negligible – as is usually true for most thermodynamic systems of practical interest – the above equation reduces to:

$$dU = \delta Q + \delta W \qquad ..(3.2)$$

One of the great strengths of the mathematical statement of the first law as codified by eqn. 3.2 is that it equates a state variable (U) with two path variables (Q, W). As a differentiator we use the symbol δ to indicate infinitesimal work and heat transfer (as opposed to d used state variables). The last equation potentially allows the calculation of work and heat energies required for a process, by simply computing the change in internal energy. As we shall see later (chapters. 4 & 5) changes in internal energy can be conveniently expressed as functions of changes in state properties such as T, P and V.

In the above equation the term δW represents any form of work transfer to or from the system. In many situations of practical interest the thermodynamic work for closed systems is typically the PdV work (eqn. 1.6). Hence in such cases one may reframe eqn. 3.2 as follows:

$$dU = \delta Q + \delta W = \delta Q - \int_{V_1}^{V_2} P dV \qquad ..(3.3)$$

In keeping with the definition of work above, we adopt the following convention:

W >0, if work is done on system

W <0, if work is done by systemQ

> 0, for heat addition to system

Q <0, for heat removal from system

The process of change in a thermodynamic system may occur under various types of constraints, which are enlisted below:

Q Constant pressure (*isobaric*)

R Constant volume (*isochoric*)

S Constant temperature (*isothermal*)

T Without heat transfer (*adiabatic*)

The mathematical treatment of each of these processes is presented

below. For a constant pressure process (fig. 3.2), we may write:

7. $Q = dU + PdV$

8. $Q = d + Ud(PV)$

9. $Q = d(U +$

$PV)$ *or* $\delta Q = dH$

where, $H=U+PV$

The term H is termed *enthalpy*. It follows that like U, H is also a state variable. On integrating the differential form of the equation above one obtains for the process:

$$\int_1^2 \delta Q = \int_1^2 dH$$

Or: $Q = \Delta H$ ..(3.4)

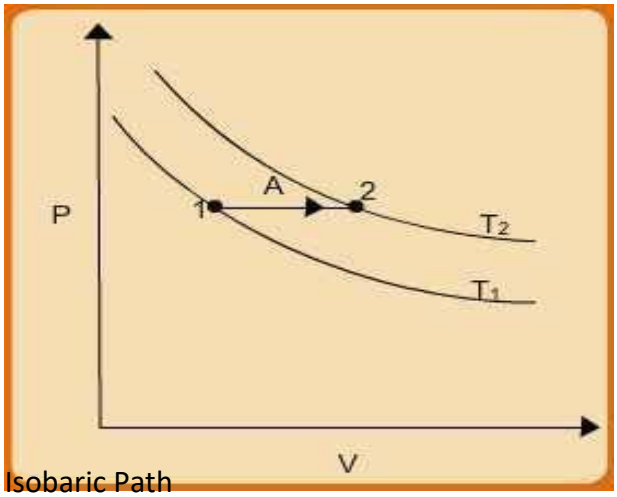


Fig. 3.2 Schematic of an Isobaric Path

On the other hand if the process occurs under isochoric (const. V) conditions (shown in fig. 3.3) the first law leads to:

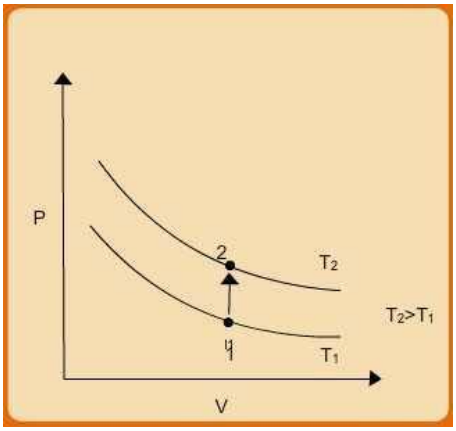


Fig. 3.3 Schematic of an Isochoric Path

$\delta Q = dU$

$\int_1^2 \delta Q = \int_1^2 dH$

$Q = \Delta U$ ..(3.5)We have already seen that a body can retain heat in the form of internal energy.

This gives rise to the concept of heat capacity C and is mathematically defined as:

$$C \equiv \frac{\delta Q}{dT}$$

It follows that using eqns. 3.4 and 3.5 two types of heat capacity may be defined:

- Constant pressure heat capacity C_P such that:

$$C_P = \frac{\delta Q}{dT} = \frac{\partial H}{\partial T_P} \quad ..(3.6)$$

- α Constant volume heat capacity C_V such that:

$$C_V = \frac{\delta Q}{dT} = \frac{\partial U}{\partial T_V} \quad ..(3.7)$$

Thus, using eqns. (3.6) and (3.7) one may rewrite eqns. (3.4) and (3.5) as follows:

$$Q = \Delta H = \int_{T_1}^{T_2} C_P \, dT \quad \text{(At const P.)} \quad ..(3.8)$$

$$Q = \Delta U = \int_{T_1}^{T_2} C_v \, dT \quad \text{(At const V.)} \qquad ..(3.9)$$

With the addition of heat to a system, the translation, vibrational and rotational (as well as subatomic) energies of the molecules are enhanced and so it may be expected that the specific heats would be dependent on temperature. On the other hand when substances are compressed intermolecular interactions begin to contribute to internal energy (as hence to enthalpy) and therefore specific heats are rendered pressure dependent. For the case of ideal gases, however, the specific heats are independent of pressure as there are no intermolecular interactions; they are only temperature dependent. Values of specific heats of ideal gases (*ig*), say at constant pressure, are available from experimental measurements and are typically expressed in the form of polynomials such as:

$$\frac{C_P^{ig}}{R} = A + BT + CT^2 + \frac{D}{T^2} \qquad ..(3.10)$$

Where, A, B, C and D are characteristic constants for a substance, and R is the universal gas constant. Values of the constants in eqn. (3.10) are readily available for a large number of pure SubstancesFig. 3.4 shows of total dependance const, pressure specific heat for select substances with temperature.

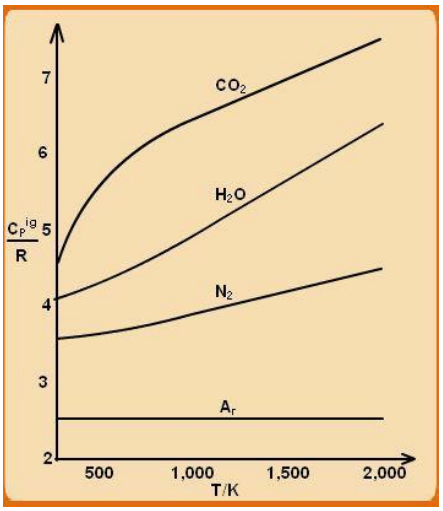


Fig. 3.4 Variation of C_P^{ig}/R vs. Temperature (Source: J. M Smith, H.C. Van Ness, M.M. Abbott, Introduction to Chemical Engineering Thermodynamics, 6th ed., McGraw-Hill, 2001)

Values of the coefficients of similar specific heat capacity polynomials for liquids and solids are available elsewhere (see for example, J.M. Smith, H.C. Van Ness and M.M. Abbott, *Introductionto Chemical Engineering Thermodynamics*, 6th ed., McGraw-Hill, 2001).

Applications to Ideal Gases

The other two types of thermodynamic processes – *isothermal and adiabatic* – in closed systems are conveniently understood by applying the first law to a system comprised of an ideal gas. For such a case the relationship between U and H may be rewritten using the EOS:

$$H = U + PV = U + RT \qquad ..(3.11)$$

Or: $H - U = RT$

(It may be noted that since the molar volume V is relatively small for both liquids and solids, one may write: $H \approx U$;hence: $C_P C_V$).

Since both H and U are only temperature dependent for ideal gases we write:

$$\frac{dH}{dT} - \frac{dU}{dT} = R$$

Now using eqns. 3.6 and 3.7 it follows that:

$$C_P - C_V = R$$

Let us now consider the relationships that obtain for an isothermal process for an ideal gas. Using eqn. 3.2, since $dU = 0$:

$$\delta Q = -\delta W$$

Or, $Q = -W$

If we consider only P-V work, the work term is calculable if the process is carried out reversibly, as the ideal gas EOS relate the P and V at all points of change, hence:

$$W = -\int_{V_1}^{V_2} P\,dV = -\int_{V_1}^{V_2} \frac{RT}{V}\,dV = RT \ln \frac{V_1}{V_2} = RT \ln \frac{P_2}{P_1} \qquad ..(3.12)$$

Thus:

$$Q = RT \ln \frac{V_2}{V_1} = RT \ln \frac{P_1}{P_2} \qquad ..(3.13)$$

Example 3.1

A gas initially at 1 MPa, 500°C is contained in a piston-cylinder arrangement of initial volume of 0.1 m^3. The gas expanded isothermally to a final pressure of 100 kPa. Determine the work.

For isobaric process: $Q = \Delta H = C_p \int dT$; $W = -\int_{V_1}^{V_2} PdV = -P(V - V)_{21} = -R(T - T)_{21}$ ..(3.14)

For *adiabatic* process $dQ = 0$

Hence : $dU = \delta W$

Or : $C_V dT = -PdV = -\frac{RT}{V} dV$

$$\frac{dT}{T} = -\frac{R}{C_v}\frac{dV}{V} \quad ..(3.15)$$

The ratio of heat capacities is defined as:

$\gamma = C_P / C_V$

Or:

$$\gamma = \frac{C_V + R}{C_V} = 1 + \frac{R}{C_V} \quad ..(3.16)$$

On integrating eqn. 3.15 and using the relationship provided by eqn. 3.16, the following set of results may be derived easily:

$$\frac{T_2}{T_1} = \left(\frac{V_2}{V_1}\right)^{\gamma - 1} \quad ..(3.17)$$

$$\frac{T_2}{T_1} = \left(\frac{P_2}{P_1}\right)^{(\gamma - 1)/\gamma} \quad ..(3.18)$$

and $P_1 V_1^{\gamma} = P_2 V_2^{\gamma} = PV^{\gamma} = const.$ ..(3.19)

Further: $dW = dU = C_V dT$

Or $W = dU = C_V dT$

Since, $W = -\int PdV$

Using eqn. 3.19 in the expression for work in the last equation:

$$W = \frac{P_2 V_2 - P_1 V_1}{\gamma - 1} \quad ..(3.20)$$

Or:

$$W = \frac{P_1 V_1}{\gamma - 1}\left[\left(\frac{P_2}{P_1}\right)^{(\gamma-1)/\gamma} - 1\right] = \frac{RT_1}{\gamma - 1}\left[\left(\frac{P_2}{P_1}\right)^{(\gamma-1)/\gamma} - 1\right] \quad ..(3.21)$$

All the above processes discussed can be captured in the form of a single *P-V* relation, which is termed a *polytropic* equation as it can be reduced to yield all forms of processes. The polytropic relations are written by generalizing eqns. 3.17 – 3.19, as follows:

$$PV^{\delta} = \text{constant} \quad ..(3.22)$$

$$TV^{\delta-1} = \text{constant} \quad ..(3.23)$$

$$TP^{(1-\delta)/\delta} = \text{constant} \quad ..(3.24)$$

The schematic of polytropic process is shown in fig. 3.5.

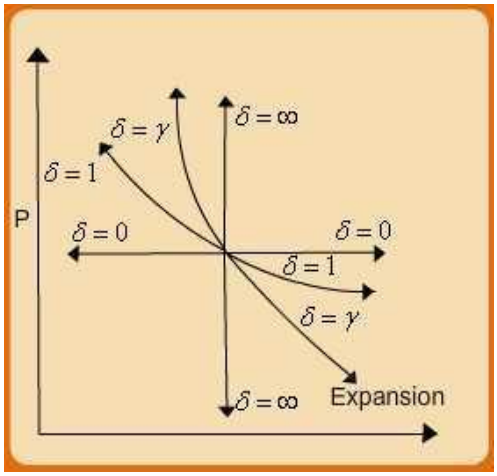


Fig. 3.5 Schematic of Polytropic Processes

As may be seen the various values of δ reproduce the isothermal, isochoric, isobaric and adiabatic processes. In line with eqn. 3.21, for such the generalized expressions for work and heat transfer may be shown to be:

$$W = \frac{RT_1}{\delta - 1}\left[\left(\frac{P_2}{P_1}\right)^{(\delta-1)/\delta} - 1\right] \qquad ..(3.25)$$

Further:

$$Q = \frac{(\delta - \gamma)RT_1}{(\delta - 1)(\gamma - 1)}\left[\left(\frac{P_2}{P_1}\right)^{(\delta-1)/\delta} - 1\right] \qquad ..(3.26)$$

Example 3.2

Helium gas expands from 125 kPa, 350 K and 0.25 m^3 to 100 kPa in a polytropic process with δ = 1.667. How much work does it give out?

3.3 Application of the First Law to Open Systems

While the last section addressed processes occurring in closed systems, the wider application of the first law involves formulating the energy balance differently in order to accommodate the fact that most thermodynamic systems, i.e., equipments, in continuous process plants are essentially open systems: they allow mass transfer across their boundaries (i.e., through inlet and outlet). Examples include pumps, compressors, reactors, distillation columns, heat exchangers etc. Since such open systems admit both material and energy transfer across their boundaries the thermodynamic analysis necessarily involves both mass and energy balances to be carried out together. Also such systems may in general operate under both steady (during normal plant operation) and unsteady states (say during startup and shutdown). As we will see the former state is a limiting case of the more general situation of unsteady state behavior.

Mass Balance for Open Systems:

For generality consider an open system with which has multiple inlets (1, 2) and outlets (3, 4). The volume enclosed by the physical boundary is the *control volume* (CV). The general mass balance equation for such a system may be written as:

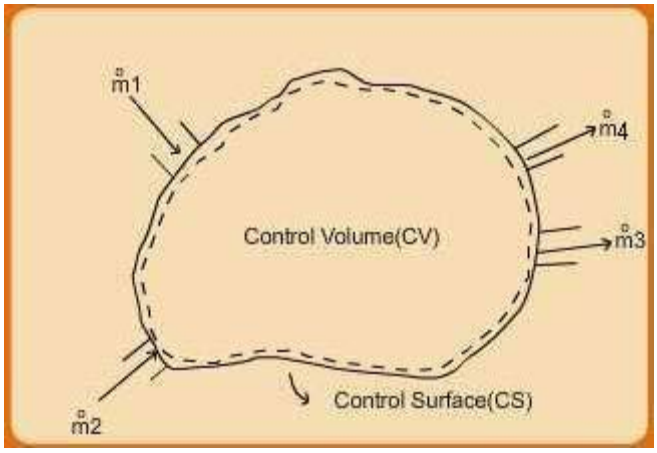


Fig. 3.6 Schematic of an open system

$$\frac{dm_{cv}}{dt} = \sum_{inlet} \dot{m} - \sum_{outlet} \dot{m} \qquad ..(3.27)$$

The mass flow rate $\dot{m}$ is given by:

$$\dot{m} = \rho u A \qquad ..(3.28)$$

Where, ρ=fluid density; u=fluid velocity; and A =aperture cross-sectional area

The first term on the left side of the eqn. (3.27) denotes sum of all flow rates over all inlets, while the second term corresponds to the summation over all outlet flow rates. For the system shown in fig. 3.5 the eqn. 3.27 may be written as:

$$\frac{dm_{cv}}{dt} = \dot{m}_1 + \dot{m}_2 - \dot{m}_3 - \dot{m}_4 \qquad ..(3.28)$$

The last equation may be reframed in a general way as follows:

$$\frac{dm_{cv}}{dt} + \Delta (\dot{m})_{fs} = 0 \qquad ..(3.29)$$

Where the symbol $\Delta \equiv$oulet-inlet; and the subscript fs stands for "flow streams".

Equation 3.29 may be recast as:

$$\frac{dm_{cv}}{dt} + \Delta (\rho u A)_{fs} = 0 \qquad ..(3.30)$$

The above equation simplifies under steady flow conditions as the accumulation term for the control volume, i.e., $\frac{dm}{dt}^{cv}=0$

In such a case for the simplest case of a system with one inlet and outlet (say: 1 and 2 respectively), which typically represents the majority of process plant equipments, the mass balance equation reduces to:

$$\dot{m} = \text{constant} \qquad \text{..(3.31)}$$

Or: $\rho_1 u_1 A_1 = \rho_2 u_2 A_2 \rightarrow u_1 A_1 / V_1 = u_2 A_2 / V_2$

Where V = specific volume

Energy Balance for Open Systems:

Consider the schematic of an open system as shown in fig. 3.7. For simplicity we assume one

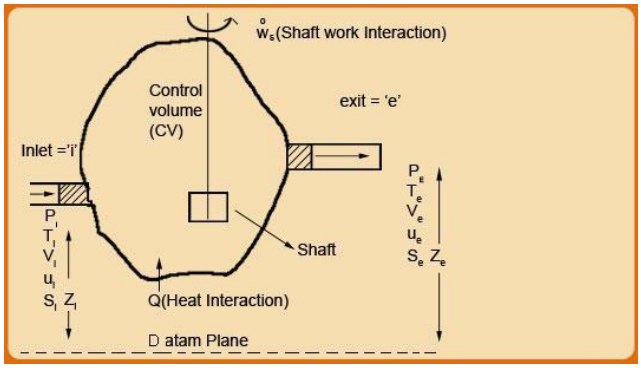


Fig. 3.7 Schematic of an open system showing flow and energy interactions

inlet and one exit ports to the control volume. The thermodynamic states at the inlet *i* and exit *e* are defined by the *P*, *V*, *T*, *u* (average fluid velocity across the cross section of the port), and *Z*, the height of the port above a datum plane. A fluid element (consider an unit mole or mass) enters the CV carrying internal energy, kinetic and potential energies at the inlet conditions (P_i, T_i, with molar volume as V_i) and leaves values of these energies at the exit state conditions (P_e, T_e, with molar volume as V_e). Thus the total specific energy of the fluid at the two ports corresponds to the sum of specific internal, potential and kinetic energies, given by: $U + \frac{u^2}{2} + gZ$. In addition, the CV exchanges heat with the surroundings at the rate $\dot{Q}$, and say a total work (in one or more forms) at the rate of $\dot{W}_{total}$

In the schematic we, however, have shown a specific work form, *shaft work*, that is delivered *to* or *by* the system by means of rotatory motion of a paddle wheel which, as we will see later in the section, is implicated in many typical process plant units. As with material balance one may write a total energy balance equation for the control volume as follows:

$$\frac{d(mU)_{cv}}{dt} = U + \frac{1}{2}(u^2) + zg)\dot{m}_i - U + \frac{1}{2}(u^2) + zg)\dot{m}_e + \dot{Q} + \dot{W}_{total}$$

Or:

$$\frac{d(mU)_{cv}}{dt} + \Delta U + \frac{1}{2}(u^2) + zg)\dot{m}_{fs} = \dot{Q} + \dot{W}_{total} \quad ..(3.32)$$

The general total work term should include all forms of work. We draw the reader's attention to the fact that the total work interaction also should include that needed to push fluid into the CV as well as that implicated in pushing it out of CV. The fluid state at the inlet or exit is characterized by a set of state properties, U, V, H, etc. Consider a unit mass (or mole) of fluid entering the CV. This fluid element obviously needs to be "pushed" by another that follows it so as to make the formed enter the CV. In essence a fluid element of (specific) volume V is pushed into the CV at a pressure P. This is akin to a P-V form of work (as in the case of a piston-in-a-cylinder system) that is done on the CV and so may be quantified as $-P_iV_i$. The same considerations apply at the exit in which case in pushing out a similar fluid element at exit conditions, i.e., $-P_eV_e$. Thus, eqn. 3.32 may be rewritten as follows:

$$\frac{d(mU)_{cv}}{dt} + \Delta U + \frac{1}{2}(u^2) + zg)\dot{m}_{fs} = \dot{Q} + \dot{W} - [PV\dot{m}]_i + [PV\dot{m}]_e \quad ..(3.33)$$

Where, $\dot{W}_{total} = \dot{W} - [PV\dot{m}]_i + [PV\dot{m}]_e$

The term $\dot{W}$ represents sum of *all other* forms of work associated with the process occurring within the CV. This residual work term may include the shaft work, P-V work resulting from expansion or contraction of the CV, electrical work, and so on. As the last two work terms on the left side of the eqn. 3.33 are associated with the flow streams we may rewrite the equation as follows:

$$\frac{d(mU)_{cv}}{dt} + \Delta\left[U + \frac{1}{2}(u^2) + zg\right]\dot{m}_{fs} = \dot{Q} + \dot{W} - \Delta[PVm]_{fs} \quad ..(3.34)$$

On rearranging:

$$\frac{d(mU)_{cv}}{dt} + \Delta\left(U + PV\right) + \frac{u^2}{2} + zg\ \dot{m}_{fs} = \dot{Q} + \dot{W} \quad ..(3.35)$$

Or: $$\frac{d(mU)_{cv}}{dt} + \Delta H + \frac{u^2}{2} + zg\ \dot{m}_{fs} = \dot{Q} + \dot{W} \quad ..(3.36)$$

It may be noted that eqn. 3.34 assumes that the CV is fixed in space and therefore no overall potential of kinetic energy terms depicting these mechanical energies for the control volume is included. This, of course, is valid for all process plant applications. In addition, For many cases of practical importance (though not all) in a chemical plant the kinetic and potential energy changes between the inlet and exit streams may not be significant, whence the last equation may be simplified as: an equipment may be neglected; hence:

$$\frac{d(mU)}{dt}_{cv} + \Delta H\dot{m}_{fs} = \dot{Q} + \dot{W} \quad ..(3.37)$$

Further, for the special case where the only shaft work is involved, the above equation may be simplified to:

$$\frac{d(mU)}{dt}_{cv} + \Delta H\dot{m}_{fs} = \dot{Q} + \dot{W}_s \quad ..(3.38)$$

For *steady state* applications the eqn. 3.34 reduces to:

$$\Delta H + \frac{u^2}{2} + zg\ \dot{m}_{fs} = \dot{Q} + \dot{W} \quad ..(3.39)$$

Further, if the kinetic and potential energy changes associated with the flow streams are insignificant, it follows that:

$$\Delta H\dot{m}_{fs} = \dot{Q} + \dot{W} \quad ..(3.40)$$

Since under steady state $\dot{m}$ is constant we may write:

$\Delta H = Q + W$ ..(3.41)

Where Q and W denote the work and heat interactions per unit mass of mole of fluid flowing through the system flowing through system. Once again if W_s is the only form of work interaction between the system and the surrounding then:

$\Delta H = Q + W_s$ ..(3.42)

Examples of process plant units to which eqn. 3.42 applies are: *pumps, compressors, turbines,fans, blowers*, etc. In all cases a rotatory part is used exchange work between the system andsurrounding.

Example 3.3

A chiller cools liquid water (Sp. Ht = 4.2 J/gmK) for air-conditioning purposes. Assume 2.5 kg/s water at 20^oC and 100 kPa is cooled to 5^oC in a chiller. How much heat transfer (kW) is needed?

Example 3.4

A piston-cylinder assembly contains 0.1 kg wet steam of quality 0.75 at 100 kPa. If 150 kJ of energy is added as heat while the pressure of the steam is held constant determine the final state of steam.

Example 3.5

An adiabatic compressor operating under steady-state conditions receives air (ideal gas) at 0.1 MPa and 300 K and discharges at 1 MPa. If the flow rate of air through the compressor is 2 mol/s, determine the power consumption of the compressor. Constant pressure specific heat for air = 1kJ/kg.

Example 3.6

An insulated piston-cylinder system has air at 400kPa & 600K. Through an inlet pipe to the cylinder air at certain temperature T(K) and pressure P (kPa) is supplied reversibly into the

cylinder till the volume of the air in the cylinder is 4 times the initial volume. The expansion occurs isobarically at 400kPa. At the end of the process the air temperature inside the cylinder is 450K. Assume ideal gas behaviour compute the temperature of the air supplied through the inlet pipe.

3.4 Measurement of Enthalpy and Internal Energy using Flow Calorimeter

The use of the first law for open or closed systems necessitates the experimental determination (or, estimation from thermodynamic relations) of internal energy and enthalpy, both being state properties. The flow calorimeter (fig. 3.8), readily allows measurement of enthalpy, which in turn can be used to compute the internal energy at the same conditions of (say) temperature and pressure. The fluid whose properties are to be measured is pumped through a constant temperature bath so that it attains a desired temperature (say T_1) prior to entry into the vessel provided with an electric heater. The corresponding pressure (P_1) may also be recorded. Heat is next provided to the passing fluid at a pre-determined and fixed rate over a period of time until the temperature and pressure at the exit of the vessel attains steady values (say, T_2, & P_2). At such a condition the calorimeter is under steady state, hence eqn. 3.41 may be applied to the heating vessel. Since there is no work transfer of any kind, the resultant energy balance yields:

$$\Delta H = Q \qquad ..(3.40)$$

Or: $H_2 - H_1 = Q$

So: $H_2 = H_1 + Q$ ..(3.41)

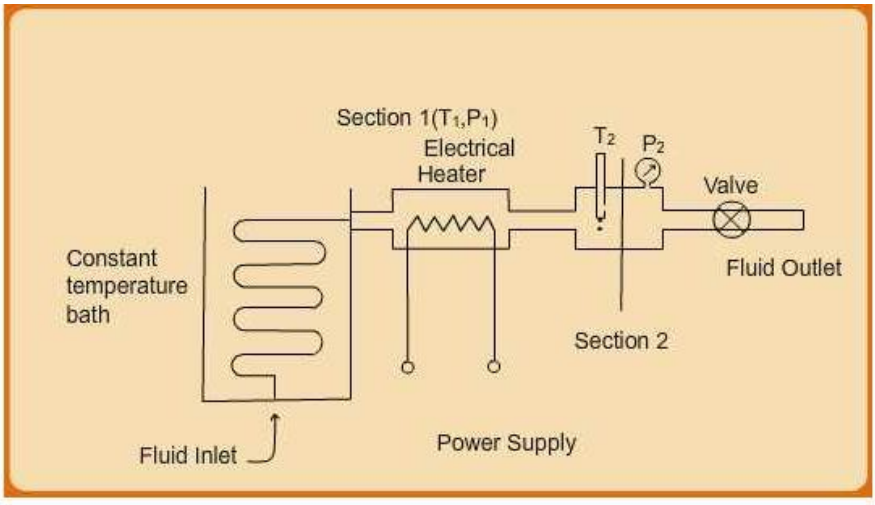


Fig. 3.8 A schematic of a flow calorimeter

Clearly, if we chose the enthalpy H_1 to be a datum state and arbitrarily assign it a zero value, then:

$H_2 = Q$

..(3.42)

The last equation then allows one to uniquely determine the value of H_2 at any condition achieved at state 2 by applying a known quantity of heat through the electric heating system. The internal energy at the same state can next be determined using the relation:

$U = H - PV$ ..(3.42)

Further we can measure the density (in terms of mass or mole) at the same state, and one may rewrite the last equation as: $U = H - P/\rho$ ..(3.43)

Thus using the above relations, U and H may be obtained experimentally at any P, T (and/or V) for any substance. The steam tables discussed at the concluding portion of section 2.1 constitutes such a tabulation of values of standard thermodynamic properties (saturation vapour pressure, internal energy, enthalpy (and entropy) of water obtained empirically over a wide range temperature and pressure.

Thermodynamic Properties of Real Fluids

It has already been demonstrated through the first and second laws, that the work and heat interactions between a system and its surroundings may be related to the state variables such as internal energy, enthalpy and entropy. So far we have illustrated the calculations of energy and entropy primarily for pure (component) ideal gas systems. However, in practice this is an *exception* rather than a rule as one has to deal with not only gases removed from ideal gas state,but also with liquids and solids. In addition, mixtures rather than pure components are far more common in chemical process plants. Therefore, computation of work and heat interactions for system comprised of *real* fluids requires more complex thermodynamic formulations. This chapter is devoted to development of such relations that can help calculate energy requirements for given changes of state for real systems. As in the case of ideal gases the goal is to correlate the energy and entropy changes for real fluids in terms of their volumetric and other easily

measurable macroscopic properties.

5.1 Thermodynamic Property Relations for Single Phase Systems

Apart from internal energy and enthalpy, two other ones that are particularly useful in depiction of thermodynamic equilibrium are Helmholtz free energy (A) and Gibbs free energy (G). We defer expanding upon the concept of these two types of energies to chapter 6; however, we state their definition at this point as they are instrumental in the development of property correlations for real fluids.

- Specific Helmholtz free energy: $A=U-TS$ ..(5.1)
- Specific Gibbs free energy: $G=H-TS$ ..(5.2)

For a reversible process in a closed system the first law gives:

$$dU = dQ + dW$$

Or:

$$dU = TdS - PdV \quad ..(5.3)$$

Using $H=U+PV$ and taking a total differential of both sides:

$$dH = dU + PdV + VdP \quad ..(5.4)$$

Putting eqn. 5.3 in 5.4 we get:

$$dH = TdS + VdP \quad ..(5.5)$$

In the same manner as above one may easily show that the following two relations obtain:

$$dA = -SdT - PdV \quad ..(5.6)$$

$$dG = VdP - SdT \quad ..(5.7)$$

Equations 5.3 to 5.7 comprise the fundamental energy relations for thermodynamic systems where there is a single phase with constant composition. In principle, they may be integrated to compute the energy changes for a system transiting from one equilibrium state to another.

5.2 Maxwell Relations

All the four types of energy relations above satisfy the mathematical condition of being continuous variables, as they are themselves functions of state variables. One can thus apply of the criterion of exact differential for these functions.

For a function of the form $P=P(X, Y)$ one can write the following total differential:

$$dP = \left(\frac{\partial P}{\partial X}\right)_Y dX + \left(\frac{\partial P}{\partial Y}\right)_X dY = MdX + NdY \quad ..(5.8)$$

Where: $M = \left(\frac{\partial P}{\partial X}\right)_Y$ and $N = \left(\frac{\partial P}{\partial Y}\right)_X$ ..(5.9)

Further, $\left(\frac{\partial M}{\partial Y}\right)_X = \frac{\partial^2 P}{\partial Y \partial X}$ and $\left(\frac{\partial N}{\partial X}\right)_Y = \frac{\partial^2 P}{\partial X \partial Y}$ ..(5.10)

It follows: $\left(\frac{\partial M}{\partial Y}\right)_X = \left(\frac{\partial N}{\partial X}\right)_Y$ ..(5.11)

Applying eqn. 5.11 to 5.3, 5.5, 5.6 and 5.7 one may derive the following relationships termed Maxwell relations:

$$\left(\frac{\partial T}{\partial V}\right)_S = -\left(\frac{\partial P}{\partial S}\right)_V \quad ..(5.12)$$

$$\left(\frac{\partial T}{\partial P}\right)_S = \left(\frac{\partial V}{\partial S}\right)_P \quad ..(5.13)$$

$$\left(\frac{\partial P}{\partial T_v}\right) = \left(\frac{\partial S}{\partial V_T}\right) \quad ..(5.14)$$

$$\left(\frac{\partial V}{\partial T_P}\right) = -\left(\frac{\partial S}{\partial P}\right)_T \quad ..(5.15)$$

5.4 Relations for Enthalpy, Entropy and Internal Energy

One may conveniently employ the general energy relations and Maxwell equations to obtain expressions for change in enthalpy and entropy and internal energy for any process, which in turn may be used for computing the associated heat and work interactions.

Let $H=H\,(T\,,P)$

Then: $dH=\left(\frac{\partial H}{\partial T}\right)_P dT + \left(\frac{\partial H}{\partial P}\right)_T dP$

But $\left(\frac{\partial H}{\partial T_P}\right) = C_P$

Thus: $dH=C_P dT+\left(\frac{\partial H}{\partial P}\right)_T dP$ ..(5.16)

Using $dH=TdS+VdP \Rightarrow \left(\frac{\partial H}{\partial P}\right)_T = T\left(\frac{\partial S}{\partial P}\right)_T + V$ ..(5.17)

From Maxwell relations as in eqn. 5.15: $\left(\frac{\partial S}{\partial P}\right)_T = -\left(\frac{\partial V}{\partial T}\right)_P$ ..(5.18)

Thus using eqns. 5.17 and 5.18 in 5.16 we get:

$$dH = C_p\,dT + \left[V - T\left(\frac{\partial V}{\partial T}\right)_P\right] dP \quad ..(5.20)$$

In the same manner starting from the general function: $U=U\,(T\,,V\,)$ an d$S=S\,(T\,,P)$ and applying appropriate Maxwell relations one may derive the following general expressions for differential changes in internal energy and entropy.

$$dU = C_v\,dT + \left[T\left(\frac{\partial P}{\partial T}\right)_V - P\right] dV \quad ..(5.21)$$

$$dS = C_P \frac{dT}{T} - \frac{\partial V}{\partial T_P} dP \quad \text{..(5.22)}$$

Or, alternately: $$dS = C_V \frac{dT}{T} + \frac{\partial P}{\partial T_V} dV \quad \text{..(5.23)}$$

Thus, eqns. 5.20 to 5.23 provide convenient general relations for computing enthalpy, internal energy and entropy changes as function of volumetric properties and specific heats. If a fluid is described by a suitable EOS, these equations may be conveniently integrated to obtain analytical expressions for energy and entropy changes.

Example 5.1

Derive an expression for enthalpy change of a gas during an isothermal process assuming using the following EOS: $P\,(V-b\,) = RT$

5.5 Residual Property Relations

An alternate method of computing energy and entropy changes for real gases involves the definition of *residual property*. The specific residual property M^R is defined as follows:

$$M^R = M\,(T\,,P\,) - M^{ig}(T\,,P) \quad \text{..(5.24)}$$

Where, $M\,(T\,,P)$ is the specific property of a real gas at a given T & P, and $M^{ig}(T\,,P)$ is the value of the same property if the gas were to behave ideally at the same T & P. Thus for example:

$V^R = V - V^{ig}$

Using the generalized compressibility factor 'Z':

$V^R = (\,ZRT/P\,) - (\,RT/P)$

$$V^R = (\,Z-1)\,RT/P \quad \text{..(5.25)}$$

The residual properties are usually used for gases *only*. Using such a property for a liquid (or solid) is inconvenient as then it would also include the *property change* of vapourization (and solidification) which generally are large in magnitude. This detracts from the advantage of working with the residual property as a measure of small corrections to ideal gas behaviour. Thus the use of residual functions is restricted to prediction of real gas behaviour only. To exploit the

concept of residual properties we take advantage of the Gibbs free energy as it can be used as a generating function for other thermodynamic properties.

Derivation of Residual functions:

We start from the generic equation: $G=H-TS$ ..(5.2)

And $dG=VdP-SdT$ ..(5.7)

Taking the total differential for the function G / RT :

$$d\frac{G}{RT} = \frac{1}{RT}dG - \frac{G}{RT^2}dT \quad ..(5.26)$$

Substituting eqn. 5.2 and 5.7 in 5.26 we get:

$$d\frac{G}{RT} = \frac{V}{RT}dP - \frac{H}{RT^2}dT \quad ..(5.27)$$

One may write the same equation specifically for an ideal gas, whence:

$$d\frac{G^{ig}}{RT} = \frac{V_{ig}}{RT}dP - \frac{H_{ig}}{RT^2}dT \quad ..(5.28)$$

Subtracting eqn. 5.28 from 5.27:

$$d\frac{G^R}{RT} = \frac{V^R}{RT}dP - \frac{H^R}{RT^2}dT \quad ..(5.29)$$

Thus we may write the following further *generative* relations:

$$\frac{V^R}{RT} = \frac{\partial(G^R/RT)}{\partial P_T} \quad ..(5.30)$$

$$\frac{H^R}{RT} = -T\frac{\partial(G^R/RT)}{\partial T_P} \quad ..(5.31)$$

And further: $\frac{S^R}{RT} = \frac{H^R}{RT} - \frac{G^R}{RT}$ ..(5.32)

From eqn. 5.29: $\int_0^P d\frac{G^R}{RT} = \left.\frac{G^R}{RT}\right|_P - \left.\frac{G^R}{RT}\right|_{P=0}$; but $\left.\frac{G^R}{RT}\right|_{P=0} = 0$

Thus: $\frac{G_R}{RT} = \int_0^P \frac{V}{RT} - \frac{RT}{P} dP$ ..(5.33)

Putting eqn. 5.25 in 5.33: $\frac{G_R}{RT} = \int_0^P (Z-1) \frac{dP}{P}$ ..(5.34)

Differentiating eqn. 5.34 w.r.t T in accordance with 5.31 gives:

$$\frac{H^R}{RT} = -T \int_0^P \frac{\partial Z}{\partial T}_P \frac{dP}{P}$$ ..(5.35)

Finally using eqns 5.32, 5.34, and 5.35:

$$\frac{S^R}{R} = -T \int_0^P \frac{\partial Z}{\partial T}_P \frac{dP}{P} - \int_0^P (Z-1) \frac{dP}{P}$$ ..(5.36)

The last two equations may be expressed in alternative forms in terms of reduced temperature and pressure:

$$\frac{H^R}{RT_C} = -T_r^2 \int_0^{P_r} \frac{\partial Z}{\partial T_r}_{P_r} \frac{dP_r}{P_r}$$ ..(5.37)

$$\frac{S^R}{R} = -T_r \int_0^{P_r} \frac{\partial Z}{\partial T_r}_{P_r} \frac{dP_r}{P_r} - \int_0^{P_r} (Z-1) \frac{dP_r}{P_r}$$ ..(5.38)

Example 5.2

Derive an expression for enthalpy change of a gas during an isothermal process assuming using the following EOS: $Z = 1 + AP_r / T_r$

5.6 Residual Property Calculation from EOS

From Virial EOS:

Using 5.35 and 2.12 one obtains:

$$\frac{H^R}{RT} = \frac{P}{RT} B - \frac{dB}{dT}$$ ..(5.39)

Next, on substituting eqns. 2.13 – 2.15 in 5.37 the following relations result:

$$\frac{H^R}{RT_C} = P_r 0.083 - \frac{1.097}{T_r^{1.6}} + \omega\left(0.139 - \frac{0.894}{T_r^{4.2}}\right) \quad ..(5.40)$$

Similarly using 5.36 and 2.12 we get:

$$\frac{S^R}{R} = -P_r \frac{dB}{dT} \quad ..(5.40)$$

Finally employing eqns. 2.13 – 2.15 in 5.38:

$$\frac{S^R}{R} = -P_r\left(0.675 + \omega 0.722\right) \quad ..(5.41)$$

From Cubic EOS:

One may use the following form of the cubic EOS presented in chapter 2:

$$P = \frac{RT}{V-b} - \frac{a}{V^2 + ubV + wb^2} \quad ..(2.21)$$

Or equivalently: $Z^3 + \alpha Z^2 + \beta Z + \gamma = 0$ ..(2.23)

While the eqns. 5.35 and 5.36 are useful for volume explicit EOS, they are unsuitable for cubic EOS which are pressure explicit. For the latter type of EOS one may show that the appropriate equations for residual enthalpy and entropy are (see S.I. Sandler, *Chemical, Biochemical andEngineering Thermodynamics*, ch. 6, 4th Edition, Wiley India, 2006):

$$\frac{H^R}{RT} = Z - 1 + \frac{1}{RT}\int_{V=\infty}^{V} \left[T\left(\frac{\partial P}{\partial T}\right)_V - P\right] dV \quad ..(5.42)$$

$$\frac{S^R}{R} = \ln Z + \frac{1}{R}\int_{V=\infty}^{V} \left[\left(\frac{\partial P}{\partial T}\right)_V - \frac{R}{V}\right] dV \quad ..(5.43)$$

The above relations may be applied to the various cubic EOSs to obtain the necessary residual property relations. The final results are shown below.

From RK-EOS:

$$\frac{H^R}{RT} = (Z-1) - \frac{3a}{2bRT}\ln\frac{Z+B}{Z}$$

$$\frac{S^R}{R} = \ln(Z-B) - \frac{a}{2bRT}\ln\frac{Z+B}{Z}$$

$$\text{where, } a = \frac{0.42748R^2T_C^2\alpha_{RK}(T_r)}{P_C};\ b = \frac{0.08664RT_C}{P_C}$$

$$\alpha_{RK}(T_r) = T_r^{-1/2}$$

For SRK-EOS:

$$\frac{H^R}{RT} = (Z-1) + \frac{1}{RT}\left[T\frac{\partial a}{\partial T} - a\right]\ln\frac{Z+B}{Z}$$

$$\frac{S^R}{R} = \ln(Z-B) - \frac{1}{bRT}\frac{\partial a}{\partial T}\ln\frac{Z+B}{Z}$$

$$\frac{\partial a}{\partial T} = -\frac{a}{\alpha_{SRK}(T_r)TT_C}(0.48 + 1.574\omega - 0.176\omega^2)$$

$$\text{where, } a = \frac{0.42748R^2T_C^2\alpha_{SRK}(T_r)}{P_C};\ b = \frac{0.08664RT_C}{P_C}$$

$$\text{and, } \alpha_{SRK}(T_r) = [1 + (0.48 + 1.574\omega - 0.176\omega^2)(1 - T_r^{1/2})]^2$$

For PR-EOS:

$$\frac{H^R}{RT} = (Z-1) + \frac{T\frac{\partial a}{\partial T} - a}{2\sqrt{2}bRT}\ln\frac{Z+B(1+\sqrt{2})}{Z+B(1-\sqrt{2})}$$

$$\frac{S^R}{R} = \ln(Z-B) - \frac{1}{2\sqrt{2}bRT}\frac{\partial a}{\partial T}\ln\frac{Z+B(1+\sqrt{2})}{Z+B(1-\sqrt{2})}$$

$$\frac{\partial a}{\partial T} = -\frac{a}{\alpha_{PR}(T_r)TT_C}(0.37464 + 1.5422\omega - 0.62992\omega^2)$$

$$where, a = \frac{0.45724 R_2 T_C^2 \alpha_{PR}(T_r)}{P_C}; b = \frac{0.07779 R T_C}{P_C}$$

$$and\ , \alpha_{PR}(T_r) = [1 + (0.37464 + 1.5422\omega - 0.62992\omega^2)(1 - T_r^{1/2})]^2$$

As part of computing the residual enthalpy or entropy (and internal energy) at any set of temperature and pressure, the molar volume or correspondingly Z^{vap} needs to be *first* computed employing the usual algorithm for solving cubic EOS described in section 2.3.3.

Example 5.3

Derive expressions for H^R, S^R from RK-EOS.

5.7 Generalized Correlations for computing *dH and dS* for a real gas:

The approach based on the use of the compressibility factors can be applied to the present instance to evolve generalized correlations for computing enthalpy and entropy changes for gases. We start with the Pitzer-type expression for the compressibility factor:

$$Z = Z^0 + \omega Z^1 \qquad ..(2.25)$$

Differentiating with respect to the reduced temperature we get:

$$\left(\frac{\partial Z}{\partial T_r}\right)_{P_r} = \left(\frac{\partial Z^0}{\partial T_r}\right)_{P_r} + \omega \left(\frac{\partial Z^1}{\partial T_r}\right)_{P_r} \qquad ..(5.55)$$

Thus using eqn. 5.54 we may recast eqns. 5.37 and 5.38 as follows:

$$\frac{H^R}{RT_c} = -T_r^2 \int_0^{P_r} \left(\frac{\partial Z^0}{\partial T_r}\right)_{P_r} \frac{dP_r}{P_r} - \omega T_r^2 \int_0^{P_r} \left(\frac{\partial Z^1}{\partial T_r}\right)_{P_r} \frac{dP_r}{P_r} \qquad ..(5.56)$$

And: $$\frac{S^R}{R} = -\int_0^{P_r} T_r \left(\frac{\partial Z^0}{\partial T_r}\right)_{P_r} + Z^0 - 1 \frac{dP_r}{P_r} - \omega \int_0^{P_r} \left(\frac{\partial Z^1}{\partial T_r}\right)_{P_r} + Z^1 \frac{dP_r}{P_r} \qquad ..(5.57)$$

Both the above equations may be rewritten individually as follows:

$$\frac{H^R}{RT}_c = \frac{(H^R)^0}{RT}_c + \omega \frac{(H^R)^1}{RT}_c \quad ..(5.58)$$

Where, $$\frac{(H^R)^0}{RT}_c = -T_r^2 \int_0^{P_r} \left(\frac{\partial Z}{\partial T}\right)_r^0 \quad ..(5.59a)$$

And:

$$\frac{(H^R)^1}{RT}_c = -T_r^2 \int_0^{P_r} \left(\frac{\partial Z^1}{\partial T}\right)_r \frac{dP}{P_r} \quad ..(5.59b)$$

Similarly:

$$\frac{S^R}{R} = \frac{(S^R)^0}{R} + \omega \frac{(S^R)^1}{R} \quad ..(5.60)$$

$$\left(\frac{S^R}{R}\right)^0 = -\int_0^{P_r} \left(\frac{\partial Z}{\partial T}\right)_r^0 + Z^0 \frac{dP_r}{P_r} \quad ..(5.61a)$$

$$\left(\frac{S^R}{R}\right)^1 = -\int_0^{P_r} \left(\frac{\partial Z^1}{\partial T}\right)_r + Z^1 \frac{dP_r}{P_r} \quad ..(5.61b)$$

The term $\frac{(H^R)^0}{RT}_c$ in eqn. 5.59a constitutes the *first order* enthalpy departure, and $\frac{(H^R)^1}{RT}_c$ (in eqn. 5.59b) the *second order* (with respect to simple fluids) enthalpy departure at specified T_r and P_r. The same is true for the corresponding entropic terms provided by eqns. 5.61a and 5.61b.

The evaluation of the integrals in eqns. 5.59 to 5.62 may be carried out assuming an EOS. The most widely used approach is that of Lee and Kesler who employed a modified form of the BWR EOS (eqn. 2.17) to extend their generalized correlation to residual property estimation. Figs. 5.1 and 5.2 respectively provide values of $(H^R)^0/RT_C$, an $(Hd^R)^1/RT_C$ respectively. Similar plots for the entropy terms $(S^R)^0/R$, an $(Sd^R)^1/R$ are also available; however, here the plot of the entire entropy term $(-S^R)$ as function of T_r and P_r is shown in fig. 5.3. Table of

values of all the above (eqns. 5.59 to 5.62) are also available as functions of T_r and P_r at discrete intervals (see for example: J.M. Smith, H.C. Van Ness and M.M. Abbott, *Introduction toChemical Engineering Thermodynamics*, 6th ed., McGraw-Hill, 2001.

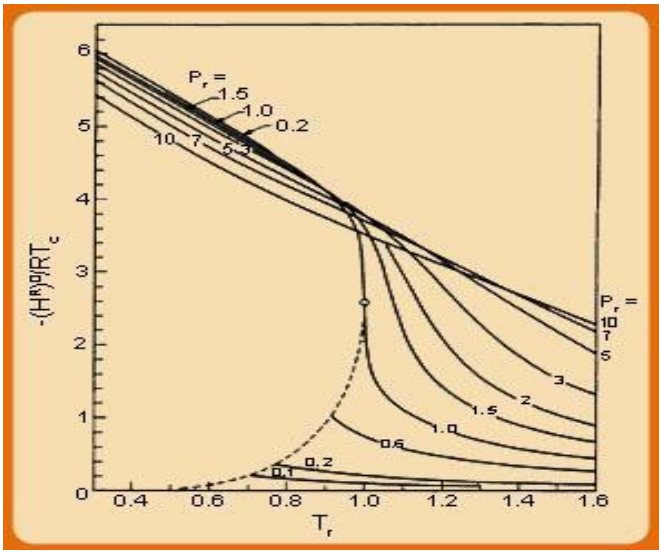


Fig. 5.1 Correlation of $-H_R{}^0 \,/\, RT_C$drawn from tables of Lee-Kesler (Source:*AIChE J.*, pp. 510-527,1975)

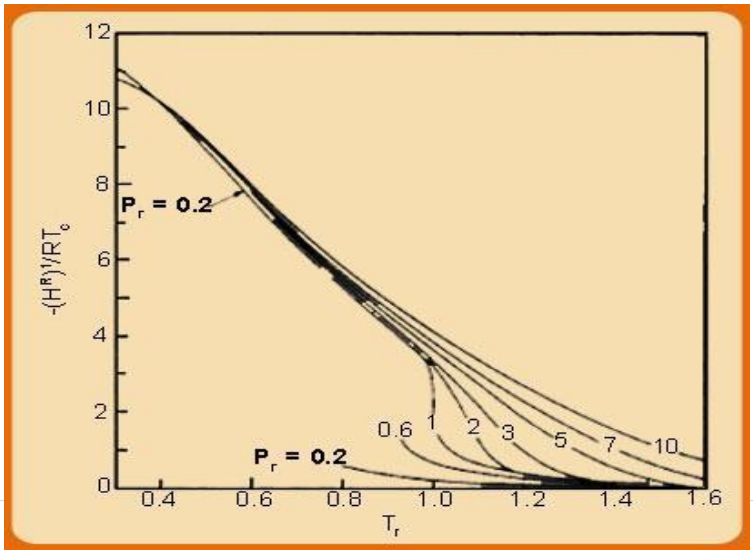


Fig. 5.2 Correlation of $-H_R{}^1 \,/\, RT_C$drawn from tables of Lee-Kesler (Source:*AIChE J.*, pp. 510-527,1975)

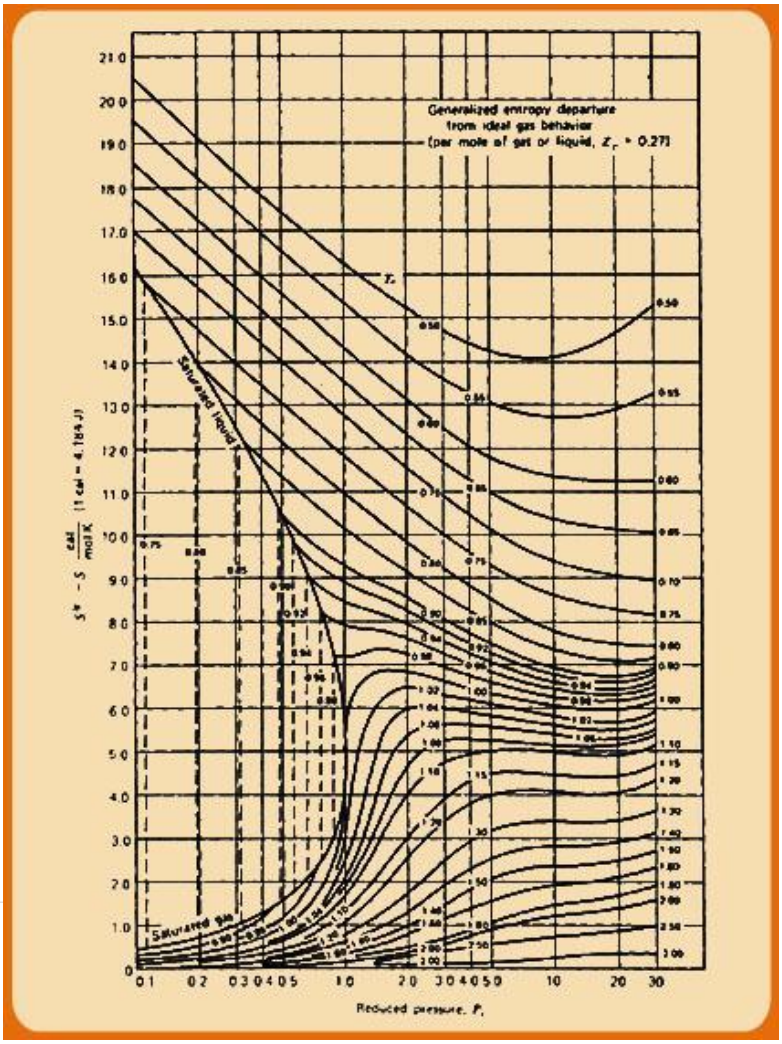


Figure 5.3 Generalized entropy departure functions using corresponding states. [Source: O.A. Hougen, K.M. Watson, and R.A. Ragatz, *Chemical ProcessPrinciples Charts,* 2nd ed., John Wiley & Sons, New York, 1960]

5.8 Computation of *H* and *S* for a Gas using Generalized Departure Functions

The residual function equations presented in the last section are particularly useful for estimating finite changes in enthalpy and entropy for real gases undergoing change in either closed or open system processes. We consider that a pure fluid changes state from (T_1,P_1) to (T_2,P_2); shown schematically in fig. 5.4.

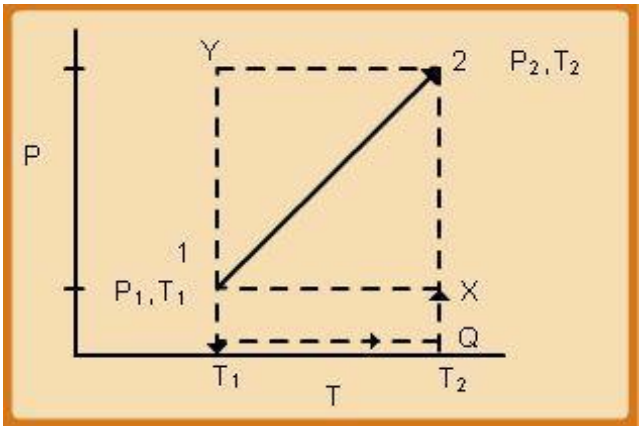


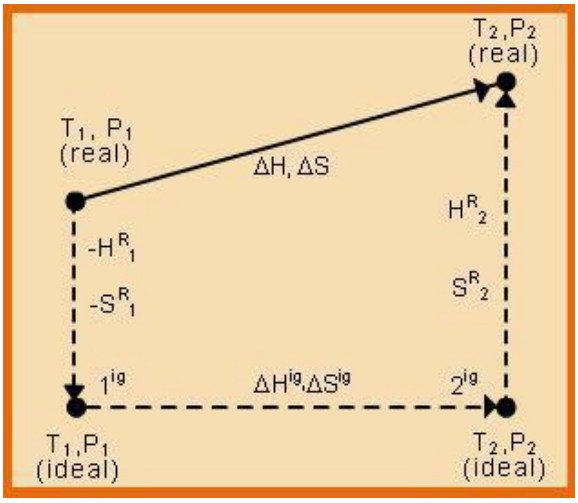
Fig. 5.4 Schematic of a General Thermodynamic Process on P – T co-ordinates

Since the departure functions H^RandS^R capture deviations from ideal gas behaviour at the same temperature as the real gas, one can conceive of the pathway between states '1' and '2' to be decomposed into following steps (see fig 5.5):

U Real gas state at (T_1,P_1) to ideal gas state (*ig*) at (T_1,P_1)

V Ideal gas state at (T_1,P_1) to ideal gas state at (T_2,P_2)

W Ideal gas state at (T_2,P_2) to real gas state at (T_2,P_2)



5.5 Pathway for calculating H and S for Real Gases

10. For step 'a' the change of enthalpy is given by: $H_1^{ig} - H_1 = -H_1^R$
11. For step 'b' the change of enthalpy is given by: $H_2^{ig} - H_1^{ig} = \Delta H^{ig}$
12. For step 'c' the change of enthalpy is given by: $H_2 - H_2^{ig} = H_2^R$

Therefore, the overall change of enthalpy is given by:

$$\Delta H = H_2 - H_1 = H_2^R - H_1^R + \Delta H^{ig} \int_{T_1}^{T_2} C^{ig}_p dT$$

Using eqn. 3.8: $H_2 - H_1 = H_2^R - H_1^R + \int_{T_1}^{T_2} C^{ig}_p dT$ ..(5.63)

The same considerations apply for computing the change of entropy between the two states:

$$\Delta S = S_2 - S_1 = S_2^R - S_1^R + \Delta S^{ig}$$

Using eqn. 4.21: $S_2 - S_1 = S_{12}^R - S_1^R + \int_{T_1}^{T_2} C^{ig}_p \, dT / T - R \ln(P_2 / P_1)$ ..(5.64)

Generalized residual property relations may be used for calculation of change in internal energy i.e. $U_2 - U_1$ for a process in the following manner:

$$U_2 - U_1 = (H_2 - P_2V_2) - (H_1 - P_1V_1)$$

Or: $U_2 - U_1 = (H_2 - H_1) - (P_2V_2 - P_1V_1)$ ..(5.65)

The term $(H_2 - H_1)$ can be calculated using eqn. 5.63, while the term $(P_2V_2 - P_1V_1)$ may be computed after obtaining V_1 and V_2 applying the generalized compressibility factor approach. One may, however, also use the generalized residual property charts for internal energy for the same purpose (fig. 5.6).

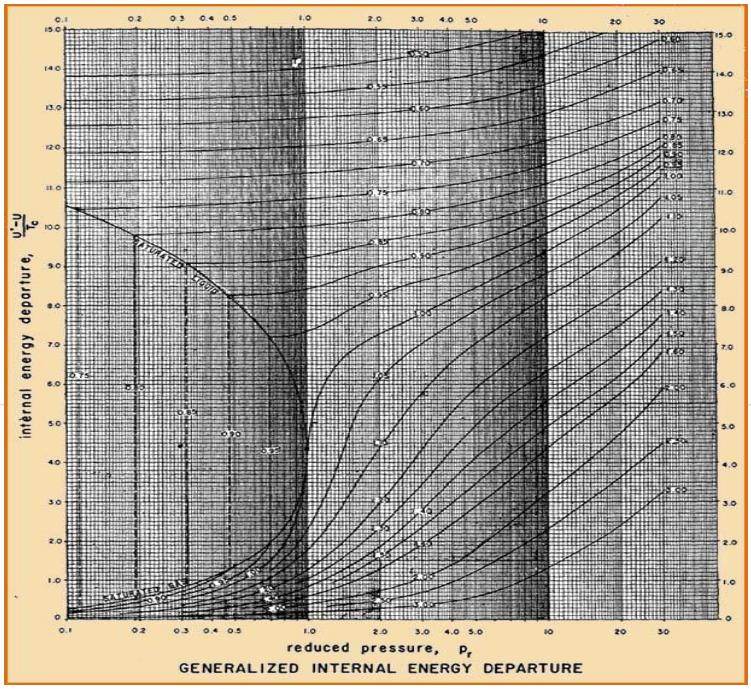


Figure 5.6 Generalized internal energy departure functions using corresponding states [Source: O.A. Hougen, K.M. Watson, and R.A. Ragatz (1960), *Chemical ProcessPrinciples Charts,* 2nd ed., John Wiley & Sons, New York]

5.9 Extension to Gas Mixtures

The generalized equations developed for ΔH and ΔS in the last section may be extended to compute corresponding changes for a real gaseous mixture. The method used is the same as that developed in section 2.4 through the definition of pseudo-critical mixture properties using linear mixing rules:

$$T_{C,m} = \sum_i y_i T_{C,i} \qquad P_{C,m} = \sum_i y_i P_{C,i} \qquad \omega_m = \sum_i y_i \omega_i \qquad ..(2.32)$$

Using the above equations pseudo-reduced properties are computed:

$T_{r,m} = T / T_{C,m}$ and $P_{r,m} = P / P_{C,m}$

Further calculations of changes in internal energy, enthalpy, and entropy follow the same principles developed in the last section.

5.10 Relations for *H* and *S* for Liquids

One starts with the generic equations for *dH* and *dS* developed in section 5.4.

$$dH = C_p\, dT + V - T \left(\frac{\partial V}{\partial P}\right)_T dP \qquad ..(5.20)$$

$$dS = C_P \frac{dT}{T} - \left(\frac{\partial V}{\partial T_P}\right) dP \qquad ..(5.22)$$

As discussed in section 2.3, for liquids it is often simpler to use *volume expansivity* and *isothermal compressibility* parameters for computing thermodynamic properties of interest. Thusfrom eqn. 2.3:

$$\text{Volume Expansivity }, \beta \equiv \frac{1}{V}\left(\frac{\partial V}{\partial T_P}\right)$$

Using eqn. 2.3 in 5.20 and 5.22 the following relations obtain:

$$dH = C_P\,dT + \left[1-\beta T\right]dP \qquad ..(5.66)$$

$$dS = C_P\,\frac{dT}{T} - \beta V dP \qquad ..(5.67)$$

The above equations may also be used to compute the properties of a compressed liquid state. Since the volumetric properties of liquids are very weakly dependent of pressure one can often use the saturated liquid phase properties as reference points and integrate the eqns. 5.66 to 5.67 (at constant temperature) to obtain enthalpy and entropy respectively. The relevant equations are:

$$H_i = H_i^{sat} + \int_{P^{sat}}^{P} V_i(1-\beta_i T)\,dP \qquad ..(5.68)$$

$$S_i = S_i^{sat} - \int_{P^{sat}}^{P} \beta_i V_i\,dP \qquad ..(5.69)$$

In the last two equations, the molar volume V_i may be set equal to V_i^{sat} (*liq.*) , and the volume expansivity approximated to that at the saturated liquid point at the given temperature.

5.11 Applications to real fluid processes in process plant equipments

In a typical process plant one encounters a variety of flow devices such pumps, compressors, turbines, nozzles, diffusers, etc. Such devices are not subject to heat transfer *by design* as are heat exchangers, condensers, evaporators, reactors, etc. However, the flow devices typically are subject to mechanical irreversibility owing to existence of dissipative forces such as fluid viscosity and mechanical friction, which results in reduction of their efficiency. In addition such devices may be subject to thermal irreversibility as their operation may not be truly adiabatic. Therefore, it is necessary to compute the efficiency of such devices in relation to a perfectly reversible (isentropic) process between their inlet and outlet.

The performance of a flow device is expressed in terms of *isentropic efficiency* in which the actual performance of the device is compared with that of an isentropic device for the same inlet conditions and exit pressure. For example, the isentropic efficiency η_T of a **turbine** (which essentially converts fluid enthalpy to shaft work, fig. 5.7) is defined as:

$$\eta_T = \frac{\textit{Power output of the actual turbine}}{\textit{Power out put of the turbine,if it were isentropic}} = \frac{H_i - H_e}{H_i - H_e^s} \qquad ..(5.69)$$

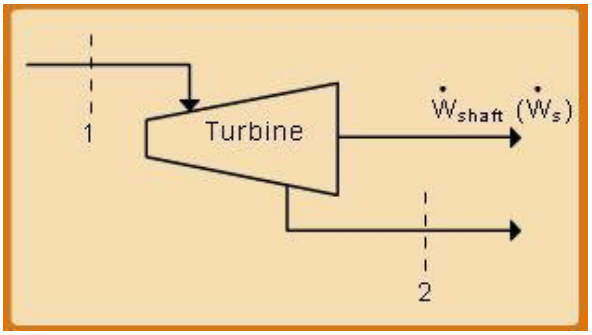


Fig.5.7 Schematic of a Turbine

Where, H_i = enthalpy of the fluid at the inlet of the turbine, H_e = enthalpy of the fluid at the exit of the actual turbine, H_e^s = enthalpy of the fluid at the exit of the turbine, if it were isentropic.

Similarly, the isentropic efficiency η_c of a **compressor** (fig. 5.8) or a pump η_p (which convert applied shaft work to fluid enthalpy) is given by:

$$\eta_c \ (or \eta_p) = \frac{(W_s)^s}{(W_s)} = \frac{H_e^s - H_i}{H_e - H_i} \qquad ..(5.70)$$

Where, H_i = enthalpy of the fluid at the inlet to the compressor (or pump), H_e = enthalpy of the fluid at the exit of the *actual* compressor (pump), and H_e^s = enthalpy of the fluid at the exit of an isentropic compressor (or pump), and W_s represents the shaft work per mole (or mass) of fluid in the two situations.

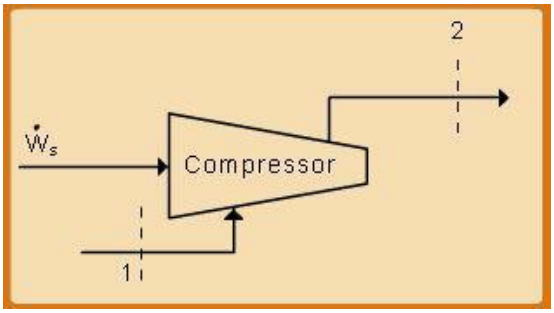


Fig.5.8 Schematic of a Turbine

The isentropic efficiency η_N of a **nozzle**, which is used to achieve high fluid velocity at exit (by conversion of enthalpy to kinetic energy) is given by:

$$\eta_N = \frac{(V_2^2)/2}{(V_2^2)^s/2} = \frac{\textit{Kinetic energy of gas leaving the actual nozzle}}{\textit{Kinetic energy of gas leaving the nozzle, if it were isentropic}} \qquad ..(5.71)$$

UNIT II NOTES

Mixtures; Partial Molar Quantities

Mixtures

We have seen that the combined first- and second laws for an open system is written,

$$dU = TdS - pdV + \mu_1 dn_1 + \mu_2 dn_2 + \cdots, \quad (1)$$

Where the initial definitions of the μ_i were given by,

$$\mu_1 = \left(\frac{\partial U}{\partial n_1}\right)_{S,V,n_2,\cdots} . \quad (2)$$

An equivalent, and more easily understood, definition of the μ_i was given by,

$$\mu_1 = \left(\frac{\partial G}{\partial n_1}\right)_{T,p,n_2,\cdots} . \quad (3)$$

μ_i is called the "chemical potential" and it can be described in words as the Gibbs free energy per mole of substance. Another name for this quantity is "partial molar Gibbs free energy." We found, using the integrated form of Equation 1 and the definition of the Gibbs free energy, that the Gibbs free energy could be written as,

$$G = \mu_1 n_1 + \mu_2 n + \cdots. \quad (4)$$

Equation 4 is an exact thermodynamic equation and contains no approximations.

Notice in Equation 3 that μ_i is intensive, it is given by the extensive G divided by the extensive n_i. Also, the variables that are being held constant are temperature and pressure along with the number of moles of the other components of the system. We can define an equation similar to Equation 3 for any extensive property. The partial molar Gibbs free energy is the most important of these because it provides a measure of the "driving force" for chemical processes. The next most important of these quantities is the partial molar volume,

$$\overline{V}_i = \left(\frac{\partial V}{\partial n_i}\right)_{T,p,n_j,\cdots} . \quad (5)$$

(We will use the bar over the symbol to indicate partial molar quantities. Some texts use a subscript, m, as in V_{mi} to indicate them.) The partial molar volume in Equation 5 can be thought of in several ways. It is the incremental volume obtained by adding a small amount of component i to the mixture while holding the temperature, pressure, and the number of moles of all the other components constant divided by the number of moles of component i. Another way to look at it is to say that it is the incremental volume obtained by adding one mole of component i to an infinite sample of the mixture. The partial molar volume is not necessarily the same as the volume of one mole of the pure component. (The intermolecular interaction between molecules of one component and

molecules of other components may be different than the interaction of molecules of a component with other molecules of the same component.)

Let us regard V as a function of temperature, pressure and composition:

$$V = V(T, p, n_1 n_2, \cdots).$$

Then

$$dV = \left(\frac{\partial V}{\partial T}\right)_{p,n} dT + \left(\frac{\partial V}{\partial p}\right)_{T,n} dp + \left(\frac{\partial V}{\partial n_1}\right)_{T,p,n_2\cdots} dn_1 + \left(\frac{\partial V}{\partial n_2}\right)_{T,p,n_1\cdots} dn_2 + \cdots$$

$$= \left(\frac{\partial V}{\partial T}\right)_{p,n} dT + \left(\frac{\partial V}{\partial p}\right)_{T,n} dp + \overline{V}_1 dn_1 + \overline{V}_2 dn_2 + \cdots.$$

(6a, b)

If we hold T and p constant we get

$$dV = \overline{V}_1 dn_1 + \overline{V}_2 dn_2 + \cdots, \qquad (7)$$

which can be integrated (similar to the way we integrated Equation 1, above) to give,

$$V = n_1\overline{V}_1 + n_2\overline{V}_2 + \cdots,$$

$$= \sum_i n_i \overline{V}_i.$$

(8a, b)

The volumes given in Equations 7, 8a, and 8b are the partial molar volumes which we have already said are not necessarily equal to the molar volumes of the pure components. Thus, Equations 8a and b tell us that volumes may not be additive. That is, if we were to mix one liter of pure ethanol with one liter of pure water the final volume of the mixture would not likely be two liters. That is because water molecules interact with ethanol molecules differently than they interact with other water molecules.

As we have said above, we can define a partial molar quantity from any extensive variable. Thus we can define the partial molar entropy as

$$\overline{S}_i = \left(\frac{\partial S}{\partial n_i}\right)_{T,p,n_j}, \qquad (9)$$

or the partial molar enthalpy as,

$$\overline{H}_i = \left(\frac{\partial H}{\partial n_i}\right)_{T,p,n_j}. \qquad (10)$$

It is also true that

$$S = n_1\overline{S}_1 + n_2\overline{S}_2 + \cdots, \qquad (11)$$

and so on.

How to measure partial molar volumes

There are several ways that partial molar volumes can be measured. One way is to begin with one mole of a compound, call it component 1, add a small amount of component 2 and measure the volume, add a little more of component 2 and measure the volume again. Keep doing this until the desired concentration range has been covered. Then fit the volume data to a curve, for example, of the form,

$$V(n_1 = 1, n_2) = \overline{V_1^\circ} + an_2 + bn_2^{3/2} + cn_2^2 + \cdots. \qquad (12)$$

(Why would we not want to include that 1/2 power of n_2 in Equation 12? People use whatever powers of n_2 they need fit the data.) The constants, a, b, c, etc are obtained from the curve fitting and the first term is the molar volume of pure component 1. Then the partial molar volume of component 2 can be obtained by direct differentiation,

$$\left(\frac{\partial V}{\partial n_2}\right)_{T,p,n_1} = \overline{V_2}$$

$$= 0 + a + \frac{3}{2}bn_2^{1/2} + 2cn_2 + \cdots. \qquad (13a, b)$$

The partial molar volume of component 1 can be obtained from,

$$V = n_1\overline{V_1} + n_2\overline{V_2}$$

$$= \overline{V_1} + n_2\overline{V_2}, \qquad (14a, b)$$

or

$$\overline{V_1} = \frac{V - n_2\overline{V_2}}{n_1}$$

$$= V - n_2\overline{V_2}. \qquad (15a, b)$$

(The last step is because, in this case we have set n_1 = 1.)

Ideal Gases

Equation 4 is also valid for gases, only here we put in the value of V for an ideal gas.

$$V = \frac{nRT}{p} \qquad (11)$$

.

With this substitution Equation 4 becomes,

$$G(p_2) = G(p_1) + \int_{p_1}^{p_2} \frac{nRT}{p} dp \qquad (12)$$

,

which is an integral we have done many times. After integration Equation 12 becomes.

$$G(p_2) = G(p_1) + nRT \ln \frac{p_2}{p_1} \quad (13)$$

It is customary (and useful) to make several changes in Equation 13. We let p_2 range over the pressures of interest to us and call it just p, we let p_1 be some standard state pressure and call it p^o, and finally we divide through by the number of moles of gas, n. With these changes equation 13 is written,

$$\frac{G(p)}{n} = \frac{G(p^o)}{n} + RT \ln \frac{p}{p^o} \quad (14)$$

One more change: the quantity G/n turns out to be so important that it is given a special symbol and its own name. Strictly speaking G/n is just the Gibbs free energy per mole of substance, but the simplicity belies its importance. This quantity is called the **chemical potential** and it is given the symbol, μ . Our final version of what used to be Equation 13 is now,

$$\mu = \mu^o + RT \ln \frac{p}{p^o} \quad (15)$$

We have replaced $G(p^o)/n$, the molar Gibbs free energy at the standard state pressure, with its chemical potential symbol, μ^o. In most cases we will set the standard state pressure equal to one atmosphere. It is not unusual to see Equation 15 written,

$$\mu = \mu^o + RT \ln p \quad (16)$$

but when it is written like this we have to remember that there is an implied p^o = 1 atm dividing the p in the ln p, otherwise the argument of the log function would not be unitless.

Nonideal Gases

Equation 15 was derived assuming the gas is ideal. It does not apply to real gases or approximations to a real gas, like the van der Waals equation of state. If we know the equation of state we can go back to Equation 4 and make the appropriate modifications in our notation. That is, we divide Equation 4 by the number of moles, n, let p_1 equal the standard state pressure, p^o and note that V/n is the molar volume to get,

$$\mu = \mu^o + \int_{p^o}^{p} \bar{V}(p')dp' \quad (17)$$

(We have also let p_2 range over the pressures of interest and called it just plain p, which means that we have to change the dummy variable of integration from p to p'.) Equation 17 would provide the correct answer in numerical calculations, but it would wreak havoc in some of the later developments of thermodynamics, namely the equilibrium constant expression, as we will see later. G. N Lewis (the same Lewis of the Lewis dot structures and Lewis acid/base theory) proposed to preserve the form of Equation 15 by writing the chemical potential as,

$$\mu = \mu^o + RT \ln \frac{f(p)}{p^o} \quad (18)$$

This equation defines a quantity $f(p)$ called the **fugacity**. The fugacity has units of pressure and it is a function of pressure. It contains all the information on the nonideality of the gas. For an ideal gas the fugacity is the same as the pressure. Since all real gases become ideal in the limit as pressure goes to zero we must have.

$$\lim_{p \to o} f(p) = p \quad (19)$$

We would like to have a way of calculating the fugacity from the equation of state for a gas. To do this go all the way back to Equation 2 and divide it by the number of moles, n,

$$d\frac{G}{n} = \frac{V}{n} dp \quad (20),$$

or, in our new notation,

$$d\mu = \overline{V} dp \quad (21).$$

We can get another expression for $d\mu$ by taking the differential of Equation 18 (Remember that R, T, p^o , and μ^o are constants.)

$$d\mu = RT d \ln f \quad (22).$$

The $d\mu$ in Equations 21 and 22 must be the same, so we can set them equal to each other

$$RT d \ln f = \overline{V} dp \quad (23).$$

Rearrange this to get,

$$d \ln f = \frac{\overline{V}}{RT} dp \quad (24)$$

We could integrate this equation directly, but that would sort of take us back to where we started from. Instead, we use a mathematical trick before we integrate it. Add and subtract $\frac{dp}{p}$ to the right hand side of Equation 24,

$$d\ln f = \frac{\bar{V}}{RT}dp - \frac{dp}{p} + \frac{dp}{p} \quad (25)$$

You can see that we didn't really change anything. Regroup the terms in Equation 25,

$$d\ln f = \left(\frac{\bar{V}}{RT} - \frac{1}{p}\right)dp + \frac{dp}{p} \quad (26)$$

Now integrate from p^o to p . (We will have to call our dummy variable of integration p' so as not to conflict with the limits of integration.) We get,

$$\ln f - \ln f_o = \int_{p_o}^{p}\left(\frac{\bar{V}(p')}{RT} - \frac{1}{p'}\right)dp' + \ln p - \ln p_o \quad (27)$$

where f_o is the fugacity at p_o. Move the $\ln f_o$ to the right hand side,

$$\ln f = \int_{p_o}^{p}\left(\frac{\bar{V}(p')}{RT} - \frac{1}{p'}\right)dp' + \ln p + \left(\ln f_o - \ln p_o\right) \quad (28)$$

Now we can take the limit where p_o goes to zero. We know that f_o goes to p_o as $p_o \rightarrow 0$ so the last two terms in parentheses on the right cancel each other in this limit. Equation 28 becomes,

$$\ln f = \int_{0}^{p}\left(\frac{\bar{V}(p')}{RT} - \frac{1}{p'}\right)dp' + \ln p \quad (29)$$

Equation 29 will suffice to calculate the fugacity, but it is customary to take the antilog of both sides to get,

$$f = p e^{\int_0^p \left(\frac{\bar{V}(p')}{RT} - \frac{1}{p'}\right)dp'} = p e^{\int_0^p \frac{1}{p'}(Z-1)dp'} \quad (30)$$

In the last segment of Equation 29, $Z = \frac{p'\overline{V}}{RT}$, is the so-called compressibility factor. In either version of Equation of 29 it is easy to see that if the gas is ideal $f = p$. It requires an equation of state or experimental data to calculate a fugacity from either Equation 30 or Equaton 29. From the right-hand side of Equation 30 we can see that the second form of the virial expansion,

(31) $$\frac{p\overline{V}}{RT} = 1 + B'p + C'p^2 + \cdots ,$$

would be the best choice for calculating fugacity.

Ideal Solutions

We will define an ideal solution as a solution for which the chemical potential of each component is given by,

$$\mu_i = \mu_i^* + RT \ln X_i , \qquad (16)$$

where μ_i^* is the chemical potential of pure component *i*, and X_i is the mole fraction of component *i* in the solution.

(Many texts define an ideal solution as a solution which obeys Raoult's law over the full range of composition,

$$p_i = X_i p_i^* , \qquad (17)$$

where p_i^* is the vapor pressure of pure component *i*.)

We will now prove that an ideal solution obeys Raoult's law (using our definition of an ideal solution).

Consider a solution of two components where the mole fraction of component 1 is X_1. We know that the chemical potential of component 1 must be the same in the solution as in the vapor in equilibrium with the solution. That is,

$$\mu_{1l} = \mu_{1g} , \qquad (18)$$

but the solution is ideal so,

$$\mu_{1l} = \mu_{1l}^* + RT \ln X_1 . \qquad (16)$$

Also, we can approximate the vapor as an ideal gas so,

$$\mu_{1g} = \mu_{1g}^\circ + RT \ln \frac{p_1}{1 \text{ atm}} , \qquad (19)$$

where p_1 is the vapor pressure (partial pressure) of component 1 above the solution. Combining Equations 16, 18, and 19 we get,

$$\mu_{1l}^{*} + RT\ln X_1 = \mu_{1g}^{\circ} + RT\ln\frac{p_1}{1\ \text{atm}}. \qquad (20)$$

Equation 20 doesn't help us very much all by itself. However we have some more information. We know that for the pure component 1 we have $X_1 = 1$, and we know that the pressure of component 1 vapor in equilibrium with the liquid is just the vapor pressure of the pure liquid, p_1^*, so that,

$$\mu_{1l}^{*} = \mu_{1g}^{\circ} + RT\ln\frac{p_1^{*}}{1\ \text{atm}}. \qquad (21)$$

Let us now subtract Equation 21 from Equation 20 to get

$$RT\ln X_1 = RT\ln\frac{p_1}{1\ \text{atm}} - RT\ln\frac{p^{*}}{1\ \text{atm}}, \qquad (22)$$

from which we conclude that,

$$\ln X_1 = \ln\frac{p_1}{p^{*}}$$

$$X_1 = \frac{p_1}{p^{*}}, \qquad (23a, b)$$

or

$$p_1 = X_1 p_1^{*}, \qquad (17)$$

which is Raoult's law.

Example calculation using Raoult's law

Benzene and toluene form a solution which is very nearly ideal. Consider a mixture of benzene (Bz) and toluene (Tol) at 60° C. At 60° C the vapor pressures of pure benzene and pure toluene are 385 Torr and 139 Torr, respectively. What are the vapor pressures of benzene and toluene in a mixture with $X_{Bz} = 0.400$, and $X_{Tol} = 0.600$, and what is the composition of the vapor in equilibrium with this solution?

Use Raoult's law to find the vapor pressures of the two species,

$$p_{Bz} = X_{Bz}p_{Bz}^{*} = 0.400 \times 385\ \text{Torr} = 154\ \text{Torr},$$

$$p_{Tol} = X_{Tol}p_{Tol}^{*} = 0.600 \times 139\ \text{Torr} = 83.3\ \text{Torr}.$$

The total pressure is the sum of these two individual pressures, 237 Torr.

The composition of the vapor phase is obtained from the vapor pressures and Dalton's law of partial pressures,

$$X_{\mathrm{Bz}} = \frac{p_{\mathrm{Bz}}}{p_{\mathrm{Tot}}} = \frac{154}{237} = 0.649,$$

$$X_{\mathrm{Tol}} = \frac{p_{\mathrm{Tol}}}{p_{\mathrm{Tot}}} = \frac{83.3}{237} = 0.351.$$

Notice that the composition of the vapor is not the same as the composition of the liquid, the vapor phase is much richer in the more volatile compound, benzene. This fact will be important when we discuss vapor pressure diagrams and two-component phase diagrams.

Properties of ideal solutions

Given the chemical potentials for the components of an ideal solution we can calculate a number of properties of ideal solutions. For example, the Gibbs free energy of mixing is the easiest to calculate,

$$\begin{aligned}
\Delta G_{\mathrm{mix}} &= G_{\mathrm{mixed}} - G_{\mathrm{unmixed}}, \\
&= n_1\mu_1 + n_2\mu_2 + \cdots - n_1\mu_1^* - n_2\mu_2^* - \cdots \\
&= n_1(\mu_1^* + RT\ln X_1) + n_2(\mu_2^* + RT\ln X_2) + \cdots \\
&\quad -n_1\mu_1^* - n_2\mu_2^* - \cdots \\
&= RT(n_1\ln X_1 + n_2\ln X_2 + \cdots).
\end{aligned} \qquad (24a, b, c, d)$$

To bring this equation into the usual form multiply and divide by the total number of moles, n, and bring the $1/n$ inside the parentheses to convert the number of moles of each component into a mole fraction,

$$\Delta G_{\mathrm{mix}} = nRT(X_1\ln X_1 + X_2\ln X_2 + \cdots). \qquad (25)$$

Notice that the Gibbs free energy of mixing is negative, as one would expect for a spontaneous process at constant temperature and pressure.

We can also calculate other properties of mixing ideal solutions. The entropy of mixing is,

$$\begin{aligned}
\Delta S_{\mathrm{mix}} &= -\left(\frac{\partial \Delta G_{\mathrm{mix}}}{\partial T}\right)_{p,n_i} \\
&= -nR(X_1\ln X_1 + X_2\ln X_2 + \cdots),
\end{aligned} \qquad (26)$$

which is the same as the entropy of mixing for ideal gases.

The volume change on mixing can be found from,

$$\Delta V_{\text{mix}} = \left(\frac{\partial \Delta G_{\text{mix}}}{\partial p} \right)_{T n_i} = 0, \tag{27}$$

so that volumes are additive for an ideal solution. That is, if we were to mix one liter of benzene and one liter of toluene the final volume of the solution would be two liters.

We can also determine whether or not there is any heat of reaction.

$$\begin{aligned} \Delta H_{\text{mix}} &= \Delta G_{\text{mix}} + T\Delta S_{\text{mix}} \\ &= nRT(X_1 \ln X_1 + X_2 \ln X_2 + \cdots) \\ &\quad + T(-nR)(X_1 \ln X_1 + X_2 \ln X_2 + \cdots) \\ &= 0. \end{aligned} \tag{28}$$

That is, there is no heat involved in the mixing of ideal solutions. If we mix several components and the mixture gets hot or cold we can be sure that the solution formed was not ideal.

Activity and Activity Coefficients

We have now seen and used several expressions for the chemical potential of a substance or component in a mixture. For a one-component ideal gas we had

$$\mu = \mu^\circ + RT \ln \frac{p}{p^\circ}, \tag{1}$$

where μ^o is the chemical potential when $p = p^o$ and p^o is usually one atmosphere.

For a mixture of ideal gases it can be shown that for each component, *i*, the chemical potential is given by,

$$\mu_i = \mu_i^\circ + RT \ln \frac{p_i}{p^\circ}. \tag{2}$$

For a nonideal gas we used the fugacity, *f*, instead of the pressure and the chemical potential for one component is,

$$\mu = \mu^\circ + RT \ln \frac{f(p)}{p^\circ}. \tag{3}$$

For a mixture of nonideal gases it can be shown that,

$$\mu_i = \mu_i^\circ + RT \ln \frac{f_i}{p^\circ}, \tag{4}$$

only now the fugacity of component *i* is a function of the pressures of all the gases in the mixture,

$$f_i = f_i(p_1, p_2, p_3, \ldots). \tag{5}$$

For ideal solutions we found that,

$$\mu_i = \mu_i^\circ + RT \ln X_i. \qquad (6)$$

These expressions for chemical potential all have the form of a reference or standard state chemical potential plus *RT* times the logarithm of something related to pressure or concentration. This form turns out to be very important, so important that G. N. Lewis used it to give the most general case of chemical potential as

$$\mu_i = \mu_i^\circ + RT \ln a_i. \qquad (7)$$

The quantity, a_i, is called the "activity" of component *i* and Equation 7 should be regarded as the definition of activity. Notice that the activity has no units.

All of the special cases we have been considering so far can be reconciled to this definition of activity. Thus, for an ideal gas mixture,

$$a_i = \frac{p_i}{p^\circ};$$

for a nonideal gas mixture,

$$a_i = \frac{f_i}{p^\circ};$$

for an ideal solution,

$$a_i = X_i,$$

and so on. In a nonideal solution we would have to just write Equation 7 again,

$$\mu_i = \mu_i^\circ + RT \ln a_i. \qquad (7)$$

In Equation 7 all the nonidealities of the solution are absorbed into the activity. We will see a more convenient way to write this below under the heading "activity coefficient."

We can find the activity of a component of a nonideal solution from measurements of the vapor pressure of that component in the vapor in equilibrium with the solution. We know that the chemical potential of a component must be the same in the vapor as in the liquid. that is, from Equations 2 and 7 we obtain,

$$\mu_{il} = \mu_{ig}$$

$$\mu_{il}^* + RT \ln a_i = \mu_{ig}^\circ + RT \ln \frac{p_i}{p^\circ}, \qquad (8a, b)$$

but for pure component *i*, we must have,

$$\mu_{il}^* = \mu_{ig}^\circ + RT \ln \frac{p_i^*}{p^\circ}. \qquad (9)$$

(Note: When dealing with liquid solutions it is customary to write the chemical potential of the pure liquid as μ^*_{il} instead of the usual μ°_{il}, which means that the standard state for a liquid is the pure liquid itself. p_i^* is the vapor pressure of the pure liquid.)

Subtracting Equation 9 from Equation 8b yields,

$$RT \ln a_i = RT \ln \frac{p_i}{p^\circ} - RT \ln \frac{p_i^*}{p^\circ}$$

$$= RT \ln \frac{p_i}{p_i^*}, \qquad (10a, b)$$

so that

$$a_i = \frac{p_i}{p_i^*}. \qquad (11)$$

If the solution were ideal p_i would be given by Raoult's law and the activity would be just the mole fraction.

Gibbs Free Energy and Pressure, Chemical Potential, Fugacity

The Gibbs free energy depends on pressure as well as on temperature. The pressure dependence of the Gibbs free energy in a closed system is given by the combined first and second laws and the definition of Gibbs free energy as,

(1) $$dG = -SdT + Vdp.$$

If we hold temperature constant and vary only the pressure we can prepare Equation 1 for integration from pressure p_1 to p_2 as follows:

(2) $$dG = Vdp,$$

Then

(3) $$G(p_2) - G(p_1) = \int_{p_1}^{p_2} Vdp$$

or

(4) $$G(p_2) = G(p_1) + \int_{p_1}^{p_2} Vdp.$$

Equation 4 is quite general and applies to all isotropic substance: solids, liquids, ideal gases, and real gases. We will apply it first to isotropic solids and liquids.

Gibbs-Duhem Equation

The Gibbs free energy can be defined in two different ways once by subtracting off combinations of entropy S, enthalpy H and temperature T and other as a sum of chemical potentials and amounts of species. The fact that they are equal gives a new relation known as "Gibbs-Duhem Relation." The Gibbs-Duhem relation helps us to calculate relationships between quantities as a system which remains in equilibrium. One example is the Clausius-Clapeyron equation which states that two phases at equilibrium with each other having equaled amount of a given substance must have exactly the same free energy i.e. it relates equilibrium changes in pressure to changes in temperature as a function of material parameters.

Deriving the Gibbs-Duhem equation from thermodynamics state equations is very easy. The Gibbs free energy G in equilibrium can be expressed in terms of thermodynamics as:

$dG = \mu_1\, dn_1 + n_1\, d\mu_1 + \mu_2\, dn_2 + n_2\, d\mu_2 \ldots\ldots\ldots\ldots \mu_j\, dn_j + n_j\, d\mu_j$

$= (\mu_1\, dn_1 + \mu_2\, dn_2 + \ldots\ldots\ldots \mu_j\, dn_j) + (n_1\, d\mu_1 + n_2\, d\mu_2 + \ldots\ldots\ldots\ldots n_j\, d\mu_j)$

At constant temperature and pressure, the above equation can be written as:

$\mathbf{n_1\, d\mu_1 + n_2\, d\mu_2 + \ldots\ldots\ldots\ldots n_j\, d\mu_j = 0}$

$\sum \mathbf{n_i\, d\mu_i = 0}$ ………………………….. (1)

Because at constant temperature and pressure, $(\mu_1\, dn_1 + \mu_2\, dn_2 + \ldots\ldots\ldots \mu_j\, dn_j) = dG$

The equation (1) is known as the Gibbs-Duhem equation.

Applications of Gibbs-Duhem equation:

(i) Gibbs-duhem equation is helpful in calculating partial molar quantity of a binary mixture by measuring the composition of the mixture which depends on the total molar quantity.

(ii) Gibbs-duhem equation is helpful in calculating the partial vapor pressures by calculating the total vapor pressure. All these calculations require a curve-fitting procedure. Using tabulated experimental data the accuracy of the calculated quantities was found to be comparable to the accuracy of the original experimental data

Excess Properties

Unlike for real gases (pure or mixtures) the EOS based approach to calculation of thermodynamic properties of real liquid solutions have not proved very successful. However, as molar *residual property*is defined for real gases, for *real* liquid solutions one may formulate a different departure function called the molar *excess property* that quantify the deviation from ideal solution property. The mathematical formalism of excess properties is, therefore, analogous to that of the residual properties.

If *M* represents the molar (or unit-mass) value of any extensive thermodynamic property(e.g., *V,**U,** H, S, G,* etc.), then an excess property M^E is defined as the difference between the actual property value of a solution and the value it would have as an ideal solution at the same temperature, pressure, and composition. Thus:

$$M^E \equiv M - M^{id} \tag{6.78}$$

The excess property bear a relationship to the property change of mixing. One may take the example of excess Gibbs free energy to illustrate the point. Thus:

$$G^E = G - G^{id} \tag{6.79}$$

Or:
$$G^E = G - \left(\sum_i x_i G_i + RT \sum_i x_i \ln x_i \right) \tag{6.80}$$

Thus:
$$G^E = \Delta G_{mix} - RT \sum_i x_i \ln x_i \tag{6.81}$$

Other relations include:

$$H^E = H - H^{id} = \Delta H_{mix} \tag{6.82}$$

$$S^E = \Delta S_{mix} + R \sum_i y_i \ln y_i \tag{6.83}$$

Also: $G^E = H - TS^E$

The non-ideality of real liquid solutions are depicted well by use of excess properties, especially through the behaviour of G^E, H^E and S^E. The excess Gibbs energy is typically obtained from low pressure vapour-liquid equilibrium data, while H^E is obtained by measuring isothermal enthalpy change of mixing. Lastly S^E is derived using the following relation:

$$S^E = \frac{H_E - G^E}{T} \tag{6.84}$$

6.5 shows the variation of each of the excess property as a function of liquid mole fraction for a number of binary solutions.

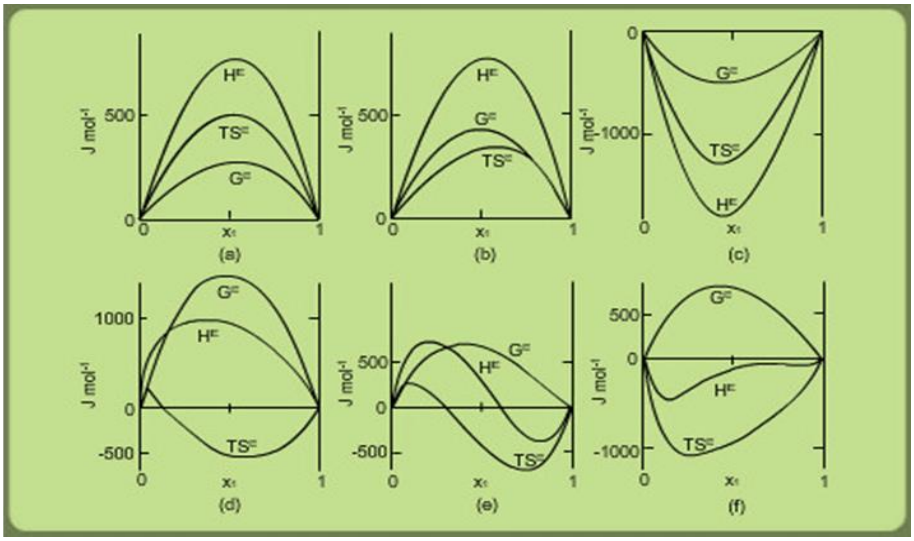


Fig. 6.5 Excess properties at 50°C for 6 binary liquid systems: (*a*) chloroform(1)/*n*-heptane(2); (*b*) acetone(1)/methanol(2);(*c*) acetone(1)/chloroform(2); (*d*) ethanol(1)/*n*-heptane(2); (*e*) ethanol(1)/chloroform(2); (*f*) ethanol(1)/water(2). (Source: H.C Van Ness and M. M. Abbott, *Perry's Chemical Engineer's Handbook* (7th ed.), McGraw Hill, 1997.

The Lewis/Randall Rule:

A simple equation for the fugacity of a species in an ideal solution follows from the following equations. In g any solution:

$$\mu_i = \Gamma_i(T) + RT \ln \hat{f}_i \tag{6.158}$$

Applying to ideal solution, $\mu_i^{id} = \bar{G}_i^{id} = \Gamma_i(T) + RT \ln \hat{f}_i^{id}$ (6.159)

However we know (eqn. 6.77) that: $\mu_i^{id} = \bar{G}_i^{id} = G_i + RT \ln x_i$ (6.160)

Also, from eqn. 6.86: $G_i = \Gamma_i(T) + RT \ln f_i$

Thus, $\mu_i^{id} = \bar{G}_i^{id} = \Gamma_i(T) + RT \ln x_i f_i$ (6.161)

On comparing eqns. 6.158 & 6.160: $\hat{f}_i^{id} = x_i f_i$ (6.162)

The last relation is known as the *Lewis/Randall rule*, and applies to each species in an ideal solution at all co temperature, pressure, and composition. It shows that the fugacity of each species in an ideal solution is prop its mole fraction; the proportionality constant is the fugacity of pure species *i* in the same physical state as th and at the same T and P.

One may write the same equation specifically for an ideal solution, whence:

$$d\left(\frac{G^{\blacksquare}}{RT}\right) = \frac{V^{\blacksquare}}{RT} dP - \frac{H^{\blacksquare}}{RT^2} dT \tag{6.165}$$

Thus subtracting eqn. 6.165 from 6.164:

$$d\left(\frac{G^E}{RT}\right)=\frac{V^E}{RT}dP-\frac{H^E}{RT^2}dT \tag{6.166}$$

Thus we may write the following further *generative* relations:

$$\frac{V^E}{RT}=\left[\frac{\partial(G^E/RT)}{\partial P}\right]_T \tag{6.167}$$

$$\frac{H^E}{RT}=-T\left[\frac{\partial(G^E/RT)}{\partial T}\right]_P \tag{6.168}$$

And further: $$\frac{S^E}{RT}=\frac{H^E}{RT}-\frac{G^E}{RT} \tag{6.169}$$

The sensitivity of the excess Gibbs free energy to changes in temperature and pressure may be estimated t
effect of pressure and temperature on liquid phase properties. For example, for an equimolar mixture of be
cyclohexane at 298K and 1 bar are **(**source: J.M. Smith, H.C. Van Ness and M.M. Abbott, *Introduction to*
Engineering Thermodynamics, 6th ed., McGraw-Hill, 2001):

$V^E = 0.65cm^3/mol$

$H^E = 800cm^3/mol$

Thus it follows:

$$\left[\frac{\partial(G^E/RT)}{\partial P}\right]_{T,x}=\frac{V^E}{RT}=\frac{0.65}{83.14x298}=2.62x10^{-5}bar^{-1}$$

$$\left[\frac{\partial(G^E/RT)}{\partial T}\right]_{P,x}=-\frac{H^E}{RT^2}=-\frac{800}{83.14x298^2}=-1.08x10^{-3}K^{-1}$$

$\Delta G^E(\text{for } \Delta T = 1K) \approx \Delta G^E(\text{for } \Delta P = 40bar)$

The above calculations suggest that to effect the same change in excess Gibbs free energy brought about a
1K, one needs to change the pressure to change by about 40bar. Hence the excess Gibbs free energy
relatively weak dependence on pressure.

Ideal Gas Mixtures and Liquid Solutions

We next explore the development of a quantitative definition of the chemical potential in terms of the
properties and composition of mixtures. We have observed earlier that just as ideal gas state is a reference f
properties, ideal gas mixtures play the same role with respect to real gas mixtures. Therefore, it is ins
establish the property relations for ideal gas mixture first.

Consider the constitution of an ideal gas mixture (containing N species) at a given temperature (T) and press
obtain n moles of the total mixture we need to bring together ni moles of each species $(n=\sum_i^N n_i)$ at temper
at a pressure pi which corresponds to the partial pressure that each species would exert in the final mixture.
total volume of the mixture, the following set of relations hold.

$P = nRT/V^t \Rightarrow p_i = n_iRT/V^t$

$Or \Rightarrow p_i/P = n_i/n = y_i$

$$n = n_i + \sum_j n_j \quad \Rightarrow \quad Hence: \left[\frac{\partial n}{\partial n_i}\right]_{T,P,n_{j\neq i}} = 1$$

$$\bar{V}_i^{ig} = \left[\frac{\partial (nV^{ig})}{\partial n_i}\right]_{T,P,n_{j\neq i}} = \left[\frac{\partial (nRT/P)}{\partial n_i}\right]_{T,P,n_{j\neq i}} = (RT/P)\left[\frac{\partial n}{\partial n_i}\right]_{T,P,n_{j\neq i}} = RT/P$$

But the molar volume of the i^{th} species $V^{ig} = RT/P$

Hence it follows:

$$\bar{V}_i^{ig} = V^{ig}$$

The last result indicates that the molar volume for a species does not change between its pure state ideal gas mixture at the same T & P. It may then be concluded that for an ideal gas mixture the pr each species are independent of that of the other ones. This may be easy to appreciate as the concept gas is premised on the idea that the intermolecular interaction is non-existent in such a state. This leads to the well-known *Gibbs theorem***:**

"Except for volume all other partial molar property of a species in an ideal-gas mixture is equal to the cor molar property of the species as a pure ideal gas at a temperature same as that of the mixture, but at a pres to its partial pressure in the mixture."

In mathematical terms: $\bar{M}_i^{ig}(T,P) = M_i^{ig}(T,p_i)$

As an example let us consider the case of enthalpy of an ideal gas mixture. By Gibbs theorem:

$$\bar{H}_i^{ig}(T,P) = H_i^{ig}(T,p_i) \qquad (6$$

But, as the enthalpy of an ideal gas is independent of pressure it follows that:

$$H_i^{ig}(T,p_i) = H_i^{ig}(T,P) \qquad (6$$

It follows: $\bar{H}_i^{ig}(T,P) = H_i^{ig}(T,P)$ (6

By the standard definition, the enthalpy of the mixture is:

$$H_{mix}^{ig}(T,P) = \sum_i y_i \bar{H}_i^{ig}(T,P) \qquad (6$$

Thus, using eqns. 6.56 – 6.59 we get:

$$H_{mix}^{ig}(T,P) = \sum_i y_i H_i^{ig}(T,P) \qquad (6$$

It follows: $\Delta H_{mix}^{ig} = H_{mix}^{ig} - \Sigma y_i H_i^{ig} = 0$ (6

Employing the same reasoning: $\Delta U_{mix}^{ig} = U_{mix}^{ig} - \Sigma y_i U_i^{ig}$ (6

The molar entropy of mixing of ideal gas mixture, however, is not zero. As stated above, the formation of mixture results from bringing together y_i moles each species at T and partial pressure p_i to form a m and P, Hence for isothermal mixing, y_i moles of each species goes from (T, p_i) to (T, P). Therefore:

$$S_i^{ig}(T,P) - S_i^{ig}(T,p_i) = -R\ln(P/p_i) = R\ln y_i \quad (6.63$$

On transposing: $S_i^{ig}(T,p_i) = S_i^{ig}(T,P) - R\ln y_i \quad (6.64$

But: $S^{ig}(T,P) = \sum_i y_i \bar{S}_i^{ig}(T,P) \quad (6.65$

So: $S^{ig}(T,P) = \sum_i y_i S_i^{ig}(T,p_i) \quad (6.66$

Using eqns. 6.63 - 6.67, one obtains:

$$S^{ig}(T,P) = \sum_i y_i S_i^{ig}(T,P) - R\sum_i y_i \ln y_i \quad (6.67$$

On applying the partial molar property operation (as given by eqn. 6.4) on 6.58, it may be shown that:

$$\bar{S}_i^{ig}(T,P) = S_i^{ig}(T,P) - R\ln y_i \quad (6.6$$

It further follows: $\Delta S_{mix}^{ig} = S^{ig}(T,P) - \Sigma y_i S_i^{ig}(T,P) = R\sum_i y_i \ln y_i \quad (6.6$

For Gibbs free energy relation we start from: $G = H - TS$

For an ideal gas mixture: $G^{ig} = H^{ig} - TS^{ig} \quad (6.7$

Taking the partial molar property derivative: $\bar{G}_i^{ig} = \bar{H}_i^{ig} - T\bar{S}_i^{ig} \quad (6.7$

On putting eqns. 6.58 and 6.59 into 6.71 we get the following relation for the chemical potential of each an ideal gas mixture:

$$\bar{G}_i^{ig} = \mu_i^{ig} \equiv G_i^{ig} + RT\ln y_i \quad (6.7$$

Using eqns. 6.51 and 6.58, it may be also shown that:

$$G_{mix}^{ig} = \sum_i y_i G_i^{ig} + RT\sum_i y_i \ln y_i \quad (6.7$$

Example 6.3

What is the change in entropy when 0.6 m^3 of CO_2 and 0.4 m^3 of N_2, each at 1 bar and 25^0C blend to f mixture at the same conditions? Assume ideal gases.

The Ideal

We have already seen that owing to the fact that pure ideal gases and mixtures are not subject to inter interactions the partial molar properties (apart from volume) of each species is the same as that o species at the same temperature and pressure. In other words each species "sees" no differenc environment in pure or mixed state. One can conceptually extend this idea to posit an ideal solution which may serve as a model to which real-solution behavior can be compared. Consider a soluti liquids, say A and B. If the intermolecular interaction in the pure species, (i.e., A-A and B-B) is equal to species interaction A-B, neither A nor B type molecules will "see" any difference in their environment b after mixing. This is in a sense the same condition as one obtains with idea gas mixtures. Hence an id of ideal solution property relations may be constructed based on the model of ideal gas mixture. By c

while describing properties of liquid solutions mole fractions yi are replaced by xi. The following therefore, ideal (liquid) solution properties (denoted by a superscript 'id'):

$$H^{id} = \sum_i x_i H_i \quad \text{and,} \quad V^{id} = \sum_i x_i V_i \tag{6.74}$$

$$S^{id} = \sum_i x_i S_i - R\sum_i x_i \ln x_i \tag{6.75}$$

Hence $$G^{id} = \sum_i x_i G_i + RT\sum_i x_i \ln x_i \tag{6.76}$$

Lastly, $$\mu_i^{id} = \bar{G}_i^{id} = G_i + RT\ln x_i \tag{6.77}$$

As we will see later the ideal solution model can also serve to describe the behaviour of mixtures of re solids.

What is the change in entropy when 0.6 m3 of CO2 and 0.4 m3 of N2, each at 1 bar and 250C blend to mixture at the same conditions ? Assume ideal gases.

For an ideal gas, mole fraction = volume CO2 (1) / N2(2); y1 = 0.6,

$$(\Delta S)_{mix} = -R\sum y_i \ln y_i = 5.5 J / molK$$

Gibbs-Duhem relation

We have found that it is often the case that relationships exist between different thermodynamic var their derivatives. Maxwell relations are good examples. Here, we show that similar relationships exis *composition variables* in mixtures.

Recall first the Gibbs free energy for a mixture, given in integrated form:

$$G(T, P, N_1, N_2, \ldots, N_M) = \sum_i N_i \mu_i(T, P, \{x\})$$

this can be derived using Euler's theorem since G is extensive in the values N_i. Take the total differential:

$$dG = \sum_i N_i d\mu_i + \mu_i dN_i \tag{12.44}$$

Consider also the differential form of the Gibbs free energy, given from the Legendre transform of the energy:

$$dG = -SdT + VdP + \sum_i \mu_i dN_i \tag{12.45}$$

Subtracting the two equations, we find

$$-SdT + VdP = \sum_i N_i d\mu_i \tag{12.46}$$

This important relationship is called the **Gibbs-Duhem** equation. It shows that differential changes in the temperature, pressure, and chemical potentials of a mixture are all interrelated. Of particular interest are the conditions of constant temperature and pressure, for which $dT = 0$ and $dP = 0$:

$$\sum_i N_i d\mu_i = 0 \qquad (\text{constant } T, P) \tag{12.47}$$

For a binary mixture of just two components, we can write

$$N_1 d\mu_1 + N_2 d\mu_2 = 0 \tag{12.48}$$

Taking the derivatives with respect to the mole fraction of the first component:

$$N_1 \frac{d\mu_1}{dx_1} + N_2 \frac{d\mu_2}{dx_1} = 0$$
$$N_1 \frac{d\mu_1}{dx_1} + N_2 \left(\frac{d\mu_2}{dx_2}\right)\left(\frac{dx_2}{dx_1}\right) = 0 \tag{12.49}$$

Simplifying using $x_1 + x_2 = 1$ and dividing through by N:

$$x_1 \left(\frac{d\mu_1}{dx_1}\right)_{T,P} = x_2 \left(\frac{d\mu_2}{dx_2}\right)_{T,P} \tag{12.50}$$

Thus in a binary mixture, the changes in chemical potentials with composition are strictly related. Clearly this relation is satisfied by an ideal solution, for which $d\mu_i/dx_i = k_B T/x_i$. For a nonideal solution,

$$x_1 \left(\frac{d \ln \gamma_1}{dx_1}\right)_{T,P} = x_2 \left(\frac{d \ln \gamma_2}{dx_2}\right)_{T,P} \tag{12.51}$$

This equation is often very useful in an experimental setting. If the activity of one component in solution is known as a function of composition, this gives a mechanism for computing the activity of

by integrating from a reference state (infinite dilution or pure phase).

> The **Gibbs-Duhem** relation places constraints on the way in which chemical potentials in a multicomponent system can vary in relation to each other.

The Activity coefficient

While we have defined fugacity coefficients of individual species in a liquid solution by 6.153, we may define yet another parameter called *activity coefficient* in order to describe the non-ideality of a liquid solution, especially at low to moderate system pressure.

We have by eqn. 6.155: $\bar{G}_i = \Gamma_i(T) + RT \ln \hat{f}_i$

And from eqn. 6.158: $\bar{G}_i^{id} = \Gamma_i(T) + RT \ln x_i f_i$

Using the above equations:
$$\bar{G}_i - \bar{G}_i^{id} = \bar{G}_i^E = RT \ln \frac{\hat{f}_i}{x_i f_i} \qquad (6.170)$$

The left side of this equation is the partial excess Gibbs energy $\bar{G}_i^E$; the dimensionless ratio $\hat{f}_i / x_i f_i$ appearing on the right is the *activity coefficient* of species *i* in solution, represented by the symbol γ_i. Thus, by definition:

$$\gamma_i \equiv \frac{\hat{f}_i}{x_i f_i}$$

$$\hat{f}_i = x_i \gamma_i f_i \qquad (6.171)$$

Whence, $\bar{G}_i^E / RT = \ln \gamma_i \qquad (6.172)$

But $G^E = \sum_i x_i \bar{G}_i^E$

On comparing the last two equations we conclude that γi is a partial molar property with respect to G^E. Thus, we have:

$$\ln \gamma_i = \left. \frac{\partial (nG^E / RT)}{\partial n_i} \right|_{T,P,n_{jk}} \qquad (6.174)$$

It follows from the definition of activity coefficient (eqn. 6.163) that for an ideal solution its value is unity for all species as $G^E = 0$. For a non-ideal solution, however, it may be either greater or less than unity, the larger the departure from unity the greater the non-ideality of the solution. The derivatives of the activity coefficient with respect to pressure and temperature can be correlated to the partial molar excess volume and enthalpy respectively.

Using eqn. 6.167 and 6.168 in conjunction with 6.173/6.174 the following results are obtained:

$$\frac{\bar{V}_i^E}{RT} = \left[\frac{\partial \ln \gamma_i}{\partial P}\right]_{T,x} \qquad (6.175)$$

$$\frac{\bar{H}_i^E}{RT^2} = \left[\frac{\partial \ln \gamma_i}{\partial T}\right]_{P,x}$$

As we have seen in the last section that the value of the function $\left[\frac{\partial(G^E/RT)}{\partial T}\right]_{P,x}$ is relatively larger compared to $\left[\frac{\partial(G^E/RT)}{\partial P}\right]_{T,x}$; thus comparing eqns. 6.167 and 6.168 with 6.175 and 6.176 respectively, one may conclude that the activity coefficients are far *more* sensitive to changes in temperature than to changes in pressure. For this reason for phase equilibria computations at low to moderate pressures, the activity coefficients are assumed invariant with respect to pressure.

Since the activity coefficients are partial molar properties, they are related by the Gibbs Duhem equation (at constant temperature and pressure) as follows:

$$\sum_i x_i d \ln \gamma_i = 0 \qquad (6.177)$$

The above equation may be used to validate or check the consistency of experimental data on isothermal activity coefficients for a binary system. The following equation may be derived from eqn. 6.177 for this purpose (by assuming negligible effect of pressure on the liquid phase properties):

$$\int_{x_1=0}^{x_1=1} \ln\left(\frac{\gamma_1}{\gamma_2}\right) dx_1 = 0$$

Thus, if the function ln (γ_1/γ_2) is plotted over the entire range of x_1, (fig. 6.8) the two areas above and below the x-axis in the resulting curve must add up to zero, if the activity coefficients are consistent. Representative values and the

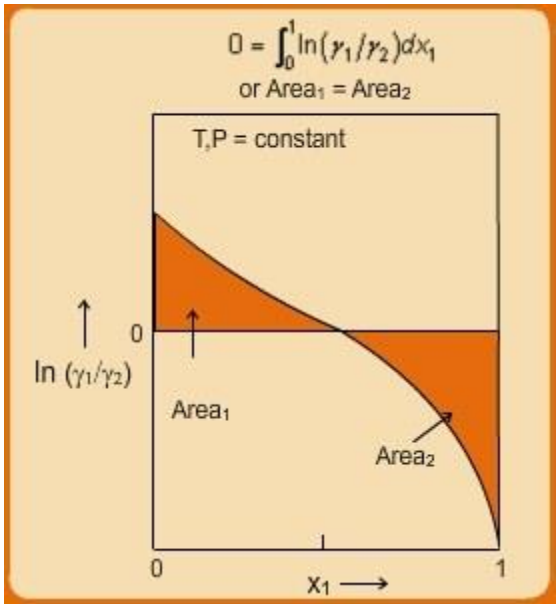


Figure 6.8 Thermodynamic consistency tests for activity coefficients in binary mixtures.

nature of variation in the magnitude of activity coefficients is shown in fig. 6.9; they correspond to the same systems for which excess property variations were depicted in fig. 6.5.

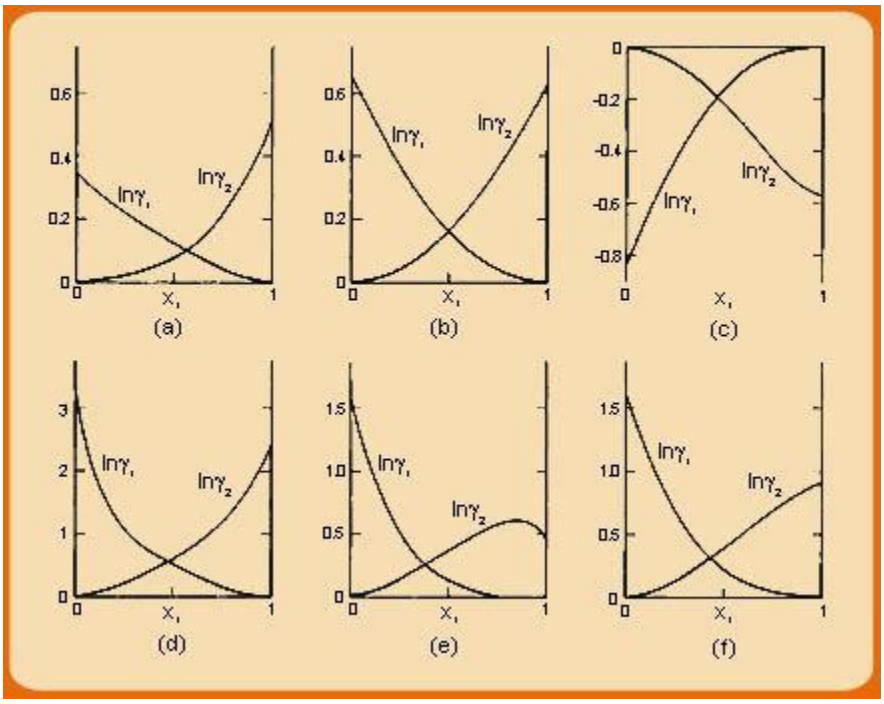


Fig. 6.9 Activity coefficients at 50°C for 6 binary liquid systems: (a) chloroform(1)/n-heptane(2); (b) acetone(1)/methanol(2); (c) acetone(1)/chloroform(2); (d) ethanol(1)/n-heptane(2); (e) ethanol(1)/chloroform(2); (f) ethanol(1)/water(2). (Source: H.C Van Ness and M. M. Abbott, Perry's Chemical Engineer's Handbook (7th ed.), McGraw Hill, 1997.

Activity Coefficient Models

In this section we present the forms of the various activity coefficient models commonly used for VLE calculations. Such models may be divided into two major groups depending upon their applicability to various types of solutions:

1. Margules/Van Laar/Regular Solution Models
2. Wilson/NRTL (Non-Random Two Liquid)/UNIQUAC (Universal Quasi Chemical) Model.

The models in Group 1 are termed as "Homogeneous Mixture" models, while those in Group 2 are termed "Local Composition" models. The reason for such distinction is as follows. The non-ideal behaviour of liquid solutions derives from two sources: (i) difference in *molecular size/shape* of constituent (ii) and the difference between the *inter-species and intra-species molecular interaction energies*. The Group 1 models generally apply to systems in which the inter-species and intra-species molecular interaction energies differ from each other, but in a relatively moderate measure. On the other hand type 2 models are principally useful for describing systems where the constituent molecular species differ on account of both size/shape as well molecular interaction energies. Thus while group I models useful for moderate deviation from ideal solution behaviour, those in group 2 represent strongly non-ideal solutions.

In case the chemical species in the solution do not differ significantly in terms of size/shape, they tend to distribute uniformly across the entire solution volume. Accordingly Group 1 models are based on the premise that molecules are "homogeneously" distributed over the solution volume in that there is no difference between the overall *macroscopic* composition and *microscopic* (local) composition around a single central molecule. The Group 2 models, however, account for both types of differences: in the size of the molecules of the chemical species as well as in their interaction energies. It is reasonable to expect that owing to such differences molecular packing at the microscopic level to be non-homogeneous. As a result, the local composition around a central molecule is likely to differ from the average macroscopic composition.

All models used for predicting species activity coefficient contain about 2 – 3 parameters. Models which employ a larger number of parameters improve the accuracy of prediction but at the same time are rendered computationally difficult. Additionally a larger number of experimentally determined values of activity coefficients are needed for fixing the values of the model parameters. In general, these parameters are much more sensitive to variations in temperature than pressure. This has already been highlighted while discussing the significance of eqns. 6.175 and 6.176. Thus, if computations of the activity coefficients occur over reasonably low range of pressures (typically about 10-20 bar) the model parameters may be assumed to remain invariant. The dependence of enthalpic quantities on temperature is usually strong. Thus, once again inspecting eqn. 6.176, we may conclude that, in general, the dependence of activity coefficients on temperature variations is relatively more significant. If the temperature variations over which activity coefficients are needed exceed ~ 100C, the effect of temperature on model parameters need to be considered. Table 6.1 presents the domain of applicability of the various commonly used activity coefficient models. For simplicity, we provide in the table 6.2 the model expressions for *binary solutions* and that of the corresponding activity coefficient formulas. In table 6.3 expressions of select models for higher order liquid mixtures are presented.

Except for the NRTL model, all others are characterized by two parameters that are essentially*adjustable* with variations of temperature. For the Wilson equation in the above table the parameters are defined as follows:

$$\Lambda_{12} = \frac{V_2}{V_1}\exp\left(-\frac{a_{12}}{RT}\right);\ \Lambda_{21} = \frac{V_1}{V_2}\exp\left(-\frac{a_{21}}{RT}\right) \qquad (6.183)$$

Here V_1 and V_2 are the pure component molar volumes at the temperature of the system; and a_{12} and a_{21} are constants for a given pair of components. However, as the expressions in 6.183 indicate, the parameters Λ_{12} and Λ_{21} are temperature dependent. The parameters for NRTL are, α, τ_{12}, and τ_{21}, the latter two are temperature dependent.

Table 6.3 Expressions for the Molar Excess Gibbs Energy and Activity Coefficients of Multi-component Systems obeying Wilson & NRTL Models

Name	Molar excess Gibbs energy	Activity coefficient for component *i*
Wilson	$\frac{G^E}{RT} = -\sum_i^N x_i \ln\left(\sum_j^N x_j \Lambda_{ij}\right)$	$\ln\gamma_i = 1 - \ln\left(\sum_j^N x_j\Lambda_{ij}\right) - \sum_k \frac{x_k\Lambda_{ki}}{\sum_j^N x_j\Lambda_{kj}}$
NRTL	$\frac{G^E}{RT} = \sum_i^N x \frac{\sum_j^N \tau_{ji}G_{ji}x_j}{\sum_k^N G_{ki}x_k}$	$\ln\gamma_i = \frac{\sum_j^N \tau_{ji}G_{ji}x_j}{\sum_k^N G_{ki}x_k} + \sum_k^N \frac{x_j G_{ij}}{\sum_k^N G_{kj}x_k}\left(\tau_{ij} - \frac{\sum_k^N x_k\tau_{kj}G_{kj}}{\sum_k^N G_{kj}x_k}\right)$

A common feature of all the models are that the parameters are basically related to the infinite dilute a coefficients for each binary. Table 6.4 provides the relevant relations for each model.

Table 6.4 Relation between the model parameters and infinite dilute activity coefficients

Model	*Relation between the model parameters and infinite dilute activity coefficients*
Two-suffix Margules	$\ln\gamma_1^\infty = \ln\gamma_2^\infty = A$
Three-suffix Margules	$\ln\gamma_1^\infty = A_{12};\ \ln\gamma_2^\infty = A_{21}$
Van Laar	$\ln\gamma_1^\infty = A_{12};\ \ln\gamma_2^\infty = A_{21}$
Wilson	$\ln\gamma_1^\infty = 1 - \Lambda_{21} - \ln\Lambda_{12};\ \ln\gamma_2^\infty = 1 - \Lambda_{12} - \ln\Lambda_{21}$
NRTL	$\ln\gamma_1^\infty = \tau_{21} + \tau_{12}\exp(-\alpha\tau_{12});\ \ln\gamma_2^\infty = \tau_{12} + \tau_{21}\exp(-\alpha\tau_{21})$

Example 6.10

Methanol (1)-acetone (2) system is described by the Van Laar activity coefficient model. At 60^0C, the model parameters are $A_{12} = 0.47;\ A_{21} = 0.78$. Estimate the activity coefficients for a solution containing 10mole% of methanol.

$$\ln \gamma_1 = \frac{A_{12}}{\left(1+\frac{A_{12}x_1}{A_{21}x_2}\right)^2};\ln \gamma_2 = \frac{A_{21}}{\left(1+\frac{A_{21}x_2}{A_{12}x_1}\right)^2}$$

For $x_1 = 0.1$, $\gamma_1 = 1.5219$, $\gamma_1 = 1.0032$

Regular Solution (RS) Model

This activity coefficient model is derived for solutions that show moderate deviations from ideal solution behaviour, and for which V^E and S^E are both zero. The model was proposed by Scatchard and Hildebrand (J. H. Hildebrand & R. L Scott. *The Solubility of Non-electrolytes*, Dover, 1964) and constitutes one of few models that are derived from theory. Known as the *Regular Soution Model*, the molar excess Gibbs free energy function is given by:

$$G^E / RT = \left(x_1V_1 + x_2V_2\right)\Phi_1\Phi_2\left(\delta_1 - \delta_2\right)^2 \qquad (6.184)$$

$$\ln \gamma_1 = V_1\Phi_2^2\left(\delta_1 - \delta_2\right)^2 \qquad (6.185)$$

$$\ln \gamma_2 = V_2\Phi_1^2\left(\delta_1 - \delta_2\right)^2 \qquad (6.186)$$

where, Φ_i = volume fraction = $\frac{x_iV_i}{V_m}$; V_i = molar volume of pure ith species, and $V_m = \sum_i x_iV_i$

Further: δ_i = solubility parameter for i^{th} species, which is given by:

$$\delta_i = \sqrt{\frac{\left(\Delta U_i^{vap}\right)}{V_i}} \cong \sqrt{\frac{\left(\Delta H_i^{vap} - RT\right)}{V_i}} \qquad (6.187)$$

Where, ΔU_i^{vap}, ΔH_i^{vap} are molar internal energy, and enthalpy of vapourization of i^{th} species at the temperature of interest.

The above relations may be extended to multi-component mixtures for which:

$$RT\ln\gamma_i = V_i\left(\delta_1 - \bar{\delta}\right)^2 \qquad (6.188)$$

$$\bar{\delta} = \sum_j \Phi_j\delta_j \qquad (6.189)$$

$$\Phi_j = \frac{x_j V_j}{\sum_j x_j V_j} \quad (6.190)$$

; (where, j R runs over all the species)

The solubility parameters for a set of select substances are provided in Appendix IV.

Example 6.11

Use of Regular Solution Model to estimate activity coefficients for an equimolar benzene (1) / cyclohexane (2) solution 350°K. The solubility parameters are: d_1=9.2 (cal/cm^3)1/2; d_2 = 8.2 (cal/cm^3)$^{1/2}$. The molar volumes: V_1^L = 88 cm^3/mol; V_2^L = 107 cm^3/mol

Volume fraction = $\Phi_1 = x_1 V_1^L / \left(x_1 V_1^L + x_2 V_2^L\right)$ = 88 / (88 + 107) = 0.45

$\Phi_2 = 1 - \Phi_1 = 0.55$

$$RT \ln \gamma_1 = V_1^L \Phi_2^2 (\delta_1 - \delta_2)^2 = 88 \; x \; 0.55^2 \left[9.2 - 8.2\right]^2$$

R = 1.987 cal/mol, T = 350°K

Hence $\ln \gamma_1$ = 0.038 $\Rightarrow \gamma_1$ = 1.04

Similarly RT $\ln \gamma_2 = V_2^L \Phi_1^2 (\delta_1 - \delta_2)^2$

Hence γ_2 = 1.03

UNIQUAC: Group Contribution Method for Evaluation of Activity Coefficients

The acronym UNIQUAC stands for *Universal Quasi Chemical Equation*. The principle that the UNIQUAC method is founded on is that of *group contribution*. The methodology is based on the postulate that any thermo-physical property of a pure substance is a *weighted sum* of the chemical groups that constitute it. Since the number of functional groups (such as $-CH_3, -CH_2, -OH, -COOH$, etc.) are few (about 50 or so) in comparison to the vast number of chemical species they can form, the approach based on group contribution constitutes an universal, and indeed a versatile method of prediction of a wide variety of properties of a very large number of substances. Properties as diverse as normal boiling point, enthalpy of vapourization, molar volume, thermal conductivity, viscosity, surface tension, etc may be estimated when no direct experimental data are available.

While estimating any property, a molecule is decomposed into the functional groups that make it up. Intermolecular interactions (in a pure fluid or a mixture) are then considered to be approximately equal to the weighted sum of group-group interactions. Characteristic quantitative values representing group interactions have been obtained from regression of experimental data for binary systems. The use of this approach for predicting a wide variety of number of physic-chemical properties has been demonstrated comprehensively by Reid et. al. (J C. Reid, J. M. Prausnitz, and B. E. Polling, *The Properties of Gases and Liquids*, 4th ed. McGraw Hill, 1987). A group contribution method is, however, necessarily approximate as it is based on the simple additivity of contributions from each group; further it assumes the contribution made by each group is independent of the nature of the adjacent group.

In extending the method to prediction of activity coefficients, the molar excess Gibbs free energy is divided into two parts: *combinatorial* and *residual*. The combinatorial part accounts for non-ideality of a mixture arising from *differences in size and shape* of constituent molecular species; whereas the residual part considers the *difference between inter-molecular and intra-molecular interaction energies.*

We provide there the final UNIQUAC activity coefficient relations for a binary. It is may be easily extended to represent ternary and higher order mixtures (J.M. Prausnitz, R.N. Lichtenthaler and E.G. Azevedo, *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed., Prentice Hall, 1998). The function $g \equiv G^E / RT$ is comprised of the combinatorial and residual parts as follows:

$$g = g^C + g^R \tag{6.191}$$

The combinatorial part represents pure species parameters, while the residual part contains two binary parameters for each pair of molecules. In general for a system comprised of N molecular species, we have the following relations:

$$g^C = \sum_i^N x_i \ln \frac{\Phi_i}{x_i} + 5\sum_i^N q_i x_i \ln \frac{\theta_i}{\Phi_i} \tag{6.192}$$

$$g^R = -\sum_i^N q_i x_i \ln \left(\sum_j \theta_i \tau_{ji} \right) \tag{6.193}$$

Where:

$$\Phi_i = \frac{x_i r_i}{\sum_j^N x_j r_j} \tag{6.194}$$

$$\theta_i = \frac{x_i q_i}{\sum_j^N x_j q_j} \tag{6.195}$$

r_i = relative molecular volume; q_i = relative molecular surface area (both are pure species parameters)

The binary interaction parameters τ_{ji} and τ_{ij} which need to be regressed from binary VLE data are temperature dependent. They are the UNIQUAC *model parameters* and are provided by:

$$\tau_{ji} = \exp\left[-\left(\frac{u_{ji}-u_{ii}}{RT}\right)\right]; \text{ and, } \tau_{ij} = \exp\left[-\left(\frac{u_{ij}-u_{jj}}{RT}\right)\right] \quad (6.196)$$

The activity coefficient is also divided into two parts:

$$\ln\gamma_i = \ln\gamma_i^C(combinatorial) + \ln\gamma_i^R(residual) \quad (6.197)$$

$$\ln\gamma_i^C = 1 - J_i + \ln J_i - 5q_i\left(1 - \frac{J_i}{L_i} + \ln\frac{J_i}{L_i}\right) \quad (6.198)$$

$$\ln\gamma_i^R = q_i\left(1 - \ln s_i - \sum_j^N \theta_j \frac{\tau_{ij}}{s_j}\right); \rightarrow (j \equiv \text{dummy variable}) \quad (6.199)$$

$$J_i = \frac{r_i}{\sum_j^N r_j x_j} \quad (6.200)$$

$$L_i = \frac{q_i}{\sum_j^N q_j x_j} \quad (6.201)$$

$$s_i = \sum_l^N \theta_l \tau_{li};\ (l \rightarrow dummy\ index)$$

The computation of the parameters J_i, L_i and s_i is based on the UNIFAC (*Universal Functional Group Activity Coefficients*) method (see eqns. 6.202 and 6.203 below), which is an extension of the UNIQUAC model. The application of the UNIFAC method is premised on the idea that a solution is composed of the sub-groups rather than molecules themselves. The groups are essentially small, self-contained chemical units, each designated as *k*, for which the relative volume and surface areas are denoted asR_k and Q_k respectively. When a molecule may be constructed from more than a single set of subgroups, the one with the least number of different subgroups is assumed as the correct one. The values of R_k and $Q_k$for various common subgroups are shown in table 1 of *Appendix V*. More exhaustive tables may be found elsewhere (J.M. Prausnitz, R.N. Lichtenthaler and E.G. Azevedo,*Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed., Prentice Hall, 1998).

Apart from the dependence on pure species properties species R_k and Q_k, the activity coefficients are functions of interaction between the various subgroups., a cluster of sub-groups are classified under a main group, which are primarily descriptive. All subgroups under a main group are identical with respect to group interactions. Accordingly the interaction between group parameters is identified with pairs of main

groups under which the former are classified. Parametric values of select pairs of main groups are provided in table 2 of*Appendix V*.

One of the distinct advantages of the UNIFAC model is that it is able to predict activity coefficients more effectively in mixtures where constituent molecules have large size differences, as in polymer solutions.

In the UNIFAC method the expression for the combinatorial part of the activity coefficient is the same as that in eqn. 6.196. However, the residual part takes a somewhat different form.

$$\ln \gamma_i^C = 1 - J_i + \ln J_i - 5q_i\left(1 - \frac{J_i}{L_i} + \ln\frac{J_i}{L_i}\right) \tag{6.202}$$

$$\ln \gamma_i^R = q_i\left[1 - \sum_k\left(\theta_k \frac{\beta_{ik}}{s_k} - e_{ki}\ln\frac{\beta_{ik}}{s_k}\right)\right] \rightarrow (k \equiv subgroup) \tag{6.203}$$

The terms J_i *and* L_i are given by eqns. 6.198 and 6.199. However, the other parameters are defined as:

$$r_i = \sum_k \nu_k^{(i)} R_k \tag{6.204}$$

$$q_i = \sum_k \nu_k^{(i)} Q_k \tag{6.205}$$

$$e_{ki} = \frac{\nu_k^{(i)} Q_k}{q_i} \tag{6.206}$$

$$\beta_{ik} = \sum_m e_{mi}\tau_{mk} \tag{6.207}$$

$\nu_k^{(i)} \equiv$ number of k^{th} subgroup in i^{th} molecular species

$$\theta_k = \frac{\sum_i x_i q_i e_{ki}}{\sum_j x_j q_j} \tag{6.208}$$

$$s_k = \sum_m \theta_m \tau_{mk} \tag{6.209}$$

$$\tau_{mk} = \exp\left(-\frac{a_{mk}}{T}\right) \tag{6.210}$$

Thus, in summary the key UNIFAC parameters are R_k, Q_k and a_{mk}. Tables containing the values of these parameters are recorded more exhaustively elsewhere (H. K. Hansen, P. Rasmusen, Aa. Fredenslund, M.Schiller, and J. Gmehling, *IEC Research*, Vol 30, pp 2352 – 2355, 1991; and R.C Reid, J, M., Prausnitz, and B.E. Poling, in Properties of Gases and Liquids, 4th ed., McGraw-Hill, 1987). All the parameters in the UNIFAC approach are essentially binary and there are effectively two parameters that need to be determined from experimental VLE data.

Example 6.12

Use UNIFAC model to estimate species activity coefficients for an equimolar diethylamine (1) /n-hexane (2) solution at 300°K.

The species are: $CH_3 - CH_2NH - CH_2 - CH_3(1) / CH_3 - (CH_2)4 - CH_3(2)$

In the following table we enlist the subgroups along with their identification numbers, and the values of the relevant UNIFAC parameters, as well as the number of each subgroup in the molecular species. (**Source**: Reid, R.C., Prausnitz, J.M., and Poling, B.E., *The Properties of Gases and Liquids*, 4th ed., McGraw Hill, 1987).

	k	R_k	Q_k	$\nu_k^{(1)}$	$\nu_k^{(2)}$
CH_3	1	0.9011	0.848	2	2
CH_2	2	0.6744	0.540	1	4
CH_2NH	33	1.2070	0.936	1	0

By eqn. 6.204 we have $r_i = \sum_k \nu_k^{(i)} R_k$

Hence:

$r_1 = 3.6836; r_2 = 4.4998$

By eqn. 6.205 we have $q_i = \sum_k \nu_k^{(i)} Q_k$

Hence:

$q_1 = 3.172; q_2 = 3.856$

By eqn. 6.206 we have:

$$e_{ki} = \frac{\nu_k^{(i)} Q_k}{q_i}$$

The foregoing parameters are next used to generate the values of e_{ki}:

$e_{ki} = \frac{\nu_k^{(i)} Q_k}{q_i}$		
k	$i=1$	$i=2$
1	0.5347	0.4398
2	0.1702	0.5602
33	0.2951	0

The interaction parameters for the various subgroups are enumerated below (**Source**: Reid, R.C., Prausnitz, J.M., and Poling, B.E., *The Properties of Gases and Liquids*, 4th ed., McGraw Hill, 1987).

$a_{1,1} = a_{1,2} = a_{2,1} = a_{2,2} = a_{33,33} = 0K$

$a_{1,33} = a_{2,33} = 255.7K$

$a_{33,1} = a_{33,2} = 65.33K$

Now we use eqn. 6.210, $\tau_{mk} = \exp\left(-\frac{a_{mk}}{T}\right)$ to obtain the following values at T = 300K:

$\tau_{1,1} = \tau_{1,2} = \tau_{2,1} = \tau_{2,2} = \tau_{33,33} = 1$

$\tau_{1,33} = \tau_{2,33} = 0.4264$

$\tau_{33,1} = \tau_{33,2} = 0.8043$

Next the eqn. 6.207 to estimate the values of β_{ik}.

β_{ik}			
i	$k = 1$	$k = 2$	$k = 3$
1	0.9418	0.9418	0.5956
2	1.0000	1.0000	0.4264

Table 6.1 Applicability of Activity Coefficient Models

System Type	Models
Species similar in size and shape	One-constant Margules
Moderately non-ideal mixtures	Two-constant Margules, Van Laar, Regular Solution
Strongly non-ideal mixtures (for example Alcohols+Hydrocarbons)	Wilson, NRTL, UNIQUAC
Solutions with miscibility gap	NRTL, UNIQUAC

Table 6.2: Select Models for the Excess Gibbs Energy and Activity Coefficients for Binary Systems

Model Name	*GE/RT*	Binary parameters	$\ln \gamma_1$ and $\ln \gamma_2$
Two-suffix Margules	$G^E / RT = Ax_1x_2$ (One-constant)	A	$\ln \gamma_1 = Ax_2^2$
			$\ln \gamma_2 = Ax_1^2$
Three-suffix Margules	$G^E / RT = x_1x_2(A_{21}x_1 + A_{12}x_2)$ (Two-constant)	A_{21}, A_{12}	$\ln \gamma_1 = x_2^2[A_{12} + 2(A_{21} - A_{12})x_1]$
			$\ln \gamma_2 = x_1^2[A_{21} + 2(A_{12} - A_{21})x_2]$
Van Laar	$G^E / RT = \frac{A_{12}A_{21}x_1x_2}{A_{12}x_1 + A_{21}x_2}$	A_{21}, A_{12}	$\ln \gamma_1 = A_{12}/(1 + \frac{A_{12}}{A_{21}}\frac{x_1}{x_2})^2$ $\ln \gamma_2 = A_{21}/(1 + \frac{A_{21}}{A_{12}}\frac{x_2}{x_1})^2$;
			Conversely: $A_{12} = \ln \gamma_1(1 + \frac{x_2 \ln \gamma_2}{x_1 \ln \gamma_1})^2$; $A_{21} = \ln \gamma_2(1 + \frac{x_1 \ln \gamma_1}{x_2 \ln \gamma_2})^2$
Wilson	$\frac{G^E}{RT} = -x_1 \ln(x_1 + x_2\Lambda_{12}) - x_2 \ln(x_2 + x_1\Lambda_{21})$	$\Lambda_{12}, \Lambda_{21}$	$\ln \gamma_1 = \ln(x_1 + x_2\Lambda_{12}) + x_2\left(\frac{\Lambda_{12}}{x_1 + \Lambda_{12}x_2} - \frac{\Lambda_{21}}{\Lambda_{21}x_1 + x_2}\right)$
			$\ln \gamma_2 = \ln(x_2 + x_1\Lambda_{21}) + x_1\left(\frac{\Lambda_{12}}{x_1 + \Lambda_{12}x_2} - \frac{\Lambda_{21}}{\Lambda_{21}x_1 + x_2}\right)$
NRTL	$G^E / RT = x_1x_2\left(\frac{\tau_{21}G_{21}}{x_1 + x_2G_{21}} + \frac{\tau_{12}G_{12}}{x_2 + x_1G_{12}}\right)$ Where, $G_{12} = \exp(-\alpha_{12}\tau_{12})$; $G_{21} = \exp(-\alpha_{12}\tau_{21})$ $\tau_{12} = \frac{b_{12}}{RT}$; $\tau_{21} = \frac{b_{21}}{RT}$		$\ln \gamma_1 = x_2^2\left[\tau_{21}\left(\frac{G_{21}}{x_1 + x_2G_{21}}\right)^2 + \frac{\tau_{12}G_{12}}{(x_2 + x_1G_{12})^2}\right]$
			$\ln \gamma_2 = x_1^2\left[\tau_{12}\left(\frac{G_{12}}{x_2 + x_1G_{12}}\right)^2 + \frac{\tau_{21}G_{21}}{(x_1 + x_2G_{21})^2}\right]$

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Unit 3 Notes
Vapour-Liquid Equilibria

Introduction

Both the general criterion of thermodynamic equilibrium as well as the specific condition of equality of chemical potential of each species which hold at equilibrium was introduced in the last chapter. We now develop the detailed relationships connecting the phase variables (T, P and composition) that originate from the concepts of chemical potential and the fugacity coefficient. The basic principle employed in all separation processes is that under equilibrium the compositions of phases differ from each other, and therefore it is possible to preferentially concentrate one species over another (or others) in one particular phase. This feature of phase equilibria is exploited in a wide variety of process equipments such as distillation, extraction, crystallization, etc.

This chapter focuses on the vapour-liquid equilibria (VLE) problem which is depicted schematically in fig. 7.1. When a multi-component, vapour and liquid phase – each (say) containing N chemical species – co-exist in thermodynamic equilibrium at a temperature T and pressure P, the phase compositions $\{ y_{1,} y_{2,} ...y_{N-1} \}$ and $\{ x_{1,} x_{2,} ...x_{N-1} \}$ remain invariant with time, and are related by a unique set of relations. If the relations are known as a function of temperature, pressure and compositions $\{ y_i \text{ and } x_i \}$ for each species, then provided some of these variables are specified the rest may be calculated. We will derive such relations for real multi-component systems; but as in all cases of thermodynamic modeling we take the ideal system as our starting point. Next the VLE of systems at moderate pressures are treated. Finally the relations for VLE at high pressures are presented. In the following section we derive the relations that hold for pure component VLE before describing those which apply to multi-component systems.

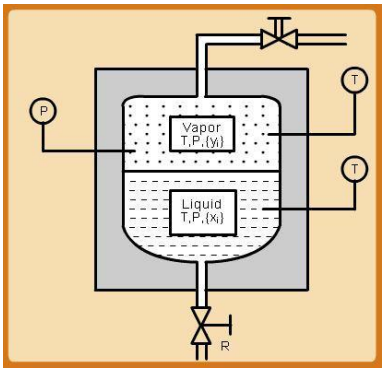


Fig. 7.1 The VLE Problem Description

Single Component System Phase Equilibria

We start with the general criterion of equality of the chemical potential in the two phases. To generalize the results we assume that any two types of phases α and β of a pure component are at equilibrium. Thus as given by eqn. 6.50:

$$\mu_i^{\alpha} = \mu_i^{\beta} \quad ..(6.50)$$

However, for a pure component the chemical potential is reduces to the pure component molar Gibbs free energy. Therefore:

$$\mu_i^{\alpha} = G^{\alpha} \text{ and, } \mu_i^{\beta} = G^{\beta} \quad ..(7.1)$$

Thus eqn. 6.50 reduces to:

$$G^{\alpha} = G^{\beta} \quad ..(7.2)$$

On taking a differential:

$$dG^{\alpha} = dG^{\beta} \quad ..(7.3)$$

Using the generic relationship in eqn. 5.7 we may write in keeping with the fact that for a given equilibrium temperature, the equilibrium pressure corresponds to the saturation vapour pressure P^{sat} :

$$V^{\alpha} dP^{sat} - S^{\alpha} dT = V^{\beta} dP^{sat} - S^{\beta} dT \quad ..(7.4)$$

On rearranging:

$$\frac{dP^{sat}}{dT} = \frac{S^{\beta} - S^{\alpha}}{V^{\beta} - V^{\alpha}} = \frac{\Delta S^{\alpha\beta}}{\Delta V^{\alpha\beta}} \quad ..(7.5)$$

Additionally using the second law we have:

$$dS = dQ / T \quad ..(7.6)$$

And that for a constant pressure process:

$$dQ = dH \quad ..(7.7)$$

Using eqns. (7.6) and (7.7) we obtain:

$$\Delta H^{\alpha\beta} = T \Delta S^{\alpha\beta} \quad ..(7.8)$$

Thus, $\Delta S^{\alpha\beta} = \Delta H^{\alpha\beta} / T$, and substitution in eqn.7.5 gives:

$$\frac{dP^{sat}}{dT} = \frac{\Delta H^{\alpha\beta}}{T \Delta V^{\alpha\beta}} \quad ..(7.9)$$

The last equation is called the *Clapeyron* equation. For the specific case of phase transition from liquid (*l*) to vapor (*v*), it translates into:

$$\frac{dP^{sat}}{dT} = \frac{\Delta H^{LV}}{T\Delta V^{LV}} \quad ..(7.10)$$

Noting that liquid phase molar volumes are relatively much lesser than vapour phase volumes, we may write, $\Delta V^{LV} = V^{V} - V^{V} \cong V^{v}$ ..(7.11) Further at low to moderate saturation pressures if we assume ideal vapour phase behaviour, then

$$V^{v} \cong \frac{RT}{P^{sat}} \quad ..(7.12)$$

Eqn. 7.10 then becomes:

$$\frac{dP^{sat}}{dT} = \frac{\Delta H^{LV}}{RT / P^{sat}}$$

Or:

$$\frac{dP^{sat} / P^{sat}}{dT / T^{2}} = \frac{\Delta H^{LV}}{R} \quad ..(7.13)$$

$$\text{Whence, } \Delta H^{LV} = -R\left[\frac{d \ln P^{sat}}{d(1/T)}\right] \quad ..(7.14)$$

This approximate equation is known as the *Clausius-Clapeyron equation*. The assumptions used in the above derivations have approximate validity only at low pressures. Integrating eqn. 7.14 we have:

$$\ln P^{sat} = A - \frac{B}{T} \quad ..(7.15)$$

On comparing eqns. 7.14 and 7.15, it follows that: $B = \Delta H^{LV} / R$, while A is the constant of integration. These are generally regarded as constants for a given species. A plot of experimental values of lnP^{sat} vs. *1/T* generally yields a line that is nearly straight between the triple and critical points. However, the validity of eqn. 7.15is questionable at relatively high pressures, and certainly in the critical region. Thus the accuracy of the Clausius-Clapeyron equation reduces at higher pressures. A modified form of eqn. 7.15, called the Antoine Equation, has proved to be more accurate (including at higher pressures), has the following form:

$$\ln P^{sat} = A - \frac{B}{T + C} \quad ..(7.16)$$

A, B, and C are readily available for a large number of species.

More complex forms of equations relation temperature and

vapour pressure of pure substances have been reported in the literature, which provide even greater accuracy. An example of such an equation is the Wagner equation, which is given by:

$$\ln P_r^{sat} = \frac{A\tau + B\tau^{1.5} + C\tau^3 + D\tau^6}{1-\tau}, \text{ where } \tau = 1 - T_r \quad ..(7.17)$$

The constants for the Wagner equation for specific substances are available in several reference texts (see R.C. Reid, J.M. Prausnitz and B.E. Poling, *Properties of Gases and Liquids*, 4th ed., McGraw-Hill, 1987).

Derivation of the Phase Rule

The phase rule was introduced in section 1.5 without proof. Here we develop its mathematical form based on the tenets of solution thermodynamics and phase equilibrium criterion presented in the last chapter. Consider a *non-reactive* system under equilibrium, with π phases each containing N independent chemical species. The degrees of freedom for the system, i.e., the number of intensive variables that may vary independently of each other would be given by:

Degrees of freedom = Total number of systemic intensive variables –number of independent equations relating all the variables.

For the system of interest here the above terms are as follows:

I. Total number of systemic intensive variables (also called the phase rule variables)= *T, P and (N-1)* species mole fractions for each of the π phases

II. Number of independent relations connecting the phase rule variables = $(\pi - 1)N$

The second relation above follows from the fact that for each of the *N* species one may use the chemical potential equality relation across all π phases, as described by eqn. 6.52. It follows that for each component there can be only $(\pi - 1)$ independent relations.

Thus the phase rule may be rewritten as:

$$F = \left[2 + (N - 1)\pi \right] - \left[(\pi - 1) N \right] = 2 + N - \pi \quad (7.18)$$

It may be noted that the actual mass of each of the species present are not considered as phase rule variables, as they cannot influence the intensive state of the system. A special case of the phase rule obtains for closed systems for which the initial mass for each species is fixed. Since no mass can

enter or leave the system, the extensive state of the system is rendered fixed along with the intensive variables. Therefore, apart from the $(\pi - 1)N$ constraining relations involving the species chemical potentials, there is an additional $[(N-1)\pi]$ constraint on the mass of each species; this follows from the fact that if a quantum of a species leaves a phase it must reappear in another or more. Thus the phase rule eqn. leads to:

$$F = [2 + (N-1)\pi] - [(\pi - 1)N] - [(N-1)\pi] = 2 \qquad ..(7.19)$$

The above equation is known as the *Duhem's theorem*. It implies that for any closed system formed initially from given masses of a number of chemical species, the equilibrium state is completely determined when any two independent variables are fixed. The two independent variables that one may choose to specify may be either intensive or extensive. However, the number of *independent intensive* variables is given by the phase rule. Therefore, it follows that when F = 1, at least one of the two variables must be extensive, and when F = 0, both must be extensive.

Description of General VLE Behavior

Before presenting the mathematical formulation of the multi-component VLE problem it is pertinent to discuss some key features of typical vapour liquid phase behaviour. The description of phase behaviour of vapour and liquid phases co-existing under equilibrium can be complex and difficult to visualize for systems containing a large number of chemical species. Thus, to clarify matters it is useful to consider a binary system. The considerations for such a system may, in principle, be generalized to understand the behaviour of multi-component systems. However, we restrict ourselves to description of VLE of multi-component systems to the corresponding mathematical formulation.

When N = 2, the phase rule yields a degree of freedom $F = 4 - \pi$. In the general VLE problem since there are at least two phases, the corresponding number of independent intensive variables become 2. In the case of extractive distillation, there are at least two liquid phases and a vapour phase, whence the degrees of freedom reduce to 1.In the following discussion we present the phase behaviour of the simplest case of a binary VLE. The more volatile of the two is designated as component (1). The phase rule variables for this case are: T, P, y_1 and x_1. Graphical plots of experimentally obtained phase behaviour can then be expressed as a functionof various combinations of these variables. For example at a given temperature one may plot two curves, *P vs.* x_1 (or in short *P-*x_1) and *P vs.* y_1. Similarly on specifying a certain pressure, one may plot *T vs.* x_1 and *T vs.* y_1. In addition if one fixes either x_1 or y_1 one may plot P-T diagrams. The combination of these diagrams lead to a 3-dimensional surface

involving pressure, temperature and phase compositions on the three axes. A typical plot is shown in fig. 7.2.

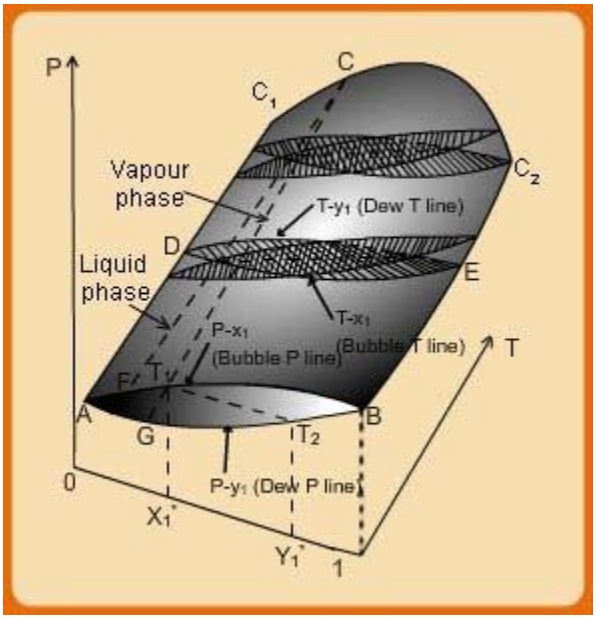


Fig. 7.2 Three dimensional VLE phase diagram for a binary system

Consider first a case of VLE phase behaviour at a constant temperature. This is in shown as the "lens" AB. The lower part of the lens corresponds to *P*-y_1 while upper curve is the *P*-x_1 plot. The values of the vapour and liquid compositions at any system equilibrium pressure are found by drawing a line parallel to the $x_1 - y_1$ axis. The point of intersection of this line with the upper curve provides the saturated liquid phase composition (x_1) and is termed the *bubble pressure line*; while that with the lower curve corresponds to the saturated vapour phase composition (y_1) and is termed the *dew pressure line*. Extending the description the entire upper face of the three dimensional surface constitutes the bubble surface. Any point above it corresponds to the state of sub-cooled liquid. In the same manner, the lower face represents the dew surface. For any point below this face the state is that of a superheated vapour. The line that connects the phase compositions (x_1^* *and* y_1^*) is called the *tie line* (shown as T_1-T_2). Such tie lines may be drawn at any other pressure and the same considerations as above are valid.

Similarly, if one considers isobaric plots (shown as the lens DE), for any equilibrium temperature the vapour and liquid phase compositions are found by drawing a line that passes through the specific temperature and is parallel to the $x_1 - y_1$ axis. The upper intersection point provides the

vapour phase composition (*dew temperature line*), while the lower one corresponds to the liquid phase composition (*bubble temperature line*). Finally if one fixes the composition at a point on the $x_1 - y_1$ axis the intersection of a vertical plane through the composition point with the 3-dimension surface yields the curve FCG. The upper part of this curve, i.e., FC corresponds to the locus of liquid phase compositions while the lower one (CG) is that of the vapour phase compositions. The point C is the meeting point of the two curves and defines the critical point of the mixture at the composition specified by the original vertical plane at a point on the $x_1 - y_1$ axis. At the two end points of the composition axis are the pure component P-T plots which terminate at the critical points, C_1-C_2, of the two substances.

If one takes a series of varying isothermal or isobaric "lenses", two types of plots result, which are shown in fig. 7.3. Consider the P-x-y plot. The lowest lens (T_X) corresponds to the situation already described in the last paragraph (i.e., the lens MN). However, the one at T_Y corresponds to a case for which $T_{C\,1} < T_Y$, but $T_{C\,2} > T_Y$. Thus at this temperature the vapour and liquid phases of the pure component '1' cannot co-exist, and hence the P-x-y plot vanishes as the composition tends to $x_1 \rightarrow 1$.
If one moves to a still higher temperature say T_Z the P-x-y "hangs" at both ends of the $x_1 - y_1$ as both $T_{C\,1} < T_Z$, and $T_{C\,2} < T_Z$. The considerations for the isobaric lenses at P_X , P_Y *and* P_Z are the same as for the isotherms, i,e., for the highest isobar, both $P_{C\,1} < P_Z$, and $P_{C\,2} < P_Z$ and so on.

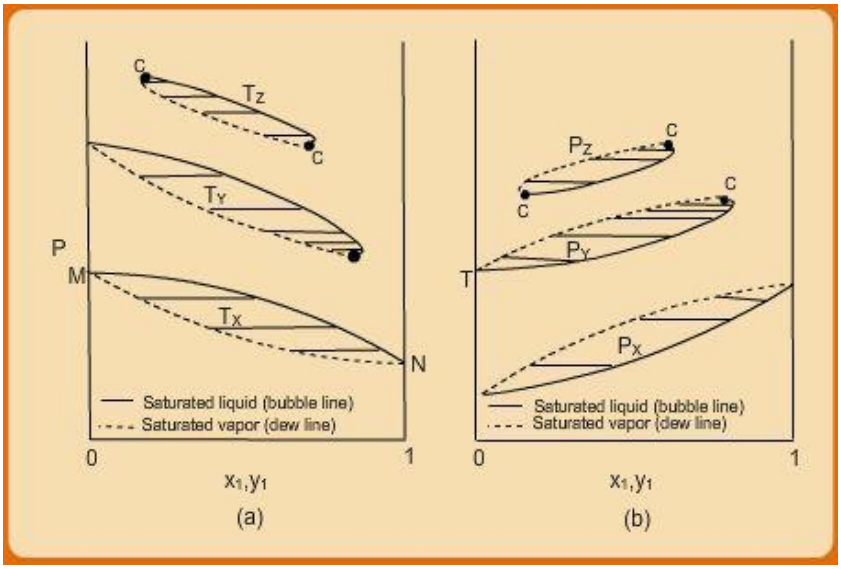


Fig. 7.3 (a) *P-x₁–y₁* diagrams for three temperatures. (b) *T-x₁-y₁* diagrams for three pressures

If one considers now a series of P-T plots they correspond to the curves shown in fig, 7.4. The lines I-J

and K-L which represent the vapour pressure-vs.-T curves for pure species 1 and 2 respectively. At other intermediate compositions the upper and lower plots (obtained by intersection of a vertical plane at constant point on the $x_1 - y_1$ axis, and the P-T-x-y surface of fig. 7.2) constitute a curve that rounds off at the *true* critical point of the mixture (as opposed to the "pseudo-critical" temperature

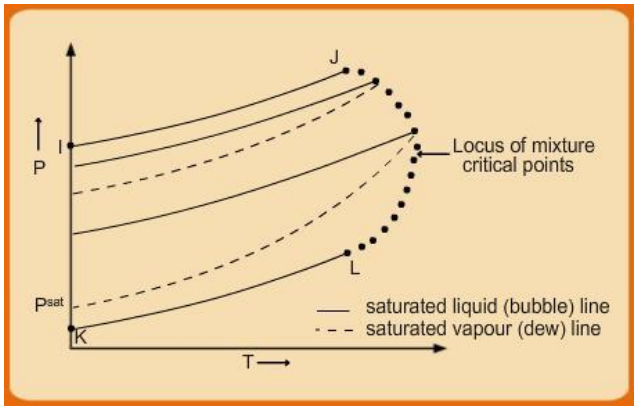


Fig. 7.4 P-T diagram for various compositions

and pressure of mixtures discussed in section 2.4). The critical points of the various mixtures of the two species thus lie along a line on the rounded edge of the surface between J and L; it is, therefore composition dependent. Each interior loop represents the *P-T* behavior of saturated liquid and of saturated vapour for a mixture of *constant composition;* the loops differ from one composition to another. It follows that the *P-T* relation for saturated liquid is different from that for saturated vapour of the same composition. This is in contrast with the behavior of a pure species, for which the bubble and dew lines are the same, as for I-J and K-L.

The above discussion suggests that the phase behaviour of even a simple binary can be relatively more difficult to interpret in its complete three-dimensional form. Reducing such behaviour to two-dimensional plots enables easier visualization of the phase behaviour and understanding their features. However, in many instances, even two dimensional plots can be more complex in nature. Examples of such curves (as are often encountered with solvent mixtures in industrial practice) are shown schematically in the form of (*P-x-y)* in fig. 7.5, for systems at relatively low pressures ($\leq$1 *atm*).

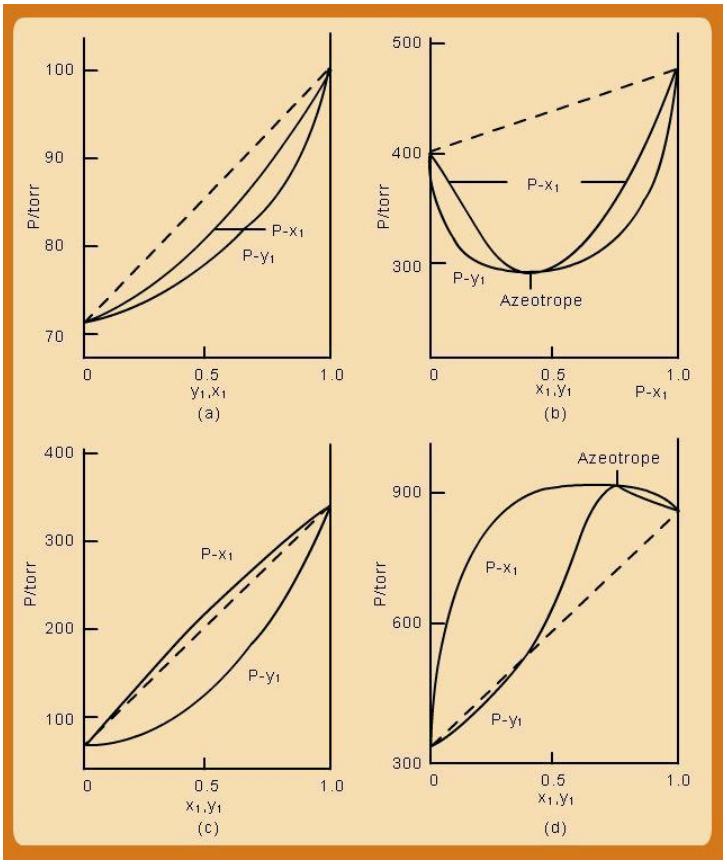


Fig. 7.5 Schematic P-x-y plots showing deviation from ideal VLE behaviour

All the four systemic VLE behaviour exhibits deviation from ideal vapour-liquid systems. As discussed in the next section ideal VLE behaviour is characterized by Raoult's Law (RL). Suffice it mention here that non-ideal VLE systems may exhibit both *negative* (figs. 7.5a & 7.5b) and *positive* deviations (figs. 7.5c and 7.5d) from that which obeys the Raoult's law (RL). The RL behaviour is typified by the dotted $P\text{-}x_1$ lines in each set in fig. 7.5. When the *actual* $P\text{-}x_1$ line lies below the RL-line, the system is said to show negative deviation from RL, while if it lies above it is indicative of a positive deviation.

From a molecular thermodynamic viewpoint, negative deviations occur if the 1-2 type (between unlike species) of molecular interactions (attraction) are *stronger* than 1-1 or 2-2 (between like species) type of interaction. As a result, the molecules of the more volatile component (1) are "constrained" by those of component 2 from transiting to the vapour phase to a *greater* extent than in the case when the former is present in a pure form. This effectively translates into an equilibrium pressure less than the RL system at the same liquid phase composition; hence the actual system displays "negative" deviation in comparison to RL for which 1-1, 2-2, and 1-2 types of interactions are

more or less the same. Appreciable negative departures from P-x_1 linearity reflect strong liquid-phase intermolecular interaction. The opposite applies to the case where a system shows a positive deviation from RL; that is, the interaction between the unlike species of molecules is *lower* than that between like molecules.

There is an additional complexity evident in figs. 7.5c and 7.5d. Considering the former we see that it is defined by two distinct types of behaviour on either sides of the point termed as the "azeotrope". At this point the *P*-x_1 and the *P*-y_1 curves converge; i.e., the two phases are identical in composition at this point. On left side of the azeotrope $x_1 > y_1$, while on the other side, $x_1 < y_1$. The reverse situation holds for the system depicted in fig. 7.4d. Such systems are not uncommon in the process industry and always pose a difficulty in purifying a mixture to compositions higher than the azeotropic point. This is because during a distillation process when the mixture composition arrives at the azeotropic point the two phases become identical in composition, and the liquid composition does not alter further during evaporation.

The phase behaviour of the same systems as in fig. 7.5 is depicted as *T*-*x*-*y* diagrams in fig. 7.6. In particular we refer to the figs 7.6b and 7.6d. As expected the P-x curves *appear inverted* on a T-x diagram. The first kind of system (fig. 7.6b) is said to show a *maximum* boiling point azeotrope, while that depicted by fig. 7.5d shows a *minimum* boiling point azeotropic behaviour.

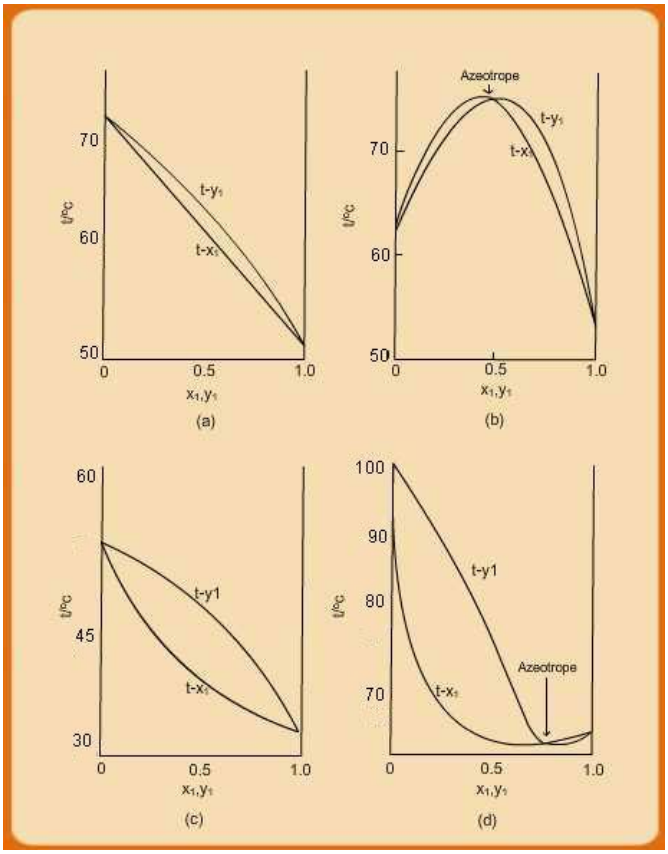


Fig. 7.6 Schematic T-x-y plots showing deviation from ideal VLE behaviour

Raoult's Law for VLE

As mentioned in the concluding part of the last section, vapour-liquid systems which are *ideal* in nature display a behaviour corresponding to Raoult's Law. In such a system, both the vapour and the liquid phases essentially behave as ideal mixtures. For describing the VLE for such systems we start by applying eqn. 6.50, to the vapour and liquid phases:

$$\mu_i^V = \mu_i^L \qquad ..(7.20)$$

For an ideal vapor mixture by eqn. 6.72 we have:

$$\mu_i^{ig} \equiv G_i^{ig} + RT \ln y_i$$

Similarly for an ideal liquid solution eqn. 6.77 provides:

$$\mu^{id} = G_i^{id} + RT \ln x_i$$

Thus rewriting eqn. 7.20 (using expressions provided by eqns. 6.72 and 6.77):

$$\mu_i^{ig} \equiv \mu_i^{id} \qquad ..(7.21)$$

$$G_i^{ig} + RT \ln y_i = G_i + RT \ln x_i \quad ..(7.22)$$

Or: $$RT \ln(y_i / x_i) = G_{ii} - G^{ig} \quad ..(7.23)$$

Since effect of pressure is negligible on liquid properties we assume that:

$$G_i^l(T, P) \; G_i^l(T, P_i^S) \quad ..(7.24)$$

Now for the gas phase: $dG_i^{ig} = V_i^{ig} dP$ (at const T) ..(7.25)

Thus: $$G_i^{ig}(T, P_i^s) - G_i^{ig}(T, P) = RT \int_{P^i}^{P^s} dP / P = RT \ln P(_i^s / P) \quad ..(7.26)$$

Combining eqns. 7.23, 7.24 and 7.26 gives:

$$RT \ln(y_{ii} / x_i) = G_i^l(T, P_i^s) - G_i^{ig}(T, P^s) + RT \ln(P^s / P) \quad ..(7.27)$$

The first two terms on the RHS in equation above correspond to the Gibbs free energy of *pure* liquid and vapour phases under equilibrium conditions, i.e., at (T, P_i^S) ; hence, as shown in section 7.1, these terms equal. Therefore, it follows that:

$$y_i / x_i = P_i^s / P \quad ..(7.28)$$

Alternately: $$y_i P = x_i P_i^S \quad ..(7.29)$$

Equation 7.29 is known as the Raoult's Law.

It may be noted that the conditions for ideal mixture behaviour for the gas and liquid phases are not the same in general. For the gas mixture to be ideal the pressures need to be close to atmospheric or less. While a liquid solution is ideal if the interaction between the *same* molecular species is identical to that between *dissimilar* molecules.

The algorithms needed for generating RL phase diagrams are discussed later in this section. But prior to that, we present examples of *typical* phase diagrams that obtain from the application of the Raoult's law (RL) equations. Consider again a binary system for which a representative *isothermal* plot is depicted in fig. 7.7. (The more volatile of the two components is designated as component '1'). The upper *straight* line represents the saturated liquid compositions, while the lower curve corresponds to the saturated vapour compositions. At any pressure, the phase compositions are found at the intersections of a line parallel to the *x-y* axis with the P-x and P-y curves. The straight line connecting these compositions is the tie line (such as A_1-B_1, A_2-B_2, etc.). The portion of the diagram enclosed by the *P-x-y* curves corresponds to the two phase region where the vapour and liquid phases co-exist. Any point lying outside of this two-phase envelope corresponds to a state where only a single phase is

present. Now consider the point L which lies above the phase envelope. At this condition the mixture exists as a *compressed* (or *sub-cooled*) state whose composition corresponds to x_1^* . Lowering the pressure at this fixed composition eventually brings the liquid mixture to the point A_3, where any further reduction of pressure leads to the formation of a vapour phase whose composition is given by B_3. Thus this point is characterized by the formation of the first bubble of vapour, and hence is termed the *bubble point*, the corresponding pressure being the *bubble pressure*, at the given composition.

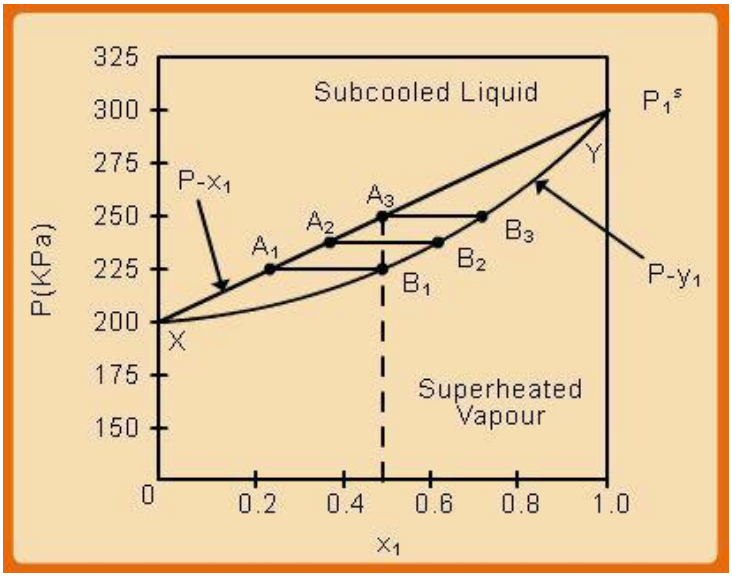


Fig. 7.7 Model P-x-y plot for a system obeying Raoult's Law

Next consider the point V in the above diagram. At this state the mixture is at the same overall composition as at L, but the state is one of single phase, *superheated vapour*. Increasing the pressure at the same composition eventually brings the mixture to the point B_1, where any further increase of pressure leads to the formation of the liquid phase, whose composition is given by the point A_1. Thus this point is said to be the *dew point* corresponding to the vapour phase composition given by y_1^* , while the pressure at this point is termed the dew pressure.

We revert to the discussion on the system state at the bubble point A_3. If one reduces the pressure progressively formation of more bubbles of vapour occurs, which coalesce and lead to the development of a bulk vapour phase. The system eventually reaches the point B_1 where practically the entire mixture exists in the vapour form; further reduction of pressure renders the mixture superheated and finally one reaches the point V (and beyond). Let us focus on what happens as the system transits through the two-phase region defined by the end points A_3 and B_1. Note that the *overall* composition of the system remains invariant as the pressure reduces. However, since now the original amounts of

each species need to be distributed across the co-existing vapour and liquid phases the actual composition in each phase must change in accordance with the following mass conservation equation: z_1 ($\equiv x_1^*$ *or* y_1^*) = $x_1 L + y_1 V$; where, z_1 = overall composition; x_1 and y_1 are compositions of the liquid and vapour phases at equilibrium, and L and V are the relative amounts of moles (per mole of the original mixture) in the liquid and vapour phases, respectively (thus $L + V = 1$) . The liquid phase composition progressively changes along the line A_3 to A_1 (*bubble curve*), while the composition of the vapour phase in equilibrium with the liquid phase transits from B_3 to B_1(*dew curve*). At each pressure between the bubble pressure (P_b) and the dew pressure (P_d) the equilibrium vapour and liquid phase compositions, as well as the relative amounts of mass in each phase are constrained by the relations: z_1 ($\equiv x_1^*$ *or* y_1^*) = $x_1 L + y_1 V$;and $L + V = 1$.

The associated, isobaric T-x-y plots for the same system are shown in fig.7.7. As is expected the dew temperature curve lies *above* the bubble temperature curve. The lens-like region corresponds to the two-phase states of the system. At the point V the system is in a single phase, super-heated state.

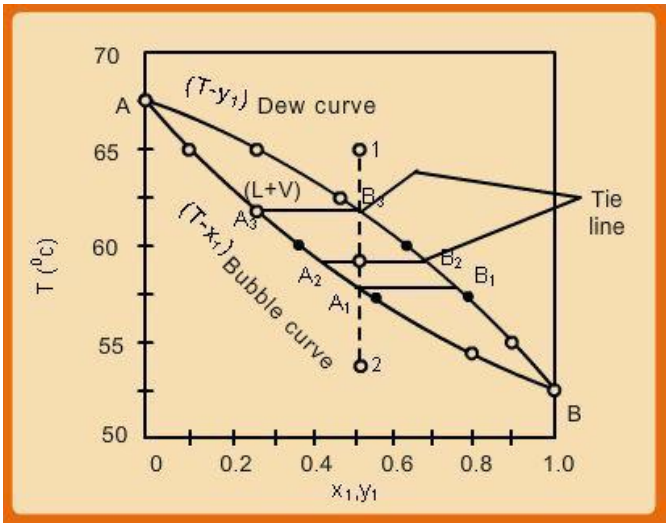


Fig. 7.8 Model T-x-y plot for a system obeying Raoult's Law

Progressive reduction of temperature brings it to the point B_3 where the *first* dew of liquid forms, whose composition is provided by the point A_3. Thus this point is referred to as the *dew point*, and the corresponding temperature called the *dew temperature*(T_d) for the given vapour phase composition (y_1^*). .On the other hand if one starts from the point L (single-phase, compressed liquid state) gradual increase of temperature brings the system to A_1, the *bubble point*, where the first bubble of vapour forms. The associated temperature then is the *bubble temperature* (T_b) for the composition x_1^*.At any

other temperature intermediate to T_d and T_b, the system contains co-existing vapour and liquid phases whose compositions are constrained by the same mass conservation relations provided above, i.e., z_1 ($\equiv x_1^*$ *or* y_1^*) = $x_1 L + y_1 V$; and $L + V = 1$. Note that as in the P-x-y plot the T-x-y plots also are characterized by horizontal tie lines that connect the compositions of the equilibrated vapour and liquid phases.

The data in figures 7.7 and 7.8 may be may be alternately displayed in the form of a *y-x* plot (fig. 7.9). It shows the equilibrium vapour and liquid phase compositions in a more immediate manner. Note that, as required by the phase rule, each pair of equilibrium *y* and *x* values correspond to a different combination of equilibrium temperature and pressure.

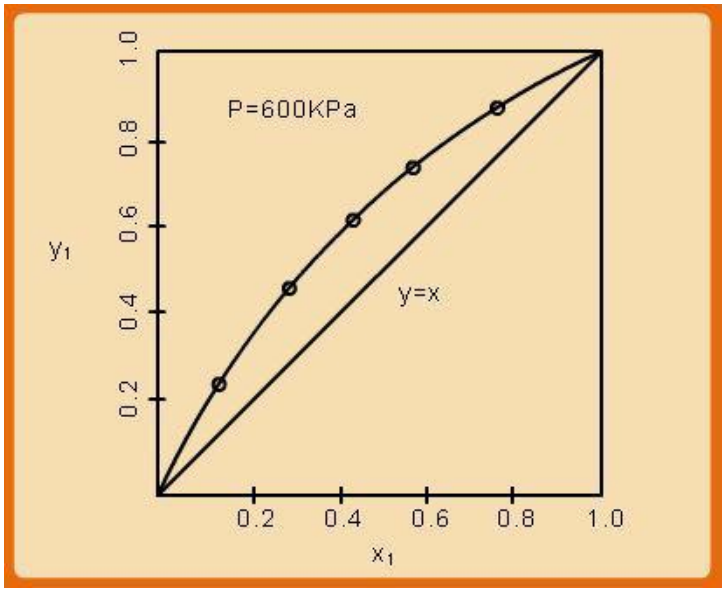


Fig. 7.9 Model y-x plot for a system obeying Raoult's Law

Multi-component VLE calculations using Raoult's Law:

For generality we consider a system containing *N* chemical species. Then by phase rule, for a VLE situation, the degrees of freedom are 2N, the phase rule variables being $\{x_i\},\{y_i\}$, T, and P. The Raoult's Law (eqn. 7.29) provides *N* constraining relations connecting these variables. Thus, for solving the VLE problem, *N* variables need to be specified, so that the values of the other *N* variables may be determined. Five types of VLE computations are commonly encountered in practice. They are enumerated below in table 7.1 first and then the relevant algorithms used are presented.

Table 7.1 Types of VLE calculations

VLE Type	Specified Variables	Computed Variables
Bubble Pressure	T and $\{x_i\}$	P and $\{y_i\}$
Dew Pressure	T and $\{y_i\}$	P and $\{x_i\}$
Bubble Temperature	P and $\{x_i\}$	T and $\{y_i\}$
Dew Temperature	P and $\{y_i\}$	T and $\{x_i\}$
Flash Distillation	T , P and $\{z_i\}$	L or V , $\{x_i\}$ and $\{y_i\}$

In the above table the notations used signify the following:

$\{x_i\} \equiv$ *overall liquid phase compoition* $\equiv \{x_1, x_2, \ldots x_{N-1}\}$ $\{$

$y_i\} \equiv$ *overall vapour phase compoition* $\equiv \{y_1, y_2, \ldots y_{N-1}\}$

$\{z_i\} \equiv$ *overall feed composition to flash vessel* $\equiv \{z_1, z_2, \ldots z_{N-1}\}$

$L \equiv$ *moles of liquid phase formed per mole of feed to flash vessel*

In summary, therefore, one specifies either T or P and either the liquid-phase or the vapor-phase composition, thus fixing 1+(N-1) or N phase rule variables as required by the phase rule for VLE calculation. The variants of Raoult's Law (eqn. 7.29) which are used are as follows:

$$y_i = x_i P_i^S / P \qquad ..(7.30)$$

Thus:

$$\sum_i^N x_i P_i^S / P = 1$$

Or:

$$P = \sum_i^N x_i P_i^S \qquad ..(7.31)$$

Also:

$$x_i = y_i P / P_i^S \qquad ..(7.32)$$

Thus:

$$\sum_i^N y_i P / P_i^S = 1$$

Or:

$$P = 1 \Big/ \sum_{i}^{N} y_i / P_i^s \qquad \text{..(7.33)}$$

For computation of vapour pressures the Antoine equation (or another suitable equation) may be used:

$$\ln P_i^s = A_i - \frac{B_i}{t + C_i} \quad ; \text{where } t = {}^0K \ (or\ {}^0C) \qquad \text{..(7.34)}$$

Values of Antoine constants for a select group of substances are tabulated in

Bubble Pressure:

Given T and $\{x_i\}$, to calculate P and $\{y_i\}$:

a) Use eqn. 7.31 to compute P

b) Next use eqn. 7.30 to obtain $\{y_i\}$

Dew Pressure:

Given T and $\{y_i\}$, to calculate P and $\{x_i\}$:

a) Use eqn. 7.33 to compute P

b) Use eqn. 7.32 to obtain $\{x_i\}$

Bubble Temperature:

Given P and $\{x_i\}$, to calculate T and $\{y_i\}$

a) For the given pressure compute $\{T_i^s\}$ using the following form of Antoine eqn.

$$T_i^s = \frac{B_i}{A_i - \ln P} - C_i$$

b) Initialize the bubble temperature as: $T_{b(i)} = \sum_{i}^{N} x_i T_i$

c) Using computed T calculate $\{P_i^s\}$

d) Use equation 7.30 to compute $\{y_i\}$

e) Is $\left|\sum_i y_i - 1\right| < \varepsilon$? (ε = pre - defined acceptable error for convergence)

f) If yes, $T_{last} = T_{b\,(f)}$; where $T_{b\,(f)}$ = final acceptable bubble temperature

g) At $T_{b\,(f)}$ compute final $\{y_i\}$

h) If $\sum_i y_i - 1 > \varepsilon$, then $T_{last} > T_{b\,(f)}$; revise to *new* T as: $T_{new} = T_{last} \dfrac{\varepsilon}{\sum_i y_i - 1}$ and return to step (c).

i) If $\sum_i y_i - 1 < -\varepsilon$, then the assumed $T < T_{b\,(f)}$; where $T_{b\,(f)}$ = final acceptable bubble temperature

Revise to *new* T using: $T_{new} = T_{last} \dfrac{\left|\sum_i y_i - 1\right|}{\varepsilon}$ and return to step (c).

Dew Temperature:

Given P and $\{y_i\}$, to calculate T and $\{x_i\}$

a) For the given pressure compute $\{T_i^{\,s}\}$ using the following form of Antoine eqn.

b) $T_i^{\,s} = \dfrac{B_i}{A_i - \ln P} - C_i$

c) Initialize the dew temperature as: $T_{d\,(i)} = \sum_i^N x_i\, T_i^{\,s}$

d) Using computed T calculate $\{P_i^{\,s}\}$

e) Use equation 7.33 to compute $\{x_i\}$

f) Is $\left|\sum_i x_i - 1\right| < \varepsilon$? (ε = pre - defined acceptable error for convergence)

g) If yes, $T_{last} = T_{d\,(f)}$; where $T_{d\,(f)}$ = final acceptable bubble temperature

h) At $T_{d\,(f)}$ compute final $\{x_i\}$

j) If $\sum_i x_i - 1 > \varepsilon$, then $T_{last} < T_{d\,(f)}$; revise to *new* T as: $T_{new} = T_{last} \dfrac{\sum_i x_i - 1}{\varepsilon}$ and return to step (c).

k) If $\sum_i x_i - 1 < -\varepsilon$, then $T_{last} > T_{d(f)}$; revise to *new* T as: $T_{new} = T_{last} \frac{\left|\sum_i x_i - 1\right|}{\varepsilon}$ and return to step (c).

Example 7.1

Consider the ternary system: Acetone (1) / Acetonitrile (2) / Nitromethane (3) for which: $\ln P_1^S = 14.5463 - \frac{2940.46}{t + 237.22}$; $\ln P_2^S = 14.5463 - \frac{2940.46}{t + 237.22}$; $\ln P_3^S = 14.2043 - \frac{2972.64}{t + 209.00}$

P_i^S (KPa); t (^0C).

Calculate: (a) P, $\{y_i\}$ for a temperature = 80^{o}C, $x_1 = 0.3$, $x_2 = 0.3$ (b) P, $\{x_i\}$, for t = 70^{o}C, $y_1 = 0.5$, $y_2 = 0.3$.

Flash Distillation Calculations

This is an operation, often exploited in the chemical industry to achieve the desired enrichment of a feedstock through a one-step distillation process. A schematic of the process is shown in fig. 7.6. A liquid at a pressure equal to or greater than its bubble point pressure is introduced into the flash by passing it through a pressure reduction valve. The abrupt reduction in pressure "flashes" or partially evaporates the feed liquid, which results in the formation of a vapour and a liquid stream which are typically assumed to leave the flash vessel in equilibrium each other.

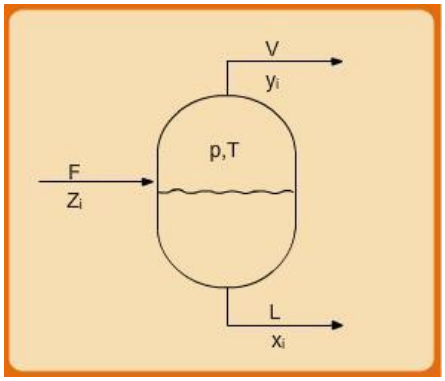


Fig. 7.10 Schematic of Flash Distillation Process

One of the common forms of flash calculation typically involves the determination of the liquid and vapour stream composition that results from process as well also the resultant liquid or vapour phase mole fractions that obtains per mole of feed. Consider a system containing one mole of mixture of chemical species with an *overall* composition represented by the set of mole fractions $\{ z_i \}$. Let L and V be the moles of liquid and vapour formed per mole of feed. The corresponding stream compositions are denoted as $\{ x_i \}$ and $\{ y_i \}$ respectively**.** The material-balance equations are:

$$F = L + V = 1 \quad z_i \qquad \text{..(7.35)}$$

$$F = x_i L + y V_i$$

$$z_i = x_i (1 - V) + y V_i \; ; \qquad \text{..(7.36)}$$

$$\text{Now: } K_i = y_i / x_i = P^s / P \qquad \text{..(7.37)}$$

Putting $x_i = y_i / K_i$ and using relations in (7.44) one obtains:

$$y_i = \frac{z_i K_i}{1 + V (K_i - 1)} \qquad \text{..(7.38)}$$

$$\sum_i y_i = 1$$

Using this condition in eqn. 7.38:

$$\sum_i \frac{z_i K_i}{1 + V (K_i - 1)} = 1 \quad (i = 1, 2 \ldots . N) \qquad \text{..(7.39)}$$

Since $x_i = y_i / K_i$, an alternative equation is:

$$x_i = \frac{z_i}{1 + V (K_i - 1)} \qquad (i = 1,2, \ldots . N) \qquad \text{..(7.40)}$$

$$\text{It follows that: } \sum_i \frac{z_i}{1 + V (K_i - 1)} = 1 \qquad \text{..(7.41)}$$

Subtracting eqn. 7.41 from 7.39and defining a function ϕ we get

$$\phi = \sum_i \frac{z_i (K_i - 1)}{1 + V (K_i - 1)} = 0 \qquad \text{..(7.42)}$$

It follows that: $\frac{d\phi}{dV} = -\sum_i \frac{z_i (K_i - 1)^2}{[1 + V(K_i - 1)]^2}$ ..(7.43)

The derivative $\frac{d\phi}{dV}$ is always negative; in other words the relation between ϕ vs. V is monotonic, and this makes for convenient application of the well-known Newton-Raphson method of solution

$$V_{n+1} = V_n - \frac{\phi_n}{\left(\frac{d\phi}{dV}\right)_n}$$ ..(7.44)

Where, the values for ϕ and $\frac{d\phi}{dV}$ may be computed using eqns. 7.42 and 7.43 respectively.

Example 7.2

A liquid mixture containing equimolar amounts of benzene (1) /toluene (2) and ethylbenzene (3) is flashed to conditions of T = 110^{O}C, P = 90 kPa, determine the equilibrium mole fractions {x_i} and {y_i} of the liquid and vapor phase formed and the molar fraction V of the vapor formed. Assume that Raoult's law applies. $\ln P^{sat}(Pa) = A - \frac{B}{t(^0K) + C}$

	A	B	C
Benzene	13.8594	2773.78	-53.08
Ethylbenzene	14.0045	3279.47	-59.95
Toluene	14.0098	3103.01	-53.36

VLE Algorithms for Low to Moderate Pressures

The next level of complexity in VLE algorithms arise when one has to account for non-ideal behaviour for both the gas and liquid phases. This may obtain at pressures away from atmospheric and if the constituent molecules form a non-ideal liquid phase. The general approach to VLE of such system involves correcting both sides of the Raoult's law to incorporate the effect of non-ideal behaviour. If the pressures are moderately high the truncated virial EOS may be used to describe the gas phase

behaviour, whereas the liquid phase non-ideality is defined by a suitable activity coefficient model. The activity coefficient based approach is preferred for moderate pressures, as under such conditions the liquid phase properties may be conveniently regarded as independent of pressure, hence only temperature effects on the activity coefficients need be accounted for. This approach, of course, is rendered inaccurate at relatively high pressures, where both the gas and liquid phases need to be described using fugacity coefficients derived typically from a cubic (or a higher order) EOS. This is dealt with in the next section. Presently the VLE algorithms for low to moderate pressure range are introduced. The starting point is the eqn. (6.126):

$$\hat{f}_i^{\alpha} = \hat{f}_i^{\psi} \qquad ..(6.126)$$

Applying it to VLE:

$$\hat{f}_i^{V} = \hat{f}_i^{L} \qquad ..(6.127)$$

For gas phase, we use eqn. 6.129:

$$\hat{f}_i^{V} = y_i \hat{\varphi}_i P$$

For liquid phase (using eqn. 6.164):

$$\hat{f}_i^{L} = x_i \gamma_i f_i$$

Applying eqn. 6.127:

$$y_i \hat{\varphi}_i P = x_i \gamma_i f_i \qquad ..(7.45)$$

From basic fugacity function for liquid phase (eqn.6.119):

$$f_i(T,P) = \varphi_i^{sat} P_i^{sat} \exp\left[\frac{V_l (P - P_i^{sat})}{RT}\right] \qquad ..(6.119)$$

Using eqn. 6.119 in 6.128 we may write the phase equilibria relation as:

$$y_i \Phi_i P = x_i \gamma_i P_i^S; \qquad (i = 1,2,\ldots N) \qquad ..(7.46)$$

Where $\Phi_i = (\hat{\varphi}_i / \hat{\varphi}_i^s)\exp[-\frac{V_{iL}(P - P_i^S)}{RT}]$

One may show that the Pontying (exponential factor) in the last equation is usually ~ 1 for low to moderate pressure range, hence one may write:

$$\Phi_i \approx (\varphi_i / \varphi_i^S) \qquad ..(7.48)$$

For a gas mixture obeying the truncated virial EOS (by eqn. 6.98):

$$\varphi_i^{sat} = \exp\left[\frac{B_{ii} P_i^s}{RT}\right] \quad ..(7.49)$$

Specifically for a binary using eqns. 6.149 and 6.150:

$$\ln \hat{\varphi}_1 = \frac{P}{RT}\left(B_{11} + y_2^2 \delta_{12}\right) \quad ..(6.149)$$

$$\hat{\varphi}_2 = \exp\left[\frac{P}{RT}\left(B_{22} + y_1^2 \delta_{12}\right)\right] \quad ..(6.150)$$

Using the last four equations it follows that:

$$\Phi_1 = \exp\left[\frac{B_{11}(P - P_1^s) + P y_2^2 \delta_{12}}{RT}\right] \quad ..(7.50)$$

$$\text{And } \Phi_2 = \exp\left[\frac{B_{22}(P - P_2^s) + P y_1^2 \delta_{12}}{RT}\right] \quad ..(7.51)$$

It may be shown that for a multi-component the general expression for Φ_i is provided by:

$$\Phi_i = \exp\frac{B_{ii}\left(P - P_i^s\right) + \frac{1}{2} P \sum\sum y_j y_k \left(\delta_{ji} - \delta_{jk}\right)}{RT} \quad ..(7.52)$$

Now, $\Phi_i = \Phi_i(T, P, y_1, ..., y_{N-1})$

And:

$$\gamma_i = \gamma_i(T, P, x_1, ..., x_{N-1}) \cong \gamma_i(T, x_1, ..., x_{N-1}) \quad ..(7.53)$$

The approximation made in eqn. 7.53 is a reasonable one, as at low to moderate pressures the dependence of γ_i γ_i on 'P' may be neglected (as at such conditions the liquid phase properties are not strongly pressure dependent).

The same five classes as provided in table 7.1 may be solved using this modified form of the Raoult's law. In all cases eqn. 7.46 provides the starting point for calculation, which may be re-written is two principal alternate forms as follows:

$$y_i = x_i \gamma_i P_i^s / \Phi_i P \quad ..(7.54)$$

$$x_i = y_i \Phi_i P / \gamma_i P_i^s \quad ..(7.55)$$

Since $\sum y_i = 1$

$$\sum x_i \gamma_i P_i^s / \Phi_i P = 1$$

Or:

$$P = \sum x_i \gamma_i P_i^s / \Phi_i \quad ..(7.56)$$

Similarly since $\sum x_i = 1 = \sum y_i \Phi_i P / \gamma_i P_i^s$; it follows that:

$$P = 1/ \sum y_i \Phi_i / \gamma_i P_i^s \quad ..(7.57)$$

We may also re-write eqn. 7.46 in terms of the K-factor (as used for Raoult's Law in eqn. 7.37) as follows:

$$K_i = \frac{y_i}{x_i} = \frac{\gamma_i P_i^S}{\Phi_i P} \quad ..(7.58)$$

Accordingly:

$$y_i = K_i x_i \quad ..(7.59)$$

Or:

$$x_i = \frac{y_i}{K_i} \quad ..(7.60)$$

From eqn. 7.59, it follows that:

$$\sum_i K_i x_i = 1 \quad ..(7.61)$$

From eqn. 7.60:

$$\sum_i \frac{y_i}{K_i} = 1$$

Note that when $\Phi_i = \gamma_i = 1$ eqn. 7.46 reduces to the ideal case of Raoult's Law.

Bubble pressure:

Given T and $\{x_i\}$, to calculate P and $\{y_i\}$:

a) Start with given T, $\{x_i\}$, Antoine constants, $\in$ (error value for convergence)

b) Set all $\{\Phi_i\} = 1.0$, Evaluate $\{P_i^s\}$, $\{\gamma_i\}$, Calculate P using eqn. 7.56

c) Calculate $\{y_i\}$ using eqn.7.54

d) Now evaluate $\{\Phi_i\}$, using eqns. 7.52

e) Calculate P_{new} using eqn. 7.56

f) Is $\delta P < \in$?

g) If 'No', go to step 'c' and calculate new $\{y_i\}$ with last $\{\Phi_i\}$

h) If 'Yes', end at last P, and $\{y_i\}$

Dew Point Pressure:

Given T and $\{y_i\}$, to calculate P and $\{x_i\}$

a) Start with T and $\{y_i\}$;Antoine constants; ε and δ (error values for convergence); start with Raoult's law by setting all $\{\Phi_i\}$= 1.0, and all $\{\gamma_i\}$= 1.0; Evaluate $\{P_i^s\}$, then calculate P using eqn. 7.57; Now evaluate $\{x_i\}$ by eqn. 7.55; Evaluate $\{\gamma_i\}$ using appropriate activity coefficient model Liquid-phase; recalculate P using eqn. (7.65), revise $\{\Phi_i\}$ using given $\{y_i\}$ and last P.

b) Calculate new set $\{x_i\}$ using eqn. 7.55

c) Normalize $\{x_i\}$ using $x_{i\,(n)} = \frac{x_i}{\sum x_i}$, and use normalized $\{x_i\}$ to compute $\{\gamma_i\}$

d) Use last $\{\gamma_i\}$ to calculate P by eqn. 7.57

e) Is $\delta P < \varepsilon$?

f) If 'Yes' then $P_{last} = P_{d\,(f)}$

Bubble Temperature:

Given P and $\{x_i\}$, to calculate T and $\{y_i\}$

a) Solve for T and $\{y_i\}$ first by assuming Raoult's Law algorithm for bubble temperature

b) Using solution in 'a' estimate $\{K_i\}$ using eqn. 7.58 with the given values of P and $\{x_i\}$; latest values of T and $\{y_i\}$

c) Next calculate $\{K_i x_i\}$

d) Calculate all $y_i = K_i x_i \Big/ \sum_i K_i x_i$

e) Using normalized $\{y_i\}$, recalculate $\{K_i\}$ and $\sum_i K_i x_i$

f) Has $\sum_i K_i x_i$ changed? If yes return to step 'd'

g) If $\sum_i K_i x_i$ has not changed between two successive iterations between steps ‘c’ and ‘d’ is $\sum_i K_i x_i = 1$?

h) If *yes*, the last values of T and $\{ y_i \equiv K_i x_i \}$ give the final bubble temperature $T_{b(f)}$, and vapour compositions.

i) If *no*, and last $\sum_i K_i x_i > 1$, then $T_{last} > T_{b(f)}$; revise to *new* T as: $T_{new} = T_{last} \frac{1}{\sum_i K_i x_i}$ and return to step (c).and return to step ‘b’.

j) If *no*, and last $\sum_i K_i x_i < 1$, then $T_{last} < T_{b(f)}$; revise to *new* T as: $T_{new} = T_{last} \sum_i K_i x_i$ and return to step (c).and return to step ‘b’.

Dew Temperature:

Given P and $\{ y_i \}$, to calculate T and $\{ x_i \}$

a) Solve for T and $\{ x_i \}$ first by assuming Raoult’s Law algorithm for dew temperature

b) Using solution in ‘a’ estimate $\{ K_i \}$ using eqn. 7.58 with the given values of P and $\{ y_i \}$; latest values of T and $\{ x_i \}$

c) Next calculate $\{ y_i / K_i \}$

d) Calculate all $x_i = (y_i / K_i) / \sum_i (y_i / K_i)$

e) Using normalized $\{ x_i \}$, recalculate $\{ K_i \}$ and $\sum_i y_i / K_i$

f) Has $\sum_i y_i / K_i$ changed? If yes return to step ‘d’

g) If $\sum_i y_i / K_i$ has not changed between two successive iterations between steps ‘c’ and ‘d’ is $\sum_i y_i / K_i = 1$?

h) If *yes*, the last values of T and $\{ x_i \equiv y_i / K_i \}$ give the *final* dew temperature $T_{d(f)}$, and liquid phase compositions.

i) If *no, and* $\sum_i y_i / K_i > 1$, then $T_{last} < T_{d\,(f)}$; revise to *new* T as: $T_{new} = T_{last} \sum_i x_i / K_i$ and return to step (b).

j) If no, *and* $\sum_i y_i / K_i < 1$, then $T_{last} > T_{d\,(f)}$; revise to *new* T as: $T_{new} = T_{last} \sum_i x_i / K_i$ and return to step (b).

Example 7.3

Methanol (1)-acetone (2) forms an azeotrope at 760 Torr with $x_1 = 0.2$, T = 55.7^0C. Using van Laar model predict the bubble pressure for a system with for $x_1 = 0.1$ at 55.7^0C.

$\log_{10} P_1^s = 8.0897 - [1582.271/(t + 239.726)]; \log_{10} P_2^s = 7.1171 - [1210.595/(t + 229.664)]$

P_i^s (*torr*); t (^0C)

Example 7.4

For a binary, the activity coefficients are $\ln \gamma_1 = Ax_2^2$ and $\ln \gamma_2 = Ax_1^2$. Show that the system forms an azeotrope when $A > \ln \left(P_2^s / P_1^s \right)$

(Click for solution)

Flash Distillation Calculations

The procedure for non-ideal systems takes a form similar to that adopted for systems obeying Raoult's Law except that one needs to additionally check for existence of both liquid and vapour phases following flash. The algorithm comprises the following steps.

a) Start with flash T, P and feed composition $\{ z_i \}$

b) At the given T, calculate dew pressure P_d by putting $\{ y_i \} = \{ z_i \}$ c) Next calculate bubble pressure P_b by putting $\{ x_i \} = \{ z_i \}$

d) Is $P_d < P < P_b$? If *no*, the vapour phase has not formed.

e) If *yes*, compute $\{\Phi_i\}$, $\{\gamma_i\}$, and V as $\frac{P_b - P}{P_b - P_d}$

f) Use $\{\Phi_i\}$, $\{\gamma_i\}$, to get $\{K_i\}$ using eqn. 7.58

g) Then use eqn. 7.42 and 7.43 to evaluate ϕ and $d\phi / dV$.

h) Using Newton-Raphson method, find V

i) With last V compute $\{x_i\}$ using eqn. 7.40 and $\{y_i\}$ by eqn. 7.38

j) Re-calculate $\{\Phi_i\}$, $\{\gamma_i\}$, and $\{K_i\}$ using eqn. 7.58

k) Check if the change in each parameter x_i, y_i, and V between steps 'e' and 'j' is within pre-defined error values chosen for convergence.

l) If *yes*, then the last values of x_i, y_i, and V constitute the solution

m) If *no*, return to step '*f*' with the last values of x_i, y_i, and V

High Pressure Vapour Liquid Equilibria

At relatively high pressures the VLE relations used in the last section lose exactness especially with respect to the activity coefficient-based approach for description of the non-ideal behaviour of the liquid phase. This is because the assumption that the activity coefficients are weakly dependent on pressure no longer remains a realistic approximation. In addition, the gas phase *P-V-T* behaviour can no longer be described by the truncated virial EOS. Under such conditions a use of a higher order EOS, which may be applied both to the gas and liquid phase is preferred. As we have seen in chapter 2, the cubic EOS provides just that advantage; besides they offer a reasonable balance between accuracy and computational complexity. We start with the general criterion for phase equilibria as applied to vapour-liquid systems, given by eqn. 6.127:

$$\hat{f}_i^V = \hat{f}_i^L \qquad (i = 1,2, \ldots, N) \qquad ..(7.62)$$

An alternative form of the last equation results from introduction of the fugacity coefficient using eqn. 6.129 and 6.130:

$$y_i \hat{\varphi}_i^V P = x_i \varphi_i^L P \qquad (i = 1,2, \ldots N) \qquad ..(7.63)$$

The last equation reduces to:

$$y_i \hat{\varphi}_i^V = x_i \hat{\varphi}_i^L \qquad ..(7.64)$$

VLE of pure species

For the special case of pure species *i*, equation 7.64 reduces to:

$$\varphi_i^V = \varphi_i^L \quad ..(7.65)$$

If both φ_i^V and φ_i^L are expressed in terms of cubic EOS as defined by any of the eqns. 6.104 to 6.107, for a given T one may obtain the saturation vapour pressure by means of suitable algorithm as shown by the worked out example below.

Example 7.5

Estimate the vapour pressure of a substance "A' using PR-EoS, at T = 428^{O}K. For the substance A: T_C = 569.4 K, P_C = 2.497 MPa, = 24.97 bar, ω = 0.398.

VLE from K-value Correlations for Hydrocarbon Systems

Using eqn. 7.64 one can write, $K_i = y_i / x_i$

Alternately: $K_i = \hat{\varphi}_i^V / \hat{\varphi}_i^L \quad ..(7.66)$

As evident from eqns. 6.155 to 6.157, the expression for species fugacity coefficients for mixtures described by cubic EOS are relatively complex, which in turn makes the estimation of the K-factors difficult as iterative solutions to obtaining T, P and/or compositions are inevitable. As demonstrated in the last section, this is true even for the fugacity and activity coefficient based formulation of the VLE problem. The use of cubic EOS for description of fugacity coefficients of species in both phases poses additional difficulty owing to the intrinsic complexity of the expressions shown in eqns. 6.155 to 6.157.

However, in the case of VLE of light hydrocarbon mixtures a reasonable simplification may be achieved by assuming *ideal solution* behaviour for both the phases. This is a relatively practical approximation as hydrocarbons being non-polar in nature, the intermolecular interactions are generally weaker than amongst polar molecules. In effect in the case of lighter hydrocarbons (C_1-C_{10}) the interactions between the same species and those between dissimilar species are not significantly different. This forms the basis of assuming ideal solution behaviour for such system. It may be noted that since equilibrium pressures in light hydrocarbon systems tend to be 'high' (as they are low-boiling) under practical conditions of distillation processes, ideal solution behaviour yields far more accurate results than would be possible by *ideal gas* assumption.

We develop next the result that obtains owing to the assumption of ideal solution behaviour.

The chemical potential of all species in an ideal solution is given by eqn. 6.77:

$$\mu_i^{id} = \bar{G}_i^{id} = G_i + RT \ln x_i \quad ..(7.67)$$

For a real solution: $d\bar{G}_i = RTd \ln f_i$ ..(7.68)

At the same time for pure species at same T&P: $dG_i = RTd \ln f_i$ ..(7.69)

From eqns. (7.68) and (7.69) it follows that:

$$\bar{G}_i - G_i = RT \ln(f_i / f_i) \quad ..(7.70)$$

From (7.67) $\bar{G}_i^{id} - G_i = RT \ln x_i$ ..(7.71)

Thus from eqns. (7.70) and (7.71):

$$\bar{G}_i - \bar{G}_i^{id} = RT \ln(f_i / x_i f_i) \quad ..(7.72)$$

For an ideal solution LHS of (7.72) is identically zero; hence for such a solution:

$$f_i = x_i f_i \quad ..(7.73)$$

For a real gas mixture the fugacity coefficient φ_i is defined by: $f_i = y_i \varphi_i P$

In analogy, for a real solution we define φ_i by: $f_i = x_i \varphi_i P$

Or: $\varphi_i = f_i / x_i P$ ..(7.74)

Using (7.74) for an ideal solution: $\hat{\varphi}_i^{id} = \hat{f}_i^{id} / x_i P$ ..(7.75)

Using (7.73) in (7.75) it follows: $\hat{\varphi}_i^{id} = x_i f_i / x_i P = f_i / P = \varphi_i$

Thus for an ideal solution: $\varphi_i = \varphi_i$ ..(7.76)

Now considering the light hydrocarbon systems, the application of eqn. 7.76 in 7.66 gives:

$$K_i = \frac{\varphi_i^L(T, P)}{\varphi_i^V(T, P)} \frac{f_i^L(T, P)}{P\varphi_i^V(T, P)} \quad ..(7.77)$$

Using eqn. 6.119 we substitute for the fugacity $f_i^L(T, P)$. Thus:

$$f_i^L = P_i^{sat} \varphi_i^{sat} \exp\left[\frac{V_i^L (P - P_i^{sat})}{RT}\right]$$

Where, V_i^l is the molar volume of pure species *i* as a saturated liquid. Thus the K-value is given by:

$$K_i = \frac{P_i^{sat}\varphi_i^{sat}(T, P_i^{sat})}{P\varphi_i^{V}(T, P)} \exp\left[\frac{V_i^{L}(P - P_i^{sat})}{RT}\right] \quad ..(7.78)$$

The advantage of eqn. 7.78 is that it is a function of the properties of the pure species only, and therefore its dependence on composition of the vapour and liquid phases is eliminated. The K-factor then is a function of temperature and pressure alone. The terms φ_i^{sat} and φ_i^{V} in eqn. 7.78 can in principle be computed using expression provided by cubic EOS (i.e., eqns. 6.104 – 6.107) or a corresponding expression from an higher order EOS, including the generalized correlation (section 6.9). This allows K-factors for light hydrocarbons to be as functions of T and P.

However, it may be noted that the computation of fugacities at high pressures (and/or temperatures) can potentially be rendered difficult as above the critical temperature the liquid state is necessarily hypothetical, while at pressures higher than the saturation pressure the vapour state is hypothetical. This is corrected for by some form of extrapolations to those hypothetical states. Various approaches have been described in the literature (T.E. Daubert, *Chemical Engineering Thermodynamics*, McGraw-Hill, 1985). The nomographs of K-factors (see figs. 7.11 and 7.12) reported by Dadyburjor (D.B. Dadyburjor, *Chem. Eng. Progr.*, vol. 74(4), 85-86, 1978) provide an example of one such approach.

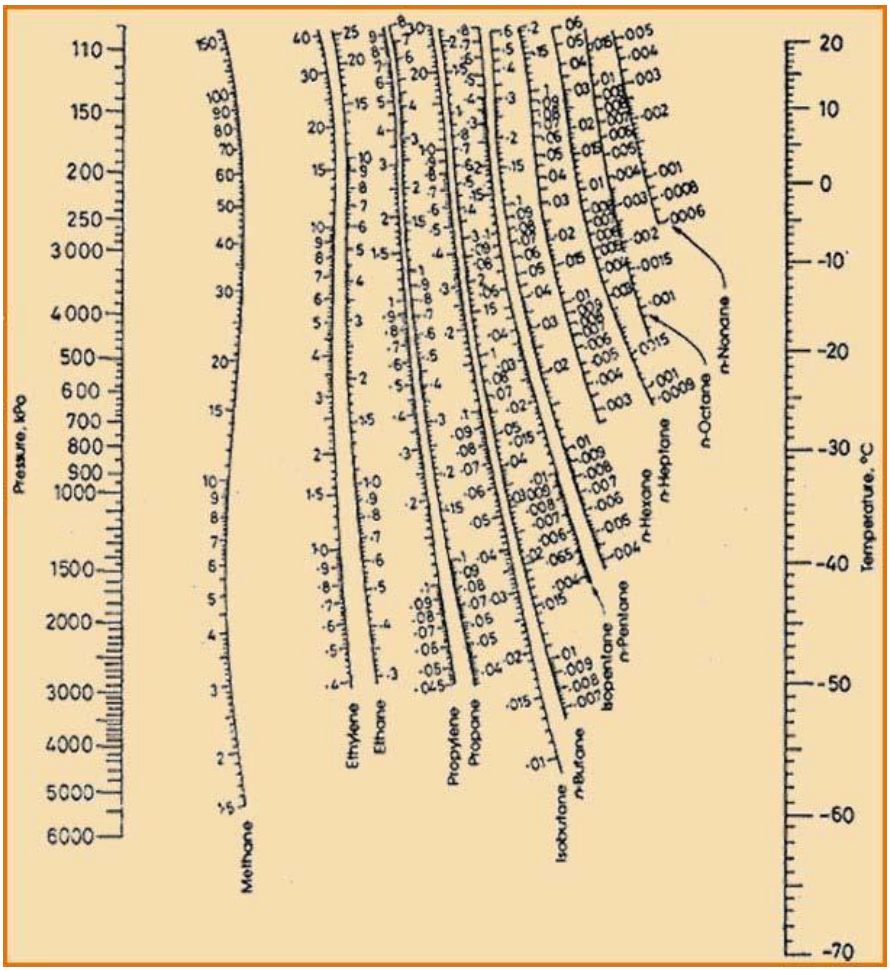


Fig. 7.11 K-factors in light hydrocarbon systems (low temperature range) [Source: Dadyburjor; D.B., *Chem. Eng.Progr.*, Vol. 74 (4) pp.85-86 (1978)].

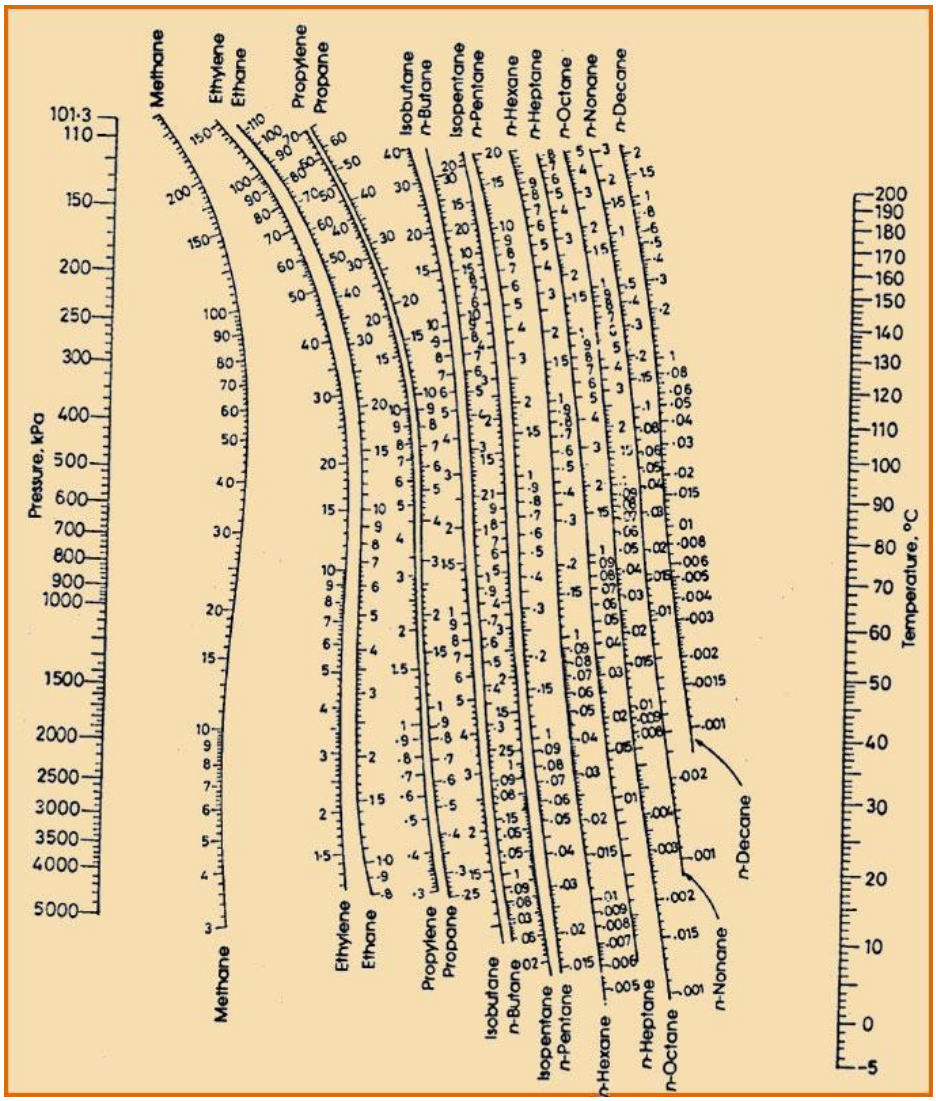


Fig. 7.12 K-factors in light hydrocarbon systems (high temperature range) [Source: Dadyburjor; D.B., *Chem. Eng.Progr.*, Vol. 74 (4) pp.85-86 (1978)].

The nomographs may be conveniently used purpose of VLE calculations in hydrocarbon systems as they the K-factors for each species can be estimated at a given T and P. This is done by drawing a straight line connecting the given temperature and pressure; the corresponding K_i value is read off from the point of intersection of this line with the K_i curve for a particular species. For *bubble point* (either T or P) calculations one uses:

$$\sum_i y_i = \sum_i K_i \, x_i = 1 \qquad ..(7.79)$$

- For ***pressure*** calculation: If $\sum_i K_i x_i > 1$, assumed pressure is *lower* than the correct value; if $\sum_i K_i x_i < 1$ the assumed pressure is *higher* than the correct pressure. Thus, pressure needs to be revised for the next step of calculation.
- Similarly, for ***temperature*** calculation: if $\sum_i K_i x_i > 1$, assumed temperature is *higher* than the correct value; if $\sum_i K_i x_i < 1$ the assumed temperature is *lower* than the correct value. Thus, temperature needs to be revised for the next step of calculation.

On the other hand the solution for **dew point** calculations derives from:

$$\sum_i x_i = \sum_i \frac{y_i}{K_i} = 1 \qquad ..(7.80)$$

- For ***pressure*** calculation: If, $\sum_i (y_i / K_i) > 1$assumed pressure is *higher* than the correct value; if $\sum_i (y_i / K_i) > 1$the assumed pressure is *lower* than the correct pressure. Thus, pressure needs to be revised for the next step of calculation.
- Similarly, for ***temperature*** calculation: if $\sum_i (y_i / K_i) > 1$, assumed temperature is *lower* than its correct value; if $\sum_i (y_i / K_i) < 1$the assumed temperature is *higher* than its correct value. Thus, temperature needs to be revised for the next step of calculation.

The use of these equations illustrated below using an example.

Example 7.6

A vapour mixture contains 20mol% methane (1), 30mol% ethane (2) , and rest propane (3), at 30^0C. Determine the dew composition.

High Pressure VLE using cubic EOS

This constitutes a generalized approach without any simplifying assumptions such as employed for light hydrocarbons. The governing relation thus is eqn. 7.66.

$$K_i = \hat{\varphi}_i^V / \hat{\varphi}_i^L$$

The fugacity of each species, either in vapour or liquid phase, is computed using the expressions that apply to use of cubic EOS (eqns. 6.155 to 6.157). For relevant VLE calculations once again the eqns. 7.79 and 7.80 are employed. The steps for computing (for example) the bubble pressure are enlisted below. The basic principle used for other types of standard calculations (such as discussed for low to moderate pressure VLE systems, table 7.1) remains the same.

Bubble pressure algorithm:

Given T and $\{x_i\}$, to calculate P and $\{y_i\}$

a) Solve for P and $\{y_i\}$ first by assuming Raoult's Law algorithm for bubble pressure

b) Using solution in 'a' estimate $\{K_i\}$ using eqn. 7.66 with the given values of T and $\{x_i\}$; and the latest values of P and $\{y_i\}$

c) Next calculate $\{K_i x_i\}$ and $\sum_i K_i x_i$

d) Calculate all $y_i = K_i x_i \Big/ \sum_i K_i x_i$

e) Using normalized $\{y_i\}$, recalculate $\{K_i\}$ and $\sum_i K_i x_i$

f) Has $\sum_i K_i x_i$ changed between steps 'c' and 'e'? If *yes* return to step 'd'

g) If $\sum_i K_i x_i$ has not changed between two successive iterations between steps 'c' and 'e' is $\sum_i K_i x_i = 1$?

h) If *yes*, the last values of P and $\{y_i \equiv K_i x_i\}$ give the final bubble temperature $P_{b(f)}$, and vapour compositions.

i) If *no*, and last $\sum_i K_i x_i > 1$, then $P_{last} < P_{b\,(f)}$; revise to *new P* as: $P_{new} = P_{last} \sum_i K_i x_i$ and return to step (c).and return to step 'b'.

j) If *no*, and last $\sum_i K_i x_i < 1$, then $P_{last} > P_{b\,(f)}$; revise to *new P* as: $P_{new} = P_{last} \sum_i K_i x_i$ and return to step (c).and return to step 'b'.

We illustrate the above methodology by a calculation of bubble pressure for an example binary system below.

Example 7.7

For the system of methane (1) and butane (2) compute the bubble pressure for a liquid phase composition of $x_1 = 0.2$ at a temperature of 310K, using the PR-EOS.

Henry's Law

The solubility of gases that are *sparingly* soluble in solvents constitutes a special application of the general VLE relations developed in sections 7.3 and 7.4. There are numerous real-life examples of such situations; for example, the solubilization of oxygen in water, which sustains aqueous life. Similarly, gases such as nitrogen, carbon dioxide, etc., display relatively low solubility (mole fraction: $10^{-5} - 10^{-2}$) in water or many solvents of industrial interest. Further, in many such instances, the solubility of a gas in a solvent is required at temperatures beyond the critical temperature of the gas. Application of vapour-liquid phase equilibria relations given by Raoult's law or its modified versions (discussed in the foregoing sections) to a solute species *i* (in a solvent) requires the saturation vapour pressure P_i^{sat} at the temperature of application. Clearly if the temperature of interest exceeds the critical temperature of the solute, the parameter P_i^{sat} is not definable, and hence such VLE relations presented in sections 7.5 and 7.6 are not appropriate in such cases.

As for any VLE problem the starting point for determining the solubility of a gaseous species 'i' in a liquid is the equality of the fugacity of the solute species and liquid (liq) phases:

$$\hat{f}_i^{gas} = \hat{f}_i^{liq} \qquad ..(7.81)$$

Using eqn. 7.45 (considering low to moderate pressures):

$$y_i \hat{\varphi}_i P = x_i \gamma_i f_i \quad ..(7.45)$$

Denoting the gaseous solute as '1' and the solvent as '2', one may write:

$$\hat{f}_1^{gas} = \hat{f}_1^{liq} \quad ..(7.82)$$

And: $\hat{f}_2^{gas} = \hat{f}_2^{liq}$ ..(7.83)

Using eqn. 7.45 the last two equations may be re-written as:

$$y_1 \hat{\varphi}_1 P = x_1 \gamma_1 f_1 \quad ..(7.84)$$

$$y_2 \hat{\varphi}_2 P = x_2 \gamma_2 f_2 \quad ..(7.85)$$

If we further assume that the gas is very sparingly soluble in the solvent, the liquid phase is essentially pure solvent and the following relations derive:

$\gamma_1 \cong \gamma_1^{\infty}$

$\gamma_2 \approx 1$

Therefore, for component 1 we may rewrite the eqn. 7.84 as:

$$y_1 \hat{\varphi}_1 P = x_1 \gamma_1^{\infty} f_1 \quad ..(7.86)$$

Or alternately:

$$y_1 \hat{\varphi}_1 P = x_1 H_1 \quad ..(7.87)$$

Where:

$$H_1 = \gamma_1^{\infty} f_1 \quad ..(7.88)$$

Equation 7.88 is termed the Henry's law, and H_1 the Henry's constant, which is defined at the system temperature. If one plots the value of $\hat{f}_1$ as a function of the gas mole fraction x_1 in the solvent phase (as shown schematically in fig. 7.13), the parameter H_1 corresponds to the slope of the tangent drawn on the curve at the limiting condition of $x_1 \rightarrow 0$.

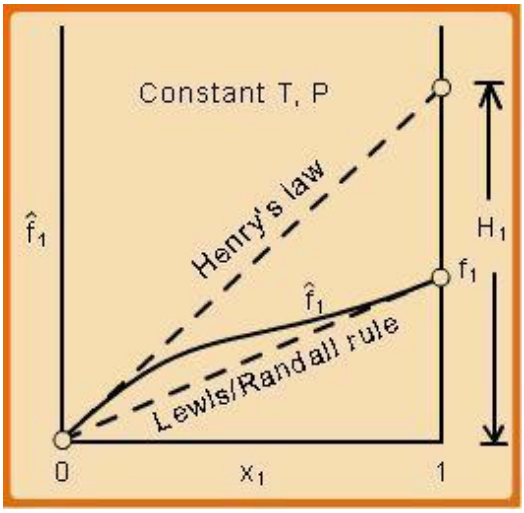


Fig. 7.13 Plot of $\hat{f}_1$ as a function of the gas mole fraction x_1

Similarly for component 2 the phase equilibrium equation 7.85 may be rewritten as:

$$y_2 \hat{\varphi}_2 P = x_2 H_2 \quad ..(7.89)$$

Where: $H_2 = \gamma_2 f_2$ ..(7.90)

Since $\gamma_2 \approx 1$, it follows that:

Where: $H_2 = f_2$ ..(7.91)

Thus: $\hat{f}_2 = x_2 f_2$ ..(7.92)

It may be noted that eqn. 7.92 is the same as 6.162 (section 6.15), which describes the Lewis -Randall rule. Thus when Henry's law is applicable for the solute then Lewis-Randall rule is applicable for the solvent. Since for a system temperature $T > T_{c,1}$ the fugacity $\hat{f}_1$ of pure liquid phase for '1' is hypothetical, it follows that the Henry's law constant H_1 (= $\gamma_1^{\infty} f_1$) is necessarily a hypothetical quantity as well. Since solubility of a gas is temperature dependent, it follows that H_i is also a function of temperature. The Henry's law constant for a large number of gases with water as the solvent has been reported in the literature. For example for acetylene the value is 1350bar, for carbon dioxide 1670bar, and for air 72950bar). Fig. 7.14 presents the value of Henry's law constant for a number of gases in water as a function of temperature.

Example 7.8

A concentrated binary solution containing mostly species 2(*but*$X_2 \neq 1$) 2 (but $x_2 \neq 1$) is in equilibrium with a vapor phase containing both species 1 and 2. The pressure of this two-phase system is 1 bar; the temperature is 298.0K. Determine from the following data good estimates of x_1 and y_1. H_1 = 200 bar; P_2^{sat} = 0.10 bar.

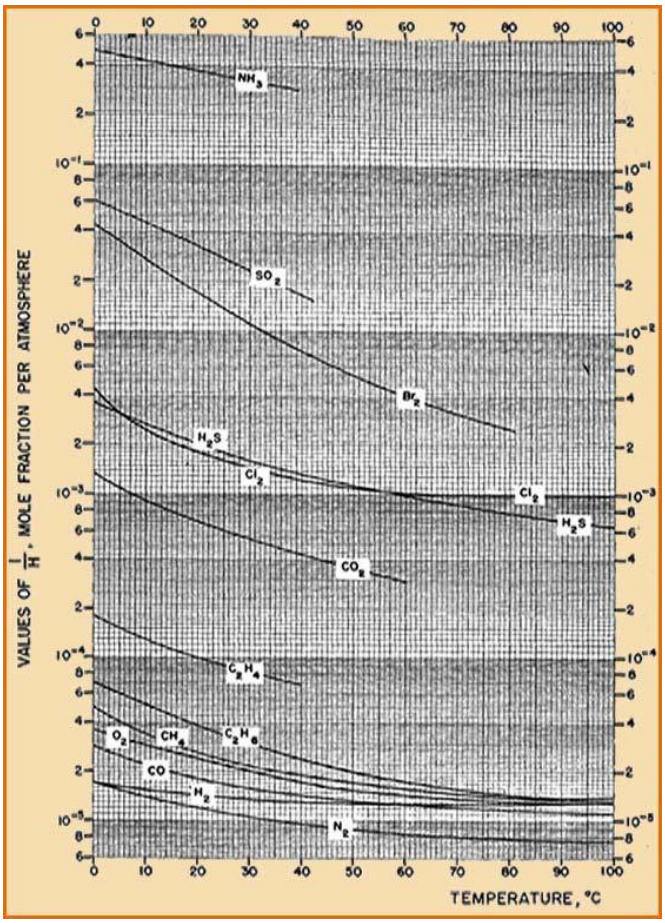


Fig. 7.14 Plot of Henry's Constant vs. Temperature, $\left[(1/H) = N\ (mole\ fraction)/P\ (atm)\right]$[Reprinted with permission from O.A. Hougen, K.M. Watson, and R.A. Ragatz (1960), *Chemical Process Principles Charts,* 2nd ed., John Wiley & Sons, New York]

Unit 4 - Chemical Reaction Equilibria

8.1 Introduction

Reaction chemistry forms the essence of chemical processes. The very distinctiveness of the chemical industry lies in its quest for transforming less useful substances to those which are useful to modern life. The perception of old art of 'alchemy' bordered on the magical; perhaps in today's world its role in the form of modern chemistry is in no sense any less. Almost everything that is of use to humans is manufactured through the route of chemical synthesis. Such reactive processes need to be characterized in terms of the maximum possible yield of the desired product at any given conditions, starting from the raw materials (i.e., reactants). The theory of chemical reactions indicates that rates of reactions are generally enhanced by increase of temperature. However, experience shows that the maximum quantum of conversion of reactants to products does not increase monotonically. Indeed for a vast majority the maximum conversion reaches a *maximum* with respect to reaction temperature and subsequently diminishes. This is shown schematically in fig. 8.1.

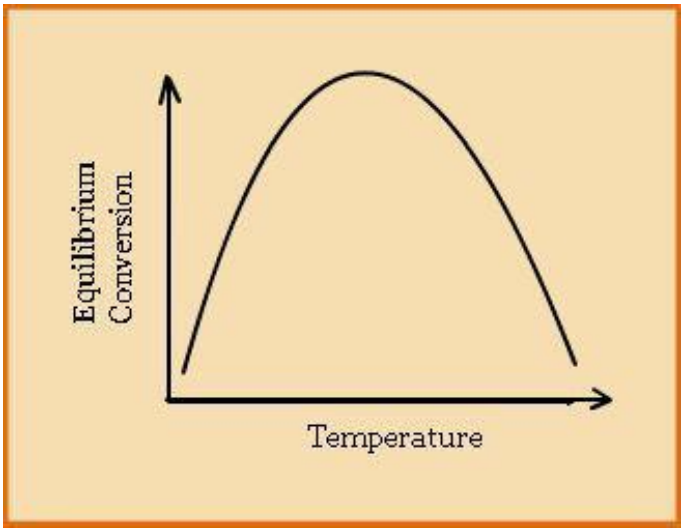


Fig. 8.1 Schematic of Equilibrium Reaction vs. Temperature

The reason behind this phenomenon lies in the molecular processes that occur during a reaction. Consider a typical reaction of the following form occurring in gas phase: $A(g) + B(g) \rightarrow C(g) + D(g)$. The reaction typically begins with the reactants being brought together in a reactor. In the initial phases, molecules of A and B collide and form reactive complexes, which are eventually converted to the products C and D by means of molecular rearrangement. Clearly then the early phase of the reaction process is dominated by the presence and depletion of A and B. However, as the process

continues, the fraction of C and D in the reactor increases, which in turn enhances the likelihood of these molecules colliding with each other and undergoing transformation into A and B. Thus, while initially the *forward* reaction dominates, in time the *backward* reaction becomes increasingly significant, which eventually results in the two rates becoming equal. After this point is reached the concentrations of each species in the reactor becomes fixed and displays no further propensity to change unless propelled by any externally imposed "disturbance"(say, by provision of heat). Under such a condition the reaction is said to be in a state of *equilibrium*. The magnitude of all measurable macroscopic variables (T, P and composition) characterizing the reaction remains constant. Clearly under the equilibrium state the percentage conversion of the reactants to products must be the *maximum* possible at the given temperature and pressure. Or else the reaction would progress furtheruntil the state of equilibrium is achieved. The principles of chemical reaction thermodynamics are aimed at the prediction of this equilibrium conversion.

The reason why the equilibrium conversion itself changes with variation of temperature may be appreciated easily. The rates of the forward and backward reactions both depend on temperature; however, an increase in temperature will, in general, have different impacts on the rates of each. Hence the extent of conversion at which they become identical will vary with temperature; this prompts a change in the equilibrium conversion. Reactions for which the conversion is 100% or nearly so are termed *irreversible*, while for those which never attains complete conversion are essentially *reversible* in nature. The fact that a maxima may occur in the conversion behaviour (fig. 8.1) suggests that for such reactions while the forward reaction rates dominate at lower temperatures, while at higher temperatures the backward reaction may be predominant.

The choice of the reaction conditions thus depends on the maximum (or equilibrium) conversion possible. Further, the knowledge of equilibrium conversions is essential to intensification of a process. Finally, it also sets the limit that can *never* be crossed in practice regardless of the process strategies. This forms a primary input to the determination of the economic viability of a manufacturing process. If reaction equilibria considerations suggest that the maximum possible conversion over practical ranges of temperature is lower than that required for commercial feasibility no further effort is useful in its further development. On the other hand if the absolute maximum conversion is high then the question of optimizing the process conditions attain significance. Exploration of the best strategy for conducting the reaction (in terms of temperature, pressure, rate enhancement by use of catalytic aids, etc) then offers a critical challenge.

This chapter develops the general thermodynamic relations necessary for prediction of the equilibrium conversion of reactions. As we shall see, as in the case of phase equilibria, the Gibbs free energy of a reaction constitutes a fundamental property in the estimation of equilibrium conversion. The next section presents method of depicting the conversion by the means of the reaction co-ordinate, which is followed by estimation of the heat effects associated with all reactions. The principles of reaction equilibria are then developed.

8.2 Standard Enthalpy and Gibbs free energy of reaction

From the foregoing discussion it may be apparent that a chemical reaction may be carried out in diverse ways by changing temperature, pressure, and feed composition. Each of the different conditions would involve different conversions and heat effects. Thus there is need to define a "standard" way of carrying out a reaction. If all reactions were carried out in the same standard manner, it becomes possible to compare them with respect to heat effects, and equilibrium conversion under the same conditions. In general all reactions are subject to heat effects, whether small or large. A reaction may either release heat (exothermic) or absorb heat (endothermic). However, it is expected that the heat effect will vary with temperature. Thus, there is a need to develop general relations that allow computation of the heat effect associated with a reaction at any temperature.

Consider a reaction of the following form:

$$|\alpha_1| A_1 + |\alpha_2| A_2 \rightarrow |\alpha_3| A_3 + |\alpha_4| A_4 \qquad ..(8.1)$$

The reactants (A_1 and A_2) and products (A_3 and A_4) may be gaseous, liquid or solid. The term α_i is the stoichiometric coefficient corresponding to the chemical species A_i. For the purpose of development of the reaction equilibria relations it is convenient to designate the stoichiometric numbers of the reactants as *negative*, while those of the products as *positive*. This is to signify that reactants are *depleted* in proportion to their stoichiometric numbers, while the products are formed in proportion to their stoichiometric numbers. Consider, for example, the following gas-phase reaction:

$$CH_4(g) + 2O_2(g) \quad CO_2(g) + 2H_2O(g)$$

The stoichiometric numbers are written as follows: $\alpha_{CH_4} = -1$; $\alpha_{O_2} = -2$; $\alpha_{CO_2} = 1$; $\alpha_{H_2O} = 2$.

The *standard enthalpy of reaction* $\Delta H_T^{o}{}_0$ at say at any temperature T is defined in the following manner: it is the *change* in enthalpy that occurs when α_1 moles of A_1 and α_2 moles of B_2 in their

standard states at temperature *T* convert *fully* to form α_3 moles of A_3 and α_4 moles of A_4 in their respective standard states at the same temperature *T*. The standard states commonly employed are as follows:

Gases: the pure substance in the ideal gas state at 1 bar *Liquids and Solids*: the pure liquid or solid at 1 bar

The conceptual schema of a standard reaction is depicted in fig. 8.1. All reactants *enter* and products *leave* the reactor in *pure* component form at the same temperature *T*, and at their respective standardstates. In the literature, data on the standard enthalpy of reaction is typically reported at a

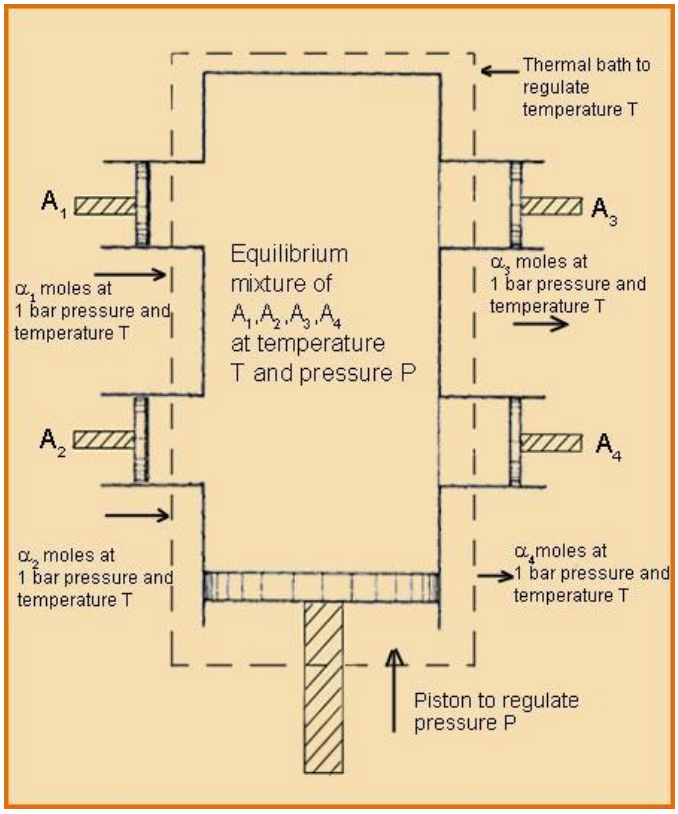


Fig. 8.2 Apparatus in which a gas-phase reaction occurs at equilibrium (van't Hoff equilibrium box)

temperature of 298^0K. Using the sign convention adopted above, the standard enthalpy of reaction at any temperature *T* may be mathematically expressed as follows:

$$\Delta H_T^0 = \sum_i \alpha_i H_i^0{}_{,T} \qquad ..(8.2)$$

Where, $H_{,T}^{0\,i}$ is the standard state enthalpy of species *'i'* at the temperature *T*, and the summation is over all the reactants and products. For example, on expansion the eqn. 8.2 takes the following form for the reaction depicted in eqn. 8.1:

$$\Delta H_T^0 = \alpha_3 H_{3,}{}^0{}_T + \alpha_4 H_{4,}{}^0{}_T - \alpha_1 H_{1,}{}^0{}_T - \alpha_2 H_{2,}{}^0{}_T \quad ..(8.2)$$

If we further consider that each molecular species '*i*' is formed from *j* elements each, an expression for the standard enthalpy of formation results:

$$\Delta H^0_{fi,T} = H_i{}^0{}_{,T} - \sum_j \alpha_j H^0_{j,T} \quad ..(8.3)$$

Where, the summation is over all *j* constituent elements that make up the i^{th} molecule, $\Delta H^0_{fi,T}$ is *standard state enthalpy of formation* of the i^{th}molecule at *T*, and$H^0_{j,T}$ the standard state enthalpy of the j^{th} atomic species. If all $H^0_{j,T}$ are *arbitrarily* set to zero as the basis of calculation then eqn. 8.3 simplifies to:

$$H_i{}^0{}_{,T} = \Delta H^0{}_{fi,T} \quad ..(8.4)$$

In such a case eqn. 1 becomes:

$$\Delta H_T^0 = \sum_{\Delta H^0_{fi,T^i}} \alpha_i \quad ..(8.5)$$

Values of Standard Enthalpy of formation of select substances are shown in Appendix VIII.

For simplicity in the subsequent equations we drop the subscript T, but implicitly all terms correspond to temperature *T*. Now writing H_i^0 in a differential form:

$$dH_i{}^0 = C_{P_i}{}^0\, dT \quad ..(8.6)$$

Where $C_P{}^0{}_i$ is the specific heat of the i^{th} species corresponding to its standard state. Note that since the standard state pressure for all substances is 1 bar in terms of pressure, for gases $C_P{}^0{}_i = C_P{}^{ig}{}_i$, while for liquids and solids it is the *actual* value of the specific heat at 1 bar ($C_P{}^0{}_i = C_{Pi}$) . Since the specific heat of liquids and solids are weakly dependent on pressure, it helps write eqn. 8.6 in the general form shown. The following summation may be applied on eqn. 8.6 to give:

$$\sum_i \alpha_i dH_i^0 = \sum_i \alpha_i C_P{}^0{}_i dT \quad ..(8.7)$$

Since eachξ_i is constant one may write:

$$\sum_i d(\alpha_i H_i^0) = d \sum_i \alpha_i H_i^0 \quad ..(8.8)$$

Or: $$d\sum_i \alpha_i H_i^0 = \sum_i \alpha_i C_P{}^0{}_i dT \quad ..(8.9)$$

Thus: $$d\Delta H^0 = \sum_i \alpha_i C_P{}^0{}_i dT = \Delta C_P{}^o dT \quad ..(8.10)$$

Where, $$\Delta C_P{}^o = \sum_i \alpha_i C_P{}^o{}_i$$

Thus on integrating eqn. 8.10, between a datum T_0 and any T, we have:

$$\Delta H_T{}^o = \Delta H_0{}^o + \int_{T_0}^{T} \Delta C_P{}^o dT \quad ..(8.11)$$

Note that since the standard state pressure is always at 1 bar, for all species one may write the general form of relation for specific heat capacity:

$$C_{P_i}{}^o = A_i + BT_i + C_i T^2 + ... \quad ..(8.12)$$

(The values of and $C_P{}^o{}_i$ thus are those shown in *Appendix III).*

Eqn. 8.12 may be substituted in eqn. 8.11 which leads to:

$$\Delta H_T{}^o = \Delta H_0{}^o + \int_{T0}^{T} (\Delta A) + (\Delta B)T + (\Delta C)T^2 + ...dT \quad ..(8.13)$$

Where: $\Delta A = \sum_i \alpha_i A_i; \Delta B = \sum_i \alpha_i B_i; \Delta C = \sum_i \alpha_i C_t; \Delta D = \sum_i \alpha_i D_t$; and so on.

The standard enthalpy of reaction is most often reported at 298^0K. Using this value as the datum, the value of the standard heat of reaction at any other temperature can be evaluated using eqn. 8.13. As evident from eqn. 8.5 the enthalpy of a reaction may be recovered from the enthalpy of formation of the individual species for a reaction. Values of standard enthalpy of formation for a select list of compounds are tabulated in

In continuance of the foregoing considerations one may also define a standard Gibbs free energy change of a reaction. As we will see in the later sections, this property is essential to computing the equilibrium constant for a reaction at any temperature. As with enthalpy of reaction (eqn. 8.2) the standard Gibbs free energy change at any temperature is given by the function:

$$\Delta G_T{}^0 = \sum_i \alpha_i G_i{}^0{}_{,T} \quad ..(8.14)$$

Thus, ΔG_T^0 is the difference between the Gibbs energies of the products and reactants when each is in its standard state as a pure substance at the system temperature and at a fixed pressure. Thus, just as the standard enthalpy of reaction is dependent *only* on temperature (the standard state pressure being fixed by definition), so is the Gibbs free energy change of a reaction. It follows that when the temperature is fixed ΔG_T^0 is *independent* of the reaction pressure or composition. Indeed extending the argument, one can define any standard property change of reaction by the same expression; all being functions of temperature alone:

$$\Delta M_T^0 = \sum_i \alpha_i M_{i,T}^0 \qquad ..(8.15)$$

Where: $M \equiv U, H, S, A, G.$

In the context of chemical reaction equlibria the relations between the standard enthalpy of reaction and the standard Gibbs energy change of reaction is of particular significance. Using the form described by eqn. 5.31, since any standard property change of a reaction is only temperature dependent, one may write:

$$H_{i,T}^o = -RT^2 \frac{d\left(G_{io,T}/RT\right)}{dT} \qquad ..(8.16)$$

Multiplying of both sides of this equation by α_i and summing over all species one obtains:

$$\sum \alpha_i H_{i,T}^o = -RT^2 \frac{d\left(\sum \alpha_i G_{i,T}^o / RT\right)}{dT}$$

This may be written as: $\Delta H_T^0 = -RT^2 \dfrac{d\left(\Delta G_T^0 / RT\right)}{dT}$ ..(8.17)

Or: $\dfrac{d\left(\Delta G_T^0 / RT\right)}{dT} = -\dfrac{\Delta H_T^0}{RT^2}$ ..(8.18)

Now substituting eqn. 8.13 in 8.18:

$$\frac{d\left(\Delta G_T^0 / RT\right)}{dT} = -\frac{1}{RT^2}\left\{\Delta H_0^o + (\Delta A)T + (\Delta B)T^2 + (\Delta C)T^3 + \ldots\right\} dT$$

If we know the standard Gibbs free energy change $\Delta G_{T_0}^0$ at a particular temperature T_0 (typically, values are reported at 298^0K) the above equation may be integrated as follows:

$$\frac{\Delta G_T^0}{RT} = \frac{\Delta G_{T_0}^0}{RT_0} - \int_{T_0}^{T}\left\langle \frac{1}{RT^2}\left\{\Delta H_0^o + (\Delta A)T + (\Delta B)T^2 + (\Delta C)T^3 + \ldots\right\} dT \right\rangle dT \qquad ..(8.19)$$

Or finally:

$$\frac{\Delta G_T^0}{T} = \frac{\Delta G_{T_0}^0}{T_0} + \int_{T_0}^{T}\left(-\frac{1}{T^2}\Delta H_0^o + \left\{(\Delta A) + (\Delta B)T + (\Delta C)T^2 + \right\}dT\right)dT \quad ..(8.20)$$

Example 8.1

Consider the reaction: $C_2H_4(g) + H_2O(g) \rightarrow C_2H_5OH(g)$. If an equimolar mixture of ethylene and water vapor is fed to a reactor which is maintained at 500 K and 40 bar determine the Gibbs free energy of the reaction, assuming that the reaction mixture behaves like an ideal gas. Assume the following ideal gas specific heat data: $C_p^{ig} = a + bT + cT^2 + dT^3 + eT^{-2}$ (J/mol); T(K).

Species	a	bx10^3	cx10^6	dx10^9	ex10^{-5}
C_2H_4	20.691	205.346	– 99.793	18.825	-
H_2O	4.196	154.565	– 81.076	16.813	-
C_2H_5OH	28.850	12.055	-	-	1.006

8.3 The Reaction Coordinate

Consider again the general chemical reaction depicted in eqn. 8.1:

$$|\alpha_1|A_1 + \alpha_2|A_2 = \alpha_3|A_3 + \alpha_4|A_4|$$

During the progress of the reaction, at each point the extent of depletion of the reactants, and the enhancement in the amount of product is exactly in proportion to their respective stoichiometric coefficients. Thus for any change dn_i in the number of moles of the ith species for a differential progress of the reaction one may write:

$$\frac{dn_1}{\alpha_1} = \frac{dn_2}{\alpha_2} = \ldots = \frac{dn_3}{\alpha_3} = \frac{dn_4}{\alpha_4} \quad ..(8.21)$$

Since all terms are equal, they can all be set equal to a single quantity $d\xi$, defined to represent *theextent of reaction* as follows:

$$\frac{dn_1}{\alpha_1} = \frac{dn_2}{\alpha_2} = \ldots = \frac{dn_3}{\alpha_3} = \frac{dn_4}{\alpha_4} = d\xi \quad ..(8.22)$$

The general relation between a differential change dn_i in the number of moles of a reacting species and $d\xi$is therefore:$dn_i = \alpha_i d\xi (i = 1,2, \ldots N)$ ..(8.23)

This new variable ξ , called the *reaction coordinate*, describe the extent of conversion of reactants to products for a reaction. Thus, it follows that the value of ξ is zero at the start of the reaction. On the other hand when ξ=1, it follows that the reaction has progressed to an extent at which point each reactant has depleted by an amount equal to its stoichiometric number of moles while each product has formed also in an amount equal to its stoichiometric number of moles. For dimensional consistency one designates such a degree of reaction as corresponding to $\Delta\xi$=1 mole.

Now, considering that at the point where the reaction has proceeded to an arbitrary extent characterized by ξ(such that $\xi > 0$), the number of moles of i^{th} species is n_i we obtain the following relation:

$\int_{n_{i0}}^{n_i} dn_i = \alpha_i \int_0^{\xi} d\eta$; where, η is a dummy variable and n_{i0}= initial number of moles of 'i'.Thus:

$$n_i = n_{io} + \alpha_i \xi; (i = 1,2,...,N) \quad ..(8.24)$$

Thus the *total* number of moles of all species corresponding to ξ extent of reaction**:**

$$n = \sum n_i = \sum n_{io} + \xi \sum \alpha_i \quad ..(8.25)$$

Or: $n = n_0 + \alpha\xi$ ..(8.26)

Where:

$$n_0 = \sum n_{io} \quad ..(8.27)$$

$$\alpha = \sum \alpha_i \quad ..(8.28)$$

Thus, $y_i = \frac{n_i}{n} = \frac{n_i + \alpha_i \xi}{n_o + \alpha\xi}$ ..(8.29)

Example 8.2

Consider the following reaction: A(g) + B(g) = C(g) + 3D(g).

Intially the following number of moles are introduced in the reactor. Obtain the mole fraction expressions in terms of reaction coordinate.

$n_{0,A}$= 2 mol, $n_{0,B}$= 1 mol, $n_{0,C}$= 1 mol $n_{0,D}$= 4 mol

---

The foregoing approach may be easily extended to develop the corresponding relations for a set of multiple, independent reactions which may occur in a thermodynamic system. In such a case each reaction is assigned an autonomous reaction co-ordinate ξ_j**(**to represent the j^{th} reaction**).** Further the stoichiometric coefficient of the i^{th} species as it appears in the j^{th} reaction is designated by $\alpha_{i,j}$.Since a species may participate in more than a single reaction, the change in the total number of moles of the species at any point of time would be the sum of the change due each independent reaction; thus, in general:

$$dn_i = \sum_j \alpha_{i,j}\, d\xi_j \qquad (i= 1,2,...N) \qquad ..(8.30)$$

On integrating the above equation starting from the initial number of moles n_{i_o} to n_i corresponding to the reaction coordinate ξ_j of each reaction:

$$\int_{n_{i0}}^{n_i} dn_i = \int_0^{\xi_i} \sum_j \alpha_{i,j}\, d\xi_j (i= 1,2,...,N) \qquad ..(8.31)$$

Or: $$n_i = n_{i_o} + \sum_j \alpha_{i,j}\, \xi_j \qquad ..(8.32)$$

Summing over all species gives:

$$\sum_i n_i = \sum_i n_{i_o} + \sum_i \sum_j \alpha_{i,j}\, d\xi_j \qquad ..(8.33)$$

Now: $\sum_i n_i = n$ and, $\sum_i n_{io} = n_0$ ..(8.34)

We may interchange the order of the summation on the right side of eqn. (8.33); thus:

$$\sum_i \sum_j \alpha_{i,j}\, d\xi_j = \sum_j \sum_i \alpha_{i,j}\, d\xi_j \qquad ..(8.35)$$

Thus, using eqns. 8.34 and 8.35, eqn. 8.33 may be written as:

$$n = n_0 + \sum_j \sum_i \alpha_{i,j} \xi_j \qquad ..(8.36)$$

In the same manner as eqn. 8.28, one may write: .

$$\alpha_j = \sum_i \alpha_{i,j} \qquad ..(8.37)$$

Thus eqn. 8.33 becomes: $n = n_0 + \sum_j \alpha_j \xi_j$ ..(8.38)

Using eqns. 8.32 and 8.38 one finally obtains:

$$y_i = \frac{n_{i_o} + \sum_j \alpha_{ij} \xi_j}{n_o + \sum_j \alpha_j \xi_j} \qquad (i = 1,2,....,N) \qquad ..(8.39)$$

Example 8.3

Consider the following simultaneous reactions. Express the reaction mixture composition as function of the reaction co-ordinates. All reactants and products are gaseous.

A + B = C + 3D ..(1)

A + 2B = E + 4D ..(2)

Initial number of moles: $n_{0,A}$ = 2 mol; $n_{0,B}$ = 3 mol

---

8.4 Criteria for Chemical Reaction Equilibrium

The general criterion for thermodynamic equilibrium was derived in section 6.3 as:

$$(dG^t)_{T,P} \leq 0 \qquad ..(6.36b)$$

As already explained, the above equation implies that if a closed system undergoes a process of change while being under thermal and mechanical equilibrium, for all incremental changes associated with the compositions of each species, the total Gibbs free energy of the system would decrease. At complete equilibrium the equality sign holds; or, in other words, the Gibbs free energy of the system corresponds to the minimum value possible under the constraints of constant (and uniform) temperature and pressure. Since the criterion makes no assumptions as to the nature of the system in terms of the number of species or phases, or if reactions take place between the species, it may also be applied to determine a specific criterion for a reactive system under equilibrium.

As has been explained in the opening a paragraph of this chapter, at the initial state of a reaction, when the reactants are brought together a state of non-equilibrium ensues as reactants begin undergoing progressive transformation to products. However, a state of equilibrium must finally attain when the rates of forward and backward reactions equalize. Under such a condition, no further change in the composition of the residual reactants or products formed occurs. However, if we consider this particular state, we may conclude that while in a macroscopic sense the system is in a state of *static* equilibrium, in the microscopic sense there is dynamic equilibrium as reactants convert to products and vice versa. Thus the system is subject to minute fluctuations of concentrations of each species.

However, by the necessity of maintenance of the dynamic equilibrium the system always returns to the state of stable thermodynamic equilibrium. In a macroscopic sense then the system remains under the under equilibrium state described by eqn. 6.36b. It follows that in a reactive system at the state of chemical equilibrium the Gibbs free energy is minimum subject to the conditions of thermal and mechanical equilibrium.

The above considerations hold regardless of the number of reactants or the reactions occurring in the system. Since the reaction co-ordinate is the single parameter that relates the compositions of all the species, the variation of the total Gibbs free energy of the system as a function of the reaction co-ordinate may be shown schematically as in fig. 8.3; here ξ_e is the value of the reaction co-ordinate at equilibrium.

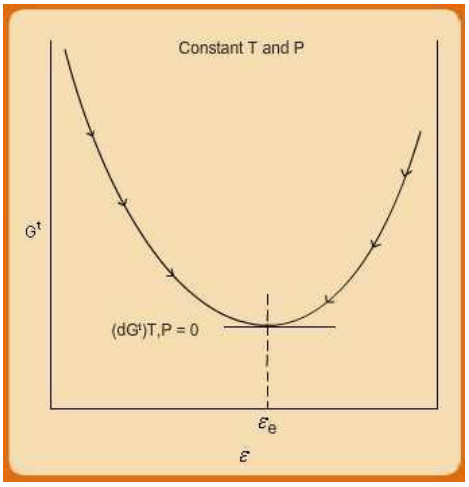


Fig. 8.3 Variation of system Gibbs free energy with equilibrium conversion

8.5 The Equilibrium Constant of Reactions

Since chemical composition of a reactive system undergoes change during a reaction, one may use the eqn. 6.41 for total differential of the Gibbs free energy change (for a single phase system):

$$d(nG) = (nV)\,dP - (nS)\,dT + \sum \mu_i\, dn_i \qquad ..(6.41)$$

For simplicity considering a single reaction occurring in a closed system one can rewrite the last equation using eqn. 8.3:

$$d(nG) = (nV)\,dP - (nS)\,dT + \sum_i \mu\alpha_i\, d\xi \qquad ..(8.40)$$

It follows that: $$\sum \alpha_i \mu_i = \frac{\partial (nG)}{\partial \xi}_{T,P} \qquad ..(8.41)$$

On further applying the general condition of thermodynamic equilibrium given by eqn. 6.36b it follows that:

$$\frac{\partial (nG)}{\partial \xi}_{T,P} \equiv \frac{\partial G^t}{\partial \xi}_{T,P} = 0 \quad ..(8.42)$$

Hence by eqn. 8.41 and 8.42:

$$\sum \alpha_i \mu_i = 0 \quad ..(8.43)$$

Since the reactive system is usually a mixture one may use the eqn. 6.123:

$d\mu_i = d\bar{G}_i = RTd \ln \hat{f}_i$; at constant T ..(6.123)Integration of this equation at constant T from the standard state of species *i* to the reaction pressure:

$$\mu_{i,T} = G^o_{i,T} + RT \ln \frac{\hat{f}_i}{f_i^0} \quad ..(8.44)$$

The ratio $\hat{f}_i / f_i^o$ is called the activity $\hat{a}_i$ of species *i* in the reaction mixture, i.e.:

$$\hat{a}_i = \frac{\hat{f}_i}{f_i^0} \quad ..(8.45)$$

Thus, the preceding equation becomes: $\mu_{i,T} = G_i^0{}_{,T} + RT \ln \hat{a}_i$ ..(8.46)

Using eqns. 8.46 and 8.44 in eqn. 8.43 to eliminate μ_i gives:

$$\sum \alpha_i (G_{i,T}^o + RT \ln \hat{a}_i) = 0 \quad ..(8.47)$$

On further re-organization we have:

$$\sum \nu_i G_i^o{}_{,T} + RT \sum \ln (\hat{a}_i)^{\alpha_i} = 0$$

$$\ln \prod (\hat{a}_i)^{\alpha_i} = - \frac{\sum \alpha_i \underline{G}_i^{\underline{0}}{}_{,T}}{RT} \quad ..(8.48)$$

Where, $\prod$ signifies the product over all species *i.* Alternately:

$$\prod (\hat{a}_i)^{\alpha_i} = \exp - \frac{\sum \alpha_i \underline{G}_{i,T}^{\underline{0}}}{RT} \quad ..(8.49)$$

$$\prod (\hat{a}_i)^{\alpha_i} = \prod \left(\hat{f}_i / f_i^0\right)^{\alpha_i} = K_T \quad ..(8.50)$$

On comparing eqns. 8.49 and 8.50 it follows: $K_T = \exp - \frac{\sum \alpha_i \underline{G}_{j,T}^{\underline{0}}}{RT}$ ..(8.51)

The parameter K_T is defined as the equilibrium constant for the reaction at a given temperature. Since the standard Gibbs free energy of pure species, $G_{i,T}^o$, depends only on temperature, the equilibrium constant K_T is also a function of temperature alone. On the other hand, by eqn. 8.50 K_T is a function of $\hat{f}_i$, which is in turn a function of composition, temperature and pressure. Thus, it follows that sincetemperature fixes the equilibrium constant, any variation in the pressure of the reaction must lead to a change of equilibrium composition subject to the constraint of K_T remaining constant. Equation (8.51)

may also be written as: ..(8.52)

$$-RT\ln K_T = \sum \alpha_i G_i^{o}{}_{,T} = \Delta G_T^0$$

..(8.53)

$$\ln K_T = -\frac{\Delta G_T^0}{RT}$$

Taking a differential of eqn. 8.53:

$$\frac{d \ln K_T}{dT} = -\frac{d\left(\Delta G_T^0 / RT\right)}{dT} \qquad ..(8.53)$$

Now using eqn. 8.18:

$$\frac{d \ln K}{dT}_T = \frac{\Delta H_T^0}{RT^2} \qquad ..(8.54)$$

On further use of eqn. 8.13:

$$\frac{d \ln K_T}{dT} = \frac{\Delta H_0^o + \int_{T_0}^{T} (\Delta A) + (\Delta B)T + (\Delta C)T^2}{RT^2}$$

Lastly, upon integration one obtains the following expression:

$$\ln K_T = \ln K_{T_0} - \frac{\Delta H_0^o + \int_{T_0}^{T} (\Delta A) + (\Delta B)T + (\Delta C)T^2 \; dT}{RT^2} \qquad ..(8.55)$$

Where, K_{T_0} is the reaction equilibrium constant at a temperature T_0.

If ΔH_T^0, is assumed independent of T (i.e. ΔH_{avg}^0, over a given range of temperature $(T_2 - T_1)$, a simpler relationship follows from eqn. 8.54:

$$\ln \frac{K_{T2}}{K_{T1}} = -\frac{\Delta H_{avg}^0}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad ..(8.55)$$

The above equation suggests that a plot of ln K_T vs. 1/ T is expected to approximate a straight line. It also makes possible the estimation of the equilibrium constant at a temperature given its values at

another temperature. However, eqn. 8.55 provides a more rigorous expression of the equilibrium constant as a function of temperature.

Equation 8.54 gives an important clue to the variation of the equilibrium constant depending on the heat effect of the reaction. Thus, if the reaction is exothermic, i.e., $\Delta H_T^0 < 0$, the equilibrium constant decreases with increasing temperature. On the other hand, if the reaction is endothermic, i.e., $\Delta H_T^0 > 0$,equilibrium constant increases with increasing temperature. As we shall see in the followingsection, the equilibrium conversion also follows the same pattern.

Example 8.4

Consider again the reaction: $C_2H_4(g) + H_2O(g) \rightarrow C_2H_5OH(g)$. If an equimolar mixture of ethylene and water vapor is fed to a reactor which is maintained at 500 K and 40 bar determine the equilibrium constant, assuming that the reaction mixture behaves like an ideal gas. Assume the following ideal gas specific heat data: $C_p^{ig} = a + bT + cT^2 + dT^3 + eT^{-2}$ (J/mol); T(K).

Species	a	$bx10^3$	$cx10^6$	$dx10^9$	$ex10^{-5}$
C_2H_4	20.691	205.346	– 99.793	18.825	-
H_2O	4.196	154.565	– 81.076	16.813	-
C_2H_5OH	28.850	12.055	-	-	1.006

8.6 Reactions involving gaseous species

We now consider eqn. 8.50 that represents a relation that connects equilibrium composition with the equilibrium constant for a reaction. The activities $\hat{a}_i$ in eqn. 8.50 contains the standard state fugacity of each species which – as described in section 8.1 – is chosen as that of pure species at 1 bar pressure. The assumption of such a standard state is necessarily arbitrary, and any other standard state may be chosen. But the specific assumption of 1 bar pressure is convenient from the point of calculations. Obviously the value of the state Gibbs free energy G_i^o of the species needs to correspond to that at the

standard state fugacity. In the development that follows we first consider the case of reactions where all the species are gaseous; the case of liquids and solids as reactants are considered following that.

For a gas the standard state is the *ideal-gas state* of pure *i* at a pressure of 1 bar. Since a gaseous species at such a pressure is considered to be in an ideal gas state its fugacity is equal to its pressure; hence at the standard state assumed at the present, f_i^o = 1 bar for each species of a gas-phase reaction. Thus, the activity and hence eqn. 8.50 may be re-written as follows:

$$\hat{a}_i = \hat{f}_i / f_i^o = \hat{f}_i \qquad ..(8.56)$$

$$K = \prod \left(\hat{f}_i\right)^{\alpha_i} \qquad ..(8.57)$$

For the use of eqn. 8.57, the fugacity $\hat{f}_i$ must be specified in bar [or (atm)] because each $\hat{f}_i$ is implicitly divided by f_i^o 1 bar [or 1(atm)]. It follows that the equilibrium constant K_T is dimensionless. This is true also for the case of liquid and/or solid reactive species, though, as is shown later, the standard state fugacity is not necessarily 1 bar, since for condensed phases the fugacity and pressure need not be identical at low pressures.

By eqn. 6.129, for gaseous species, $\hat{f}_i = \hat{\varphi}_i y_i P$.Thus eqn. 8.57 may be rewritten as:

$$K_T = \prod \left(\hat{\varphi}_i y_i P\right)^{\alpha_i} \qquad ..(8.58)$$

On further expanding the above equation:

$$K_T = \left\{\prod \left(\hat{\varphi}_i\right)^{\alpha_i}\right\}\left\{\prod (y_i)^{\alpha_i}\right\}\left\{\prod (P)^{\alpha_i}\right\} \qquad ..(8.59)$$

Or:

$$K_T = K_\varphi K_y P^{\alpha} \qquad ..(8.60)$$

Where:

$$K_\varphi = \left\{\prod \left(\hat{\varphi}_i\right)^{\alpha_i}\right\} \qquad ..(8.61)$$

$$K_y = \left\{\prod (y_i)^{\alpha_i}\right\} \qquad ..(8.62)$$

$$\left\{\prod (P)^{\alpha_i}\right\} = P^{\sum_i \alpha_i} = P^{\alpha} \qquad ..(8.63)$$

An alternate from of eqn. 8.60 is:

$$K_\varphi K_y = K_T P^{-\alpha} \qquad ..(8.64)$$

Both the terms K_φ and K_y contain the mole fraction y_i of each species. As given by eqn. 8.29 or 8.39, all the mole fractions may be expressed as a function of the reaction co-ordinate ξ of the reaction(s). Hence, for a reaction under equilibrium at a given temperature and pressure the only unknown in eqn. 8.64 is the equilibrium reaction co-ordinate ξ_e . An appropriate model for the fugacity coefficient (based on an EOS: virial, cubic, etc.) may be assumed depending on the pressure, and eqn. 8.64 may then solved using suitable algorithms to yield the equilibrium mole fractions of each species. A relatively simple equation ensues in the event the reaction gas mixture is assumed to be ideal; whence $\varphi_i = 1$.Thus, eqn. 8.64 simplifies to:

$$K_y = K_T P^{-\alpha} \qquad ..(8.65)$$

$$\prod (y_i)^{\alpha_i} = P^{-\alpha} K$$

$$K_\varphi = \left\{ \prod (\varphi_i)^{\alpha_i} \right\}$$

Or:

..(8.66)

Yet another simplified version of eqn. 8.64 results on assuming *ideal solution* behavior for which (by eqn.7.84): $\hat{\varphi}_i = \varphi_i$. Thus:

..(8.67)

This simplification renders the parameter K_φ independent of composition. Once again a suitable model for fugacity coefficient (using an EOS) may be used for computing each φ_i and eqn. 8.64 solved for the equilibrium conversion.

Example 8.5

Consider the reaction: $C_2H_4(g) + H_2O(g) \rightarrow C_2H_5OH(g)$. If an equimolar mixture of ethylene and water vapor is fed to a reactor which is maintained at 500 K and 40 bar determine the degree of conversion, assuming that the reaction mixture behaves like an ideal gas. Assume the following ideal gas specific heat data: $C_p^{ig} = a + bT + cT^2 + dT^3 + eT^{-2}$ (J/mol); T(K).

Species	a	$bx10^3$	$cx10^6$	$dx10^9$	$ex10^{-5}$
C_2H_4	20.691	205.346	– 99.793	18.825	-
H_2O	4.196	154.565	– 81.076	16.813	-
C_2H_5OH	28.850	12.055	-	-	1.006

8.7 Reaction equilibria for simultaneous reactions

While we have so far presented reaction equlibria for single reactions, the more common situation that obtains in industrial practice is that of multiple, simultaneous reactions. Usually this occurs due to the presence of 'side' reactions that take place in addition to the main, desired reaction. This leads to the formation of unwanted side products, necessitating additional investments in the form of purification processes to achieve the required purity of the product(s). An example of such simultaneously occurring reaction is:

$$CH_4(g) + 2O_2(g) \rightarrow CO_2(g) + 2H_2O(g)$$

$$CH_4(g) + H_2O(g) \rightarrow CO(g) + 3H_2(g)$$

Clearly the challenge in such cases is to determine the reaction conditions (of temperature, pressure and feed composition) that maximize the conversion of the reactants to the desired product(s). Essentially there are two methods to solve for the reaction equilibria in such systems.

Method 1: Use of reaction-co-ordinates for each reaction

This is an extension of the method already presented in the last section for single reactions. Consider, for generality, a system containing *i* chemical species, participating in *j independent* parallel reactions, each defined by a reaction equilibrium constant K_j and a reaction co-ordinate ξ_j . One can then write a set of *j* equations of the type 8.64 as follows:

$(K_\varphi)_j (K_y)_j = K_{T,j} P^{-\alpha_j}$

Where, α_j and y_i are given by eqns. 8.37 and 8.39 respectively (as follows):

$$\alpha_j = \sum_{i,j} \alpha_{i,j}$$

And, $y_i = \dfrac{n_{i_o} + \sum_j \alpha_{ij} \xi_j}{n_o + \sum_j \alpha_j \xi_j}$ $(i = 1,2,....,N)$

Therefore there are j unknown reaction co-ordinates which may be obtained by solving simultaneously j equations of the type 8.68.

Example 8. 6

The following two independent reactions occur in the steam cracking of methane at 1000 K and 1 bar: $CH_4(g) + H_2O(g) \rightarrow CO(g) + 3H_2(g)$; and $CO(g) + H_2O(g) \rightarrow CO_2(g) + H_2(g)$. Assuming ideal gas behaviour determine the equilibrium composition of the gas leaving the reactor if an equimolar mixture of CH_4 and H_2O is fed to the reactor, and that at 1000K, the equilibrium constants for the two reactions are 30 and 1.5 respectively.

Method 2: Use of Lagrangian Undetermined Multipliers

This method utilizes the well-known Lagrangian method of undetermined multipliers typically employed for optimizing an objective function subject to a set of constraints. As outlined in section 8.3 at the point of equilibrium in a reactive system, the total Gibbs free energy of the system is a minimum. Further, during the reaction process while the total number of moles may not be conserved, the total mass of each atomic species remains constant. Thus, in mathematical terms, the multi-reaction equilibria problem amounts to minimizing the total Gibbs free energy of the system subject to the constraint of conservation of total atomic masses in the system. The great advantage that this approach offers over the previous method is that one does not need to explicitly determine the set of independent chemical reactions that may be occurring in the system.

We formulate below the set of equations that need to be solved to obtain the composition of the system at equilibrium. Let there be N chemical (reactive) species and p (corresponding) elements in a system; further, n_i= initial no of moles of species i; β_{ik}= number of atoms of k^{th} element in the i^{th} chemical species; β_k = total number of atomic masses of k^{th} element as available in the initial feed

Use of p number of Lagrangian multipliers (one for each element present in the system) give:

$$\lambda_k \sum_i n_i \beta_{ik} - \beta_k = 0; \quad (k=1,2\ldots,p) \qquad ..(8.71)$$

These equations are summed over p, giving:

$$\sum_p \lambda_k \sum_i n_i \beta_{ik} - \beta_k = 0 \qquad ..(8.72)$$

Let G^t be the total Gibbs free energy of the system. Thus, incorporating p equations of the type 8.72 one can write the total Lagrangian L for the system as follows:

$$L = G^t + \sum_p \lambda_k \sum_i n_i \beta_{ik} - \beta_k \qquad ..(8.73)$$

It may be noted that in eqn. 8.73, L always equals G^t as the second term on the RHS is identically zero. Therefore, *minimum* values of both $L$and G_t occur when the partial derivatives of L with respect to all the n_i and λ_k are zero.

$$\text{Thus: } \left(\frac{\partial F}{\partial n_i}\right)_{T,P,n_{j\neq i}} = \left(\frac{\partial G^t}{\partial n_i}\right)_{T,P,n_{j\neq i}} + \sum_k \lambda_k \beta_{ik} = 0; \quad (i = 1,2,\ldots,N) \qquad ..(8.74)$$

However, the first term on the RHS is the chemical potential of each reactive species in the system; thus eqn. 8.74 may be written as: $\mu_i + \sum_k \lambda_k \beta_{ik} = 0; (i=1,2,\ldots,N)$ ..(8.75)

But by eqn. 8.44:

$$\mu_{i,T} = G_{i,T}^o + RT\ln\left(\hat{f}_i / f_i^o\right)$$

..(8.44)

Once again, we consider, for illustration, the case of gaseous reactions for which the standard state pressure for each species is 1 bar, whence, $f_i^0 = 1 bar$.

$$\mu_{i,T} = G_{i,T}^o + RT\ln\left(\hat{f}_i\right) \qquad ..(8.76)$$

$$\mu_{i,T} = \Delta G^o_{f_i,T} + RT\ln\left(y_i \hat{\varphi}_i P\right) \qquad ..(8.77)$$

In the above equation $G^o_{i,T}$ may be equated to $\Delta G^o_{f_i,T}$, the latter being the standard Gibbs free energy of formation of the 'i' species (at temperature T). In arriving at this relation, the standard Gibbs free energy of formation of the elements comprising the i^{th} species are arbitrarily set to zero (for

convenience of calculations). Thus combining eqns. 8.75 and 8.77 one obtains:

$$\Delta G^{o}_{f_i,T} + RT\ln\left(y_i\hat{\varphi}_i P\right) + \sum_k \lambda_k \beta_{ik} = 0; \qquad (i=1, 2,\ldots,N); \qquad ..(8.78)$$

In eqn. 8.78, the reaction pressure P needs to be specified in bar (as $f_{i\ 0} = 1bar$).Also, if the i^{th} species is an element, the corresponding $\Delta G^{0}_{f_i,T} = 0$

Further taking the *partial* derivative of the Lagrangian L (of eqn. 8.73) $(\partial L/\partial\lambda_k)_{n_{i,\lambda_{n\neq k}}}$ with respect to each of the p undetermined multipliers, an additional set of p equations of type 8.70 obtains. Thus there are a total of (N+p) equations which may be solved simultaneously to obtain the complete set of equilibrium mole fractions of N species.

Example 8.7

The gas n-pentane (1) is known to isomerise into neo-pentane (2) and iso-pentane (3) according to the following reaction scheme: $P_1P_2; P_2P_3; P_3P_1$. 3 moles of pure n-pentane is fed into a reactor at 400^{o}K and 0.5 atm. Compute the number of moles of each species present at equilibrium.

Species	ΔG^{0}_{f} at 400^{o}K (Cal/mol)
P_1	9600
P_2	8900
P_3	8200

8.8 Reactions involving Liquids and Solids

In many instances of industrially important reactions, the reactants are not only gaseous but are also liquids and / or solids. Such reactions are usually *heterogeneous* in nature as reactants may exist in separate phases. Some examples include:

- Removal of CO_2 from synthesis gas by aqueous solution of potassium carbonate
- Removal of H_2S by ethanolamine or sodium hydroxide
- Air oxidation of aldehydes to acids
- Oxidation of cyclohexane to adipic acid
- Chlorination of benzene

• Decomposition of $CaCO_3$ to CaO and CO_2

In all such instances some species need to dissolve and then diffuse into another phase during the process of reaction. Such reactions therefore require not only reaction equilibria considerations, but that of phase equilibria as well. For simplicity, however we consider here only reaction equilibria of instances where liquid or solid reactive species are involved. The thermodynamic treatment presented below may easily be extended to describe any heterogeneous reaction. The basic relation for the equilibrium constant remains the starting point. By eqn. 8.50 we have:

$$K_T = \prod (\hat{a}_i)^{\alpha_i} \qquad ..(8.50)$$

On expanding (by eqn. 6.171):

$$\hat{a}_i = \hat{f}_i(T, P, x_i)/f_i^0 \qquad ..(8.79)$$

As already mentioned in section 8.1 above, for solids and liquids the usual standard state is the *pure* solid or liquid at 1 bar [or 1(atm)] and at the temperature (T) of the system. However, unlike in the case of gaseous species, the value of f_i^o for such a state cannot be 1 bar (or 1 atm), and eqn.(8.50) cannot be reduced to the form simple form of eqn. 8.57.

Liquid-phase reactants

On rewriting eqn. 8.79:

$$\hat{f}_i(T, P, x_i) = x_i \gamma_i f_i$$

Thus:

$$\hat{a}_i = x_i \gamma_i f_i(T, P) / f_i^0(T, 1bar) \qquad ..(8.80)$$

By eqn. 6.115:

$$RT d\ln f_i = V_i \, dP$$

Thus on integrating:

$$\int_{f_i^0(T,1bar)}^{f_i(T,P)} d\ln f_i = \int_1^P \frac{V_i}{RT} \, dP \qquad ..(8.81)$$

As we have already seen in section 6.10, the liquid phase properties, such as molar volume, are weakly dependent on pressure; hence their variation with respect to pressure may be, for most practical situations, considered negligible. Thus, if one considers that in the last equation the molar volume V_i is constant over the range 1 – P bar, one obtains:

$$\ln \frac{f_i}{f_i^0} = \frac{V_i(P-1)}{RT} \quad ..(8.82)$$

$$\therefore f_i / f_i^o = \exp \frac{V_i (P-1)}{RT} \quad ..(8.83)$$

Thus, using eqn. 8.53 in 8.50:

$$K_T = \prod_{(a_i)}^{\alpha_i} = \prod (x_i \gamma_i)^{\alpha_i} \prod (f_i / f_i^o)^{\alpha_i} \quad ..(8.84)$$

Or: $\prod (f_i / f_i^o)^{\alpha_i} = \exp \frac{P-1}{RT} \sum \alpha_i V_i$

Thus:

$$K_T = \prod (x_i \gamma_i)^{\alpha_i} \exp \frac{(P-1)\sum \alpha_i V_i}{RT} \quad ..(8.85)$$

Except for very high pressure the exponential term on the right side of the above equation: $(P-1)\sum \alpha_i V_i << RT$.

Thus one may approximate:

$$\exp \frac{(P-1)\sum \alpha_i V_i}{RT} \approx 1.0$$

Whence:

$$K_T \prod (x_i \gamma_i)^{\alpha_i} \quad ..(8.86)$$

To use the above equation for prediction of the equilibrium composition one needs to employ a suitable activity coefficient model. The activity coefficient models described in section 6.18 are based on physical interactions between molecules. Hence, their use is not expected to be adequately representative of a situation where molecules are subject to specific chemical forces and are chemically transformed due to formation of intermediate reactive complexes. While it is possible to write models for physical interactions it is generally not feasible to establish quantitative relations that describe the microscopic-level interactions between reactive molecular species. Discussions on approaches to solve such problems may be found elsewhere (J.M. Prausnitz, R.N. Lichtenthaler and E.G. Azevedo, *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed., Prentice Hall, 1998). Nevertheless, for

the purpose of illustrating an approximate solution, one may simplify eqn. 8.86 by assuming ideal solution behavior, wherein $\gamma_i = 1.0$. Hence:

$$K = \prod (x_i)^{\alpha_i} \qquad ..(8.87)$$

However, since reactive solutions can never be ideal, one way to overcome the difficulty is by defining a reaction equilibrium constant based on molar concentration (say in moles/m^3), rather than in terms of mole fractions. Thus:

$$K_C = \prod (C_i)^{\alpha_i} \qquad ..(8.88)$$

Where, C_i = molar concentration of each species.

It is generally difficult to predict the equilibrium constant K_C, and one needs to use experimentally determine values of such constants in order to predict equilibrium compositions.

Example 8.8

Consider the liquid phase reaction: A(l) + B(l) → C(l) + D(l). At 50^{o}C, the equilibrium constant is 0.09. Initial number of moles, $n_{A,0}$ = 1 mole; $n_{B,0}$ = 1 mol Find the equilibrium conversion. Assume ideal solution behaviour.

Solid-phase reactants

Consider a solid reactive species now, for which one again starts from eqn. 8.80:

$$\hat{a}_i = f_i(T, P, x_i) / f_i^0 \qquad ..(8.80)$$

Thus as for a liquid reactant one has

$$\hat{a}_i = (x_i \gamma_i)\left(f_i / f_i^0\right) = \hat{a}_i = (x_i \gamma_i) \exp \frac{(P-1)V_i}{RT}$$

As it is for liquid species, V_i for solids is also small and remains practically constant with pressure, thus:

$$\exp \frac{(P-1)\sum \alpha_i V_i}{RT} \approx 1.0$$

In addition, the solid species is typically 'pure' as any dissolved gas or liquid (for a multi-phase reaction) is negligible in amount.

Thus $x_i \sim 1.0$, $\rightarrow \gamma_i = 1$.

Therefore, for solids $\hat{a}_i = (x_i \gamma_i) \exp \frac{(P-1)V_i}{RT} \approx 1.0$ ..(8.89)

APPLIED THERMODYNAMICS FOR BIOTECHNOLOGISTS
Unit V notes

Metabolic stoichiometry and energetics

A living cell is a complex chemical reactor in which more than 1000 independent enzyme-catalyzed reactions occur. Still, the material and energy balance restrictions and thermodynamic principles familiar from analysis of chemical process systems apply equally well to biological systems. In this chapter, we shall examine the cell as a chemical reactor, focusing on the stoichiometric rules and energy flows which characterize living organisms.

The total of all chemical reaction activities which occur in the cell is called *metabolism*. A simplified diagram of some of the major elements of metabolism in the bacterium *Escherichia coli* is shown in Fig. 5.1. We see there that metabolic reactions tend to be organized into sequences called metabolic pathways, and that there is some connection between the pathways by virtue of circular, closed pathways feeding back on themselves and because of pathway branches which connect one reaction sequence with another. All of the arrows in Fig. 5.1 denote one or more enzyme-catalyzed reactions which convert cell constituents (*metabolites*) to different compounds.

A cell produces order (itself and its offspring) from its disorderly surroundings. We now know in some detail how energy from the environment is used to drive this process. Finally, and perhaps of greatest importance in bioprocess engineering, the study of energy exchanges helps explain the major distinction between cell function in the presence and absence of oxygen. As we have already

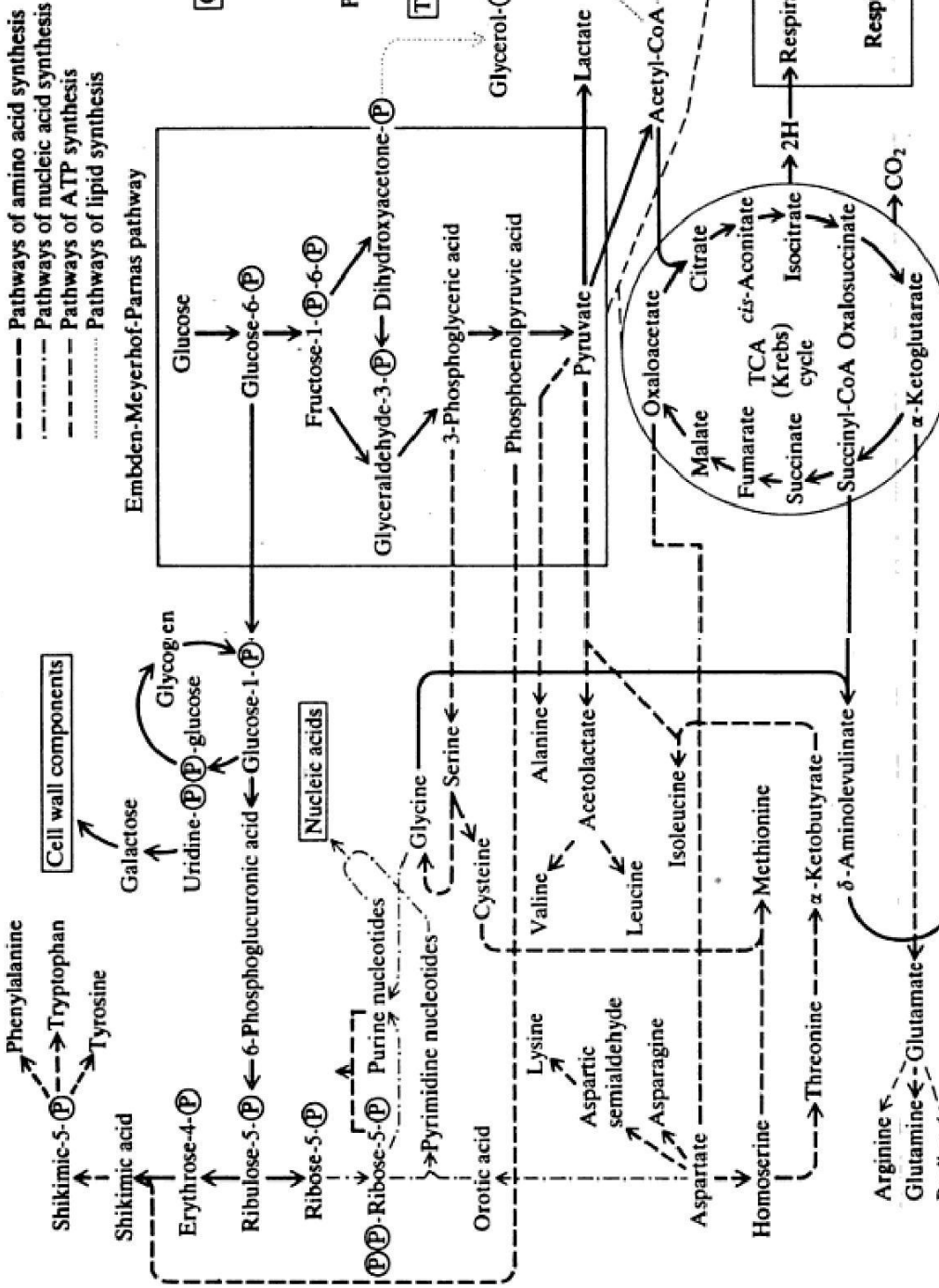


Figure 5.1 A summary of a few of the major metabolic pathways in the bacterium *E. coli*. *(Reprinted by permission from J. D. Watson, "Molecular Biology of the Gene," 2d ed., pp. 96–97, W. A. Benjamin, Inc., New York, 1970.)*

Table 5.2 Classification of organisms by carbon and energy source

In fact, material balance constraints and thermodynamic requirements give useful working relationships within a very simplified view of cellular activity, one which is entirely macroscopic and which does not utilize any information about the internal chemical workings of the cell. This macroscopic view, considered further in Sec. 5.10, examines the utilization of substrates by cells to produce more cells and certain products. Based on this perspective, we can develop equations for inferring certain biological reactor conditions from available measurements and for testing the consistency of experimental data.

In this chapter, we will build from fundamental detail to overall macroscopic views following the sequence of the preceding outline. We begin by looking in some detail at the role of ATP, the energy shuttle in the cell, and the types of reactions in which energy is transferred to and taken from this carrier. Also, the importance of oxidation-reduction reactions in the cell is considered along with the electron carrying reagents NADH and NADPH. Then, we turn to certain catabolic reaction pathways—reaction pathways involving the breakdown of certain nutrients, here typically glucose, to determine their energetic and chemical stoichiometry. Photosynthetic mechanisms are reviewed briefly. Subsequently, some of the synthetic pathways of the cell are considered from the same viewpoint. Next, we examine coupling between energy and electron generating pathways and those pathways which utilize these cellular commodities, and, finally, we examine the overall stoichiometric constraints which apply to cell growth. To conclude the chapter, the stoichiometry of product formation and its relationship to cell growth stoichiometry is considered.

5.1 THERMODYNAMIC PRINCIPLES

To get an idea of whether a certain chemical reaction in the cell will run forward or backward, we will use a number of approximations since full analysis of the entire metabolic network is not practical. First, we note that the free-energy change $\Delta G'$ of the single chemical reaction

$$\alpha A + \beta B \rightarrow \gamma C + \delta D \tag{5.1}$$

can be written in the form

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln \left(\frac{c^{\gamma} d^{\delta}}{a^{\alpha} b^{\beta}} \right) \tag{5.2}$$

As before, lowercase letters a, b, ... signify molar concentrations of compounds A, B, ... In writing Eq. (5.2), we have substituted concentrations for activities since biological solutions are typically dilute. Here, primes denote evaluation in aqueous solution at pH 7. Accordingly, under these conditions, the concentrations of water and H^+ are not included in the last term of Eq. (5.2) even when H_2O and H^+ appear in reaction (5.1). The standard free energy change $\Delta G^{\circ\prime}$ thus denotes the free energy change of reaction (5.1) in neutral aqueous solution with all other reactants and products present at 1 M concentration (see Ref. 1).

of chemical or light energy utilized by the cell.

In a closed system, the reaction will proceed from left to right if and only if $\Delta G'$ is negative. Accordingly, $\Delta G'$ is zero at equilibrium giving the well-known relationship

$$\Delta G^{\circ\prime} = -RT \ln K'_{eq} \tag{5.3}$$

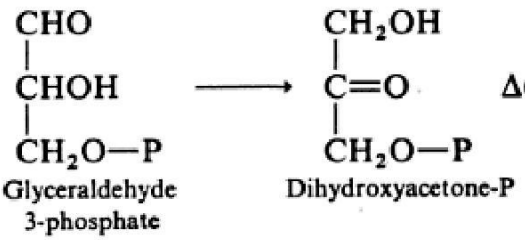
where

$$K'_{eq} = \frac{c_{eq}^{\gamma} d_{eq}^{\delta}}{a_{eq}^{\alpha} b_{eq}^{\beta}} \tag{5.4}$$

Remember that if water or H^+ are involved in the reaction, their concentrations do not enter into the calculation of the right-hand side of Eq. (5.4); the value of K'_{eq} already includes the water and H^+ concentrations (for pH 7).

Occasionally in the following discussion, we will associate negative values of $\Delta G^{\circ\prime}$ with reactions which occur from left to right. This is clearly an approximation since a cell is not a closed system and since reactants are generally not present at 1 M concentration. The utility of this approximation in gaining a general understanding of bioenergetic principles is presented lucidly in Lehninger's book *Bioenergetics* [1], which is recommended additional reading.

However, we should recognize possible pitfalls of such a limited approach. In coupled-reaction networks of the kind indicated in Fig. 5.1, the direction of a major reaction path in cell metabolism is often not indicated properly by examining an isolated reaction. For example, consider the reaction between two isomers in the Embden-Meyerhof pathway for glucose breakdown



(5.5)

where P denotes phosphate. Because of the negative free-energy change, equilibrium favors the dihydroxyacetone by a 22:1 ratio. However, as Fig. 5.1 indicates, when this reaction occurs within the EMP pathway, glyceraldehyde phosphate is continually removed by reactions leading ultimately to pyruvate. As the glyceraldehyde phosphate is tapped off, reaction (5.4) is forced to proceed from right to left in an attempt to maintain equilibrium.

Many biological reactions and energy conversion processes involve oxidation-reduction reactions such as

$$A_{ox} + B_{red} \rightleftharpoons A_{red} + B_{ox} \tag{5.6}$$

The tendency for this type of reaction to occur is described frequently using the standard potential change $\Delta E^{\circ\prime}$, which in turn may be written

$$\Delta E^{\circ\prime} = E^{\circ\prime}_{(A_{ox}/A_{red})} - E^{\circ\prime}_{(B_{ox}/B_{red})} . \tag{5.7}$$

where $E^{\circ\prime}_{(A_{ox}/A_{red})}$ is the standard half-cell potential for the half-reaction

$$A_{ox} + 2e^- \longrightarrow A_{red} \tag{5.8}$$

As a reference point for half-cell potential values, the hydrogen half-cell (at pH = 0) is assigned a value of zero:

$$2H^+ + 2e^- \longrightarrow H_2 \qquad E_0 = 0.00\ \text{V (pH = 0)} \tag{5.9}$$

Free energy changes and corresponding potential changes are related by

$$\Delta G' = -n\mathcal{F}\Delta E' \tag{5.10}$$

where n is the number of electrons transferred and Faraday's constant $\mathcal{F}$ is equal to 23.062 kcal/V mol. Equation (5.10) indicates that only redox reactions with positive $\Delta E'$ values proceed from left to right. If we require the value of $\Delta E'$ for nonstandard conditions, Eqs. (5.2) and (5.10) may be used.

5.2 METABOLIC REACTION COUPLING: ATP AND NAD

In Chaps 3 and 4 the kinetics of isolated enzyme systems and the industrial utilization of free enzymes and enzyme preparations have been considered. The majority of the kinetic studies mentioned in Chap. 3 and most of the current important usages cited in Chap. 4 involve hydrolytic enzymes which, by definition, split or degrade larger molecules into smaller components by consuming water as a second substrate. Such degradations proceed with a decrease in free energy of the system and are therefore spontaneous in a closed system. The present section introduces the mechanisms by which an open system, the living cell, is able to couple energy-yielding (*exergonic*) reactions with chemical reactions and other functions which do not occur appreciably unless energy is supplied (*endergonic* processes).

5.2.1 ATP and Other Phosphate Compounds

The structure and some properties of adenosine triphosphate (ATP) have been mentioned already (see Sec. 2.3.1 and Fig. 2.8). As noted there, the enzymatic hydrolysis of ATP to yield ADP and inorganic phosphate has a large negative free-energy change

$$ATP + H_2O \longrightarrow ADP + P_i \qquad \Delta G^{\circ\prime} = -7.3\ \text{kcal/mol} \tag{5.11}$$

where P_i denotes inorganic phosphate. Thus, a substantial amount of free energy may be released by the hydrolysis and, by reversing the reaction and adding phosphate to ADP, free energy can be stored for later use. Let us next examine how the latter situation can occur using coupled chemical reactions.

Another example from the Embden-Meyerhof-Parnas pathway serves to illustrate the concept of a *common chemical intermediate*. Conversion of an aldehyde in an aqueous medium to a carboxylic acid results in a free-energy decrease

In a closed system, the reaction will proceed from left to right if and only if $\Delta G'$ is negative. Accordingly, $\Delta G'$ is zero at equilibrium giving the well-known relationship

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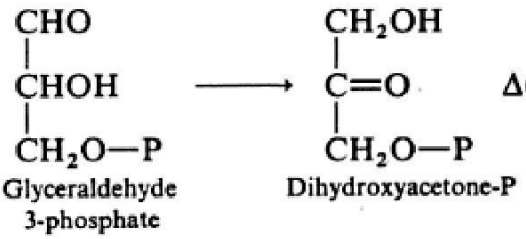

Glyceraldehyde 3-phosphate — Dihydroxyacetone-P

$$\Delta G^{\circ\prime} = -1830 \text{ cal/mol} \tag{5.5}$$

where P denotes phosphate. Because of the negative free-energy change, equilibrium favors the dihydroxyacetone by a 22:1 ratio. However, as Fig. 5.1 indicates, when this reaction occurs within the EMP pathway, glyceraldehyde phosphate is continually removed by reactions leading ultimately to pyruvate. As the glyceraldehyde phosphate is tapped off, reaction (5.4) is forced to proceed from right to left in an attempt to maintain equilibrium.

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where P_i denotes inorganic phosphate. Thus, a substantial amount of free energy may be released by the hydrolysis and, by reversing the reaction and adding phosphate to ADP, free energy can be stored for later use. Let us next examine how the latter situation can occur using coupled chemical reactions.

Another example from the Embden-Meyerhof-Parnas pathway serves to illustrate the concept of a *common chemical intermediate*. Conversion of an aldehyde in an aqueous medium to a carboxylic acid results in a free-energy decrease

of about 7000 cal/mol. As summarized in Fig. 5.3, this chemical free energy would be completely dissipated in an isolated solution. This does not occur in the living cell. In biochemical glucose oxidation, when 3-phosphoglyceraldehyde is converted into a carboxylic acid (3-phosphoglycerate), ATP is simultaneously regenerated from ADP (see reaction 2 of Fig. 5.3). The free-energy decrease resulting from aldehyde oxidation is coupled by the cell enzymes to the simultaneous regeneration of ATP. Since reaction 2 results in approximately no free-energy change, the free-energy released in oxidation of 3-phosphoglyceraldehyde has been transformed into a so-called *high-energy phosphate bond* in adenosine triphosphate.

The elementary reaction sequence by which the conversions actually occur is shown at the bottom of Fig. 5.3. The central important features of these last two reactions are: (1) the appearance of a common intermediate; the same compound

1 Isolated oxidation of aldehyde to carboxylic acid (aqueous solution),

$$RCHO + H_2O \longrightarrow 2H + RCOO^- + H^+, \qquad \Delta G_1^{\circ\prime} \simeq -7 \text{ kcal/mol}$$

2 Same reactions, coupled to ATP generation (glucose oxidation),

$$RCHO + HPO_4^{2-} + ADP^{3-} \longrightarrow 2H + RCOO^- + ATP^{4-},$$

$$\Delta G_2^{\circ\prime} \simeq 0 \text{ kcal}$$

3 Evidently, *2* − *1* yields,

$$ADP^{3-} + HPO_4^{2-} + H^+ \longrightarrow ATP^{4-} + H_2O, \qquad \Delta G_3^{\circ\prime} \simeq +7 \text{ kcal/mol}$$

The elementary reactions occurring in *2* are,

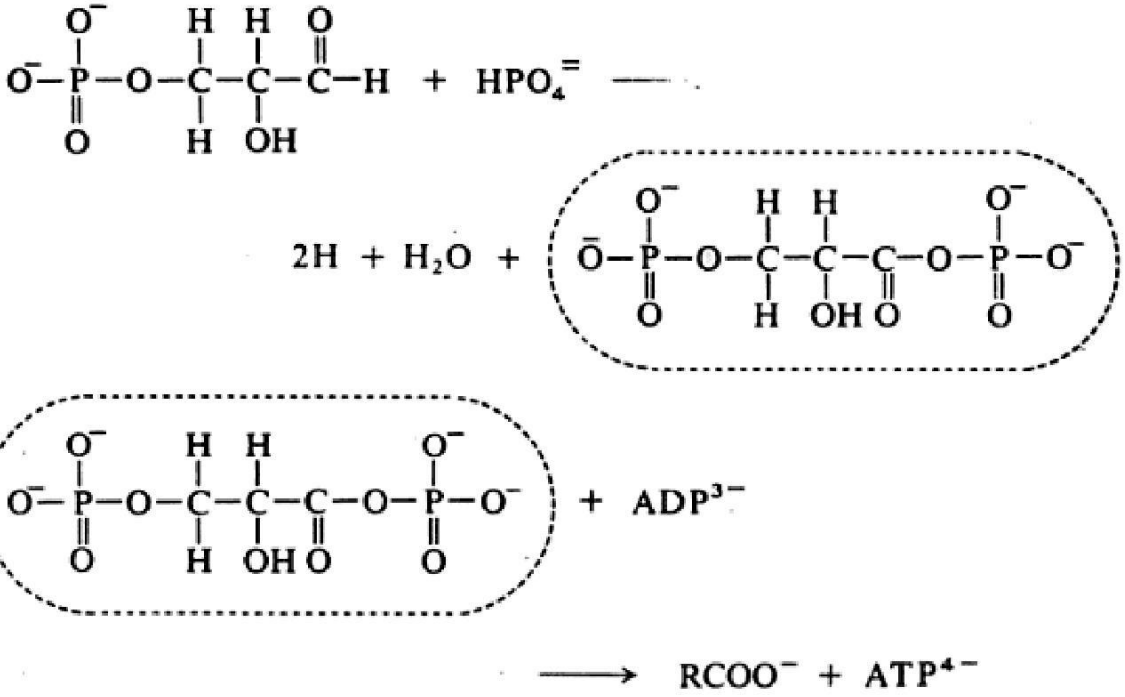
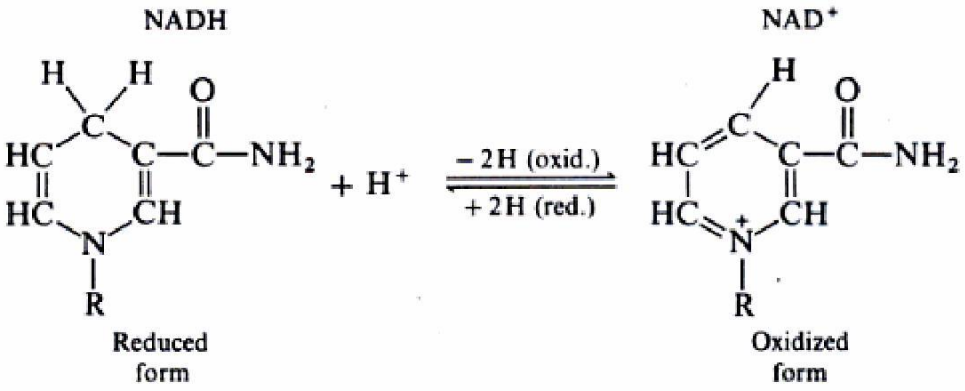


Figure 5.3 Example of reactions coupled via a common intermediate.

(Pyruvic acid is the same compound as pyruvate in Fig. 5.1. Pyruvate refers to the ionized form CH_3COCOO^-, which predominates at biological pH.)

Pairs of hydrogen atoms freed during oxidations or required in reductions are carried by nucleotide derivatives, especially nicotinamide adenine dinucleotide (NAD) (see Fig. 2.9) and its phosphorylated form NADP. These compounds were classified as coenzymes earlier since they usually must be present when an oxidation or reduction is conducted. When hydrogen atoms are needed, for example, the nicotinamide group of reduced NAD can contribute them by undergoing the oxidation



This oxidation is readily reversible, so that NAD can also accept electrons (H atoms) when they are made available by oxidation of other compounds. As indicated above, we shall denote the oxidized and reduced forms of NAD as NAD^+ and NADH, respectively.

In its role as electron shuttle, NAD serves two major functions. The first is analogous to one of ATP's jobs: reducing power (=electrons ≈ H atoms) made available during breakdown of nutrients is carried to biosynthetic reactions. Such a transfer of reducing power is often necessary because the oxidation state of the nutrients to be used for construction of cell components is different from the oxidation state of biosynthesis products. As observed already, the oxidation state of carbon within the cell is approximately the same as carbohydrate (CH_2O). Thus, autotrophic organisms, which employ CO_2 as their carbon source, use considerable reducing power when assimilating carbon

$$CO_2 + 4H \longrightarrow (CH_2O) + H_2O$$

While carbon dominates reducing-power requirements, nitrogen and sulfur assimilation often also demands some of the cell's carrier-bound hydrogen. To estimate these needs, we may assume that cell material contains nitrogen at the oxidation level of ammonia (NH_3), while sulfur's oxidation state is approximately that of sulfide (S^{2-}). For example, use of sulfate as a sulfur source requires considerable reducing power, as suggested by

$$SO_4^{2-} + 8H \longrightarrow S^{2-} + 4H_2O$$

Furthermore, certain microbes can degrade toxic compounds such as phenols and polychlorinated biphenyls. Obviously, such organisms and the enzymes they synthesize have exciting process potential for specific chemical catalysis and for environmental protection. An introduction to the enzymes and pathways encountered in hydrocarbon metabolism is provided in Doelle's excellent book [3].

5.4 RESPIRATION

Respiration is an energy-producing process in which organic or reduced inorganic compounds are oxidized by inorganic compounds. As Table 5.4 indicates, various bacteria conduct respiration using several different reductants and oxidants. When an oxidant other than oxygen is involved, the process is called *anaerobic respiration*, the term *aerobic respiration* being reserved for the situation typical of eucaryotes and many bacteria where O_2 is the oxidant. Recall that lithotrophs are organisms employing inorganic reductants. Several lithotrophs are evident in Table 5.4. So far as is known today, all lithotrophs are also autotrophs; they obtain carbon from CO_2.

In the most common forms of respiration, an organic compound is oxidized using oxygen. We consider only this case in the remainder of this section, and the term respiration will be used to describe this process. It is convenient to decompose the overall process of respiration into two phases. In the first, organic compounds are oxidized to CO_2, and pairs of hydrogen atoms (electrons) are transferred to NAD. Next, the hydrogen atoms are passed through a sequence of reactions, during which ATP is regenerated from ADP. At the end of their journey, the hydrogen atoms are combined with oxygen to give water. These two phases of biological oxidation will now be examined in greater detail.

Table 5.4 Reductants and oxidants in bacterial respirations†

Reductant	Oxidant	Products	Organism
H_2	O_2	H_2O	Hydrogen bacteria
H_2	SO_4^{2-}	$H_2O + S^{2-}$	*Desulfovibrio*
Organic compounds	O_2	$CO_2 + H_2O$	Many bacteria, all plants and animals
NH_3	O_2	$NO_2^- + H_2O$	Nitrifying bacteria
NO_2^-	O_2	$NO_3^- + H_2O$	Nitrifying bacteria
Organic compounds	NO_3^-	$N_2 + CO_2$	Denitrifying bacteria
Fe^{2+}	O_2	Fe^{3+}	*Ferrobacillus* (iron bacteria)
S^{2-}	O_2	$SO_4^{2-} + H_2O$	*Thiobacillus* (sulfur bacteria)

† From W. R. Sistrom, *Microbial Life*, 2d ed., table 4-2, p. 53, Holt, Rinehart, and Winston, Inc., New York, 1969.

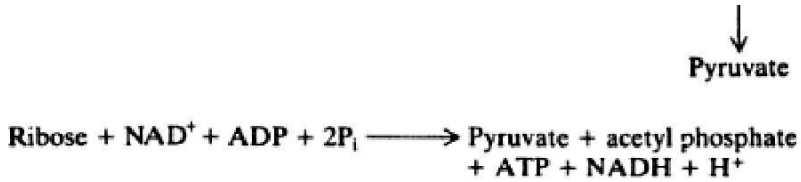


Figure 5.6 Simplified diagram of ribose catabolism via the phosphoketolase pathway.

energy yield of the EMP pathway.

processed, the overall effect is phosphorylation of ATP. As indicated in Fig. 5.4, dehydrogenation of glyceraldehyde 3-phosphate is coupled with reduction of

5.4.1 The TCA Cycle

For the moment let us continue the story of carbohydrate metabolism started in Sec. 5.3. All the pathways to pyruvate described there can also operate during respiration. The reactions peculiar to respiration start with pyruvate. In respiration metabolism, pyruvate is not reduced to some end product using the hydrogen atoms obtained during glucose breakdown. Instead, this reducing power is saved for other uses, to be discussed shortly. Moreover, additional reducing power is generated from pyruvate by converting it to an acetic acid derivative (acetyl CoA)

$$CH_3COCOOH + NAD^+ + CoA{-}SH \xrightarrow{\text{pyruvic dehydrogenase complex}} CH_3CO{-}S{-}CoA + CO_2 + NADH + H^+ \quad (5.18)$$

Acetyl CoA† is also a key intermediate in the catabolism of amino acids and fatty acids. Consequently, three classes of biomolecules can be oxidized through acetyl CoA.

The first phase of this oxidation is carried out in a cyclic reaction sequence called the *tricarboxylic acid* or *TCA cycle* (also, Krebs cycle and citric acid cycle; Fig. 5.7). Notice that the two remaining carbon atoms from pyruvate (one was lost as CO_2 in the reaction to acetyl CoA) enter the cycle to create a six-carbon acid from one with four carbons. In one pass around the TCA cycle, however, two-carbon atoms are expelled as CO_2. Thus, this first phase of respiration consumes all the carbon atoms from the original pyruvate substrate.

The overall stoichiometry of the TCA cycle plus reaction (5.12) is

$$C_3H_4O_3 + ADP + P_i + 2H_2O + FAD + 4NAD^+ \longrightarrow 3CO_2 + ATP + FADH_2 + 4(NADH + H^+) \quad (5.19)$$

The TCA cycle as shown in Fig. 5.7 appears to serve a strictly catalytic function: no input of carbon other than the substrate is indicated. As Fig. 5.1 shows, however, the TCA cycle serves a very important function as a pool of precursors for biosynthetic reactions. Consequently, some intermediates in the cycle are constantly being tapped off and must be replaced. This is accomplished by synthesis of oxaloacetic acid from pyruvate or another three-carbon acid. Alternatively, in some microorganisms TCA cycle intermediates are replenished by the *glyoxylate cycle* which has the net effect of producing one molecule of succinate by condensation of two acetate molecules.

5.4.2 The Respiratory Chain

Leaving carbon metabolism, let us next follow the reactions in which hydrogen atoms are oxidized to water, the process from which aerobic cells derive most of

† For coenzyme A see Fig. 2.9.

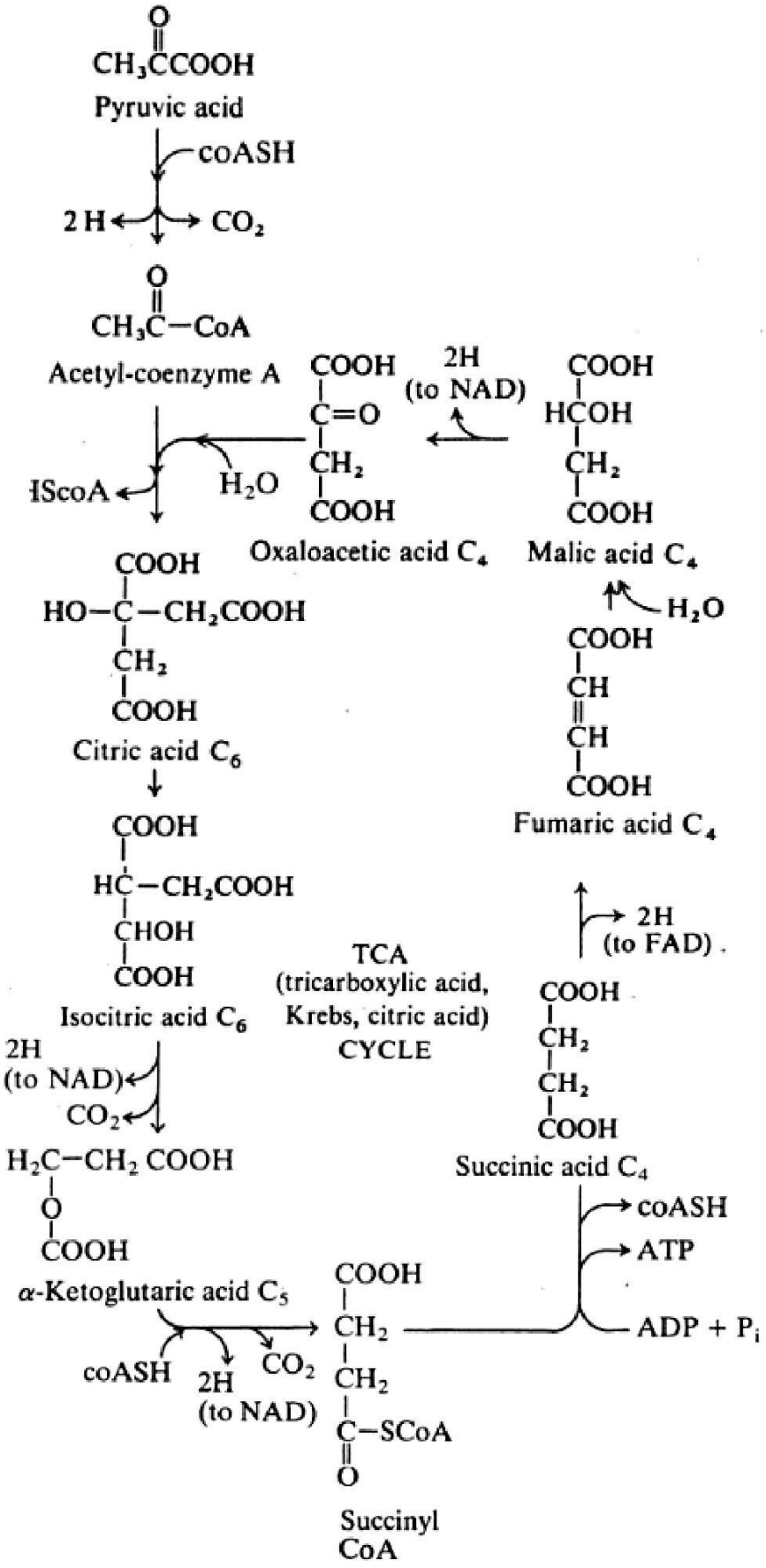


Figure 5.7 The tricarboxylic acid cycle.

their energy. In each pass around the TCA cycle, four pairs of hydrogen atoms are liberated. Three pairs are transferred to NAD and, as outlined in Fig. 5.7 the hydrogen atoms resulting from succinic acid dehydrogenation are transferred to flavin adenine dinucleotide (FAD) (see Fig. 2.9). Some of the reducing power derived from the TCA cycle may be needed in biosynthesis reactions; the rest is used to generate ATP.

In the following discussion of ATP generation during respiration, we shall concentrate on the case in which all the hydrogen atoms obtained during glucose breakdown are available for reaction in the *respiratory chain*. This is the situation which provides the most stored energy for the cell in the form of ATP. Figure 5.8 shows an abbreviated diagram of this reaction sequence and how it ties with the ultimate breakdown of pyruvate via acetyl CoA and the TCA (Krebs) cycle. In this diagram, FP_1 and FP_2 denote two different flavoproteins, which are enzymes containing FAD for transport of electrons. Electrons from NADH ($=FP_1$) are passed to coenzyme Q (designated as Q in the figure), and in this process one molecule of ADP is regenerated to ATP for each pair of electrons passed. The electrons obtained from succinate dehydrogenation in the TCA cycle are carried by FAD in FP_2 directly to coenzyme Q.

From there, all electrons enjoy the common fate of passing through a sequence of *cytochromes*, proteins containing heme groups which are designated *b*, *c*, *a* and a_3 in Fig. 5.8. Along the way, ATP is generated twice for each electron pair. The process of ATP regeneration in the respiratory chain is called *oxidative phosphorylation*. Ultimately, the hydrogen atoms are combined with dissolved oxygen to yield water as the second final product of the oxidation. If we examine the free-energy changes along the respiratory chain, we find that ATP is regenerated at each point where there is a sufficiently large decrease in electron free energy to more than offset the 7.3 kcal/mol needed to phosphorylate ADP.

The net reactions, then, which describe the respiratory chain are

$$NADH + H^+ + \tfrac{1}{2}O_2 + 3ADP + 3P_i \longrightarrow NAD^+ + 4H_2O + 3ATP \quad (5.20)$$

and

$$FADH_2 + \tfrac{1}{2}O_2 + 2ADP + 2P_i \longrightarrow FAD + 3H_2O + 2ATP \quad (5.21)$$

Respiration potentially makes available much more energy for use by the cell than glycolysis since $\Delta G^{\circ\prime}$ is -686 kcal/mol for the reaction

$$Glucose + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad (5.22)$$

Let us examine how efficiently respiring living systems tap this large source of energy. For comparison with the above reaction, we presume here that glucose is completely oxidized to CO_2 and water via the EMP, TCA, and respiratory chain pathways. We should remember, however, that the EMP pathway and TCA cycle are both amphibolic pathways and that some intermediates are constantly being drawn off in biosynthetic side streams so that glucose substrate is not always

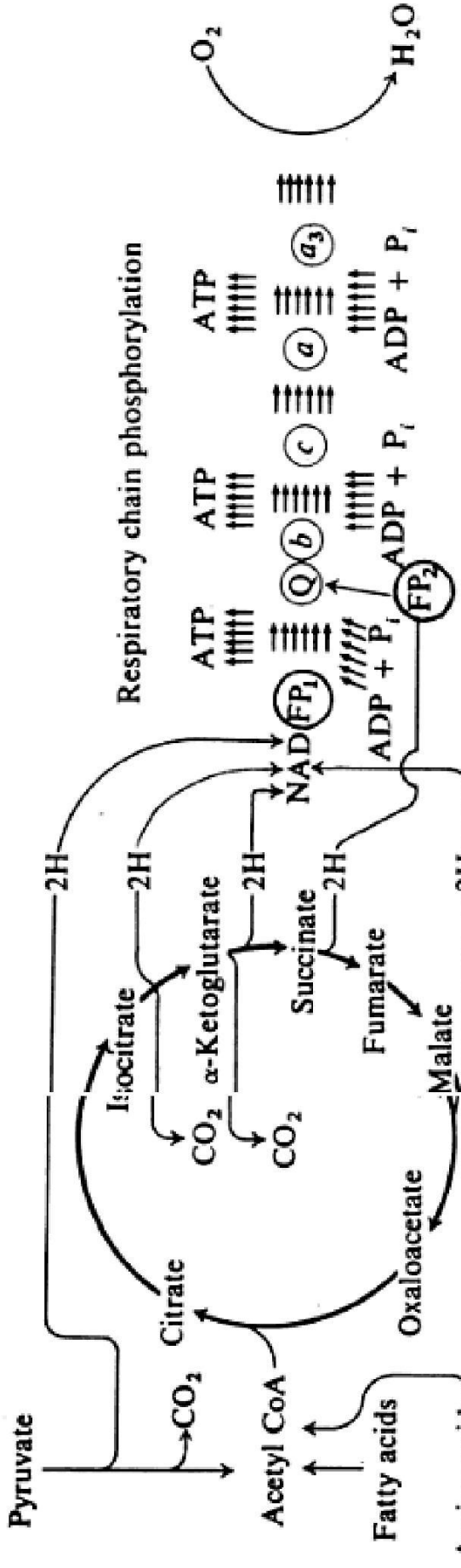


Figure 5.8 High-energy electrons released in oxidation of carbohydrates, fatty acids, and amino acids drive ADP phosphorylation as they move through the respiratory chain: FP_1 (NADH), FP_2 (succinate dehydrogenase), Q (coenzyme Q), *b*, *c*, *a* and a_3 (the cytochromes). *(Adapted from A. Lehninger, "Bioenergetics," 2d ed., p. 74, W. A. Benjamin, Inc., Palo Alto, CA, 1974.)*

processed as we now assume. The following calculation gives an upper bound on the ATP yield from glucose in a respiring cell.

Adding Eq. (5.12) (EMP reactions) with two times Eq. (5.19) (TCA cycle) with ten times Eq. (5.20) [oxidation of all NADH + H^+ generated in the EMP (two) and TCA reactions (four)] and twice Eq. (5.21) [$FADH_2$ oxidation] gives

$$C_6H_{12}O_6 + 38\,ADP + 38\,P_i \longrightarrow 6CO_2 + 38\,ATP + 44\,H_2O \quad (5.23)$$

Since ATP hydrolysis has a standard free-energy change of -7.3 kcal/mol, the free-energy change of reaction (5.23) is approximately

$$\Delta G^{\circ\prime} \approx (38 \text{ mol ATP/mol glucose})(7.3 \text{ kcal/mol ATP}) = -277 \text{ kcal/mol glucose} \quad (5.24)$$

This is 19 times the energy which the cell captured during glycolysis. As in glycolysis, energy retention is very efficient:

$$\text{Energy capture efficiency} = \frac{277}{686} \approx 40\% \quad (5.25)$$

If this figure is corrected for the nonstandard concentrations within the cell, a rather astounding efficiency estimate of greater than 70 percent results. Most of the remaining energy is dissipated as heat, which must be removed in some fashion to keep the temperature in the physiologically suitable range.

Since *chemical* engines are not commonly studied in engineering thermodynamics, it may be helpful to draw an analogy with the classical example of compressing a gas in a cylinder with a piston. If the gas is compressed rapidly, much energy is lost as heat and therefore cannot be recovered in a subsequent expansion. An analogous process would be burning glucose in air, which is quite inefficient. On the other hand, the reversible compression ideal can be approached by pushing the piston very slowly, so that heat generation is minimized. Similarly, by carrying out glucose oxidation in many steps, where each has a relatively small free-energy change, the living cell is able to approach reversibility and to maximize efficiency for extraction of energy.

The above description of respiratory chain operation applies to a typical eucaryotic cell. In procaryotes, electron transport to oxygen may result in fewer than three phosphorylations. Furthermore, the cytochrome pathway differs in detail from one species to another, and phosphorylation efficiency may vary with growth conditions. The *P/O ratio*, the number of ADP phosphorylations per atom of O_2 consumed, is the parameter used to describe the energy storage activity of the respiratory chain.

Later in this chapter we shall examine how the oxidation state of the substrate and the cell's biosynthetic requirements interact to provide stoichiometric constraints on the activities of amphibolic pathways. Next, however, we should conclude our overview of energy-yielding metabolism with a brief examination of photosynthesis, the energy transduction process on which all life on earth depends.

Lactose H^+

to changing environmental conditions. Cell-cell recognition important in both tissue organization and response to disease is mediated by surface receptors in higher eucaryotic cells.

Membranes are generally asymmetric, exhibiting different functions and components on the interior and exterior sides. Diffusion of lipids and many membrane proteins in the plane of the membrane is rapid; the lipid diffusivity in several membranes has been estimated as approximately 10^{-8} cm^2/s.

5.8 METABOLIC ORGANIZATION AND REGULATION

The cell, like any other chemical plant, must have controls on its complex array of chemical reactions so that supply and demand for materials, energy, and electrons are balanced and so that resources are used efficiently. Also, the reaction systems must be organized or structured in a fashion consistent with effective control. In this section we examine the ways in which modulation of the activities of key enzymes controls flows through the cell's many branched and looped metabolic reaction pathways. Also, we consider key structural features of the metabolic network. We can gain an appreciation for the importance and efficiency of these controls and structures by examining metabolic activities in an *E. coli* bacterium. In a rich medium, the cells divide every 20 minutes, and they conduct an amazing array of chemical functions with great precision, productivity, and balance (see Table 5.6).

Table 5.6 Biosynthetic activity during a 20-min cell-division cycle of *E. coli*[†]

Chemical component	Percent of dry weight	Approximate mol wt	Number of molecules per cell
DNA	5	2,000,000,000	1
RNA	10	1,000,000	15,000
Protein	70	60,000	1,700,000
Lipids	10	1,000	15,000,000
Polysaccharides	5	200,000	39,000

Chemical component	Number of molecules synthesized per second	Number of molecules of ATP required to synthesize per second	Percent of total biosynthetic energy required
DNA	0.00083	60,000	2.5
RNA	12.5	75,000	3.1
Protein	1,400	2,120,000	88.0
Lipids	12,500	87,500	3.7
Polysaccharides	32.5	65,000	2.7

[†] A. L. Lehninger, *Bioenergetics*, 2d ed., p. 123, W. A. Benjamin, Inc., Palo Alto, Ca., 1965.

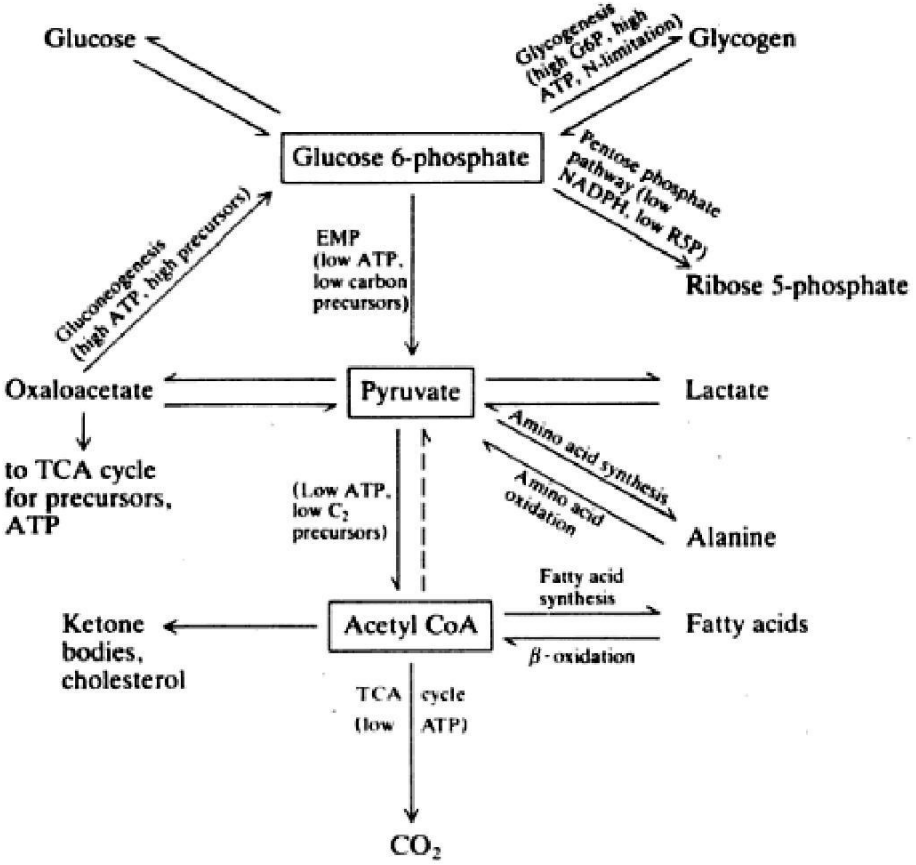


Figure 5.24 Summary of the key crossroads of carbon metabolism. The conversion of acetyl CoA into pyruvate is not possible in mammals.

5.8.2 Enzyme Level Regulation of Metabolism

Allosteric enzymes control flows through metabolic pathways. The concentrations of ATP, NADH, NADPH, and certain key precursors and intermediates are the regulating signals which govern the activities of these controlling enzymes. The influences of some of these signals on flow division at branch points are indicated in general terms in Fig. 5.24. Here we consider representative examples of metabolic regulation at the molecular level. Also, we shall see here that different pathways are sometimes controlled in a coordinated fashion.

Clearly, maintaining a suitable ATP level in the cell is a necessity. A useful indicator of the energetic state of a cell is the adenylate *energy charge* [4], defined as the ratio of ATP concentration to the sum of AMP, ADP, and ATP concentrations:

$$\text{Energy charge} = \frac{[\text{ATP}]}{[\text{AMP}] + [\text{ADP}] + [\text{ATP}]} \tag{5.43}$$

The energy charge of most cells is in the range 0.87 to 0.94.

To see how the ATP level is maintained so precisely, we shall begin with the EMP pathway (Fig. 5.25). The primary regulatory enzyme is phosphofructokinase, which is activated by ADP and inhibited by ATP. Thus, this enzyme slows the flow through the EMP pathway if the energy charge is high and vice versa. Pyruvate kinase, activated by AMP, acts similarly. We recall that glucose phosphorylation and the reactions of F6P and PEP were the three irreversible reactions of the EMP pathway. Now we can see why: the first step gives a charged derivative which is retained effectively by the cell envelope and the other two irreversible steps are control points.

The energy charge also affects the activity of the TCA cycle. Isocitrate dehydrogenase is activated by ADP and inhibited by ATP. Since one of the major

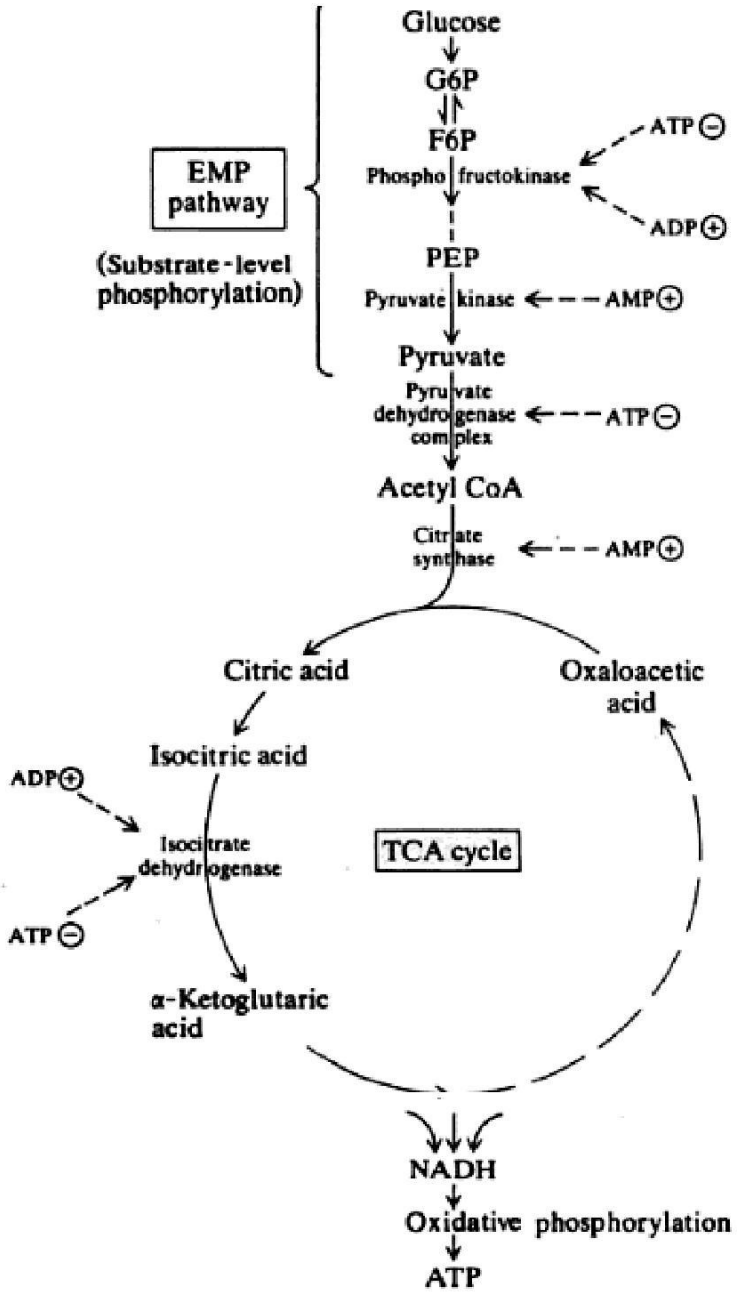
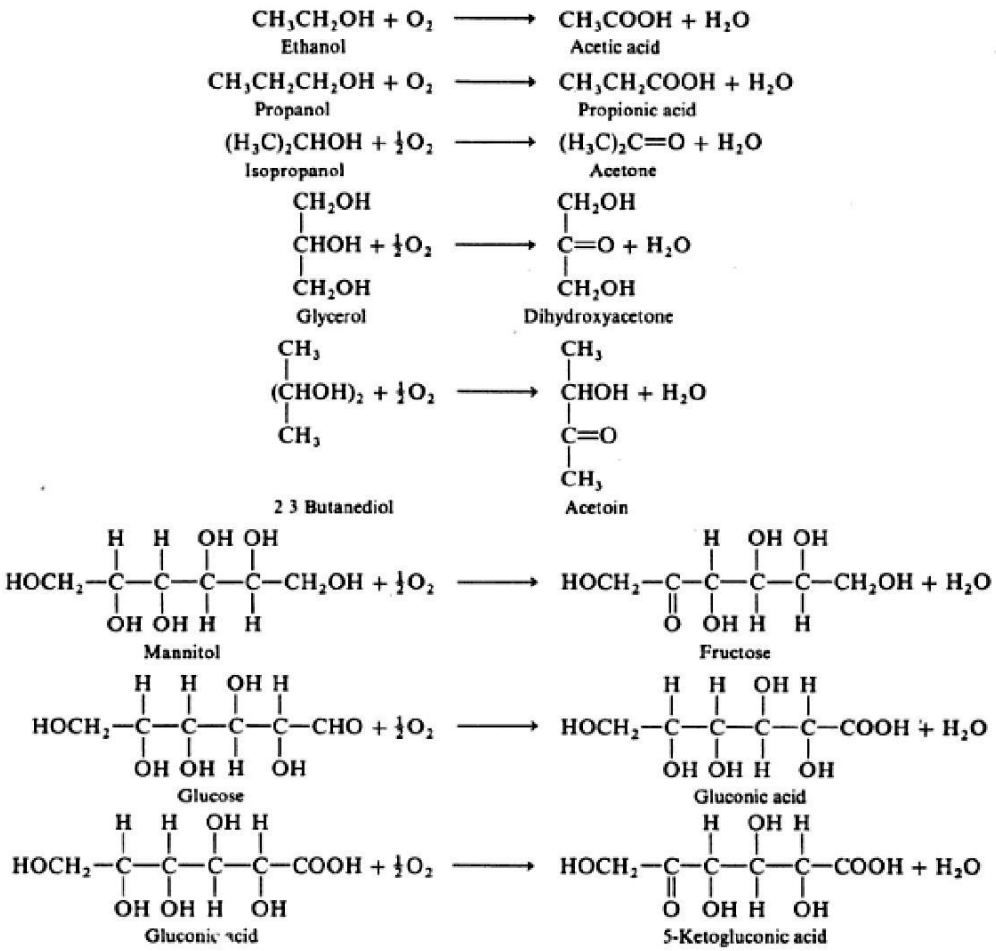


Figure 5.25 Regulation of the EMP pathway and TCA cycle by adenosine phosphate compounds. Dots denote allosteric effects on the indicated enzymes. ⊕ = activation, ⊖ = inhibition.

5.10 STOICHIOMETRY OF CELL GROWTH AND PRODUCT FORMATION

Cell growth, although an exquisite and complex process, obeys the laws of conservation of matter. Atoms of carbon, nitrogen, hydrogen, oxygen, and the other elements of life discussed in Chap. 2 are rearranged in the metabolic processes of the cell, but the total amounts of each of these elements incorporated into cell material is equal to the amounts removed from the environment. Further, the amount of some metabolic product formed or the amount of heat released by cell growth is often proportional to the amount of consumption of some substrate or the amount of formation of another product such as CO_2. In this section we

Table 5.9 Useful end products obtained by partial oxidations conducted by the acetic acid bacteria†



shall examine rigorous material balance constraints as well as approximate, empirical stoichiometric relationships which have proven useful. As we shall see, these stoichiometric considerations have broad implications in biochemical technology ranging from growth medium formulation to computer control and cooling requirements in bioreactors. Also, as in reactor analysis in general, knowledge of stoichiometric relationships is critical in formulating bioreactor material balances and making most effective and systematic use of reaction kinetics (Chap. 7).

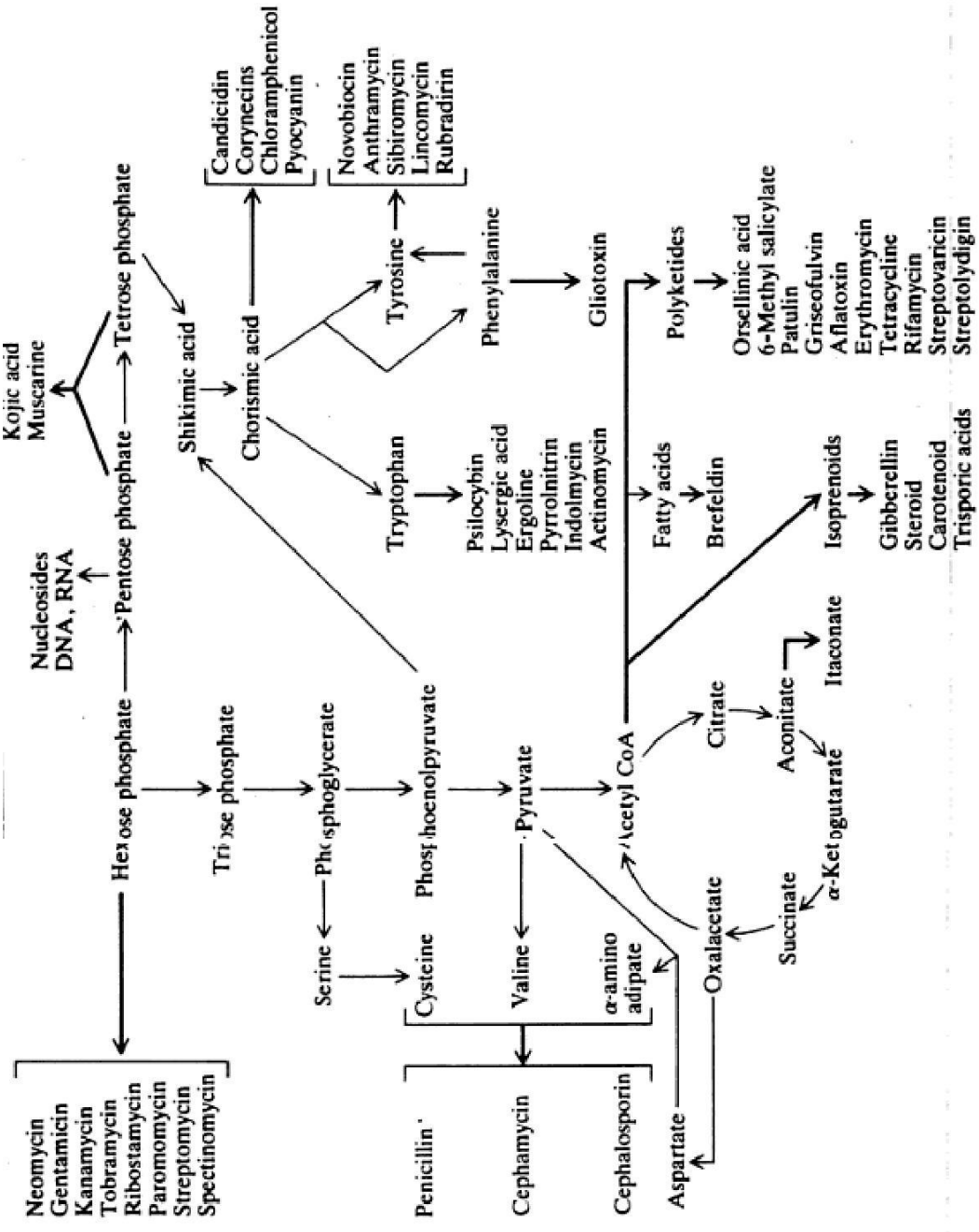


Figure 5.27 Summary of different metabolic routes to secondary metabolites. The heavy arrows denote enzyme-catalyzed reaction sequences and the products at the termini of these arrows are secondary metabolites. *(Reprinted by permission from V. S. Malik, "Microbial Secondary Metabolism," Trends Biochem. Sci., vol. 5, p. 68, 1980.)*

5.10.1 Overall Growth Stoichiometry: Medium Formulation and Yield Factors

Cell growth involves consumption of substrates which provide energy and raw materials required for the synthesis of additional cell mass. Viewed in strictly macroscopic terms, this process requires that the cell environment contains elements needed to form additional cell mass and that the free energy of substrates consumed should exceed the free energy of cells and metabolic products† formed (see Fig. 5.28). Clearly, this implies that the free energy of products formed must be less than the free energy of substrate consumed.

Also, the compounds supplied as nutrients must be compatible with the available enzymatic machinery of the cell for catabolism and biosynthesis. However, in examining cell growth from a strictly macroscopic viewpoint, which is the outlook we wish to develop in much of this section, it can be confusing to interject considerations of metabolic mechanisms. The whole point of the macroscopic perspective indicated in Fig. 5.28 is that certain stoichiometric constraints apply to this growth process *independent of* the particular mechanism or reaction pathways which the "system" of cells employs to effect the overall growth reaction. Consequently, we shall for the moment resolve the question of chemical suitability of certain nutrients by simply restricting our attention to those nutrients which are known to be acceptable substrates for growth of the cell strain of interest.

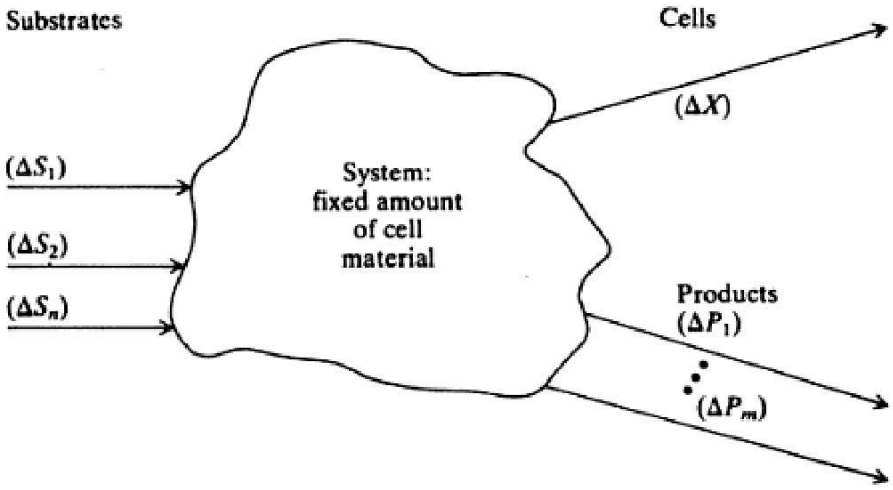


Figure 5.28 Simplest macroscopic view of cell growth. The "system" is defined as some fixed amount of cell material. In the process of growth, with this "system" cell material as a catalyst, substrates are converted into more cells and metabolic products.

† Here we shall adopt the following nomenclature convention; *metabolic product* or *product* refers to a substance, typically an organic compound, different from cell material that is released into the medium, and *nutrients* or *substrates* denote compounds which are depleted from the medium as a result of cell growth or product formation.

Table 5.10 Data on elemental composition of several microorganisms. μ denotes specific growth rate = mass of cells formed per unit time/mass of cells (see Chap. 7)

(Reprinted by permission from B. Atkinson and F. Mavituna, "Biochemical Engineering and Biotechnology Handbook," p. 120, Macmillan Publishers Ltd., Surrey, England, 1983).

Microorganism	Limiting nutrient	μ (h^{-1})	Composition (% by wt)							Empirical chemical formula	Formula "molecular" weight
			C	H	N	O	P	S	Ash		
Bacteria			53.0	7.3	12.0	19.0			8	$CH_{1.666}N_{0.20}O_{0.27}$	20.7
Bacteria			47.1	7.8	13.7	31.3				$CH_2N_{0.25}O_{0.5}$	25.5
Aerobacter aerogenes			48.7	7.3	13.9	21.1			8.9	$CH_{1.78}N_{0.24}O_{0.33}$	22.5
Klebsiella aerogenes	Glycerol	0.1	50.6	7.3	13.0	29.0				$CH_{1.74}N_{0.22}O_{0.43}$	23.7
K aerogenes	Glycerol	0.85	50.1	7.3	14.0	28.7				$CH_{1.73}N_{0.24}O_{0.43}$	24.0
Yeast			47.0	6.5	7.5	31.0			8	$CH_{1.66}N_{0.13}O_{0.40}$	23.5
Yeast			50.3	7.4	8.8	33.5				$CH_{1.75}N_{0.15}O_{0.5}$	23.9
Yeast			44.7	6.2	8.5	31.2	1.08	0.6		$CH_{1.64}N_{0.16}O_{0.52}P_{0.01}S_{0.005}$	26.9
Candida utilis	Glucose	0.08	50.0	7.6	11.1	31.3				$CH_{1.82}N_{0.19}O_{0.47}$	24.0
C. utilis	Glucose	0.45	46.9	7.2	10.9	35.0				$CH_{1.84}N_{0.2}O_{0.56}$	25.6
C. utilis	Ethanol	0.06	50.3	7.7	11.0	30.8				$CH_{1.82}N_{0.19}O_{0.46}$	23.9
C. utilis	Ethanol	0.43	47.2	7.3	11.0	34.6				$CH_{1.84}N_{0.2}O_{0.55}$	25.5

carefully to ensure consistent application of the yield factor parameter. Considering different substrates S_i in the medium, different corresponding cell growth yield factors Y_{X/S_i} may be defined. Table 5.11 lists yield factors for several different microorganisms grown in media with different carbon sources. Different values listed for each case are based upon substrate change in units of mass, moles, or grams of carbon per mole of substrate.

If the yield factor is approximately constant for a particular cell cultivation system, the relationship it provides is extremely useful, for knowledge either of the substrate or the cell mass concentration change suffices to determine the other quantity based on stoichiometry alone. This means that one of these variables can be expressed in terms of the other, eliminating one variable in reactor

Table 5.11 Summary of yield factors for aerobic growth of different microorganisms on various carbon sources. Y_{X/O_2} is the yield factor relating grams of cells formed per gram of O_2 consumed

[Reprinted by permission from S. Nagai, "Mass and Energy Balances for Microbial Growth Kinetics" in Advances in Biochemical Engineering vol. 11 (T. K. Ghose, A. Fiechter, and N. Blakebrough (eds.), Springer-Verlag, New York, p. 53, 1979.]

		$Y_{X/S}$			Y_{X/O_2}
Organism	Substrate	$\frac{g}{g}$	$\frac{g}{mole}$	$\frac{g}{g-C}$	$\frac{g}{g}$
Aerobacter aerogenes	Maltose	0.46	149.2	1.03	1.50
	Mannitol	0.52	95.5	1.32	1.18
	Fructose	0.42	76.1	1.05	1.46
	Glucose	0.40	72.7	1.01	1.11
Candida utilis	Glucose	0.51	91.8	1.28	1.32
Penicillium chrysogenum	Glucose	0.43	77.4	1.08	1.35
Pseudomonas fluorescens	Glucose	0.38	68.4	0.95	0.85
Rhodopseudomonas spheroides	Glucose	0.45	81.0	1.12	1.46
Saccharomyces cerevisiae	Glucose	0.50	90.0	1.25	0.97
Aerobacter aerogenes	Ribose	0.35	53.2	0.88	0.98
	Succinate	0.25	29.7	0.62	0.62
	Glycerol	0.45	41.8	1.16	0.97
	Lactate	0.18	16.6	0.46	0.37
	Pyruvate	0.20	17.9	0.49	0.48
	Acetate	0.18	10.5	0.43	0.31
Candida utilis	Acetate	0.36	21.0	0.90	0.70
Pseudomonas fluorescens	Acetate	0.28	16.8	0.70	0.46
Candida utilis	Ethanol	0.68	31.2	1.30	0.61
Pseudomonas fluorescens	Ethanol	0.49	22.5	0.93	0.42
Klebsiella sp.	Methanol	0.38	12.2	1.01	0.56
Methylomonas sp.	Methanol	0.48	15.4	1.28	0.53
Pseudomonas sp.	Methanol	0.41	13.1	1.09	0.44
Methylococcus sp.	Methane	1.01	16.2	1.34	0.29
Pseudomonas sp.	Methane	0.80	12.8	1.06	0.20
Pseudomonas sp.	Methane	0.60	9.6	0.80	0.19
Pseudomonas methanica	Methane	0.56	9.0	0.75	0.17

design calculations. The stoichiometric relationship is also potentially useful for bioreactor monitoring: measurement of substrate concentration implies a corresponding estimate of the cell concentration if initial values of both quantities are known. This is quite significant since substrate levels are often much easier to determine than is biomass concentration. Actually, some product concentration levels, such as CO_2 leaving an aerobic bioreactor, are the most convenient to measure in practice. Here, too, stoichiometry provides a useful structure for relating different bioreactor process variables. Before turning to these points, however, we need to mention yield factor variability and the concept of maintenance metabolism.

There is no guarantee that a yield factor, an empirically defined, apparent stoichiometric ratio, is a constant for a given organism in a given medium. Variations in yield factors, for example with respect to growth rate, are commonly observed. To understand these variations it is sometimes useful to break down substrate consumption into three parts: assimilation into cell mass, provision of energy for cell synthesis, and provision of energy for maintenance. *Maintenance* here refers to the collection of cell energetic requirements for survival, or for preservation of a certain cell state, which are not directly related to or coupled with the synthesis of more cells. Such activities include active transport of ions and other species across cell membranes and replacement synthesis of decayed cell constituents.

For chemoheterotrophs, a single substrate serves as both carbon and energy source, so that total substrate utilization may be written as

$$(\Delta S) = (\Delta S)_{\text{assimilation}} + (\Delta S)_{\text{growth energy}} + (\Delta S)_{\text{maintenance energy}} \tag{5.47}$$

Dividing the equation by (ΔX) gives

$$\frac{1}{Y_{X/S}} = \frac{(\Delta S)_{\text{assimilation}}}{(\Delta X)} + \frac{(\Delta S)_{\text{growth energy}}}{(\Delta X)} + \frac{(\Delta S)_{\text{maintenance energy}}}{(\Delta X)} \tag{5.48}$$

While the true growth yield factor $\Delta X/(\Delta S)_{\text{assimilation}}$ is a relatively constant, stoichiometrically well-defined quantity, the overall yield factor $Y_{X/S}$ is not. The allocation of substrate used into the three components indicated in Eq. (5.48) is variable. A rapidly growing cell population will use more substrate for assimilation and growth energy, while a cell population in a resting or stationary state often consumes substrate for maintenance without any growth (here $Y_{X/S} = 0$).

In the next section we will develop an alternative and more rigorous formulation of cell growth stoichiometry based upon material balances for C, H, N, and O. Also, we shall examine a more detailed view of cell growth that, although still basically in macroscopic form, considers the central role of NADH and ATP in growth metabolism.

5.10.2 Elemental Material Balances for Growth

In order to describe cell growth and related metabolic activities in the same way as used for simple chemical reactions, it is first necessary to establish a chemical

formula for dry cell material. If the elemental composition of a particular strain growing under particular conditions is known (see, for example, Table 5.10), the ratios of subscripts in the empirical cell formula $C_\theta H_\alpha O_\beta N_\delta$ are determined. In order to establish a unique cell formula and corresponding formula weight, it has proved convenient to employ a formula which contains one gram-atom of carbon. That is, we choose $\theta = 1$ and then fix α, β, and δ so that the formula is consistent with known relative elemental weight content of the cells. The cell formulas in Table 5.10 have been written using this convention. One *C-mole of cells* is by definition the quantity of cells containing one gram-atom (12.011 grams) carbon, and corresponds to the cell formula weight with the carbon subscript θ taken as unity.

As the simplest illustration of the elemental balance approach to stoichiometry, we consider first aerobic growth without product formation, that is, where the only products of the growth reaction are cells, CO_2 and H_2O. Then, writing the carbon source and nitrogen source chemical formulas as CH_xO_y and $H_lO_mN_n$, respectively, the growth reaction equation is

$$a'CH_xO_y + b'O_2 + c'H_lO_mN_n \longrightarrow CH_\alpha O_\beta N_\delta + d'H_2O + e'CO_2 \quad (5.49)$$

Without loss of generality (since stoichiometric coefficients may always be multiplied by the same constant), the coefficient of cells is taken as unity.

Balances on the four elements in Eq. (5.49) provide four relationships among the five unknown stoichiometric coefficients a', b', c', d', and e':

$$\begin{aligned} &\text{C: } a' = 1 + e' \\ &\text{H: } a'x + c'l = \alpha + 2d' \\ &\text{O: } a'y + 2b' + c'm = \beta + d' + 2e' \\ &\text{N: } c'n = \delta \end{aligned} \quad (5.50)$$

An additional relationship may be provided by experimental determination of the *respiratory quotient*, or RQ, for the growth reaction. The respiratory quotient is defined as the molar ratio of CO_2 formed to O_2 consumed:

$$\text{Respiratory quotient} = \text{RQ} = \frac{\text{moles } CO_2 \text{ formed}}{\text{moles } O_2 \text{ consumed}} \quad (5.51)$$

For the growth reaction in Eq. (5.49) above,

$$\text{RQ} = e'/b' \quad (5.52)$$

Thus, if the RQ is known, Eq. (5.52) plus Eqs. (5.50) provide five equations for the five unknown stoichiometric coefficients. Possible difficulties with extreme sensitivity of the calculated stoichiometry to small errors in the RQ measurement are considered in Prob. 5.16.

The chemical formula for cell composition and the stoichiometric coefficients in Eq. (5.49) in general change as a function of cell environment. We must remember that this is a phenomenological relationship, used here to describe the combined effects of a large number of independent chemical reactions. Insights

into the influences on overall stoichiometry and bridges to our earlier discussions of metabolism can be obtained by decomposing the overall growth reaction into several reactions steps which correspond to different metabolic functions (but not necessarily to specific metabolic pathways).

We shall next consider a more detailed stoichiometric representation for aerobic growth of a chemoheterotrophic organism. To each reaction we associate a reaction involving ATP generation or utilization. These have been written separately here, and not simply added to the associated reaction involving C, H, O, and N rearrangement, because of practical difficulties with ATP stoichiometry which will be discussed shortly.

Energy source dissimilation

$$a\mathrm{CH}_x\mathrm{O}_y + \text{coefficient}\cdot\mathrm{H_2O} + \frac{\gamma_s}{2}a\mathrm{NAD}^+ \longrightarrow a\mathrm{CO_2} + \frac{\gamma_s}{2}a(\mathrm{NADH} + \mathrm{H}^+) \quad (5.53a)$$

$$\varepsilon_s a(\mathrm{ADP} + \mathrm{P}_i) \longrightarrow \varepsilon_s a(\mathrm{ATP} + \mathrm{H_2O}) \quad (5.53b)$$

Oxidative phosphorylation

$$2b(\mathrm{NADH} + \mathrm{H}^+) + b\mathrm{O_2} \longrightarrow 2b\mathrm{NAD}^+ + 2b\mathrm{H_2O} \quad (5.54a)$$

$$2b(\mathrm{P/O})(\mathrm{ADP} + \mathrm{P}_i) \longrightarrow 2b(\mathrm{P/O})(\mathrm{ATP} + \mathrm{H_2O}) \quad (5.54b)$$

Biosynthesis

$$(1+\sigma)\mathrm{CH}_x\mathrm{O}_y + \frac{\delta}{n}\mathrm{H}_l\mathrm{O}_m\mathrm{N}_n + \tfrac{1}{2}\left(\gamma_B - \gamma_S(1+\sigma) - \frac{\delta}{n}\gamma_N\right)(\mathrm{NADH} + \mathrm{H}^+) \longrightarrow \mathrm{CH}_\alpha\mathrm{O}_\beta\mathrm{N}_\delta + \sigma\mathrm{CO_2} + (\text{coefficient})\cdot\mathrm{H_2O} \quad (5.55a)$$

$$\frac{MW_B}{Y_{\mathrm{ATP}}^{\max}}(\mathrm{ATP} + \mathrm{H_2O}) \longrightarrow \frac{MW_B}{Y_{\mathrm{ATP}}^{\max}}(\mathrm{ADP} + \mathrm{P}_i) \quad (5.55b)$$

Maintenance and dissipation

$$c(\mathrm{ATP} + \mathrm{H_2O}) \longrightarrow c(\mathrm{ADP} + \mathrm{P}_i) \quad (5.56)$$

Interconnections among these reactions are provided by the constraints that, under the conditions of interest here, it may be assumed that no ATP and no NADH accumulates. Formation must be balanced by utilization.

The cost of a more complex but more revealing representation of chemical changes accompanying cell growth is a dramatic increase in the number of stoichiometric parameters. First, notice that the stoichiometric coefficients are written taking into account atom balances for each reaction. Next, notice that the stoichiometric coefficient of biomass ($\mathrm{CH}_\alpha\mathrm{O}_\beta\mathrm{N}_\gamma$) has been taken to be unity. The

coefficients a, b, and c which appear throughout other reactions are therefore used to denote the extent to which these reactions occur *relative to* the growth reaction. Rather than writing out the value in detail, the stoichiometric coefficients of water have been written simply as "coefficient" in two cases above since water consumption or production is usually not significant relative to the amount present. Here ε_s denotes the number of substrate-level phosphorylations per carbon mole passing through dissimilation metabolism.

The parameters γ_B, γ_S, and γ_N denote the *degrees of reductance* of biomass, carbon source, and nitrogen source, respectively. The reductance degree is the number of equivalents of available electrons per g atom carbon, based on carbon = +4, hydrogen = +1, oxygen = −2 and nitrogen = −3. Thus, the degree of reductance of a compound with formula $CH_rO_sN_v$ is $\gamma = 4 + r - 2s - 3v$. Based on this convention, the reductance degrees of CO_2, H_2O, and NH_3 are all zero.

The yield coefficient Y_{ATP}^{max} is the mass of cells formed per mole of ATP consumed for biosynthesis. Efforts have been made to evaluate this quantity based upon known cell composition and known biosynthetic pathways (see Ref. 13). Such calculations indicate, for example, that Y_{ATP}^{max} is 28.8 g cells/(gmol ATP) for growth of *E. coli* on glucose and inorganic salts. The molecular weight (B denotes biomass) in Eq. (5.55*b*) is needed for conversion from mass to molar units.

As noted earlier, P/O is the number of ADP phosphorylations per atom of O_2 consumed; this parameter characterizes the efficiency of oxidative phosphorylation and in general varies depending on cultivation conditions. Calculations based upon an average microbial composition estimate maximum theoretical P/O values of 2.25, 2.50, and 3.00 for growth on acetate, malate, and glucose, respectively.

Direct experimental measurement of these quantities is complicated by the difficulty of isolating experimentally a particular ATP generation or utilization pathway and the dependence of the relative extents of ATP formation and use on culture environment. The major problems here arise because of ATP utilization to drive membrane transport processes, to synthesize molecules which are degraded by intracellular hydrolases, and other not fully characterized processes sometimes called futile cycles. All of these ATP demands, grouped together here under the title "maintenance and dissipation" and indicated above by the single "reaction" (5.56), influence other ATP generation and utilization processes.

Since ATP generation must match ATP utilization, alteration in the relative extent or weight of maintenance ATP consumption, indicated above by the coefficient c, forces shifts in other reaction extents. Experimental evidence shows that maintenance requirements are not dictated by stoichiometric considerations but depend instead on the rates of metabolic processes. Accordingly, we must defer quantitative treatment of maintenance until we explore cell kinetics in Chap. 7.

It may offer useful perspective at this point to mention the following observations: the overall cell mass yield based on ATP utilization is quite similar for many substrates and organisms grown anaerobically. The average value is about

Y_{ATP} = 10.7 g cells/(mol ATP). In chemoheterotraphic anaerobes, typically much more substrate is consumed for energy production than for assimilation into biomass. In one study of anaerobic growth of bakers' yeast, *Saccharomyces cerevisiae*, in rich medium with glucose carbon source, 98 percent of the carbon consumed is used for energy production and only 2 percent for cell mass. It has proved quite difficult to determine accurately ATP generation in aerobic growth. Available data suggests P/O ratios in the range 0.5 to 1.8—much lower than theoretical estimates. Studies of the bacterium *Aerobacter cloacae* in aerobic minimal medium showed that 55 percent of the glucose consumed was assimilated, and 45 percent of the glucose utilized was used for energy production. As these figures suggest, the cell mass yield per amount of substrate consumed is typically greater under aerobic conditions. For example, for *Streptococcus faecalis* grown in glucose medium, the yield factor $Y_{X/S}$ [g cells/mol glucose)] is 21.5 under anaerobic conditions and 58.2 for aerobic growth. We will examine the implications of these allocations of substrate for product formation stoichiometry in the next section.

5.10.3 Product Formation Stoichiometry

A variety of metabolic end products is released into the growth medium or accumulated intracellularly. The pertinent stoichiometries for product formation may be classified usefully into the following four classes; the first three correspond to the fermentations classification formulated by Elmer L. Gaden, Jr. in 1955.

1. The main product appears as a result of primary energy metabolism. Example: ethanol production during anaerobic growth of yeast.
2. The main product arises indirectly from energy metabolism. Example: citric acid formation during aerobic mold cultivation.
3. The product is a *secondary metabolite*. Example: penicillin production in aerated mold culture.
4. Biotransformation. The product is obtained from substrate through one or more reactions catalyzed by enzymes in the cells. Example: steroid hydroxylation.

Class 1 processes have a relatively simple stoichiometric description. Product appears in relatively constant proportions as cell mass accumulates and substrate is consumed. Here, the processes of substrate utilization, cell mass synthesis, and product formation are linked as in a simple, single chemical reaction. This may be obtained by adding product, denoted CH_vO_w, to the right-hand side of Eq. (5.49), giving

$$a'CH_xO_y + b'O_2 + c'H_lO_mN_n \longrightarrow CH_\alpha O_\beta N_\delta + d'H_2O + e'CO_2 + f'CH_vO_w \quad (5.57)$$

(Extension of the formalism to include nitrogen-containing products is straightforward.)

A corresponding yield factor may be written, taking into account the use of molar units in the yield factor parameter. Thus, if the substrate is glucose which contains six carbon atoms per molecule, the number of glucose molecules used when reaction (5.57) occurs is $a'/6$. Similarly, if the product contains n_p carbon atoms per molecule, f'/n_p product molecules are formed. Thus, the molar yield factor $Y_{P/S}$ (moles product formed/moles substrate consumed) may be written

$$Y_{P/S} = \frac{f'}{a'}\frac{n_s}{n_p} \tag{5.58}$$

Here, n_s denotes the number of carbon atoms in a substrate molecule.

For class 2 situations the simple stoichiometry of Eq. (5.57) does not apply. Product formation is not necessarily proportional to substrate utilization or cell mass increase. Representation of the stoichiometry in such a case requires an independent reaction equation for product formation. It is instructive to consider the addition of a product formation step to the growth reactions given in Eqs. (5.53) through (5.56) above:

Product formation

$$d\mathrm{CH}_x\mathrm{O}_y + (\text{coefficient})\mathrm{H}_2\mathrm{O} + \tfrac{1}{2}(\gamma_s - z\gamma_p)\mathrm{NAD}^+ \longrightarrow$$
$$d\mathrm{CH}_y\mathrm{O}_w + d(1-z)\mathrm{CO}_2 + d\tfrac{1}{2}(\gamma_s - z\gamma_p)(\mathrm{NADH} + \mathrm{H}^+) \tag{5.59a}$$

$$\varepsilon_p d(\mathrm{ADP} + \mathrm{P}_i) \longrightarrow \varepsilon_p d(\mathrm{ATP} + \mathrm{H}_2\mathrm{O}) \tag{5.59b}$$

Here, z denotes the carbon fraction of substrate used for product formation which is found in the product. The number of ATPs generated by product formation is denoted ε_p; this coefficient may be negative, indicating that ATP is utilized rather than generated in connection with product formation metabolism. Similarly, if the coefficient $\gamma_s - z\gamma_p$ is negative, reducing equivalents stored as NADH (or NADPH—at this low level of metabolic detail there is no reason to distinguish between these two carriers) are utilized in concert with product formation.

The stoichiometric descriptions appropriate for classes 3 and 4 cases depend on the particular substrates and products involved. Product formation in these cases is typically completely uncoupled from cell growth. Secondary metabolite accumulation is dictated by kinetic regulation and activity of the cells.

Examination of product formation stoichiometry offers several useful insights for engineering analysis. One application is estimation of upper bounds for product yields. These numbers can be very useful in preliminary economic analysis (see Chap. 12). Consider the anaerobic fermentation of glucose to ethanol as an initial example. (The reactions given above for aerobic metabolism may be adapted for anaerobic situations by deleting O_2 everywhere and dropping oxidative phosphorylation reactions.) The best possible case is utilization of all

11.4 Calculation of the Operational Stoichiometry of a Growth Process at Different Growth Rates, Including Heat Using the Herbert–Pirt Relation for Electron Donor

Using the three thermodynamic correlations for growth and maintenance (Equations 11.6, 11.7, and 11.10) one can calculate for any growth system (with specified C, N-source, electron donor and acceptor, temperature) a value for Y_{DX}^{max} and m_D. The presence of maintenance causes that the measured (operational) biomass yield Y_{DX} is smaller than the theoretical maximum Y_{DX}^{max}, because part of the consumed electron donor is spent for maintenance. This is expressed in the Herbert–Pirt relation for electron donor consumption.

$$-q_D = 1/Y_{DX}^{max}\ \mu + (-m_D) \tag{11.11}$$

q_D, which is negative, is the biomass specific rate of electron donor consumption (mol electron donor/CmolXh). $(1/Y_{DX}^{max})\mu$ is the rate of electron donor spent on growth and m_D is the rate of electron donor consumed for maintenance. The operational yield Y_{DX} (CmolX/mol electron donor) is defined as the ratio of μ and $(-q_D)$:

$$Y_{DX} = \mu/(-q_D) = Y_{DX}^{max}\left[\frac{\mu}{(\mu+(-m_D Y_{DX}^{max}))}\right] \tag{11.12}$$

Equation 11.12 shows that the operational biomass yield Y_{DX} is a hyperbolic function in μ. At high μ ($\mu \gg (-m_D Y_{DX}^{max})$) the operational biomass yield approaches asymptotically the maximal yield. At low μ ($\mu \ll (-m_D Y_{DX}^{max})$ the yield Y_{DX} drops to zero. Equation 11.12 shows that, knowing m_D and Y_{DX}^{max} allows to calculate the operational biomass yield on electron donor Y_{DX} in CmolX/mol donor for any specified value of μ. Summarizing, when beside C, N-source, the electron donor and electron acceptor, also μ and temperature is specified one can calculate the operational biomass yield Y_{DX} and the effect of μ on Y_{DX}!!

Example 8: Calculation of the Effect of μ on Y_{dx}

Consider Example 7, aerobic growth on glucose. At 37°C $m_D = -0.0046$ mol glucose/CmolXh. In Example 6 it was found that $Y_{DX}^{max} = 3.87$ CmolX/mol gluxose. This gives (Equation 11.12) for the biomass yield : $Y_{DX} = 3.87\ \mu/(\mu + 0.018)$

This result shows that for large μ-values Y_{DX} becomes constant at 3.87 CmolX/mol glucose. For $\mu < 0.1$ h^{-1} Y_{DX} starts to drop significantly. Consider now anaerobic growth on glucose with ethanol production (Examples 6 and 7). Using $m_D = -0.058$ mol glucose/CmolXh and $Y_{DX}^{max} = 0.82$ CmolX/mol glucose gives: $Y_{DX} = 0.82\mu/(\mu + 0.047)$.

Having calculated for a specified growth system with a chosen T and μ, the value for Y_{DX} allows to obtain the complete growth stoichiometry which belongs to the specified value of μ and T. This is obtained by solving the element/charge conservation relations and/or the balance of degree of reduction, as already used before. Having the complete reaction one can also use energy conservation (1st law thermodynamics, the enthalpy balance) to obtain the heat production. The procedure is shown in Example 9.

Example 9: Calculation of Complete Growth Process Stoichiometry Including Heat

Consider anaerobic growth of an organism which produces ethanol (C_2H_6O) from glucose ($C_6H_{12}O_6$). Also assume that the cultivation temperature is 37°C and $\mu = 0.1$ h^{-1}. Example 8 shows that for $\mu = 0.1$ h^{-1} the

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MOLECULAR BIOLOGY

UNIT – I

PART – A

1. Write the contribution of Johan Gregar Mendel.

Johan Gregor Mendel (1822-1884) is called as Father of Genetics. He conducted crossing experiments in garden peas and found that, as certain factors are responsible for carrying morphological and behavioural characters from parent to offspring. Later the factors are called as genes. The pair of genes are called as alleles.

2. Briefly write the Mendelian law of principles of segregation.

This law is otherwise called as principles of segregation in which the separation of pairs of determiners resulted in a purity of gametes. The determiners are gene. The paired genes (allelic pairs) separate from one another and are distributed to different sex cells. This is otherwise called segregation of genes.

3. What are genes?

Genes are unit of characters i.e. the traits are controlled by physical entities present in living organisms, which is present always in pairs. In the pair, one factor is derived from male parent, and the other is from the female parent. Hence, gene is a genetic factor, which control one trait or characteristic of organism. Allele is one of the pair or more of different from of given gene.

4. Describe phenotypes and genotypes.

The appearance of physical structure or morphology of the organisms, which is derived through parent to off spring, is called phenotype. The genetic composition responsible for the phenotypes are called genotype, which is influenced by the gene pair.

5. What are alleles?

Allele is the one of the pair or more of different forms of given gene. Alleles are set of genes responsible for the morphology of the organisms. Alleles are found as several types as single or multiple alloles which involve in determination of heredity.

6. Define homozygous and heterozygous.

The pair of characters or genes responsible for the phenotypic of organisms derived from different kinds of alleles i.e. the maternal and paternal are different. Homozygous is the pair of characters which posses same type of alleles i.e maternal and paternal is same. Heterozygous is the pair of characters which posses different type of alleles. i.e., maternal and paternal is different.

7. Compare recessive and dominant.

Dominance is the expression of phenotypic characters belongs to the alleles that are always dominant. The alleles, which are not able express phenotypically, are recessive or otherwise suppressive. In Homozygous, receive express phenotypically.

8. Explain the principles of Independent assortment.

In the dihybrid cross experiments, Mendel again found that the pair of alleles or each gene pair was independently transmitted to the gamete during sex-cell formation. As per this second law, the all four possible gametes are produced with equal frequency. There is no tendency of genes arising from one parent to stay together. The allelic pairs of different genes behaved independently with each other i.e. assorted independently into gametes.

9. What is linkage and linked genes?

There is certain pair of genes or combination of genes that are present in particular chromosome of the parent remain together on the chromosome of the progeny. Such genes are called linked genes or linked gene markers which remain together during gametic formation or sexual reproduction. I.e. they do not exhibit independent assortment. The transmission pattern called linkage.

10. Write brief note on crossing over.

The reciprocal exchange of linked genes of the chromosomes between the homologous pairs is performed by some special genetic process, which is termed as crossing over. By this process, new combination of genes occurred by interchanging the segments of chromosomes between non-sister chromatics of homologous chromosomes.

11. What are recombinants?

Recombinants are the progeny that have new blended combination of gene pairs or allelic pairs obtained from the both the parents as male and female. These recombinant genes are obtained from the non-sister chromatids through crossing over. Recombinants are genetically importants that are useful in breeding program.

12. What are hybrids?

Hybrids are new progenies, which carry new combination ;of characters obtained from both the male and female parents. Hybrids possess new combination of characters that are helpful in breeding program and genetically important.

13. Write short notes on mutation.

Mutation is the genetic processes in which sudden changes are occurred in the gene pattern of chromosome due to various factors include physical, chemical and biological means. The mutation may be deletion of genes or addition of genes in the particular chromosome fragment, which will get change due to mutation.

14. Define map unit used for chromosome mapping.

All the genes in a given chromosome are located on a line. The gene arrangement was strictly linear and never branched. Thus, the distances between genes on such a map are measured in map units, which are related to the frequency of recombination between genes.

15. What is genetic map?

The location of genes or relative position of genes on a chromosome is more significant to measure the linkage or crossing over. Thus the relative positioning of genes on chromosome produce a genetic map. It is useful identify the location of genes and distant between the genes which are responsible for linkage and crossing over.

16. What are mutant genes?

The gene pattern of the particular chromosome may be changes due to mutational factors like radiation, chemical and others. Such new pattern of genes or changed pattern of genes due to mutation is called mutant genes. The new gene types were obtained through mutation.

17. What are wild genes?

The wild genes are the gene pattern of chromosome naturally presents which posses for some good phenotypic characteristics. Such unchanged natural gene pattern is called wild genes. Wild genes also sometimes possess good advantage characteristic feature.

18. Write short notes on crossing over and its importance.

Crossing over are the genetic process in which the new gene combination were obtained through exchange of sister chromatids fragments.

19. Write briefly on checkerboard.

The checkerboard is other wise called as a punnet square which is used to derive the possible gene pattern from the parents. By this checkerboard, one can identify or expect the genotypic and photypic.

20. Define test cross and back cross.

Test cross are the progeny obtained from parent through hybrid experiments. The ratio of phenotypic expression of progeny will be mostly 1:3.

In back cross, the test cross is once again the experiment is conducted between the test cross versus any one parent.

21. Describe incomplete dominance or semi dominance.

When a dominant allele does not mask completely the phenotypic expression of recessive allele in heterozygote, then blending both dominant and recessive trait takes place in the F1 heterozygous. This will be appearing in new blended phenotype. Then in F2, 1:2:1 will be dominant, semi dominance and recessive expression. E.g. Red dominant pea and white recessive pea gives pink F2 incomplete dominance.

22. What is co-dominance?

When the dominant and recessive alleles lack their relationships and both have the capacity to express them phenotypically in the heterozygous (F1). The dominant and recessive alleles occur side by side or express equally. The F2 progeny in the phenotype and genotypic ratios of 1:2:1occur like incomplete dominance.

E.g. the white coat dominant cattle and red coat recessive crossed, F1 heterozygous occur with reddish gray or roan colour. Then the F2 progeny in the phenotypic and genotypic ration of 1:2:1 is White dominant, Roan colour and Red recessive respectively.

23. Define super dominance or heterodominance or overdominance.

When two homozygous crossed, the heterozygous have a more extreme phenotypic expression than the either of the corresponding homozygous parent in F1 progeny. For example the heterozygote Aa pair of factors which control size in bigger than the homozygous AA or aa. It implies interaction between the alleles, or factors of the genotype, which produce qulitative characters such as size, production and vigor etc.

24. Define complete and incomplete linkage.

Genes located so close is linked together and inherit together for several generation continuous and regular fashion is called complete linkage.

The widely located genes or linked genes are having chances of separation by crossing over. This type genes locating at varying lengths are said to be incompletely linked genes and the phenomena is called incomplete linkage.

25. What are the types of crossing over?

I. Meiotic crossing over: which is occurring in reproductive cells or gametes during development. It has universal occurrence and has great genetic significance.

ii. Mitotic crossing over or somatic crossing over: Sometimes the crossing occurs in somatic cells and it is least significance.

26. Write the features of crossing over?

i. Crossing over involves the breakage of each two homologous chromosomes and exchange of parts.
ii. CO occurs at zygotene and pachytene after synopsis of the homologous chromosomes in prophase I.
iii. Cromosomes with recombinant combination linked genes are formed after crossing over.
iv. The probability that CO will occur in between two loci increases with increasing distance.

27. Mention the role of sex pili or F pili.

Sex pili or F pili are the appendages occur on the surface of bacteria, which is responsible for the conjugation of two bacteria. In which the sex pili of both the bacteria having contact by fusion and provide a path for transfer of bacterial chromosome strand from one organism to other organism. This F pili are synthesized by special genes present in the bacterial mini chromosome.

28. What is bacterial F factor?

F factor or fertility factor is the minichromosome, which is, appears in small, circular DNA with 9.4 kb size, which is responsible for the synthesis of F pili. F factors exist in 2 forms like autonomous state and integrated state. The F factor is like extrachromosome called episomes.

29. What are Hfr cells?

Hfr cells are otherwise called High frequency recombinant bacterial cell, which occurs through conjugation process of bacteria. This Hfr bacterial cell is having recombinant characteristics features due to the integration of foreign chromosome during the conjugation process.

30. Write short notes on bacterial transformation.

Bacterial transformation process is the active energy requiring process in which the uptake of DNA molecules from one bacterium called donor by the recipient bacteria. This process occur only in few bacteria species, which has the enzyme machinery to perform the above active process.

31. Write short notes on bacterial conjugation.

Conjugation is the process of transfer DNA strand from one bacterium to another bacteria by means of contact conjugation tube by sex pili. During the conjugation process, new bacterial cells are produced which is called as a recombinant that possess with new genetic characters.

32. What is bacterial transduction?

Bacterial transduction process is the transfer of bacterial genes or chromosomal structures from one bacterium to another bacteria through certain vectors like viruses. Here, the donor gives the genes and recipient receives the genes. In which the new bacteria cells produced are called recombinants.

33. Mention the bacterial transforming principle.

The transforming principle proves that, as the DNA is the genetic material. In which the uptake of DNA molecule from donor to recipient bacteria by active energy requiring process. Avery, McLeod, Mc Carty in 1944, conducted the set of experiments proved the transforming principle.

34. What is transfection?

Transfection is otherwise indicates the bacterial transduction process. The transfer of DNA molecule from donor to recipient bacteria through bacterial virus (Bacteriophage) is called transfection. Since the viruses are involved in this process, the name transfection is derived from the term infection of virus on bacteria.

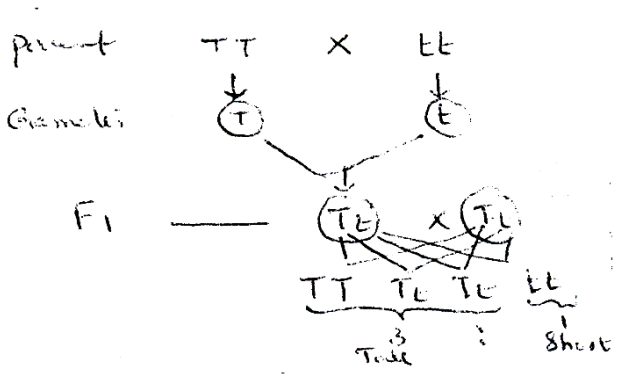
35. What are heteroduplexes?

In the bacterial transformation process, the insertion of single stranded DNA stand is occurs which resulted to produce the transformant chromosome have 2 different alleles called mismatch pairs or duplex of DNA hybrid. This is called heteroduplexes and these are important in mutation, recombination and DNA repair process.

36. What are lethal genes? Give one example.

Genes present in the organism, which is affect, the viability or visible traits of an organism are otherwise called as lethal genes or killer genes. This is due to impaired biochemical changes and physical functioning. This effect is prenatal or postnatal prior to sexual maturity.

37. Prove the Mendelian monohybrid ratio as 3:1 in F2 by using Tall (T) and short (t) plants using checkerboard.



	T	t
T	TT tall	Tt Tall
t	Tt Tall	tt Short

TT – Tall – 1 3
Tt - Tall – 2 :
tt - Short – 1 1

38. Prove the Mendelian incomplete dominance ration as 1:2:1 in F2 using Red (R) and white (r) using checkerboard.

	Red		White
Parent	RR	X	rr
Gamete	R		r
F_1		X	
R	r		

	R	r
R	RR Red	Rr Pink
r	Rr Pink	rr White

RR red -1
Rr pink - 2
rr white - 1

PART – B

1. Write an essay on linkage and its implication in genetics.

Genetics markers or genes located on the same chromosome remain together during gametes formation or sexual reproduction without any change of their location or loci. That is, they do not exhibit independent assortment. Those markers or genes are remain together and segregate together during successive generations.

Such markers or genes are called **linked genes** and their transmission pattern is called as l**inkage.**

Generally, more than 50% of progeny with parental combination and less than 50% progeny with recombinant pattern from both the parents. Therefore, the linked genes are present in parents, whenever over 50% of gametes produce parental combination and less than 50% recombinant genes.

Commonly, the genes located in the same chromosome assort independently with linked genes. So, the genes located in same chromosome are close together. It was first observed in sweet peas by Bateson and Punnet in 1906. T.H.Morgan reported linkage in homologous chromosomes and crossing over in the fruit fly Drosophila in 1911. The following figures indicate the pattern of linked genes in homologous chromosomes.

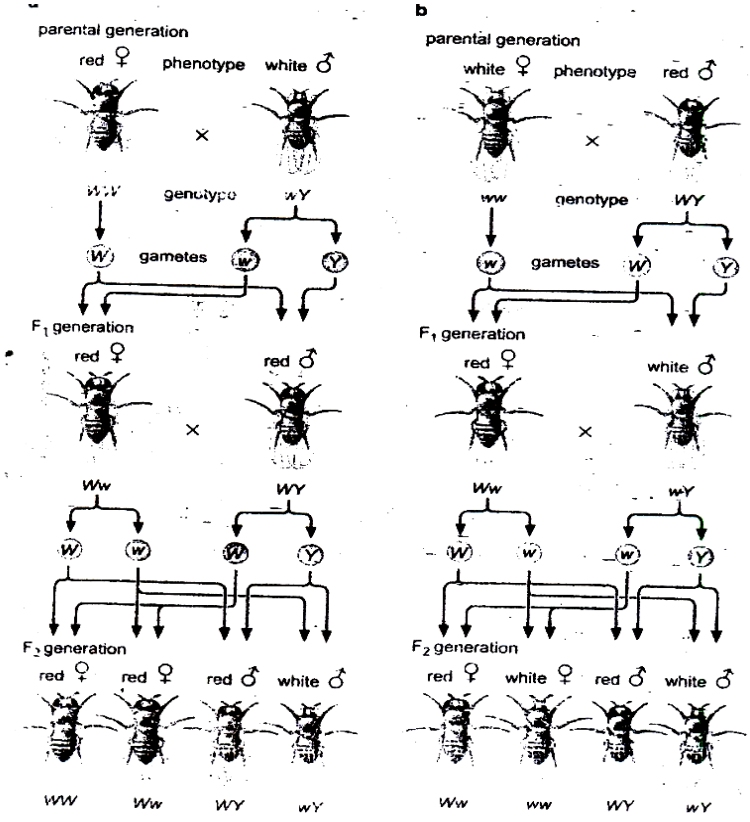


Figure: The inheritance of a sex-linked gene in Drosophila: Genes located on sex chromosomes can express themselves differently in male and female progeny, because if there is only one X chromosome present, recessive genes on this chromosome are always expressed. Here are two crosses, both involving a receissive gene (W· for white eye) located on the X chromosome. (a) The male parent is a white – eyed (WY) fly, and the female is homozygous for red eye (WW). (b) The male has red eyes (WY) and the female white eyes (WW). The letter Y stads here not for an allele, but for the Y chromosome, present in male Drosophila in place of a homologus X chromosome. There is no gene on the Y chromosome corresponding to the W or W gene on the X chromosome.

The linked genes said to be linked together and belong to the same linkage group. In inheritance, the chromosomes inherited as units, they pair and segregate in meiosis and form to gametes. All the genes specify different phenotypic traits located in any given chromosomes tend to be inherited together.

E.g. In Dihybrid cross

Parent type - AABB X aabb
↓
AB X ab
F1 progeny – AaBb
Test cross between F1 and Homozygous recessive
Genotype – AaBb X aabb
↓
Gametes AB,Ab,aB,ab X ab

F2 → AaBb, Aab, aaBb, aabb

1 : 1 : 1 : 1

Parent AB/ab X ab/ab

Gamets (AB) (ab) X (ab) (ab)

F1 - (AB)(ab) : ab ab
1 : 1 ----Test cross ratio

e.g. In Drosophila

In second chromosome alleles of two genes are considered.

1. One gene for body colur and
   recessive allel b – Black body
   Domant allele b+ - grey body
2. Second gene for the wing, wild type homozygous recessive vg(s) for short wings and
3. Dominant allele s+ for long wings.

Homozygous – Short and black body
- Long and grey body

Cross between shor & black X Long & grey
Gives in F1 Grey & long wings

In the test cross 82% exhibit totally (41% each of) the parental combinations & 18% showed recombinant organisms.
This indicates the allelic forms of 2 genes that are tend to remain together on the chromosomes of the progeny.

Linkage classified into 2 types as

i. Complete linkage: In which the gene located so close is linked together and inherit together for several generations. i.e. complete linkage. E.g Drasophila.

Incomplete linkage: In which, the exchange of non-sister chromatids at varying length during meiotic prophase by crossing over. The widely located genes (linked genes) in chromosomes have chances of separation by crossing over. This is said to be incomplete linkage and these type of genes are incompletely linked genes. E.g. In Maize

2. Briefly discuss on crossing over and its importance.

Crossing over is some special genetic process, which is termed as crossing over, performs the reciprocal exchange of linked genes of the chromosomes between the homologous pairs.

This phenomena is termed by T.H.Morgan. Through this process, new combination of genes (recombination ;of genes) occurred by interchanging the segments of chromosomes between non-sister chromatids of homologous chromosomes.

New combination of genes are called cross over. Crossing over and independent assortment are mechanisms that produce new combination of genes. This is mainly important for evolution of organisms.

The following diagram explains the events of crossing over occurs in chromosomes.

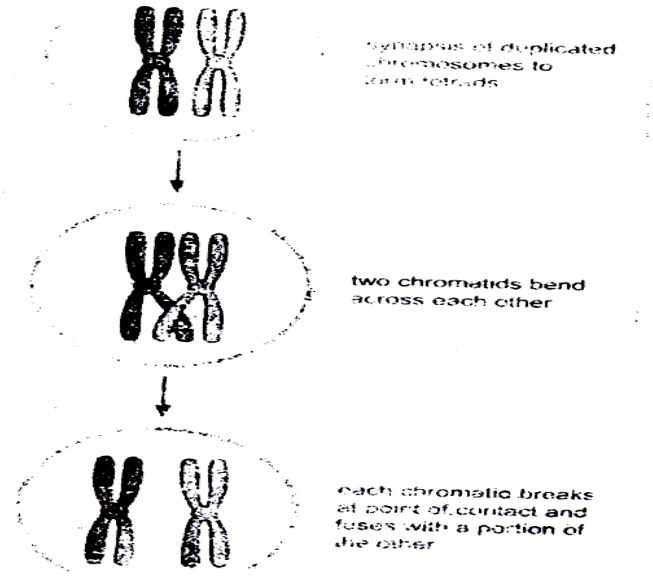


Figure: Janssens's hypothesis of crossing over

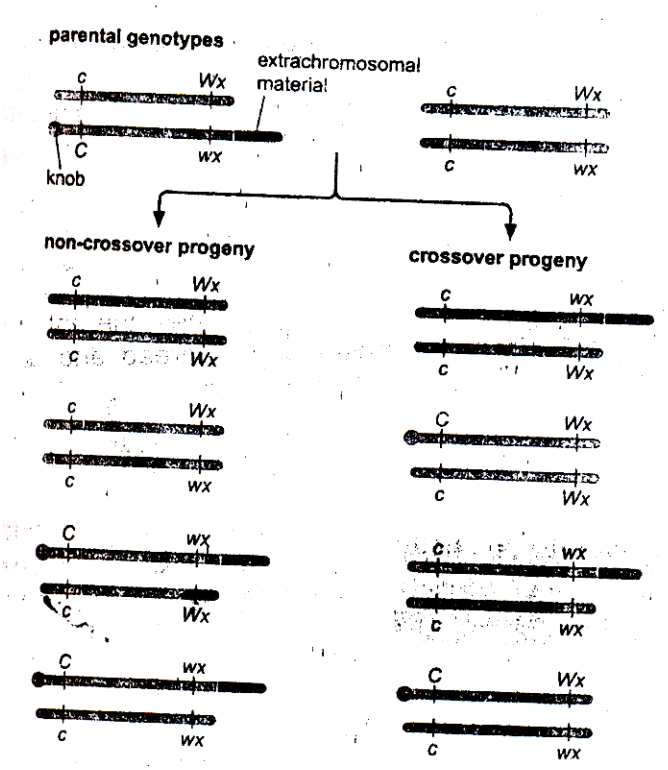


Figure: Demonstration of Physical exchanges between homologous chromosomes. In most organisms, pairs of homologous chromosomes have identical shapes. Occasionally, however, the two members of a pair are not identical, one is marked by he present of extrachromosomal material or compacted regions that reproducibly form knob – like structures. McClintock and Creighton found one such pair and used it to show that crossing over involves actual physical exchanges between the paird chromosomes. In the eperiment shown here, the homozygous c, wx progeny had to arise by crossing over between the C and wx loci. When such c, wx offspring were cytologically examined, knob chromosomes were seen, showing that a knobless W x region had been physically replaced by a knobbed wx region. The colored box in the figre identifies the chromosomes of the homzygous c, wx offspring.

There are two types of crossing over have been studied in living organisms.

i. Miotic crossing over: Meiotic crossing over occurs in reproduction cells or gametes during development. It has universal occurrence and has great genetic significance.

ii. Mitotic crossing over or Somatic crossing over: Some times the crossing over occurs in somatic cells of the organisms. It is generally least significance. This is called as somatic or mitotic crossing over. Scientist Stern found this type of crossing over in Drosophila and Pantecorao in Aspergillus nidulans.

Features and concept of Crossing over:

i. The location of gene is called locus (loci). The loci of genes are arranged in a linear fashion.

Two alleles of the gene in heterozygote occupy corresponding position, in the homologous chromosomes. i.e. Allele 'A' in same position of homologous similar and Allele ' occupies in homologous 2.

Crossing involves the breakage of each of two homologous chromosomes (chromotids) and exchange of parts.

Crossing over occurs at zygotene and pachytene after synopses of the homologous chromosomes in prophase I. Chromosome replication in interphase. Crossing over occurs in post replication of tetrad stage. Crossing over that involves sister chromatids occurs (2 chromatids off homology).

Chromosomes with recombinant combination of linked genes are formed after crossing over.

The probability that crossing will occur in between two loci increases with increasing distance.

Mechanism of crossing over:

I. Synopsis of chromosomes:

During meiosis of prophase I of zygotene stage, pairing of chromosomes, the entire length gene by gene called synapsis. At pachytene, paired chromosome get thread and divide equally. Duplication of chromosome in to 4 chromatids is termed as tetrad stage.

II. Crossing over:

The two non-sister chromatids first get breaks on any of the point with help of endonucleases. The fusion of segments of non-sister chromatids on the break points by the use of enzyme called ligase. The crossing over of 2 chrmoatids is called chiasma formation and the portion is called chiasmata.

III. Terminalization:

After exchange of nonsister chromatids and repairing of broken points of segments, the chromatids or the force of synapsis is decreased and move back to the original stage. The homologous chromosomes are separated. Chiasma frequencies are correlated as recombinant frequencies.

Kinds of crossing over:

i. Single crossing over: In this event, only one chiasma is formed. It produces 50% non crossovers and 50% cross over chromatids.
ii. Double crossing over Chiasma (2 Nos.) at 2 points are produced in this type of crossing over. It produces 4 cross over in this process.
iii. Multiple crossing over: In this type, more than 2 Nos. of chiasma are formed.

Frequency of crossing over:

i. Larger the chromosomes, greater the chiasma formation.
ii. The number of chiasma formation is also a role.
iii. The closely located genes are less affected by chiasma formation.

Limits of recombination:

Limits of recombination are 50% normal and 50% recombinants.

Significance

i. Crossing over is great use in constricting the genetic maps.
ii. It provides direct evidence and linear arrangement of linked genes.
iii. Increases the frequency of genetical variations.

3. Prove the transforming principle of DNA by using Avery, Macleod and McCarty experiments.

In 1944, Avery, Macleod, McCarty published "Transforming Principle" by the results of set of laborious experiments. They rose how the DNA may be pure and as genetic material. It is difficult to have one macromolecule component with high pure state. The preparation of DNA molecules with protein of contaminating proteins was responsible for transformation. The proof of DNA was the transforming principle involved in the use of enzymes. (Proteins that catalyze the specific metabolic reactions).

The experimental set up and the results are presented below:

1. Type II R live cells were cultured in agar plates and found type II R colonies in the plate.

2. The DNA extracts of type III S heat killed bacteria were cultured in the agar plates and found no colonies on the agar plate.

3. The type II R live cells + DNA extract of type III S heat killed bacteria + Serum precipitate of type II R cells mixture were grown in agar plates. The result was type III S bacteria colonies in agar plate.

4. The type II R live cells + DNA extract of type III S heat killed bacteria + Serum precipitate of type II R cells mixture _ RNAse were grown in agar plates. The result was type III S bacteria colonies in agar plate. There is no effect on transformation by RNAse was observed.

5. The type II R live cells + DNA extract of type III S heat killed bacteria + Serum precipitate of type II R cells mixture + DNAse were grown in agar plates. The result was No bacteria colonies in agar plate. The observation was the DNAse treatment destroyed the transforming activity of DNA preparation.

6. The type II R live cells + DNA extract of type III S heat killed bacteria + Serum precipitate of type II R cells mixture + Protease were grown in agar plates. The result was type III S bacteria colonies in agar plate. The observation was as there is no effect by protease on transformation.

So, they concluded that the DNA carries the genetic information for synthesis of type III capsule physically integrated into type II R cells recipient by specific recombinant process.

When DNAse is treated, it digests DNA fragments type III S bacteria. Hence there is no transformation of type III S to the type II R. But, RNAse and protease had no effect on synthesis of type III S DNA and digests RNA and Protein respectively.

The observation is other protein and enzymes are not involved in DNA transformation and DNA is the responsible material involve in transformation. Thus, it was observed from the above experimental study, as the DNA is the genetic material.

R – Rough colories (virulent)
S – Smooth colories (non-virulent)

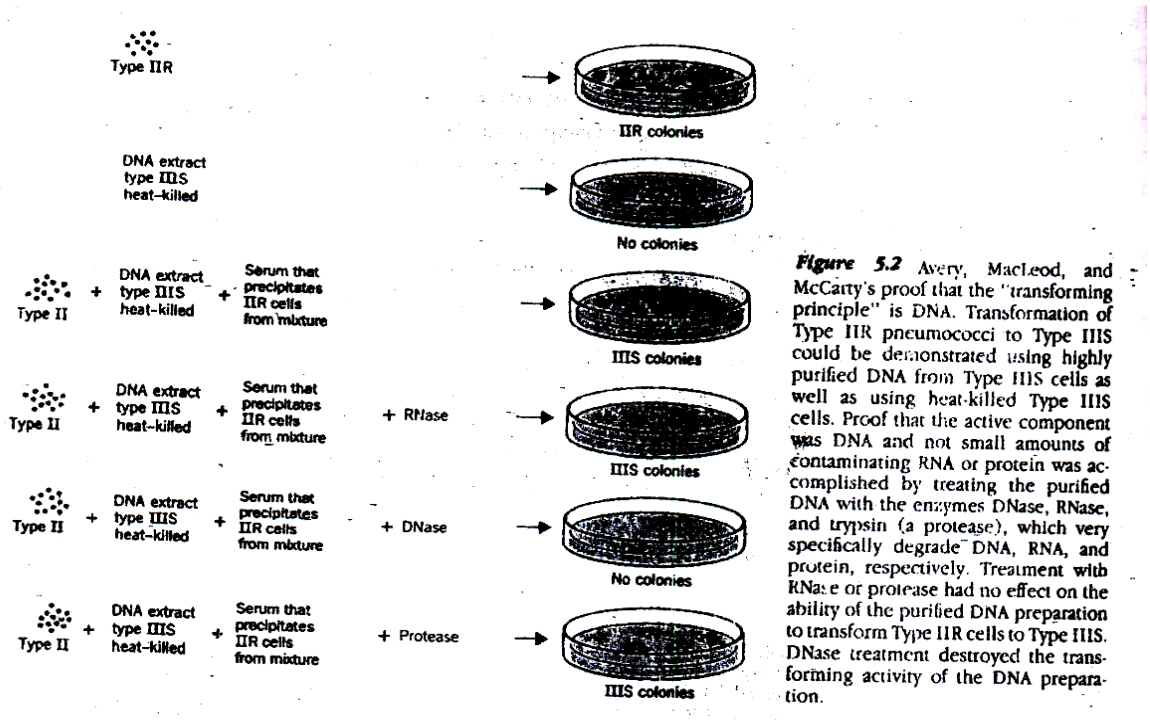


Figure 5.2 Avery, MacLeod, and McCarty's proof that the "transforming principle" is DNA. Transformation of Type IIR pneumococci to Type IIIS could be demonstrated using highly purified DNA from Type IIIS cells as well as using heat-killed Type IIIS cells. Proof that the active component was DNA and not small amounts of contaminating RNA or protein was accomplished by treating the purified DNA with the enzymes DNase, RNase, and trypsin (a protease), which very specifically degrade DNA, RNA, and protein, respectively. Treatment with RNase or protease had no effect on the ability of the purified DNA preparation to transform Type IIR cells to Type IIIS. DNase treatment destroyed the transforming activity of the DNA preparation.

4. Demonstrate the 'Hershey-Chase' experiment used to proving DNA as a genetic material.

A.D.Hershey (Nobel Lauratee) and M.Chase in 1952, published a papers for DNA as the genetic material. They have made the experiments and provided with additional evidence for proving the DNA is the genetic material. Nobel prize was awarded for this invention to A.D.Hershey.

The experiments were carried out in bacteria virus (T2 phage) for the above invention. Since the viruses are smallest particles, rapid reproduction and contain very simple structures like nucleic acids and protein coat , it was selected for study of DNA in viruses with host bacterial cell for its synthesis. Generally, T2 phage infects bacteria E.coli.

Hershey and Chase showed that in T2 phage reproduction, phage DNA enters in the Host cell and the protein observed outside of Host cell. This implies that DNA as the genetic material. The basis is the DNA contains phosphorus and the protein contains sulphur.
They done the following procedure in their experimental sturdy and continue observation.

They labeled phage DNA by radioactive phosphorus 32p in place of normal P is 31P.

1. The protein coat of the phage is labeled with radioactive sulphur 35S in place of normal S, 32S in growth medium.

They cultured the phage with bacteria E.coli in culture medium and allow the infection with Host in their study.

The experimental set up is as given below;

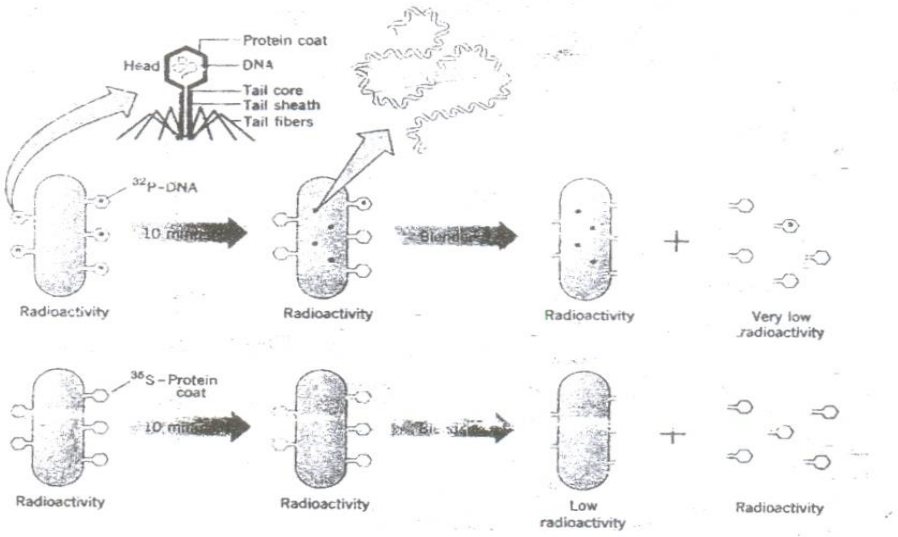


Figure 5.3 The "Hershey–Chase experiment": evidence that DNA is the genetic material in bacteriophage T2. *Escherichia coli* cells were infected with ^{32}P-labeled phage (DNA-labeled), and after being allowed time for infection, they were agitated in a blender, which sheared off the phage coats. The phage coats and the infected cells were then separated by centrifugation. Radioactivity was measured in the cell pellet (the sediment) and in the phage coat suspension. Most of the radioactivity was found in the cells. When the same experiment was done using phage with ^{35}S-labeled proteins, the results were very different. Most of the radioactivity was found in the suspension of phage coats; very little entered the host cells. Since phage reproduction (both DNA synthesis and new-coat protein synthesis) occurs inside the infected cells, and since only the phage DNA enters the host cell, the DNA, not the protein, must carry the genetic information. (Based on R. Sagar and F. J. Ryan, *Cell Heredity*, Wiley, New York, 1961.)

I. Experiment I:

1. The phage labeled with 32P (DNA) was infected in the culture medium and allowed it for 10 mins.
2. The host cells with phage radioactive P were blended to shear to remove the coats and centrifuged . The pellet containing DNA were removed and supernatant containting phage coats were collected.
3. The observation was, the host cells were radioactive and the phage coats were found less radioactive.

II. Experiment II:

1. The phage labeled with 35S for protein coat infected in E.coli for 10 mins in a culture medium.
2. Host cells with radioactive were blended to shear to separate protein coats and DNA. Centrifuged to remove DNA and supernatant with protein coat.
3. The observation was the phage coats are found more radioactive and host cells with DNA were less radioactive.

During phage synthesis, the DNA and protein synthesis occur inside the host cells. The phage DNA enters in host cell and not the coat protein. Hence, the DNA must carry the genetic information. In the progeny of virus, the DNA contains 32P but none of the phage contains 35 s.

However, the above experiment did not provide strong proof (unambiguous proof) for T2 phage DNA as the genetic material because some of 35s found in host cells DNA. Hence, small amount of genetic information in coat proteins. The process of infection of E.coli with phage DNA is called "Transfection".

5. Write an essay on bacterial conjugation and its importance in recombination.

Bacterial conjugation is a type of mechanism of recombination process occur in bacteria. This was invented by J.Lederberg and E.L.Tautum in 1946 in E.coli bacteria of K12 strains. For this invention, Lederberg and Tautum were awarded Nobel prize in the year 1958.

In this process, the transfer of DNA from donar bacteria to recipient bacteria is referred as male and female respectively. It is one way transfer of genetic material (DNA) and no reciprocal exchange between the above two organisms. Donor differs with surface appendages in pili. Synthesis of 'F' pili controlled by several genes. The genes carried by small circular DNA called Minichromosome. This is called 'F' factor i.e. Fertility factor or Sex factor. Conjugation and DNA transfer initiates after contacts of F- cells.

So, F+ cells (Donors) contain F factor and F- cells are not contain F factor (recipient.

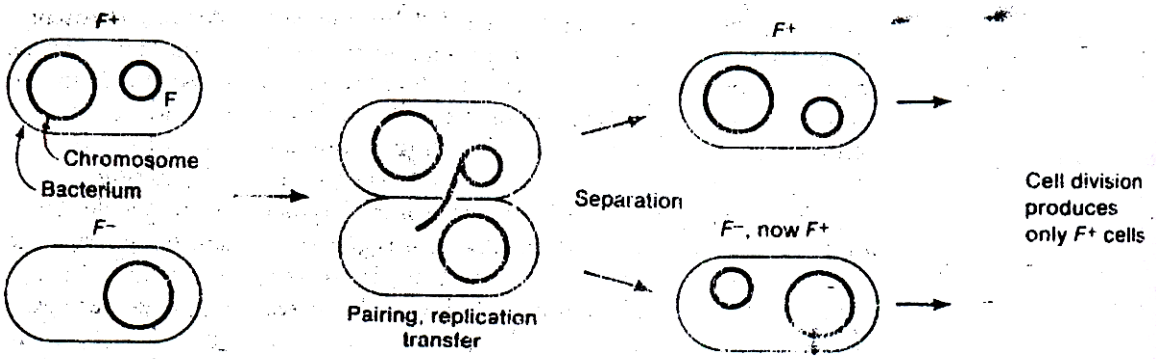


Figure 2-7 Diagram showing the events in an $F^+ \times F^-$ mating.

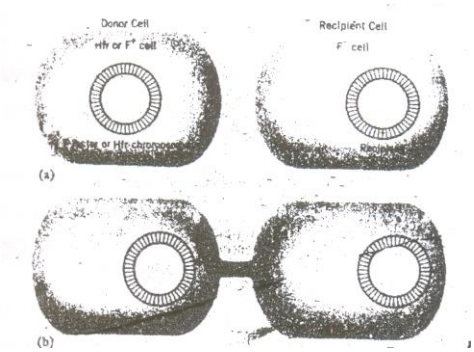
In the conjugation process, there are two stages of conjugation process and transfer of DNA occurs.

i. Donor contain F factor is F+ cell. F+ conjugates with F_ cell and the autonomous F factor is transferred both exoconjugates become F+ cell. F factor replicates during transfer. The new popultation become F+ cells i.e. recombinants.

iii. The integration of F factor is at any specific sites is appeared. The F factor integration is mediated by IS elements. I.e. short DNA sequences approximately 800 to 1400 bop. Cells carry integrated F factor is called Hfr cells (High frequency recombinants).

In the integrated state, F factor mediates the transfer of chromosome of the Hfr cells to recipient. Usually, a portion of the Hfr chromosome transferred and rarely entire Hfr transferred. Transfer is by endonucleotic nick in one strand of specific site on the factor. The 5′ end of the nicked strand is transferred through conjugate tube. The 5′ send of nick strand is replicated in recipient cell. The remaining part of the F factor is the last part to be transferred. The F- cell acquires complete F factor. In rare cases, entire integrated chromosome is transferred.

The following diagramme demonstrate the typical conjugation process.



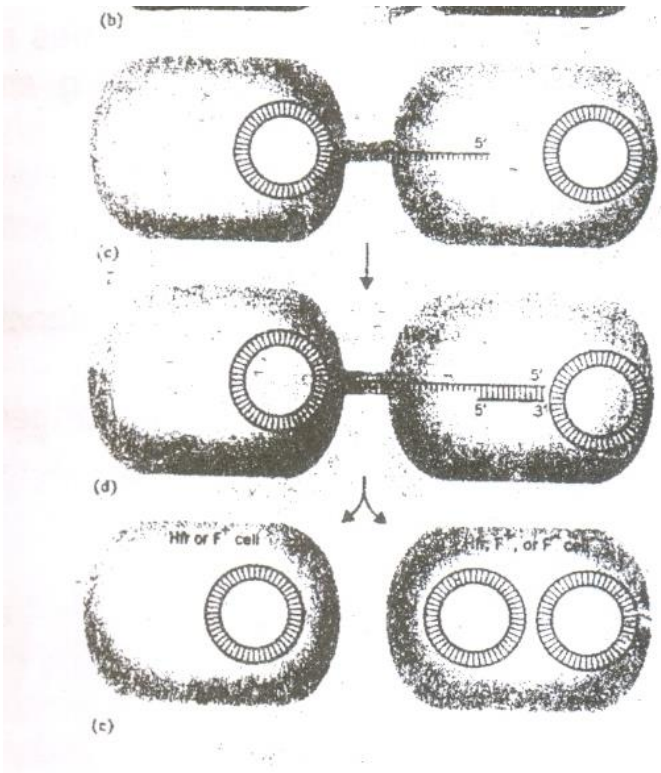


Figure 8.11 Mechanism of DNA transfer during conjugation. (a) The recipient chromosome is shown in greenish color. The chromosome shown (reddish color) in the donor is either carrying the integrated F factor (Hfr cell) or is the F factor (F^+ cell). (b) After cell contact occurs, a conjugation tube forms between the two cells. An endonuclease cleaves one strand of DNA at a unique site (the "origin" of transfer) on the F factor (either integrated in the case of an Hfr or autonomous in the case of an F^+ cell). (c) The 5′ end of the cleaved strand is then displaced, as in normal rolling circle replication, except that during conjugation the 5′ end moves through the conjugation tube into the recipient cell. (d) Transfer occurs concurrently with, possibly driven by, rolling circle replication, with the intact circular strand serving as a template for the synthesis of a complementary strand in the donor cell and the transferred linear strand being replicated discontinuously (by the synthesis of short 5′→3′ Okazaki fragments that are then joined by DNA ligase in an overall 3′→5′ reaction) in the recipient cell immediately after transfer. (DNA strands synthesized during the conjugation process are shown in black; parental DNA strands are shown in reddish color.) (e) In F^+ by F^- matings, both exconjugants will be F^+, since both will have a complete copy of the F factor. In Hfr by F^- matings, the donor cell will remain an Hfr and the recipient cell will usually remain F^-, since a portion of the integrated F factor is the last segment of DNA to be transferred. Although the diagram shows complete chromosome transfer to be comparable to F factor transfer in F^+ by F^- matings, the conjugation tube and chromosome usually break spontaneously in Hfr by F^- matings before the entire chromosome is transferred. In the rare cases where the entire Hfr chromosome is transferred, the recipient cell becomes an Hfr after conjugation.

Hence, from the above phenomena it is observed that the genes present in one bacterium type is transferred to another type and the new recombinants are obtained.

6. Explain the bacterial transformation process.

Griffith first discovered the bacterial transformation process in 1928 in Pneumococcus (Diplococcus pneumoniae). Then, Avery, Macleod, McCarty, 1944, proved that the DNA as the genetic material i.e. transforming principle. Further work in transforming of bacteria also was done by Hershey and Chase.

After 60 years, the Griffith discovery was completely studied and found, as the transformation is "uptake of DNA molecules from donor to recipient bacterium" by active energy requiring process. There is no passive entry through cell membrane was studied and found. The transformation process is not occur in all the species of bacteria instead only few specific bacteria contain enzymatic machinery involved in active uptake of DNA or transformation process.

Following species of bacteria were mostly studied for its transformation process:

i. Diplococcus pneumoniae
ii. Bacillus subutilis
iii. Haemophilus influenzae.

Even in the above, all the cells are not capable of transforming the DNA. These few cells capable of transforming DNA are called as competent cells and it posses competence factors.

The transformation steps involve in the following:

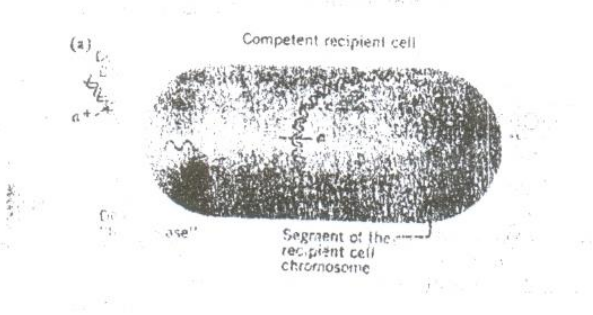
1. Reversible binding of dsDNA molecules to receptor sites of the cell surface occur in the initial stage.

2. Irreversible uptake of the donar DNA, at which time the donar DNA becomes resistant to DNAse in the medium. Transfer pull one strand DNA in to cell by using energy from degradation of other strand.

3. Conversion of dsDNA to ssDNA by nucleolytic degradation of one strand.

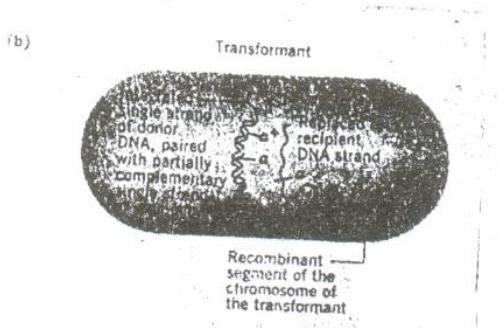
4. Integration (covalent insertion) of all or part of the ssDNA strand of donar DNA to chromosome of the recipient bacterium.
5. The segregation and phenotypic expression of the integrated donar gene or genes in the recombiant cells (transferred cells).

The following figure represents the outline of transformation process:



The integration or DNA recombination is specific for homologous DNA. In heterologous very less frequency. The donar DNA fragment are approximately 20,000 bp i.e. 1/200 of chromosome. Sometimes, double transformation also occurs with two different genes segment.

The sequential events of transformation are represented in the following diagramme.



After insertion of Donar ssDNA strand, the transformant chromosome has two different alleles called mismatch pairs (duplex of DNA hybrid) or heteroduplexes, which are important in mutation, recombination and DNA repair process.

7. Discuss in detail about transduction process in bacteria.

Transduction is a another type of bacterial DNA transformation process, which is discovered by N.Zinder and J.Lederberg, in 1052. Transduction process can be described as the transfer of DNA fragment from donar to recipient bacterium through bacterial virus (Bacteriophage) and recombination of genetic markers of two cells.

There are two types of phage particles involved in this process are;

i. Virulent bacteriophage which is multiplies and lyse the host cell.
ii. Temperate bacteriophage which has two choices as either inter in lytic or lysogenic cycle.

The transduction process in bacteria is occurring in 2 types as;

1. Generalised transduction (Non-specific) i.e. any part of host DNA will be transformed in random manner. In generalized transduction, the DNA segment is integrated into the virus or in place of it.

A random or nearly random DNA segment packed with phage DNA in place of phage DNA progeny called transducing particles. The segment may be 1/100 or 1/50 of the total host DNA (donor).

Therefore, in lytic cycle, transducing particles contain only bacterial DNA or both phage and host DNA produced by both virulent and temperate phage. T even phages (T2, T4, T6) degrade the host DNA and utilize the nucleotides for synthesis of phage DNA. Hence host DNA not available. I.e. transduced particles are produced. The given below diagram represent the generalized transduction.

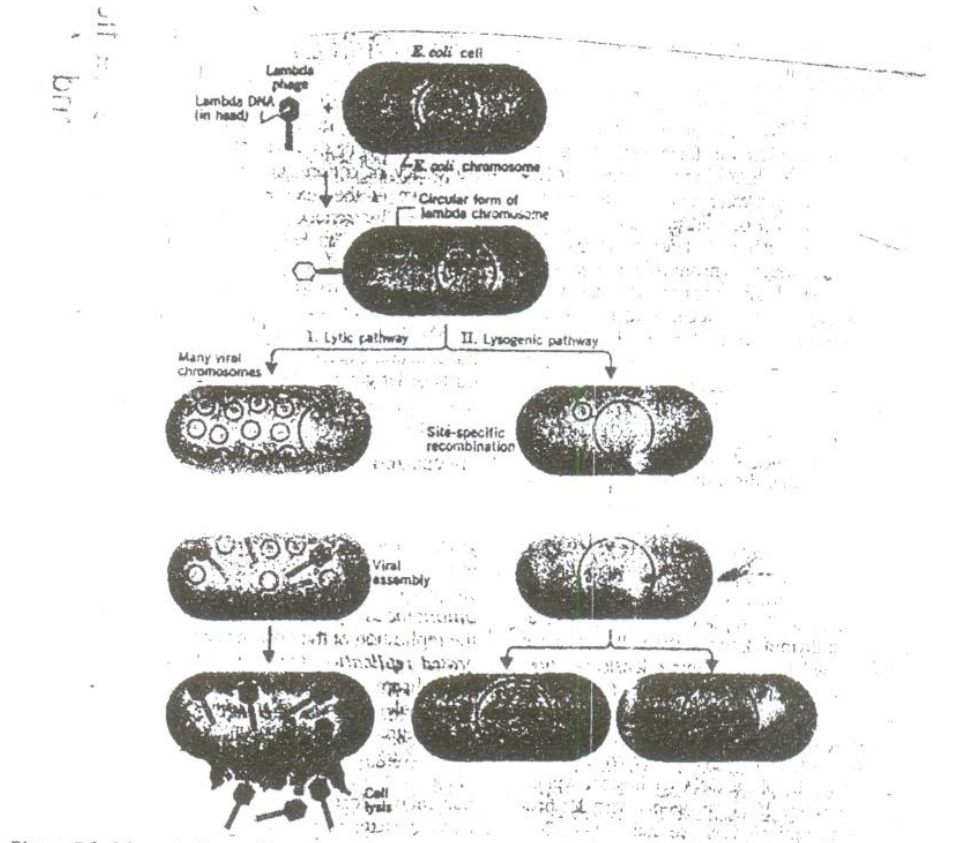


Figure 8.3 Schematic diagram illustrating the two alternative life-styles of temperate bacteriophages such as the *E. coli* phage lambda (λ). After the injection of the linear lambda chromosome into an *E. coli* cell, the chromosome is converted to its circular form. It then either (I) commences replicating autonomously and lyses the host cell, releasing 100 or more progeny viruses (the *lytic pathway*), or (II) integrates via a site-specific recombination event into the *E. coli* chromosome (the *lysogenic pathway*). In the integrated *prophage* state, the lambda chromosome replicates as part of the host chromosome and is transmitted to both progeny cells at each cell division. With a low probability, the prophage may be spontaneously excised from the host chromosome and enter the lytic pathway. It can be induced to enter the lytic pathway with a high frequency by irradiation with ultraviolet light.

In lysogenic cycle, after infection of phage DNA, it may be integrated with recipient DNA or remain freely in cytoplasm. If it is not integrated, it will not replicate and transmitted to only one cell of progeny in each cell division.

The genes present in non-integrated chromosome also expressed and which fragments are called abortive transductants. The probability is very low as 10-6 to 10-8. The conclusion is

i. certain phages carry only bacterial DNA, no phage DNA based on results of density transfer experiment.

2. Specialised transduction (restricted transduction) in which the transfer of specific DNA fragment from host cell. E.g. gal and bio genes of E.coli are best known sequences. In pecialized transduction, some specific genes of phage (virus) are replaced by host cell. These sites are specified attachment sites otherwise called prophage site. E.g. lambda phage. The lytic genes are repressed in prophage state. Lambda transduced the specific genes of the 'gal' (galactose utilized genes), 'bio' (biotin synthesis) operons. Phage Q 80 integrates the genes coding for tryptophan synthesis called trp genes.

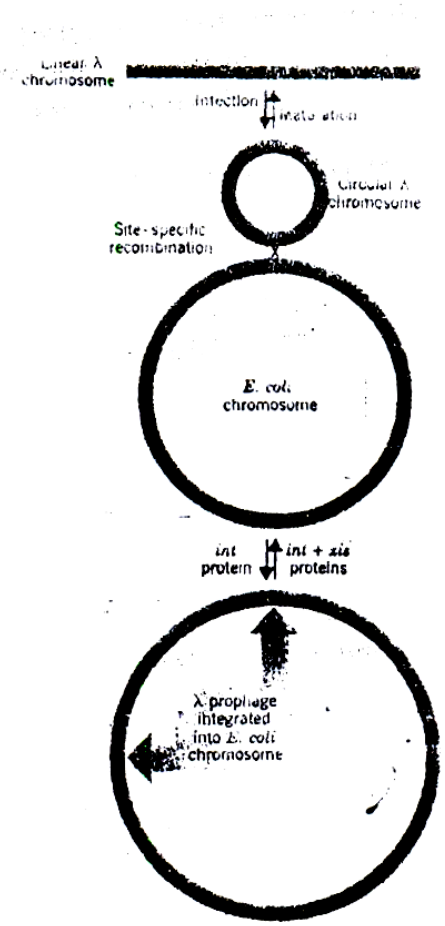
In recipient, genetic integration is takes place. The specific gene sites (DNA fragment) will carry to the recipient genome of the bacterial cell.

Figure 8.4 Integration and excision of the phage lambda chromosome. When the lambda chromosome is packaged inside the phage head it exists as a linear molecule with gene sequence *A* through *R*, as shown at the top (step 1). After infection, it is converted to its circular intracellular form by the mechanism described in Fig. 5.17. When lambda enters the lysogenic pathway (Fig. 8.3), it undergoes a site-specific recombination event between the *bp'* site on the phage chromosome and the lambda attachment site *bb'* on the *E. coli* chromosome (yellow-green). This integrative recombination step is catalyzed by the lambda *int* gene-product. In the prophage state, the lambda gene sequence is *p'-cIII-R-A-J-p*. This sequence is a circular permutation of the gene sequence in the linear form packaged in the lambda head (top), which results from a specific endonuclease cut between genes *A* and *R* during phage maturation.

During spontaneous or UV-induced excision, essentially the reverse of integration occurs. We can visualize this as the lambda prophage "looping out" to form a "figure-8" structure; this step is followed by a site-specific recombination event between the *bp'* sequence and *pb'* sequence. The site-specific excision step requires the product of the *int* gene plus the product of another lambda gene called *xis*. Normal excision produces an intact circular lambda chromosome plus an intact circular *E. coli* chromosome. Landy and Ross have sequenced both the lambda chromosome attachment site (*pp'*) and the *E. coli* chromosome lambda attachment site (*bb'*), as well as the lambda prophage-*E. coli* DNA junctions (*bp'* and *pb'*), and found that all four contain the identical 15 nucleotide-pair sequence

```
--GCTTTTTTATACTAA-
-CGAAAAAATATGATT-
```

For this to be true, the site-specific recombination events involved in integration and excision must occur within or at one end of these 15 nucleotide-pair sequences.



In both the above cases, the transducing phages are usually defective in some respects e.g. often they lose the ability of lyse the host cells. Transduction process is studied in the following bactera;

Salmonella
Shigella
Escherichia spp.
Bacillus
Psuedomonos
Streptococcus
Vibrio
Rhizobacterium
E.coli

8. Mendel crossed pea plants that produced round seeds with those that produced wrinkled seeds. From a total of 7324 F2 seeds, 5424 were round and 1850 were wrinkled. Using symbols W and w for dominant & recessive genes respectively;

i. Symbolize the original parent cross.
ii. The gametes
iii. F1 progeny
iv. Represent a cross between two F1 plants sympolize the gametes and
v. Summarize the expected F2 results under the following heading, phenotypes, genotypes and
vi. Summarize the expected F2 results under the following headings, phenotypes, genotypic frequency and phenotypic ratio.
vii. Briefly explain dominance with suitable example Mendelian genetics?

PRINCIPLE OF INDEPENDENT ASSORTMENT

Mendel also crossed plaints that differed in two pairs of alleles (Fig). In this cross, designed to clarify the relation of different pairs of alleles, he crossed plaints having round, yellow seeds with plants having wrinkled, green seeds. The F_1 progeny from such a cross between homozygous parents are hybrids (heterozygote) for two gene pairs. The F_1 progeny (Gg Ωω) are dihybrids, and by extension, the GGΩΩ x ggωω cross is a **dibybrid cross.** Alleles for both round and yellow were known from previous studies to be dominant over their respective alleles, producing wrinkled and green seeds.

Phenotypes	Genotypes	Genotypic Frequency	Phenotypic Ratio
Yellow, round	GG ΩΩ GG Ω w Gg Ω Ω Gg Ω w	1 2 2 4	9
Yellow, wrinkled	GG ww Gg Ω w	1 2	3
Green round	Gg ΩΩ Gg Ω w	1	3
Green, wrinkled	Gg ww	1	1

Figure: Diagram and summary of a cross between a variety of garden peas with yellow, round seeds and a variety with green, wrinkled seeds. The F_1 X F_1 represented illustrates a dihybrid cross.

All the F_1 seeds resulting from the cross were round and yellow, as expected. When the F_1 hybrids were allowed to self-fertilize, four F_2 phenotypes were observed in a definite pattern. From a total of 556 seeds, the following distribution was obtained: 315 round, yellow; 108 round,

green; 101 wrinkled, yellow; and 32 wrinkled, green. These results closely fit a ratio of 9:3:3:1 (i.e., 315/556 ≅ 9/16, 108/556 ≅ 3/16, 101/556 ≅ 3/16, 32/556 ≅ 1/16). Mendel recognized this as the result of **two monohybrid** crosses, each expected to result in a 3 : 1 ratio, operating together. The product of the two monohybrid ratios $(3:1)^2$ was equal to the **dibybrid ratio** $(3+1)^2 = (9+3+3+1)$, thus conforming to the **law of probability,** called the "product rule", which states: **The chance of two or more independent events occurring together is the product of the their separate occurrences.**

The results were those excepted from the assortment of two independent pairs of alleles, each showing dominance of one member. Not only did the members of each pair of alleles segregate, but the allelic pairs of different genes behaved independently with respect to each other. Mendel therefore drew another conclusion: Members of different pairs of alleles **assort independently into gametes.** This concept of independent assortment of different pairs of alleles is designated as his second principle. It is a simple corollary of meiosis (Chapter 3). Mendel's two principles were set for the in a paper entitled "Experiments in Plant Hybridization", which was read before the Brunn Natural History Society in 1865 and published in the proceedings of that society in 1866.

An understanding of Mendel's principle of independent assortment is one of the key components considered in the design of many experiments in plant and animal breeding aimed at improving the quantity or quality of agricultural products. Desirable traits carried in different varieties can be combined and maintained in a single type. A variety of barley resistant to rust, for example, was needed in a rust-resistant variety, however, like most barley varieties, had hulls on the seeds and did not thresh well. Another variety had no hulls and threshed out clean like wheat, but had poor rust resistance. These two varieties were combined by appropriate crosses, and a valuable new strain with rust resistance and no hulls was obtained.

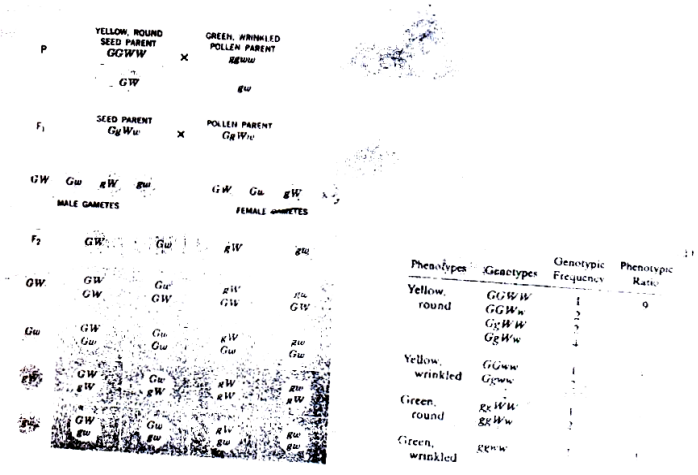


Diagram and summary of a cross between a variety of garden peas with yellow, round seeds and a variety with green, wrinkled seeds. The F_1 X f_2 represented illustrates a dihybrid cross.

9. Explain in detail on Watson & Crick model of DNA.

J.D.Watson and F.H.C.Crick first deduced the physical structure of DNA in 1953 very elaborately. Their model of DNA proposal is called as Double-Helix model, which is based on the structural elucidation by i. chemical composition and ii. X ray diffraction patterns.

According to Watson & Crick model, two polynucleotide chains are coiled about one another in spiral. Each chain consists of a sequence of nucleotides linked together by phosphodiester bonds joining deoxyribose moieties. The nucleotide strands are held together in their helical configuration by hydrogen bonding between bases in opposing strands. The base pairs perpendicular to axis like steps of a spiral staircase. Base pairing is specific as adenine always thiamine. Guanine always to cytosine. All base pairs consist of one purine and one pyrimidine. Base pairing results from hydrogen bonding. As adenine and thymine from two hydrgogen bonds and G & C form three hydrogen bonds.

The following diagram represents the Watson & Crick model.

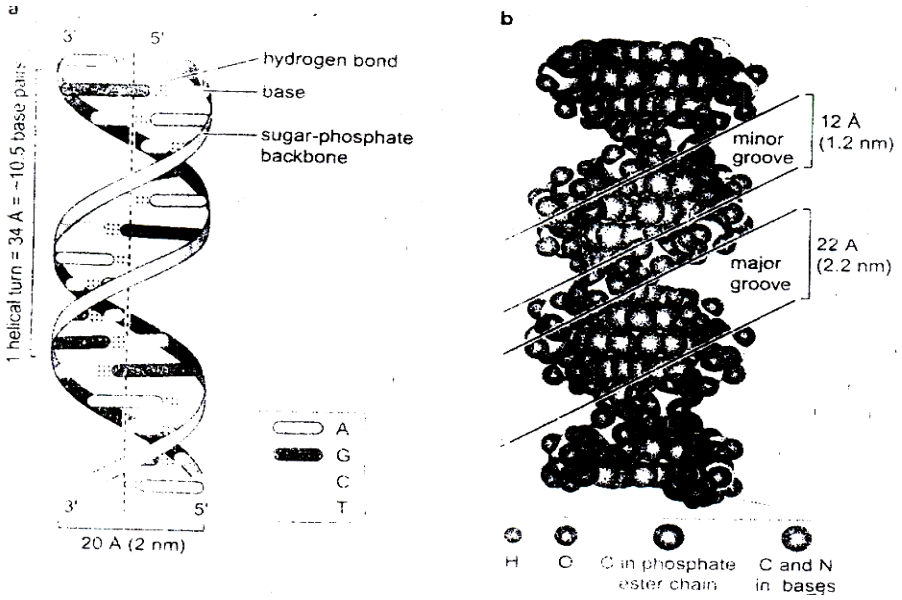


Figure: The helical structure of DNA: (a) Schematic model of the double helix. One turn of the helix (34 Å or 3.4 nm) spans approximately 10.5 base pairs. (b) Space – filling model of the double helix. The sugar and phosphate residues in each strand form the backbone, which are traced by the yellow, gray, nd red circles, showing the helical twist of the overall molecule. The bases project inward but are accessible through major and minor grooves.

The sequence of bases of both strands complementary each other. I.e. not identical. This is otherwise called antiparallel strand. This complementary suits to store and transmit the genetic information. The base pair of DNA stacked at 3.4 Ao apart with 10 base pair per turn (360 o. The sugar moieties of 2 strands are antiparallel i.e. opposite chemical polarity. So, one goes unidirectionally along DNA helix, the 'P' bonds is one strand go from 3' C of one nucleotide to 5'C of adjacent nucleotide. Whereas the complementary a 5'C to 3'C. The opposite polarity of complementary is important in replication mechanism of DNA.

The high stability of DNA strand is based on large number of 'H' bonds between the base pairs i.e. hydrophobic. This is otherwise water-soluble. The stacked base pairs for stability of DNA molecules in aqueous protoplasm of living cells.

UNIT – II

PART – A

1. Write the differences between eukaryotic and prokaryotic chromosome.

Prokaryotic chromosome:

Lacking nuclear membrane to cover DNA. Single in number and very large, circular in shape. DNA naked and no histone proteins present.

Eukaryotic chromosome:

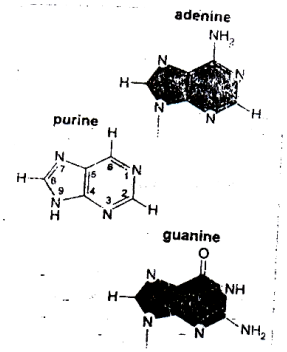
Surrounded with nuclear membrane. More than one in number or multiples and slender. DNA consist of histone proteins.

2. Mention any 2 differences between DNA & RNA.

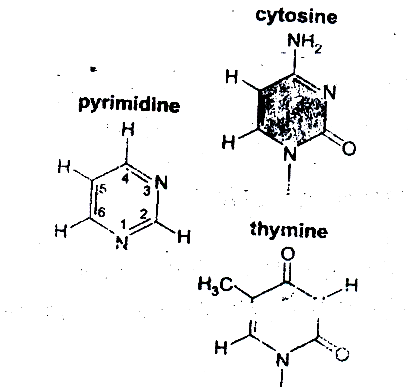
DNA: Contain histones on the surface structures. DNA is double helix. The pyrimidine bases contain cytosine and thymine.

RNA: Generally single strand. The pyrimidine bases contain cytosine and uracil.

3. Write the structure of any one purine base.



4. Write the structure of any one Pyrimidine base.



5. What are purines and pyrimidines?

Purine and pyrimidines are the base units or nucleotides of DNA or RNA. There are two types of base units as purine and pyrimidines. The purine bases consist of double ring structure and pyrimidine bases consist of single ring structure. Purine contains adenine and guanine and pyrimindine contain thymine, cytosine and uracil.

6. What is G+C ratio? Write it importance?

G and C pairs fraction is important for expression of composition of DNA molecule.

i.e. [(G)+(C)]/all bases

that is termed G+C content or % G+C. Generally the G+C ratio is 0% for higher organism and the range of 0.49 to 0.51 in lower organism.

7. Write short note on DNA double helix model.

DNA double helix model is invented by Watson and Crick and awarded Nobel Prize in the year of 1981. According to this model, two polynucleotide chains are coiled about one another in spiral. Each chain consists of a sequence of nucleotides linked together by phosphodiester bonds joining deoxyribose moieties. Hydrogen bonding is involved between bases in the helix.

8. What are the different forms of DNA?

The following types of DNA were found in cells.

iii. B – form DNA: This is a normal type of DNA found in all the organisms.
iv. A – form DNA: In dehydrate state this form of DNA exists.
v. Z – form DNA: This left-handed helix. Certain organism has this model.

9. Explain the hypochromic & hyperchromic effect of DNA.

The absorbance of double stranded DNA and Single stranded DNA by UV spectrometer at 260 nm is measured and the OD value is referred for the DNA denaturation property. The intensity of solution less absorbance of DNA at 260 nm is hyporchromic effect and less absorbance of DNA is called hypochromic effect. dsDNA are hypochromic and ss DNA are hyperchromic effect.

10. Describe the DNA denaturation.

The transition state of native DNA to distrubted state is called denaturation. In denaturation, the ds DNA are getting disturbed or converted into ss DNA. The bonding of ds DNA is weakened and the strands are separated to single strand, which is called denatured DNA.

11. What is renaturation of DNA.

The renaturation demonstrates that the ability of reformation of denatured ss DNA to native form as ds DNA state. This renaturation will be influenced by several factors like temperature, pH and various other chemical factors. It is useful in study of DNA bases and base sequences of RNA.

12. What is melting temperature T_M of DNA?

The temperature, which favours the increase of broken pairs of DNA or ss DNA from the native state of DNA, is called melting temperature or denaturation temperature. The melting temperature (Tm) is the critical temperature that last pair broken and strands separate completely. In which the rise in A260 is half complete.

13. Describe the DNA denaturation.

DNA melting curve is the value of DNA showing melting temperature before rise of temperature of ds DNA and the occurrence of ss DNA molecules after melting temperature.

14. What are DNA melting proteins or helix destablishing proteins?

The protein molecules involve in unwinding of DNA helix are called helix destabilizing proteins or melting proteins. E.g. T4 32 proteins i.e. 32 gene of T4 phage. These protein are useful when the DNA duplex is unwind during synthesis of DNA molecules.

15. What are DNA heteroduplexes?

The occurrence of single standed DNA molecules, which resulted to produce the transformant chromosome, has 2 different alleles called mismatch pairs or duplex of DNA hybrid. This is called heteroduplexes and these are important in mutation, recombination and DNA repair process.

16. What are cot values?

Cot values are the values to measure the rate of formation of ds DNA from ss DNA from the denaturation state of DNA based on the concentration of DNA in solution (S) respect time (t) of reaction. Otherwise it is measure of renaturation kinetics.
This is done based on the measure of OD of DNA at 260 nm (A260).

17. Mention about cot curves.

The Cot curves provide the information on DNA of prokaryotes and eukaryotes with respect of renaturation values. The renaturation value is inversely proportional to the number of times occur for repetition of repetitive base sequences. The presentation of data on hybridization

kinetics in a plot of C/C_o versus Cot.

18. Define DNA kinetic complexity.

Kinetic complexity describes the length of different sequences of genome, which is less than the length of total DNA of genome when there is a replication process.

i.e. Cot ½ value of genome is used for calculating the kinetic complexity of the same genome.

$$\text{Kinetic complexity} = \frac{4.2 \text{ X } 10\text{^}6}{9} \times \text{Cot ½ of genome}$$

19. Describe tRNA.

RNA are mainly 3 types. One among that is tRNA, which is otherwise called as transfer RNA. These are containing in cell about 10-15% of total RNA content. This type of RNA molecule is work as adopter molecule for carrying amino acids to the site of the protein synthesis.

20. Write short notes on mRNA.

This is otherwise called as messenger RNA, which contain 5-10% of total RNA of the cell. It carries genetic information or instruction from DNA in correct order of amino acids during protein synthesis. mRNA is short lived and rapid turn over. The molecular weight of mRNA from 5,00,000 to 20,00,000 Daltons.

21. What are RAS proteins (RNAse associated proteins)?

The protein molecules including enzymes bind with RNA are called RNAse associated protection. The protein include transcription factors, translation factors and the protein molecules associate with RNAse during replication, transcription & translation are belong to this group.

22. What is satellite RNA?

In some plants and animals viral RNA act as genetic material. This virusoids or small RNAs about 350 bp independent in function, which is not covered by coat proteins. These are called as satellite RNA that are encapsidated with plant viruses and packaged with plant genome. These are replicate regularly.

23. Describe rRNA.

Ribosomal RNA (rRNA) are most stable form of RNA in the cell. These are associated with ribosomes, which consist about 40-60% in weight and form as 80% of the total RNA in the cell. This type of RNA involve in catalyzing the assembly of amino acids into polypeptides. This also binds with tRNA and other accessory proteins for protein synthesis. All ribosomes associated with rRNA molecules.

24. Write the importance of DNA replication.

DNA is a genetic material, which carries genetic information of the organisms. Nucleotides sequences of DNA are translated in to structures of protein that performs all the cell functions properly. Hence, it is necessary to copying of DNA during cell division otherwise called as DNA replication. The genetic informations are passed from parent to offsprings accurately with the help of DNA synthesis or replication, which passes to generation. By proper DNA replication, the cells perform various metabolic activities without much deviation.

25. What are primers?

Primers are nothing but the short preexisting fragment of DNA or RNA molecule which help in begin or initiate the chain growth or continuous formation of DNA or RNA during synthesis or replication. The primers base paired and the nucleotide added to the free hydroxyl groups at 3' end of primer.

26. Write short notes on primase.

Primase are the enzymes or RNA polymerases forms short RNA primers complement to DNA template. The primer paired and elongated to its complement DNA by use of DNA polymerase and than new daughter strands formed.

27. What is helicase?

Helicases are also enzymes, which involve in unwinding of ds DNA molecule i.e. double strand of DNA. During replication initiation, the double strands unwind at the specific sites of DNA called origin of replication. During the replication process, only after the unwinding of DNA by helicases, the DNA will open.

28. Write the significance of DNA polymerase.

DNA polymerases are the group of enzymes, which are, involve in the synthesis of DNA molecues. With the help of DNA primases, the daughter strands are formed. The prokatyotic and eukaryotic polymerases are independatly function with the enzyme subunits. DNA polymerases

have different subunits, which involve in various steps of DNA replication process.

29. What is replication fork?

Replication fork is a region (or) junction of DNA template between the newly seperated template strands and unreplicated duplex DNA. The replication fork moves continuously toward the duplex region of unreplicated DNA, leaving its wake two SSDNA template that direct the formation of two daughter DNA duplexes.

30. Write short notes on topoisomerase.

Topoisomerases also a class of enzymes, which involve in maintenance of DNA structure of unwind DNA molecule from torsioral stress. Topoisomerase work with helicase to maintain in unwinding of coils.

31. Describe DNA gyrase.

DNA gyrase is also a class of enzymes which involve in maintain geometry of DNA strand with the help of topoisomerases during synthesis. Rolling or coiling effect of entire genome during unwinding of DNA of ENA during synthesis is establishes by DNA gyrase.

32. What are okazaki fragments?

Okazaki fragments are the discontinuous short DNA segments occur during synthesis. This discontinuos segments are occur in the direction of lagging strand, which are called as okazaki fragments. The RNA primer of each okazaki fragment is removed and replaced by DNA repair.

33. Mention briefly on leading and lagging strands.

Synthesis of DNA continuously from a single RNA primer as 5′ to 3′ direction in the same direction of replication fork movement called as leading strand.

Lagging strand are the synthesis of DNA at the 5′ to 3′direction of strand. But the copying form template strand occurs at opposite direction.

34. Write the role of DNA ligase.

DNA ligase is the enzymes, which work in the DNA synthesis and DNA repair, DNA ligase are particularly work with Okazaki fragments and join the discontinuous process. In general, the DNA ligase involve in DNA repair enzymes.

35. Write brief note on klenow fragment.

In prokaryotic polymerases, the pol I sub unit consist of 2 fragments as;

1. Klenow fragment (38 KD) and
2. Small fragment.
3. The klenow fragment consist of 38 KD molecular weight proteins. Klenow fragment consit 3′ – 5′ exonuclease activity with 5′ – 3′ poly nucleasing activity. Pol I synthesized by gene of pol A of E.coli.

36. What are replicons?

In prokaryotes and eukaryotes, a discrete units contain site of origin involved in DNA replication is called Replicons. Replicons vary in genome size fro 500 bp to several thousands. The replicons could be isolated and cloned in plasmids to autonomous replication of plasmid DNA. That is called artificial chromosome.

37. What are 'D' loops?

'D' loops or displacement loops are occurring in circular DNA molecules. In de novo (fresh) initiation, synthesis of leading strand before lagging strand occurs. i.e. before synthesis of the first precursor fragment begins. Replication bubble exists which consist one ds branch made up of one parental strand paired with leading strand and one ss branch which is unreplicated parental branch. This D loops are temporary or transient, until synthesis of precursor of fragement.

38. Write short notes on primosome.

Primosome is a complex of protein molecules consist of enzymes, which are help in initiation of DNA synthesis. This enzyme complex is mobility in nature and function. The enzymes present in this primosome complex are helicase and primase, which are collectively called as primosome.

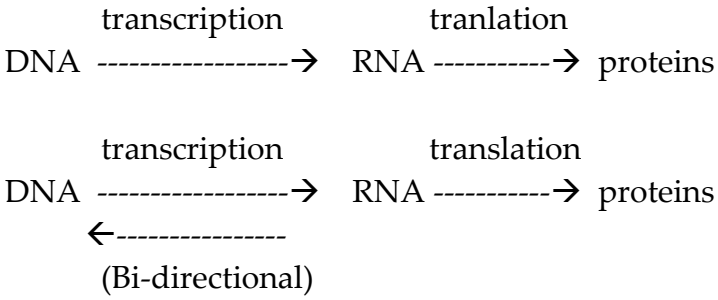
39. What are replisome?

Replisome are mobile protein complex, which are involve in DNA replication. This mobile complex consist of helicase, primase and polymerase enzyme in the replication fork, which are lead in DNA replication collectively called replisome.

40. What is central dogma?

DNA is a genetic material, which contain the entire genetic information of the organism or cell. The information of genetic material for biosyntheis of proteins is stored in polynucleotides of DNA, which are called sequences of bases. The sequences are bases of nucleotides determine the

sequence of amino acid polypeptide, which is known as Central Dogma.



41. What are telomeres?

Telomere is a highly conserved elements of the chromosome occur in all the eukaryotic cells which are uniform in structure and function. This sequence portion located in the terminal or ending portion of the eukaryotic chromosome. This consist simple, randomly repeated sequences by cluster of G & C pairs. I.e. G rich in one strand and C rich in other strand. Telomere is useful in segregation of eukaryotic chromosome through shortening of chromosomes.

42. Write short notes on telomerase.

Telomerease is an enzyme molecule, which involves in synthesis of telomeres in eukaryotic chromosome. It is a large RNA protein involve in synthesis of template for telomeric DNA and protein component, which has reverse transcriptase activity. The G rich strand overhang works as primer and for its elongation of telomeric DNA template of telomerease.

43. What are the three enzymatic activities of DNA pol – I?

DNA polymerase I is a one of the 3 sub units of the DNA polymerases. Pol I contain 5 active sites as i. Template site, ii. Primer site, iii. 5′-3′ cleavage site, iv, nucleoside triphosphate site and v, 3′-5′ cleavage site. These enzymes moves along with okazaki fragment, which involve in removal of mispaired base pairs i.e. called as proof reading. Polymerase contain i. Klenow fragment (38 KD) and ii. Smaller fragment (35KD) . Pol I will involve in nicked DNA in vitro called nick translation.

44. Write short notes on prokaryotic DNA polymerases.

The prokaryotic DNA polymerases contain 3 kinds of subunits as i. Pol I, ii. Pol II and pol III.
Pol I contain 5 active sites involve in initiation and proof reading of DNA synthesis.
Pol II involve in DNA repair, which resembles like pol I brings growth of 5′ → 3′ direction.
Pol III is a holoenzyme, which is essential in DNA replication, which contain 10 sub units.

45. Write short notes on eukaryotic DNA polymerases.

Eukaryotic DNA polymerase contains 5 kinds of units as;

i.Pol α – high molecular weight and cytoplasmic polymerase.

ii. pol β – Nuclear polymerase and small

iii. Pol γ – mitochondrial polymerase encoded by nucleus

iv. pol δ – noval enzymes involve for DNA synthetic processivity PCNA dependant

v. Pol ɘ – It is involve in longtime process for eukaryotic DNA replication.

46. What is nick translation?

The 5′ → 3′ exonuclease activity at a single-strand break (a nick) can occur simultaneously with polymerization. That is, as a 5′ – P nucleotide is remove, a replacement can be made by the polymerizing activity. Since pol I cannot form a bond between a 3′- OH group and a 5′ – monophosphate, the nick moves along the DNA molecule in the direction of synthesis. This movement is called nick translation.

PART – B

1. Discuss in detail on various forms of DNA and it significance.

There are three types of DNA molecules reported in cells of the organisms. The most widely distributed form of DNA is occurring as B – from of DNA. The following three types of DNA molecules are reported:

i. B – for DNA
ii. A – form DNA
iii. Z – form DNA

i. B – form of DNA:

Most of the DNA molecules present in the cell is belongs to B form of DNA. This B form DNA is exist as Watson-Crick model studied earlier. This is a normal stage of DNA found in all the cells.

B - form DNA conform physiological conditions in aqueous solutions containing low salt concentration at 92% humidity. DNA structure changes of their environment. B – form DNA have average of 10.4 nucleotide pairs per turn of helix. But normally 10 nucleotides as precise level. This is 36o away in turn. The following diagramme shows the structure of B- form DNA.

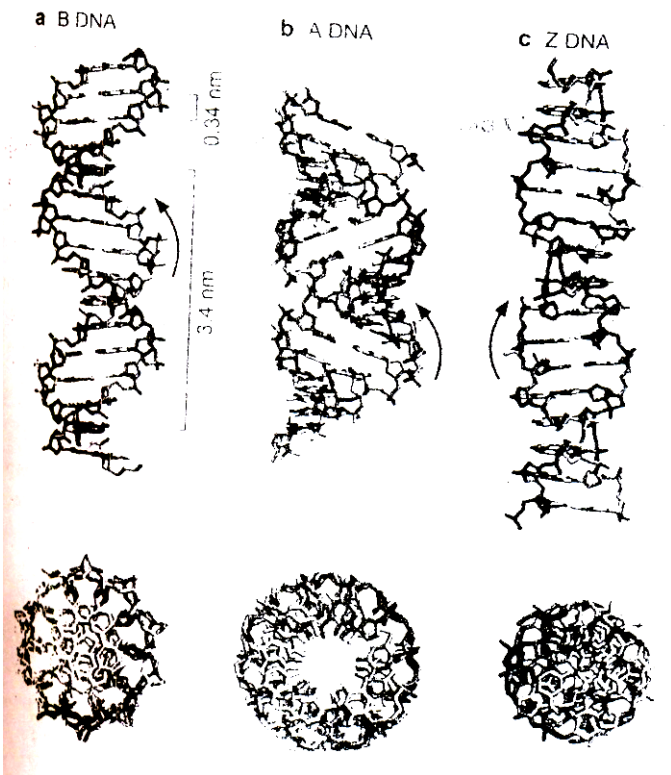


Figure: Models of the B, A, and Z forms of DNA. The sugar – phosphate backbone of each chain is on the outside in all structures (one purple and one gree) with the bases (silver) oriented inward. Side views are shown at the top, and views along the helical axis at the bottom. (a) The B form of DNA the usual form found in cells, is characterized by the helical turn every 10 base pairs. (3,4 nm); adjacent stacked base pairs are 0.34 nm apart. The major and minor grooves are also visible. (b) The more comapct A form of DNA has 11 base pairs per turn and exhibits a large tilt of the base pairs with respect to the helix axis. In addition, the A form has a central hole (bottom). This helical form is adopted by RNA – DNA and RNA – RNA helicals. (c) Z DNA is a left – handed helix and has a zigzag (hence "Z") appearance. (source: Courtesy of C keilkopf and P.B Dervan)

ii. A form DNA:

A – form DNA is the other form of DNA occurs in cells in some environmental conditions. In high concentration of salt or in dehydrated state (i.e. 66% humidity) the form of DNA exist is as A – form. It can be prepared in 'in vitro'. It has 11 nucleotide pairs and 20o away in each turn of helix. This structure is of interesting one. This is conformation DNA-RNA duplexes in 'in vitro' or RNA-RNA duplexes 'in vitro'.

iii. Z – form DNA:

The other important form of DNA is Z – form of DNA. The Z – form of DNA reported in the form of left handed helix pattern. Hence, the name Z form for zigzagged path of S-P. The A and B forms of DNA are right handed manner but the Z – form is in left handed manner. DNA containing alternative purine and pyrimidine residues can fold into left-handed as well as right-handed helices. To understand how DNA can form a left-handed helix, we need to consider the glycosidic bond connects the base to the 1′ position of 2′ –deoxyribose. This bond can be in one of two conformations called ***syn*** and ***anti***. The following figure shows the left-handed pattern of Z form of DNA. In right-handed DNA, the glycosidic bond is always in the anti conformation. In the left-handed helix, the fundamental repeating unit usually is a purine-pyrimidine dinucleotide, with the glycosidic bond in the anti conformation at pyrimindine residues and in the syn conformation at purine residues. It is this syn conformation at the purine nucleotides that is responsible for the left-handedness of the helix. The change to the syn position in the purine residues to alternating anti-syn conformations gives the backbone of left-handed DNA a zigzag look. The following figure indicates the Z form DNA structure.

ILE 6-2 A Comparison of the Structural Properties of A, B, and Z DNAs as Derived from Single-Crystal X-Ray Analysis

	Helix Type		
	A	B	Z
Overall proportions	Short and broad	Longer and thinner	Elongated and sl
Rise per base pair	2.3 Å	3.32 Å	3.8 Å
Helix-packing diameter	25.5 Å	23.7 Å	18.4 Å
Helix rotation sense	Right-handed	Right-handed	Left-handed
Base pairs per helix repeat	1	1	2
Base pairs per turn of helix	~11	~10	12
Rotation per base pair	33.6°	35.9°	−60° per 2 bp
Pitch per turn of helix	24.6 Å	33.2 Å	45.6 Å
Tilt of base normals to helix axis	+19°	−1.2°	−9°
Base-pair mean propeller twist	+18°	+16°	−0°
Helix axis location	Major groove	Through base pairs	Minor groove
Major-groove proportions	Extremely narrow and very deep	Wide and of intermediate depth	Flattened out on t surface
Minor-groove proportions	Very broad but shallow	Narrow and of intermediate depth	Extremely narrow very deep
Glycosyl-bond conformation	*anti*	*anti*	*anti* at C, *syn* at G

The conformational changes of Z – form DNA to B – form DNA is occur at lower and

higher salt concentrations with positively charged ions. This conformational changes also reported by binding of certain regulatory proteins.

The structural variations of DNA in cells play an important role in biological activities. It is not invariant. Comparison of structural properties of A, B and Z form of DNA are given in the following table (Ref: Mol Biol of the gene by Watson et al.)

2. Explain in detail on various steps and mechanism involved in replication of DNA.

DNA is a genetic material occurs in cells. It carries genetic information, which is most important for hereditary factors from parent to progeny. Nucleotide sequences of DNA in translated in to structures of protein, that performs cell function properly. Hence, it is necessary to copying of DNA during cell division and DNA replication. The cells perform various metabolic activities without deviation. Hence, there is an accurate process or mechanism is required for DNA replication.

Both in prokaryotic and eukaryotic cells, the general mechanism of DNA replication is more or less same. The method of replication is observed as semiconservative process in which the parental strand and daughter involve in construction of new DNA strand.

Replication process

New strands of DNA are synthesized by using existing strands / parental strands. Parent strands are called templates and the daughter strands are new strands complementary to parental strands. The pattern of synthesis is called semiconcervative method.

Semi conservative method:

By this method of replication, the parental strands are permanently separated and each strand paired with new daughter strand and duped (double helix) is formed. In this one strand from parent and other is newly synthesized i.e. semiconservative of parental strand for new DNA strands. Evidences were obtained to prove semiconservative method.

Mechanism

DNA polymerases are enzyme, which actively involve in DNA replication process. DNA polymerases require primers to initiate replication process. Primers are short sequenced fragments, which initiate the process of DNA synthesis.

The DNA polymerase need **primers** – a short preexisting RNA on DNA strand to begin

chain growth. The primer base paired and nucleotide added to the free hydroxyl groups at 3′ end of primer. DNA unwound and daughter strands formed at the replication fork.

Helicases are involve in initiation of the unwinding at replication origins of specific sites of DNA molecules. Origins are the initiation sites or opening sites, which is one in prokaryotic cell and multiple in eukaryotic cell. Once the heliacses unwound, DNA open and allow for synthesis of new strands.

Primases (RNA polymerase) forms short RNA primer complement to DNA template. Primers will be paired and elongated to its complementary DNA.

DNA polymerase involve in elongation of DNA molecule during synthesis after primer is nictitate the process. DNA polymerase then involve continuously in development of new daughter strands.

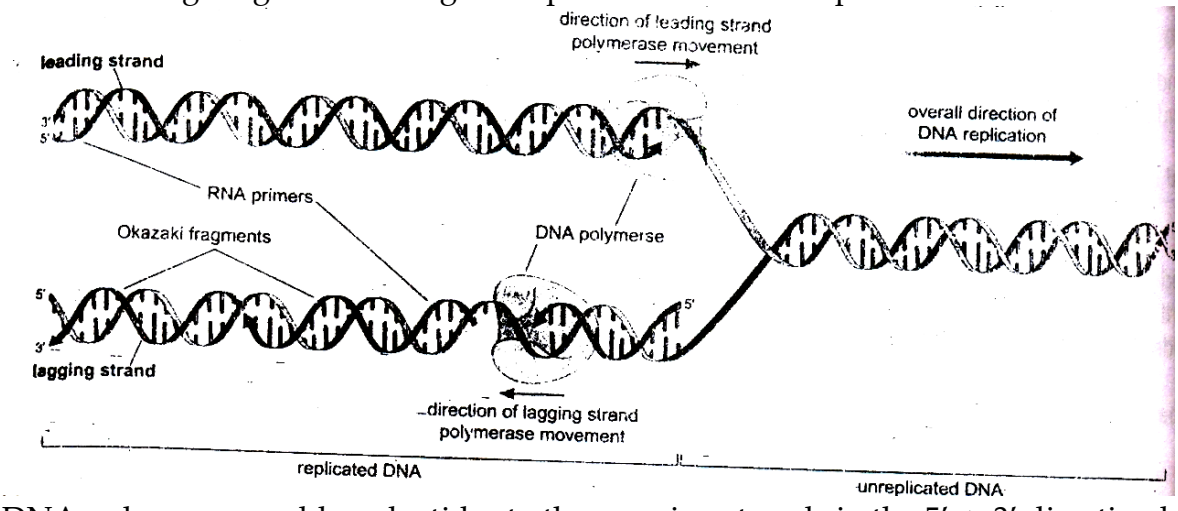
Replication fork:

Replicating fork is the region of DNA at which the initiation and synthesis of daughter DNA strands takesplace. All the protein and enzymes complex together with the DNA site involve in elongation of DNA and it is as growing fork.

Topoisomerase

Topoisomerase is the enzyme involve in DNA replication and it is involve in maintenance of the structure of unwind DNA from torsiorel strand stress. **Helicase** and Topoisomerse maintain the unwinding of coils. Here, the **gyrase** also involve in rotation of coiling.

The following diagram showing the replication fork and replication action of DNA.



DNA polymerases add nucleotides to the growing strands in the 5′ -> 3′ direction by witch leading

strand and lagging strands are produced which helps in replication.

Leading strand occurs during replication or synthesis of DNA continuously from a single RNA primer in 5′ -> 3′ direction. In the same direction of replication fork movement.

Lagging strand – The growth occur must be at 5′ -> 3′ direction of strand. But, copying from template strand occurs at opposite direction from the movement of replication fork. So, the unwound strand exposed some time before the RNA primer base paired but elongated in 5′ -> 3′ direction.

Okazaki fragments - The discontinuous short segments in the lagging strand are called okazaki fragments. The RNA primer of each Okazaki fragments is removed and replaced by DNA chain.

DNA ligase - The short fragments of DNA called okazaki fragments are joined together to form a new DNA chain is done by the enzyme DNA ligase. The DNA ligase enzyme involve in bridging of short fragments to a chain of DNA molecule.

DNA replication occurs in Birdectional

In both prokaryotes and eukaryotes, the replication of DNA is proved as bidirectional direction. In the replication fork, the origin of strand and synthesis of new strands are in 5′ - > 3′ direction, that means in opposite direction. In lagging strand, short RNA-DNA strand formed first and extended in 3′ end by polymerase alpha enzyme. By which it resulting of short 5′ RNA – 3′ DNA short strands. Replication factor C proteins (Rfc proteins) are involved in this process.

In most eukaryotes, DNA synthesized by; polymerase sigma (pol5) which takes over from pol alpha and continuous elongation in 5′ -> 3′ direction. In this process, the PCNA protein (Proliferating cell nuclease antigen) is involved.

The following diagram showing the bidirectional replication mechanism fof DNA synthesis occurs in both prokaryotes as well as eukaryotes.

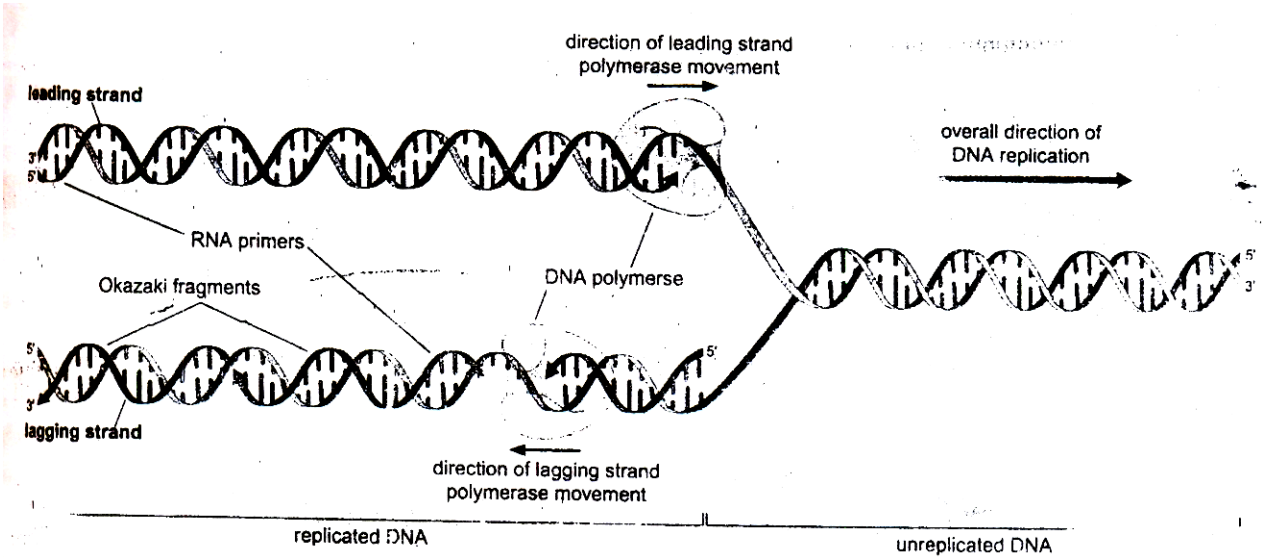


Figure. The replication fork. Newly synthesized DNA is indicated in red and RNA primers are indicated in green. The Okazaki fragments shown are artificially short for illustrative purposes. In the cell, Okazaki fragements can vary between 100 to greater than 1,000 bases.

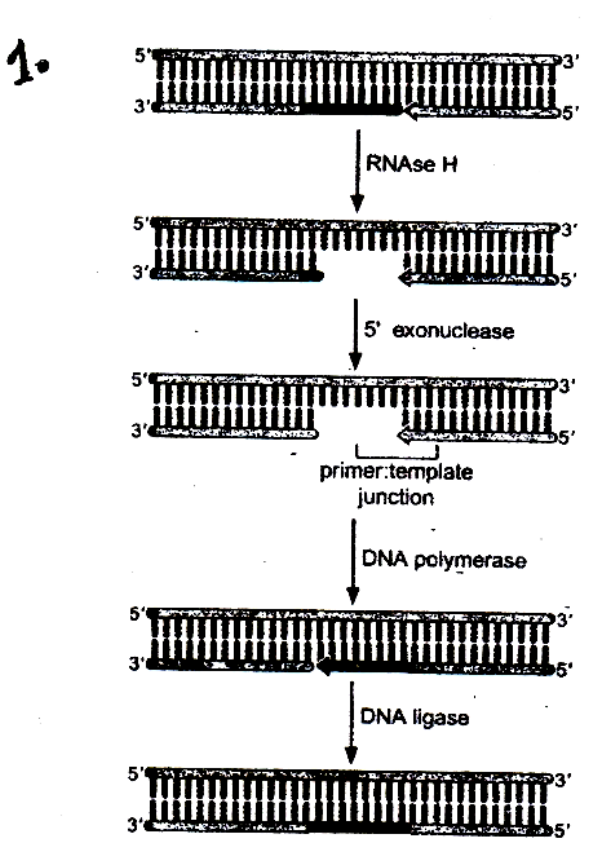
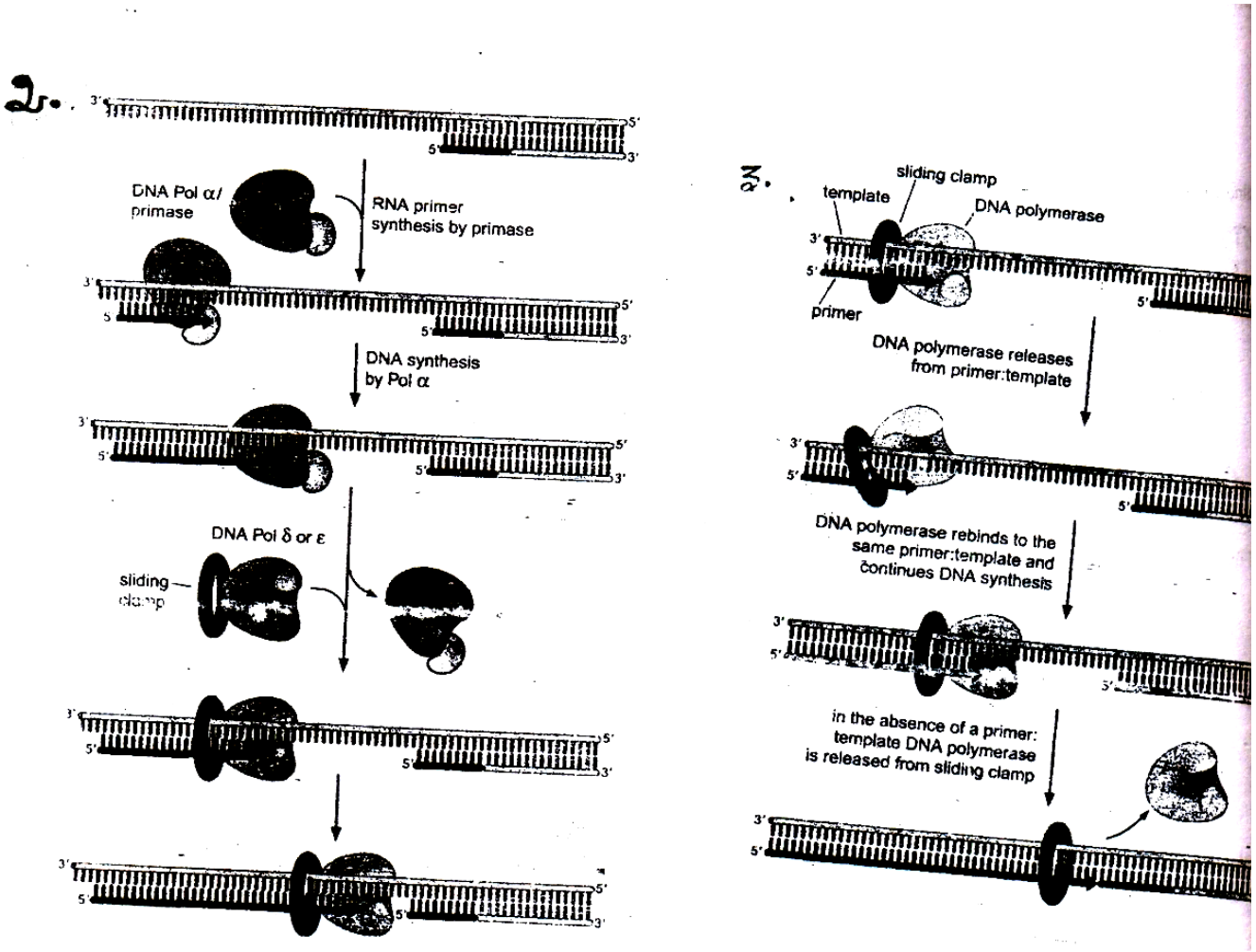


Figure 1: Removal of RNA primers from newly synthesized DNA. The sequential function of RNAse H, 5' exonuclease, DNA polymerase, and DNA ligase during the removal of RNA primers is illustrated. DNA present prior to RNA primer removal is shown in gray the RNA primer is shown in green, and the newly synthesized DNA that replaces the RNA primer is shown in red.

Figure 2: DNA polymerase switching during eukaryoic DNA replication. The order of DNA polymerase function is illustrated. The length of the DNA synthesized is shorter than in reality for illustrative purposes. Typically the combined DNA Pol α / primase product is between 50 – 100 bp and the further extension by Pol ϵ or Pol δ is between 100 and 10,000 nucleotides. Although both DNA pol δ and ϵ can substitude for DNA Pol α / primase, it is likely that they function in the replication of specific DNA strands (leading or lagging). Current studies have yet to determine whixh polymerase functions on which strand, however.

Figure 3: Sliding DNA clamps increase the processivity of associated DNA polymerases.



3. Describe different forms of RNA and their functions.

RNA is differ from DNA in three aspects as given below:

i. The backbone of RNA contain s ribose rather than 2′ –deoxyribose. That is, ribose has a hydroxyl group at the 2′ position.

ii. RNA contains uracil in place of thymine in DNA. Uracil has the same single-ringed structure as thymine, except that it lacks the 5 methyl group. Thymine is in effect 5 methyl-uracil.

iii. RNA is usually found as a single polynucleotide chain.

Except for the case of certain viruses, RNA is not the genetic material and does not need to be capable of serving as a template for its own replication. Rather, RNA functions as the intermediate, the mRNA, between the gene and the protein-synthesizing machinery. Another function of RNA. RNA found in small quantities. Based on the role of RNA, it is classified into thio groups as i. Genetic RNA and ii. Non-genetic RNA.

i. Genetic RNA:

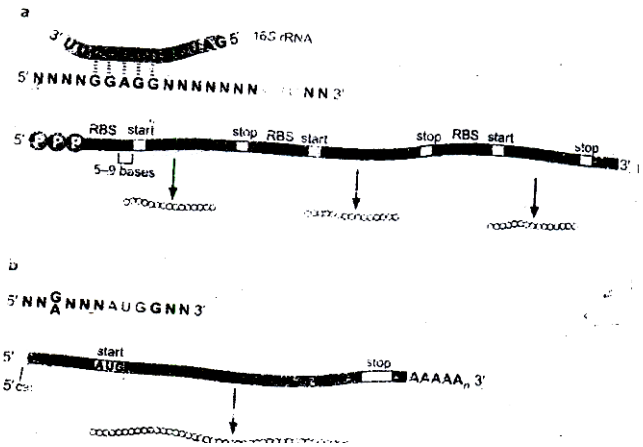
In some plants, animals and bacteriophage RNA act as genetic material. In bacteriophage, the RNA found as in different forms as ssRNA and dsRNA. In viroids or virusoids, the small RNAs, which about 350 bp is independent in function and not covered by coat proteins. Virusoides RNA are otherwise called as satellite RNA. Which is encapsulated with plant genome.

ii. Non-genetic RNA:

RNA does not have genetic role but contain genetic information, which is transmitted through DNA. These are synthesized on DNA template. The different types of RNA studied in this type are as given below:

a. mRNA (Messenger RNA)

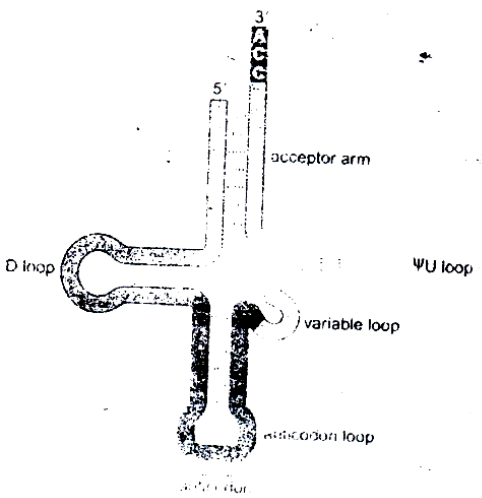
Messenger RNA is occur 5 to 10% of total RNA in cell mRNA is carries genetic information and instruction from DNA in correct order of amino acids during protein synthesis. mRNAs are short lived and rapid turn over involve in translation of proteins by accurate, stepwise assembly of amino acids. The molecular weight of mRNA form 5,00,000 to 20,00,000 daltons.



b. tRNA (Transfer RNA)

Transfer RNA (tRNA) or otherwise called as soluble RNA (sRNA). TRNA contain about 10-15% of total RNA in cell. These are smallest molecules and work as adopter molecules for carrying amino acids to site of protein synthesis. tRNA recognizes base sequences and plays major role in protein synthesis. This is about 74-95 base pairs present in on tRNA molecules. In its structure 'G' residue present in 5' terminal and unpaired 'CCA' sequence at the 3' end in all tRNAs. The structure of t RNA is explained in the following figure.

FIGURE 14-4 Cloverleaf representation of the secondary structure of tRNA. In this representation of a tRNA, the base-pairing between different parts of the tRNA are indicated by the dotted red lines.



c. rRNA (Ribosomal RNA)

Ribosomal RNA (rRNA) is most stable form of RNA, which is associated with ribosomes. It contain about 40-60% of the cell and 80% of the total RNA in the cell. This kind of RNA involve in catalyze the assembly of amino acids into polypeptides. This also binds with tRNA and other accessory proteins for protein synthesis. All ribososmes contain rRNA molecules. The structure of the rRNA is presented below:

Other forms of RNA

Other forms of RNA also reported in the cell, which is given below;

(i) Antisense RNA – It involve in inhibiting complementary RNA.
(ii) Hn RNA – It is synthesized from split genes in eukaryotes.
(iii) Small nuclear RNA (SnRNA) – Found in nucleus and used for formation of spliceosome.
(iv) Small nucleolar RNA (SnoRNA) –Found in nucleolus.
(v) Small cytoplasmic RNA (ScRNA) – It is found in cytoplasm.
(vi) Guide RNA (gRNA) – it is useful in synthesis for minicircle mitochondrial DNA.

4. Write in detail about the role of DNA polymerases involved in replication of DNA.

DNA polymerase is the polymerizing enzymes, which involve in synthesis of DNA strands. Arthur Kornberg in 1957, invented the polymerase I from the extracts of E.coli. DNA polymerase able to synthesize DNA from four precursor molecules. The four precursor molecules namely the four deoxynucleoside 5′ –triphosphates (dNTP), dATP, dGTP, dCTP, and dTTP sd s DNA molecule to be copied is provided. It is occur in both prokaryotes and eukaryotic organisms.

The DNA polymerases of prokaryotic and eukaryotic cell are slightly different, but the functions are same.

I. Prokaryotic DNA polymerase:

In prokaryotic DNA replication process, there are 3 kinds of DNA polymerases are reported. Following text deals about the prokaryotic DNA polymerases.

i. Polymerase I
ii. Polymerase II and
iii. Polymerase III

The DNA pol I and pol II are involve in DNA repair mechanism. The Pol III polymerase is involve in DNA replication.

Polymerase I

DNA polymerase I contain 5 active sites. Which are

ii. Template site
iii. Primary site
iv. 5′ – 3′ cleavage site
v. Nucleoside triphosphate site
vi. 3′ – 5′ cleavate site.

The enzyme moves along with okazaki fragment. This involve in removal of mispaired base pairs is called proof reading.

Pol I consist of 2 fragments as

i. Kelenow fragment which is about 38 KD which consist of 3′ – 5′ endonuclease activity with 5′->3′ polymerizing activity.

ii. Smaller fragment (35 KD) contain 5′ – 3′ nuclease activity.

Pol I involve in nicked DNA in vitro called nick translation. Pol I synthesized by gene Pol A of E.coli.

Polymerase II

Pol II involved in DNA repair mechanism. Pol II resembles like Pol I in its activity which bring growth of 5′ → 3′ direction using free 3′ – OH groups. This can not be used for nicked duplexes.

Polymerase III

Pol III is a holoenzymes which is essential in DNA replication. Pol III contain 10 units with 4 major components as

i. core enzymes contain α, β, θ each one.

ii. Sliding clamp consist of dimer (β2) binds DNA in an ATP reaction catalysed by 'γ' complex.

iii. γ complex consist of 6 polypeptidase with 5 sub units. (γ2,δ1, δ1, χ1, Y1). It is also called as a match maker.

iv. γ sub unit (refer as dimmer T2). The preinitiation complex is formed by binding of enzyme with ATP. An initiation complex is formed in an ATP independent reaction.

II. Eukaryotic DNA polymerases

In the eukaryotic DNA polymerase, there are 5 kinds of polymerases involved in DNA replication. They are detailed below.

i. DNA Polymerase α

This is high molecular weight proteins involved replication. This occurs in cytoplasmic area and this is large polymerase. This enzyme is believed to be involved in long time process as for eukaryotic DNA replication.

II. DNA Polymerase β

This is small polymerase, which has low molecular weight proteins. This is available in nucleus, hence it is nuclear polymerase.

III. DNA Polymerase γ

This is mitochondrial polymerase involved in replication. This enzyme is encoded in nucleus.

IV. DNA Polymerase δ

This is noval enzyme, which is depending on PCNA (proliferating cell nuclear antigen processivity) for DNA synthesis processivity.

V. DNA Polymerase ε

This is DNA Polymerase δ II. This also involve in synthesis of DNA, which is independent to PCNA.

5. Explain in detail on replication of Eukaryotic DNA with suitable diagrams.

The replication of DNA in eukaryotic cells is limited to 'S' phase of cell cycle. In synthesis the DNA replication and Histone proteins synthesis are duplicated. In eukaryotes, multiple origins of replication sites occur up to several thousand basepairs i.e. 10^3 to 10^5 and the replication events in each cell cycle in a coordinated manner.

Mechanism of synthesis

In eukaryotic cells there are two different DNA polymerases required as given below:

i. DNA polymerase α and
ii. DNA polymerase δ

DNA pol sigma synthesis of leading strand and DNA pol alpha synthesis lagging strand. It is believed to be 8 components are involved in DNA replication. Those components as given below:

i. T-antigen
ii. Replication protein A or RP-A or Replication factor (RFA) or eukaryotic SSB protein
iii. Topoisomerase I
iv. Topoisomerase II
v. DNA polymerase α (pol α), which is associated with primase and complex, formed.
vi. DNA polymerase δ (pol δ)
vii. Proliferating cell nuclear antigen (PCNA) known as cyclin
viii. Replication factor C (RF-C) or (ATPse)

T-Antigen is SV 40 encoded. Others are from mammalian cells.

Tag has DNA binding domain have ATPase and helicase activities 3 sites are present Tag as

1. Imperfect invert repeat
2. Site II of 27 bp
3. Site I – 17 bp, which A-T rich sequence.

DNA replication

1. Presynthesis stage of 8-10 mins duration in which formation unwound DNA DNA complex. It requires 3 proteins as I) T-antigen ii) RP – A and iii) Topoisomerase I & II.

Multisubunit complex forms with ATP in site I & II. RP-A, Topoisomerase, DNA helicase and extension of unwinding of DNA. The topoisomerase help in unwinding of DNA by altering topology of DNA and replication fork. Here RP-A & SSB binds on unwound ssDNA.

2. The primase – pol α complex undertakes primer synthesis (RNA primer). RNA primer synthesis and

3. DNA polymerase α extend into the synthesis of short DNA sequence (3 – 4 bp long) i.e. called iDNA from RNA primers.

4. RF – C binds 3′ end of iDNA and lead DNA pol δ and fPCNA. PCNA is a cyclin works as a processivity factor for polymerase δ. Therefore it resemble sliding clamp in E.coli. RF-C and pCNA switching of DNA polymerases. So, polymerae α replaced by polymerase δ. This precedes the continuous synthesis of leading and lagging strands. At one time DNA pol α synthesis lagging strand and DNA pol δ synthesis leading strand. So, it is observed that DNA pol α initiates DNA replication. Polymerase δ elongates DNA replication.

5. Another I DNA molecule is synthesis by RNA primer by pol α primase complex and the steps is repeated again and again till the completion of replication of entire DNA. One pol α involve in synthesis of one okazaki fragment, two pol δ involve in elongation of synthesis by produce one for lagging strand and one leading strand.

6. RNA primers are removed after completion of replication by MF1 (5′-3′ exonuclease) and gaps are filled by DNA polymerase δ as in E.coli polymerase I. DNA ligase seals the sticky ends. This switching of DNA polymerase αand DNA polymerase δ involved in all eukaryotic DNA replication. In another study, i.e. in yeast other one DNA polymerse called DNA polymerase ε also found. εyeast the polymerase α involve in initiation and polymerase ε and δ involve in elongation of replication as synthesis of lagging and leading strands.

6. Write the rolling circle model replication of chromosomes.

Rolling circle replication is a replication mechanism occurs in phage DNA and during bacterial mating.

In phage DNA

The circular DNA gives rise to linear daughter molecules in which the base sequences repeated numerous forming a concatemer. Concatemers are necessary for phage production. Concatemers are consisting of repetitive unit of sequences.

In bacterial mating

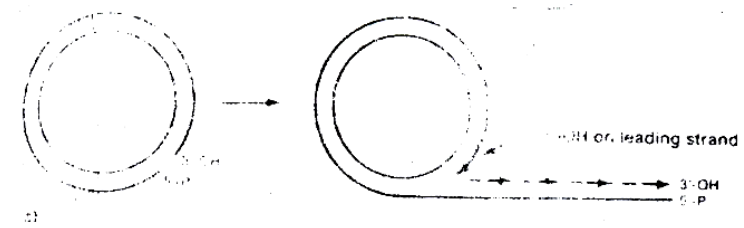
The linear DNA molecule is transformed in a recipient cell for replication present.

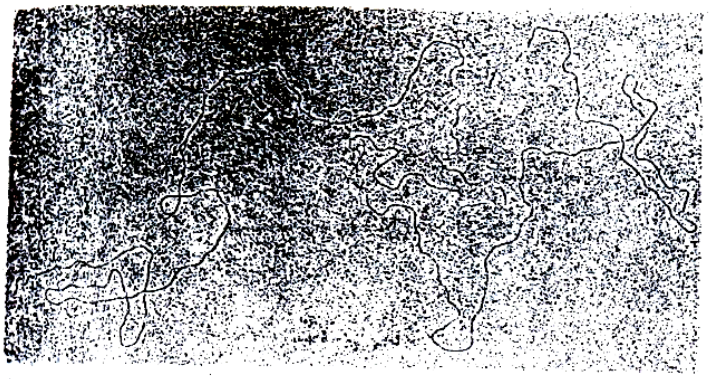
In both the above are, given the initiation of covalent extension, this gives rise to a replications made known as rolling circle replication.

Mechanism of rolling model replication

In a circular duplex, during initiation process, nick is made having 3′ –OH and 5′ –P termini by helicase and ssb protein and replication fork generated. Synthesis of nucleotide and elongation of leading strand occur from 3′ –Oh group termini and displaces to 5′ –P termini. The enzyme polymerase III holoenzymes is involved. At the same time. The parental strand for lagging strand is displaced due to presence of the reaction between, helicase, SSB protein and pol III holoenzyme. The displaced parental strand involve in replication process in a usual way by means of precursor fragments. The strand elongation is copied in 5′ → 3′ direction and roll out as a free tail. These tails become double strand and give circular DNA. It also called as sigma replication, because look like Greek letter **'δ'.**

The following diagram explain the rolling circle replication.





(b)

Figure 9-33 (a) Rolling circle or σ replication. Newly synthesized DNA is shown in ——→ (b) An electron micrograph of a rolling circle of phage λ DNA isolated from infected *E. coli*. The thin regions are partially denatured, as described in Figures 9-36 and -37, and served as references in the original experiment (*Proc. Nat. Acad. Sci.* (1975) 70: 1768). [Courtesy of Ross Inman.]

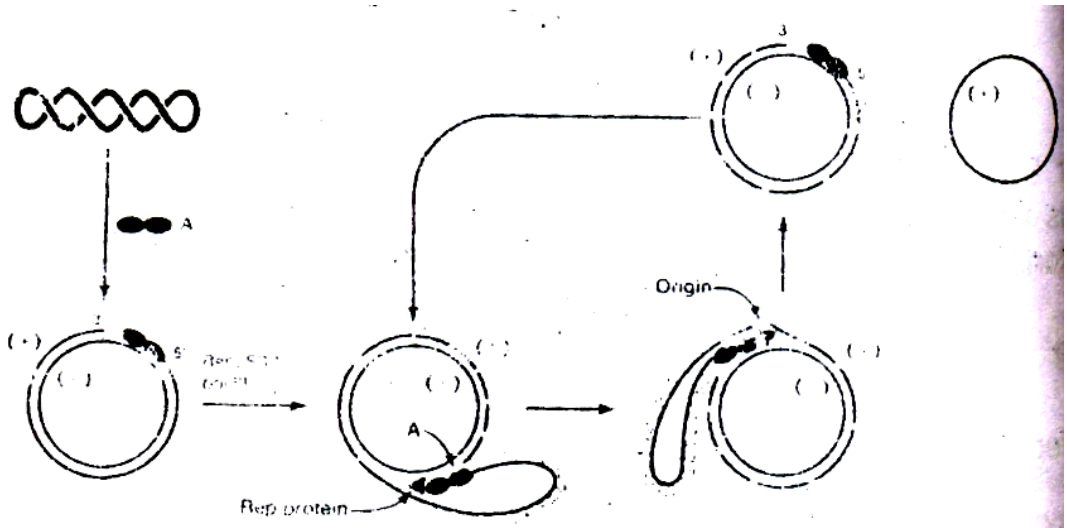


Figure 9-34 A diagram of looped rolling circle replication of phage ϕX174. The gene-A protein nicks a supercoil and binds to the 5' terminus of a strand (known as the + strand) whose base sequence is the same as that of the DNA in the phage particle. Rolling circle replication ensues to generate a daughter strand (———) and a displaced + single strand that is coated with SSB protein and still covalently linked to the A protein. When the entire + strand is displaced cleaved from the daughter + strand catalyzed by the nicking activity of the protein. The cycle is ready to begin. Note that the — strand is never clea

There are 4 significant feature of rolling circle mechanism.

i. The leading strand is covalently linked to the parental template for the lagging strand.
ii. The linear branch has free 5′ -P termini, before precursor fragment synthesis begins.
iii. The rolling circle replication continues unabated, generating a concatemeric branch.
iv. The circular template leading strand synthesis never leaves the circular part of the molecule.

A variant of the rolling circle model called looped rolling circle replication, is used to generate a progeny of single stranded circle form as double stranded circular template.

e.g. phage φ X 174, gene A protein makes nicks on ds DNA circle i.e. covalently linked with 5′-P terminus. Replicon, SSP proteins and pol III holozyme are used in replication and chain growth from 3-OH group and displace the broken parental strand.

7. Elaborate the formation of D-loops with suitable diagrams.

D loops are otherwise displacement loops that are occur in circular DNA molecule. In de novo initiation, synthesis of leading strand before lagging strand occurs. Before the synthesis of the first precursor fragment beings, a replication bubbles exists. This bubble consist one ds branch, which is made up of one parental strand paired with leading strand. Here, one single stranded branch, which is unreplicated parental strand when the bubble is called a 'D', loop i.e. displacement loop. The following diagram shows the D loop structure in circular DNA.

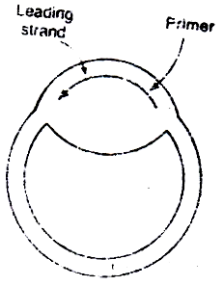


Figure: Circular DNA molecule with a D loop.

This D loop is temporary or transient until synthesis of precursor of fragment. The precursor require to synthesis leading strand for release of specific sequences (single stranded form), which can be used for prepriming. In some circumstances, when the replication system doesn't employ DNA gyrase, a 'D' loop may be long lived.

In the circular DNA

In the initial stages of replication, advance of replication fork doesn't require DNA gyrase because such circular DNA initially is negatively super coiled and negative twists componsate positive turns introduced by movement of the fork. E.g. mouse mitochondrial DNA.

Once the fork is completed, the negative twists are used up, a topological constraint relieving system, as gyrase is needed. The superhelix density is 0.05% in all DNA molecule i.e. if the positive superhelicity is forbidden, the leading strand move 5% distance along circular, negative superhelical strand before gyrase is needed. The replication complex not move along ds of movement produce one or two turns of over winding. If neither gyrase nor any other system is present, replication would be ceased and unreplicated portion lost its supercoiling. The molecular

coformation bubble would depend on the first precursor fragment had been initiated. If it not, the 'D' loops would result.

The following diagram shows the occurrence of D loops in mouse mitochondrial DNA.

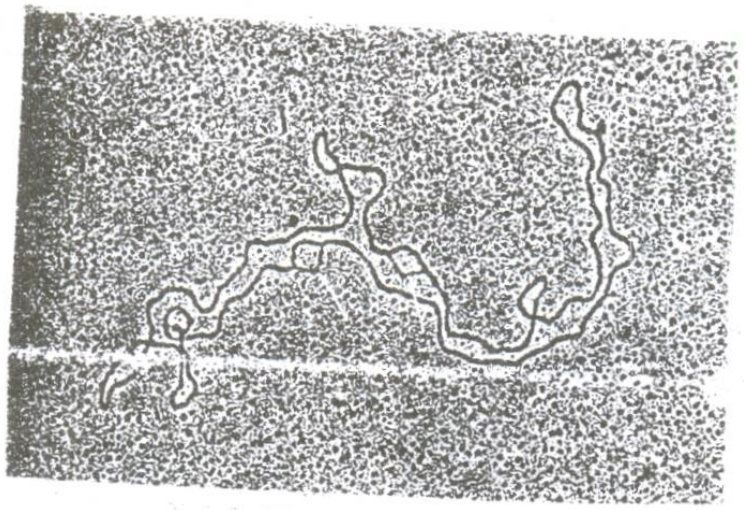


Figure: An electron micrograph of a dimer of mouse mitochondrial DNA showing diametrically opposing D loops (arrows). The single strand in each D loop appears thinner than the double – stranded DNA. The total length of the molecule is 10μm. (Courtesy of David Clayton).

The ssDNA of loop thinner than dsDNA of circular form. In circular DNA like mouse mitochondrial DNA about 4% of DNA (leading strand) is replicative and 70% of all replicating mitochondrial DNA molecules with D loops, which indicates the replication has ceased at the point.

These assumes that, either DNA gyrase is highly inefficient, or a simple nicking –sealing system is present. The system does not work continuously; it may be active in response to slightly over wound DNA.

8. Write the structural organization of eukaryotic DNA.

Eukaryotic chromosomes are more complex than prokaryotic chromosome. Eukaryotic chromosome contain much more genetic informations. That is two to ten times as many genes as E.coli, but have orders of magnitude more DNA. In eukaryotes, DNA is not a single unit like prokaryotes, but as many as units called chromosomes. The number is always instant in every species. The DNA is packaged in several chromosomes. Each chromosome is two or more copies i.e diploid or polyploidy.

The continuous length of E.coli chromosome is 1100 nm. The haploid chromosome complement or genome of human contain 1000 nm of DNA or about 2000 nm per 2n cell. Each chromosome contain 15 to 18 mm of DNA.

Eukaryotes are diploid and each c ell one set of chromosomes from maternal and paternal. The number of chromosome in dual set is called diploid number (2n). The sperm and ovum contain half a number is called haploid (n) cells.

Mophology:

Eukaryoteic chromosome differ from prokaryotes in morphology, chemical composition and molecular structure.

The shape also varies in phase to phase of cell growth and cell division.

Eukaryotic chromosomes are thin, coiled, elastic, contactile, anastomose, thread like structures during interphase. Chromotin threads look like compact, stable mass often called chromatin substance or material.

In metaphase stage of mitosis and prophase of meiosis, the chromosome though become coiled and folded to form compact and individually distinct ribbon shaped chromosme. This clear zone is called kinetocore or centromere along their length. Number and position of centromere is variable, but defineite in chromosome of all cells and individual of species. Centromere has small granules or spherules and divides the chromatin in to two or more equal or unequal arms i.e. chromosome arms.

According to position of chromosome, it may be rod shaped (telocentric or acrocentric), J shaped (sub metacentric) and V shaped (metacentric).

Centromere during cell division, move towards the opposite poles of cell and spindles and microtubules are attached with it. Chromosome also bears terminal unipolar segments called telomeres. Some chromosome contain additional segment nucleolus organises, which is associated with the nucleolus.

According to cassified cytological studies, chromosome structurally consist limiting membrane called pellicle, an amorphous matrix and two very thin highly coiled filaments called chromonema or chromonemata.

Each chromonemata is 800oA thick and contain 8 microfibrils and each of which contain two double helix of DNA. Both chromonemata intimately coiled in spiral manner each other and have bead like swelling along it length called chromomeres. Chromomeres are the region of superimposed coils. The following figure gives the structure of eukaryotic chromosome.

Chemical structure

Normally the light microscope is not sufficient to view for the structure of chromosomes of interphase chromosomes. EM, X-ray diffraction and chemical analysis showed some information. The structure gives the following details.

i. Deoxyribonucleic acid (DNA) – primarily
ii. Protein
iii. RNA – less amounts
iv. Certain metallic ions.

The proteins are two major classes as;

i. The histones or basic proteins, which are positively charged at neutral pH.
ii. Heterogenous (non-histones) – largely acidic which are negatively charged and neutral pH.

Histones play a major role in chromatin and they present equal to DNA. The histones are 5 different types as H1, H2, H3, H4 and H5 which are present almost in all the cells. In sperm cells, other class of small basic proteins called protamines replaces histones. Histones are specifically compiled with DNA to produce basic structure subunits of chromatin, small ellipsoidal beads called nucleosomes.

Some non-histone proteins have enzymatic activities, phosphoproteins, DNA polymerase, RNA polymerase, and DPN pyrophosphorylase. The metal ions as Ca+ and Mg+ are present to maintain the chromosome organsiation intacts.

Molecualr structure

Each eukaryotic chromosme is composed of single, greatly elongated and highly folded nucleoprotein fiber of 100oA thick. Nucleoprotein composed of single, linear, double standard DNA that wrapped with equal amounts of histone and non-histone proteins. This is folded fiber model. The following diagrams show the structure of chromosomes highly folded in nature.

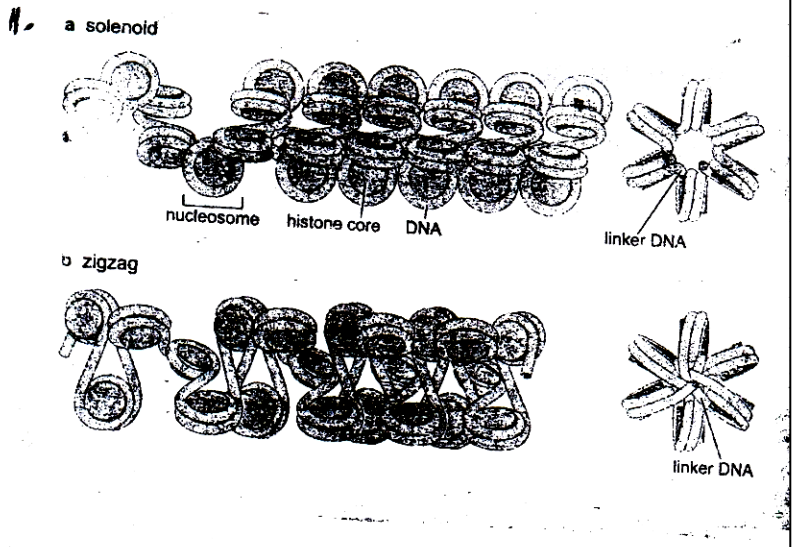
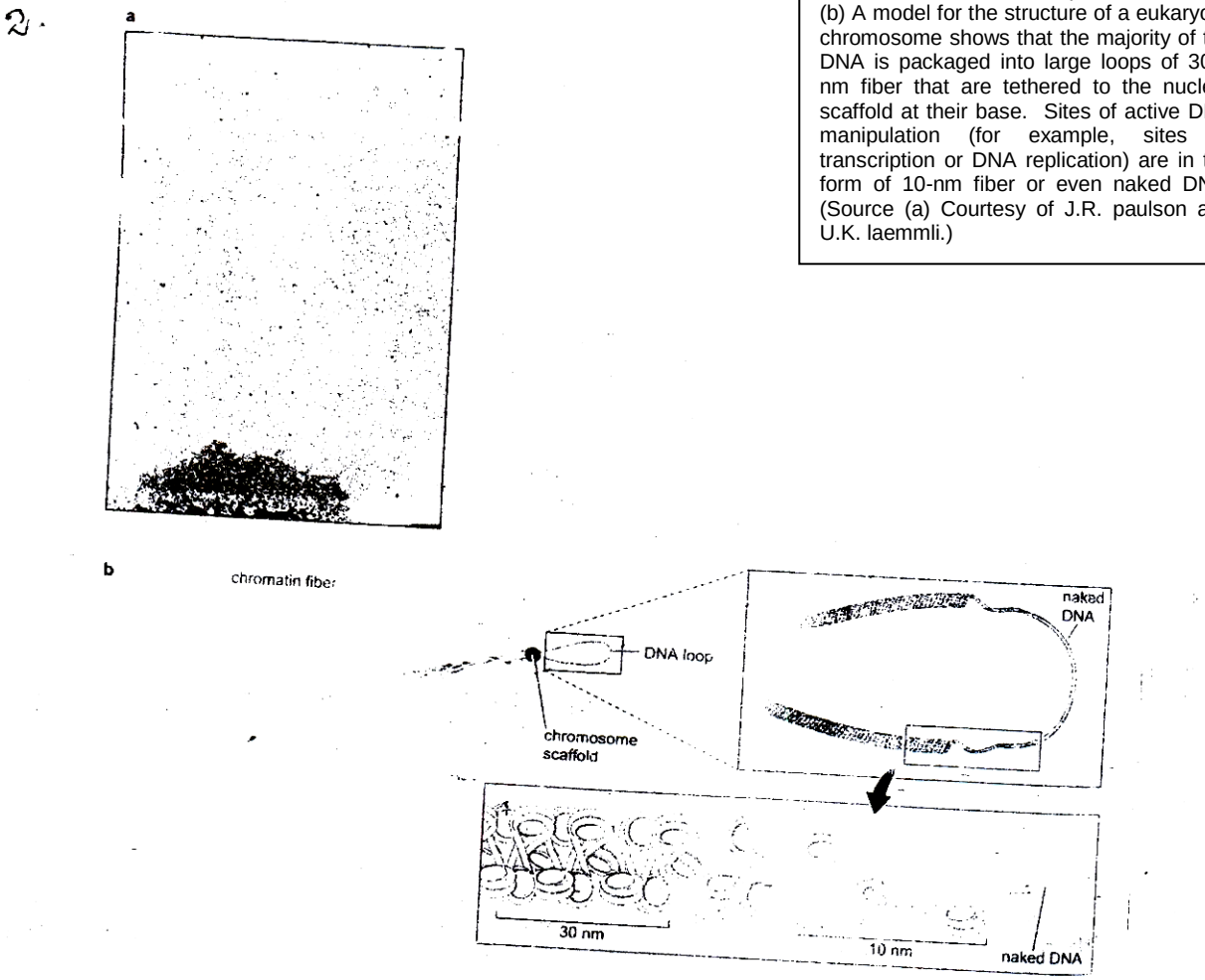


Figure 1: Two models for the 30 – nm chromatin fiber. (a) The solenoid model. Note that the linker DNA does not pass through the central axis of the superhelix and that the sides and entry and exit points of the nucleosomes are relatively inaccessible. (b) The "zigzag" model. In this mode, the linker DNA frequently passes through the central axis of the fiber and the sides and even the entry and exit points are more accessible. (Source: Pollard T. and Earnshaw W.2002. Cell biology, 1st Edition, p.202, f13 – 6 Copyright © 2002. Reproduced by permission of W.B. Saunders Inc)

Figure 2: The higher – order structure of chromatin: (a) A transmission electron micrograph shows chromatin emerging from a central structure of a chromosome. The electron – dense regions are the nuclear scaffold that acts to organize the large amounts of DNA found in eukaryotic chromosomes. The bar represents 200 nm. (b) A model for the structure of a eukaryotic chromosome shows that the majority of the DNA is packaged into large loops of 30 – nm fiber that are tethered to the nuclear scaffold at their base. Sites of active DNA manipulation (for example, sites of transcription or DNA replication) are in the form of 10-nm fiber or even naked DNA. (Source (a) Courtesy of J.R. paulson and U.K. laemmli.)



Non-histone proteins are believed o be more role in regulation of expression of genes or sets of genes.

The eukaryotic chromosomes are broadly classified into 2 types as i. Euchromatin and ii. Heterochromatin.

i. Euchroamtins are stained lightly by feulgen staining . t composed of less tightly packed forms the major portion of chromosomes. Euchromatin are genetically more active. This DNA molecule involve in RNA synthesis.
ii. Heterochromatin are highly condensed inter coiled state of chromatin. These are genetically inactive. Not synthesis of RNA molecules. This is 2 or 3 times more DNA than euchromatin.

Eukaryotic chromosomes are classified into i. Autosomes and ii. Sex chromoses based the determination of phenotypic and genotypic expression.

9. Discuss in detail on structural conformation of DNA.

The stable or native structure of DNA is stabilized by many physiological or structural factors. Otherwise the stable DNA will get fluctuate and denaturation or structural disturbances occur due to various factors like temperature, pH and ionic concentration of the external medium. Various factors responsible for the structural stability of DNA will be discussed in detail in the following text.

Evidence for H bonds in DNA

Variation of melting temperature (Tm) with different base composition was observed. i.e. with G+C and A+T pairs. E.g. in bacteria G+C is 20% to 80%. So, the Tm also were plotted against the above different G+C samples. i.e. G.C pair is 3 H bond and A.T pair is with 2 H bond. So, higher temperature required for G.C pair than A.T pair.

Evidence of Hydrophobic interaction

Any reagent would enhance either weakly soluble substances with water or distrupt the water shall and weaken the hydrophobic interactions.

The first is methanol which increases the solubility of the bases. The second is sodium trifluoroacetate. The addition of both the above reduce the Tm enormously suggests the hydrophobic interactions also important in stability of DNA. By experiments it was concluded that as, 3 dimensional the highly water-soluble 'P' group is outside, bases are less constant with water surface.

Base stacking

The base stacking was studied by optical rotatory dispersion (ORD) and circular dichroison (CD) applied to ssDNA and RNA. This showed large fraction of the bases is ss polynucleotides arranged in helix. A parallel arrangement of bases also detected. This further concluded as

i. Base stacking in elimination by reagents addition weak hydrogen bonds.
ii. If a DNA is heated, base stacking is refaced and the absorbance at 260 is increased.
iii. Reagents break H bonds has effect on base stacking of dsDNA but no ssDNA. This concludes, the base stacking is dsDNA is more than ssDNA.

Co-operativity of base stacking

The orientation of bases paring also play in role of base stacking. So, the orientation and hydrogen bonding are co-operatively forming the stable structure. If one is eliminated, other gets weakened. That is why melting temperature markedly differs after addition of reagents.

Ionic concentration – effect of DNA

Intrastand electrostatic repulsion between PO4-. This strong force drive strands apart if the changes are not neutralilzed.

i. Tm decreases as salt concentration decreases indeed DNA denatures at room temperature in distilled water. i.e. in the absence of salt solution, the DNA strand repel. Addition of salt (Na+) change around PO4- and shield the phosphates. That is at 0.2 M.

ii. Second effect is positive (+) ions are bound by DNA. The molecular weight of DNA measured in NaCl and CsCl are varied and the ration is 0.75.

Fluctuation of structure of DNA

In the presence of formaldehyde react with NH2 group of bases and eliminate their hydrogen bond. This is slow and irreversible denaturation breaking and deforming of H bonds. Similar is triturated water (H2O). Rapid exchange of H bond protons and 3H+ in the water.

These indicate, the DNA is a dynamic structure in which ds region frequently open to become single stranded bubbles. The phenomenon is called breathing. This enable the specialized protein interact with DNA and to read its encoded information. Breathing occurs only in A.T pairs. i.e. two hydrogen bonds.

10. Give an comparative account on DNA denaturation and renaturation.

At elevated temperature, the three dimensional structures of DNA are disrupted. In the disrupted state, the molecules are in random coil conformation, which is said to be denatured. But the original state without any disruption is called native or native state. The transition from native state of DNA to disrupted state is called denaturation. When heating of the DNA molecules, the bonding forces are distrupted and the denatured DNA is single stranded DNA. The structure and stabilization interactions are obtained by denaturation are studied by UV absorption spectrum. Reagents involve are either breakdown H-bonds or weakened the hydrophobic interactions are powerful denaturants. The study of various concentration of denaturants at constant temperature is based on the DNA solution absorbs the UV light at 260 nm strongly.

Denaturation and strand separation

Changes in the physical properties of DNA that accompany denaturation .e.g. decrease in viscosity and rotate polarized light indicate when hydrogen and hydrogen interactions are eliminated. The helical structure is disrupted and the rigidity is lost. This collapse the ordered structure. Once it was thought, the long DNA strand are impossible to separate, but several evidence, indicate it is possible.e.g. CsCl solution. Ss DNA absorb CS and density is increased. Before denaturation the dsDNA is single band. After the denaturation it shows 2 bands. The density ;of ssDNA is more with Cs than native dsDNA. The following graph showing the absorption of UV light before and after denaturation.

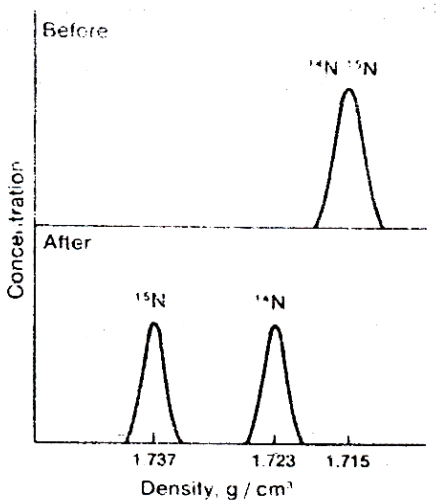


Figure 4-10 Demonstration of strand separation by equilibrium centrifugation in CsCl. Before denaturation, [$^{14}N^{15}N$]DNA gives a single band. After denaturation two bands result: the density of the [^{14}N]DNA is greater than that of [$^{14}N^{15}N$]DNA because the density of single-stranded DNA is greater than that of double-stranded DNA by 0.014 g/cm^3, even if the isotopic composition is the same.

Factors cause denaturation

i. Helix-destablishing proteins – Many proteins unwind a DNA helix are called Helix destabilizing protein or melting proteins. E.g. T4 32 proteins i.e. 32 gene of T4 phage.

ii. Denaturation in Alkali – Alkali is an other method of denaturation of DNA. DNA also denatured by alkali action by raising of pH 11.3 of DNA solution in which all H bands are eliminated. The DNA is completely denatured. These procedures are method of choice wherever it is necessary for application.

Structure of Denatured DNA

Denaturation of DNA complete above 90oC temperature. Mostly A260 at UV is increase 37% and the solution consist only ssDNA. When the bases are unstacked. If the ssDNA kept at low salt concentration it remain ss strands. If the salt concentration increased above 0.05Mand above reformation occurs. After denaturation, the DNA kept cooled and in low salt i.e. less than 0.01M the DNA are single strands.
After cooling in high salt concentraion as > 0.05M, the value of A260 not significant increase. It reforms the H bonds reformation.

Renaturation

The reformation of ssDNA, which is otherwise, denatured DNA to formation dsDNA by any factor is called renaturatoin or reannealing. The reformed DNA is called a renatured DNA.

The renaturation is very important in molecular biology and the tool is significant for study the relatedness of DNA bases and base sequences of RNA.

Methods involve in renaturation

i. High salt concentraion as 0.15 to 0.5 M NaCl influence the renaturation process.
ii. Optimal temperature as 20 – 25oC, it should below the melting temperature values.

Renaturation is slow process and it may take several hours.

Mechanism of Renaturation process

1. The repeated short sequences (4 to 6 bases) are provide random base pairing.
2. As soon as two or few sequences are paired.
3. The entire strand reformed or paird with in few strands.
4. This is by random manner.
5. The ss DNA are freely mix and strands join at random and the result of joining occur random manner. I.e hybridization.

Renaturation rate

Renaturation (R) is collision and the reaction rate or the R rate obeys law of mass action and increases with DNA concentration. The concentration dependance is used to determine DNA properties in eukaryotes and prokaryotes. This concentration values are otherwise called Cot values. Cot values are the values to measure the formation rate or renaturation or reassociation of double stranded DNA from ssDNA.

Based on concentration of DNA in solution © respect time (t) of reaction. Otherwise the measure of renaturation kinetics. This done based on measure of OD of DNA solution at 260 nm i.e. A260.

The renaturation f any DNA can be described by the rate constant K (in units as, nucleotide moles/ lit/second) or it reciprocal as Cot1/2 (which as nucleotides mole A sec/lit). Therefore, the re association depends on concentration (Co) and time of incubation (t) i.e. Cot.

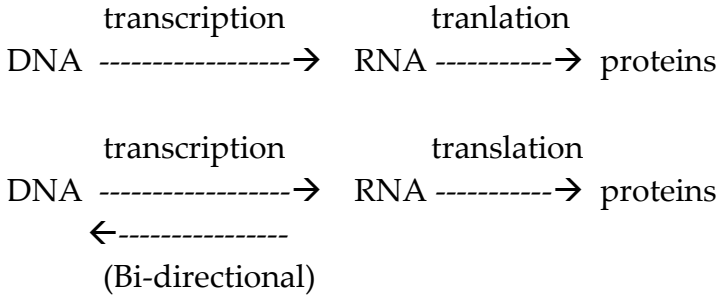
A higher Cot ½ value – Slower reaction and lower reassociation dsDNA. A lower Cot1/2 – higher reaction and higher reassociation of ds DNA.

UNIT – III

PART – A

1. Describe central dogma.

DNA is a genetic material, which contain the entire genetic information of the organism or cell. The information of genetic material for biosynthesis of proteins is stored in polynucleotides of DNA, which are called sequences of bases. The sequences are bases of nucleotides determine the sequence of amino acid polypeptide, which is known as Central Dogma.



2. Define transcription process.

Flow of genetic information from DNA to the messenger RNA (mRNA) to express the genome for biosynthesis of proteins. The transcription process is otherwise called as synthesis of mRNA from DNA template either of the DNA strand by using enzyme RNA polymerase.

3. Write short notes on prokaryotic RNA polymerases.

RNA polymerase is the single enzyme present in the nucleus which is responsible for the synthesis of all kinds of RNAs like mRNA, tRNA and rRNA. RNA polymerase present in prokaryotic as well as eukaryotic cells is slightly differing in their structure and has many subunits. This cluster of enzyme is responsible for different functions during the mRNA synthesis or transcription process.

4. Mention briefly on sigma (δ) factor.

The sigma subunits present in the RNA polymerase of prokaryotic cell helps in recognition of start signals during mRNA synthesis. The sigma subunits are otherwise called sigma factors, which directs RNA polymerase in selecting the initiation sites. Once the RNA synthesis initiated, the sigma factors dissociate from the DNA and combine in the next transcription cycle.

5. Write on promoter and terminator region.

The promoter region or site is the special locations of DNA where the special region contain specific nucleotides are present in which the RNA polymerase binds for initiation of transcription process. This is otherwise called promoter site.

The terminator region is the special site of DNA, which contain specific nucleotide sequences, which are responsible for terminating or stop the transcription process. This is otherwise called terminator region.

6. Write short notes on prokaryotic promoter.

The RNA polymerase binding site of the prokaryotic DNA region is called promoter region or promoter site. This region contain 41 – 44 bp in E.coli.

Promoter is the start site, which contains 90% purine bases. The upstream from the promoter site is a 6 bp region described as TATAAT sequence or TATAAT box. This is otherwise called as pribnow box. This lies 10 bp region as – 10 sequence. I.e. –18 to –12 region. Another sequence is TTGACA is lying –35 sequences on upstream is called recognition region. The typical prokaryotic DNA use –35 and –10 sequences for transcription.

7. Describe pribnow box.

The pribnow box is otherwise described as the promoter region of DNA. The upstream from the promoter site is a 6 bp region described as TATAAT sequence or TATAAT box. This is otherwise called as pribnow box. This lies 10 bp region as – 10 sequence. I.e. –18 to –12 region.

8. Where is consensus TATA sequence seen? Write the significance of it.

The consensus TATA sequence is seen in the promoter region of prokaryotic DNA. This is more helpful in recognition of RNA polymerase to bind for transcription process. This consensus sequences occur at –10 bp of promoter region.

9. What is upstream and downstream site?

The upstream and downstream sites are occurring at the transcription region of DNA. The Upstream is the sequence region, which is prior to the start point of the promoter (from –1 sequence). The Downstream site is the region after the start point of the promoter region (from +1 sequence).

10. What is promoter complex?

The promoter complex is described as the combine product occurred after the holoenzymes (RNA polymerase unit) binds at promoter site. When DNA is in double helix stage, it is called closed promoter complex. Once the double helix is unwinded, then it is called open promoter complex. After formation of promoter complex, the transcription state is begin to start.

11. What is abortive initiation?

The initial base pairing of RNA chain up to 9 bp length without the movement of enzyme RNA polymerase is called 'Abortive initiation'.

12. Describe the inchworm model of elongation.

The elongation of mRNA synthesis during transcription is proved as inchworm like movement. The translocation of RNA polymerase along the DNA template is like inchworm like movement. The RNA polymerase is assumed to 2 sites of RNA binding sites and one site for DNA binding. This is proved with evidence.

13. Write short notes on elongation complex.

The elongation complex otherwise called as RNA-DNA hybrid or RNAP-DNA interaction during transcription process. Elongation compiled in E. coli consists of RNA polymerase, DNA template and Nascent DNA. This combined form is called as elongation complex, which helps in elongation during transcription process.

14. What are Rho factors?

Rho factors are nothing but the protein molecules with molecular weight of 55 KD (in E.coli) involve in termination of transcription process. Rho causes the termination of mRNA in vitro at low ionic strength. The termination process may depend on Rho factors or independent to Rho factors.

15. How the elongation of mRNA is getting arrested?

I. Pause sites, ii, retard the elongation of transcription process. Arrest sites or dead ends and iii. Terminators or releases ends. Pause sites induce temporary reversible block to nucleotide addition. The arrest site stop elongation, which is resumed in the presence of elongation factors like Gre A & Gre B. The release sites lead release of RNA and RNAP either intrinsically or upon activation by factors as Nus A, Rho and Tau.

16. What is Rohr dependent and Rho independent termination?

The elongation chain recognized by a protein factor known as rho (9) factor, which cause Rho dependant termination. When the rho factors are not need for termination process or release process, it is called rho independent termination of elongation process.

17. What are palindronic sequences?

In both rho dependant and rho independent near termination sites there is a similarity in secondary structures called palindronic sequences.

e.g., 5′ GGTACC 3′
3′ CCATGG5′

The palindronic means, in both the strands when read in 5′ – 3′ direction it is same as in sequences. Palindrome ie GGT represent in invert repeats giving dyed symmetry.

18. What are terminator proteins?

The proteins involved inn the termination process and they help in termination process. The main terminator proteins involve are Rho factors, based on which rho dependent or rho independent proteins. Nus G proteins are also needed for rhodependant termination inside the cells.

19. Write short notes on Nus genes.

Nus genes are the sequences responsible for the production of terminator proteins. This also may be rho dependant and rho independent termination process. e.g., Nus gene A also produce the terminator proteins, which is helpful in termination process.

20. Write short notes on Rho independent termination.

Rho independent terminators differs from rho dependant as the presence of a ong run o f 'U' residues in stem and hairpin of mRNA. The sites contain G-C rich regions in stem and harirpin of mRNA. Polidronic sequences in mRNA synthesis cause formation of hairpins, which cause RNA-p per chain [arise on transcript).

21. Write any two differences between prokaryotic and eukaryotic transcription.

In prokaryotic DNA, only one type of RNA polymerase responsible for synthesis of all RNA.

In eukaryotic DNA, the RNA polymerases are classified into 3 types as RNA pol Pol II, pol iii and I.

22. Write short notes on eukaryotic RNA polymerase II.

RNA polymerase II of eukaryotic RNA polymerase is a one among the 3 classes of RNAP. RNAP II located at nucleoplasm. Its function is synthesis of mRNA and hn RNA.. It is sensitive for alpha amanitin.

23. Mention the features of eukaryotic mRNA.

Eukaryotic mRNA contain 3 classes of RNAPs.
Both 3′ and 5′ termini are modified in eukaryotes as

i. Complex structure, i.e. cap is found at the 5′ end.
ii. Long sequence upto 200 bp fo polyadenylic acid is called poly A is present.
iii. Small fraction of transcript of mRNA is used for protein synthesis.
iv. It is monocistronic (single protein synthesis)
v. It contain introns and exons.
vi.mRNA molecules are very long lived.

24. Describe eukaryotic promotrs.

eukaryotic DNA, more than one promoter sites are available for transcription. Different promoter sites are for different RNA polymerase enzyme like RNAP I, RNAP II and RNAP III.

A core promoter is sequence, -40 base pairs long, which is sufficient to direct transcription of RNAP II. Several core promoters are known as,

i. TATA box (A_T rich sequence located –30 upstream of start site at which TATA binding protein (TAB) sub unit of TFIID binds.
ii. BRE (TFIIB recognition element), which is located immediately after the upstream of the TATA box of some TATA containing promoters.
iii. Inr (Initiator) – It is a conserved sequence encompases in start site.
iv. DPE (Downstream promoter element)

25. Describe eukaryotic enancers.

Eukaryotic DNA enhancers are Upstream activator sites (UAS), which elements located at 100 or 200 base pairs upstream, interact with protein other than RNA polymerase. These elements

moves several hundreds to several thousands and increase 200 fold transcription rate. These elements are called enhancers.

26. What are eukaryotic silencers?

Eukaryotic silencers are specific gene or regulatory elements which repress gene expression. It functions at great distance for gene. E.g. in Yeast cells. The enkaryotic silenears are either completely suppress the gene expression or minimize the gene expression activity.

27. What is Hogness Box?

In the eukaryotic DNA, the promoter region contain the universal sequence about 25 bp upstream from transcription start site as TATAAT is known as 'TATA' or Hogness box. This is otherwise called as TATA driven promoters. Hogness box is similar to that of pribnow box in prokaryotes. This TATA box is flanked by high G+C sequences.

28. Write on eukaryotic promoter for RNA polymerase III.

RNA polymerase III is responsible for synthesis of a 120 base rRNA molecule called 5S RNA and for tRNA molecules. Its promoters differ significantly from RNA polymerase II promoters, as they are downstream from the transcription start site and within the transcribed DNA. TRNA gene promoters have 2 elements each 10 bp long spaced 30-120 bp located at downstream region of start point called box A and Box B. U6 Sn RNA gene promoters. RRNA promoters i.e. 5S RNA gene promoter 50bp downstream of start site called box C.

29. Write short notes on eukaryotic transcription factors (TF).

Transcription factors (TF) of eukaryotic DNA are protein molecules involve in initiation of transcription of mRNA. Each RNA polymerase requires its own transcription factors. Transcription factors are not a part of rNAP. Many transcription factors binding DNA sequences are at upstream of start point. The formation of preinitiation complex (PIC) takes place and the transcription process occurs.

30. What is preinitiation complex (PIC)?

Pre-initiation complex is the combined form or combination of I) Transcription factors at ii) DNA binding sites of DNA with III) RNA polymerases (RNA polymerase II) in eukaryotic transcription process. The pre-initiation complex helps to start the transcription process.

31. Write short notes on eukaryotic ribosomes.

Eukaryotic ribosome is a multicomponent particle contains several of the enzymatic activities needed for protein synthesis and serves to bring together single mRNA molecule and charged tRNA molecule in a proper position. It is larger (80S) contain about 80 proteins and additional RNA molecule. Eukaryotic ribosomes are highly basic in nature.

32. Define elongation factors.

Once RNA pol II is initiated the transcription process in eukaryotes, it shits to elongation phase. Elangation factors protein molecules, which are involve in elangation in synthesis of mRNA in eukaryotic DNA. Elongation factors stimulate the elongation of RNA processing. These factors favours the phosphorylation of CTD which leads exchange of initiation factors required for elongation and RNA processing.

33. What is mRNA splicing?

After transcription of eukaryotic DNA for mRNA synthesis, the coding sequence (Exons) is interrupted periodically by streches of non-coding sequences (Introns). Therefore, the removal of non-coding sequences (Introns) are said to be mRNA splicing. I.e. the conversion of premature mRNA to mature mRNA is called splicing.

34. What are spliceosome?

Spliceosome is a complex protein molecules about 150 proteins and 5 RNAs similar to size in ribosome. It is involve in splicing reaction and hydrolyzes the ATP molecules and catalyze the RNA cleavage. Hence, the removal of introns are achieved and mature mRNA occurred.

35. Mention briefly on mRNA capping.

The mRNA capping is one of the post transcriptional modification of eukaryotic transcription. This is the first process of modification. The addition of modified quanine base at other 5′ end of mRNA. The quanine is methylated and is unusual 5′-5′ linkage involving 3 phosphates. This is nucleotide modified methylated group in mRNA.

36. Describe mRNA polyadenylation.

Polyadenylation of mRNA is the another process of post transcriptional modification of mRNA processing. Here, the mRNA linked with 3′ end linked with the termination of transcription. The CTD of RNAP II recruits enzymes for polyadenylation. The addition of many adenine residues to

the mRNAs 5'end and termination of transcription occurs. Polyadenylation mediated by poly-A polymerase which adds 200 adenine to 3' of mRNA.

37. Write short notes on ribozymes.

Ribozymes are enzyme complex which present in nucleoplasm as well as in cytoplasm. Many RNAPs involve in RNA processing is composed of 86% RNA and 14% protein. The enzyme or protein involve in RNA folding in order to catalyze the activity of RNA synthesis and processing. The RNA molecules with enzymes and enzymatic activity are termed as ribozymes.

38. What are 'exons' and 'introns'?

After transcription of eukaryotic DNA for mRNA synthesis, the coding sequence (Exons) is interrupted periodically by streches of non-coding sequences (Introns). Therefore, the 'exons' are the nucleotide sequence of mRNA, which are responsible for coding and wanted sequences. The term 'introns' indicates that the sequences, which are non-coding or unwanted, sequences that interrupt the mRNA expression.

39. Write short notes on eukaryotic RNA polymerase.

Eukaryotic RNA polymerase is the enzyme complex required for the transcription of mRNA. The eukaryotic RNA polymerase consist 3 classes of enzymes as;

I. RNA polymerase – located at nucleoplasm, synthesis of rRNA.
II. RNA polymerase II – Located at nucleoplasm, synthesis of mRNA.
III. RNA polymerase III – Location in nucleoplasm, synthesis of tRNA.

40. Describe SnRNA and snRNPs.

SnRNA means small nuclear RNAs which are U1, U2, U4, U5 and U6. Each SnRNA is between 100 to 300 nucleotides long and complexed with several proteins.

The RNA-protein complexes are called as Small nuclear ribonuclear proteins (SnRNPS). SnRNPs ahs 3 role in splicing.

41. Write the function of reverse transcriptase.

Reverse transcriptase is the enzyme, which is incapable of initiating the polymerization and transcription process. It requires oligonucleotide sequences that is hydrogen bonded to the RNA and that bears a 3'OH group. The oligonucleotide is called as primers.

PART – B

1. Explain briefly on the transcription process of prokaryotic cells with suitable diagram.

Synthesis of messenger RNA (mRNA) from DNA template either of the DNA strand by using enzyme RNA polymerase is called as Transcription process. In the transcription process, the flow of genetic information from DNA to RNA occur or biosynthesis of proteins. It is the expression of genetic information process from DNA through mRNA for synthesis of polypeptides.

In prokaryotic cells, for example in E.coli, the single enzyme RNA polymerase (RNAP) is responsible for synthesis of all RNAs (mRNA, tRNA and rRNA). The bacterial RNA polymerase a core enzyme consist of 5 sub units as;

i. Two identical α sub units (α I and α II) – 2 X 40 KD
ii. One β sub unit (β unit) – 155 KD
iii. One β' sub unit (β unit) – 160 KD
iv. One δ sub unit (sigma factor) – 32 – 90 KD.

Total molecular weight of 4,65,000 daltons (465 KD). The structure of prokaryotic RNAP is given below:

Figure: Comparison of the crystal structures of prokaryotic and eukaryotic RNA polymerases. (a) Structure of RNA polymerase core enzyme from T. aquaticus. The sub units are colored as follows: β is shown in purple, β' in blue, the two α subunits in yello and green, and ω in red. (Seth Darst, The Rockefeller University, personal communication)

The prokaryotic promoter and terminator sites (Upstraeam and down stream sites)

The RNA polymerase binds in special region called promoter at the start of the gene of template Dante start point of the promoter surrounds the first base pairs i.e. transferred into RNA. From this point RNAP move along template and synthesis mRNA.

Terminator site or sequences are the ending region of the template strand where the synthesis of mRNA stops or ceases.

The action defines transcription unit extends from promoter to terminator.

Upstream means the sequence prior to start point of promoter and the Down stream define the sequence after the start point. Following diagram shows the promoter and terminator region of template DNA that involve in mRNA synthesis.

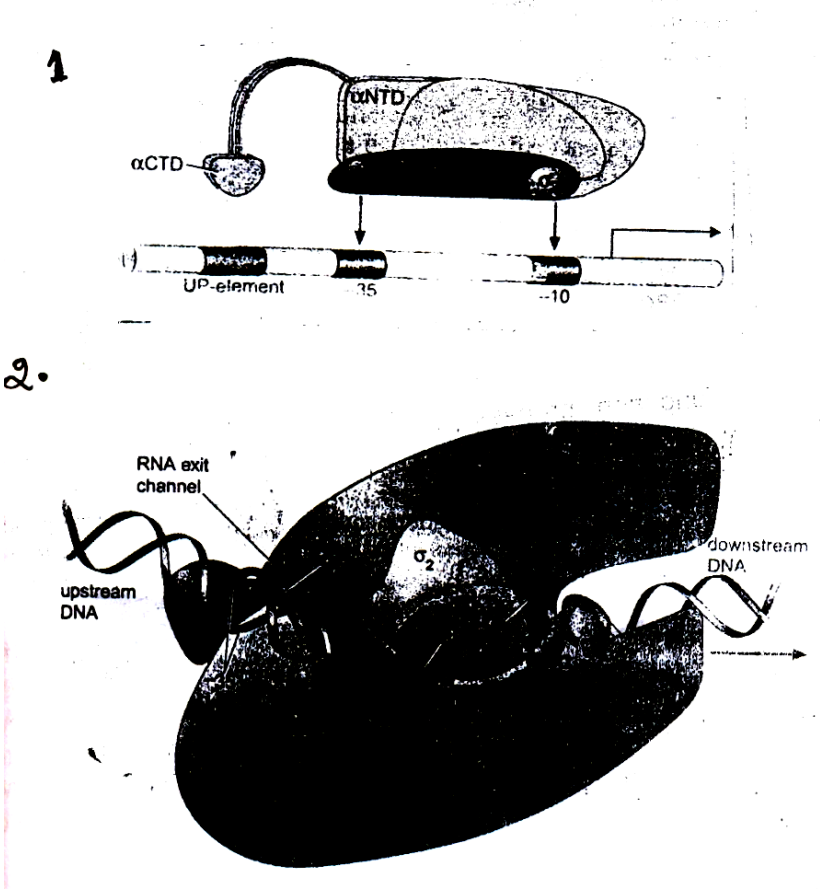


Figure (1): σ and α subunits recruit RNA polymerase core enzyme to the promoter. The C-terminal domain of the α subunit (αCTD) recognizes the UP-element (where present), while σ regions 2 and 4 recognize the – 10 and -35 regions respectively (see figure). In this figure, RNA polymerase is shown in a rather different schematic form than presented in earlier figures. This form is particularly useful for indicating surfaces that touch DNA and regulating proteins and we use it again in some figures in we consider regulation of transcription in bacteria. **(2) Channels into and out of the open complex.** This figure shows the relative positions of the DNA strands (template strand in gray, nontemplate strand in orange); the four regions of σ, the -10 and -35 regions of the promoter and the start site of transcription (+!). The channels through which DNA and RNA enter or leave the RNA polymerase enzyme are also shown. The only channel not shown here is the nucleotide entry channel, through which nucleotides enter the active site cleft for incorporation into the RNA chain as it is made. As drawn, that channel would enter the active site down into the page at about the position shown as "+1" on the DNA. Where a DNA strand passes underneath a protein, it is drawn as a do

The prokaryotic transcription process described as following stages;

1. Initiation – The start of mRNA synthesis takesplace
2. Elongation – The extension or elongation of mRNA synthesis takesplace to complete the mRNA strand.
3. Termination – The ending of mRNA synthesis or stop of mRNA synthesis takesplace.

1. Initiation process

The initiation process of started by RNAP which contain 2 binding sites with DNA template in its enzyme. These are

I. Initiation site and
II. Elongation site

1. First the holozyme binds at promoter site and forms closed promoter complex. Here the DNA remains double helix.

2. The closed promoter complex isomerises and cause unwinding and separation of DNA to form open promoter complex. I.e. melting of DNA occurs and strand gets unwind.
3. After unwinding, only one strand of DNA is copied in mRNA. The initial base pairing of RNA chain upto 9 bp length without movement of enzyme (RNAP) that is called 'Abortive initiation'. Here the RNAP lost the δ sub unit after addition of nucleotides.
4. Once, the initiation is succeeds the δ factor dissociates and marks the entry of NusA protein which helps in elongation. The promoter pausing and termination at specific sites.

Elongation process

Ternary elongation complex formed by conformation of core enzyme. Ternary elongation complex moves along DNA and synthesis of mRNA occur. The rate of elongation is 40bp per second at 37oC in bacteria. In T3, T7 phages it is 200 bp per second. Elongation proceeds till the termination complex formed.

The movement pattern of elongation process was demonstrated as Inchworm like translocation of RNAP along the DNA template. The RNAP has 2 binding sites as leading product site and lagging product site. First the leading product site gets filled with 10 newly added nucleotides and the lagging site occupies in new RNA. RNAP translocates 10 nucleotide forward. In the next cycle of elongation process, the same pattern occurs. This is called 'Inchworm like movement model' which is proved by evidence.

Elongation complex in E.coli consist of RNAP, DNA template and nascent DNA. The strength of RNA-DNA (8-12 bp) in the transcription bubble plays a major role. The modern view explains as the stability depends on the front (F) interaction i.e. growing end of RNA and RNAP processivity. This is no-ionic and has 7-9 bp of intact DNA duplex ahead of the transcription fork. The rear (R) interaction i.e. ionic and 5 bp of DNA behind active site. The F-interaction involve Sn-finger motif in NH2 domain. The beta' subunit of RNAP and R-interaction involves COOH of beta sub unit.

The following diagram explains the typical prokaryotic transcription process:

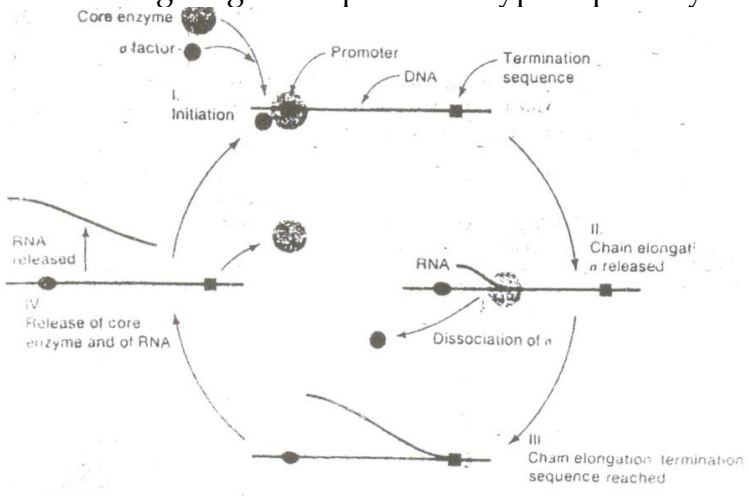


Figure: The transcription cycle of – E coli RNA polymerase showing dissociation of the σ subunit shortly after chain clongation begins, dissociation of the coreenzyme during termination, and re-formation of the holoenzyme from the coreenzyme and the σ subunit. A previously joined core enzyme and σ subunit will rarely become rejoined, instead, reassociation occurs at random.

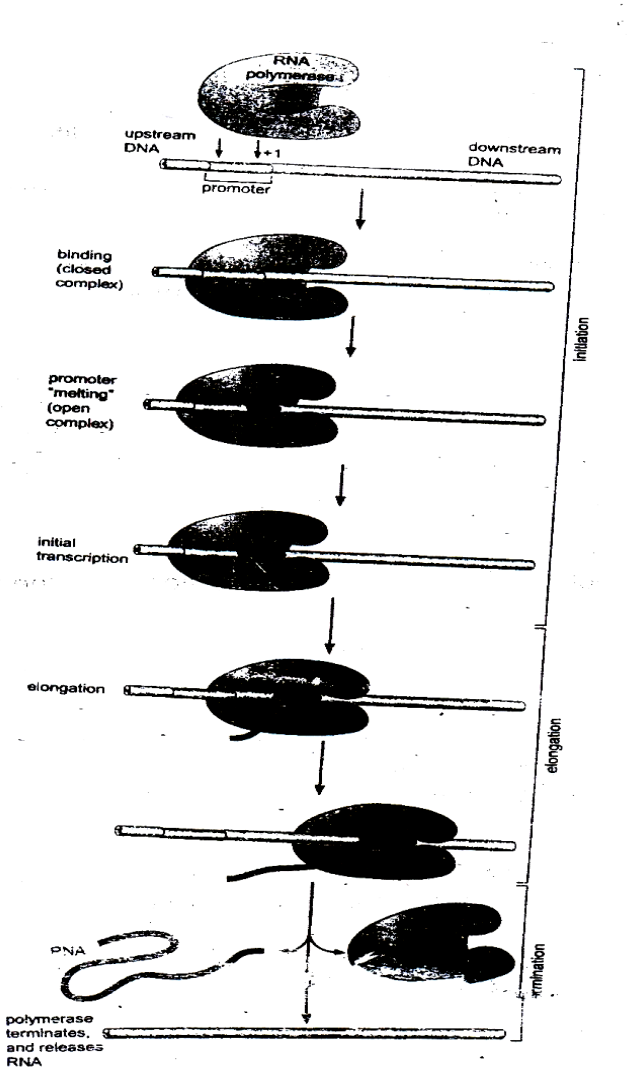


Figure: The phases of the transcription cycle: Initiation, elongation, and termination. The figure shows the general schme for the transcription cycle. The features shown hold for both bacterial and eukaryotic cases. Other factors required for initiation, elongation, and termination are not shown here, but are described later in the text. The DNA nucleotide encoding the beginning of the RNA chainis called the transcription start site and is designted the "+1" position. Sequences in the direction in which transcription proceeds are referred to as downstream of the start site. Likewise, sequences preceding the start site are referred to as upstream sequences. When referring to a specific position in the upstream sequence, this is given a negative value. Down stream sequences are allotted positve values.

Termination process

Elongation process of prokaryotic transcription is retarded by

1.Pause sites – pause site induce temporary reversible block to nucleotide addition.

2. Arrest sites or dead ends – the arrest site stop elongation and which is resumed in the presence of elongation factors GreA and GreB proteins.

3. Terminators or release ends – The release sites led release of RNA and RNAP binding either intrinsically or upon activation factor as NausA, Rho or Tau proteins.

The transcription arrest may result when, the catalytic site of RNAP is misplaced from the f3′ end of growing transcription. Then again it restored to normal transcription process.

Antitermination factors like GreA and GreB also play a major role in transcription arrest and reverse.

Termination and antitermination

Terminators are DNA sequences give signals for termination process.
Antitemiinators – Factors interact with RNA polymerases prevent termination. So, the termination depends not only DNA template also the products of RNA.

Generally 2 types of termination factors are reported to involve in transcription termination in E.coli.

i. Rho factor: The termination is recognized by a protein factor known as rho factor, which cause Rho dependant terminatin. When no need of rho factor that is called rho independent termination. Nus G is a another transcription factor which is also needed for rho dependant termination inside the cell.

ii. Palindronic sequences: near termination site, there is similarity in secondary structure called palindronic sequences, which are also responsible for termination of transcription. The following sequence pattern is observed;

5′ GGTACC 3′

3′ CCATGG 5′

The palindromic means in the both strands when read in 5′ to 3′ direction, it is same as in sequences. The palindrom GGT

CCA

represents in invert repeat giving dyed symmetry.

iii. Nus genes: In E.coli, the nus loci is occur in several which mutations at nus loci. This prevent antitermination protein pN to perform it function. Hence, termination is occurs. These are nusA, nusB and nusG.Nus stand for N utilization, these are solely for termination of transcription. These are bind RNA at box A sequence . Nus factors form complex and involve in termination.

2. Write short notes on the following.

i. Prokaryotic RNA polymerase
ii. Inchworm model of elongation of mRNA

i. Prokaryotic RNA polymerase:

In prokaryotic transcription process , a single enzyme RNA polymerase is responsible for synthesis of all RNAs like mRNA, tRNA and rRNA. For example Escherishia coli.

The bacterial RNA polymerase core enzyme consist of 5 sub units as given below:

i. Two identical α sub units (α I and α II) – 2 X 40 KD
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iv. One δ sub unit (sigma factor) – 32 – 90 KD.

In some E.coli mutants, the omega (ω) sub unit also present.

Total molecular weight of 4,65,000 daltons (465 KD). The structure of prokaryotic RNAP is given below:

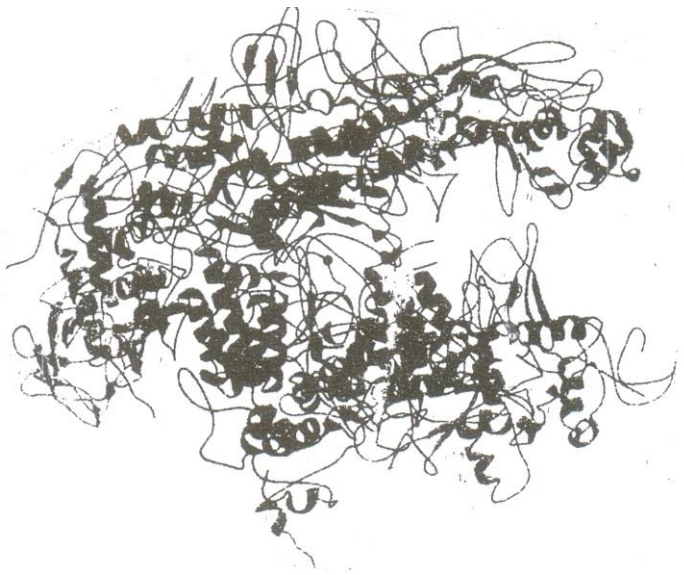


Figure: Comparison of the crystal structures of prokaryotic and eukaryotic RNA polymerases. (a) Structure of RNA polymerase core enzyme from T. aquaticus. The sub units are colored as follows: β is shown in purple, β' in blue, the two α subunits in yello and green, and ω in red. (Seth Darst, The Rockefeller University, personal communication)

The β andβ' sub units are involve in catalysis of reactions.
αI and αII sub units involve in synthesis of assembly and transcription regulation.

β, αI and αII are acts as an assembly intermediate. The omega (ω) facilitates to form the intermediate with β' and for core enzyme as β, αI, αII and ω. αI, αII are identical sequences but

differ in location with 9in RNAP. The δ helps in recognition of start signals on DNA strand and direct RNAP in selecting the initiation sites. In the absence of sigma, the core enzyme initiates RNA synthesis. Once RNA synthesis initiated, the sigma dissociates after RNA is 8-0 bp long. Then the core enzyme brings elongation of mRNA. The dissociated sigma again combines with core enzyme to form RNAP holoenzyme. There are more than one sigma factors found in E.coli. These are δ70, δ32, δ41.5, δ54, δ28.

δ70 are giving in normal conditions of growth of the cell. Others are in adverse conditions as high temperature, starvation, nitrogen deficiency and chemotaxis.

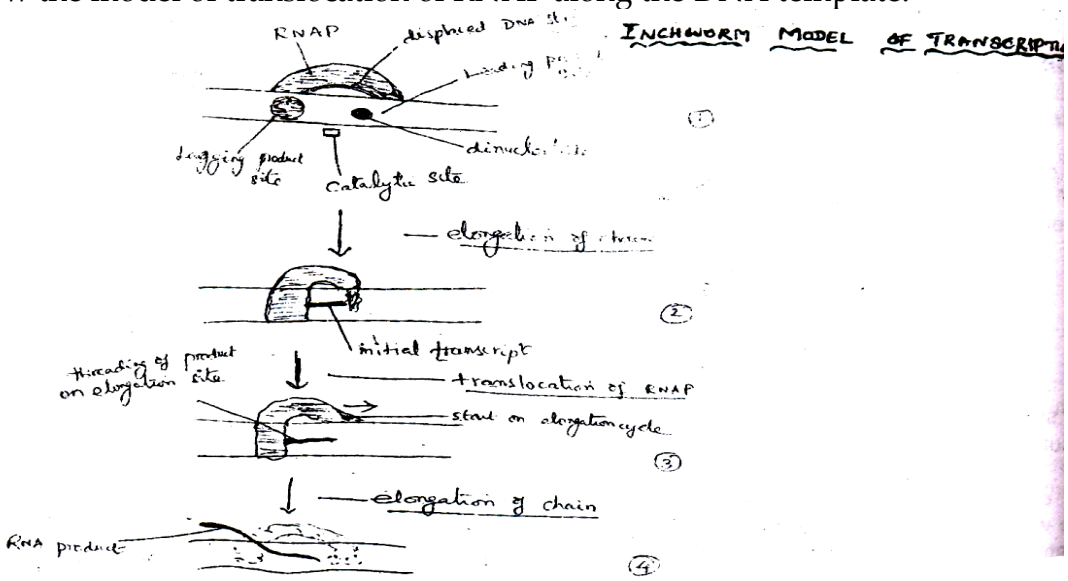
II. Inchworm model of elongation of mRNA

M.J.Chamberlin in1 993, proposed the elongation process of prokaryotic transcription process during mRNA synthesis as Inchworm like model. Inchworm like model of translocation of RNAP along the DNA template.

1. RNAP is assumed to 2 sites for RNA binding as
    i. Leading product site (Product site)
    ii. Lagging product site (product site) and

2. RNAP has one site for DNA binding.

In the first cycle, the leading product site gets filled with 10 newly added nucleotides. Then the lagging site occupies in new RNA. RNAP translocated 10 nucleotide forward. Then next cylce of elongation is occurs. The stability of elongation complex is provided. The DNA interaction with lagging site. It is called 'Inchworm model' and it is proved by evidence. The following diagram show the model of translocation of RNAP along the DNA template.



3. Discuss in detail about the termination of transcription process in prokaryotic cells.

Termination of transcription process in prokaryotic transcription requires special proteins and mechanism.

The **terminators** are the special DNA sequences give signals for termination of transcription process in prokaryotic DNA.

The **antiterminators** are the factors which interact with RNA polymerase prevent termination of transcription process. So, the termination depends not only DNA template, also the products of RNA.

Rho dependant and Rho independent termination in E.coli

There are 2 types of termination factors found on DNA as;

i. The termination factors recognized by a protein factor known as rho (ρ) factor which is called rho dependant termination.

iii. When no need of rho (ρ) factor which is called rho independent termination process.

Other proteins called NusG transcription factor, which also needed for rho dependant termination inside the cells.

Rho (ρ) factor

It is a protein molecules with molecular weight of 55 KD isolated from E.coli. It causes termination of mRNA in vitro at low ionic strength also not needed in some of mRNAs. In both rho dependant or rho independent termination, near termination sites there is a similarity in secondary structure called palindromic sequences.

Palindromic sequences are as given below;

5′ GGTACC 3′

3′ CCATGG 5′

The palindromic means in the both strands when read in 5′ to 3′ direction it is same as in sequences. In palindrome GGT
CCA represents in invert repeats giving dyed symmetry. This causes complementary between bases of same strand to form a hairpin in mRNA or cruciform in DNA.

Rho independent terminators are differs from rho dependant on the presence of long run of 'U' residues in mRNA (A in DNA) and of G-C rich regions in stem of hairpin of mRNA.

Palindromes in mRNA cause formation of hairpins, which cause RNAP slow down or cause pause in transcription.

The events through rho factor are;

i. Rho act as a tetramer and binds to the 5'end of mRNA (nascent) and moves along the length.
ii. When RNAP pause due to hairpin structure rho factor catches up RNAP and releases mRNA from DNA template accompanied with dissociation with RNAP and rho factor.

The following diagram showing the mechanism of action of rho (ρ) factor termination of transcription

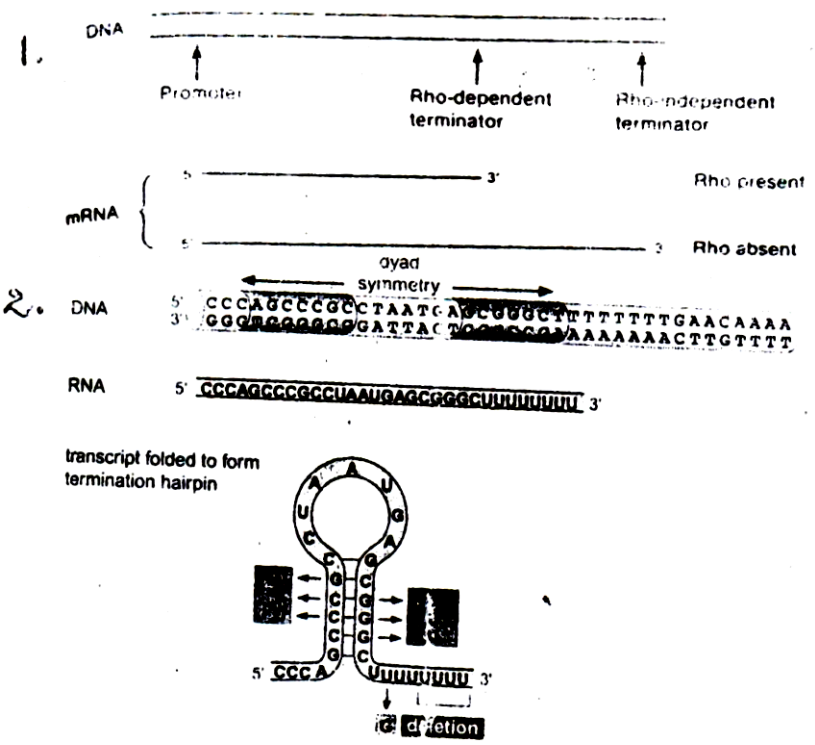


Figure 1: The effect of Rho on termination of RNA synthesis. This effect is easily demonstrated in vitro. If Rho is added to a reaction mixture after RNA polymerase has passed the Rho-dependent terminator. RNA synthesis continues until the Rho-independent terminator is reached. The bottom lines represent the completed mRNA molecules.

Figure 2: Sequence of a rho-independent terminator: At the topis the sequences, in the DNA, of the terminator. Below is shown the sequence of the RNA, and at the bottom the structure of the terminator hairpin. The terminator in question is from the trp attenuator, discussed. The boxes show mutations isolated in the sequence that disrupt the terminator.

Rho independent

At rho independent termination, the long run of 'U' bases in mRNA and 'A' bases in DNA template. There will be a very weak rU-d'A base pairs need very little energy to break. Since RNAP slows down due to hair pin structure, rU-dA bond breaks at any point causing release of mRNA.

Nus genes

In E.coli, nus genes are occur on DNA occur at nus loci. In mutations at nus loci, it prevent antitermination protein pN to perform it function. These N proteins form a tight complex with RNA polymerse and other cellular proteins which involve in termination process of mRNA synthesis. These genes are nusA, nusB, nusG and the nus stand for N utilization. These nus proteins are solely for termination of transcription. These nus proteins are bind RNA at Box A sequence and nus factors form complex which involve in termination. They may act by directly controlling the kinetics of elongation i.e. by acting as pausing suppressors, or they could specifically interact with the termination apparatus, by preventing arrest or directly controlling template structure.

4. Describe the initiation of transcription in eukaryotic DNA with highlights to promoters, enhancers and silencers.

The basic features of transcription process of eukaryotic DNA are similar to that of prokaryotic DNA. But, in eukaryotic DNA, the transcription process is very complex. The major differences are;

1. Eukaryotic DNA contain 3 classes of nuclear RNA polymerase and
2. Both 3′ and 5′ termini are modified, as i. Complex structure, cap is found at the f5′ end. Ii. Long sequence (upto 200bp) of polyadenylic acid poly A found in 3′ end.
3. Small fraction of transcript of mRNA is used for protein synthesis. Interrvening sequences or introns are excised and surrounding fragments are joined during mRNA synthesis.
4. mRNA are moncistronic (single protein synthesis).
5. Most RNA molecules are very long lived.

Initiation of transcription of eukaryotes

i. Promoter

Different promoters (sequences) sites are involved in different RNA polymerases.

Promoters for RNAP-I

It has elements that are a GC-rich upstream (-180 to –107) control element (UCE). Acore region that overlaps the transcription start site as –45 to +20 region.

Promoters for RNA-II

The structure of eukaryotic promoter is complexd. The difference is lengthy seqauences i.e. hundreds of base pairs, which are upstream from start site. This control the ratre of initiation called upstream sites.

A core promoter is sequence, -40 base pairs which is sufficient to direct transcription of RNAP –II with the help of transcription factors called general transcription factors (GTF).

There are several core promoters are known as;

(i) TATA Box (A-T rich sequence) located –30 upstream of start site at that TATA binding protein (TBP) sub unit of TF II D binds.

(ii) BRE (TFIIB recognition element) this is located immediately after the upsteam of the TATA Box of some TATA containing promoters. The BRE increasing the affinity of TF-IIB for core promoter.

(iii) Inr (Initiator)

It is a conserved sequence, which encompass in start site. Function of initiator is initiation by itself or in conjunction with TATA or dPE motif.

(iv) DPE (Downstream promoter element)

It is conserved sequence (as common as TATA box) located –30 bp downstream of start site which oconsist of Inr and DPE motifs and lacks a TATA box.

Therefore, based on the above, the eukaryotic promoters can be classified into

i. TATA – driven promoters and
ii. DPE – driven promoters

e.g. Drosophila.

Further upstream ;of their box are present GC box (-60 or –100 bp and CAAT box (-80 bp).

Enhancers or Upstream activator sites (UAS)

The elements or sequences located at 100 or 200 bp upstream interact with proteins other than RNA polymerase. These move several hundreds to several thousands and increase 200 fold transcription rate.

Eukayotic silencers

These are regulatory elements repress gene expression. It functions at great distance for gene. E.g. Yeast

The following diagram showing the upstream and downstream regions of eukaryotic promoters and elangation regions.

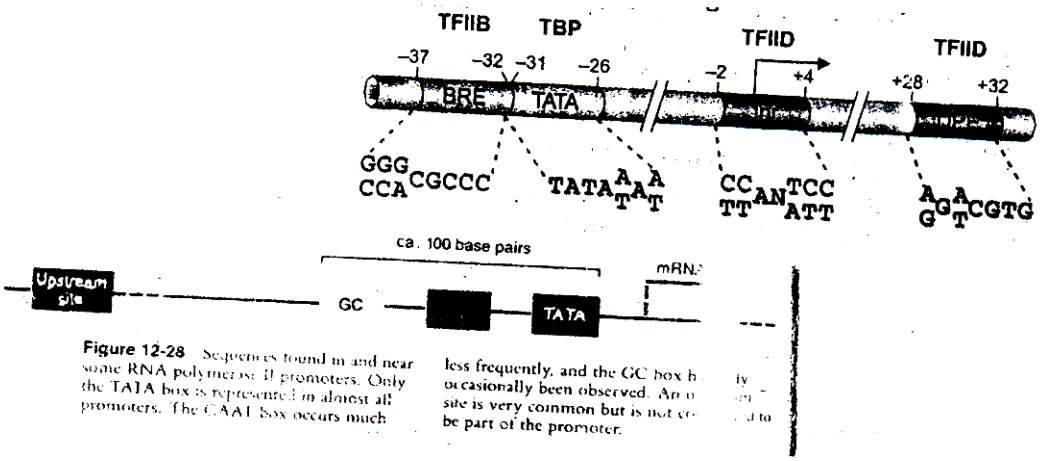


Figure 12-28 Sequences found in and near some RNA polymerase II promoters. Only the TATA box is represented in almost all promoters. The CAAT box occurs much less frequently, and the GC box h... occasionally been observed. An o... site is very common but is not co... be part of the promoter.

The DNA segment in eukaryotic showing promoter sites (TATA box and CAAT box) and enhancer sites.

The highlights are

1. Universal sequence, about 25 bp upstream from transcription start site as TATAAT is known as TATA **orHogness box.**

2. In many, but not at all, another common sequence in the –75 up region with consequence as GG(T/C) CAATCT.

   T & C are equally present and called CAAT box.
   Other elements GC box which consumes GGGCGG.

3. The TATA box and other sequences if present are sites of binding for trancription factors, but not of RNA polymerase.

4. RNA pol II, does not interact directly with any part of the promoters and dependent o9n the other protein.

   It is a major difference but EU and Prokaryotes.

5. The TATA box probably determines the base tht is first recommended.

Promoters of RNA polymerase III

There are 3 classes of enzymes are found as;

i. tRNA gene promors :

It has 2 elements each 10bp long spaced 30 – 120 bp which is located is spaced 30-120 bp which is located at downstream refgion of start point called box A and box B.

i. U6SnRNA gene promoters
ii. rRNA i.e. 5S RNA gene promoter.

About 50 bp downstreaming of start site called box C. However, the location of downstream promoters varies in different gens.

RNA pol III attached +55 to +80 bp sequence. It can start transformation from start point.

5. Explain the role of transcription factors in initiation and formation of initiation complex in transcription process of eukaryotic cells.

In eukaryotic DNA, the transcription factors (TF) play a major role in transcription process. Each RNA polymerase (RNAP) of DNA requires its own transcription factors. But Transcription factors II D and part of TBP is required by all the RNA polymerases.

Transcription factors (TF) are the proteins needed for initiation of transcription process, but not a part of the RNAP. DNA sequences and TF for RNAP II may be either common for many genes or specific for tissue specific expression in basal transcription or activator dependant transcription. Many TFs binding DNA sequences are at upstream of start point. The TFs increases when variety of genes transcribed by RNAPs increases.

Preinitiation complex (PIC)

Preinitiation complex is formed or initiation complex (IC) by help of TF in DNA binding on RNAP. After the complex formation (PIC/IC), the transcription occurs. The steps involve in PIC or IC formation is as the 4 steps given below;

1. Nucleation – It is recognition of promoter and binding of TF.
2. Association of TF links RNAP with promoter.
3. RNAP entry on transcription process.
4. Complex formation of PIC & IC.

Formation of PIC with RNAP II

The TFs of the eukaryotic DNA is equal to that of δ factor in prokaryotes. The general transcription factors (GTFs) involve are;

i. Help RNAP to bind to the promoter and melt the DNA and open the DNA strand equal to open promoter complex in bacteria.

ii. It helps polymerase to escape from promoter and emboss on the elongation phase.

The GTF and RNAP bound together at the promoter and poised for initiation called Preinitiation complex (PIC). PIC begins in TATA box. The steps involve are as follow:

1. TATA recognizes the GTF as TFIID with individual factors as A, B etc.) TF IID is a multisubunit complex. The complement is TBP (TATA binding protein). Other subunit in this complex is TAFs (TBP associated factors). TAFs helps bind the DNA and controls the DNA-binding activity of TBP.

2. The TBP-DNA complex provide platform to recruit other GTF and polymerase itself to the promoter in the following order;

TF II A
TF II B
TF II F

Together with RNAP II as complex.

3. Then, TF II E and TF II H bind upstream of Pol II. The above containing PIC followed by promoter melting. The melting requires hydrolysis of ATP by TF II H, which is comparable to helicase like activity in bacteria.

The series of steps presented in the following diagram show the initiation of eukaryotic DNA.

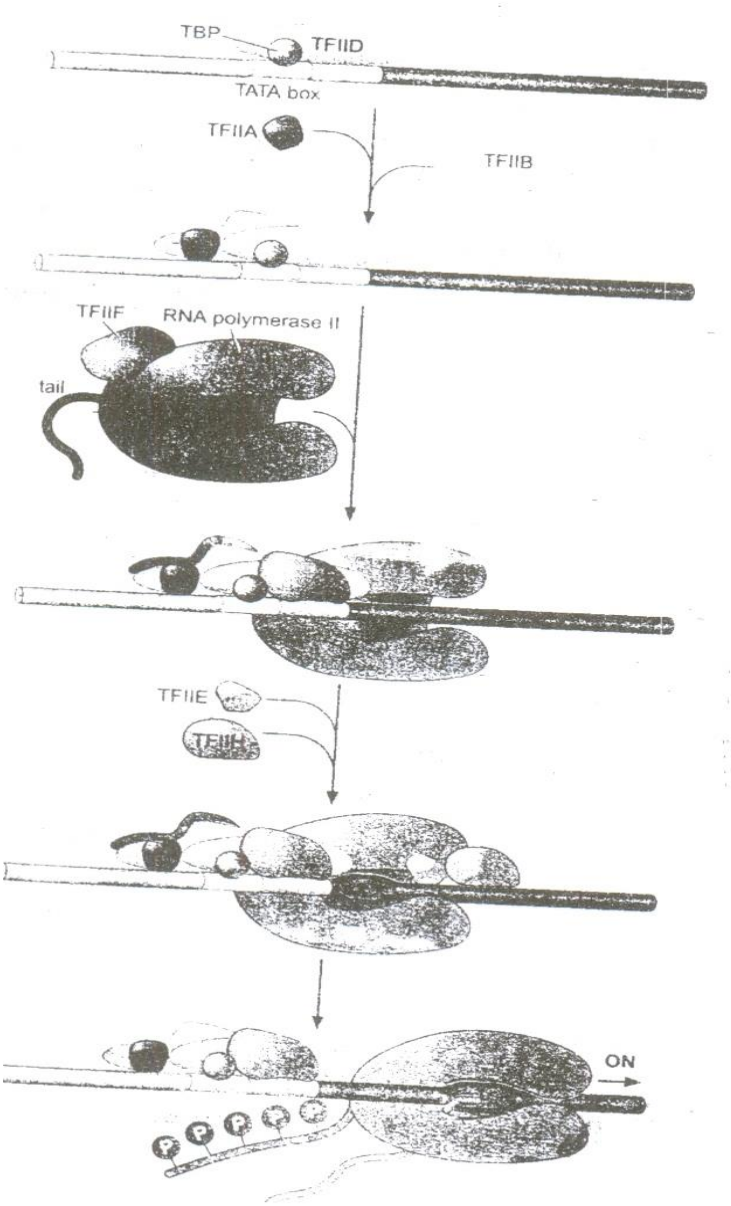


Figure: Transcription initiation by RNA polymerase II: The step-wise assembly of the Pol II pre-initiation complex is shown here, and described in detail in the text. Once assembled at the promoter, Pol II leaves the pre – initiation complex upon addition of the nucleotide precursors required for RNA synthesis, and after phosphorylation of ser resides within the enzyme's "tail", The tail contains multiple repeats of the heptapeptide sequence: Tyr – Ser – Pro – Thr – Ser – Pro – Ser

Now the RNAPII escapes the promoter and enters the elongation phase.

Phosphorylation of the CTD (C-terminal domain) of RNAP II

The large sub unit of RNAP II has a C-terminal domain (CTD) extends as tail. CTD contain series of repeats of the hepapeptide sequence as Tyr-Ser-Pro-Thr-Pro-Ser. These are 27 repeats in Yeast RNA II and 52 in human. Each repeat contain sites for phosphorylation by specific kinase including that of TF II H sub unit. Number of other kinases have been identified that act on CTD as well as a phosphatase that removes the phosphates.

Formation of PIV by RNAP I and III

RRNA genes transcribed by RNAP I by different steps are;

1. The promoter (UCE) is nucleated by upstream binding factor (UBF) or assembly factor.
2. UBF + DNA and RNAP I is brought above to TBF + TAFs complex.

In RNAP II, the tRNAs formation of complex in promoter like Box A, Box B, TFIII C complex in case of tRNAs genes. TF III B facilitates the bridging between each of these complexes and RNAP III.

Other general Transcription factors

Transcription factors (TFs) includes TAFs – TBp associated factors involve transcription process. There are 10 TAFs reported in eukaryotic cell. Two of the DNA element bind TAFs is Inr (initiator element). Several TAFs are structural homology with Histone proteins. So, it might bind as histone marker with DNA. E.g. TAF 42 and TAF 62 of Drosophila similar to H3 and H4 tetramer. Another TAF appear regulate binding of TBP to DNA.

TF II B

This is single polypeptide chain enters in PIC after TBP. The ternary complex TFII B-TBP-DNA is crystal structure include base specific interactions with major groove of upstream and minor groove of downstream of TATA element. This factor bridge between TATA-TBP-RNAP complex. Structural studies suggest, the N-terminal domain of TF II B inserts to the RNA exit channel of RNAP II.

TF II F

This contain two subunit which associates with RNAP II and is recruited to be promoter together with RNAP II. TF II F – RNAP II complex stabilizes the DNA-TNBP – TF II B complex and require before TF II E and TF II H, which are recruited for PIC formation.

TF II E and TF II H

TF II E consists of 2 subunits, which binds and has role in recruit of TF II H. TF II H controls ATP-dependant transition to open complex of PIC. This is the largest and most complex of GTF, which has 9 sub units. The molecular mass comparable to RNA polymerase itself. The two subunits of TF II H works as i. ATPase and ii. Protein Kinase. These are role in promoter melting and RNAP escapes.

6. Discuss in detail about mRNA processing in eukaryotic transcription process.

Once RNA polymerase II initiated, it shifts to elongation phase of mRNA synthesis. Elongation factors required or recruited for the elongation process, which stimulates the elongation. Other factors also required for RNA processing .By the way once mRNA described, it has to be processed before being exported from the nucleus to the translation location. This process is otherwise called as posttranscriptional mRNA modification.

The main events of mRNA processing are as follows;

1. mRNA capping 5′ end
2. mRNA splicing and
3. Polyadenylation of the 3′ end.

Two proteins are mainly involved as 1.LSPT 5 which recruits and stimulates the 5′ capping enzyme and TAT-SF1 which recruits components for splicing machinery.

1. mRNA capping

After the transcription of eukaryotic mRNA takes place, the mRNA needs post transcriptional modification to give processed mRNA for translation. The first process of event in posttranscriptional modification is mRNA capping. In mRNA capping , the addition of modified quinine base to the 5′ end of RNA. The quinine is methylated quinine and it is unusual 5′ – 5′ linkage involving of 3 phosphates. There are three kinds of steps are involved in this process as given below:

1. Phosphate group is removed from 5′ of t transcript

2. The guanine tri phosphate (GTP) ism added to the above and

3. Methyl group modifies nucleotide.

The following diagram represents the mRNA capping process.

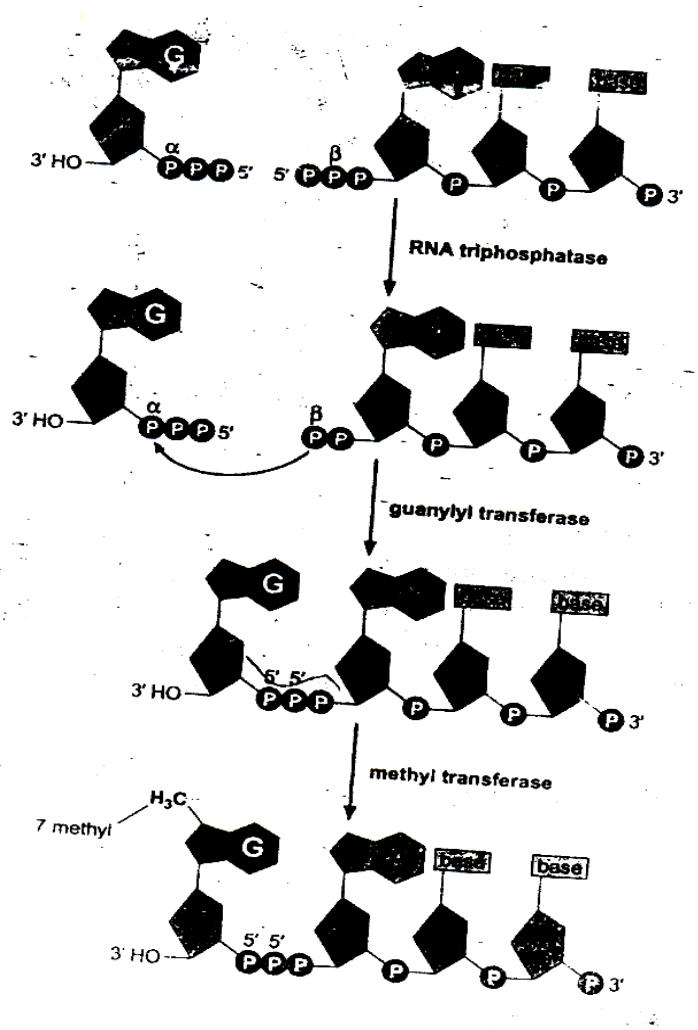


Figure: The structure and formation of the 5' RNA cap: In the first step, the γ-phosphate at the 5' end of the RNA is removed by an enzyme called RNA triphosphatase (the initiating nucleotide of a transcript initially retains its α-, β-, and γ-, phosphates). In the next step, the enzyme guanylyl transferase catalyzes the nucleophilic attack of the resulting terminal β-phosphate on the α-phosphoryl group of a molecule of GTP, with β- and γ-phosphates of the GTP serving as a pyrophosphate leaving group. Once this linkage is made, the newly added guanine and the purine at the original 5' end of the mRNA are further modified by the addition of methyly group by methyl transferase. The resulting 5' cap structure later recruits the ribosome to the mRNA for translation to being.

mRNA capping is occurring when 20-40 nucleotides long strand. This occurs when the transition occur between initiation and elongation phases. After RNA capping, dephosphorylation of serine 5 within tail repeats leads to dissociation of capping machinery. Further phosphorylation causes recruitment of machinery needed for RNA capping.

II. mRNA Splicing

In eukaryotic mRNA, after the transcription, the coding sequence is interrupted periodically by stretches of non-coding sequence. Thus, eukaryotic genes are mosaics, consisting blocks of coding sequences separated from other blocks of non-coding sequences. Coding sequences are called **Exons**. Non-coding sequences are **called Introns.** The genes bearing noncoding sequences are said to be 'in pieces' or 'split'.

The number of intron varies in gene in different organisms. This is found more in yeast and few in human and 50 in chicken pro X2 gene.

The following diagram shows the mRNA splicing process.

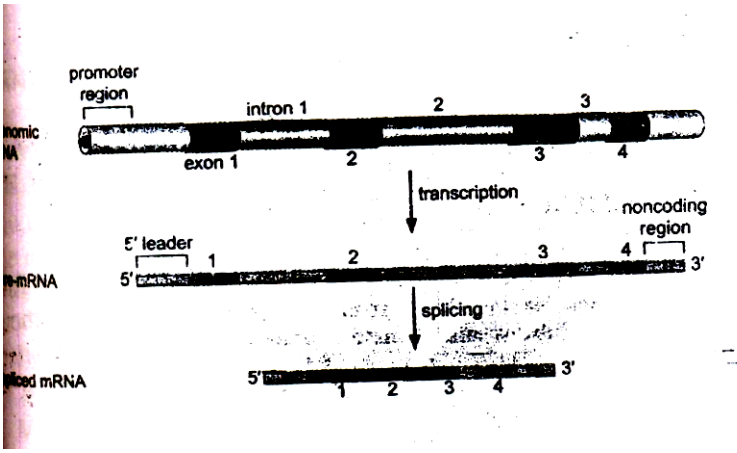


FIGURE 13-1 Typical eukaryotic gene. The depicted gene contains four exons separated by three introns. Transcription from the promoter generates a pre-mRNA, shown in the middle line, that contains all the exons and introns. Splicing removes the introns and fuses the exons to generate the mature mRNA that, once processed further (see polyadenylation, Chapter 12) and exported from the nucleus, can be translated to give a protein product.

So, the removal of introns is mRNA splicing.

Introns are much longer and exons are shorter comparatively. Exons are in order of 150 nucleotides and introns are as long as 800 kb in length. Thus eukaryotic gene, the coding region is less than 10% of the total RNA. e.g. in mammalian gene for enzyme didydrofolate reductase, the length is < 31 kb and the coding region is 2 kb of mRNA.

Spliceosome is a complex proteins consist about 150 proteins and 5 RNAs, which similar in size to ribosome involve in splicing mechanism. The spliceosome hydrolyses the ATP molecules. The intron and exon borders are likely participating in catalysis reaction. So, RNA components are involved in reaction. SnRNAs (Small nuclear RNAs) as 5 RNAs (U1, U2, U4, U5 and U6). Each SnRNA between 100 to 300 nucleotides long and complexed with several proteins. These RNA-Proteins complexes are called small nuclear ribonuclear proteins (SnRNPs). SnRNPs has 3 roles in splicing as

i. Recognize the 5′ splice site and ranch site

ii. Bring these sites together as required and

iii. Catalyze the RNAP cleavage and joining reaction for this RNA-RNA, RNA-Protein and protein-protein interactions are important.

III. mRNA Polyadenylation

mRNA polyadenylation is the process where the 3′ end linked with the termination of transcription. The CTD of Pol II recruits enzymes for polyadenylation. Once RNAP II reaches the end of a gene, particular sequences trigger the transfer of polyadenylation enzymes to the RNA. It leads three events as mention below:

1. Cleavage of the message
2. Addition of many adenine residues to its 5′ end and termination of transcription.

In this process, two protein complexes are involved with CTD of RNAP II.

i. CPSF (Cleavage and polyadenylation specificity factor) and
ii. CstF (Cleavage stimulation factor)

When the sequences transcribed in to RNA, trigger transfer of these factors called poly-A signals. Once, CPSF and CstF bound to RNA, other protein recruited leading cleavage first and then plyadenylation.

Polyadenylation mediated by poly-A polymerase, which add 200 adenine (A) to 3′ of RNA and produced by the cleavage. M Poly-A polymerase uses ATP as a precursor. It does without DNA template. As long tail of Adenines is found in RNA. The mature mRNA is transported from nucleus.

The following diagram shows the mechanism of polyadenylaion of RNA processing.

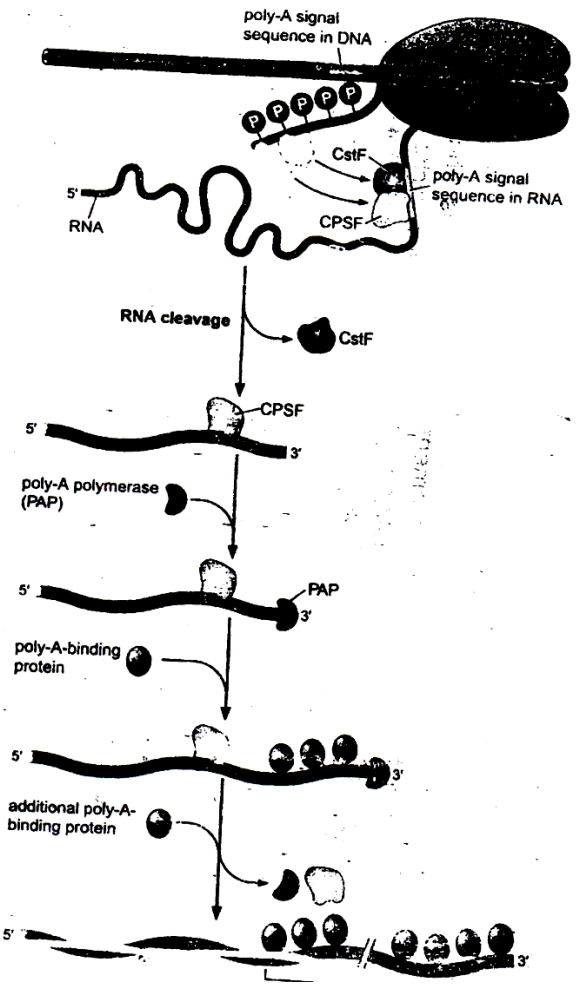


FIGURE 12-20 Polyadenylation and termination. The various steps in this process are described in the text.

7. Write short notes on the following;

i. mRNA capping

ii. mRNA polyadenylation

i. mRNA capping

After the transcription of eukaryotic mRNA takesplace, the mRNA needs post transcriptional modification to give processed mRNA for translation. The first process of event in posttranscriptional modification is mRNA capping. In mRNA capping, the addition of modified quinine base to the 5′ end of RNA. The quinine is mehtylated quinine and it is unusual 5′ – 5′ linkage involving of 3 phosphates. There are three kinds of steps are involved in this process as given below:

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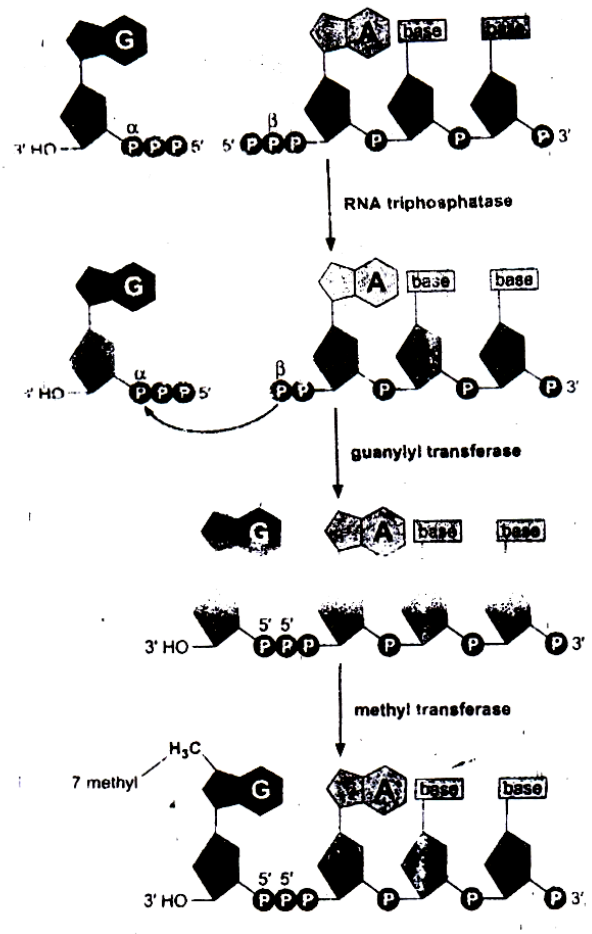

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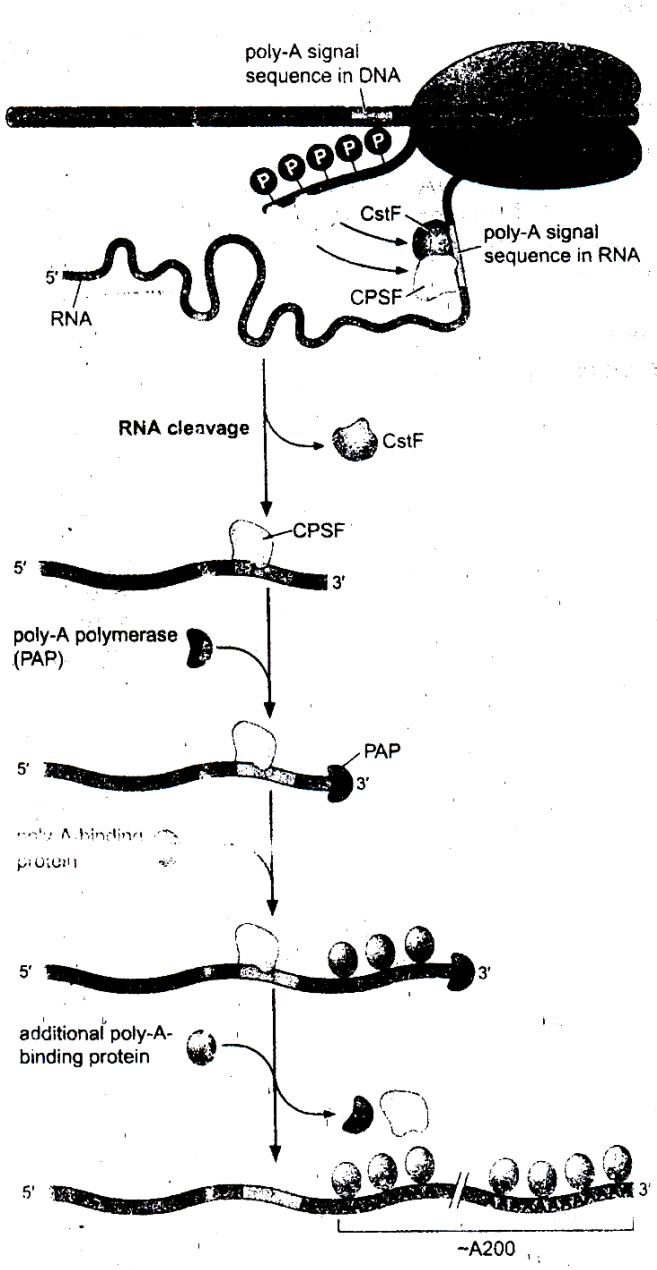


Polyadenylat
termination. The various steps ir
are described in the text.

8. Discuss the features of promoters and enhancers that involve in Eukaryotic mRNA synthesis.

I. Eukaryotic promoters

In eukaryotic DNA transcription, the RNA polymerase II cannot initiate transcription itself, but is absolutely dependent on some transcription factors. The enzyme along with these factors

constitutes the basic transcription apparatus. Additional factors are required to recognize specific promoters or group of promoters.

1. Class I promoters which are required for initiation of transcription;
2. Class II promoters which participate late in elongation of RNA transcripts; and
3. Class III promoters, which assist in the formation of an active pre-initiation complex but may not be required after initiation of transcription.

The first two types are called general transcription factors that are required for all the genes transcribed by a particular RNA polymerase. The factors belonging to the third category help in regulation of transcription of particular genes. Promoter elements usually serve as recognition sequences for DNA-binding proteins and constitute DNA sequence, which is recognized by RNA polymerase. The structure of eukaryotic promoters is quite complex and their identification considerably difficult.

RNA pol II promoters

Since there are three different RNA polymerases, it is natural to expect different regulatory sequences to initiate transcription. Each enzyme is endowed with specific regulatory sequences located with respect to start sites on the template DNA strand. At the same time each RNA polymerase requires different transcription factors or accessory protein that bind to these sequences. The specific nucleotide sequences located upstream of the transcription unit determine the transcription by RNA polylmerase II and I.

Before we describe the location of promoter structure for RNA pol II, it will be helpful to understand the gene arrangement in eukaryotic chromosome. The protein coding genes in eukaryotics are discontinuos and are interrupted by noncoding segments. The noncoding regions are called intron, and range in the size from 80 bp to 10,000 bp. Histone mRNAs are devoid of introns because histone is a protein and non-genetic material.

Class II promoters that correspond to RNA pol II comprise of two to three short conserved regions in their upstream sequences from the start site, whose consensus is TATAAAT, commonly known as TATA or Hogness box, CAAT box, and GC box. TATA box is about 25 bp upstream from the transcription start site, and is usually flanked by G+C sequences. The Hogness box is comparable to the Pribnow box. The consensus sequence of the second module is GCCCAATCT also called as CAAT box is about -75 bp upstream, while the third one called GC box, others lack a CAAT box or GC boxes. The choice of start site depends upon the TATA box and its elimination causes the start site error prone. The promoters of RNA pol II and I are located upstream.

The following diagram shows the eukaryotic promoter region of DNA.

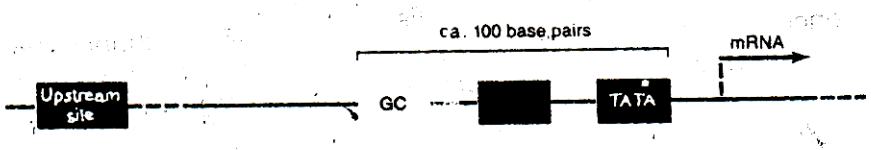


Figure 12-28 Sequences found in and near some RNA polymerase II promoters. Only the TATA box is represented in almost all promoters. The CAAT box occurs much less frequently, and the GC box has only occasionally been observed. An upstream site is very common but is not considered to be part of the promoter.

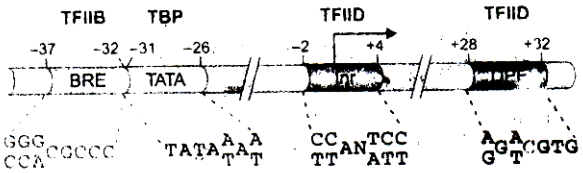


FIGURE 12-12 **Pol II core promoter.** The figure shows the positions of various DNA elements [illegible] indicated by the arrow above the DNA). These elements, described [illegible] 1995 ("Fifs recognition element"); TATA (TATA Box); Ini (initiator element); and [illegible] element). Also shown (below) are the consensus sequence for each element [illegible] as described for the bacterial promoter elements, see Box 12-1); and (above) the name of the general transcription factor that recognizes each element. (Source: Butler J.E.F. e [illegible] 2002 Genes and Development 16: 2583 – 2592, Fig. 1.)

Promoters of RNA Polymerase III

RNA polymerase III promoters are located downstream of the start site, hence called as internal promoters. Responsible for synthesis of %S RNA and tRNA molecules, RNA polymerase III uses promoters that are different from RNA pol II promoters. RNA pol III also relies on TATA-binding protein (TBP) for initiation together with other factors such as TFIID. The activity of RNA pol III has been studied in the transcription of %S RNA gene in Xenopus, where three transcription factors are required. The enzyme RNA pol III binds to TFIID for initiation and the factor TFIIA (a 40 KD protein) binds to the internal promoter on the DNA and recruits other binding sites. There is a negative feedback control in this process in which %S rRNA will bind to RFIIA when produced in excess. However, the enzyme will function even when a TATA box is absent. The exact mechanism is still not clear.

Inducible Promoters

The promoters, which have short sequence motifshaving TATA binding, associated factors (TAFs) form a part of a constitutive promoter. On the other hand, there are inducible promoters that may contain sequence motifs upstream, and induce activation of genes for transcription in response to various signals, which may be exogenous or endogenous. The exogenous signals comprise of temperature, light, pathogenic infections or metal ions, whereas endogenous signals are generated by hormones and chemicals produced in various tissues. The sequences responding

to these signals are called upstream regulatory sequences (URS) and can be delinked from the constitutive part of the promoter. Structure of a typical class II gene and its upstream sequence motifs.

Transcription Enhancers

Another type of sequences has been identified which greatly enhance the transcription of a gene. These sequences whose locations may be changed without damaging their effectiveness are c called enhancers. They differ from the promoters or upstream regulatory sequences in the following ways.

1. The position of the enhancer relative to the promoter of start site is not fixed and can vary substantially:
2. The enhancers can function in either orientation;
3. Though enhancers are also modular like promoters, their modules are contiguous rather than spaced apart.

Enhancer sequences were first discovered in a mammalian virus, SV 40, in which enhancer is located 200 bp upstream of the start site of a transcription unit and contains two identical sequences of 72 bp each. The position of the enhancer can be moved upstream or downstream and in any location, i.e. to other side of the promoter without any loss of activity. Experimentally, the endonulceases can be removed and inserted subsequently elsewhere, demonstrated that enhanced transcription was sustained so long the enhancer is present any where on the DNA molecule. In another experiment, a globulin gene was placed on a DNA molecule that contained 72 bp repeat. It was found that its transcription is increased in vivo more than 200 times even when the 72 bp repeat. It was found that its transcription is increased in vivo more than 200 times even when the 72 bp sequences is several thousand base pairs upstream or downstream of the start point in either orientation.

Cellular enhancers have been discovered in the form of sequences in the genome that stimulate the nearby promoter in a specific tissue. The enhancers are present in immunoglobulin genes within the transcription unit, and these appear to be active in the B lymphocytes only where immunoglobulin genes are expressed. Looping of the DNA allows activator proteins to bind to distant enhancers to interact with TFIID, other TFII proteins, and RNA polymerase II. Thus, enhancers have no promoter activity of their own, but exert stimulatory effects on promoters thousands of base pairs away.

Important feature of the transcription signals in rRNA genes (class I genes) is that they are species-specific and the rRNA genes of the closely related species can be distinguished by the specificity of their own transcription machinery, as revealed from the studies on Drosophila.

There is, however, no conservation of nucleotide sequences preceding or beginning the transcription units in eukaryotes studied so far.

The situation with the class II genes is different where genes within a class or in widely divergent species have highly conserved nucleotide sequences in their transcription control unit. On the basis of studies in humans and rodents, it has been suggested that two regions on DNA, which are; accomplish regulation of transcription by RNA polymerase;

i. Sequences between -20 and -45, called core promoter essential for promoting transcription though at a low level;
ii. Sequences between -107 and -156, called upstream control region, which enhances the transcription activity of the core promoter.

9. Write short notes on the following;

i. Prokaryotic promoters
ii. Eukaryotic promoters

i. Prokaryotic promoters

promoters are relatively simple. Initiation of transcription is crucial to controlling gene expression. The promoter is a DNA sequence, which is recognized by RNA polymerase in order to initiate transcription, this is the DNA region where the RNA polymerase bind and start the transcription. The type of promoter occur in prokaryotic cell is called constitutive, because the promoter site is recognized by a single holoenzme (E.coli contain single RNA polymerase enzyme) and transcription takes place directly. It not requires any accessible proteins. Binding of RNA polymerase is followed by initiation of transcription at the start site or promoter region, which is referred to as Pribnow box, then RNA polymerase continues long the template until it reaches a terminator sequences.

In E.coli promoters, the site consists of two consensus motifs, the -10 sequences, which is otherwise Pribnow's ox, and the -35 sequnce, which interact directly with the sigma factor. The following diagram showing the prokaryotic promoter region of DNA template of transcription region.

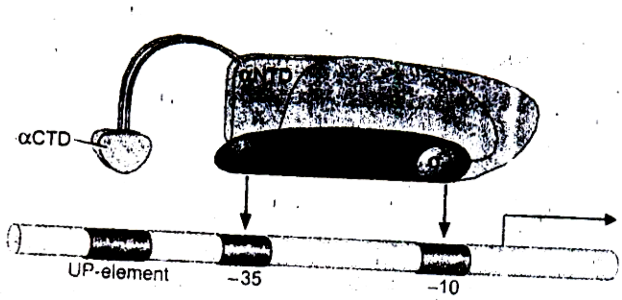


FIGURE 12-7 σ and α subunits recruit RNA polymerase core enzyme to the promoter. The C terminal domain of the α subunit (αCTD) recognizes the UP-element (where present), while σ regions 2 and 4 recognize the −10 and −35 regions respectively (see Figure 12-6). In this figure, RNA polymerase is shown in a rather different schematic form than presented in earlier figures. This form is particularly useful for indicating surfaces that touch DNA and regulating proteins and we use it again in some figures in Chapter 16 when we consider regulation of transcription in bacteria.

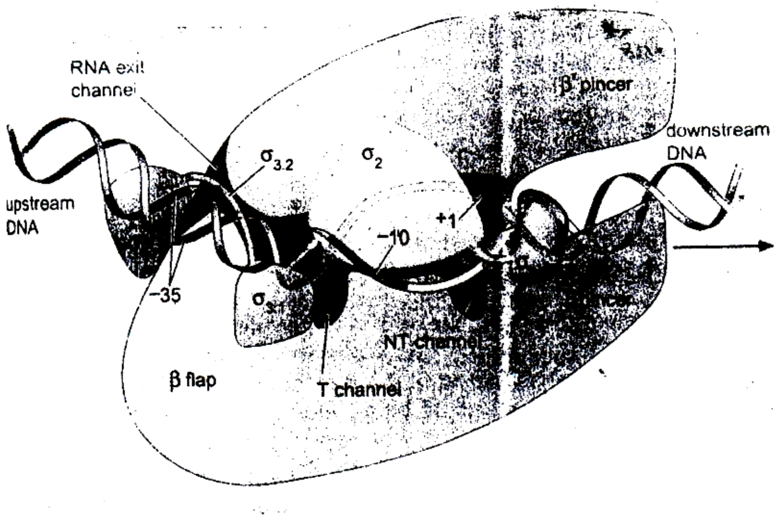


FIGURE 12-8 Channels into and out of the open complex. [illegible] relative positions of the DNA [illegible] strand in gray, nontemplate strand [illegible] the four regions of σ, the −10 and [illegible] of the promoter and the start site of transcription (+1). The channels through which DNA and RNA enter or leave the RNA polymerase enzyme are also shown. The only channel not shown here is the nucleotide entry channel through which nucleotides enter the active site cleft for incorporation into the RNA [illegible] made. As drawn, that channel [illegible] active site down into the page [illegible] tion shown as "+1" on the [illegible] strand passes underneath [illegible] as a dotted ribbon. [illegible] linker region between [illegible]

Sequences prior to the start site are called upstream, and those after the start site are known as downstream sequences. The upstream sequences are denoted y a minus sign, ie. -1, -2 and so on. The downstream sequences are denoted by a plus sign, such as +1, +2 and so on and conventionally written from 5′ to 3′ in the transcription proceeds in this direction.

Many promoters have been reported in E.coli but only two sites are conserved for transcription. Between two prokaryotic promoters, one is located about 10 nucleotides upstream of where the transcription begins and the other one located about 35 nucleotides upstream. Just upstream of the start site, a 6 bp sequence is identified in almost all the prokaryotic promoters. The center of the hexamer is close to 10 bp upstream of the start site, the distance varies from position -18 to -19. The hexamer is often called TATA box or Pribnow box. The -10 sequence is TATAAT or consensus sequence. The sequence of nucleotides at each position is conserved, and the percentage varies from 45 to 96%.

Another conserved sequence is located around -35 upstream of the start site, which is TTGACA nucleotide sequence. In 90% of the promoters, a distance of 16 and 18 bp separates -35 and -10 sites. The following diagram shows the prokaryotic promoter region.

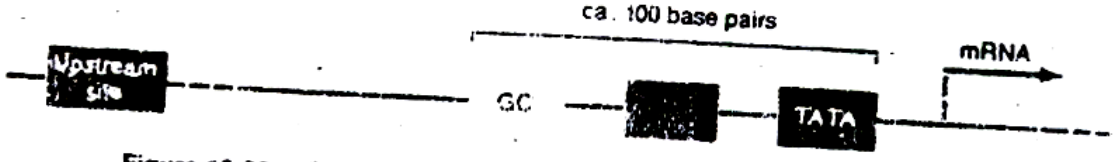


Figure 12-28 Sequences found in and near some RNA polymerase II promoters. Only the TATA box is represented in almost all promoters. The CAAT box occurs much less frequently, and the GC box has only occasionally been observed. An upstream site is very common but is not considered to be part of the promoter.

iii. Eukaryotic promoters

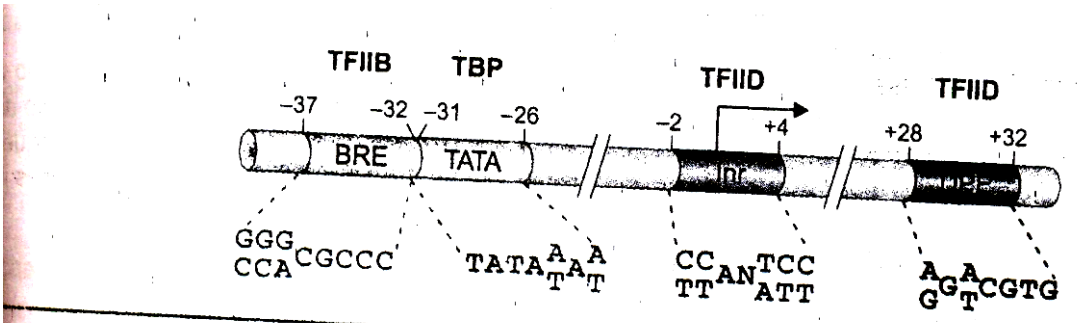
In eukaryotic DNA transcription, the RNA polymerase II cannot initiate transcription itself, but is absolutely dependent on some transcription factors. The enzyme along with these factors constitutes the basic transcription apparatus. Additional factors are required to recognize specific promoters or group of promoters.

1. Class I promoters which are required for initiation of transcription;
2. Class II promoters which participate late in elongation of RNA transcripts; and
3. Class III promoters, which assist in the formation of an active pre-initiation complex but may not be required after initiation of transcription.

The first two types are called general transcription factors that are required for all the genes transcribed by a particular RNA polymerase. The factors belonging to the third category help in regulation of transcription of particular genes. Promoter elements usually serve as recognition sequences for DNA-binding proteins and constitute DNA sequence, which is recognized by RNA polymerase. The structure of eukaryotic promoters is quite complex and their identification considerably difficult.

Class II promoters that correspond to RNA pol II comprise of two to three short conserved regions in their upstream sequences from the start site, whose consensus is TATAAAT, commonly known as TATA or Hogness box, CAAT box, and GC box. TATA box is about 25 bp upstream from the transcription start site, and is usually flanked by G+C sequences. The Hogness box is comparable to the Pribnow box. The consensus sequence of the second module is GCCCAATCT also called as CAAT box is about -75 bp upstream, while the third one called GC box, others lack a CAAT box or GC boxes. The choice of start site depends upon the TATA box and its elimination causes the start site error prone. The promoters of RNA pol I and II are located upstream.

The following diagram shows the eukaryotic promoter region of DNA.



Pol II core promoter. The figure shows the positions of various DNA elements transcription start site (indicated by the arrow above the DNA). These elements, described as follows: BRE (TFIIB recognition element); TATA (TATA Box); Inr (initiator element); and dam promoter element). Also shown (below) are th

UNIT – IV

PART – A

1. Describe translation process.

The flow of genetic information from DNA (Nucleotides) through mRNA (messenger RNA) containing nucleotides for synthesis of proteins. The nucleotides of mRNA, which generate the linear sequence of amino acids in, turn to synthesis of proteins, which perform cell functions. The release of proteins in cytoplasm and various cell locations. This sequential process is called as translation.

2. What are ribosomes?

Ribosomes are macromolecular proteins occurred in cytoplasm. Ribosomes are larger in size and more complex in nature. Ribosomes involve and direct the protein synthesis. Generally, 3 types of ribosome molecules are occurring in cell, which are actively, involve in vital cell functions like synthesis of proteins.
Ribosomes of prokaryotes and eukaryotes are in 50S and 60S in size respectively.

3. What are the primary components involved in the translation machinery?

The primary components involve in the translation machinery are;

i. messenger RNA
ii. tRNA
iii. Ribosmes and
iv. amino acyl tRNA.

4. Write brief note on tRNA.

The t RNA is an important molecule require for translation process or amino acid synthesis. This is otherwise called as adopter molecules. The tRNA contain site for amino acid attachment and region called anticodon. This anticodon recognizes the appropriate base sequence (codon) in the mRNA for proper selection of the amino acid. The tRNA attach with ribosomes in 2 sites as 'P' site and the 'A' site.

5. Define codons and anticodons.

Codons are a base sequences (3 nucleotide bases) arranged in the mRNA and is otherwise called as genetic code is the collection of base sequences in mRNA. These codons are corresponds to each amino acid and stop signals for translation.

Anticodons are also a base sequence (3 nucleotide bases) occur in tRNA where the site is recognize the codon appropriate to the amino acid synthesis.

6. What are different RNA molecules involve in protein synthesis?

There are 3 types of RNA molecules involve in protein synthesis. These 3 kinds of RNA are;

i. mRNA
ii. ii. tRNA and
iii. amino acyl tRNA.

7. What is genetic code?

The Genetic code is the collection of base sequences (codons) that are corresponds to each amino acid and stop signals for translation. Genetic codes are 3 letter based or triplets of nucleotides of purine and pyrimidine bases.
e.g. AUG – methionine as well as start codon

8. Write short notes on start codons.

The protein synthesis require for starting the translation process at special code or site as AUG. AUG is the most common start codon. In few cases the GUG also act as start codons. AUG will be code for methionine and GUG will code for methionine when it is in initiating position, otherwise code for valine.

9. What are stop codons?

The protein synthesis or translation process will be stopped once, the particular protein is synthesized. Stop codons are triplet codes used for stop the translation or protein synthesis when it is reached to desired level. Generally, UAA act as stop codons. Sometimes, UAG and UGA also act as stop codons.

10. What is meant by redundant or degenerate code?

More than one type of codes involve for synthesis of one amino acid is called degenerant code. This is otherwise called as synonymous codes or degenerant codes. It is described, as the code is degeneracy. The non-degenerant code would be where there is one to one relationship between amino acid.

11. Write the characteristic feature of genetic code?

The properties of genetic codes are as follows;

1. the code is triplet
2. the code is degenerant
3. the code is non-overlapping
4. the code is commaless
5. it is nonambiguous
6. the code is universal

12. The genetic code is non-ambiguous – explain.

There is no ambiquity about a particular codon. A particular codon always code for same amino acid. In ambiquous, the codon give more than one amino acid and such is not in a case. i.e the code is degenerant as several codons give same amino acid.

13. The genetic code is non-overlapping – justify.

Non-overlapping of genetic code means the base in an mRNA is not used for 2 different codons. E.g. is overlapping code coding 4 amino acid from 6 bases. But, actually 6 bases code only 2 amino acid. Hence, more number of protein can be synthesized from minimum bases. But, mutation in single base affect 3 amino acids. Mutations are deleterious.

14. Mention the synthesis of phenylalanine.

When polyuridylic acid (poly U) is incubated the Polyphenylalanine can be synthesized. The triplet letter 'UUU' responsible for synthesis of phenylalanine.

15. Write short notes on fMet-tRNAf^{met}.

The initiator tRNA requires for initiation, the base pair is 'AUG' or 'GUG' of codons. The start codon 'AUG' is charged on tRNA as modified methionine (N-formyl met) i.e. formyl group attached to NH2 group. This is referred as fMet-tRNAf^{met}.

16. Write the significance of redundancy of code.

Several codons involve in synthesis of same amino acid is called redundancy of genetic code. The biological significance of redundancy of code is minimizing the deleterious effects of mutations. If redundancy is not the case, then 44 of 64 codons would not code for an amino acid –

that is, they would be stop codons. Thus, two thirds of the base changes could not possibly lead to improvement of an organism and the evolutionary process would be seriously limited.

17. What are synonymous codons? Write the features of synonymous codons?

Synonymous codons are more than one codons involve for synthesis of one amino acid. Those codons are called synonymous codons. This is described as the code is degeneracy. The non-degenerate code would be where there is one to one relationship between amino acid. Few nonsense codons are use as stop codons.

e.g. UUU, UUG synonymous for phenylalanine. In fact, when the first two nucleotides are identical, the third nucleotide can be either cytosine or uracil and the codon will still code for the same amino acid.

18. What is adaptor RNA molecule? Why it is called as adaptor RNA molecules?

The tRNA molecule is otherwise called as adaptor molecule.

The tRNA molecules are found to be act as a mediator for amino acid synthesis after recognize the respective site of sequence in mRNA. Hence, it is called as adaptor molecule identified by Crick.

19. Write about the tRNA molecule.

The tRNA appears in cloverleaf structure. It has 74-95 bases. It has one amino acid attachment site, which is 3′ end. Next to that is ribosomal binding site or loop and anticodon loop or anticodon binding site. The third loop is called amino acyl sunthetase binding loop. The ration of A:U and G:C are near unity which suggest the formation double helical segments like DNA.

20. Define ORF (open reading frame).

Open reading frame (ORF) is a nucleotide sequence present in the mRNA, which are translatable for protein synthesis. Each mRNA contains protein-coding sequence, which is composed of contagious, no-overlapping string of codons, which are called an open reading frame. Each ORF specifies a single protein and starts and ends at internal sites within the mRNA.

21. What is polycistronic mRNA?

When the messenger RNA (mRNA) contain more than one Operon reading frame, it is called as polycistron mRNA. Example for polycistronic mRNA is generally prokaryotic and this codes multiple polypeptide chains. E.g. E.coli.

22. What is monocistronic mRNA?

When the messenger RNA (mRNA) is contain single open reading frame (ORF), it is called as monocistronic mRNA. Example is almost all the eukaryotic cells, which contain one ORF, which encode only one polypeptide chain.

23. Write short notes on initiator tRNA.

Initiator tRNA are specialized tRNA charged with modified methionine is binds with small sub unit. The typically charged tRNA first binds on 'A' site and reach 'P' site after a round of peptide bond synthesis. The initiator tRNA requires for initiation of the bas pairs is AUG or GUG codons. These start codon AUG is charged on tRNA as modified methionine (N-formyl met) i.e. formyl group attached to NH2 group. This is referred as fMet-tRNAfmet.

24. Write short notes on eukaryotic translation elongation factors.

The eukaryotic translation elongation factors are three kinds of non-ribosomal proteins involve in chain elongation. These are;

i. EF-Tu – carrier protein
ii. EF-Ts – binary complex protein
iii. EF-G – control the translation process

These comprise in cell about 5-10% soluble protein.

25. Define 'Shine Delgarno site'.

The prokaryotic mRNA containing short sequene upstream (on the 5′ site) of the start codon in the open reading frame is referred ribosome binding site (RBS) for recruiting ribosome for protein synthesis. This ribosome binding site or element also referred to as a Shine-Dalgarno sequence after the scientists who discovered.

26. Define circle pattern of protein synthesis on mRNA

The circle pattern of protein synthesis on mRNA is due to the close association of initiation factors with 3′ end of mRNA through its poly – A tail. This is mediated by interaction between eIF4F and the poly A binding proteins that coats poly A tail. Circular configuration is the bridge between the 5′ and 3′ end of the molecules.

27. What are chain release factors?

When stop codons enters in the 'A' site, stimulates the Release Factors (RF) by ribosome in unknown way. These release factors are otherwise called as chain release factors, which are responsible for chain termination during translation process. There are three types of chain release factors were studied as;
RF1, RF2 and RF3.

28. Write short notes on initiation factors of translation process.

Three initiation factors (IF) are direct the assembly of initiation complex contain mRNA and initiator tRNA. The ribosome small sub unit catalyzed by 3 translation initiation factors called as;
IF1 – prevents tRNA from binding to the portion of the small sub unit and become part of A site.
IF2 – is a GTP ase (binds and hyrolyse the GTP)
IF3 – binds to the small sub unit and block its from reassociating with a large sub unit.

29. Which of the two amino acids have single codon? Give their codon sequence.

The amino acids Leucine and Serine are have single codon. The codon sequence is CUG.

30. Write short notes on initiator tRNA of E.coli.

The initiator tRNA of Prokaryotic cell (E.coli) have formyl group on their mehtionine. The enzyme deformylase removes formyl group after synthesis of polypeptide chain. Amino peptidases after remove amino tertiary methionine. The start codon AUG is charged on tRNA as modified methionine i.e. formyl group attached to NH2 group.

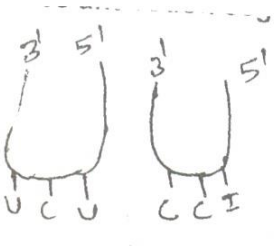
31. What is formylation?

Formylation is nothing but the charge of start codon with modified methionine (N-formyl methionine) i.e. formyl group attached to NH2 group. This is referred as formylated methionine charged on tRNA formylated methionine.

32. What is frameshift mutation?

Frameshit mutation is a kind of point mutation. This kind of mutations is insertions or deletions of one or a small number of base pairs that alter the reading frame.

33. Write three bases anticodon segment in the tRNAs specific for adenine.



UCU (or)

CCI can pair with adenine

34. Mention the factors that affect translation accuracy.

i. Charged tRNA
ii. Positioning of ribosome over start codon
iii. Translation initiation factors
iv. GTPase binding proteins
v. Elangation factors

35. Explain in vitro translation system with two examples.

i. If poly uridiylic acid (poly U) is incubated, the polyphenylalanine was synthesized. This proves, the triplet 'UUU' codon corresponds to amino acid phenylalanine. This basic experiment allows to identify all 'U' containing bases.
ii. If a single guanine (G) is added to poly 'U' chain, it was observed as polyphenylalanine as terminated by leucine. Thus, UUG must be leucine codon.

36. Write short notes on amino acyl tRNA synthetase.

Amino acyl synthetase are the enzymes which catalyze the formation of amino acyl tRNA. Each enzyme recognizes specific sites (recognition site and amino acid attachment site) of each tRNA molecule and each one of 20 amino acids. Therefore, appropriate amino acid will be attached to the correct tRNA molecule with the help of this enzyme.

37. Write the difference between eukaryotic ORF and prokaryotic ORF.

In Eukaryotic mRNA, only one open reading frame (ORF) is present. It is called as monocistronic mRNA.

In prokaryotic mRNA, many open reading frame (ORF) are present, which is called as polycistronic mRNA.

38. Write the role of GTP in translation process.

During elongation process of translation, the ribosomal proteins having GTPase activity cause hydrolysis of GTP-EF-Tu to yield bound GDP and free Pi. The GDP converted as GTP for the next

round of reaction in the internal metabolic reaction. The sole function of EF-Ts complex is regeneration GTP or conversion of GDP to GTP. The EF-Ts is otherwise called as GTP exchange factor.

39. What are suppressor mutations?

Often, the effects of harmful mutations can be reversed by a second genetic change. Some of these subsequent mutations are easy to understand, being simple rears (back) mutations, which change an altered nucleotide sequence back to its original arrangement. More difficult to understand are the mutations occurring at different locations on the chromosome that suppress the change due to a mutation at site A by producing an additional genetic change at site B. Such suppressor mutations fall into two main categories as intragenic suppression and intergenic suppression.

40. Write short notes on mis-sense suppressor mutations.

Misense suppressor mutation is a kind of point mutation in which an alteration that changes a codon specific for one amino acid to a codon specific for another amino acid. As a consequence, a gene bearing a missense mutation produces a protein product in which a single amino acid has been substituted for another. E.g. sickle cell anemia, in which glutamate 6 in the beta globin subunit of hemoglobin has been replace with a valine.

41. Write short notes on non-sense suppressor mutations.

This is a kind of point mutation. A more drastic effects results from an alteration causing a change to a chain- termination codon, which is known as a nonsense or stop mutation. When a nonsense mutation arises in the middle of a genetic message, an incomplete polypeptide is released from the ribosome owing to premature chain termination. The size of the incomplete polypeptide chain depends on the location of the nonsense mutation.

42. Write brief note on wobble hypothesis.

Crick explained wobbling of genetic code. It describes the response of same tRNA molecules to several codons and pattern of no redundancy code. I.e. is differing in the third phase (position). If 'U; is in the first position of anticodon it can pair with either A or G at the third position of codon. Wobblilng pairs are;

Anticodon I position	Codon III position
U	A,G
C	G
A	U
G	U,C
I	U,C,A

PART – B

1. Support the genetic coding system with suitable experimental evidences.

Genetic code is the collection of base sequences (codons) that are corresponds to each amino acid and stop signals for translation. There are 20 amino acids available in organism. So, more than 20 codons to be included in us for

i. Start
ii. Stop and
iii. For particular protein synthesis

So, minimum of 20 codons to be involved for 20 amino acid synthesis. The following aspects to be known before understanding the genetic code.

i. Single base can not be codon because only 4 bases
ii. Pair of bases also cannot, because (4^2 = 16) only 16 codons available.
iii. Triplets may be possible, because 4^3 = 64, triplets which more than adequate.

It is proved all the 64 codons involve in translation process and carry some meaning as;

i. Stop codons
ii. Start codons and
iii. Protein synthesis codons.

Several codons carry same meaning and code for same amino acid that is called redundant or degenerate code.

Properties of Genetic code

The properties of genetic code are given below which are proved by evidence. These are;

1. the code is triplet
2. the code is degenerate
3. the code is non-overlapping
4. the code is commaless
5. the code is non-ambiguous and
6. the code is universal.

1. **The code is triplet**

Triplet code system is minimum required and if it is more, than e excess and no meaning. The excess 44 codes out of 20 amino acids is also utilized for mean the same amino acid. The tiplet letters are responsible for synthesis of individual amino acids. That is the nucleotide letters are arranged in a regular fashion and every 3 letters (codon) of messenger RNA are involve in pairing with tRNA anticodons site and synthesis of amino acids. E.g. UUU for phenylalanine.

2. **The code is degenerate**

More than one code involve from one amino acid (Synonymous). This is described, as the code is degeneracy. The non-degenerate cod would be where there is one to one relationship between amino acid. So few nonsense codons are as stop codons.

3. **The code is non-overlapping**

Non-overlapping means, the base in a mRNA is not used for 2 different codons. E.g. in overlapping code, coding 4 amino acid from 6 bases. But, actually 6 bases code only 2 amino acid. The following scheme represents the non-overlapping of codes.

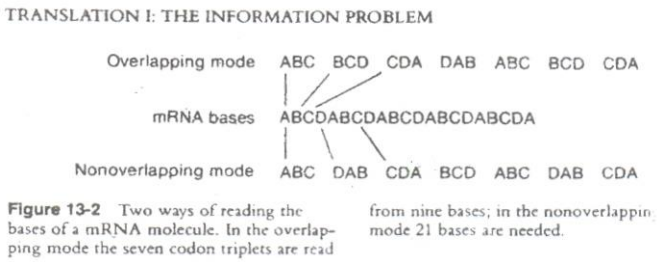


Figure 13-2 Two ways of reading the bases of a mRNA molecule. In the overlapping mode the seven codon triplets are read from nine bases; in the nonoverlapping mode 21 bases are needed.

The advantage of overlapping is more number of proteins can be synthesized from minimum bases. But mutation in single base affect 3 amino acids. Mutations are deleterious. But, the experimental evidence shows that the mutation affects only one protein, not 3 proteins. Hence it proves, the non-overlapping of codes.

4. **The code is commaless**

No punctuation is needed between any 2 words. Otherwise, after one amino acid is coded, the second amino acid will automatically coded by next 3 letters and there is no letter is wasted for some purposes.

CAT GAT - non-overlapping without comma

CAT GAT - non-overlapping with comma.

5. **The code is ambiquous**

There is no ambiquity about a particular codon. A particular codon always code same amino acid. In ambiquous, the same codon give more than one amino acid. Such is not in a case. I.e. the code is degenerate as several codons give same amino acid. Exceptions are AUG and GUG. Both code for methionine as initiating or start codons although GUG is meant for valine. Moreover, different codes exist in mitochondria of some eukaryotes. So, that is cytoplasm and mitochondria same codon code for different amino acids. This is not ambiquity. In mitochondria, there is separate genetic code which is differs from universal code in some essential repects.

6. **The code is universal**

Based on experimental evidences, the genetic code is same as in microorganisms, (Bacteria, yeast), microorganisms like non flowering plants (algae, bryophytes, Pteridophytes), Plants and animals including mammals. In this, numerous genes, as if protein products, the base sequences , amino acid sequences core were compared. However, different and more primitive genetic code exists in mitochondria of some organisms.

UCAG X UCAG X UCAG = 64 Codons yield 20 amino acids including stop and start codons.

2. How will you elucidate the genetic coding system.

Nuremberg Khorana and Mathieu initiated the first step on elucidation of genetic coding system in 1961 for artificial synthesis of amino acid. That is for elucidation of Genetic code. The requirements were, mixer of ribosomes, tRNA molecules, radio active amino acids, a cell fraction of E.coli for protein synthesis.

I. The synthetic polynucleotide – polyuridylic acid (poly U) was incubated and polyphenylalanine was synthesized. This proves, the triplet 'UUU' codon corresponds to amino acid phenylalanine. This was a great new all over the world for scientific community in 1961. This basic experiment allows to identify all 'U' containing codons.

II. For example, if a single quanine (G) is added to poly 'U' chain, it was observed as polyphylalanine was terminated by leucine. Thus, 'UUG' must be leucine codon. If many 'G' were added at the terminus, the polyphenylalanine is terminated sometimes by glycine. Thus 'GGG' corresponds 'glycine'. At low frequency leucine and tryptophan also found COOH-terminus of the polyphenylalanine. These must be come true 'UUG' and 'UGG' at the transition point between 'U' and 'G'. Thus UUG is for leucine and UGG is for typtophan codons.

III. Similar experiments also carried out with mixed polymers of uridine, adenosine and cystidine. Poly U,A and poly U,C to identify the remaining U- containing codons.

IV. Other homopolymers were also studied. Poly (A) results in the formation of polylysine. This indicates that 'AAA' is lysine codon. Mixed polymers of this type just also were studied to identify 'A' containing codons.

V. Similarly poly 'C' was found to produce, polyproline.

VI. Poly 'G' fails to act as synthetic mRNA, because it form 'triple stranded helix' and cannot be translated.

Other experiments were also performed to identify codons that couldn't be identified as earliest procedure.

VII. For instance alternating polymer, poly 'AC' that is as …ACACAC….this read in groups of 3 codons, as

ACA CAC ACA CAC ACA C.. – This make alternate amino acid as threonine and histidine as Thr, His, Thr, His.

VIII. From other experiments, it is known that threonine has a 2A and 1C codon and histidine has a 2C and 1A codon. So, codon assignment as Thr = ACA and His = CAC, could be made.

IX. In another type of experiments, synthetic trinucleotides were observed as to stimulate and binding of tRNA to ribosome. If mixer is prepared consist of ribosomes, all of the tRNA molecules, and the 'UUU' trinucleotide, and than ribosomes are isolated and it is found that only phenlylanyl-tRNA ^phe is associated with the ribosomes in any appreciable amount. These types of experiments were repeated for all the 64 amino acids (nucleotides). In most cases, it is found unambiquous results were obtained and 50 codon – confirmed in that way.

3. Genetic code is univeral – explain.

The results of large-scale sequencing of genomes have largely confirmed the expected universality of the genetic code. The universality of the code has had a huge impact on our understanding of evolution as it made it possible to directly compare protein-coding sequences among all organisms for which a genome sequence is available. As we shall know, powerful computer programs are available that can search for and identify similarities among predicted coding sequences from a wide range of organisms. The universality of the code also helped to create the field of genetic engineering by making it possible to express cloned copies of genes encoding useful protein products in surrogate host organisms, such as the production of human insulin bacteria.

To understand the conservative nature of the code, consider what might happen if a mutation changed the genetic code. Such a mutation might, for example, alter the sequence of the serine tRNA molecule of the class that corresponds to UCU, causing them to recognize UUU sequences instead. This would be a lethal mutation in haploid cells containing only one gene directing the production of tRNA^ser, for serine would not be inserted into many of its normal positions in proteins. Even if there were more than one gene for tRNA^ser (as in diploid cell), this type of mutation would still be lethal since it would cause the simultaneous replacement of many phenylalanine residues by serine in cell proteins.

In view of what we have just said, it was completely unexpected find that in certain subcellular organelles, the genetic code is in fact slightly, different from the standard. This realization came during the elucidation of the entire DNA sequence of the 16,569-base pair human mitochondrial genome but is observed for mitochondria in yeast, the fruit fly, and higher plants. Sequences of the regions known to specify proteins have revealed the following differences between the standard and mitochondrial genetic codes.

i. UGA is not a stop signal but codes for tryptophan. Hence, the anticodon of mitochondrial tRNA^trp recognizes both UGG and UGA, as if obeying the traditional wobble rules.

ii. Internal methionine is encoded by both AUG and AUA.

iii. In mammalian mitochondria, AGA and AGG are not arginine codons (of which there are six in the "universal" code) but specify chain termination. Thus, there are four stop codons like UAA, UAG, AGA and AGG in the mammalian mitochondrial code.

iv. In fruit fly mitochondria, AGA and AGG are also not arginine codons but specify serine.

Perhaps not surprisingly, mitochondrial tRNAs are unusual with respect to the rules by which they decode mitochondrial messages. Only 22 tRNAs are present in mammalian mitochondria , whereas a minimum of 32 tRNA molecules are required to decode the 'universal'' code according to the wobble rules. Consequently, when an amino acid is specified by four codons (with the same first and second positions), only a single mitochondrial tRNA is involved. Such mitochondrial tRNAs all have in the 5′ (wobble) position of their anticodons a U residue, which is able to engage in pairing with any of the four nucleotides in the third codon position. In cases where purines in the third position of the codon correspond to different amino acids from pyrimidines in that position, a modified U in the firs t position of the anticodon of the mitochondirial tRNA restricts wobble to pairing with the two purines only.

Exceptions to the "universal" code are not limited to mitochondria but also found in several prokaryotic genomes and in the nuclear genomes of certain eukaryotes. The bacterium Mycoplasma capricolum uses UGA as a tryptophan codon rather than a chain-termination codon. Likewise, some unicellular protozoa use UAA and UAG, which are stop codons in the "universal"

code, as glutamine codons. Finally, a codon (CUG) for one amino acid (leucine) in the "universal" code has become a codon for another amino acid (serine).

4. Write briefly on aminoacyl synthetases (aas).

Aminoacyle-tRNA synthetases are xtremely divers group of proteins with respect to both size and number of their polypeptide subunits. The enzyme normally has a molecular weight of 1,00,000 except the one for phenylalanine, which has a molecular weight of 1,80,000. All enzymes have an absolute requirement for mg^2+ and all contain one or more –SH groups that are essential for the activity.

Aminoacyl-tRNA synthetases are enzymes catalyze the formation of amino acyl-tRNA. Each enzyme recognizes specific sites (recognition site and amino acid attachement site) of each tRNA molecule and each one of 20 amino acids. So, appropriate amino acid will be attached to the correct tRNA molecule. The tRNA molecules has to recognize i. nucleic acids ie. Ribosomal RNA in the ribosome and codons in mRNA, ii. Proteins a translation factors and amino acyl synthetase (qqRs). The amino acyl synthetase recognition is more important because it gives an aminoacyl tRNAs. The sub unit of synthetase is holoenzyme is 54 to 380 KD, which vary from 33 KD to 113 KD. These may monomeric, Dimeric or terameric i.e heterogenous proteins. The active siste of an aminoacyl-tRNA synthetase is a rather small part of the enzyme.

Aminoacyl-tRNA synthetases are divided into two classes as;

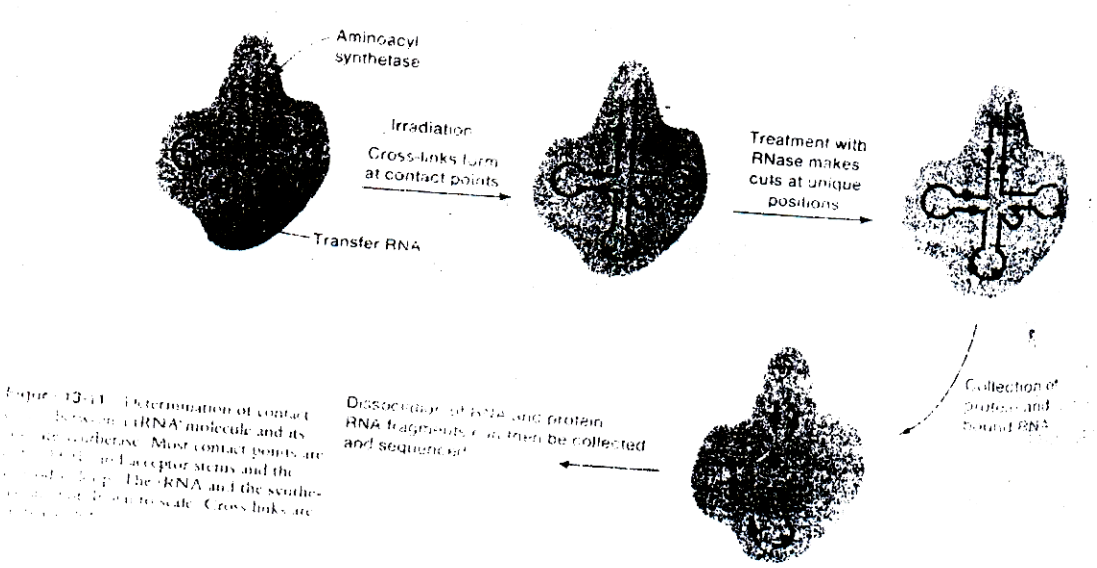
1. Class I recogises larger amino acids (10 amino acids), and
2. Class II recognizes the smaller amino acids (10 amino acids).

Each class has different methods of recognizing tRNA and different folding (amino acid sequence). The reactin mechanism of aminoacyl-tRNA synthetase involves 12 hydrogen bonds in the case of tyrosine, with the ezyme to substrate that position the amono acid R group, alpha amino, alpha-carboxyl, phosphodiester linkage and adenine moiety. The reaction occurs by the attack of the carboxyl group of trrosine on the alpha-phosphate of ATP, releasing PPi and forming aminoacyladenylate (amino acyl-AMP). Subsequently, the amino acyl portion of the adenylate is transferred to the tRNA.

Class I synthetases have N-terminal catalytic domain consist 2 conserved sequences describe as 'Signature sequences'. They useful for ATP binding or amino acid sites. The C-terminus of this class useful for binding of anticodon of tRNA between N-terminus and C-terminus, a domain found for binding of acceptor helix of tRNA.

Class II synthetase has C-terminal catalytic domain consist of lare antiparallel beta strand surrounded by alpha helix N-termina domain is meant for anticodon binding.

The following diagram represents the amino acyl tRNA-synthetse.



The aminn;acyl-tRNA synthetases are so specific in discriminating among the naturally occurring amino acids that there is much less than one error in 10,000 reactions. The enzyme itself proof reads mistakenly coupled amino acids and hydrolyses them from the tRNA. The two catalytic activities, coupling the aino acid to the tRNA and hydrolysis of mistakes, are located very close to each other, and recognition of the mistakes is dictated by the sizxe and shape of the substrate binding motifs.

The tRNA molecules can recognize more than one codon due to the wobble position, such as GCU, GCC and GCA in yeast alanine tRNA. The codons that differe in either of the first two bases are recognized by different tRNAs.

5. Explain the translation process of prokaryotic cells with sitable diagram.

The mechanism of translation process in prokaryotic cells is mainly found as three evens as;

1. Initiation
2. Elongation and
3. Termination

Initiation

In initiation process, three evens mainly occurred as
i.ribosome must be recruit to mRNA;
ii. Charged tRNA must be placed in 'P' site of the ribosome
iii. The ribosome positioned over the 'start codon'

The correct positioning is very critical and is regulated by various molecules occur in cells.

Assembly of sub unit of ribosome is one at a time. First small sub unit associate by base-pairing interactions between ribosome binding site of mRNA and 16 S rRNA. In the correctly positoned ribosome, the start codon will be on 'P' site. Then the large sub unit joined to form a complex.

The specilised tRNA charged with modified methionine is binds with small subunit. The typically charged tRNA first binds on 'A' site and reach 'P' site after a round of peptide bond synthesis.

Iniitator tRNA requires for initiaton, the bas pairs is 'AUG' or 'GUG' of codons. The start codon 'AUG' is charged on tRNA as modified methionine (N-formyl methionine). I.e. formyl group attached to NH2 group. This is referred as fMet-tRNAf^met. All prokaryotes have formyl group on their methionine.

The enzyme deformylase removes formyl group after synthesis of polypeptide chain. Amino peptidases then remove amino terminus methionine.

Three initiaion factors (Ifs) direct the assembly of initiation complex contain mRNA and initiator tRNA. The small ribosome sub unit catalysed by 3 translation initiation factors called IF1, IF2 and IF3. IF1 prevents tRNAs from binding to the potion of the small subunit and become part of 'A' site. IF2 is a GTPase (binds and hydrollyse the GTP) that interacts with 3 key components as; i. The small sub unit, ii. IF1 and iii. The charged initiator tRNA (fMet-tRNAf^fMet). IF3 binds to the small sub unit and block its from reassociating with a large sub unit. Among the 3 potential tRNA binding sites on a small sub unit, only the 'P' site is capable of binding tRNA in the presence of initiation factors.

With all three initiatin factors bound the small sub unit prepared to bind to the mRNA and initiator tRNA. Binding mRNA mainly involves, base pairing between ribosome binding site and the 16S RNA in the small subunit.

Meanwhile binding of fMet-tRNAi^fMet to the small sub unit is facilitated by its interactin with IF2 and bound to GTP and base pairing between the anticodon and start codon of ('AUG') of mRNA.

The last step is the association of the large subunit ribosome and create 70S initiation complex. The binding of large subunits stimulates the GTPase activity of IF2-GTP and IF1 from the ribosome. The IF2-GTP reduced the affinity and rease of IF2GTP and IF1 from ribosome.

The net result is formation of 70S ribosome assembled at start site of mRNA with fMet-tRNAi^fmet in the 'P'site and empty 'A' site. The ribosome-mRNA complex enhances polypeptide synthesis.

Elongation

The elongation of translation in both prokaryotes and eukaryotes are more or less same. There are mainly 3 events occur in the elongation of prokaryotes.

1. The correct amino acyl-tRNA is loaded into the 'A' site of ribosome by 'A' site codon. The formation of peptide bond between amino acyl tRNA and peptidyl tRNA of 'A' site and 'P' site. Peptyl transferease reaction occurs in this process. Translocation of peptidyl tRNA to 'P' site involve in another cycle of peptide bond formation. Three non-ribosomal proteins called elongation factors, which are involved in chain elongation, are i. EF-Tu, ii. EF-TS and iii. EF-G. These comprise in cell about 5-10% soluble proteins. EF-Tu, a carrier protein needed for the fbinding of charged tRNA to 'A' site of an active 70S ribosome. Interaction of GTP with EF-Tu to form binary complex and allows charged tRNA to bind and for ternary complex. Ternary complex bind on 'A' site and stabilization of this complex on 'A' site. Ribosome proteis having GTPase activity cause hydrolysis of GTP-EF-Tu to yield boiynd GDP and free Pi. The GDP-(EF-Tu) co;mplex lead dissociation from tRNA and leaves acylated tRNA in the 'A' site. Removal of EF-Tu is necessary for peptide bond formation.

Then, EF-TS binds with EF-Tu and displaces the GDP. The (EF-TS) – (EF-Tu) intermediate complex forms. The GDP converted as GTP for next round of reaction in the internal metabolite reaction. The GTP joins in the binary complex formation in the next cycle of elongation. The sole function of EF-TS is regeneration of GTP or conversion of GDP to GTP. EF-TS otherwise called GTP exchange factor. Now both the P and A sites are occupied and a peptide bond cans forms.

Peptidyl transferase activity involved the reaction occurs at 50S ribosomal sub unit. It is known as peptidyl transferease center. About 10 different 50S ribosomal protein are involved in the reaction and formation of active site. Then cleavage of bond connecting fMet and tRNA^fMet, the enzyme tRNA deacylase in a ribosomal component. Deacylated tRNa poorly bind with 'P' site and finally leaves ribosome immediately. Then peptidyl-tRNA situated in 'A' site is weakened and strong with P site hence movers from 'A' site to 'P' site. The movement is transloaction does occur. This dissociation not random. So, the third elongation factor EF-G controls their translocation process. Here one molecule of ATP and two molecules of GTP conserved.

6. Describe the translation system of eukaryotic cells with suitable diagram.

The flow of genetic information from DNA (nucleotides throughmRNA (messenger RNA) containing nucleotides which generate the linear sequence of maino acids in turn to synthesis of protein which perform cell functions is called translation process. That is otherwise called as synthesis of protein molcecules in a cell is directed by intracellular DNA through mRNA and release of proteins in cytoplasm and various cell locations.

There are two aspects mainly involve in translation process as; i. information or coding problem and ii. The chemical problem. The information problem means, the base sequence in a DNA molecule is translated into amino acid sequence of a; polypeptide chain. The chemical problem is the actual process of synthesis of; proteins.

Generally, the translation process involved with three major evens as;

a. Initiation
b. Elongation and
c. Termination.

Eukaryotic translation

The small sub unit is already bind with an initiator tRNA and then it is recruited by 5′ cap end of the mRNA. This complex scans mRNA in 5′ – 3′ directin until it reaches 5′ – AUG 3′. The first 'AUG' is a start site and single polypeptide is synthesized. So, most of eukaryotic RNAs are monocistronic and encode single polypeptide.

More than 30 polypeptides or proteins include many initiation factors like IF1, IF2 and IF3 etc. are required for initiation process. The other initiatin factors are eIF1, eIF2, eIF3.

Two GTP binding protein as eIF2 and eIF5B mediate the recruitment of initiator tRNA . The tRNA is the Met-tRNAi. The eIF2-GTP and EiF5B-GTP are associate with small subunit on eIF1A dependant manner on MetttRNAi^Met position in 'P' site of small sub unit and forms formation 43 S pre-iitiation complex.

The preinitiatin complex joined with eIF4B, which activates RNA helicase, which unwind hairpin structure of mRNA. The start codons is found by scanning downstream for 5′ end of mRNA of the small sub unit and its complex move along mRNA in a 6′ → 3′ directin in an ATP-dependant process driven by eIF4F associated with helilcase.

The first 'AUG' recognized thrugh base pairing between anticodon of tRNA and start codon of mRNA. Then the base pairing of large sub unit with small sub unit and release of other iF5 by stimulating GTP hydrolysis. As a result MettRNAi^Met is place in the 'P' site of resulting 80S initiation complex. Then the formation of first peptide bond takesplace.

The translation initiation factors hold eukaryotic mRNA in cirles pattern.

The initiation factors are closely associated with 3′ end of mRNA through its ply-A tail. This is mediated by interaction between eIF4F and the poly A binding proteins that coats poly A tail. Circular configuration is the bridge between the 5′ end of the molecules on the same mRNA, the ribosome repeatedly involve in translation process.

The elongation of translation process of both prokaryotic and eukaryotic DNA is more or less same mechanism.

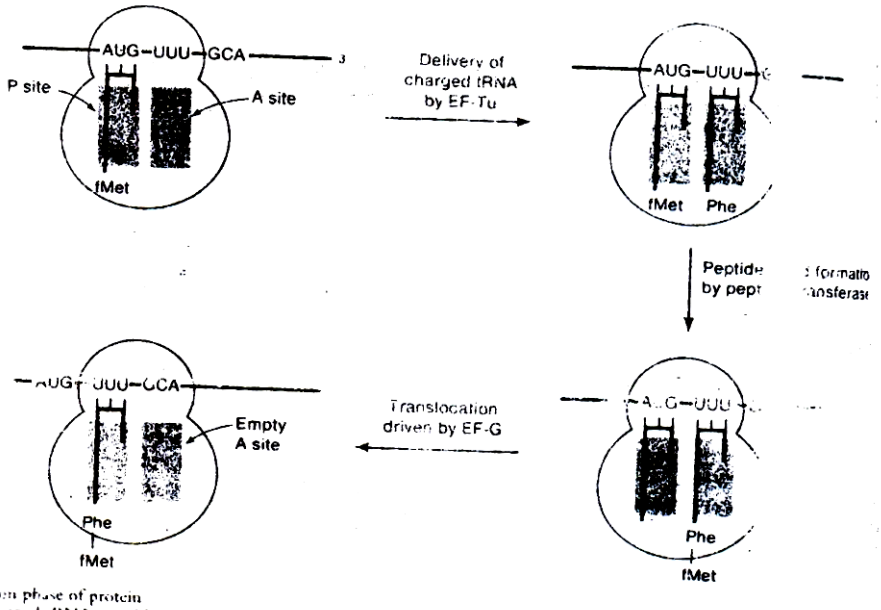


Figure 14-8 Elongation phase of protein synthesis: binding of charged tRNA, peptide bond formation, and translocation

Chain termination

In the termination of translation chain, when stop codons enter in the 'A' site, stimulates the release factors (RF) by ribosome in unknown way. There are different release factors like class I (RF1 & RF2), which activates the hydrolysis of polypeptide from the peptidyl-tRNA. RF1 recognizes the 'UAG' stop codons and RF2 recognizes the 'UGA' stop codons. Both the above class I release factors recognizes 'UAA' stop codons.

The class ii release factors as RF3 stimulate the dissociation of the calls I release factors from ribosome after release of the polypeptide chain. In both prokayotes and eukaryotes, only one-class II factors called RF3 or eRF3. RF1 contain 323 amino acids and RF2 contain 339 amino acids and totally about 35% homologous. These bind ribosome and recognize stop codons and interact with peptidyl transferease complex.

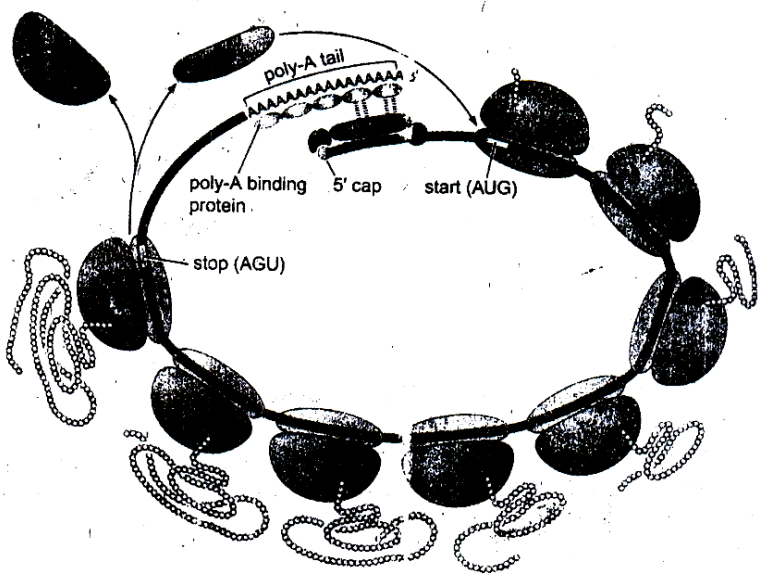
The GTP is an energy rich molecule facilitates the binding of proeins factors either to tRNA or ribosome. Hydrolysis of GTP to GDP and Pi always dissociation of bound factor. GTP is allosteric effectors make confirmation changes of complex. GTP is used in binding reaction and in translocation. But ATP is used for peptide bond formation.

7. Write short notes on circling of translation initiation factors on eukaryotic mRNA.

Translation initiation factors hold eukaryotic mRNAs in cirles during translation process. In addition binding to the 5′ end of eukaryotic mRNAs, the initiation factors are closely associated with the 3′ end of the mRNA through its poly-A tail. This is mediated by an interaction between eIF4F and the poly-A binding protein that coats the poly-A tail. A consistant interaction between the two ends occurs because both eIF4F and the poly-A binding protein are bound to the mRNA through multiple rounds of translation. The interaction between these proteins results in the mRNA being held in a circular configuration via a protein bridge netween the 5′ and 3′ ends of the molecule. It has long been known that the poly-A tail contributes to efficient translation of mRNA. The finding that translation initiation factors"circularize" mRNA in a poly-A dependent manner provides a simple rationale for this observation: once a ribosome finishes translating an mRNA that is circularized via its poly-A tail, the newly released ribosome is ideally positioned to re-initiate translation on the same mRNA.

The following diagram indicates the process steps of circularization of eukaryotic mRNA.

29 A model for the . of eukaryotic mRNA. Circu- osed to be mediated by an in- n the eIF4G subunit of eIF4F nding protein.



8. Write in detail on posttranslational modification of proteins.

Once translation process is completed, the necessary proteins for the cell functions are synthesized. However, the cell need modification of proteins from which is synthesized.

Proteolytic cleavage

In prokaryotes fMet is never retained as the nH2-terrminal amino acid. In roughly half of all proteins the fromyl group is removed by the enzyme deformylase, which leaves methionine as the fNH2-terminal amino acid.

In both prokaryotes and eukaryotes the fMet or Met and other amino acids are often removed by aminopeptidase by hydrolysis. This hydrolysis may sometimes occur while the chain is being synthesized and sometimes after the chain is released from the ribosome. The choice of deformylation versus removal of fMet usually depends on the identity of the adjacent amino acids. That is deformylation predominates if the second amino acid is arginine, asperageine, aspartic acid, glysine, isoluesine or lysine, whereas fMet is usually removed if the adjacent amino acid is alanine, glycine, proline, Threonine and valine.

Protein acetylation and methylation

Newly created NH2-terminal and amino acids are sometimes acetylated.

Post translation methylation occurs at lysine residues in some proteins such as calmodulin and cytochrome C. S-adenosylmethionine is the active methyl donor.

Phosphorylation

Modification of Aamino aciid chain occurred in Collagen – which contains that large fraction of proline and lysine are hydroxylated. Phosphorylation occurs in serine, tyrosine and threonine. Also various sugars may be attached to the free hydroxyl group of serine or thronine to form glycoproteins. Finally a variety of prosthetic fgroups such as fheme and biotin are covalently attached to some enzymes.

Oxidation

Oxidation of sulfhydryl group of cysteine to form disulfide bond.

Cleavage of polypeptide chain at specific sites. Conversion of Chymotripnogen to chymostrypsa digestive enzyme by removal of 4 amino acids in 2 different sites. Some caes uncleaved proteins used as storage for future. .i.e. pepsinogen to pepsin.

Polyprotein is cleaved proteins of infected polioviruses produced huge proteins in host and cleaved. Poly proteins are active proteins.

Vitamin – dependant modifications

Certain vitamins function as cofactors and modify proteins. For example, vitamins C-dependant modifications include hydroxylation of proline and ysine, and amidation carboxy terminal. The enzymes proly hydroxylase and lysyl hydroxylase hydroxylate these amino axids, and gllycine acts as a donor of amide group at the C-terminal. Some examples of hydroxylated proteins are collagens, and that amidation at the C-terminal are oxytocin and vasopressin.

Vitamin K-dependant modification includes carboxylation of glutamine residues as in case of g-carboxyglutamate.

9. Write the structure and features of tRNA.

Generally all tRNA molecules are small, single-stranded nucleic acids ranging in size from 73 to 93 nucleotides. The base sequence of a tRNA, namely, yeast tRNA^ala was first determined in 1965. It was noticed that several segments of this molecule could form double-stranded regions that would give the molecule a cloverleaf structure in which open loops are connected to one another ny double-stranded stems. Follwing diagram represents the structural pattern of tRNA.

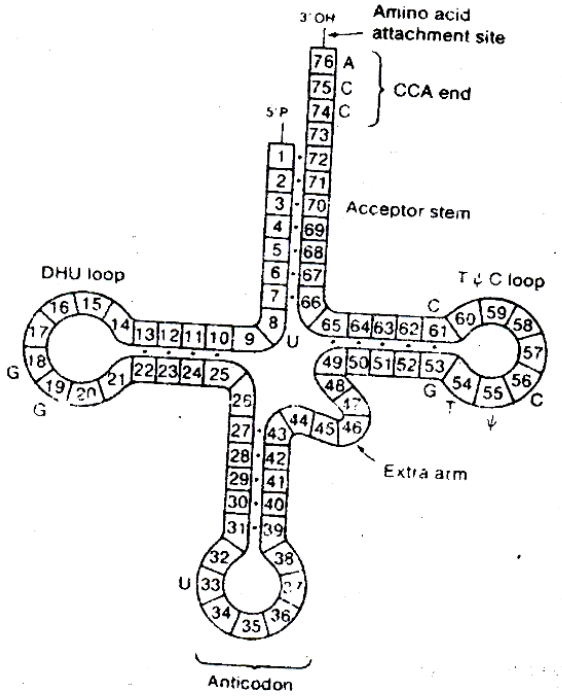


Figure 13-8 The currently accepted "standard" tRNA cloverleaf with its bases numbered. A few bases present in almost all tRNA molecules are indicated.

More than 200 different tRNA molecules from bacteria, yeast, plants and animals have been sequenced, and for each sequence base pairing can be arranged to produce the cloverleaf conformation; study of many ofthese molecules by physical methods has shown in each case that the cloverleaf is the correct conformation. By careful comparison of these sequences certain features have been found to be common to almost all of the molecules. This has led to the idea of a "consensus" tRNA molecue consisting of 76 nucleotides arranged in a cloverleaf form. By convention, the nucleotides are numbered 1 through 76 starting from the 5′-P terminus. This 76-base sequence is sufficiently fundamental that in tRNA molecules having more than 76 bases the additional ones can usually be recognized as additions to the standard sequence. The most common additions follow standard bases 17, 20, and 47; the "extra" bases are numbered 17:1, 17:2, 20:1, 20:2, 47:1, 47:2 and so forth.

The standard tRNA molecule has the following features:

Bases in positions 8, 11, 14, 15,18, 19, 21, 24, 32, 33, 37, 48, 53, 54, 55, 57, 58, 60, 61, 74, 75, and 76 are invariant in that they are the same in nearly all tRNA molecules whose sequences are known.

The 5′-P terminus is always base-paird. It is thought that this contributes to the stability of tRNA.

The 3′-OH terminus is always a four-base single-stranded region having the base sequence XCCA-4′-OH, in which X can be any base. This is called CCA or acceptor end. The adenine in the CCA sequence is the site of attachment of the amino acid by the cognate synthetase.

There are many so-called "modified" in tRNA. A few of these, dihyrouridine (DHU), ribosylthemine (rT), Pseudouridine (ψ), and inosine (I) occur frequently and in particular regions. Others are found in a variety of ;positions. In some cases the substsituents of the purine and pyrimidine rings are so numerous and complex that the base is called "hypermodified". In only a few cases is the signifcance of these unusual bases known.

There are three large single-stranded loops. The lowermost or anticodon loop contains severn bases. The anticodon occupies positions 34 through 36. It is almost always preceded by two ;pyrimidines (usually base 33 is uridine) and follwed by a modified purine. Thus the anticodon loop has the general sequence

5′-Py-U-XYZ-Pu(modified)-variable base
Anticodon

The loop containing bases 14 through 21 is called the DHU loop. It is not constant in size in diffeent tRNA molecules but may contain up to three extra bases between bases 17 and 18 and one to three extra bases following base 19. The loop containing bases 54 through 60 almost always contains the sequence TψC and is called the TψC loop.

There are four double-stranded regions called stems, or arms. The stems have no invariant bases and often contain base pairs other than G.C and A.T such as G.U. The three stems attached to each loop are given the fname of the corresponding loop, as for example, the anticodon stem.

An additional loop, containing bases 44 through 48, is also present. In the smallest tRNA molecules it contains four bases, lacking base 47, whereas in the lartest tRNA molecule it contains 21 bases with 16 bases present between bases 47 and 48. This highly variable loop is called the extra arm.

TRNAs are adaptors between codons and amino acids

At the heart of protein synthesis is the "translation" of nucleotide sequence information (in the form of codons) into amino acids. This is accomplished by tRNA molecules, which act as adaptors between codons and the aminoa cids they spcify. There are many types of trNA molecules, but each is attached to a specific amino acid and each recognizes a particular codon or codons, n the mRNA (most tRNAs recognize more than one codon). TRNA molecues are between 75 to 95 ribonucleottidesin length. Although the exact sequence varies, all tRNAs have certain features in common. First, all tRNAs end at the 3′ terminus with the sequence 5′-CCA-3′. This is the site that is attached to the cognate amino acid by the enzyme aminoacyl tRNA synthetase.

10. Describe suppressor mutations and wobble hypothesis.

Supressor mutations

When mutations occur in organisms, it behaves like the wild type in certain circumstances and mutant type in other circumstances. In suppressor sensitive mutations behave like wild type when a suppressor molecule is present. For example, the phage mutant, whom grow in one strain of bacteria and fails to grow on other strain i.e. the sup+ genes are present. The suppressor containing bacterium or a suppressor mutant, contains an altered tRNA molecule- one that has the anticodon CUA, which can pair with UAG. This mutant tRNA is called suppressor tRNA. This suppressor is based on the changes in the decoding system.

There are 2 types of suppressor sensitive mutations have been reported as i. Non-sense or chain termination mutation and ii. Misense or amino acid substitution mutation.

I. Non-sense suppressor mutation or Chain termination mutation

This kind of mutation is very common and arises in many caes. E.g. the single base change in the codons AAG, CAG. GAG, UUG, UGG, UAC (not UAA) give rise to the amber chain termination codon UAG. There is no ;protein function or little will occur because, no trNA acticodon complementary to UAG. Thus, a fragment of the wild type protein produce and fail to function unless the mutation is near the COOG- terminal.

In certain bacterial mutants, the chain termination mutations do not cause termination. E.g.Lac gene

I Wild type AUG UAG UAA

Wild prtein Met Tyr .

II Mutant UAG UAA

Mutant prptein.

The lac- bacteria by mutation revert bact to lac+ after several generations. It gives 3 classes of reverted as

1. Wild type class I reerter UAG back to UAC and come to correct protein
2. Silent mutant class II revertant as

UCG UAA
Ser .

3. Suppresed mutant class III revertant

UAG UAA

+

Mutant tRN_{AAG}^{Lys} that responds to UAG codon to Lys is suppressed mutant class IIIrevertant. I.e. nonsense suppreor mutant tRNA called suppressor mutatnt. This contain altered tRNA molecule, has anticodon CUA, which can pair with uAG and the mutant called suppressor mutant that translate amino acid (lysine).

II. Misense mutations or base substitution amino acid mutations

Substitution of one amino acid for other amino acid by tRNA is called misense or base substitution amino acid mutatins. For example valine is mutated to aspertic acid and loss its activity. This is restored by wild type phenotype by missense suppressor that substitutes alanine (nonpolar) for aspartic acid. This is by 3 ways as;

i. a mutatnt tRNA molefue may recognize 2 codons by change in the anticodon loop.

ii. a mutant tRNA can be recognized by noncognate aminoacyl synthetase and be misacylated.

iii. a mutant synthetase may change a noncognate trNA molecule.

There are many examples for the first 2 classes were reported. Misense suppressors are inefficient and usually occur 1% effieciency. The misense suppressors grow slowly and are generally unhealthy.

Wobble hyposthesis

Francis Crick in 1966, explained the wobbling of genetic code. It explains the response of same tRNA molecules to several codons and pattern of the redundancy code. That is differeing in

the third phase (position). If 'U' is in the first position of anticodon it can pair with either A or G at the theird positon of codon.

It states that the base at the 5' end of the anticodon is not as spatially fonfined as the other two, allowing it to form hydroten bonds with any of seeral bases located at the 3' end of a codon. Not all combinations are possible, with pairing restricted to those shown in the following table. For example, U at the wobble position can pair with either A or G, while I can pair with U, C or A. The pairings permitted hy the wobble rules are those that give ribose-ribose distances close to that of the standard A:U or G:C base pairs. Purine-purine (with the exception of I:A pairs) or ;pyrimidine:pyrimidine pairs would give ribose-ribose distances that are too long or too shor, respectively.

Base in anticodon (I position)	Codon (III position)
G	U or C
C	G
A	U
U	A or G
I (Inosine resembles G)	A, U, or G

Wobbling is economic to recognize several codons and amino acids. In mitochondria example, IGA – anticodon recognize GCU, GCC, GCA. In mitochondria, extent of wobbling increased sothat 22 tRNA instead of 55 tRNA are needed.

The wobble rules do not permit any single tRNA molecule to recognize fur different codons. Three codons can be recognized only when inosine occupies the firs(5') position of the anitcodon.

Almost all the evidence gathered since 1966 supports the wobble concept. For example, the concept correctly predicated that at least three tRNAs exist for the six seine codons (UCU, UCC, UCA, UCG, AGU, and AGC). The other two amino acids (leucine and arginine) that are encoded by six codons also have different tRNAs for the sets of codons that differ in the first or second positioon.

UNIT – V

PART – A

1. Describe gene expression.

The gene properties present in the DNA of the cell of particular organism are expressed for synthesis of proteins for cellular functions. Synthesis of particular protein by the specific gene present in the DNA is controlled and expressed only by the above said respective gene. This translation mechanism is called gene expression. All the proteins are not needed at the same time and the different proteins are synthesized at different intervals as and when required.

2. Write the significance of gene regulation.

Many proteins including enzyme machinery are required by the cell to perform various cellular activities or metabolic activities. But, all the proteins synthesized by mRNA of the cell are not required at the same time. However, these proteins are synthesized as and when require by the cell by systematic control of genes or the DNA. There are various factors involved to control and express the gene activity. This mechanism of regular control of gene expression is much more important to regularize the cellular activity.

3. What is temporal and spatial gene regulation?

In a particular time or period, the desired genes are in function and other genes are not in function or to be restricted. This action requires time controlled or periodical regulation of individual genes called temporal gene regulation. Varity of mechanisms are known to regulate gene expression in different levels of cellular functions with respective environment is called spatial (in space) gene regulation.

4. What are the major steps involved in gene regulation?

The major steps involved in gene regulations are as follows.

i. Transcription
ii. Processing of mRNA and
iii. Translation of genetic information.

5. Define lytic and lysogenic cycle of bacteriophage.

Bacteeriophage infects E.coli bacteria and it undergoes propagation in 2 ways as i. Lytic cycle and ii. Lysogenic cycle.

In lytic cycle, the replication of the phage DNA and synthesis of new coat proteins, which are, combine to form new phage particles. This new phage particle released from bacteria after complete lysis of bacteria.

In lysogenic cycle, the integration of the phage DNA into the bacterial chromosome where it is passively replicated at each cell division –just as though it were a legitimate part of the bacterial genome.

6. What are regulatory proteins?

The regulation of gene expression in both prokaryotic and eukaryotic cells is controlled by special kind of protein molecules, which are called as regulatory proteins. These protein molecules act as extra cellular signals and control the gene expression. There are two types of regulatory proteins studied in cell as,

i. Positive regulators or activators and
ii. Negative regulators or repressors. Regulatory proteins are DNA binding proteins that recognize specific sites at or near the genes.

7. Write short notes on activators and repressors.

Activators are otherwise called as positive regulator proteins, which increase the transcription rat of gene.

Repressors are negative regulatory proteins, which decrease or eliminate the transcription rate of gene.

8. Write short notes on polymerase-promoter-complex in gene regulation.

The mechanism of the gene regulation is the binding of regulatory proteins with DNA on promoter site (closed complex) and polymerase enzyme, which activate the transcription mechanism or control the regulation of gene expression. This complex is called polymerase-promoter complex on DNA start site. The activators that help in RNA polymerase binding with DNA and repressors that block the binding of RNA polymerase with DNA.

9. Write short notes on lac operon.

The lac operon is a lactose utilizing operator genes, which is studied in Bacteria (E.coli). This lac operon has three genes as;

i. lac Z – encodes enzyme beta galactosidase.
ii. lac Y – encodes enzyme lactose permease
iii. lac A – encode enzyme thiogalactocide transacetylase.

These 3 genes are expressed when the lactose is available in high level.

10. Describe trp genes in operator region.

The 'trp' genes otherwise contain in 'trp opron' is also studied in bacteria like E.coli and Salmonella sp. which are responsible for synthesis of tryptophan.
There are 5 trp genes are reported as given below;
Trp E & trp D – encodes for anthranilate synthetase
Trp C – encodes for indol glycerol phosphate synthetase
Trp B & trp A – encodes tryptophan synthetase.

11. What are CAP proteins?

The activator proteins of the lac operon are otherwise called as CAP (catabolic activator proteins). The CAP proteins can bind DNA and activate the lac genes only in the absence of glucose and regulate the lactose metabolism.

CAP and lac repressor have opposing effects on RNA polymerase binding to the lac promoter.

12. Write short notes on tryptophan repressor.

The tryptophan repressor is the repressor protein in tryptophan synthesis, which is coded by the gene trp R. The gene trp R is not linked with trp operon. This tryptophan repressor has low specificity with the operator and remains inactive and active when associating with tryptophan and which is called as co-repressor.

13. Write the feed back inhibition of tryptophan synthesis.

The trryptophan synthesis functions through feedback inhibition. When tryptophan combines with first enzyme of tryptophan synthesis, inhibits its activity through allosteric modification. Thus, the synthesis of tryptophan is self-regulatory. When tryptophan is plenty in cell, first the feed back inhibition takes place subsequently co-repression and stop the synthesis of tryptophan.

14. What are retrons?

The retron is otherwise called as reverse transcriptase operon, which is reported in bacteria. The retron is the locus, which contains genes, encodes for synthesis of msDNA (multiple copy single-stranded DNA) and msdRNA (msDNA associated RNA), which are synthesized in transposable retroelements type of chromosomal structures.

15. What is conditional mutant? Give example?

A conditional mutant carries a (usually missense) mutation whose effects manifest only under certain restrictive conditions. Under normal permissible conditions, the wild-type phenotype is

displayed. Important classes of conditional mutation include temperature-sensitive mutations, which display the mutant phenotype under conditions of elevated temperature, and cold-sensitive mutations, which display the mutant phenotype at low temperature. In each case, the properties of the mutant are likely to involve an increased tendency of the protein to denature at restrictive temperatures.

16. How do lactose molecules first enter an uninduced lac A+, Lac Z+. Lac y+ cell to induce synthesis of beta-galactosidase?

When lactose enters in the cell, it is converted into allolactose. It is allolactose that control & lac repressor. Paradoxically the conversion of lactose to alloctose is catalysed by β - galacto sidaze, itself enclosed by one fo the lacgenes, hence it induce the synthesis of β- galactosidase.

17. What is point mutation? What are types of point mutations?

Point mutations occur at a single site and involve a small number of nucleotide residues. If point mutations occur within a gene, the consequences depend on any change to the structure or expression of the encoded polypeptide, and range from neutral to severely deleterious.
The different types of point mutations are;

i. missense mutation
ii. nonsense mutation
iii. read through mutation
iv. frameshift mutation
v. nonframeshifting ideal
vi. intragenic noncoding regions
vii. untranslated region

18. Write short notes on polycistronic mRNA.

Polycistronic mRNA describe the messenger RNA contain more than one or several open reading frame (ORF). Example is prokaryotic cell, in which the mRNA has more than one ORFs.

19. What is lac repressor?

Lac repressor is the one among the regulatory proteins for utilization of lactose by prokaryotic cell example E.coli. The lac repressor bind DNA and repress or inactivate the transcription process only in the absence of lactose in the cell.

20. What are attenuator proteins?

Attenuation is a bacterial regulatory mechanism controlling the expression of several operons concerned with amino acid biosynthesis and in E.coli, pyr BI operon that encodes enzymes for pyrimidine synthesis. The attenuator proteins are the protein molecules which involve and responsible for the control of protein synthesis or amino acid synthesis or transcription itself.

21. Describe briefly on lambdaphage lytic cycle.

Bacteriophage infects E.coli bacteria and it undergoes propogation in 2 ways as i. Lytic cycle and ii. Lysogenic cycle.

In lytic cycle, the replication of the phage DNA and synthesis of new coat proteins, which are, combine to form new phage particles. This new phage particle released from bacteria after complete lysis of bacteria.

22. Describe briefly on lambda phage lysogenic cycle.

Bacteriophage infects E.coli bacteria and it undergoes propagation in 2 ways as i. Lytic cycle and ii. Lysogenic cycle.

In lysogenic cycle, the integration of the phage DNA into the bacterial chromosome where it is passively replicated at each cell division –just as though it were a legitimate part of the bacterial genome.

23. What is lysogenic induction?

The switch over of lysogenic bacteriophage to lytic bacteriophage due to the agents present internal as well as external of the phage. A lysogen extremely stable under normal condition. But the phage can efficientely change from lysogenic to lytic growth if the cell is exposed to agents that damage DNA. This will threaten the host cell for its continued existence.

24. Write short notes on lambda phage genome.

The lampda phage genome is linear in the phage head. But upon infection, it circularizes at the cos site. The lampda phage genome is about 50 kb in size and some 50 genes are present. Most of the encode proteins and the proteins involve in DNA replication, recombination and lysis.

25. Mention briefly on phage repressor.

Phage (lambda phage) repressor is a protein of two domains joined by a flexible linker region. The N terminal domain contains the DNA-binding region (a helix-turn-helix domain). As with the

majority of DNA binding proteins, lambda phage repressor binds DNA as a dimmer; the main dimerization contacts are made between the C-terminal domains. A single dimmer recognizes a 17 bp DNA sequence, each monomer recognizing one half-site, again just as in the lac system. But the lambda phage repressor can both activate and repress transcription process of lambdaphage.

26. What are CI and Cro genes?

The CI gene encodes lambda phage repressor, a protein of two domains joined by a flexible linker region. The phage repressor will bind with DNA and involve in both activation and repression of transcription.

The Cro gene (stands for Control of repressor and other things) only involve in the precision of transcription, like Lac repressor in lambda phage. It is a single domain protein and again binds as a dimmer to 17 bp DNA sequences.

27. Compare positive and negative autoregulation.

When the levels of repressor protein are occurring in too high the negative regulation is takes place. When the concentration is too high, repressor will bind to O_{R3} as well, and repress P_{RM} (in a manner analogous to Cro binding Or3 and repressing PRM during lytic growth). This prevents synthesis of new repressor until its concentration falls to a level at which it vacates OR3.

28. Write the function of lambda CII activator gene.

CII is a transcriptional activator. It binds to a site upstream of a promoter called PRE (for repressor establishment) and stimulates transcription of the CI (repressor) gene from that promoter. Thus the repressor gene can be transcribed from two different promoters (PRE and PRM). CII protein binds to a site that overlaps the -35 region but is located on the opposite face of the DNA helix by directly interacting with polymerase, CII helps polymerase bind to the promoter.

29. What is antitermination?

Antitermination is type of positive transcriptional regulation. The transcripts controlled by lambdphage N and Q proteins are initiated perfectly well in the absence of those regulators. But the transcripts terminate a few hundred to a thousand nucleotides downstream of the promoter unless the regulator has modified RNA polymerase. Lambdaphage N and Q proteins are called antiterminators and their transcriptional control is called antitermination.

30. Describe retraregulation.

In the lambdaphage transcription process, the RNA initiated at P1 stops at a terminator about 300 nucleotides after the end of the it gene; it has a typical stem-and-loop structure followed by six uridine nucleotides; This longer stem can form as stem that is a substrate for nucleases. Because the site responsible for this negative regulation is downstream of the gene it affects, and because degradation proceeds backward through the gene, this process in called retroregulation.

31. What are DNA micro satellites?

The point mutations are prone to give sequences are repeats of simple dinucleotide, trinucleotide or tetranucleotide sequences. These short sequences occur due to various point mutations are called DNA microsatellites. The well-known example for the dinucleotide sequence is with CA. Stretches of CA repeats are found in the chromosomes of human and other eukaryotes.

32. How replication errors escape proofreading?

The proof reading of exonuclease is not however, foolproof. Some misincorporated nucleotides escape detection and become a mismatch between the newly synthesized strand and the template strand. Three kinds of nucleotides in the template strand (for example, T, G or C opposite a Tin the template) for a total of 12 possible mismatches (T:T, T:G, T:C, and so forth). If the misincorporated nucleotide is not subsequently detected and replaced, the sequence change will become permanent in the genome. At this point, the mismatch will no longer exist; instead it will have a resulted in a permanent change (a mutation) in the DNA sequence.

33. What are Mut S, Mut L & Mut H?

A dimmer of the mismatch repair protein MutS detects the mismatches during the point mutations. MutS scans the DNA, recognizing mismatches from the distortion they cause in the DNA backbone. MutL is a second protein component of the repair system. MutL in turn, activates Mut, an enzyme that causes an incision or nick on one strand near the site of the mismatch.

34. Write short notes on Dam methylases.

Dam methylase is otherwise the enzyme DNA adenine methylase that are reported in E.coli. In E.coli, adenine residues in the sequence GATC are methylated at the N6 position by the enzyme Dam methylase. Dam methylation play a role in modification of methylation in adenine residues of both the strands, which allow the cell to discriminate between the parent and daughter strands

following replication when the newly synthesized strand is transiently unmethylated. This is useful in repair synthesis of ENA strands.

35. How DNA get damage?

The DNA damage is influenced generally by mutations, which cause changes in the nucleotide sequences. Many kind of mutations are responsible for DNA damage.

The environmental factors such as radiation effect and chemical agents also were responsible for the DNA damage. The chemical reactions like hydrolysis, deamination, alkylation, oxidation, and radiation are influence the DNA damage.

36. What is ames test? Give the application?

Ames test is a simple test to check the potency of carcinogenic effects of chemicals based on their capacity to cause mutations in the bacterium Salmonella typhimurium. The Ames test uses a strain of S.typhimurium that is mutant for the operon responsible for the biosynthesis of the amino acid histidine. Due to mutation in the bacterium cells, the mutant fails to grow and form colonies on solid medium lacking histidine.

37. How base analogs and intercalating agents cause mutation?

Mutations are also caused b7 compounds that substitute for normal bases (base analogs) or slip between the bases (intercalating agents) to cause errors in replication. Base analogs are structurally similar to proper bases but differ in ways that make them treacherous to the cell. Thus, base analogs are similar enough to the proper bases to get taken up the cells, converted into nucleoside triphosphates, and incorporated into DNA during replication.

Intercalating agents are chemicals contain polycyclic rings that bind to the equally flat purine or pyrimidine bases of DNA. Example is proflavin, acridine, and ethidium, which are causes addition, or deletion of nucleotide sequences lead to mutation.

38. What is the role of 5-bromouracil?

Five bromouracil is one of the most mutagenic base analogs that are an anlog of thymine. The presence of the bromo substituent allows the base to mispair with quinine via the enol tautomer. The keto tautomer is strongly favoured over the enol tautomer, but more so for thymine than for 5- bromocil.

39. Write short notes on clastogenic.

The ionizing radiation like gamma radiation and X-rays and anticancer drugs like bleomycin cause double strand breakage in DNA, which are difficult to repair. These attack the deoxyribose in the DNA backbone. The mutagenic agents like ionizing radiations and anticancer drugs like bleomycin that cause DNA to break are said to be clastogenic.

40. What is excision repair system?

The excision repair system is a type of DNA repairing process found in the cell. In this system, the damaged nucleotide is not repaired but removed from the damaged DNA. In excision repair systems, the other, undamaged, strand serves as a template for reincorporation of the correct nucleotide by DNA polymerase.

41. Describe the direct reversal of DNA damage photreactivation.

The photoreactivation directly reverses the formation of pyrimidine dimmers that result from ultraviolet irradiation. In photoreactivation, the enzyme DNA photolyase captures energy from light and uses it to break the covalent bonds linking adjacent pyrimidines. In other words, the damaged bases are mended directly.

42. Write short notes on double strand break (DSB) repair pathway.

Excision repair uses the undamaged DNA strand as a template to replace a damaged segment of DNA on the other strand. How do cells repair double-strand breaks in DNA in which both strands of the duplex are broken? This is accomplished by the double-strand break (DSB) repair pathway. The DSB retrieves sequence information from the sister chromosome. Because of its central role in general, homologous recombination as well as in repair.

43. Explain shortly on transcription-coupled repair.

The nucleotide excision repair is not only capable of mending damage through the genome, but is also capable of rescuing RNA polymerase, the progression of which has been arrested by the presence of a lesion in the transcribed (template) strand of a gene. This phenomenon is known as transcription-coupled repair, involves recruitment to the stalled RNA polymerase of nucleotide excision repair proteins.

44. What is translesion synthesis?

This is the method of repairing the damaged DNA during DNA synthesis. In this method, the DNA is resynthesised using an undamaged template. During the synthesis, the DNA polymerase encounters lesions, such as pyrimidne dimmer or apurinic site that has not been repaired. The replication machinery must attempt to copy across the lesion by bypass these sites of damage by special mechanism. This type of mechanism is called translesion synthesis.

PART – B

1. How replication errors occur in DNA and how it is rectified?

The perpetuation of the genetic material from generation to generation depends on maintaining rates of mutations at low levels. High rates of mutation in the germ line would destroy the individual. Living cells require the correct functioning of thousands of genes, each of which could be damaged by a mutation at many sites in its protein-coding sequence or in flanking sequences that govern its expression or the processing of its messenger RNA.

The nature of mutations and changes in DNA

Mutations include almost every conceivable change in DNA sequence. The simplest mutations are switches of one base for another. There are two kinds as;

i. Transitions, which are pyrimidine-to-pyrimidine and purine –to- purine substitutions, such as T to C and A to G;
ii. Transversions, which are pyrimidne to purine and purine to pyrimidine substitutions, such as T to G or A and A to G or T.

Other simple mutations are insertions or deletions of a nucleotide or a small number of nucleotides. Mutations that alter a single nucleotide are called point mutations.

Kinds of mutations cause more drastic changes in DNA, such as extensive insertions and deletions and gross rearrangement of chromosome structue. Such changes might be caused, for example, by the insertion of a transposon, which typically places many thousands of nucleotides of foreign DNA in the coding or regulatory sequences of a gene or by the aberrant actions of cellular recombination processes.

Other kind of sequenece that is particularly prone to mutations merits special comment because of its importance in human genetics and disease. These mutation-prone sequences are repeats of simple di-, tri-, or tetranucleotdie sequences, wfhich are known as DNA micosatellits. One well kown example involves reapeats of the dinucleotide sequence GA stretches of CA repeats are found at many widely scattered sites in the chromosomes of humans and some other eukaryotes. The replication machinery has diffulty copying such repeats accurately, frequenctly-undergoing slippage. This slippage increases or reduces the number of copies of the repeated sequence. As a result, the CA repeats length at a pparticular site on the chromosome is often hughly polymorphic in the population.

Some replication errors escape proofreading

The replication machinery achieves a remarkably high degree of accuracy using a proofreading mechanism, the 3′ -> 5′ exonuclease componenet of the replisome, which removes wrongly incorporated nucleotides. Hoever, some misincorporaated nucleotides escape detection and become a mismatch between the newly synthesized strand and the template strand. Three different nucleotides can be misincorporated opposite each of the four kinds of nucleotides in the template strand. If the misincorporated nucleotide is not subsequently detected and replaced, the sequence change will become permenant in the genome; during a second round of replication, the misincorporated nucleotide, now part of the template strand, will direct the incorporation of its complementary nucleotide into the newly synthesized strand.

Mismatch repair removes errors that escape proofreading

There is a mechanism exists for detecting mismatches and repairing them. Final responsibility for the fidelity of DNA replication rests with this mismatch repair system, which increases the accuracy of DNA synthesis by an additional two to three orders of magnitude. The mismatch repair system faces two challenges. First it must scan the genome for mismatches Second , the system must correct the mismatch accurately; that is , it smust replace the misincorporated nucleotide in the newly synthesized strand and not the correct nucleotide in the parental strand.

2. Discuss in detail about mismatch repair pathway with suitable diagram for the repair of replication errors in DNA.

There is a special mechanism exists for detecting mismatches and repairing the DNA have been reported. Final responsibility for the fidelity of DNA replication rests with this mismatch repair system, which increases the accuracy of DNA synthesis by an additional two to three orders of magnitude. The mismatch repair system faces two challenges. First, it must scan the genome for mismatches. Because mismatches are transient (they are eliminated following a second round of replication when they result in mutations), the mismatch repair system must rapidly find and reapir mismatches. Second, the system must correct the mismatch accurately; that is, it must replace the misincorporated nucleotide in the newly synthesized strand and not the correct nucleotide in the parental strand.

In E.coli, a dimmer of the mismatch repair protein MutS detects mismatches. MutS scans the DNA, recognizing mismatches from the distortion they cause in the DNA backbone. MutS embraces the mismatch-containing DNA, inducing a pronounced kink in the DNA and a conformational change in MutS itself. A key to the specificity of MutS is that DNA containing a mismatch is much more readily distorted than properly base-pairedDNA. This complex of MutS and the mismatch-containing DNA recruits MutL, a second protein componenet of the repair system. MutL, in turn, activates MutH, an enzyme that causes an incision or nick on one strand near the site of the mismatch. Nicking is followed by the action of a specific helicase (UVrD) and

one of three exonucleases. The helicase unwinds the DNA, starting from the incision and moving in the direction of the site of the mismatch, and the exonuclease progressively didgests the displaced single strand, extending to and beyond the site of the mismatched nucleotide. This actin roduces a single-stranded gap, which is then filled in by DNA polymerase III and sealed with DNA ligase. The overall effect is to remove the mismatch and replace it with the correctly base-paired nucleotide.

The following diagram shows the mismatch repair pathway for the repair of replication errors.

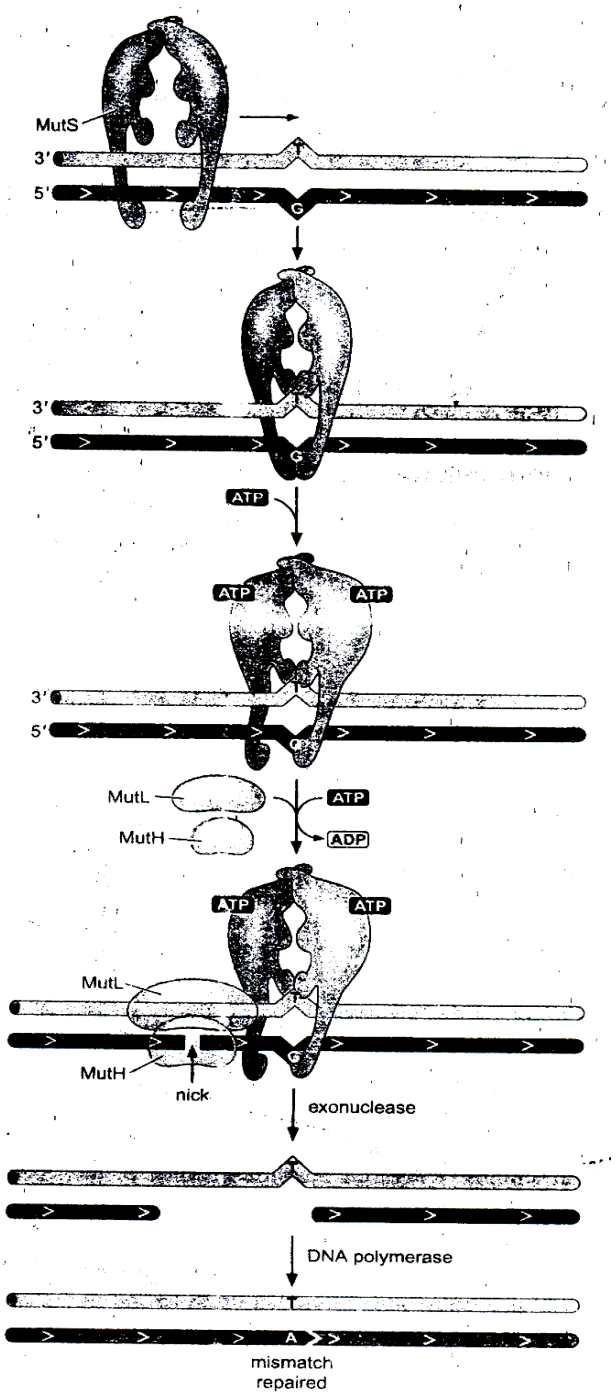


FIGURE 9-3 Mismatch repair pathway for the repair of replication errors.

3. Write the methods involved in repair of DNA damage.

The damage to DNA can have two consequences. Some kinds of damage, such as thymine dimmers or nicks and breaks in the DNA backbone, create impediments to replication or transcription. Other kinds of damage create altered bases that have no immediate structural consequence on replication but cause mispairing; these can result in a permanent alteration to the DNA sequence after replication. For example, the conversion of cytosine to uracil by deamination creates U:G mismatch, which, after a round of replication, becomes a C:G to T:A transition mutation on one daughter chromosome. These considerations explain why cells have eveolved elaborate mechanisms to identify and repair damage before it blocks replication or causes a mutation. Cells would not endure long without such mechanisms.

The most direct of the DNA repair system is a repair enzyme simpley reverses the damage. One more elaborate step involves excision repair systems, in which the damaged nucleotide is not repaired but removed from the DNA. In excision repair systems, the other, undamaged, strand serves as a template for reincorporation of the correct nucleotide byk DNA polymerase. There is two kinds of excision repair exist, one involving the removal of only the damaged nuceotide and the other, involving the removal of only the damaged nucleotide and the other, the removal of a short stretch of single-stranded DNA that contains the lesion.

The more elahborate is recombninational repair, which is employed when both strands are damaged as when the DNA is broken. In such situations, one strand cannot serve as a template for the repair of the other. Hfence in recombinational repair known as double-strand break repire, sequence information is rerieved from a second undamaged copy of the chromosme. Finally, when progression of a replicating DNA polymerae copies across the site of the damage in a manner that does not depend on base pairing between the templates and newly synthesized DNA strands. This mechanism is a system of last resort because tranlesion synthesis is inevitably highly error-prone (mutagenic).

Direct reversal of DNA damage

An example of repair by simple reversal of damage is ;photoreactivation. Photoactivation directly reverses the formation f pyrimidine dimmers that result from ultraviolet irradiation. In photoreactivatin, the enzyme DNA photolyase capturesf energy from light and uses it to break the co-valents bonds linking adjacent pyrimidines. In other words, the damaged sbaess are mended directly.

Another example of direct reversal is the removal of the methyl group from the methylated bases “O -methylguanine. In this casse, a methyltransferease removes the methyl group from the guanine residues b7y transferring it to one of its own cysteine residues. This is very costly to the

cell because the methyltransferease is not catalytic having onceaccepted a methyl group, it cannot be used again.

Base excision repair, enzymes remove damaged by a by a base-flipping mechanism.

This is the most prevalent way of DNA repairing mechanism in which the DNA is cleansed of damaged bases is by repair systems that remove and replace the altered bases. The two principal repair systems are

i. Base excision repair and
ii. Nucleotide excision repair.

In the base excision repair, an enzyme called a glycosylase recognizes and removes the damaged base by hydrolyzing the glycosidic bond. The resulting abasic sugar is removed from the DNA back bone in a further endonucleolytic sstep. Endonucleotytic cleavage also removes apurinic and apyrimidinic sugars that arise by spontaneous bydrollysis. After the eedamaged nucleotide has been entirely removed from the backbone, a repair DNA polymerase and DNA ligase restore an intact strand using the undamaged strand as a template.

DNA glycosylases are lesion –spedific and cells have multiple DNA glycosylases with diffeent specificities. Thus, a specific glycosylase recognizes uracil and another is responsible for removing oxoG. A total of eight different DNA glycosylases have nbeen identified in the nuclei of human cells.

Nucleotide excision repair enzymes cleave damaged DNA on either side off the lesion

Unlike base excision repair, the nucleotide excision repair enzymes do not recognize any particular lesion. Rather, this system workds by recognizing distortions to the shape of the double helix, such as those caused by a thymine dimmer or by the presence of bulky chemical adduct on a base. Such disotations trigger a chain of events that lead to the removal of a short single-stranded segment that includes the lesion. This removal creates a single-stranded gap in the DNA, which is filled in by DNA polymerase using the undamaged strand as a template and therby restoring the original nucleotide sequences.

Not only is nucleotide excision repair capable of mending damate throught the genome, ut it is also capable of rescuing RNA polymerase, the progression of which has been arrested by the presence of a lesion in the transcribed strand of a gene. This phenomenon, known as trancription-coupled repair, involved recruitment to the stalled RNA polymerase of nucleotide excison repair proteins. The significance of transcription-coupled repair is that it focuses repair enzymes on DNA being actively transcsribe in the cell. Central to transcription-coupled repair in eukaryotes is the general transcssription factor TFIIH. As TFIIH unwinds the DNA template during the initiation of transcription.

Recombination repairs DNA breaks by retrieving sequence information from undamaged DNA

Excision repair uses the undamaged DNA strand as a template to replace a damaged segment of DNA on the other strand. The repairing of double strands in damaged DNA, which were broken, is by the method of double-strand break (DSB) repair pathway. In which it retrieves sequence information from the sister chromosome. Because of its central role in general, homologous recombination as well as in repair, the dSB-repair pathway is important.

DNA recombination also helps to repair errors in DNA replication. Consider a replication fork that encounters a lesion in DNA (such as thiamine dimmer) that has not been corrected by nucleotide excision rrepair. The DNA polymerase will sometimes stall attempting ot replicateover the lesion. Although the template strand cannot be used, the sequence information can be retrieved from the other daughter molecule of the replication fork by recombination.
The DSB-repair pathway can only operate when the sister of the broken chromosome is present in the cell.

Translesion DNA synthesis enables replication to proceed across DNA damage

This is the method of repairing the damaged DNA during DNA synthesis. In this method, the DNA is resynthesised using an undamaged template. During the synthesis, the DNA polymerase encounters lesions, such as pyrimidne dimmer or apurinic site that has not been repaired. The replication machinery must attempt to copy acroos the lesion by bypass these sites of damage by special mechanism. This type of mechanism is called translesion synthesis. Thils is highly error prone and thus likely ot intorudce mutations, translesion synthesis spares the cell the worse fate of an incompletely replicated chromosome.

Translesion synthesis is catalyzed by a spcecialilzed class of DNA polymeerases that synthesize DNA directly across the site of the damage. A complex of the fproteins UmuC and UmuD carries out Translesion synthesis in E.coli. UmcC is a member of a distinct family of DNA polymerases found in many organisms known as he Y-family of DNA polymerases.

4. Write short notes on the following.
i. Translesion DNA synthesis
ii. Repair mechanism by Recombination in DNA breakage

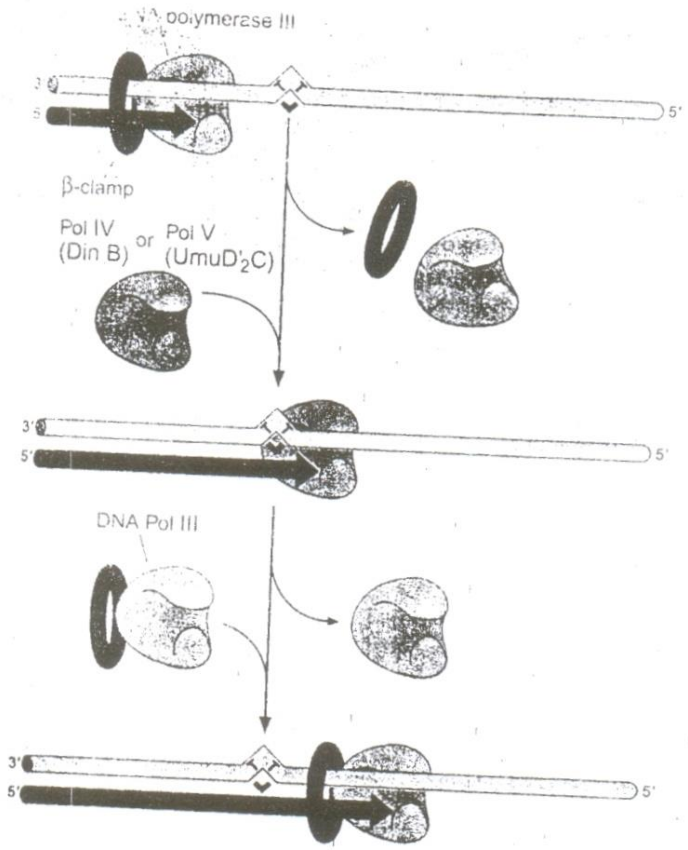
i. Translesion DNA synthesis

Translesion DNA synthesis enables replication to proceed across DNA damage. This kind of repair systems do not operate with complete efficiency and sometimes a replicating DNA polymerase encounters a lesion, such as a pyrimidine dimmer or an apurinic site, that has not been repaired. Because such regions are obstacles to progresin of the DNA polymerse, the

replication machining must attempt to copy across the lesion be forced to cease replicating. Even it sells can not repair these lesions, there is a fail-safe mechanism that allows the replication machinery to bypass these siter of damage. This mechanism is known as translesion synthesis.

Translesion synthesis is catalysed by a specialized class of DNA polymerse that synthesize DNA directly across the sit of the damage (refer following diagram). An important feature of these polymerse is that, although thay are template dependent incorporate nucleotides in a manner that is independent of base pairing.

The following diagram shows the translesion DNA synthesis repair system of DNA.



Translesion DNA synthes

Upon encountering a lesion in the template during replication, DNA polymerase III with its sliding clamp dissociates from the DNA and is replaced by the translesion DNA polymerase, which extends DNA synthesis across the thymine dimer on the template (upper) strand. The translesion polymerase is then replaced by the DNA polymerase III. (Source: R. Woodgate.)

ii. Repair mechanism by Recombination in DNA breakage

In this type of DNA repair mechanism, the recombination repairs DNA breaks by retrieving sequence information from undamaged DNA. Excision repair uses the undamaged DNA strand as a template to replace a damaged segment of DNA on the other strand. How do cells repair double-strand breaks in DNA in which both strands of the duplex are broken? This is accomplished by the double-strand break (DSB) repair pathway, which retrieves sequence information from the sister chromosome. Because of its central role in general, homologous recombination as well as in repair, the DSB-repair pathway is an important area of mechanism.

DNA recombination also helps to repair errors in DNA replication. Consider a replication fork that encounters a lesion in DNA (such as a thymine dimmer) that has not been corrected by nucleotide excision repair. The polymerase will sometimes stall attempting to replicate over the lesion. Although the template strand cannot be used, the sequence information can be retrieved from the other daughter molecules of the replication fork by recombination. Once this recombinational repair is complete, the nucleotide excision system has another opportunity to repair the thymine dimmer. Indeed, he sensitive to ultraviolet light knows the mutants defective in recombination. Consider also the situation in which the replication fork encounters a nick in the DNA template. Passage of the fork over the nick will create a DNA break, repair of which can only be accomplished by the double-strand break repair pathway. Although we generally consider recombination as an evolutionary device to explore new combinations of sequences, it may be that its original function was to repair damage in DNA.

5. Explain the regulation of gene expression in prokaryotic system with the example of lac operon.

J.Monod and F.Jaccob, who studied the induction of beta-galactosidase activity in E.coli, first propounded the molecular and genetic relationships between enzyme induction and enzyme reression. They concluded that there are three different loci in the genetic map of E.coli that influence the formation of beta-galactosidase, designated as z, o and i. The z locus specifies the amino acid sequence of the beta-galactosidase molecule. Mutation in this locus lead to synthesis of a faulty or an inactive enzyme for the amino acid sequence of a specific protgein such as beta-galactosidase. The second locus i functions as an inhibitory gene and determines whether the structural gene for betea-galactosidase will be transcribed. Such genes are called regulatory genes. When these genes undergo mutation and become defective, they can no longer inhibit the transcription of the structural gene. The regulator gene (i) encodes a repressor that binds to the operator locus (o), thereby preventing transcription of mRNA from the structural gnes z, y and a. Inducer metabolite combines with the repressor so that it no longer binds to the operator locus. The structural genes are then free to be transcribed to yield a polycistronic mRNA, which is translated into three proteins-the enzymes. P is the promoter gene can no longer inhibit the transcription of the structural gene. In such cases beta-galactosidase is synthesized regardless of the inducer. The following diagram shows the gene location on the chromosome.

Operon model

This is a mechanism of corordinate induction in prokaryotes. According to Jacob and Monod, in the absence of an inducer the repressor protein is made by the cell following transcription from the I gene. In the absence of an iinducer the repressor occurs in its active state and combi9nes with the operator (o) locu, thus prev3enting the transcsription of structural gene for Beta-galactosidase. When the inducer is added, it combines with the repressor protein to form

a repressor-inducred complex, rendering the repressor incapable of binding to the o locus. The stsructural gene for beta-galactosidase thus becomes freef for transcription to yield mRNA and the enzyme is then synthesized. The interaction between the repressor and the inducer is recersible and accounts for the fact that when the inducer is removed from the medium or is used up by the enzyme, the repressor reverts to its inhibitory from and binds to the operator, preventing the synthesis of enzyme.

Jacob and Monod extended their hyposthesis ffor the regulation of proetein synthesis to provide a mechanism for coordinate induction in which a sequence of enzymes can be induced as sgroup by asingle inducer. We have seen the beta-galactosidase coordinately induces a set of three enzyme proteins namely beta-galactosidase, permease and acetytransfserease, which are coded by three gens, z, y and a respectively. Therse three structural genes are regulated by the same I gene and the same o locus. These were named as lac operon. An operon can be defined as a collection of functionally related strutral genes close to each other forming a single operator.

Lac operon in Bacteria (E.coli) has 3 genes as

i. Lac z – 3150 bp
ii. Lac y and – 780 bp
iii. Lac a – 825 bp

These are arranged in genome adjacently.

The lac promoter locatd at 5′ end of lac z, which direct transcription of all 3 genes (polycistronic).

The following diagram shows the representation of lac operon including lac promoter genes.

These 3 genes are expressed when the lactose is available in high level.

An acivator and repressor together control the lac genes

i. In lac operon, the three lac genes lac a, lac y and lac a – are arranged adjacently in the genome. The lac promoter located at the 5′ end of lac Z, directs transcription of all three genes as a single mRNA (polycistronic) ; this mRNA is translated to give the three protein products.

i. The lac z gene lac Z encodes enzyme beta-
The cell uses galactosidase, which cleaves the sugar lactose into galactose and glucose, both of wich, as energy sources.
ii. lac Y encodes enzyme lactose permease, a protein that inserts into the cell membrane and transports lactose into the cell and
iii. iii. lac A encodes enzyme thiogalactoxide transacetylase which rids the ell of toxic thiogalactosides that also get transporteded in by lac y.

These genes are expressed at high levels only when lactose is available and glucose – the preferred energy source – is not. Two regulatory proteins are involved;

i. an activator called **CAP (catabolic activator protein) and**
ii. the other repressor called the **lac repressor** which is encodes by i gene.

Both CAP and lac repressor is DNA binding proteins and each binds to a specific site on DNA at or near the lac promoter.

Following diagram represents the structural pattern of the lac operon system.

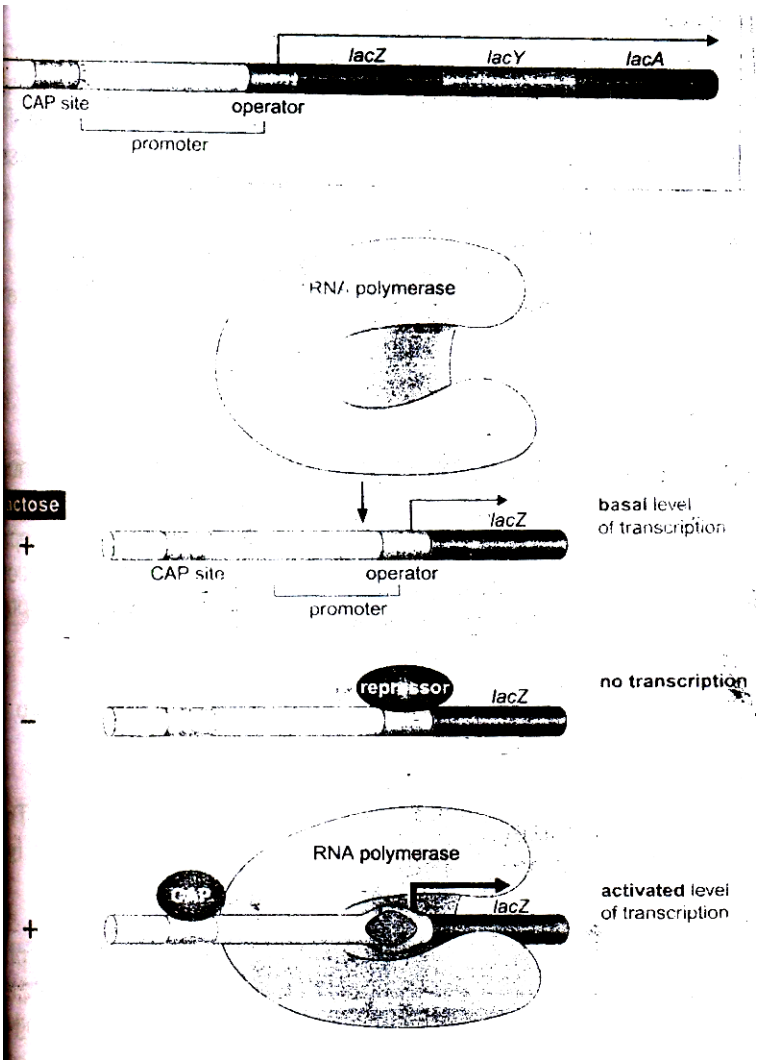


Figure 1: The lac operon: The three genes (lac Z, Y, and A) are transcribed as a single mRNA from the promotor (as indicated by the arrow). The CAP site and the operator are each about 20 bp. The operator lies witin the region bound by RNA polymerase at the promoter, and the CAP site lies just upstream of the promotor (For more details of the relative arrangements of these binding sites and the text for a description of the proteins that bind to them). The picture is simplified in that there are two additional, weaker, lac operators located nearly but we do not need to consider those at present.

Figure 2: Expression of the lac genes: The presence or absence of the sugars lactose and glucose control the level of expression of the lac genes. High levels of expression require the presence of lactose (and hence the absence of functional lac repressor) and absence of the preferred energy source, glucose (and hence presence of the activator CAP). When bound to the operator, Lac repressor excludes polymerase whether or not active CAP is present. CAP and Lac repressor are shown as single units, but CAP actually binds DNA as a dimer, and Lac repressor binds as a tetramer. CAP recruits polymerase to the lac promoter where it spontaneously undergoes isomerization to the open complex. (the state shown in the bottom line)

Each of these regulatory proteins responds to one environmental signal and communicates it to the lac genes. Thsu, CAP mediates the effect of glucose, whereas lac repressor mediates the lactose signal. This regulatory system works in the following way. Lac repressor can bind DNA

and repress transcription ony in the absence of lactose. In the presence of that sugar, lthe repressor is inactive and the genes de-repressed (expressed). CAP can bind DNA and activate the flac genes only in the absence of glucose. Thus, the combined effect of these two regulators ensures that the genes are expressed at significant levels only when lactose is; present and glucose absent.

Mechanism of enzyme repression

The enzyme whose amount is reduced by the presence of their end products are called repressibnle enzymes. In histidine biosynthesis, for example, histidine is the end product. The end-product metabolites when indteoduced into a agrowth medium specifically decrease the amount of a particular enzuyme. These are known co-repressors. To account for such an end-product repression of an enzyme or enzymes system. Jacob and Monod postulated that the repressor molecule in ksusch cases is inactive by itself, bu binds the repressing metabolite to form a repressor-co-repressor complex. Thus complex then binds to the operator (o) and prevents transcsripiton of the corresponding structural genes. There are two classes of repressor molecules, one operative in the indudction of enzyme activity, and the other in repression of the en-product. Induction and repression are, thus, based on similar principles and no covalent bond is formed betweefn repressor and their inducers or corepressors. A portion of each repressor molecule is complementary in shape of a specific portion of tis innducer or corepressor, and weak bonds join the two. These weak bonds are readily made and broken, allowing the active or inactive repressor to adjust quickly to the fphysiological need.

The promoter

The fpromoter represents a short sequence of bases, normally less than 100 nucleotides, which is recognized by a DNA-dependant RNA polymerase. It has been identified by genetic and kbiochemical studies that the fpromoter lies between the regulatory gnes (i) and the fopreator (o) of the flac operon. The promoter-RNA polymerase binding initiates the trascription of the neighbouring gene. The promoter is also the binding site for another specific tuype of protein, the cyclic AMP receptor protein (CRP) or catabolite gene activator protein (CAP).

CAP and Lac repressor have opposing effects on RNA polymerase binding to the flac promoter

In the DNA region, the site bound by Lac repressor is called the lac operator. The 21 bp sequence is twofold symmetric and is recognized by two subunits of Lac repressor, one binding to each half-site. The CAP and Lac repressor bind DNA using a common structural motif.

The lac operator overlaps the promoter, and so repressor bound to the operator physically prevents RNA polymerase from binding to the promoter and thus initiating RNA synthesis.

Protein binding sites in DNA can be identified, and their location mapped, using DNA foot printing and gel mobility assays.

The RNA polymerase binds the lac promoter poorly in the absence of CAP, even when there is no active repressor present. This is because the sequence of the –35 region of the lac prom0ter is not optimal for its binding, and the promoter lacks an UP-element. This is typical of promoters that are controlled by activators.

CAP binds as a dimere to a site similar in length to the lac operator, but different in sequence. This siste is located some 60 bp upstream of the start site of transcription. When CAP binds to that site, the activator helps polymerase bind to the promoter by interacting with the enzyme and recruiting it to the promoter. This cooperatie binding stabilizes the binding of polymerase to the promoter.

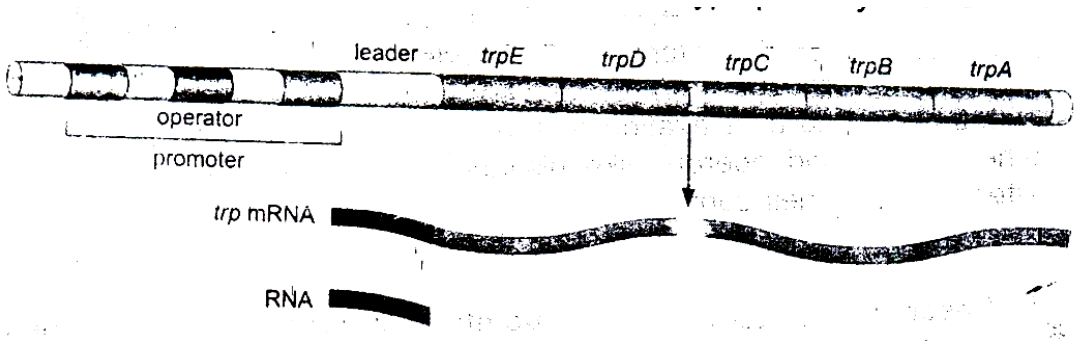
6. Discuss the regulation of tryptophan system with trp operon system.

This is an example of gene regulation in prokaryotes at the steps after transcription initiation. The amino acid biosynthetic pathway is explained for the synthesis of tryptophan. The expression of operon genes for the synthesis of tryptophan in E.coli is demonstrated.

Amino acid biosynthetic operons are controlled by premature transcription termination

In E.coli the five contiguous trp genes encode enzymes that synthesize the amino acid tryptophan. These genes are expressed efficiently only when tryptophan is limiting. A repressor controls the genes, just as the lac genes are, but in this case the ligand that controls the activity of that repressor (tryptophan) acts not as an inducer but as a corepressor. That is, when tryptophan is present, it binds the trp refpressor and induces a coforamtional change in that protein, enabling it to b9ind the trp operator, allowing the synthesis of trp mRNA to commence from the adjacent promoter.

The given below diagram shows the pattern of genes arrangement in tryptophan synthesis.



The TRP operon. The tryptophan operon of E. coli, showing the relation of the leader synthetase (product of trpE), phosphoribosyl anthranilate transferase (trpD), phosphoribosyl anthranilate isomerase-indole glycerol phosphate synthetase (trpC), tryptophan synthetase β (trpB), an, I tryptophan.

On the above genes,

i. trp E and trp D encode for anthranilate synthatase
ii. trp c encodes for indol glucero-(P) synthatase
iii. trp B & trp A encode tryptophane synthetase

Repressor is a tryptophan repressor, which is encoded by gene trp R – that is not linked with operon. Repressor has low speficity with the operator and remains inactive and active when association with tryptophan and this is called as co-repressor.

The tryptophan function through feed back inhinition, when trptopjhan combnines with first enzyme of tryptophan synthesis inhinits its activity thorugh allosteric modification. Thus the synthesis of tryptophan is self-regulatory. When tryptophan is plenty, i. From feeed back inhibition; ii. Co-repression and iii. Stop synthesis of ltryptophan.

Surprisingly, howeve, once polymerase has initiated a trp mRNA molecule it does not always fomplete the full transcript. Indeed, most messages are terminated prematurely before they include even the first trp gene (trp E), unless a second and novel device confirms that little tryptophan is available to the cell.

This second mechanism overcomes the premature transcription termination, called attenuation. When tryptophan levels are high, RNA polymerase that has initiated trancription pauses at a specific site, and then terminates before getting to trpE, as we just described. But when tryptophan is limiting, polymerase does not terminate, and instead reads through the trp genes. Attenuatin, and the way it is overcome, rely on the close link between transcription and translation in bacteria, and on the ability of RNA to form alternative structures through intramolecular base pairing, as we now escribe.

The key to understanding attenuation came from the tryptophan promoter before RNA polymerase encounters the first codon of trp E. Near the end of this leader sequence, and before trpE, is a transcssription terminator, composed of a characterestic hairpin loop in the RNA 139 nucleotides long. This is the RNA product seen in the presence of high levels of tryptophan.

The use of both repression and attenuation to control expression allows a finer tuning of the legel of intracellular tryptophan. It provides a two-stage response to progressively more stringent

tryptophan staravation – the initial response being the cessation of repressor binding, with greater staration leaeing to relaxation of attenuation. But attenuation alone can prove robust regulation; other amino acid operons like his and leu have no repressors; instead, they 4ely entirely on attenuation for their control.

7. Describe the stages in the lytic life cycle of typical lambda phage and the role of genes involved in this process.

Bacteriophage lambda is a virus that infects E.coli and multiplies inside the bacteria cell. Up pon infection, the phages can prophagate generally in the bacterial cell by either of two ways. As i. Lytic method and ii. Lysogenic method. In both the methods the virus get multipliled more number and release out of the cell by either affecting or not affecting the bacteria cell.

Lytic life cycle

In lytic life cycle the virus growth requires replication of the phage DNA and synthesis of new coat proteins. These components combine to form new phage particles that are released by lysis of the host cell. The given below diagram shows the diagrammatic representation of lytic life cycle of Bacteriophage.

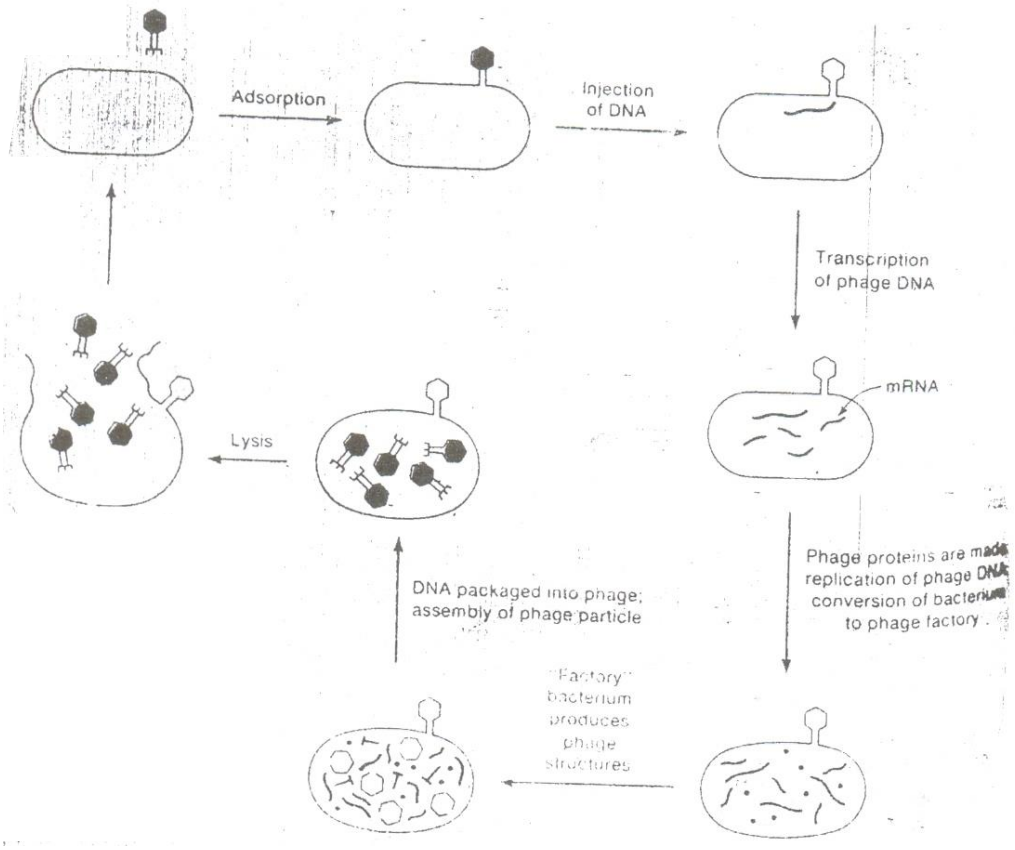


Figure schematic diagram of the lytic cycle of a typical phase

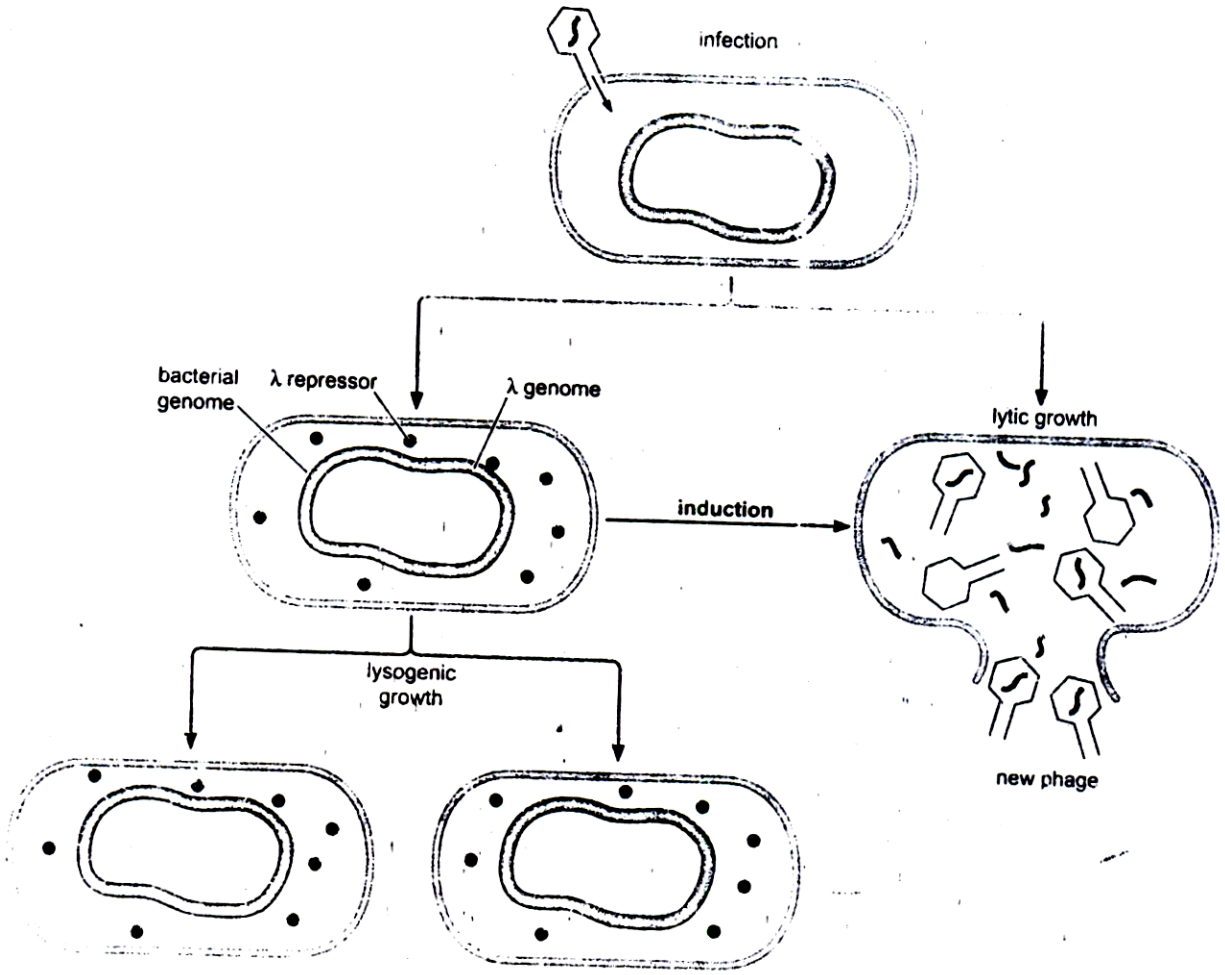


FIGURE 16-24 Growth and induction of λ lysogen. Upon infection, λ can grow either lytically or lysogenically. A lysogen can be propogated stably for many generations, or it can be induced. Following induction, the λ genes are expressed in proper order, leading to the production of new phage particles.

The choice of developmental pathway depends on which of two alternative programs of gene expression is adopted in that cell. The program responsible for the lylsogenic state can be maintained stably for many generations, but then, upon indudction, switch over to the lytic program with great efficiency.

Gene expression control Lytic cycle

Lambdaphage has a 50-kb genome and some 50 genes. Most of these encode coat proteins, proteins invoved in DNA replication, recombination and lysis. The produdcts of these genes are important in making new phage particles during the lytic cycle, lbut our concern here is restricted to the regulatory protein, and where they act. We can, therefore, concentrate on just a few of them, and start by considering a very small area of the genome, shown in the following diagram.

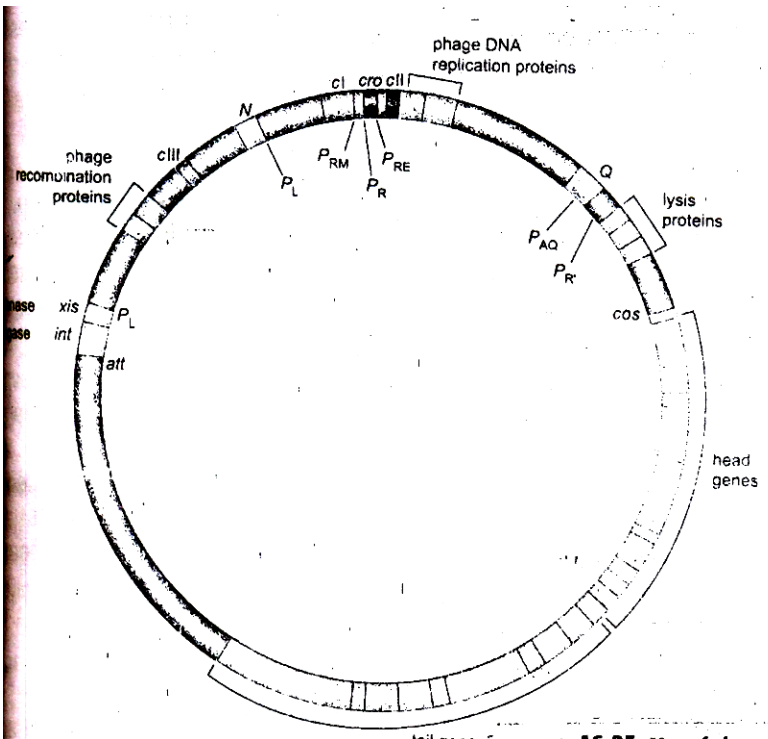


FIGURE 16-25 Map of phage λ in the circular form. λ genome is linear in the phage head, but upon infection, circularizes at the *cos* site. When integrated into the bacterial chromosome it is in a linear form, with ends at the *att* site (see Chapter 11 for a description of integration).

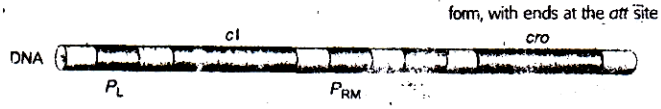


FIGURE 16-26 Promoters in the right and left control regions of phage λ.

The depicted region contains two genes as cI and cro and three promoters lilke PR, pL and PRM. All the other phage gens except one (minor one) are outside this region and are transcribed directly from PRand PL (which stand for rightward and leftward promoter, respectively), or from other promoters whose activities are controlled by products of genes transcribed from pR and PL. PRM (promote for repressor maintenance) transcsribes only the cI gene. PR and PL are strong, constitutive promters – that is, they bind RNA polymerase efficiently and direct transcription without help from an activator. PRM, in contrast, is a weak promoter and only directs efficient transcription when an activator is bound just upstream. PRM resembles the lac promoter in this regard.

There are two arrangements of gene expression depicted in the following figure. One renders growth llytic, the other lysogenic. Lytic growth proceeds when pL and PR remain switched on, while PRM is kept off.

Regulatory proeins and their binding sites

The lambda repressor encoded by cI gene is a protein of two domains joined by a flexible linker region. The N-terminal domain contains the DNA-binding region. Lambda repressor bind

with DNA as a dimmer; the main dimerization contacts are made between the c-terminal domains. A single dimmer recognizes a 17 bp DNA sequence, each monomer recognizing one half-site as in the lac system.

Despite its name, lambda repressor can both activate and repress transcription. When functioning as repressor, it works in the same way as does lac repressor – it binds to sites that overlap the promoter and excludes RNA polymerase. Lambda repressor and Cro can each bind to any one of six operators. These sistes are recognized with different affinities by each of the proteins. Three of those sites are found in the left-control region, and three in the right. The three binding sites in the right operator are called OR1, OR2 and Or3; these sites are similar in sequence, but not identical, and each one – if isolated from the others and examined separately – can bind either a dimmer of repressor or a dimmer of Cro. The affinities of these various interactions, however, are not all the same. Thus, repressor binds OR1. OR3 binds repressor with about the same affinity as does OR2, Cro, on the other hand, bind OR3 with highest affinity, and only binds OR2 and OR1 when present at tenfold higher concentration. The significance of these differences will become apparent presently.

Repressor and Cro bind in different patterns to control lytic growth

For llytic growth, a single Cro dimmer is bound to OR3; this site overlaps PRM and so Cro represesses that promoter. As neither repressor nor Cro is bound to OR1 and OR2, PR binds RNA polymerase and directs transcription of lytic genes; PL does likewise. Recall that both PR and PL are strong promoters that need no activator.

Another activator λcII, controls the decision between llytic and lysogenic growth upon infection of a new host

Here the critical genes are cII and cIII from λgenes. cII is on the right of cI and is transcribed from PR; cIII, on the left of cI is transcribed from PL. Like λ repressor, CII is a transcriptional activator. It binds to a site upstream of a promoter called PRE (for repressor establishment) and stimulates transcription of the cI (repressor) genes from that promoter. Thus, the repressor gene can be transcsribed from two different promoters (PRE and PRM).

Growth condition of E.coli control the stability of CII protein and thus the Lytic and Lysogenic choice

The efficiency with which CII directs transcription of the cI gene – and hence the rate at which repressor is made – is the critical step in deciding how λ will develop. When the phage infects the population of bacterial cells that are heathly and growing vigourously, it tends to propagate ytically, releasing progeny into an environment rich in fresh host cells. When

conditions are poor for bacterial growth, however, the phage is more likely to form lysogens and sit tight; there will likely be few host cells in the cicinity for any progeny phage to infect.

CII is a very unstable protein in E.coli; it is degraded by a spcifc protesase calle FtsH, encoded by the jfl gene. The speed which CII cn direct synthesis of repressor is thus determined by how quicklyl it is being degraded by FtsH. Cells lacking the hfl gene almost always form lysogens upon infection by λ; in the absence of the protease, CII is stable and directs snthesis of ample repressor. FtsH activity is itself regulated by the growth conditions of the bacterial cell, and, although it is not understood exactly how that is achieved. We can say as, if FtsH is very active, the growth is good. CII is destroyed efficiently, repressor is not made, and the phages tend to grow llytically. In poor growth conditions the opposite happens. Low ftsH activity, slow degradation of CII, repressor accumulation, and tendency toward lysogenic development. The phage protein CIII also modulates levels of CII. CIII stabizes cII, probably because it acts as an alternative substrate fr FtsH.

A second cII protein-dependant promoter, PI, ihas a sequence similar ot that of PR and is located in front of the phage gene int; this gene encodes the integrase enzyme that catalyzes site-spefific recombination λ DNA into the bacterial chromosome to form the prophage. A third cII-dependant promoter, PAQ, located in the middle of gene q, acts to retard llytic development and thus to promote lysogenic development. This is because the PAQ RNA acts as an antisense message, binding to the Q message and promoting its degradation. Q is another regulator, one that promotes the late stages of ytic growth.

8. Discuss in detail on the role of gene regulation and life cycle of lysogenic bacteria phage.

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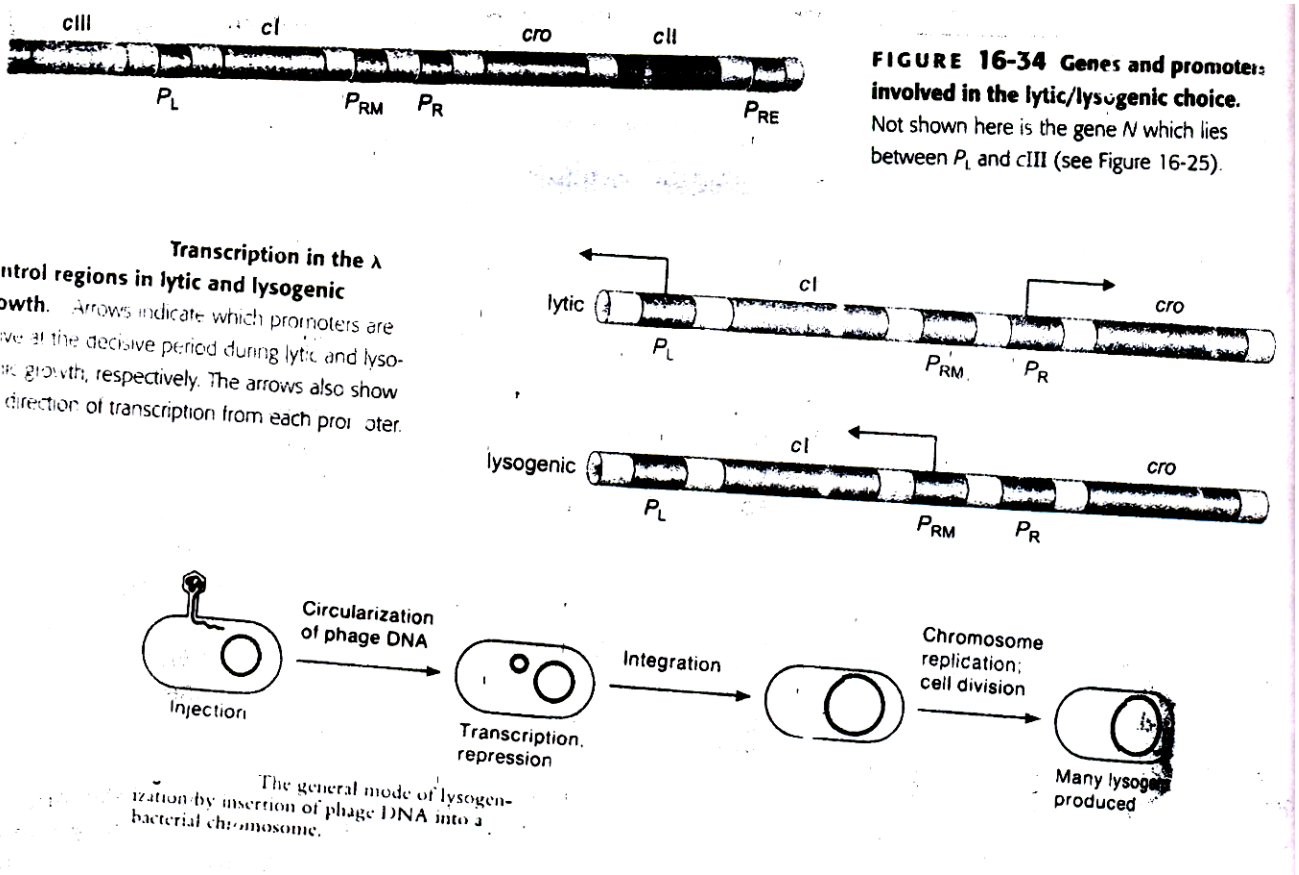

FIGURE 16-34 Genes and promoters involved in the lytic/lysogenic choice. Not shown here is the gene *N* which lies between P_L and cIII (see Figure 16-25).

Transcription in the λ control regions in lytic and lysogenic growth. Arrows indicate which promoters are active at the decisive period during lytic and lysogenic growth, respectively. The arrows also show the direction of transcription from each promoter.

The general mode of lysogenization by insertion of phage DNA into a bacterial chromosome.

The depicted region contains two genes as cI and cro and three promoters lilke PR, pL, and PRM. All the other phage gens except one (minor one) are outside this region and are transcribed directly from PRand PL (which stand for rightward and leftward promoter, respectively), or from other promoters whose activities are controlled by products of genes transcribed from pR and PL. PRM (promote for repressor maintenance) transcsribes only the cI gene. PR and PL are strong, constitutive promters – that is, they bind RNA polymerase efficiently and direct transcription without help from an activator. PRM, in contrast, is a weak promoter and only directs efficient transcription when an activator is bound just upstream. PRM resembles the lac promoter in this regard.

When lysogenic growth is proceeds when PL and PR genes are switched of and PRM is switched on.

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Repressor and Cro bind in different patterns to control lysogenic growth

During lysogeny method, PRM is on, while PR and Pl are swithed off. Repressor bound cooperatively at OR1 and OR2 blocks RNA polymerase binding at PR, repressing transcription from that promoter. But repressor bound at OR2 activates transcsription from PRM.

Lysogenic induction requires proteolytic cleavage of lambda repressor

E.coli senses and responds to DNA damage. It does this by activating the function of protein called RecA. This enzyme is involved in recombination but it has another function. That is, it stimulates the proteolytic autocleavage of certain proteins. The primary substrate for this activity is a bacterial repressor protein called LexA that represses genes encoding DNA repair enzymes. Activated RecA stimulates autocleavage of LexA, releasing repression ofthose genes. This is called SOS response.

If the cell is a lysogen, it is in the best interests of the prophage to escape under these threatening circumstances. To this end, lambda repressor has evolved to resemble LexA, ensuring that lambda repressor too undergoes autocleavage in response to activated RecA. The cleavagte reaction removes the C-terminal domain of repressor, and so dimerization and cooperativity are immediately lost. As these functions are critical for repressor binding to OR1 and OR2, loss of cooperativity ensures that repressor dissociates from those sites . Loss of repression triggers transcription from PR anf PL leading to lytic growth.

For inductin to work efficiently the level of repressor in a lysogen must be tightly regulated. If the levels were to drop too low, under normal conditions, the lysogen might spontaneously induce. Of levels reose too high, appropriate inductin would be inefficient. The regulation system is called positive and negative regulatin.

Another activator λcII, controls the decision between llytic and lysogenic growth upon infection of a new host

Here the critical genes are cII and cIII from λgenes. cII is on the right of cI and is transcribed from PR; cIII, on the left of cI is transcribed from PL. Like λ repressor, CII is a transcriptional activator. It binds to a site upstream of a promoter called PRE (for repressor establishment) and stimulates transcription of the cI (repressor) genes from that promoter. Thus, the repressor gene can be transcsribed from two different promoters (PRE and PRM).

Growth conditions of E.coli control the stability of CII protein and thus the Lytic and Lysogenic choice

The efficiency with which CII directs transcription of the cI gene – and hence the rate at which repressor is made – is the critical step in deciding how λ will develop. When the phage infects the population of bacterial cells that are heathly and growing vigourously, it tends to propagate ytically, releasing progeny into an environment rich in fresh host cells. When conditions are poor for bacterial growth, however, the phage is more likely to form lysogens and sit tight; there will likely be few host cells in the cicinity for any progeny phage to infect.

CII is a very unstable protein in E.coli; it is degraded by a spcifc protesase calle FtsH, encoded by the jfl gene. The speed which CII cn direct synthesis of repressor is thus determined by how quicklyl it is being degraded by FtsH. Cells lacking the hfl gene almost always form lysogens upon infection by λ; in the absence of the protease, CII is stable and directs snthesis of ample repressor. FtsH activity is itself regulated by the growth conditions of the bacterial cell, and, although it is not understood exactly how that is achieved. We can say as, if FtsH is very active, the growth is good. CII is destroyed efficiently, repressor is not made, and the phages tend to grow llytically. In poor growth conditions the opposite happens. Low ftsH activity, slow degradation of CII, repressor accumulation, and tendency toward lysogenic development. The phage protein CIII also modulates levels of CII. CIII stabizes cII, probably because it acts as an alternative substrate for FtsH.

A second cII protein-dependant promoter, PI, ihas a sequence similar to that of PR and is located in front of the phage gene int; this gene encodes the integrase enzyme that catalyzes site-spefific recombination λ DNA into the bacterial chromosome to form the prophage. A third cII-dependant promoter, PAQ, located in the middle of gene q, acts to retard llytic development and thus to promote lysogenic development. This is because the PAQ RNA acts as an antisense message, binding to the Q message and promoting its degradation. Q is another regulator, one that promotes the late stages of lytic growth.

B.E./B.TECH DEGREE EXAMINATION, APRIL / MAY 2008

Fourth Semester

Biotechnology

BT 1255 – MOLECULAR BIOLOGY

PART – A

1. What is the correlation between replication slippage and DNA repeats number?
2. What is C value paradox?
3. Draw a ribosome with three major RNA binding sites.
4. List the various mechanism of DNA repair.
5. List proteins that participate in DNA replication.
6. Mention two techniques that help in identification of 5′ end of mRNA.
7. Differentiate foot printing and finger printing techniques.
8. What is meant by codon usage pattern/codon preference of an organism?
9. Differentiate monocistronic and polycistronic mRNA.
10. How will you differentiate a promoter and enhancer element?i

PART - B

11. (a) (i) Discuss the rationale relating Mender's monohybrid results to his postulates.

    (ii) What advantages were provided by Mendel's choice of garden peas in his experiments?

    Or

    (b) Discuss the concept of Hershey – Chase experiment and its inferences with illustrations?

12. (a) (i) Discuss about catalytic RNAs.

    (ii) Elaborate on Splicesome mediated splicing of nuclear mRNA.

    Or

(b) Briefly explain the following post-transcriptional modifications such as

(i) RNA editing.

(ii) Self splicing introns.

(iii) Processing of rRNA and tRNA.

(iv) mRNA turnover/decay.

13. (a) Narrate transcription in prokaryotic system and the factors involved in initiation elongation and termination.

Or

(b) Expound Lac operon and its regulation in prokaryotic system?

14. (a) (i) Elaborate on tRNA structure, wobbling and decoding.

(ii) Explain how suppressor mutants and synthetic lethal mutants help in genetic studies.

Or

(b) (i) Narrate the cycle of peptide chain elongation during translation in eukaryotes.

(ii) How translation is terminated and ribosome recycling increases the efficiency of translation?

15. (a) (i) Describe the semi conservative mechanism of DNA replication.

(ii) The experiment which was used to confirm the above mechanism?

Or

(b) Discuss the various stages of attenuation in trp operon with suitable diagram.

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B.E./B.TECH DEGREE EXAMINATION, MAY/JUNE 2006

Fourth Semester

Biotechnology

BT 1255 – MOLECULAR BIOLOGY

PART – A

1. Differentiate prokaryotic and eukaryotic promoters.
2. What are the three enzymatic activities for DNA polymerase I?
3. One of the complementary strands of two DNA molecules is given. Which DNA molecule would denature at low temperature? Why?

   (a) AGTTGCGACCATGATCTG
   (b) ATTGGCCCCGAATATCTG

4. Explain DNA foot printing.
5. What is the fundamental difference between the initiation of θ replication and of rolling circle replication?
6. Differentiate heterochromatin from euchromatin.
7. Write a note on types of RNA splicing.
8. What is conditional mutant? Give example.
9. How do lactose molecules first enter an uninduced $lacI^+$ $lacZ^+$ $lacy^+$ cell to induce synthesis of β-galactosidase?
10. What is the reading frame of an mRNA? What additional features would you expect an mRNA to have?

PART - B

11. (i) Discuss spliceosome role in nuclear pre mRNA splicing.

    (ii) Explain the structure and role of RNA polymerase.

12. (a) Describe in detail the molecular regulation involved in tryphtophan synthesis.

Or

(b) Explain in detail how conjugation and transduction are used in gene mapping.

13. (a) Explain in detail tRNA role in translation.

Or

(b) Outline the mechanisms for excision and recombinational repair in E.Coli.

14. (a) Write in detail the various uses of mutant.

Or

(b) Discuss in detail the stages of life cycle of typical lytic and lysogenic phages.

15. (a) Explain the events taking place in replication fork with a neat sketch.

Or

(b) Discuss in detail the physical and chemical structure of DNA. Add a note on alternate DNA structures.

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B.E./B.TECH DEGREE EXAMINATION, NOVEMBER /DECEMBER 2006

Fourth Semester

Industrial Biotechnology

IB 241 – MOLECULAR BIOLOGY

PART – A

1. What are sigma factor and holoenzyme?
2. What is an Insertion Sequence or IS element?
3. Define a Open Reading frame.
4. Mention the factors that affect translation accuracy.
5. Explain how DNA is packed into chromosomes.
6. Give two examples for invitro translation systems.
7. What is meant by C value paradox.
8. Mention the role of methylation in replication and cell division control.
9. State whether transcription is on or off in a prokaryotic system where deletion analysis involves the removal of sequences in the following way.

   (a) - 1 to – 5 removed (b) - 10 to – 15 removed
   (c) - 16 to – 19 removed (d) - 80 to – 100 removed

10. What are retrons?

PART - B

11. (a) (i) Formation of Open complex in E. Coli and its significance.

    (ii) A bacterial medium contains Lactose and Glucose, describe the preferred metabolism and the molecular mechanism behind it.

    Or

    (b) (i) Describe briefly the functions of nucleosome and ribosome.

    (ii) Describe the function of RNA polymerase II and tRNA.

12. (a) (i) With the aid of suitable diagrams describe the process of translation initiation.

(ii) Explain what is a promoter and enhancer, their role in gene regulation and mention how they can be identified.

Or

(b) (i) Describe the mechanisms that minimize error in DNA replication.

(ii) Give the roles of the key players in DNA replication Topoilsomerase, helicase, polymerase, ligase, RNA primase and Single strand binding protein.

13. (a) (i) Mention the different types of point mutation and their consequences.

(ii) Describe the modules in an eukaryotic promoter and Motifs of the DNA binding protein which are essential for eukaryotic transcription.

Or

(b) (i) List the post transcriptional modifications in eukaryotic mRNA and comment on Central Dogma and RNA editing.

(ii) With the aid of a diagram/s describe how introns are removed from eukaryotic genes.

14. (a) (i) Explain how Tryptophan levels are regulated in a prokaryotic system.

(ii) Explain the following – Secondary structure of DNA, and Events of the replication fork.

Or

(b) (i) Describe nucleic acid chain elongation with suitable diagram.

(ii) List the translation elongation factors and Sn RNPs and their function.

15. (a) (i) What are mobile genetic elements give a report on various types?

(ii) What are tumor suppressor genes? Illustrate your answer with two examples.

Or

(b) (i) Describe how cyclin and P53 are involved in cell cycle regulation.

(ii) Give a brief account on excision repair systems in E.Coli.

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B.E./B.TECH DEGREE EXAMINATION, APRIL /MAY 2003

Fourth Semester

Industrial Biotechnology

IB 241 – MOLECULAR BIOLOGY

PART – A

1. Enumerate the differences between B-DNA and Z-DNA.
2. How is an initiator tRNA different from $tRNA^{met}$ in E. coli?
3. Which two amino acids have a single codon? Give their codon sequence.
4. How is specificity of binding of various transcription factors to specific DNA sequences achieved?
5. What is the role of cAMP in the regulation of lac operon?
6. Why is ATP hydrolysis required for the unwinding of DNA by the DNA B gene product of E. coli?
7. How is the kinetic complexity of eukaryotic DNA related to its chemical complexity?
8. A nuclear localization signal is inserted in the middle of the sequence of an ER protein. Where will this 'hybrid' protein be localized in the cell?
9. Write briefly on retroviruses.
10. I have generated a strain of S. cerevisiae lacking a functional guanylyl transferase enzyme that caps the 5′ ends of mRNAs. Would this strain be viable? Explain.

PART - B

11. Explain the mechanism of splicing of eukaryotic nuclear genes.
12. (a) Detail the steps involved in the targeting, translocation and localization of an endoplasmic reticulum lumen protein.

Or

(b) Detail the steps involved in initiation and elongation phases of translation in prokaryotes.

13. (a) Outline the mechanism of replication of the ends of linear eukaryotic DNA.

Or

(b) Explain how the origin is fired and replication is regulated in E. coli.

14. (a) Explain in detail the regulation of the tryptophan operon in E.coli.

Or

(b) Detail the mechanism of genomic imprinting in mammals.

15. (a) Write notes on:

(i) Mutagenesis by deoxybromouridine.

(ii) Nucleotide excision repair.

Or

(b) Write notes on:

(i) Regulation of CI gene expression in lambda phage.

(ii) Zinc-finger proteins.

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B.E./B.TECH DEGREE EXAMINATION, NOVEMBER /DECEMBER 2003

Fourth Semester

Industrial Biotechnology

IB 241 – MOLECULAR BIOLOGY

PART – A

1. Write the peptide sequence for the DNA sequence 5′ – GTTTGGTTTCTT-3′.
2. What are protooncogenes? Give two examples.
3. What is point mutation? What are the types of point mutation?
4. Where is the consensus TATA sequence seen? Explain its probable significance.
5. What is the role of a repressor in the transcription process?
6. What is nick translation?
7. Write a brief notes on histones.
8. What is the major function of reverse transcriptase?
9. Write the three bases-anticodon segment in the tRNAs specific for alanine.
10. What is a catenane?

PART - B

11. Describe the three different forms of RNA and their functions.
12. (a) Explain the steps involved in the translation processes in the prokaryotic system.

Or

(b) What is a gene mutation? Explain different types of gene mutations. Describe the role of chemical mutagens in the mutagenesis.

13. (a) What are the regulations in a transcription process of prokaryotes? Explain these regulations using Lac operon as a model.

Or

(b) What are oncogenes? How does the activation of oncogenes lead to carcinogenesis? Explain with examples.

14. (a) Write the functions of the pol I, pol II, pol III that are involved in the replication of DNA.

Or

(b) Describe codons and anticodons. Explain start, stop and redundant codons.

15. (a) Describe the RNA splicing mechanisms in eukaryotic transcription.

Or

(b) Describe the Watson-Crick DNA double helix model.

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B.E./B.TECH DEGREE EXAMINATION, MARCH 2006

Fourth Semester

Industrial Biotechnology

BT 1255 – MOLECULAR BIOLOGY

PART – A

1. What are the differences between DNA and RNA?
2. What are the different forms of DNA?
3. Write a note on hyperchromic effect.
4. Define genotypic and phenotypic expression.
5. What are heteroduplexes?
6. What is mean by reverse transcription?
7. What is meant by splicing?
8. Write a brief account on the structure and role of ribosome?
9. Define codon.
10. What is mean by suppressor mutation?

PART - B

11. Write in detail about the structure of DNA and RNA.

Or

12. (a) Explain the different experiments which prove DNA is the genetic material.

Or

(b) Give a detailed account on discontinuous replication and rolling circle model of replication.

13. (a) What is replication? Discuss about the replication of telomeres in eukaryotes.

Or

(b) Write a detailed account of the prokaryotic transcription.

14. (a) What are the features of promoters, enhancers and transcription factors?

Or

(b) What are the post translational modifications? Explain with their significance.

15. (a) Explain in detail about the genetic code.

Or

(b) Explain the different steps involved in the translation process.

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Important Part B Questions

1. Essay on lethality and various lethal genes with examples.
2. Explain linkage with examples.
3. Essay on Crossing over.
4. Features of crossing over.
5. Mechanism of crossing over.
6. Any problem solving for Monohybrid cross ratio.
7. Any problem solving for Dihybrid ratio.
8. Essay on Bacterial conjugation.
9. Essay on Bacterial Transformation.
10. Essay on Bacterial Transduction.
11. Classical Experiment of Avery, Macleod & Mc (arty DNA is the transforming principle)
12. Grifith's Experiment.
13. Hershey and chase experiment is direct evidence for DNA is a genetic material.
14. Chemical structures of DNA & RNA.
15. Physical structure of DNA.
16. Watson and crick model of DNA (Double helix)
17. Various forms of DNA. (Conformational changes of DNA)
18. Essay on Denasturation and melting curve of DNA.
19. Various conformational changes / flexibilities of DNA.
20. Effect of Ionic concentration on DNA.
21. Essay on DNA Renaturation process.
22. Kinetics of DNA Renaturation.
23. DNA kinetic complexity.
24. Different types of RNA – Essay.
25. Explain DNA Replication is Prokaryotic cell.

26. DNA Replication in Eukaryotic cell.
27. DNA replication by semiconservative method (general)
28. Short notes on Prokaryotic DNA polymerases.
29. Short notes on Eukaryotic DNA polymerases.
30. Short notes on 'D' loops (or) Essay.
31. Short notes on rolling circle model Replication.
32. Replication (Rolling model) in phage DNA.
33. Telomeric synthesis in Eukaryotic DNA.
34. Explain prokaryotic transcription process.
35. Short notes on 'Inch warm' model of Elongation in transcription process.
36. Explain the stability of Elongation complex.
37. Write in detail on Termination process of prokaryotic transcription process.
38. Discuss in detail on Eukaryotic transcription process.
39. Eukaryotic RNA polymerases. (10 marks)
40. Eukaryotic Transcription factors. (16 marks)
41. Nuclear RNA processing in Eukaryotic transcription.
42. Discuss in detail the initiation process of Eukaryotic transcription process.
43. Support the genetic coding system with experiments (or) How will you Elucidate the genetic coling system?
44. Genetic code is triplet and universal Explain.
45. Short notes on Amino acyl synthetasis. (Aas)
46. Translation process in prokaryotic system.
47. Translation in Eukaryotic system.
48. Post translational modifications in prokaryotes.
49. Poor translational modifications in Eukaryotes.
50. mRNA processing. (mRNA splicing, polyadinylation and mRNA capping).
51. Gene regulation in prokaryotic cell.

52. Regulation of gene expression in phage life cycle as lytic and lysogenic cycle.
53. Write an essay on repair synthesis of DNA.
54. Short notes on Repressor mutation and it effect in Genetic expressivity.
55. Describe codons and Anticodons? Explain start, stop and redundant codons.
56. Role of DNA polymerases in DNA replications?

PART – A

UNIT - I

1. Bacterial 'F' factor.
2. IS elements.
3. 'Hfr' cells (High frequency recombination)
4. Short notes on Bacterial transformation.
5. Hetero duplexes / Mimatch pairs.
6. Short notes on Transduction.
7. Short notes on Transfection
8. Virulent and Non-virulent organisms.
9. Write the transforming principle.

UNIT – II

1 Difference between Eukaryotic and prokaryotic chromosome.
2 Difference between RNA & DNA.
3 Structure of any one RNA base.
4 Structure of any one DNA base.
5 What are purines and pyrimidines.
6 G/C ratio.
7 DNA double helix structure.

8 Hypochrimic / Hyper chromic effect of DNA.

9 DNA denaturation.

10 Melting temperature (™) of DNA.

11 Melting curve for DNA.

12 DNA melting proteins / Helix destabilizing proteins.

13 What is Renaturation of DNA.

14 DNA Hetero duplexex.

15 Cot values

16 Cot curves

17 Short notes on DNA kinetic complexity

18 Short notes on tRNA.

19 Short notes on mRNA

20 Short notes on Satellite RNA.

21 Short notes on rRNA.

22 RNA splicing.

23 RAS proteins (RNAse Associated proteins)

24 What is DNA replication?

25 What are Primers?

26 Short notes on primase.

27 Helicase.

28 DNA polymerase

29 Short notes on Replication fork.

30 Short notes on Topoisomerase.

31 DNA Gyrase.

32 Leading strand / cynthesis

33 Lagging strand synthesis.

34 Okazaki fragments

35 DNA Ligase

36 Eukaryotic DNA polymerase

37 Prokaryotic DNA polymerase

38 Klenow fragment

39 What are replicons?

40 What are 'D' loops?

41 Short notes on Primosome.

42 Replisome.

43 'Tus' proteins (or) Termination Binding Proteins.

44 Histone proteins (Protein associated with Eukaryotic chromosomes)

45 Origin of Replication.

46 Replication bubbles.

47 RNA primers

48 What are Telomeres?

49 Significance of Telomeres.

50 Short notes on Telomerase.

51 Short notes on Telemeric binding proteins.

UNIT – III

1. Central Dogma.
2. Prokaryotic RNA polymerases.
3. Upstream and Downstream site of Transcription.
4. Short notes on Promotor site.
5. Prokaryotic promoter.
6. Short notes on Pribnow box.
7. Write about typical E.Coli promotor.

8. What is Abortive initiation?
9. Promotor complex.
10. Rho factors.
11. Terminator proteins
12. What are palindromic sequences?
13. Rho dependant termination.
14. Rho independent termination.
15. Short notes on Eukaryotic RNA polymerases.
16. Features of Eukaryotic mRNA
17. mRNA splicing / Nuclear RNA splicing.
18. mRNA capping.
19. What is polyadynation?
20. What is methylation?
21. Short notes on Eukaryotic promotor.
22. Short notes on Eukaryotic Transcription factors.
23. What are Eukaryotic silencers?
24. What is 'Hogness Box'.
25. Short notes on Initiation complex in Eukaryotic transcription.
26. Short notes on Spliceosome.

UNIT – IV

1. Short Notes on Ribosomes.
2. What is tRNA?
3. Define codon and Anticodon.
4. What are start codons.
5. What is amino acyl + RNA synthatase.

6. What are stop codons?
7. Define degenerant code.
8. What are the characteristic features of Coli.
9. The genetic code is non-ambiguous – How?
10. The genetic code is non-overlapping – Justify?
11. Synthesis of Phenylatanine?
12. Short notes on $tRNA^{met}$.
13. Short notes on ftRNA; ^{melf}
14. What is the significance of Redundancy of code?
15. What are synonymous codons? Write the features of synonymous codons?
16. What is adoptor RNA molecule? Why it is called as adoptor molecules?
17. Features of tRNA molecules.
18. Define ORF.
19. What are Polycistronic mRNA?
20. What are monocistronic mRNA?
21. Short notes on Initiator tRNA.
22. Eukaryotic translation factors.
23. Circle pattern of mRNA synthesis.
24. Chain release (Translation) factors.
25. Short notes on Delfano site.
26. Short notes on Initiation factors.
27. Translation elongation factors.
28. Short notes on Suppressor mutation.
29. Explain Wobble hypothesis briefly.
30. What is DNA breathing?
31. Short notes on Okazaki fragments.
32. Role of GTP molecule in Translation.

33. What are Kozak sequence? Write it importance.
34. What are catenanes?
35. Which two amino acids have single codon? Give their codon sequence.
36. Short notes on kinetic complexity.
37. Short notes on chemical complexity.
38. Short notes on initiator tRNA in E.Coli.
39. What is formylation?
40. Short notes on formylated tRNA.
41. What is nick translation?

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MADHA
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ENGINEERING COLLEGE

DEPARTMENT OF BIOTECHNOLOGY

BT3452-INDUSTRIAL ENZYMOLOGY

LECTURE NOTES

REGULATION 2021

II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

INDUSTRIAL ENZYMOLOGY

Unit - I

Enzyme Technology - Enzymes are proteins that catalyze the chemical reactions, biosynthetic and degradative, occurring in living cells. Some RNA molecules also have catalytic action; these are called ribozyme. Enzymes increase, by a factor of 103 - 1016, the rates of such reactions that would naturally occur, at a very slow rate, but, they can not induce a reaction that would not happen normally. The use of purified enzymes for generating a useful product or service constitutes enzyme technology.

History of Enzymes - The term 'enzyme' was introduced by Kiihne in 1878, although the first observation of enzyme activity in a test tube was reported by Payen and Persoz in 1833. During 1890s, Fisher suggested the 'lock and key' model of enzyme action, while a mathematical model of enzyme action was proposed by Michaelis and Menten in 1913. in 1926, Sumner crystallized, for the first time, an enzyme (urease).

Coenzymes and Cofactors - Enzymes mayor may not have a nonprotein molecule attached to them. Some enzymes contain covalently bound carbohydrate groups, which do not affect the catalytic activity, but may influence enzyme stability or solubility. Many enzymes have metal ions, while some others possess low weight organic molecules; these are called cofactors, and are essential for enzyme activity.

The transition state theory of enzyme action was put forth by Pauling in 1948, and in 1951 Pauling and Corey discovered, the (X-helix and sheet structures of enzymes. Sanger, in 1953, determined the amino acid sequence of a protein (insulin). In 1986, Cech discovered catalytic RNA, while Lerner and Schutlz developed catalytic antibodies.

Enzymes mayor may not have a nonprotein molecule attached to them. Some enzymes contain covalently bound carbohydrate groups, which do not affect the catalytic activity, but may influence enzyme stability or solubility. Many enzymes have metal ions, while some others possess low weight organic molecules; these are called cofactors, and are essential for enzyme activity.

An organic cofactor is commonly known as coenzyme. Cofactors and coenzymes may be covalently or noncovalently attached to the protein molecule, called apoenzyme.
An apoenzyme lacks catalytic activity in the absence of its specific cofactor. When a cofactor is so tightly bound to the apoenzyme that it is difficult to remove it without damaging the enzyme, the cofactor is often called a prosthetic group.
Both coenzymes and cofactors generally contribute to enzyme activity as well as stability. The complex of an apoenzyme and the cofactor is known as holoenzyme.

Enzymes and Catalyst - Enzymes are similar to catalysts in the following respects:

(i) they lower the activation energy of reaction,
(ii) they do not participate in the reaction, and return to their original form at the end of reaction; and
(iii) they only increase the reaction rate. But
(i) enzymes increase the rate of reaction at a phenomenal scale. They are

(ii) highly specific in that they can distinguish between similar parts of the substrate molecule (regiospecificity), and between optical isomers of the substrate (streospecificity). In addition, enzymes
(iii) are subject to a variety of regulations, and
(iv) their reaction rates show substrate saturation, which is not the case with catalysts (nonbiological). Enzymes are attractive because
(v) they operate under mild conditions of temperature, pressure and pH, which save energy, and
(vi) undesirable by products are not produced by enzymes.

Enzymes and Whole Cells - Purified enzymes offer certain advantages over whole cells.
(i) An enzyme catalyses only a specific reaction, and there is no risk of by product formation or product breakdown as may be the case with whole cells.
(ii) Whole cells convert a proportion of the substrate into biomass, which is not the case with enzymes.
(iii) The optimum conditions for an enzyme may be different from those for the organism producing it; a purified enzyme permits the operations to be performed under the optimal conditions.
(iv) Often purification of the product is much easier than when whole cells are used; this is because there is no interference from the molecules present in the complex medium used for production using whole cells, the by products produced by the cells and the constituents of the cells (whim the products accumulate within cells).
However, purified enzymes have limitations of
(i) high cost and
(ii) instability. In addition,
(iii) an enzyme catalyzes only a single reaction, while most industrial products are produced after a series of biochemical reactions, which can be performed only by whole cells.
(iv) In some cases, the by products produced by whole cells may add to the quality of the product, e.g., in case of wines various aldehydes, ketones, acids and tannins, etc. enhance its qualities.
Therefore, whole cells are preferred to enzymes in such processes where the use of latter does not offer an economic advantage.

Comparison Between Enzymes and Nonbiological Catalysts -

Feature	**Enzymes**	**Nonbiological catalysts**
Specificity	Highly specific; binding to substrate	Nonspecific

	may be less specific but the catalyzed reaction is highly specific	
Rate of reaction	Enhanced by a factor of 10^3-10^6	Enhancement only a fraction of that by enzymes
Regulation	Enzymes are subject to a variety of regulations, which increase/ decrease reaction rate	They are not subject to such regulations
Saturation	Enzymes have a maximum reaction rate with respect to substrate concentration	Most catalysts do not show substrate staturation
Chemical nature	Enzymes are proteins (RNA with enzymatic action is called ribozyme)	Metal and nonmetal inorganic molecules
Temperature, pressure and pH	Mild, biologically compatible	Often high temperature and high pressure
Side reactions	Do not occur	Occur

Production of Enzymes - Enzymes are obtained from animal tissues, plants, bacteria and fungi, including yeast. The bulk of enzymes, both in terms of quantity and variety, are derived from microorganism, higher plants being the distant second and animals being the least important.
The only animal enzyme to be produced in quantities greater than 2 ton/year is rennet or chymosin obtained from calf stomach. The bulk of plant enzymes are hydrolytic enzymes, e.g., α - and β-amylases, β -glucanase, papain, etc. Most of the enzymes are used by food industry.
Therefore, initially, plant and animal enzymes were preferred over microbial enzymes mainly for considerations of safety and the fear of contamination by microorganisms, toxins, etc.

But increased demands, shortages in supplies of enzymes from plant and animal sources, and difficulties in maintaining a continued supply of these enzymes prompted a much closer and more pragmatic evaluation of the microbial enzymes.
These enzymes have found increasing applications even in such areas where enzymes of animal origin were once exclusively used, e.g., cheese production. The rennet (= aspartic proteinase) produced by Mucor miehei is now widely used for cheese production.

Abzymes - Abzymes are antibodies that catalyze specific chemical reactions, i.e. function as enzymes. Antibodies by definition have evolved to recognise and bind to the ground states of the molecules they are specific to. In contrast, enzymes have binding sites that preferentially bind to the transition state of their substrate molecules.
For this reason abzymes are not produced naturally. A catalytic antibody is produced in response to molecules that have a structure similar to the

proposed/expected transition state of the substrate of the reaction to catalyse which the antibody is sought.

The fact that such catalytic antibodies are in fact produced is the strongest evidence in support of the transition theory of enzyme action.

Ribozymes - RNA molecules that have the capability of catalysing chemical reactions are called rhizomes. Ribozymes are true catalysts in that they enhance the rate of chemical reactions without any net change to themselves.
They are capable of turnover, i.e., recycling, and show kinetics typical of enzymes. Ribozymes are known to catalyze reactions like cleavage of RNA, cleavage of DNA, etc. Ribozymes are able to cut and splice themselves into a form that can catalyze the cleavage of other RNA/DNA molecules.

Enzyme Engineering - Improvement in the activity and usefulness of an existing enzyme or creation of a new enzyme activity by making suitable changes in its amino acid sequence is called enzyme engineering. When this approach is used to modify the properties of any protein, whether enzyme or nonenzyme, it is termed as protein engineering.

Since enzymes are proteins, enzyme engineering is a part of the larger activity of protein engineering. Enzyme engineering utilizes recombinant DNA technology to introduce the desired changes in the amino acid sequences of enzymes.

Recombinant DNA technology is also used to transfer genes encoding useful enzymes from dangerous, unapproved, slow growing or low producing microorganisms into safe, fast growing and high producing microorganisms.
In addition, the level of production of an enzyme may be increased by introducing more copies of the gene into the concerned organism. Such applications of recombinant DNA technology are enzyme or protein engineering, which must rest on modification of the amino acid sequence of the concerned enzyme or protein.

Objectives of Enzyme Engineering - The chief objective of enzyme engineering is to produce an enzyme that is more useful for industrial and/or other applications. The various properties of an enzyme that may be modified to achieve this objective are as follows:

(i) improved kinetic properties,
(ii) elimination of allosteric regulation,
(iii) enhanced substrate and reaction specificity,
(iv) increased thermostability,
(v) alteration in optimal pH,
(vi) suitability for use inorganic solvents,
(vii) increased/decreased optimal temperature, etc.

Principles of Enzyme Engineering - The structure and function of an enzyme molecule, for that matter of any protein molecule, are chiefly determined by its amino acid sequence, i.e., its primary structure. Therefore, any change in the properties of an enzyme is always reflected in its primary structure.
Conversely, a change in the amino acid sequence should alter the properties of the enzyme. But this is not always the case because the enzymatic properties, etc. are changed only when amino acid changes are introduced in certain critical regions of the protein.

Therefore, it is of great importance to know the critical regions for the various functions of an enzyme, and to be able to predict the effect, of specific amino acid changes in these areas on the various functions.
However, the present know ledge of the relationships between amino acid sequence, and three dimensional structure and properties of enzymes, obtained from a large database, is only partially operative.
It allows an explanation of the changes in structure and function on the basis of the changes in amino acid sequence, but it does not allow a dependable prediction of the influences of specific amino acid changes on the structure and function of enzymes.

It may; however, be reasonable to anticipate that as more elaborate databases and improved software become available, it should become possible to predict with a far greater confidence the structural and functional changes in enzymes produced by the specified changes in their amino acid sequences.
The effectiveness of enzyme engineering will be greatly enhanced then, and, this activity may have a tremendous influence on enzyme technology.

Steps in Enzyme Engineering - The strategies for enzyme engineering and their theoretical considerations are quite involved. The steps involved in enzyme engineering are briefly described in simple terms.
I. The first step consists of isolation of the concerned enzyme and determination of its structure and properties. Both amino acid sequence and the three dimensional structure of the enzyme are determined. Information on three dimensional structure are usually obtained from X-ray diffraction, nuclear magnetic resonance (NMR), etc.

2. The data so obtained are analyzed together with the database of known and putative structural effects of amino acid substitutions on enzyme structure and function. Molecular modelling is performed to determine a possible change in amino acid sequence for the desired improvement in function/structure of the enzyme.
3. The next step consists of constructing a gene that will encode the amino acid sequence specified at the end of step 2. This is best achieved by isolation and cloning of the endogenous gene encoding the concerned enzyme, and using this gene for site-directed mutagenesis.
In case of site directed mutagenesis, specified changes in the base sequence are introduced at specified sites of genes. There are several different approaches to

achieve this, but the most successful and rather simple approach uses PCR.

This approach uses primers that are complementary to the site/sequence in which the base sequence is to be changed. Obviously, the base sequence of primer oligonucleotides includes the proposed change(s). Alternatively, the entire gene may be synthesized using a gene machine/oligonucleotide synthesizer.
4. Once the appropriate gene is constructed, it is introduced and expressed in a suitable host, e.g. E. coli.
5. The recombinant or mutant enzyme so produced is isolated, purified and used for determination of its structure and properties. The information so obtained is added to the database. If the enzyme structure and function are not altered as desired, the next cycle of experimentation (steps 2-5) is undertaken.

Examples of Enzyme Engineering - So far, a commercial example has not emerged out of enzyme engineering. However, the technique has been extensively used for studies on the relationships between amino acid sequences, and structures and functions of various enzymes.
Many studies have focussed on modifications of industrial enzymes to enhance their usefulness. The results from these studies demonstrate the power of the technique, have generated valuable data on sequence structure function relationships and also revealed the gaps in our knowledge.

The value of enzyme engineering can be depicted by using the example of subtilisin (from B. amyloliquefaciens), a proteolytic enzyme used in detergents. It is desirable to enhance its stability at higher temperatures; pH and oxidant (bleach) strengths in order to improve its activity in detergents.
The P I cleft of this enzyme holds the amino acid residue on C-terminal side of the targeted peptide bond. Amino acid substitutions in the PI cleft enhance the specificity of the enzyme for specific peptide bonds, while that for others is reduced.
This change can be predicted fairly accurately. Increases in relative specificities may be useful for some applications. It should be kept in mind that subtilisin is unusual in that it is fairly nonspecific in activity, and has a large hydrophobic site, which can be made more specific relatively easily.

Subsitisin is inactivated by oxygen produced by bleaches; the inactivation is due to oxidation of the methionine residue at position 222 (Met 222) to its sulphoxide. Engineered enzymes in which the Met 222 residue was replaced by serine or alanine were much less sensitive to oxygen than the native enzyme; however, these enzymes showed somewhat reduced activity.
The lactate dehydrogenase from Bacillus stearothermophilus was used for enzyme engineering. When glutamine residue at position 102 (Gin 102) was replaced by arginine, the engineered enzyme became specific to malate to the same extent to which the native enzyme has specificity for lactate. This dearly shows that enzyme engineering may be able to create enzymes with new substrate specificities

Industrially Important Enzymes and their Applications-

Enzyme	Source	Annual production (tons/yr)	Application
Animal enzyme			
Catalase	Liver	<1	Food
Lipase	Pancreas	<1	Food
Rennet (= chlymosin)	Pancreas	>1	Cheese
Trypsin	Pancreas	<1	Leather
Plant enzymes			
α-Amylase	Malted barley	>100	Brewing
β-Amylase	Malted barley	>100	Brewing
β-Glucanase	Malted barley	>10	Brewing
Ficin	Fig latex	<1	Food
Papain	Papaya latex	>10	Meat
Bacterial enzymes			
α-Amylase	Bacillus	>100	Starch
β-Amylase	Bacillus	>1	Starch
Glucose (xylose) isomerase	Bacillus, Streptomyces	>10	Fructose syrup
Pencillin amidase	Bacillus	<1	Pharmaceutical
Protease	Bacillus	>100	Detergent
Fungal Enzymes			
α-Amylase	Aspergillus	>10	Baking
Glucoamylase	Aspergillus, Rhizopus	>100	Starch
Rennet (aspartic proteinase)	Mucor miehei	>10	Cheese
Pectinase	Aspergillus	>10	Drinks
Protease (aspartic proteinase)	Aspergillus	>10	Baking
Yeast Enzymes			
Invertase	Saccharomyces	<1	Confectionery
Lactase (β-galactosidase)	Kluyveromyces	<1	Dairy
Raffinase(α-galactosidase)	Saccharomyces	<1	Food

Enzymes Classification and Nomenclature- Enzymes are classified and named by the Commission on Biochemical Nomenclature of the International Union of Biochemistry.

An enzyme may be denoted in one of the following three accepted ways:

(i) a four number code following the letters EC (for Enzyme Commission), e.g., EC 1.1.1.3 (the first number refers to enzyme class, the second to subclass, the third to sub-subclass and the fourth to the serial number of the enzyme within a sub-subclass),

(ii) its systematic name based on the above classification, and

(iii) its recommended name. The various enzymes have been classified in the following six classes.

1. **Oxidoreductases** are involved in redox reactions, i.e., transfer of hydrogen or oxygen atoms between molecules. This class includes: dehydrogenases (hydride transfer), oxidases (e- transfer to O2), oxygenase (oxygen atom transfer from O2), and peroxidases (e- transfer to peroxides). Example, glucose oxidase (EC 1.1.3.4).
2. **Transferases catalyse** the transfer of an atom or group of atoms (like acyl-, alkyl- and glycosyl groups) between two molecules. The transferred groups are different from those transferred by the other classes of enzymes like Oxidoreductases, etc. Example, aspartate aminotransferase (EC 2.6.1.1)
3. **Hydrolases** are those enzymes, which catalyze hydrolytic reactions (and their reversals); this class includes esterases, glycosidases, proteases and lipases. Example, chymosin or rennin (EC 3.4.23.4).
4. **Lyases** are involved in elimination reactions resulting in the removal of a group of atoms from the substrate molecule. This class includes aldolases, decarboxylase, dehydratases and some pectinases. Example, histidine ammonia lyase (EC 4.3.1.3).
5. **Isomerases** catalyse the formation of isomers of molecules; they include epimerases, racemases and intramolecular transferases. Example, xylose isomerase (EC 5.3.1.5).
6. **Ligases or synthetases** catalyze the formation of covalent bonds between two molecules utilizing the energy obtained from hydrolysis of a nucleoside triphosphate like A TP or GTP. Example, glutathione synthase (EC 6.3.2.3).

Chemical Energetics- All chemical reactions are theoretically reversible, i.e., they proceed in both the directions, although at different rates. For example, the chemical reaction A + B=C + D utilizes reactants A and B to yield the products C and 0 (forward reaction). The reverse reaction, i.e., formation of A and B from C and D, will also occur simultaneously.

The rates of forward and reverse reactions depend on the concentrations of the reactants (e.g., A and B) and the products (e.g., C and D), and on the "rate constants" for the forward and backward reactions. (These rate constants differ for every reaction).

Therefore, the rate of forward reaction is the highest at the start of a reaction when the concentrations of the reactants are the highest and those of the products are zero. As the reaction proceeds, reactant concentrations progressively decline, while those of the products increase correspondingly.

As a result, the rate of forward reaction declines gradually, while that of the reverse reaction increases till both the rates become equal, i.e., the reaction reaches an 'equilibrium'. At equilibrium, the forward and reverse reactions proceed at identical rates and there is no net formation of either the products or the reactants.

All chemical reactions possess two major sets of properties:
(i) thermodynamic and
(ii) kinetic properties.

Thermodynamic Properties of Products- Thermodynamic predictions are based on the stabilities of products and reactants, and indicate the extent to which the reactants will be converted into products.
The stability of a compound depends on its free energy; a lower free energy is more stable and thermodynamically favoured state.The most useful thermodynamic value in the determination of whether a reaction will occur or not is the change in Gibbs free energy denoted by ΔG.
In general, the free energy of reactants is higher than that of the products. Therefore, the products of a reaction are more stable and at equilibrium their concentrations are much higher than those of the reactants.
Kinetic Properties of Molecules- According to transition state theory, for chemical reaction to occur between two reactant molecules, their free energy level must be raised above a threshold level to take them to a very unstable, high energy state called transition state.
The free energy needed to elevate a molecule from its stable ground state to the unstable transition state is known as activation energy (denoted by ΔG*). As a rule, very few molecules possess enough energy to reach the transition state.

Therefore, the magnitude of ΔG* is the major factor limiting the rate of a chemical reaction: the higher the value of the G: lower the rate of reaction. Enzymes markedly reduce the value of ΔG: for a reaction, thereby phenomenally increasing the rate of reactions.

Mechanism of Enzyme Action- An enzyme (E) molecule has a highly specific binding site or active site to which its substrate(s) bind to produce enzyme substrate complex (ES). The reaction proceeds at the binding site to produce the products (P), which remain associated briefly with the enzyme (enzyme-product complex).
The product is then liberated and the enzyme molecule is freed in an active state to initiate another round of catalysis. Apparently, the affinity of binding site for the product is much lower than that for the substrate.
E+S = ES = EP = E+P

Enzymes reduce the overall level of activation energy or ΔG:. Even a modest reduction in the value of ΔG leads to very large increases in the reaction rates. The various mechanisms used by enzymes to lower the activation energy are briefly described below.

1. Stabilization of the transition state of the substrates reduces the activation energy of the overall reaction; this is the most important feature of enzymatic catalysis. The binding sites of enzymes bind more strongly to the substrate molecules in the transition state than to those in the ground or stable state.
In addition, when a substrate molecule in ground state binds to the binding site, it is forced into a configuration closer to that of transition state; this lowers the energy needed for reaching the transition state.
2. When the substrate binds to an enzyme, it is held close to another chemical group and in the optimal orientation for the reaction to proceed; this is called catalysis by approximation. In contrast, substrate molecules in the transition state must collide with each other, a random process, for the reaction to occur in uncatalysed systems.
3. The chemical groups, e.g., amino groups, in the side chains of amino acids present in the active site become covently bound to a group in the substrate molecule; such substrate molecules are much more reactive than the nonbound molecules.
Another substrate may then react with the substrate bound to the enzyme molecule to form the product. The product they become free from the enzyme. This strategy of enzyme action is called covalent catalysis.
4. The amino acid side chains may act as acids, i.e., donors of protons, or bases, that is, acceptors of protons, and thereby catalyze reactions. In addition, electrons may also be transferred during the course of a reaction; such reactions are called redox reactions.
The term 'redox' is derived from 'reduction' (gain of an electron by a molecule) and 'oxidation' (loss of an electron by a molecule). The electron transfers generally involve transition metals or coenzymes present as a component of such enzymes. These I mechanisms are called acid base and redox catalysis.

Enzyme Kinetics- The rate of reaction catalyzed by an enzyme increases linearly with the substrate concentration up to a point, but it soon reaches the maximum value called Vmax beyond which there is no further increase in reaction rate; this is called substrate saturation. The phenomenon of substrate saturation is described by the Michaelis-Menten equation given below.
$V = V\ max\ [S] / [S] + Km$
Where, Vmax is the maximum rate of reaction, which occurs when the enzyme is saturated with substrate. V is the rate of reaction, [S] is the substrate concentration and Km is Michaelis constant. This equation can be used to predict the rate of reaction catalyzed by an enzyme at any substrate concentration provided the values of Vmax and K", are known.
Kill is the substrate concentration needed to obtain a reaction rate equal to 1/2Vmax. The substrate concentrations and reaction rates considered in the above equation are always the initial values, i.e., the values at the start of reaction.
Although few enzymes follow the Michaelis-Menten equation over a wide range of experimental conditions, it is still the most generally applicable equation for describing enzyme catalyzed reactions. The value of Km is characteristic for each enzyme substrate combination, but is it dependent of the enzyme and/or substrate concentrations. However, Km may be influenced by pH, temperature, ionic strength and other factors.

http://www.molecular-plant-biotechnology.info/enzyme-technology/enzyme-technology.htm

Why enzymes?

Catalysts increase the rate of otherwise slow or imperceptible reactions without undergoing any net change in their structure. The early development of the concept of catalysis in the 19th century went hand in hand with the discovery of powerful catalysts from biological sources. These were called enzymes and were later found to be proteins. They mediate all synthetic and degradative reactions carried out by living organisms. They are very efficient catalysts, often far superior to conventional chemical catalysts, for which reason they are being employed increasingly in today's high-technological society, as a highly significant part of biotechnological expansion. Their utilization has created a billion dollar business including a wide diversity of industrial processes, consumer products, and the burgeoning field of biosensors. Further applications are being discovered constantly.

Enzymes have a number of distinct advantages over conventional chemical catalysts. Foremost amongst these are their specificity and selectivity not only for particular reactions but also in their discrimination between similar parts of molecules (regiospecificity) or optical isomers (stereospecificity). They catalyse only the reactions of very narrow ranges of reactants (substrates), which may consist of a small number of closely related classes of compounds (e.g. trypsin catalyses the hydrolysis of some peptides and esters in addition to most proteins), a single class of compounds (e.g. hexokinase catalyses the transfer of a phosphate group from ATP to several hexoses), or a single compound (e.g. glucose oxidase oxidises only glucose amongst the naturally occurring sugars). This means that the chosen reaction can be catalysed to the exclusion of side-reactions, eliminating undesirable by-products. Thus, higher productivities may be achieved, reducing material costs. As a bonus, the product is generated in an uncontaminated state so reducing purification costs and the downstream environmental burden. Often a smaller number of steps may be required to produce the desired end-product. In addition, certain stereospecific reactions (e.g. the conversion of glucose into fructose) cannot be achieved by classical chemical methods without a large expenditure of time and effort. Enzymes work under generally mild processing conditions of temperature, pressure and pH. This decreases the energy requirements, reduces the capital costs due to corrosion-resistant process equipment and further reduces unwanted side-reactions. The high reaction velocities and straightforward catalytic regulation achieved in enzyme-catalysed reactions allow an increase in productivity with reduced manufacturing costs due to wages and overheads.

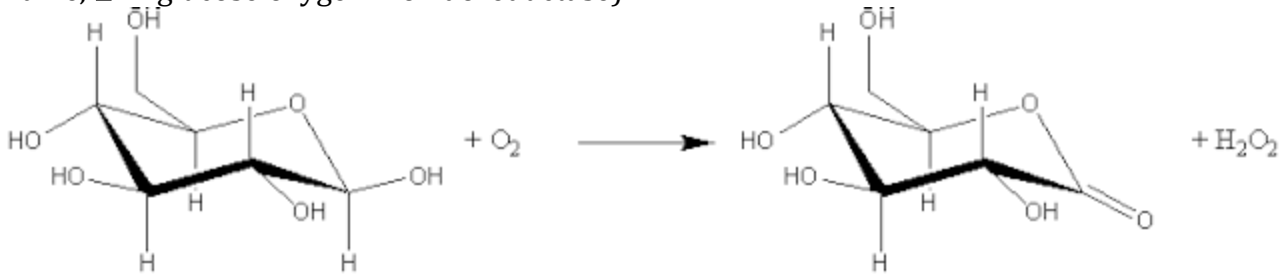
There are some disadvantages in the use of enzymes which cannot be ignored but which are currently being addressed and overcome. In particular, the high cost of enzyme isolation and purification still discourages their use, especially in areas which currently have an established alternative procedure. The generally unstable nature of enzymes, when removed from their natural environment, is also a major drawback to their more extensive use.

Enzyme nomenclature

All enzymes contain a protein backbone. In some enzymes this is the only component in the structure. However there are additional non-protein moieties usually present which may or may not participate in the catalytic activity of the enzyme. Covalently attached carbohydrate groups are commonly encountered structural features which often have no direct bearing on the catalytic activity, although they may well effect an enzyme's stability and solubility. Other factors often found are metal ions (cofactors) and low molecular weight organic molecules (coenzymes). These may be loosely or tightly bound by noncovalent or covalent forces. They are often important constituents contributing to both the activity and stability of the enzymes. This requirement for cofactors and coenzymes must be recognised if the enzymes are to be used efficiently and is particularly relevant in continuous processes where there may be a tendency for them to become separated from an enzyme's protein moiety.

Enzymes are classified according the report of a Nomenclature Committee appointed by the International Union of Biochemistry (1984). This enzyme commission assigned each enzyme a recommended name and a 4-part distinguishing number. It should be appreciated that some alternative names remain in such common usage that they will be used, where appropriate, in this text. The enzyme commission (EC) numbers divide enzymes into six main groups according to the type of reaction catalysed:

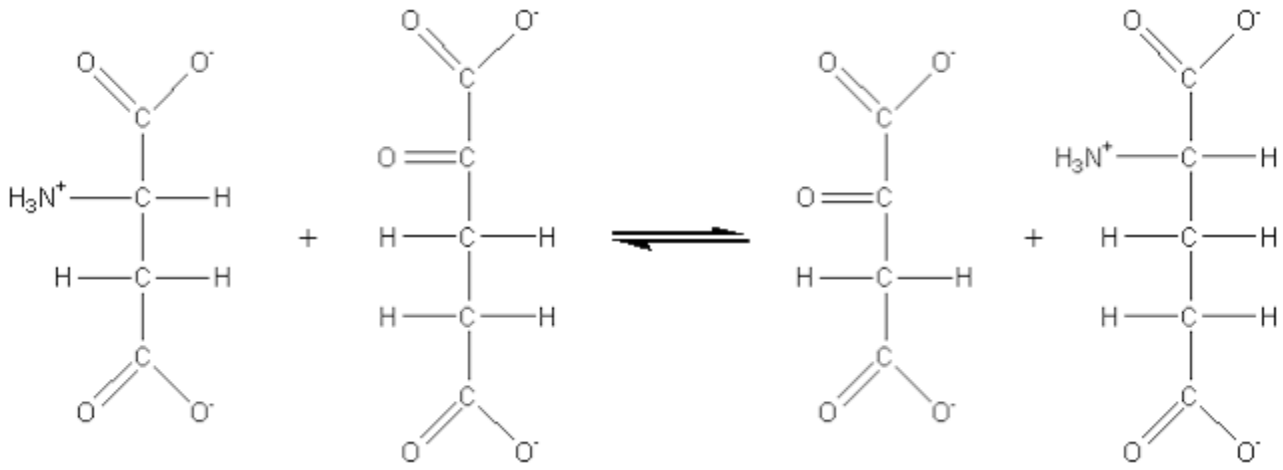
(1) Oxidoreductases which involve redox reactions in which hydrogen or oxygen atoms or electrons are transferred between molecules. This extensive class includes the dehydrogenases (hydride transfer), oxidases (electron transfer to molecular oxygen), oxygenases (oxygen transfer from molecular oxygen) and peroxidases (electron transfer to peroxide). For example: glucose oxidase (EC 1.1.3.4, systematic name, □-D-glucose:oxygen 1-oxidoreductase).



[1.1]

□-D-glucose + oxygen ⟶ D-glucono-1,5-lactone + hydrogen peroxide

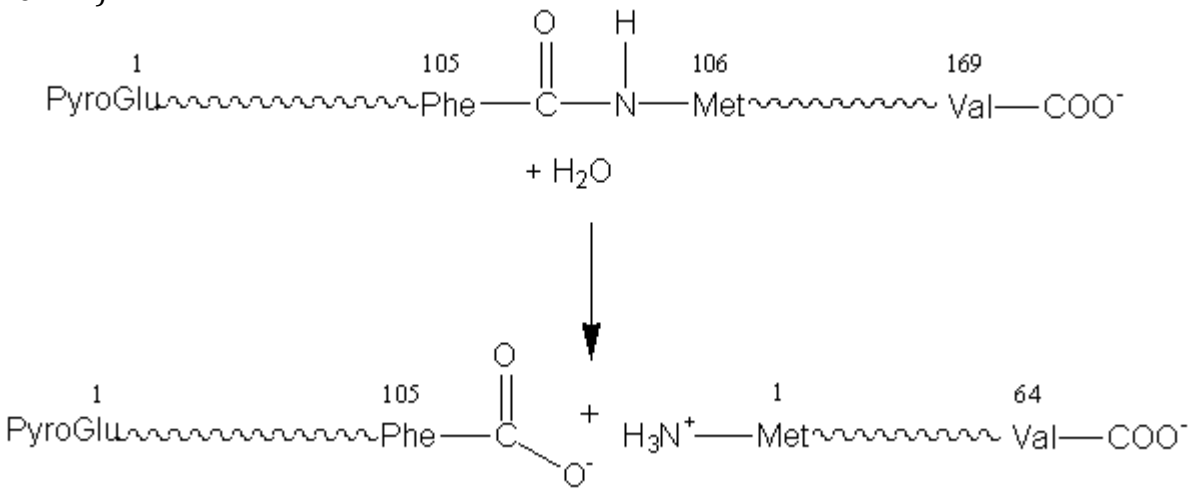
(2) Transferases which catalyse the transfer of an atom or group of atoms (e.g. acyl-, alkyl- and glycosyl-), between two molecules, but excluding such transfers as are classified in the other groups (e.g. oxidoreductases and hydrolases). For example: aspartate aminotransferase (EC 2.6.1.1, systematic name, L-aspartate:2-oxoglutarate aminotransferase; also called glutamic-oxaloacetic transaminase or simply GOT).



[1.2]

L-aspartate + 2-oxoglutarate ⇌ oxaloacetate + L-glutamate

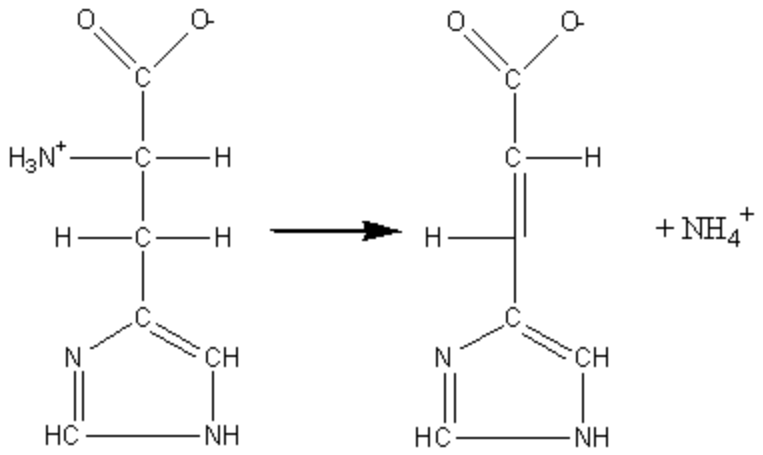
(3) Hydrolases which involve hydrolytic reactions and their reversal. This is presently the most commonly encountered class of enzymes within the field of enzyme technology and includes the esterases, glycosidases, lipases and proteases. For example: chymosin (EC 3.4.23.4, no systematic name declared; also called rennin).



[1.3]

κ-casein + water ⟶ *para*-κ-casein + caseino macropeptide

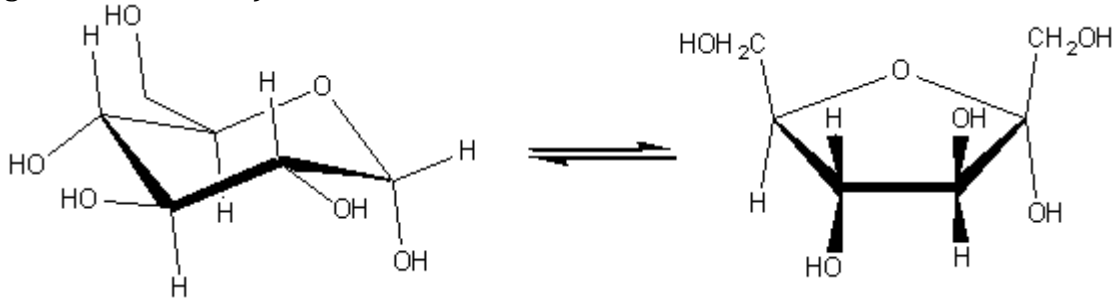
(4) Lyases which involve elimination reactions in which a group of atoms is removed from the substrate. This includes the aldolases, decarboxylases, dehydratases and some pectinases but does not include hydrolases. For example: histidine ammonia-lyase (EC 4.3.1.3, systematic name, L-histidine ammonia-lyase; also called histidase).



[1.4]

L-histidine ⟶ urocanate + ammonia

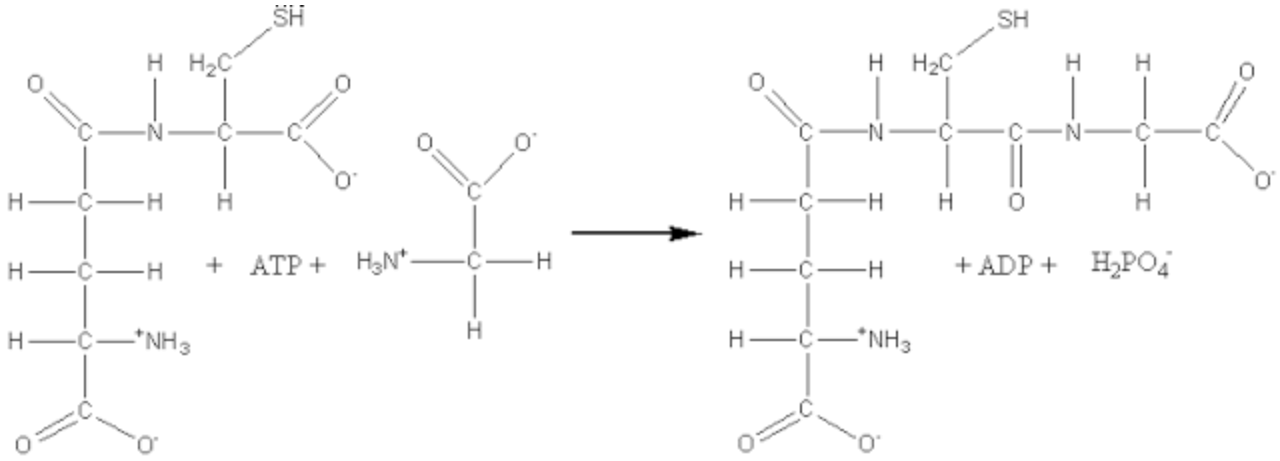
(5) Isomerases which catalyse molecular isomerisations and includes the epimerases, racemases and intramolecular transferases. For example: xylose isomerase (EC 5.3.1.5, systematic name, D-xylose ketol-isomerase; commonly called glucose isomerase).



[1.5]

⍺-D-glucopyranose ⇌ ⍺-D-fructofuranose

(6) Ligases, also known as synthetases, form a relatively small group of enzymes which involve the formation of a covalent bond joining two molecules together, coupled with the hydrolysis of a nucleoside triphosphate. For example: glutathione synthase (EC 6.3.2.3, systematic name, ⍺-L-glutamyl-L-cysteine:glycine ligase (ADP-forming); also called glutathione synthetase).



[1.6]

ATP + □-L-glutamyl-L-cysteine + glycine ⟶ ADP + phosphate + glutathione

Enzyme units

The amount of enzyme present or used in a process is difficult to determine in absolute terms (e.g. grams), as its purity is often low and a proportion may be in an inactive, or partially active, state. More relevant parameters are the activity of the enzyme preparation and the activities of any contaminating enzymes. These activities are usually measured in terms of the activity unit (U) which is defined as the amount which will catalyse the transformation of 1 micromole of the substrate per minute under standard conditions. Typically, this represents 10^{-6} - 10^{-11} Kg for pure enzymes and 10^{-4} - 10^{-7} Kg for industrial enzyme preparations. Another unit of enzyme activity has been recommended. This is the katal (kat) which is defined as the amount which will catalyse the transformation of one mole of substance per second (1 kat = 60 000 000 U). It is an impracticable unit and has not yet received widespread acceptance. Sometimes non-standard activity units are used, such as Soxhet, Anson and Kilo Novo units, which are based on physical changes such as lowering viscosity and supposedly better understood by industry. Rightfully, such units are gradually falling into disuse. The activity is a measure of enzyme content that is clearly of major interest when the enzyme is to be used in a process. For this reason, enzymes are usually marketed in terms of activity rather than weight. The specific activity (e.g. U Kg^{-1}) is a parameter of interest, some utility as an index of purity but lesser importance. There is a major problem with these definitions of activity; the rather vague notion of "standard conditions". These are meant to refer to optimal conditions, especially with regard to pH, ionic strength, temperature, substrate concentration and the presence and concentration of cofactors and coenzymes. However, these so-termed optimal conditions vary both between laboratories and between suppliers. They also depend on the particular application in which the enzyme is to be used. Additionally, preparations of the same notional specific activity may differ with respect to stability and be capable of very different total catalytic productivity (this is the total substrate converted to product during the lifetime of the catalyst, under specified conditions). Conditions for maximum

initial activity are not necessarily those for maximum stability. Great care has to be taken over the consideration of these factors when the most efficient catalyst for a particular purpose is to be chosen

The mechanism of enzyme catalysis

In order for a reaction to occur, reactant molecules must contain sufficient energy to cross a potential energy barrier, the activation energy. All molecules possess varying amounts of energy depending, for example, on their recent collision history but, generally, only a few have sufficient energy for reaction. The lower the potential energy barrier to reaction, the more reactants have sufficient energy and, hence, the faster the reaction will occur. All catalysts, including enzymes, function by forming a transition state, with the reactants, of lower free energy than would be found in the uncatalysed reaction (Figure 1.1). Even quite modest reductions in this potential energy barrier may produce large increases in the rate of reaction (e.g. the activation energy for the uncatalysed breakdown of hydrogen peroxide to oxygen and water is 76 kJ M^{-1} whereas, in the presence of the enzyme catalase, this is reduced to 30 kJ M^{-1} and the rate of reaction is increased by a factor of 10^8, sufficient to convert a reaction time measured in years into one measured in seconds).

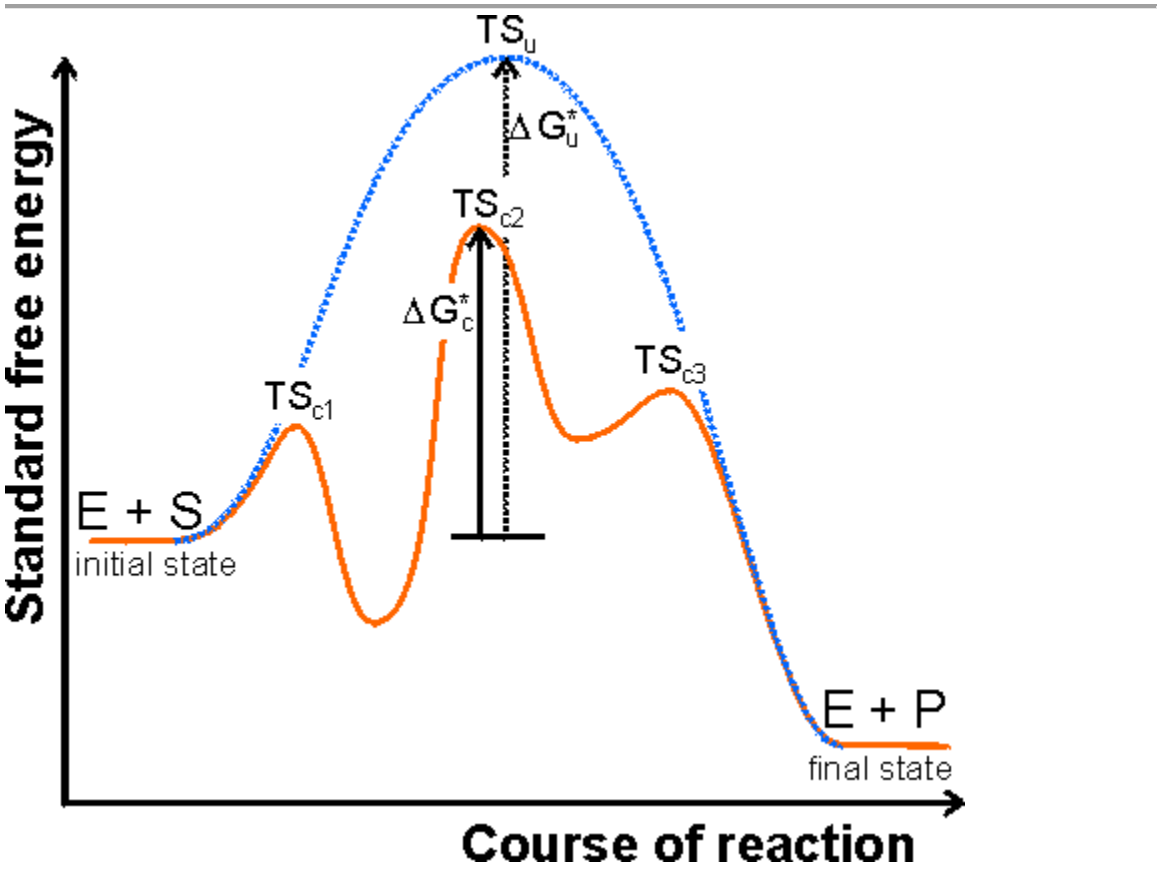
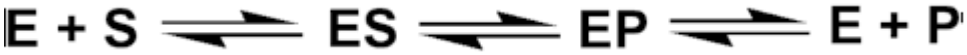


Figure 1.1. A schematic diagram showing the free energy profile of the course of an enzyme catalysed reaction involving the formation of enzyme-substrate (ES) and enzyme-product (EP) complexes, i.e.



The catalysed reaction pathway goes through the transition states TS_{c1}, TS_{c2} and TS_{c3}, with standard free energy of activation ΔG_c^*, whereas the uncatalysed reaction goes through the transition state TS_u with standard free energy of activation ΔG_u^*. In this example the rate limiting step would be the conversion of ES into EP. Reactions involving several substrates and products, or more intermediates, are even more complicated. The Michaelis-Menten reaction scheme [1.7] would give a similar profile but without the EP-complex free energy trough. The schematic profile for the uncatalysed reaction is shown as the dashed line. It should be noted that the catalytic effect only concerns the lowering of the standard free energy of activation from ΔG_u^* to ΔG_c^* and has no effect on the overall free energy change (i.e.. the difference between the initial and final states) or the related equilibrium constant.

There are a number of mechanisms by which this activation energy decrease may be achieved. The most important of these involves the enzyme initially binding the substrate(s), in the correct orientation to react, close to the catalytic groups on the active enzyme complex and any other substrates. In this way the binding energy is used partially in order to reduce the contribution of the considerable activation entropy, due to the loss of the reactants' (and catalytic groups') translational and rotational entropy, towards the total activation energy. Other contributing factors are the introduction of strain into the reactants (allowing more binding energy to be available for the transition state), provision of an alternative reactive pathway and the desolvation of reacting and catalysing ionic groups.

The energies available to enzymes for binding their substrates are determined primarily by the complementarity of structures (i.e. a good 3-dimensional fit plus optimal non-covalent ionic and/or hydrogen bonding forces). The specificity depends upon minimal steric repulsion, the absence of unsolvated or unpaired charges, and the presence of sufficient hydrogen bonds. These binding energies are capable of being quite large. As examples, antibody-antigen dissociation constants are characteristically near 10^{-8} M (free energy of binding is 46 kJ M^{-1}), ATP binds to myosin with a dissociation constant of 10^{-13} M (free energy of binding is 75 kJ M^{-1}) and biotin binds to avidin, a protein found in egg white, with a dissociation constant of 10^{-15} M (free energy of binding is 86 kJ M^{-1}). However, enzymes do not use this potential binding energy simply in order to bind the substrate(s) and form stable long-lasting complexes. If this were to be the case, the formation of the transition state between ES and EP would involve an extremely large free energy change due to the breaking of these strong binding forces, and the rate of formation of products would be very slow. They must use this binding energy for reducing the free energy of the transition state. This is generally achieved by increasing the binding to the transition state rather than the reactants and, in the process, introducing an energetic strain into the system and allowing more favourable interactions between the enzyme's catalytic groups and the reactants.

Introduction to Enzymes

The following has been excerpted from a very popular Worthington publication which was originally published in 1972 as the Manual of Clinical Enzyme Measurements. While some of the presentation may seem somewhat dated, the basic concepts are still helpful for researchers who must use enzymes but who have little background in enzymology.

Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

- Absolute specificity - the enzyme will catalyze only one reaction.
- Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor. Among them are the alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

Enzyme Concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. Graphically this can be represented as:

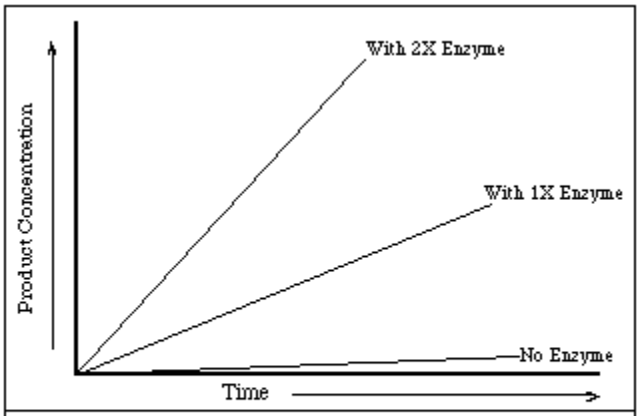


FIGURE 4
"Zero order" reaction rate is independent of substrate concentration.

These reactions are said to be "zero order" because the rates are independent of substrate concentration, and are equal to some constant k. The formation of product proceeds at a rate which is linear with time. The addition of more substrate does not serve to increase the rate. In zero order kinetics, allowing the assay to run for double time results in double the amount of product.

Table I: Reaction Orders with Respect to Substrate Concentration

Order	**Rate Equation**	**Comments**
zero	rate = k	rate is independent of substrate concentration
first	rate = k[S]	rate is proportional to the first power of substrate concentration
second	rate = $k[S][S]=k[S]^2$	rate is proportional to the square of the substrate concentration
second	rate = $k[S_1][S_2]$	rate is proportional to the first power of each of two reactants

The amount of enzyme present in a reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, etc. An enzyme assay must be designed so that the observed activity is proportional to the amount of enzyme present in order that the enzyme concentration is the only limiting factor. It is satisfied only when the reaction is zero order.

In Figure 5, activity is directly proportional to concentration in the area AB, but not in BC. Enzyme activity is generally greatest when substrate concentration is unlimiting.

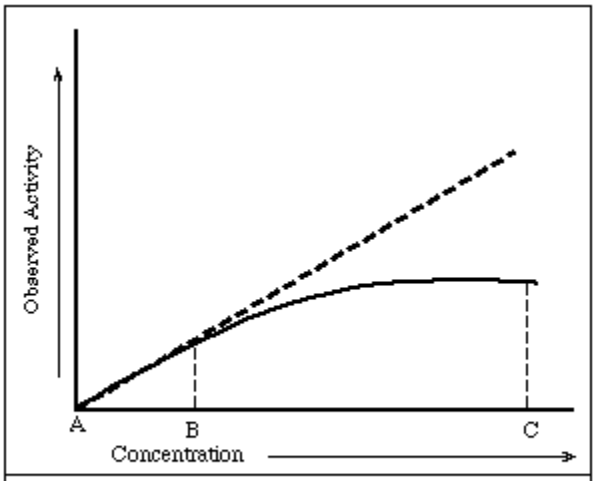


FIGURE 5
Activity vs. Concentration

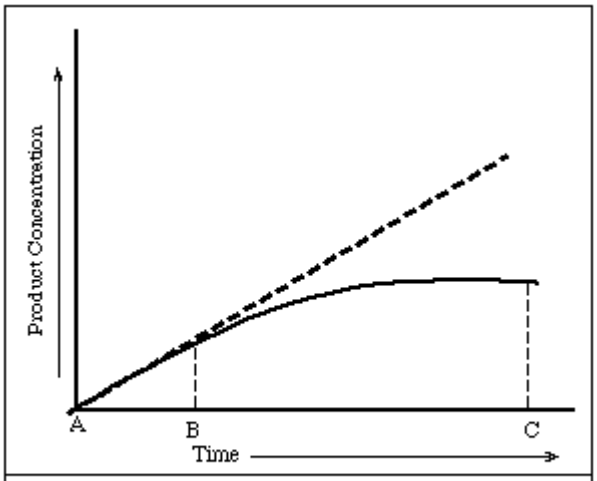


FIGURE 6
Reaction rate limited by substrate concentration

When the concentration of the product of an enzymatic reaction is plotted against time, a similar curve results, Figure 6.

Between A and B, the curve represents a zero order reaction; that is, one in which the rate is constant with time. As substrate is used up, the enzyme's active sites are no longer saturated, substrate concentration becomes rate limiting, and the reaction becomes first order between B and C.

To measure enzyme activity ideally, the measurements must be made in that portion of the curve where the reaction is zero order. A reaction is most likely to be zero order initially since substrate concentration is then highest. To be certain that a reaction is zero order, multiple measurements of product (or substrate) concentration must be made.

Figure 7 illustrates three types of reactions which might be encountered in enzyme assays and shows the problems which might be enountered if only single measurements are made.

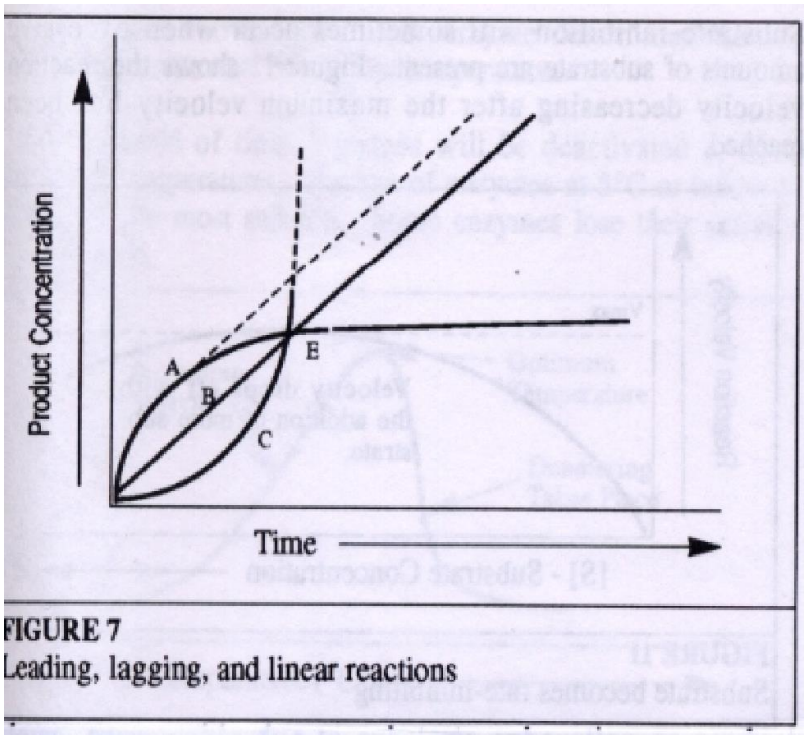


FIGURE 7
Leading, lagging, and linear reactions

B is a straight line representing a zero order reaction which permits accurate determination of enzyme activity for part or all of the reaction time. A represents the type of reaction that was shown in Figure 6. This reaction is zero order initially and then slows, presumably due to substrate exhaustion or product inhibition. This type of reaction is sometimes referred to as a "leading" reaction. True "potential" activity is represented by the dotted line. Curve C represents a reaction with an initial "lag" phase. Again the dotted line represents the potentially measurable activity. Multiple determinations of product concentration enable each curve to be plotted and true activity determined. A single end point determination at E would

lead to the false conclusion that all three samples had identical enzyme concentration.

Substrate Concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity (delta A/delta T). This is represented graphically in Figure 8.

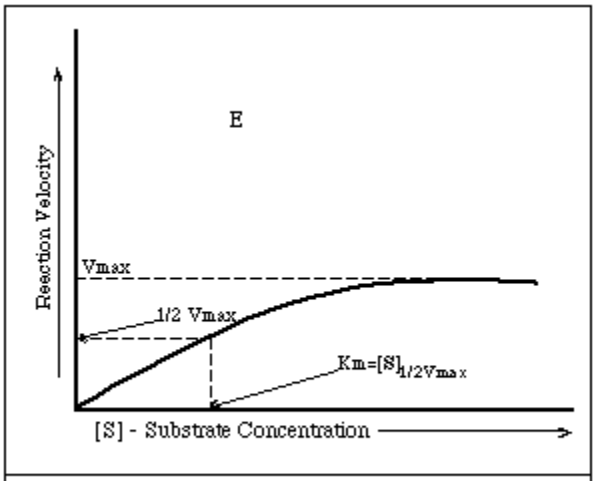


FIGURE 8
Effect of substrate concentration

It is theorized that when this maximum velocity had been reached, all of the available enzyme has been converted to ES, the enzyme substrate complex. This point on the graph is designated Vmax. Using this maximum velocity and equation (7), Michaelis developed a set of mathematical expressions to calculate enzyme activity in terms of reaction speed from measurable laboratory data.

$$E + S \underset{K_{-1}}{\overset{K_{+1}}{\rightleftarrows}} ES \underset{K_{-2}}{\overset{K_{+2}}{\rightleftarrows}} P + E \qquad [7]$$

The Michaelis constant Km is defined as the substrate concentration at 1/2 the maximum velocity. This is shown in Figure 8. Using this constant and the fact that Km can also be defined as:

$K_m = K_{-1} + K_2 / K_{+1}$

K^{+1}, K^{-1} and K^{+2} being the rate constants from equation (7). Michaelis developed the following

$$V_i = \frac{V_{max}[S]}{K_m + [S]}$$

where

V_t = the velocity at any time

[S] = the substrate concentration at this time

V_{max} = the highest under this set of experimental conditions (pH, temperature, etc.)

K_m = the Michaelis constant for the particular enzyme being investigated

Michaelis constants have been determined for many of the commonly used enzymes. The size of Km tells us several things about a particular enzyme.

- A small Km indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations.
- A large Km indicates the need for high substrate concentrations to achieve maximum reaction velocity.
- The substrate with the lowest Km upon which the enzyme acts as a catalyst is frequently assumed to be enzyme's natural substrate, though this is not true for all enzymes.

Effects of Inhibitors on Enzyme Activity

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition.

Most theories concerning inhibition mechanisms are based on the existence of the enzyme-substrate complex ES. As mentioned earlier, the existence of temporary ES structures has been verified in the laboratory.

Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.

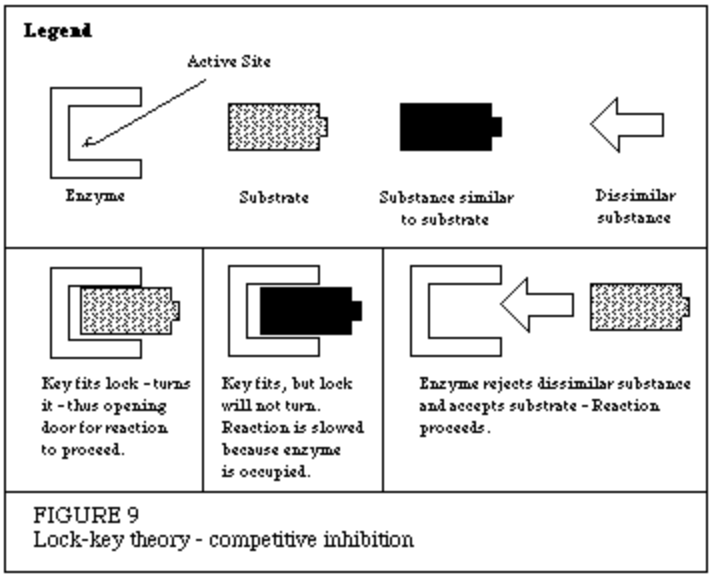


FIGURE 9
Lock-key theory - competitive inhibition

The lock and key theory utilizes the concept of an "active site." The concept holds that one particular portion of the enzyme surface has a strong affinity for the substrate. The substrate is held in such a way that its conversion to the reaction products is more favorable. If we consider the enzyme as the lock and the substrate the key (Figure 9) - the key is inserted in the lock, is turned, and the door is opened and the reaction proceeds. However, when an inhibitor which resembles the substrate is present, it will compete with the substrate for the position in the enzyme lock. When the inhibitor wins, it gains the lock position but is unable to open the lock. Hence, the observed reaction is slowed down because some of the available enzyme sites are occupied by the inhibitor. If a dissimilar substance which does not fit the site is present, the enzyme rejects it, accepts the substrate, and the reaction proceeds normally.

Non-competitive inhibitors are considered to be substances which when added to the enzyme alter the enzyme in a way that it cannot accept the substrate. Figure 10.

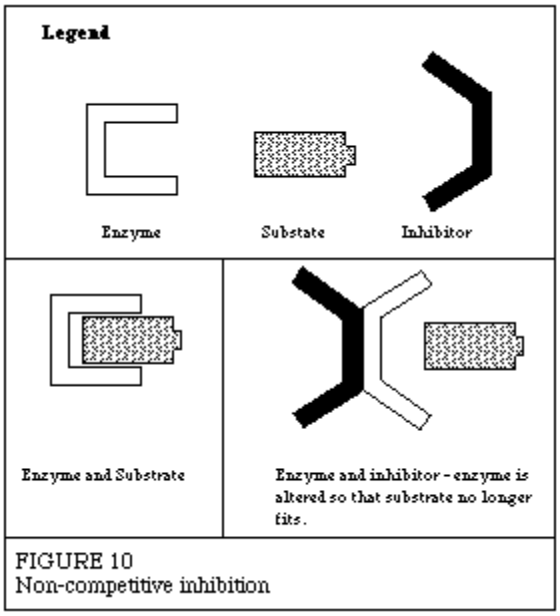


FIGURE 10
Non-competitive inhibition

Substrate inhibition will sometimes occur when excessive amounts of substrate are present. Figure 11 shows the reaction velocity decreasing after the maximum velocity has been reached.

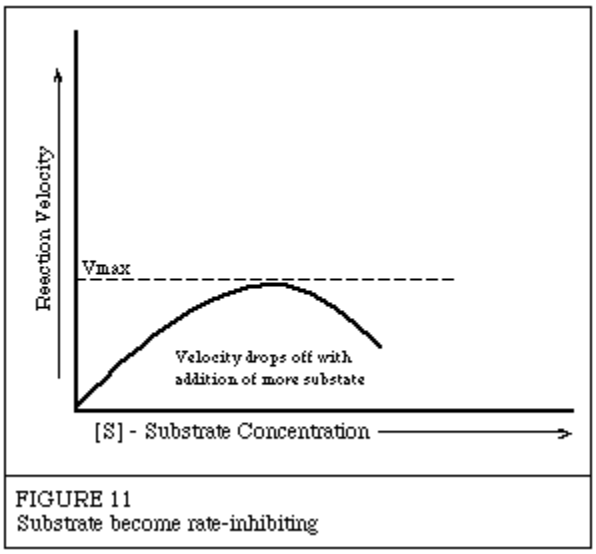


FIGURE 11
Substrate become rate-inhibiting

Additional amounts of substrate added to the reaction mixture after this point actually decrease the reaction rate. This is thought to be due to the fact that there are so many substrate molecules competing for the active sites on the enzyme surfaces that they block the sites (Figure 12) and prevent any other substrate molecules from occupying them.

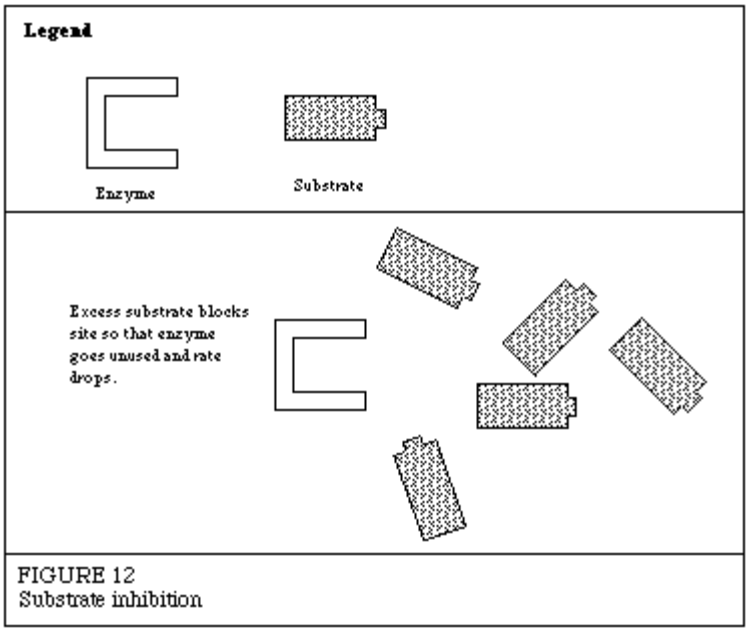


FIGURE 12
Substrate inhibition

This causes the reaction rate to drop since all of the enzyme present is not being used.

Temperature Effects

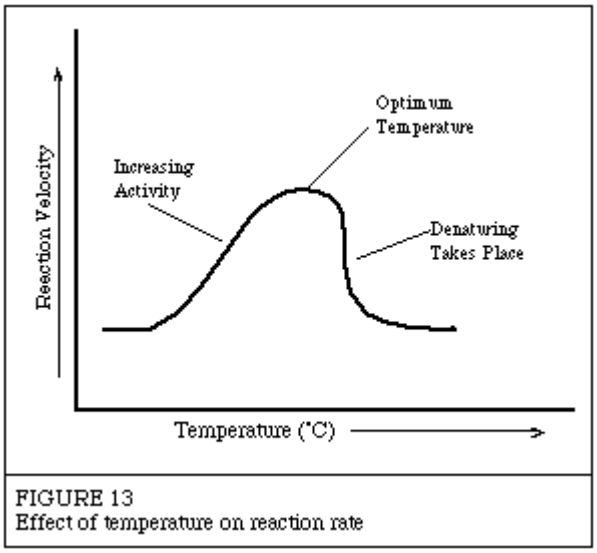


FIGURE 13
Effect of temperature on reaction rate

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. As shown in Figure 13, the reaction rate increases with temperature to a maximum level, then abruptly declines with further

increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.

Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable. Some enzymes lose their activity when frozen.

Effects of pH

Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH. This is graphically illustrated in Figure 14.

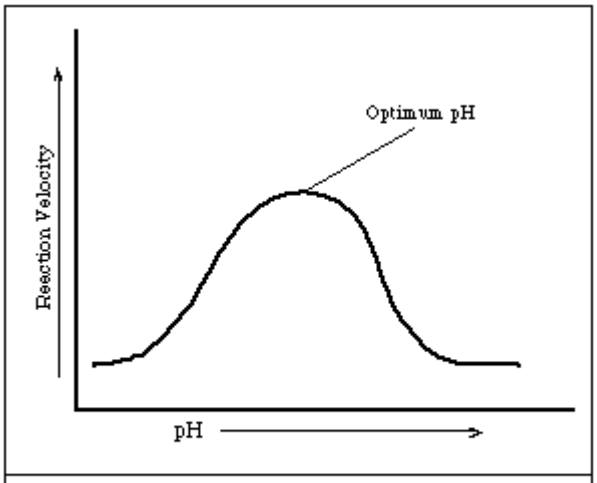


FIGURE 14
Effect of pH on reaction rate

Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

The optimum pH value will vary greatly from one enzyme to another, as Table II shows:

Table II: pH for Optimum Activity

Enzyme	**pH Optimum**
Lipase (pancreas)	8.0
Lipase (stomach)	4.0 - 5.0

Table II: pH for Optimum Activity

Enzyme	**pH Optimum**
Lipase (castor oil)	4.7
Pepsin	1.5 - 1.6
Trypsin	7.8 - 8.7
Urease	7.0
Invertase	4.5
Maltase	6.1 - 6.8
Amylase (pancreas)	6.7 - 7.0
Amylase (malt)	4.6 - 5.2
Catalase	7.0

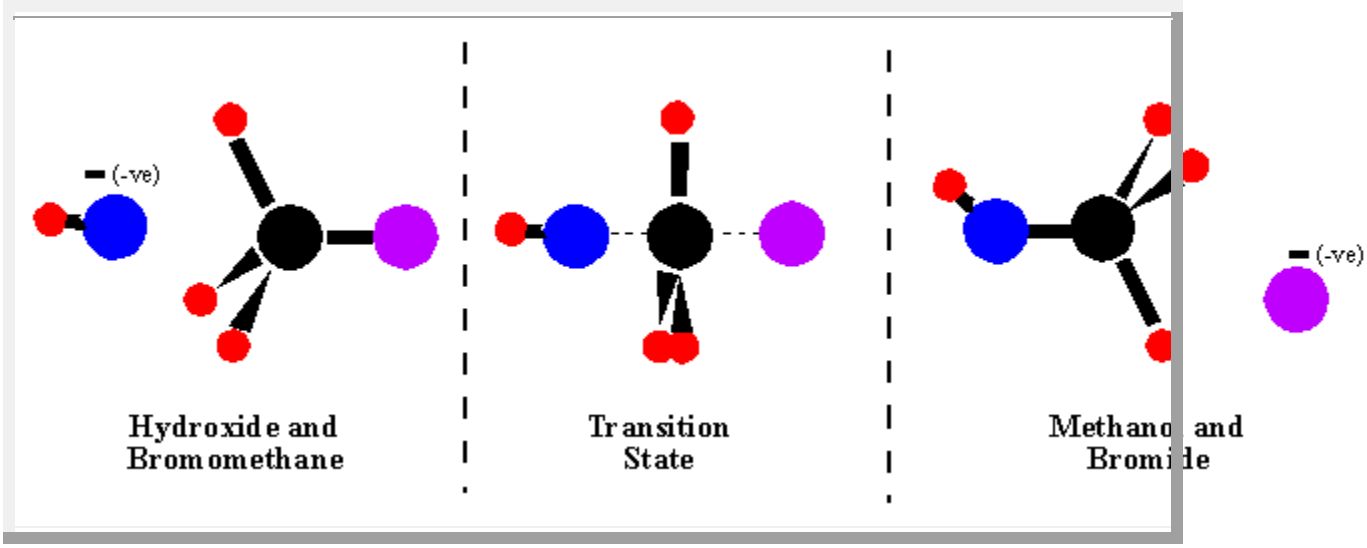
In addition to temperature and pH there are other factors, such as ionic strength, which can affect the enzymatic reaction. Each of these physical and chemical parameters must be considered and optimized in order for an enzymatic reaction to be accurate and reproducible.

Transition State Theory

Theory

In the transition state theory, the mechanism of interaction of reactants is not considered; the important criterion is that colliding molecules must have sufficient energy to overcome a potential energy barrier (the activation energy) to react.

For a bimolecular reaction, a transition state is formed when the two molecules' old bonds are weakened and new bonds begin to form or the old bonds break first to form the transition state and then the new bonds form after. The theory suggests that as reactant molecules approach each other closely they are momentarily in a less stable state than either the reactants or the products. In the example below, the first scenario occurs to form the transition state:



It takes a lot of energy to achieve the transition state, so the state is a high-energy substance. The potential energy of the system increases at this point because:

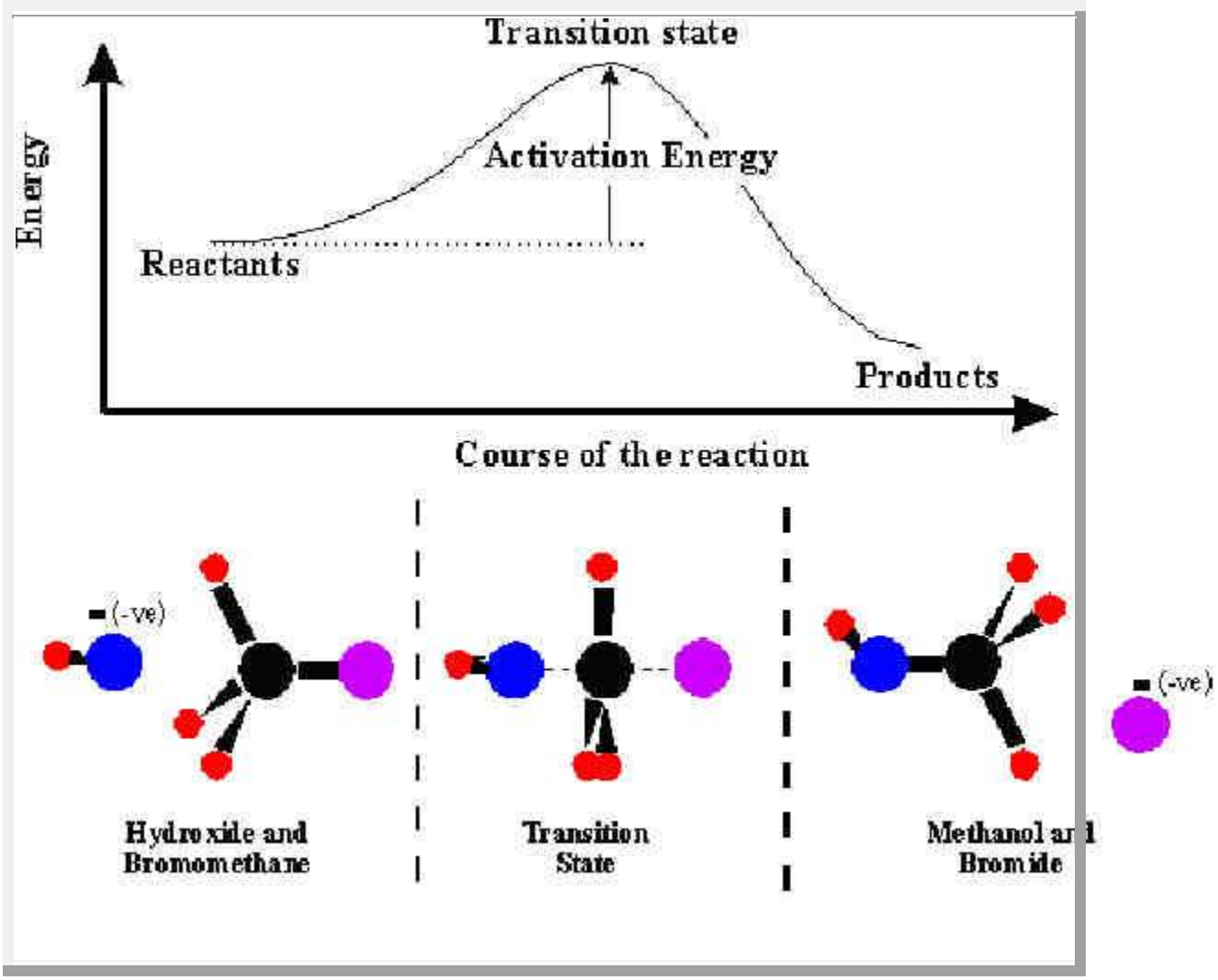
- The approaching reactant molecules must overcome the mutual repulsive forces between the outer shell electrons of their constituent atoms
- Atoms must be separated from each other as bonds are broken

This increase in potential energy corresponds to an energy barrier over which the reactant molecules must pass if the reaction is to proceed. The transition state occurs at the maximum of this energy barrier.

- The transition state is an unstable transitory combination of reactant molecules that occurs at a potential energy maximum

The combination can either go on to form products or fall apart to return to the unchanged reactants.

- The energy difference between the reactants and the potential energy maximum is referred to as the activation energy



The equation for an enzymatic reaction is:

$$E + S \xleftrightarrow{K^{\ddagger}} (ES)^{\ddagger} \rightarrow E + P$$

$K^{\ddagger}$ is the concentration equilibrium constant, defined as:

$$K^{\ddagger} = \frac{[ES]^{\ddagger}}{[E][S]}$$

Use the following equation to find the rate constant *(k)*:

$$k = \left(\frac{k_B T}{h}\right) K^{\ddagger}$$, where kB is Boltzmann's constant, h is Planck's constant and T is the temperature

Thermodynamics

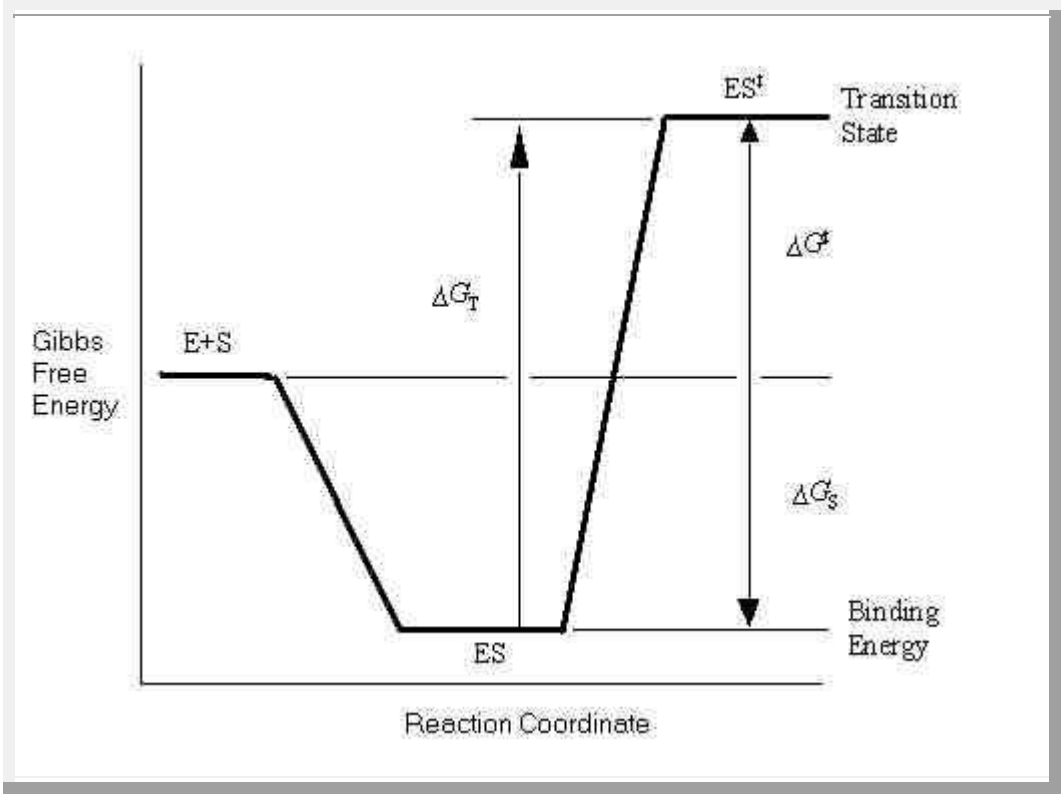
$K^{\ddagger}$ resembles the equilibrium constant used to describe Gibbs free energy, defined as:

$$\Delta G^{\ddagger} = -RT \ln [K^{\ddagger}(c^{o})^{-1}]$$

where c^{o} is the standard state concentration. ΔG^{t} can be defined as the Gibbs energy of activation. The Gibbs energy difference between the ground and transition state can be used to predict the rate of reaction. The binding energy associated with the specific substrate-enzyme interaction is a significant factor in lowering the Gibbs free energy change required for reaction. The large binding energies of substrates are due in part to the complementary shape of the active site of the enzyme. The Gibbs energy can be considered to be composed of two terms, ΔG_{t}, the binding energy and $\Delta G_{s,}$ the activation energy involved in the making and breaking of bonds leading to the transition state $(ES^{\ddagger})$ from enzyme-substrate intermediate (ES). They are related as follows:

$$\Delta G^{t} = \Delta G_{t} + \Delta G_{s}$$

This can be seen on the energy diagram below:



The above equation can be substituted into the equation for the rate constant k, and k is defined as a second order constant (k_{cat}/K_M).

$$RT \ln (k_{cat}/K_M) = RT \ln (k_B T/h) - \Delta G_S - \Delta G_T$$

Entropy is composed of translational, rotational, and internal entropies. When two molecules react without a catalyst there is a loss of rotational and translational entropies. An enzyme brings together the reactants as an effective intramolecular adduct that will not suffer the previous losses. There will only be a small loss of internal entropy. Therefore this reaction will be entropically favored.

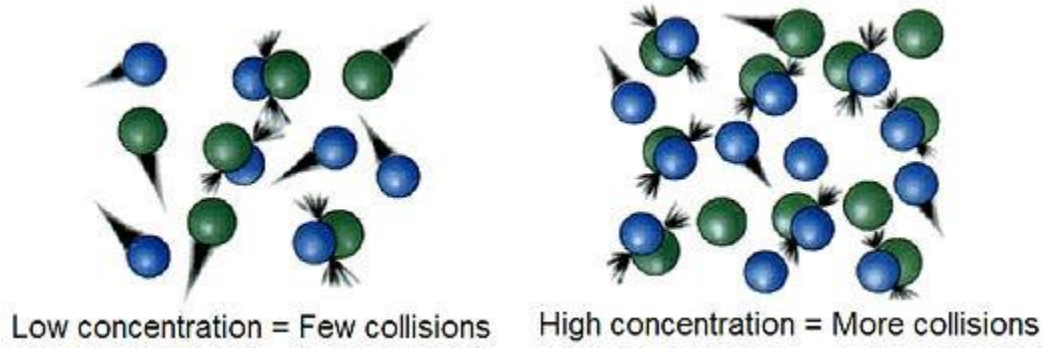
A sample calculation can be done below to find the entropic contribution to a reaction for the following equation:

$$k = (k_B T/h) \exp (\Delta S^{\ddagger}/R) \exp (-\Delta H^{\ddagger}/RT)$$

http://en.wikipedia.org/wiki/Collision_theory

The **Collision theory**, proposed by Max Trautz[1] and William Lewis in 1916 and 1918, qualitatively explains how chemical reactions occur and why reaction rates differ for different reactions.[2] This theory is based on the idea that reactant particles must collide for a reaction to occur, but only a certain fraction of the total

collisions have the energy to connect effectively and cause the reactants to transform into products. This is because only a portion of the molecules have enough energy and the right orientation (or "angle") at the moment of impact to break any existing bonds and form new ones. The minimal amount of energy needed for this to occur is known as activation energy. Collision theory is closely related to chemical kinetics. collision theory is where particles from different elements react with each other by releasing activation energy as they hit each other, which if is big enough will cause the elements to react with each other this is called a successful collision but if the concentration of the potassium iodide is lower there will be less particles for the other elements to react with causing the reaction to happen much slower.



Reaction rate tends to increase with concentration - a phenomenon explained by **collision theory**

Contents

[hide]

[edit] Rate constant

The rate constant for a bimolecular gas phase reaction, as predicted by collision theory is:

.

- Z is the collision frequency.[3]
- is the steric factor.[4]
- E_a is the activation energy of the reaction.
- T is the temperature.
- R is gas constant.

And the collision frequency is:

- N_A is Avogadro's number
- σ_{AB} is the reaction cross section
- k_B is Boltzmann's constant
- μ_{AB} is the reduced mass of the reactants

[edit] Qualitative overview

Fundamentally collision theory is based on kinetic theory and therefore it can only be applied strictly to ideal gases, otherwise approximations are used. Qualitatively, it assumes that the molecules of the reactants are *rigid*, *uncharged spheres* that physically collide prior to reacting. Moreover, it postulates that the majority of collisions do not lead to a reaction, but only those in which the colliding species have:

- A kinetic energy greater than a certain minimum, called the activation energy, E_a
- The correct spatial orientation (steric factor) with respect to each other.

These collisions which lead to reaction are called *effective collisions*. The reaction rate, may be defined as the number of effective collisions per unit time.

According to collision theory, two significant factors determine reaction rates:

- **Concentration:** Increase in concentration of reactants increases the collision frequency between the reactants. Thus the effective collision frequency also increases.
- **Temperature:** The kinetic energy of particles follows the Maxwell-Boltzmann distribution. An increase in temperature not only increases the average speed of the reactant particles and the number of collisions, but also the fraction of particles having kinetic energy higher than the activation energy. Thus, the effective collision frequency increases.

If a heterogeneous reaction takes place, then the **surface area** of the solid is also important: the more reactive centers exposed on the surface (due to the porosity of the solid and how finely divided it is), the more collisions with reacting molecules.

[edit] Quantitative insights

[edit] Derivation

Collision theory can only be applied quantitatively to bimolecular reactions, of the kind:[5]

$$A + B \rightarrow C$$

In collision theory it is considered that two particles A and B will collide if their nuclei get closer than a certain distance. The area around a molecule A in which it can collide with an approaching B molecule is called the cross section (σ_{AB}) of the reaction and is, in principle, the area corresponding to a circle whose radius (r_{AB}) is the sum of the radii of both reacting molecules, which are supposed to be spherical. A moving molecule will therefore sweep a volume per second as it moves, where is the average velocity of the particle.

From kinetic theory it is known that a molecule of A has an average velocity (different from root mean square velocity) of . Where is Boltzmann constant and is the mass of the molecule.

The solution of the two body problem states that two different moving bodies can be treated as one body which has the reduced mass of both and moves with the velocity of the center of mass, so, in this system μ_{AB} must be used instead of m_A.

Therefore, the total **collision frequency**,[3] of all A molecules, with all B molecules, is:

From Maxwell Boltzmann distribution it can be deduced that the fraction of collisions with more energy than the activation energy is . Therefore the rate of a bimolecular reaction for ideal gases will be:

Where:

- Z is the collision frequency.
- is the steric factor, which will be discussed in detail in the next section.
- E_a is the activation energy of the reaction.
- T is the absolute temperature.
- R is gas constant.

The product Zρ is equivalent to the preexponential factor of the Arrhenius equation.

[edit] Validity of the theory and steric factor

Once a theory is formulated, its validity must be tested, that is, compare its predictions with the results of the experiments.

When the expression form of the rate constant is compared with the rate equation for an elementary bimolecular reaction, , it is noticed that .

That expression is similar to the Arrhenius equation, and gives the first theoretical explanation for the Arrhenius equation on a molecular basis. The weak temperature dependence of the preexponential factor is so small compared to the exponential factor that it cannot be measured experimentally, that is, *"it is not feasible to establish, on the basis of temperature studies of the rate constant, whether the predicted $T^{½}$ dependence of the preexponential factor is observed experimentally"*[5]

[edit] Steric factor

If the values of the predicted rate constants are compared with the values of known rate constants it is noticed that collision theory fails to estimate the constants correctly and the more complex the molecules are, the more it fails. The reason for this is that particles have been supposed to be spherical and able to react in all directions; that is not true, as the orientation of the collisions is not always the right one. For example in the hydrogenation reaction of ethylene the H_2 molecule must approach the bonding zone between the atoms, and only a few of all the possible collisions fulfill this requirement.

A new concept must be introduced: the **steric factor**, ρ. It is defined as the ratio between the experimental value and the predicted one (or the ratio between the frequency factor and the collision frequency, and it is most often less than unity(one).[4]

Usually, the more complex the reactant molecules, the lower the steric factor. Nevertheless, some reactions exhibit steric factors greater than unity: the harpoon reactions, which involve atoms that exchange electrons, producing ions. The deviation from unity can have different causes: the molecules are not spherical, so different geometries are possible; not all the kinetic energy is delivered into the right spot; the presence of a solvent (when applied to solutions), etc.

Experimental rate constants compared to the ones predicted by collision theory for gas phase reactions			
Reaction	A (Arrhenius frequency factor)	Z (collision frequency)	Steric factor
$2ClNO \rightarrow 2Cl + 2NO$	$9.4\ 10^{9}$	$5.9\ 10^{10}$	0.16
$2ClO \rightarrow Cl_2 + O_2$	$6.3\ 10^{7}$	$2.5\ 10^{10}$	$2.3\ 10^{-3}$

$H_2 + C_2H_4 \rightarrow C_2H_6$	$1.24\ 10^6$	$7.3\ 10^{11}$	$1.7\ 10^{-6}$
$Br_2 + K \rightarrow KBr + Br$	10^{12}	$2.1\ 10^{11}$	4.3

Collision theory can be applied to reactions in solution; in that case, the *solvent cage* has an effect on the reactant molecules and several collisions can take place in a single encounter, which leads to predicted preexponential factors being too large. ρ values greater than unity can be attributed to favorable entropic contributions.

Experimental rate constants compared to the ones predicted by collision theory for reactions in solution[6]

Reaction	Solvent	A 10^{-11}	Z 10^{-11}	Steric factor
$C_2H_5Br + OH^-$	C_2H_5OH	4.30	3.86	1.11
$C_2H_5O^- + CH_3I$	C_2H_5OH	2.42	1.93	1.25
$ClCH_2CO_2^- + OH^-$	water	4.55	2.86	1.59
$C_3H_6Br_2 + I^-$	CH_3OH	1.07	1.39	0.77
$HOCH_2CH_2Cl + OH^-$	water	25.5	2.78	9.17
$4\text{-}CH_3C_6H_4O^- + CH_3I$	ethanol	8.49	1.99	4.27
$CH_3(CH_2)_2Cl + I^-$	$(CH_3)_2CO$	0.085	1.57	0.054
$C_5H_5N + CH_3I$	$C_2H_2Cl_4$	-	-	$2.0\ 10^{-6}$

Enzyme Kinetics- The rate of reaction catalyzed by an enzyme increases linearly with the substrate concentration up to a point, but it soon reaches the maximum value called Vmax beyond which there is no further increase in reaction rate; this is called substrate saturation. The phenomenon of substrate saturation is described by the Michaelis-Menten equation given below.

$V = V\ max\ [S] / [S] + Km$

Where, Vmax is the maximum rate of reaction, which occurs when the enzyme is saturated with substrate. V is the rate of reaction, [S] is the substrate concentration and Km is Michaelis constant. This equation can be used to predict the rate of reaction catalyzed by an enzyme at any substrate concentration provided the values of Vmax and K", are known.

Kill is the substrate concentration needed to obtain a reaction rate equal to 1/2Vmax. The substrate concentrations and reaction rates considered in the above equation are always the initial values, i.e., the values at the start of reaction.

Although few enzymes follow the Michaelis-Menten equation over a wide range of experimental conditions, it is still the most generally applicable equation for describing enzyme catalyzed reactions. The value of Km is characteristic for each enzyme substrate combination, but is it dependent of the enzyme and/or substrate concentrations. However, Km may be influenced by pH, temperature, ionic strength and other factors.

Simple kinetics of enzyme action

It is established that enzymes form a bound complex to their reactants (i.e. **substrates**) during the course of their catalysis and prior to the release of products. This can be simply illustrated, using the mechanism based on that of Michaelis and Menten for a one-substrate reaction, by the reaction sequence:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} P + E \qquad \textbf{[1.7]}$$

where k_{+1}, k_{-1} and k_{+2} are the respective rate constants, typically having values of 10^5 - 10^8 M^{-1} s^{-1}, 1 - 10^4 s^{-1} and 1 - 10^5 s^{-1} respectively; the sign of the subscripts

indicating the direction in which the rate constant is acting. For the sake of simplicity the reverse reaction concerning the conversion of product to substrate is not included in this scheme. This is allowable (1) at the beginning of the reaction when there is no, or little, product present, or (2) when the reaction is effectively irreversible. Reversible reactions are dealt with in more detail later in this chapter. The rate of reaction (v) is the rate at which the product is formed.

$$v = \frac{d[P]}{dt} = k_{+2}[ES] \qquad (1.1)$$

where [ ] indicates the molar concentration of the material enclosed (i.e. [ES] is the concentration of the enzyme-substrate complex). The rate of change of the concentration of the enzyme-substrate complex equals the rate of its formation minus the rate of its breakdown, forwards to give product or backwards to regenerate substrate.

therefore:

$$\frac{d[ES]}{dt} = k_{+1}[E][S] - (k_{-1} + k_{+2})[ES] \qquad (1.2)$$

During the course of the reaction, the total enzyme at the beginning of the reaction ($[E]_0$, at zero time) is present either as the free enzyme ([E]) or the ES complex ([ES]).

i.e. $[E]_0 = [E] + [ES]$ (1.3)

therefore:

$$\frac{d[ES]}{dt} = k_{+1}([E]_0 - [ES])[S] - (k_{-1} + k_{+2})[ES] \qquad (1.4)$$

Gathering terms together,

$$\frac{d[ES]}{dt} = k_{+1}[E]_0[S] - k_{+1}[ES][S] - (k_{-1} + k_{+2})[ES]$$

$$\frac{d[ES]}{dt} = k_{+1}[E]_0[S] - (k_{+1}[S] + k_{-1} + k_{+2})[ES]$$

this gives:

$$\frac{\frac{d[ES]}{dt}}{k_{+1}[S]+k_{-1}+k_{+2}}+[ES]=\frac{k_{+1}[E]_0[S]}{k_{+1}[S]+k_{-1}+k_{+2}} \qquad (1.5)$$

The differential equation 1.5 is difficult to handle, but may be greatly simplified if it can be assumed that the left hand side is equal to [ES] alone. This assumption is valid under the sufficient but unnecessarily restrictive steady state approximation that the rate of formation of ES equals its rate of disappearance by product formation and reversion to substrate (i.e. d[ES]/dt is zero). It is additionally valid when the condition:

$$\frac{\frac{d[ES]}{dt}}{k_{+1}[S]+k_{-1}+k_{+2}}<<[ES] \qquad (1.6)$$

is valid. This occurs during a substantial part of the reaction time-course over a wide range of kinetic rate constants and substrate concentrations and at low to moderate enzyme concentrations. The variation in [ES], d[ES]/dt, [S] and [P] with the time-course of the reaction is shown in Figure 1.2, where it may be seen that the simplified equation is valid throughout most of the reaction.

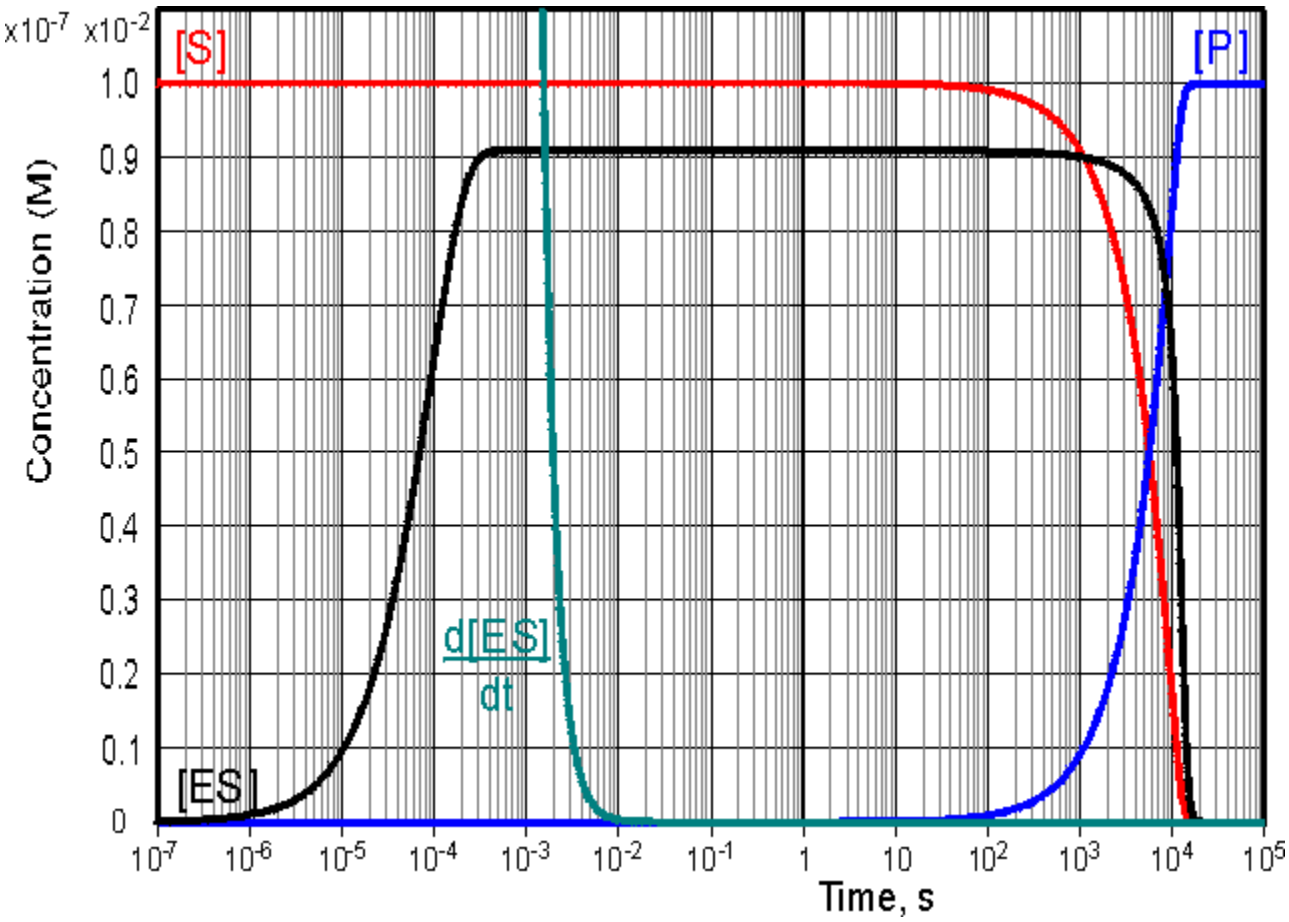


Figure 1.2. Computer simulation of the progress curves of d[ES]/dt (0 - 10^{-7} M scale), [ES] (0 - 10^{-7} M scale), [S] (0 - 10^{-2} M scale) and [P] (0 - 10^{-2} M scale) for a reaction obeying simple Michaelis-Menten kinetics with $k_{+1} = 10^6$ M^{-1} s^{-1}, $k_{-1} = 1000$ s^{-1}, $k_{+2} = 10$ s^{-1}, $[E]_0 = 10^{-7}$ M and $[S]_0 = 0.01$ M. The simulation shows three distinct phases to the reaction time-course, an initial transient phase which lasts for about a millisecond followed by a longer steady state phase of about 30 minutes when [ES] stays constant but only a small proportion of the substrate reacts. This is followed by the final phase, taking about 6 hours during which the substrate is completely converted to product.

$\frac{\frac{d[ES]}{dt}}{k_{+1}[S] + k_{-1} + k_{+2}}$ is much less than [ES] during both of the latter two phases.

The Michaelis-Menten equation (below) is simply derived from equations 1.1 and 1.5, by substituting $\boldsymbol{K_m}$ for $\frac{k_{-1} + k_{+2}}{k_{+1}}$. K_m is known as the **Michaelis constant** with a

value typically in the range 10^{-1} - 10^{-5} M. When $k_{+2} << k_{-1}$, K_m equals the dissociation constant (k_{-1}/k_{+1}) of the enzyme substrate complex.

$$v = k_{+2}[ES] = \frac{k_{+2}[E]_0[S]}{[S] + K_m} \qquad (1.7)$$

or, more simply

$$v = \frac{V_{max}[S]}{[S] + K_m} \qquad (1.8)$$

where $\boldsymbol{V_{max}}$ is the maximum rate of reaction, which occurs when the enzyme is completely saturated with substrate (i.e. when [S] is very much greater than K_m, V_{max} equals $k_{+2}[E]_0$, as the maximum value [ES] can have is $[E]_0$ when $[E]_0$ is less than $[S]_0$). Equation 1.8 may be rearranged to show the dependence of the rate of reaction on the ratio of [S] to K_m,

$$v = \frac{V_{max}}{1 + \frac{K_m}{[S]}} \qquad (1.9)$$

and the rectangular hyperbolic nature of the relationship, having asymptotes at v = V_{max} and [S] = $-K_m$,

$$(V_{max}-v)(K_m+[S])=V_{max}K_m \qquad (1.10)$$

The substrate concentration in these equations is the actual concentration at the time and, in a closed system, will only be approximately equal to the initial substrate concentration ($[S]_0$) during the early phase of the reaction. Hence, it is usual to use these equations to relate the initial rate of reaction to the initial, and easily predetermined, substrate concentration (Figure 1.3). This also avoids any problem that may occur through product inhibition or reaction reversibility (see later).

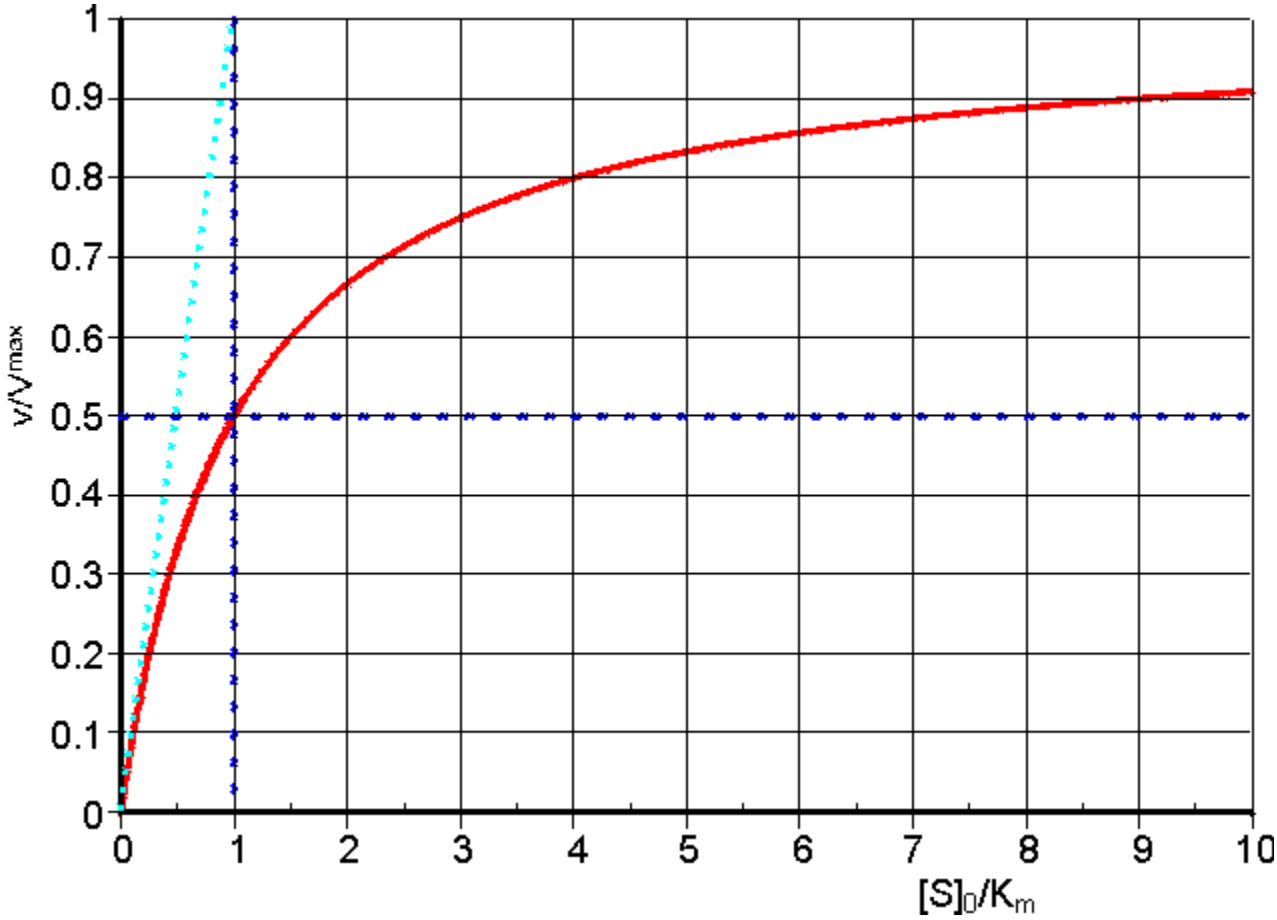


Figure 1.3. A normalised plot of the initial rate (v_0) against initial substrate concentration ($[S]_0$) for a reaction obeying the Michaelis-Menten kinetics (equation 1.8). The plot has been normalised in order to make it more generally applicable by plotting the relative initial rate of reaction (v_0/V_{max}) against the initial substrate concentration relative to the Michaelis constant ($[S]_0/K_m$, more commonly referred to as □, the dimensionless substrate concentration). The curve is a rectangular hyperbola with asymptotes at $v_0 = V_{max}$ and $[S]_0 = -K_m$. The tangent to the curve at the origin goes through the point ($v_0 = V_{max}$),($[S]_0 = K_m$). The ratio V_{max}/K_m is an important kinetic parameter which describes the relative specificity of a fixed amount of the enzyme for its substrate (more precisely defined in terms of k_{cat}/K_m). The substrate concentration, which gives a rate of half the maximum reaction velocity, is equal to the K_m.

It has been established that few enzymes follow the Michaelis-Menten equation over a wide range of experimental conditions. However, it remains by far the most generally applicable equation for describing enzymic reactions. Indeed it can be realistically applied to a number of reactions which have a far more complex mechanism than the one described here. In these cases K_m remains an important

quantity, characteristic of the enzyme and substrate, corresponding to the substrate concentration needed for half the enzyme molecules to bind to the substrate (and, therefore, causing the reaction to proceed at half its maximum rate) but the precise kinetic meaning derived earlier may not hold and may be misleading. In these cases the K_m is likely to equal a much more complex relationship between the many rate constants involved in the reaction scheme. It remains independent of the enzyme and substrate concentrations and indicates the extent of binding between the enzyme and its substrate for a given substrate concentration, a lower K_m indicating a greater extent of binding. V_{max} clearly depends on the enzyme concentration and for some, but not all, enzymes may be largely independent of the specific substrate used. K_m and V_{max} may both be influenced by the charge and conformation of the protein and substrate(s) which are determined by pH, temperature, ionic strength and other factors. It is often preferable to substitute $\boldsymbol{k_{cat}}$ for k_{+2} , where $V_{max} = k_{cat}[E]_0$, as the precise meaning of k_{+2}, above, may also be misleading. k_{cat} is also known as the **turnover number** as it represents the maximum number of substrate molecules that the enzyme can 'turn over' to product in a set time (e.g. the turnover numbers of □-amylase, glucoamylase and glucose isomerase are 500 s^{-1}, 160 s^{-1} and 3 s^{-1} respectively; an enzyme with a relative molecular mass of 60000 and specific activity 1 U mg^{-1} has a turnover number of 1 s^{-1}). The ratio k_{cat}/K_m determines the relative rate of reaction at low substrate concentrations, and is known as the **specificity constant**. It is also the apparent 2nd order rate constant at low substrate concentrations (see Figure 1.3), where

$$v = \frac{k_{cat}}{K_m}[E]_0[S] \qquad (1.11)$$

Many applications of enzymes involve open systems, where the substrate concentration remains constant, due to replenishment, throughout the reaction time-course. This is, of course, the situation that often prevails *in vivo*. Under these circumstances, the Michaelis-Menten equation is obeyed over an even wider range of enzyme concentrations than allowed in closed systems, and is commonly used to model immobilised enzyme kinetic systems (see Chapter 3).

Enzymes have evolved by maximising k_{cat}/K_m (i.e. the specificity constant for the substrate) whilst keeping K_m approximately identical to the naturally encountered substrate concentration. This allows the enzyme to operate efficiently and yet exercise some control over the rate of reaction.

The specificity constant is limited by the rate at which the reactants encounter one another under the influence of diffusion. For a single-substrate reaction the rate of encounter between the substrate and enzyme is about 10^8 - 10^9 M^{-1} s^{-1}. The specificity constant of some enzymes approach this value although the range of determined values is very broad (e.g. k_{cat}/K_m for catalase is 4 x 10^7 M^{-1} s^{-1}, whereas it

is 25 M^{-1} s^{-1} for glucose isomerase, and for other enzymes varies from less than 1 M^{-1} s^{-1} to greater than 10^8 M^{-1} s^{-1}).

Reversible reactions

A reversible enzymic reaction (e.g. the conversion of glucose to fructose, catalysed by glucose isomerase) may be represented by the following scheme where the reaction goes through the reversible stages of enzyme-substrate (ES) complex formation, conversion to enzyme-product (EP) complex and finally desorption of the product. No step is completely rate controlling.

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E + P \qquad \textbf{[1.12]}$$

Pairs of symmetrical equations may be obtained for the change in the concentration of the intermediates with time:

$$\frac{d[ES]}{dt} = k_{+1}[E][S] + k_{-2}[EP] - (k_{-1} + k_{+2})[ES] \qquad (1.36)$$

$$\frac{d[EP]}{dt} = k_{-3}[E][P] + k_{+2}[ES] - (k_{+3} + k_{-2})[EP] \qquad (1.37)$$

Assuming that there is no denaturation, the total enzyme concentration must remain constant and:

$$[E] + [ES] + [EP] = [E]_0 \qquad (1.38a)$$

therefore:

$$\frac{d[ES]}{dt} = k_{+1}([E]_0 - [ES] - [EP])[S] + k_{-2}[EP] - (k_{-1} + k_{+2})[ES] \qquad (1.38b)$$

gathering terms in [ES]

$$\frac{d[ES]}{dt} = k_{+1}([E]_0 - [EP])[S] + k_{-2}[EP] - (k_{+1}[S] + k_{-1} + k_{+2})[ES] \qquad (1.39a)$$

$$\frac{\frac{d[ES]}{dt}}{k_{+1}[S]+k_{-1}+k_{+2}}+[ES]=\frac{k_{+1}([E]_0-[EP])[S]+k_{-2}[EP]}{k_{+1}[S]+k_{-1}+k_{+2}} \qquad (1.39b)$$

and,

$$\frac{d[EP]}{dt}=k_{-3}[([E]_0-[EP]-[ES])[P]+k_{+2}[ES]-(k_{+3}+k_{-2})[EP] \qquad (1.38c)$$

gathering terms in [EP]

$$\frac{d[EP]}{dt}=k_{-3}[([E]_0-[ES])[P]+k_{+2}[ES]-(k_{-3}[P]+k_{+3}+k_{-2})[EP] \qquad (1.40a)$$

$$\frac{\frac{d[EP]}{dt}}{k_{-3}[P]+k_{+3}+k_{-2}}+[EP]=\frac{k_{-3}([E]_0-[ES])[P]+k_{+2}[ES]}{k_{-3}[P]+k_{+3}+k_{-2}} \qquad (1.40b)$$

Under similar conditions to those discussed earlier for the Michaelis-Menten mechanism (e.g. under the steady-state assumptions when both d[ES]/dt and d[EP]/dt are zero, or more exactly when

$$\frac{\frac{d[ES]}{dt}}{k_{+1}[S]+k_{-1}+k_{+2}}\langle\langle[ES] \qquad (1.41)$$

and

$$\frac{\frac{d[EP]}{dt}}{k_{-3}[P]+k_{+3}+k_{-2}}\langle\langle[EP] \qquad (1.42)$$

are both true. The following equations may be derived from equation 1.39b using the approximation, given by equations 1.41 and collecting terms.

$$k_{+1}([E]_0-[EP])[S]+k_{-2}[EP]=(k_{+1}[S]+k_{-1}+k_{+2})[ES] \qquad (1.43a)$$

$$-k_{+1}[EP][S]+k_{-2}[EP]=(k_{+1}[S]+k_{-1}+k_{+2})[ES]-k_{+1}[E]_0[S] \qquad (1.43b)$$

$$[EP] = \frac{(k_{+1}[S] + k_{-1} + k_{+2})[ES] - k_{+1}[E]_0[S]}{k_{-2} - k_{+1}[S]} \qquad (1.43c)$$

Also, the following equations (symmetrical to the above) may be derived from equation 1.40b by using the approximation, given by equation 1.42, and collecting terms.

$$k_{-3}([E]_0 - [ES])[P] + k_{+2}[ES] = (k_{-3}[P] + k_{+3} + k_{-2})[EP] \qquad (1.44a)$$

$$-k_{-3}[ES][P] + k_{+2}[ES] = (k_{-3}[P] + k_{+3} + k_{-2})[EP] - k_{-3}[E]_0[P] \qquad (1.44b)$$

$$[ES] = \frac{(k_{-3}[P] + k_{+3} + k_{-2})[EP] - k_{-3}[E]_0[P]}{k_{+2} - k_{-3}[P]} \qquad (1.44c)$$

Substituting for [ES] from equation 1.44c into equation 1.43a

$$k_{+1}([E]_0 - [EP])[S] + k_{-2}[EP] = \frac{(k_{+1}[S] + k_{-1} + k_{+2})\{(k_{-3}[S] + k_{+3} + k_{-2})[EP] - k_{-3}[E]_0[P]\}}{k_{+2} - k_{-3}[P]}$$

(1.43d)

$$(k_{+2} - k_{-3}[P])\{k_{+1}([E]_0 - [EP])[S] + k_{-2}[EP]\} = (k_{+1}[S] + k_{-1} + k_{+2})\{(k_{-3}[P] + k_{+3} + k_{-2})[EP] - k_{-3}[E]_0[P]\}$$

(1.43e)

$$\begin{aligned} &k_{+1}k_{+2}[E]_0[S] - k_{+1}k_{+2}[EP][S] + k_{+2}k_{-2}[EP] - k_{+1}k_{-3}[E]_0[S][P] + k_{+1}k_{-3}[EP][S][P] - k_{-2}k_{-3}[EP][P] \\ &= k_{+1}k_{-3}[EP][S][P] + k_{+1}k_{+3}[EP][S] + k_{+1}k_{-2}[EP][S] - k_{+1}k_{-3}[E]_0[S][P] + k_{-1}k_{-3}[P][EP] + k_{-1}k_{+3}[EP] \\ &+ k_{-1}k_{-2}[EP] - k_{-1}k_{-3}[E]_0[P] + k_{+2}k_{-3}[P][EP] + k_{+2}k_{+3}[EP] + k_{+2}k_{-2}[EP] - k_{+2}k_{-3}[E]_0[P] \end{aligned}$$

(1.43f)

Removing identical terms from both sides of the equation:

$$\begin{aligned} &k_{+1}k_{+2}[E]_0[S] - k_{+1}k_{+2}[EP][S] + k_{+2}k_{-2}[EP] - k_{-2}k_{-3}[EP][P] \\ &= k_{+1}k_{+3}[EP][S] + k_{+1}k_{-2}[EP][S] + k_{-1}k_{-3}[P][EP] + k_{-1}k_{+3}[EP] \\ &+ k_{-1}k_{-2}[EP] - k_{-1}k_{-3}[E]_0[P] + k_{+2}k_{-3}[P][EP] + k_{+2}k_{+3}[EP] + k_{+2}k_{-2}[EP] - k_{+2}k_{-3}[E]_0[P] \end{aligned}$$

(1.43g)

Gathering all the terms in [EP]:

$$\begin{aligned} &k_{+1}k_{+2}[EP][S] - k_{+2}k_{-2}[EP] + k_{-2}k_{-3}[EP][P] + k_{+1}k_{+3}[EP][S] + k_{+1}k_{-2}[EP][S] + k_{-1}k_{-3}[P][EP] + k_{-1}k_{+3}[EP] + k_{-1}k_{-2}[EP] + k_{+2} \\ &+ k_{+2}k_{+3}[EP] + k_{+2}k_{-2}[EP] = k_{-1}k_{-3}[E]_0[P] + k_{+2}k_{-3}[E]_0[P] + k_{+1}k_{+2}[E]_0[S] \end{aligned}$$

(1.43h)

$$[EP] = \frac{k_{+1}k_{+2}[E]_0[S] + (k_{-1} + k_{+2})k_{-3}[E]_0[P]}{(k_{-1}k_{+3} + k_{-1}k_{-2} + k_{+2}k_{+3}) + (k_{+2} + k_{-2} + k_{+3})k_{+1}[S] + (k_{+2} + k_{-2} + k_{-1})k_{-3}[P]}$$

(1.43i)

Also, substituting for [EP] from equation 1.43c into equation 1.44a

$$k_{-3}([E]_0 - [ES])[P] + k_{+2}[ES] = \frac{(k_{-3}[P] + k_{+3} + k_{-2})\{(k_{+1}[S] + k_{-1} + k_{+2})[ES] - k_{+1}[E]_0[S]\}}{k_{-2} - k_{+1}[S]}$$

(1.44d)

$$(k_{-2} - k_{+1}[S])\{k_{-3}([E]_0 - [ES])[P] + k_{+2}[ES]\} = (k_{-3}[P] + k_{+3} + k_{-2})\{(k_{+1}[S] + k_{-1} + k_{+2})[ES] - k_{+1}[E]_0[S]\}$$

(1.44e)

$$\begin{aligned} &k_{-3}k_{-2}[E]_0[P] - k_{-3}k_{-2}[ES][P] + k_{+2}k_{-2}[ES] - k_{+1}k_{-3}[E]_0[S][P] + k_{+1}k_{-3}[ES][S][P] - k_{+1}k_{+2}[ES][S] \\ &= k_{+1}k_{-3}[EP][S][P] + k_{-1}k_{-3}[ES][P] + k_{+2}k_{-3}[ES][P] - k_{+1}k_{-3}[E]_0[S][P] + k_{+1}k_{+3}[S][ES] + k_{-1}k_{+3}[ES] \\ &+ k_{+2}k_{+3}[ES] - k_{+1}k_{+3}[E]_0[S] + k_{-2}k_{+3}[S][ES] + k_{-1}k_{-2}[ES] + k_{+2}k_{-2}[ES] - k_{+1}k_{-2}[E]_0[S] \end{aligned}$$

(1.44f)

Removing identical terms from both sides of the equation:

$$\begin{aligned} &k_{-3}k_{-2}[E]_0[P] - k_{-3}k_{-2}[ES][P] + k_{+2}k_{-2}[ES] - k_{+1}k_{-3}[E]_0[S][P] + k_{+1}k_{-3}[ES][S][P] - k_{+1}k_{+2}[ES][S] \\ &= k_{+1}k_{-3}[EP][S][P] + k_{-1}k_{-3}[ES][P] + k_{+2}k_{-3}[ES][P] - k_{+1}k_{-3}[E]_0[S][P] + k_{+1}k_{+3}[S][ES] + k_{-1}k_{+3}[ES] \\ &+ k_{+2}k_{+3}[ES] - k_{+1}k_{+3}[E]_0[S] + k_{-2}k_{+3}[S][ES] + k_{-1}k_{-2}[ES] + k_{+2}k_{-2}[ES] - k_{+1}k_{-2}[E]_0[S] \end{aligned}$$

(1.44g)

Gathering all the terms in [ES]:

$$\begin{aligned} &k_{-3}k_{-2}[ES][P] - k_{-2}k_{+2}[ES] + k_{+2}k_{+1}[ES][s] + k_{-3}k_{-1}[ES][P] + k_{-3}k_{+2}[ES][P] + k_{+3}k_{+1}[S][ES] + k_{+3}k_{-1}[ES] + k_{+3}k_{+2}[ES] + k_{-2} \\ &+ k_{-2}k_{-1}[ES] + k_{-2}k_{+2}[ES] = k_{+3}k_{+1}[E]_0[S] + k_{-2}k_{+1}[E]_0[S] + k_{-3}k_{-2}[E]_0[P] \end{aligned}$$

(1.44h)

$$[ES] = \frac{k_{-3}k_{-2}[E]_0[P] + (k_{+3} + k_{-2})k_{+1}[E]_0[S]}{(k_{-1}k_{+3} + k_{-1}k_{-2} + k_{+2}k_{+3}) + (k_{+2} + k_{-2} + k_{+3})k_{+1}[S] + (k_{+2} + k_{-2} + k_{-1})k_{-3}[P]}$$

(1.44i)

The net rate of reaction (i.e.. rate at which substrate is converted to product less the rate at which product is converted to substrate) may be denoted by *v* where,

$$v = k_{+2}[ES] - k_{-2}[EP] \qquad (1.45)$$

Substituting from equations 1.43i and 1.44i

$$v = \frac{k_{+2}\{k_{-3}k_{-2}[E]_0[P] + (k_{+3} + k_{-2})k_{+1}[E]_0[S]\} - k_{-2}\{k_{+1}k_{+2}[E]_0[S] + (k_{-1} + k_{+2})k_{-3}[E]_0[P]\}}{(k_{-1}k_{+3} + k_{-1}k_{-2} + k_{+2}k_{+3}) + (k_{+2} + k_{-2} + k_{+3})k_{+1}[S] + (k_{+2} + k_{-2} + k_{-1})k_{-3}[P]}$$

(1.46a)

Simplifying:

$$v = \frac{k_{+1}k_{+2}k_{+3}[E]_0[S] - k_{-1}k_{-2}k_{-3}[E]_0[P]\}}{(k_{-1}k_{+3} + k_{-1}k_{-2} + k_{+2}k_{+3}) + (k_{+2} + k_{-2} + k_{+3})k_{+1}[S] + (k_{+2} + k_{-2} + k_{-1})k_{-3}[P]}$$

(1.46b)

Therefore,

$$v = \frac{\frac{V^f[S]}{K_m^S} - \frac{V^r[P]}{K_m^P}}{1 + \frac{[S]}{K_m^S} + \frac{[P]}{K_m^P}} \qquad (1.47)$$

where:

$$V^f = \frac{k_{+2}k_{+3}[E]_0}{k_{+2} + k_{-2} + k_{+3}} \qquad (1.48)$$

$$V^r = \frac{k_{-2}k_{-1}[E]_0}{k_{-2} + k_{+2} + k_{-1}} \qquad (1.49)$$

$$K_m^S = \frac{k_{-1}k_{-2} + k_{-1}k_{+3} + k_{+2}k_{+3}}{k_{+1}(k_{+2} + k_{-2} + k_{+3})} \qquad (1.50)$$

$$K_m^P = \frac{k_{+3}k_{+2} + k_{+3}k_{-1} + k_{-2}k_{-1}}{k_{-3}(k_{-2} + k_{+2} + k_{-1})} \qquad (1.51)$$

At equilibrium:

$$v = 0 \qquad (1.52)$$

and, because the numerator of equation 1.47 must equal zero,

$$\frac{V^f[S]_\infty}{K_m^S} = \frac{V^r[P]_\infty}{K_m^P} \qquad (1.53)$$

where $[S]_\infty$ and $[P]_\infty$ are the equilibrium concentrations of substrate and product (at infinite time). But by definition,

$$K_{eq} = \frac{[P]_\infty}{[S]_\infty} \qquad (1.54)$$

Substituting from equation 1.53

$$K_{eq} = \frac{V^f K_m^P}{V^r K_m^S} \qquad (1.55)$$

This is the **Haldane** relationship.

Therefore:

$$V = \frac{\frac{V^f}{K_m^S}\left([S] - \frac{[P]}{K_{eq}}\right)}{1 + \frac{[S]}{K_m^S} + \frac{[P]}{K_m^P}} \qquad (1.56)$$

If K_m^S and K_m^P are approximately equal (e.g. the commercial immobilised glucose isomerase, Sweetase, has K_m(glucose) of 840 mM and K_m(fructose) of 830 mM at 70°C), and noting that the total amount of substrate and product at any time must equal the sum of the substrate and product at the start of the reaction:

$$[S] + [P] = [S]_0 + [P]_0 \qquad (1.57)$$

Therefore:

$$V = \frac{V^f\left([S] - \frac{[P]}{K_{eq}}\right)}{K_m^S + [S]_0 + [P]_0} \qquad (1.58)$$

Therefore:

$$V = K'\left([S] - \frac{[P]}{K_{eq}}\right) \qquad (1.59)$$

where:

$$K' = \frac{V^f}{K_m^S + [S]_0 + [P]_0} \qquad (1.60)$$

K' is not a true kinetic constant as it is only constant if the initial substrate plus product concentration is kept constant.

Also,

$$[S] + [P] = [S]_\infty + [P]_\infty \qquad (1.61)$$

Substituting from equation 1.54,

$$[P] = K_{eq}[S]_\infty + [S]_\infty - [S] \qquad (1.62)$$

Let [S#] equal the concentration difference between the actual concentration of substrate and the equilibrium concentration.

$$[S^\#] = [S] - [S]_\infty \qquad (1.63)$$

Therefore:

$$[P] = K_{eq}[S]_\infty - [S^\#] \qquad (1.64)$$

Substituting in equation 1.47

$$V = \frac{\frac{V^f[S^\#]}{K_m^S} + \frac{V^f[S]_\infty}{K_m^S} - \frac{V^r K_{eq}[S]_\infty}{K_m^P} + \frac{V^r[S^\#]}{K_m^P}}{1 + \frac{[S^\#]}{K_m^S} + \frac{[S]_\infty}{K_m^S} + \frac{K_{eq}[S]_\infty}{K_m^P} - \frac{[S^\#]}{K_m^P}} \qquad (1.65)$$

Rearranging equation 1.55,

$$\frac{V^f}{K_m^S} = \frac{V^r K_{eq}}{K_m^P} \qquad (1.66)$$

Therefore:

$$V = \frac{\left(\frac{V^f}{K_m^S} + \frac{V^r}{K_m^P}\right)[S^\#]}{1 + \frac{[S]_\infty}{K_m^S} + \frac{K_{eq}[S]_\infty}{K_m^P} + \frac{(K_m^P - K_m^S)[S^\#]}{K_m^S K_m^P}} \qquad (1.67)$$

Therefore:

$$V = \frac{\boldsymbol{V}[S^\#]}{\boldsymbol{K} + [S^\#]} \qquad (1.68)$$

Where

$$\boldsymbol{V} = \frac{K_m^P V^f + K_m^S V^r}{K_m^P - K_m^S} \qquad (1.69)$$

Therefore:

$$\boldsymbol{V} = \frac{K_m^P V^f + \frac{K_m^P V^f}{K_{eq}}}{K_m^P - K_m^S} \qquad (1.70)$$

Therefore:

$$\boldsymbol{V} = \left(\frac{K_{eq} + 1}{K_{eq}}\right) x \left(\frac{K_m^P}{K_m^P - K_m^S}\right) x V^f \qquad (1.71)$$

and:

$$\boldsymbol{K} = \frac{K_m^S K_m^P + (K_m^P + K_m^S K_{eq})[S]_\infty}{K_m^P - K_m^S} \qquad (1.72)$$

As in the case of K' in equation 1.59, ***K*** is not a true kinetic constant as it varies with $[S]_{\square}$ and hence the sum of $[S]_0$ and $[P]_0$. It is only constant if the initial substrate plus product concentration is kept constant. By a similar but symmetrical argument, the net reverse rate of reaction,

$$v_{rev} = \frac{V_{rev}[P^{\#}]}{K_{rev} + [P^{\#}]} \qquad (1.73)$$

with constants defined as above but by symmetrically exchanging K_m^P with K_m^S, and V^r with V^f.

Both equations (1.59) and (1.68) are useful when modelling reversible reactions, particularly the technologically important reaction catalysed by glucose isomerase. They may be developed further to give productivity-time estimates and for use in the comparison of different reactor configurations (see Chapters 3 and 5).

Although an enzyme can never change the equilibrium position of a catalysed reaction, as it has no effect on the standard free energy change involved, it can favour reaction in one direction rather than its reverse. It achieves this by binding strongly, as enzyme-reactant complexes, the reactants in this preferred direction but only binding the product(s) weakly. The enzyme is bound up with the reactant(s), encouraging their reaction, leaving little free to catalyse the reaction in the reverse direction. It is unlikely, therefore, that the same enzyme preparation would be optimum for catalysing a reversible reaction in both directions.

Determination of V_{max} and K_m

It is important to have as thorough knowledge as is possible of the performance characteristics of enzymes, if they are to be used most efficiently. The kinetic parameters V_{max}, K_m and k_{cat}/K_m should, therefore, be determined. There are two approaches to this problem using either the reaction progress curve (integral method) or the initial rates of reaction (differential method). Use of either method depends on prior knowledge of the mechanism for the reaction and, at least approximately, the optimum conditions for the reaction. If the mechanism is known and complex then the data must be reconciled to the appropriate model (hypothesis), usually by use of a computer-aided analysis involving a weighted least-squares fit. Many such computer programs are currently available and, if not, the programming skill involved is usually fairly low. If the mechanism is not known, initial attempts are usually made to fit the data to the Michaelis-Menten kinetic model. Combining equations (1.1) and (1.8),

$$\frac{d[P]}{dt} = v = \frac{V_{max}[S]}{K_m + [S]} \qquad (1.99)$$

which, on integration, using the boundary condition that the product is absent at time zero and by substituting [S] by ($[S]_0$ - [P]), becomes

$$t = \frac{[P]}{V_{max}} - \frac{K_m}{V_{max}} Ln\left(\frac{[S]_0 - [P]}{[S]_0}\right) \qquad (1.100)$$

If the fractional conversion (***X***) is introduced, where

$$\boldsymbol{X} = \frac{[S]_0 - [P]}{[S]_0} \qquad (1.101)$$

then equation (1.100) may be simplified to give:

$$t = \frac{\boldsymbol{X}[S]_0}{V_{max}} - \frac{K_m Ln(1-\boldsymbol{X})}{V_{max}} \qquad (1.102)$$

Use of equation (1.99) involves the determination of the initial rate of reaction over a wide range of substrate concentrations. The initial rates are used, so that $[S] = [S]_0$, the predetermined and accurately known substrate concentration at the start of the reaction. Its use also ensures that there is no effect of reaction reversibility or product inhibition which may affect the integral method based on equation (1.102). Equation (1.99) can be utilised directly using a computer program, involving a weighted least-squares fit, where the parameters for determining the hyperbolic relationship between the initial rate of reaction and initial substrate concentration (i.e.. K_m and V_{max}) are chosen in order to minimise the errors between the data and the model, and the assumption is made that the errors inherent in the practically determined data are normally distributed about their mean (error-free) value.

Alternatively the direct linear plot may be used (Figure 1.10). This is a powerful non-parametric statistical method which depends upon the assumption that any errors in the experimentally derived data are as likely to be positive (i.e. too high) as negative (i.e. too low). It is common practice to show the data obtained by the above statistical methods on one of three linearised plots, derived from equation (1.99) (Figure 1.11). Of these, the double reciprocal plot is preferred to test for the qualitative correctness of a proposed mechanism, and the Eadie-Hofstee plot is preferred for discovering deviations from linearity.

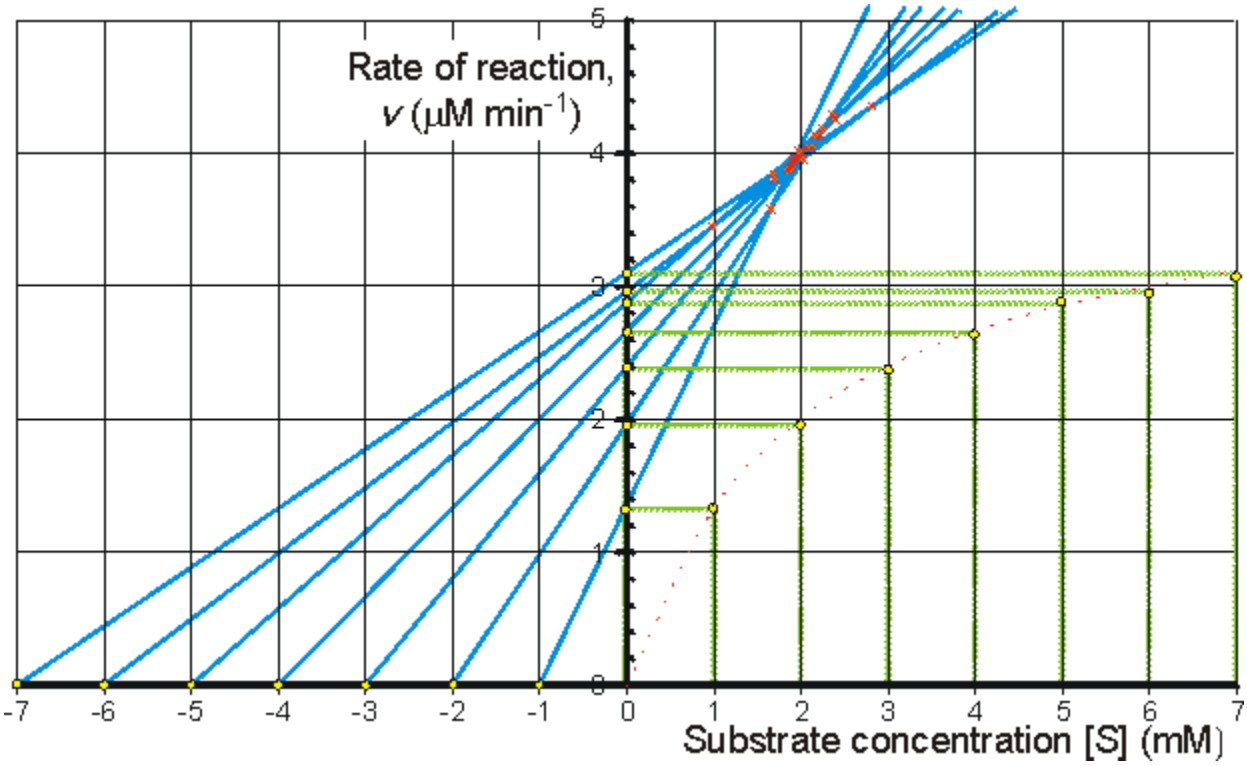


Figure 1.10. The direct linear plot. A plot of the initial rate of reaction against the initial substrate concentration also showing the way estimates can be directly made of the K_m and V_{max}. Every pair of data points may be utilised to give a separate estimate of these parameters (i.e. n(n-1)/2 estimates from n data points with differing $[S]_0$). These estimates are determined from the intersections of lines passing through the (x,y) points $(-[S]_0,0)$ and (0,v); each intersection forming a separate estimate of K_m and V_{max}. The intersections are separately ranked in order of increasing value of both K_m and V_{max} and the median values taken as the best estimates for these parameters. The error in these estimates can be simply determined from sub-ranges of these estimates, the width of the sub-range dependent on the accuracy required for the error and the number of data points in the analysis. In this example there are 7 data points and, therefore, 21 estimates for both K_m and V_{max}. The ranked list of the estimates for K_m (mM) is 0.98,1.65, 1.68, 1.70, 1.85, 1.87, 1.89, 1.91, 1.94, 1.96, **1.98**, 1.99, 2.03, 2.06, 1.12, 2.16, 2.21, 2.25, 2.38, 2.40, 2.81, with a median value of 1.98 mM. The K_m must lie between the 4th (1.70 mM) and 18th (2.25 mM) estimate at a confidence level of 97% (Cornish-Bowden *et al.*, 1978). The list of the estimates for V_{max} (□M.min^{-1}) is ranked separately as 3.45, 3.59, 3.80, 3.85, 3.87, 3.89, 3.91, 3.94, 3.96, 3.96, **3.98**, 4.01, 4.03, 4.05, 4.13, 4.14, 4.18, 4.26, 4.29, 4.35, with a median value of 3.98 □M.min^{-1}. The V_{max} must lie between the 4th (3.85 □M.min^{-1}) and 18th (4.18 □M.min^{-1}) estimate at a confidence level of 97%. It can be seen that outlying estimates have little or no influence on the results. This is a major advantage over the least-squared statistical procedures where rogue data points cause heavily biased effects.

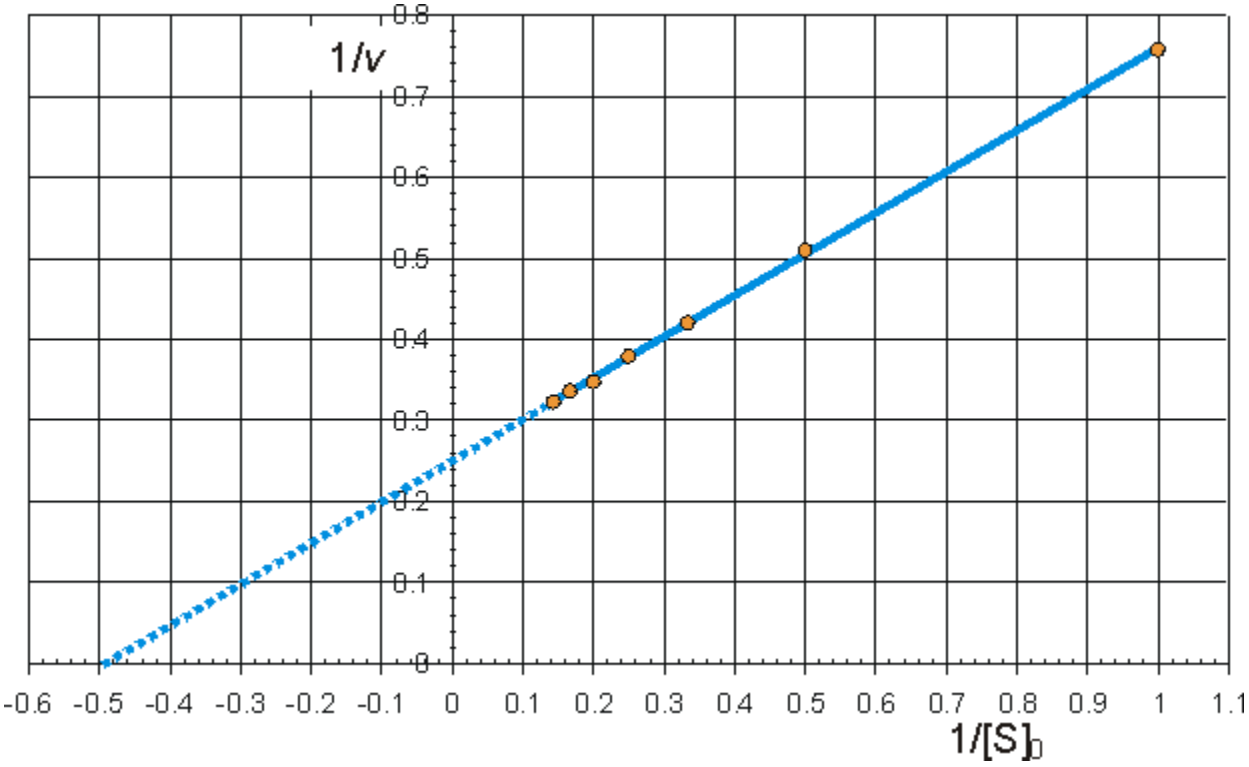
Figure 1.11. Three ways in which the hyperbolic relationship between the initial rate of reaction and the initial substrate concentration

$$v = \frac{V_{max}[S]_0}{K_m + [S]_0}$$

can be rearranged to give linear plots. The examples are drawn using K_m = 2 mM and V_{max} = 4 □M min^{-1}.

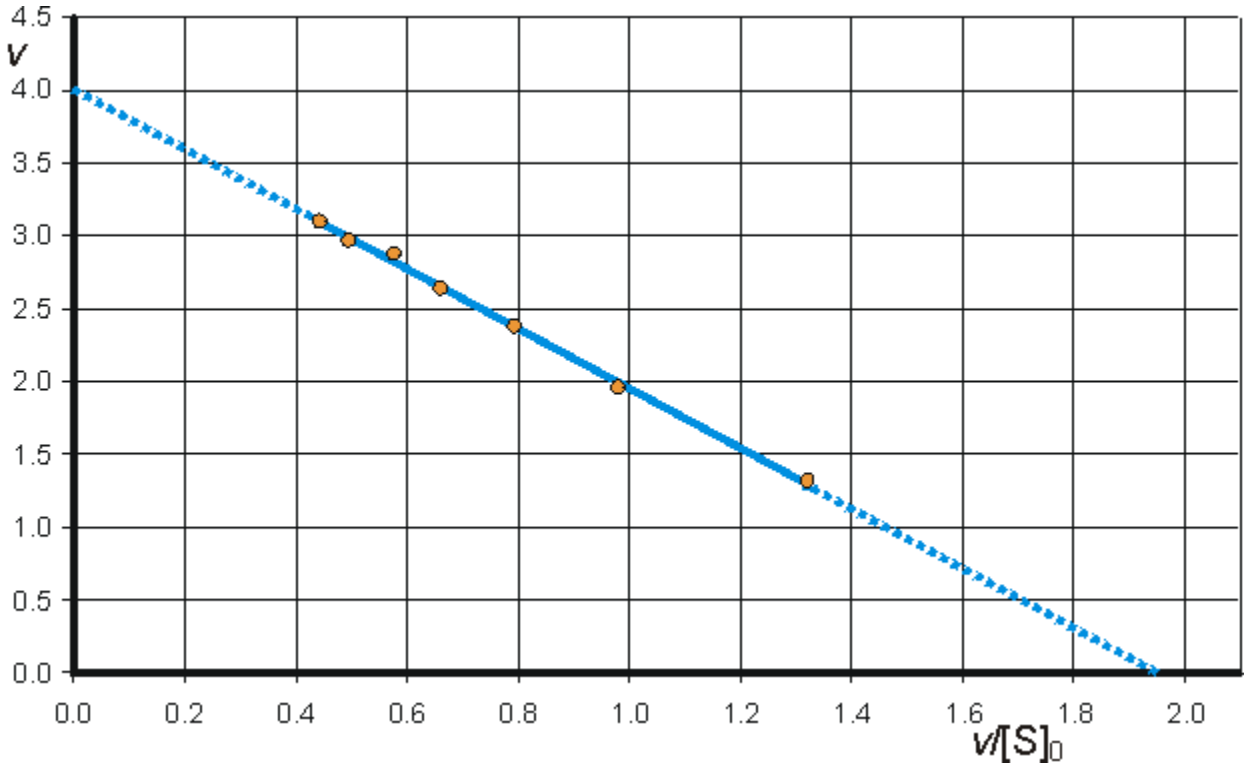
(a) Lineweaver-Burk (double-reciprocal) plot of 1/v against $1/[S]_0$ giving intercepts at $1/V_{max}$ and $-1/K_m$

$$\frac{1}{v} = \frac{K_m}{V_{max}} x \frac{1}{[S]_0} + \frac{1}{V_{max}} \qquad (1.103)$$



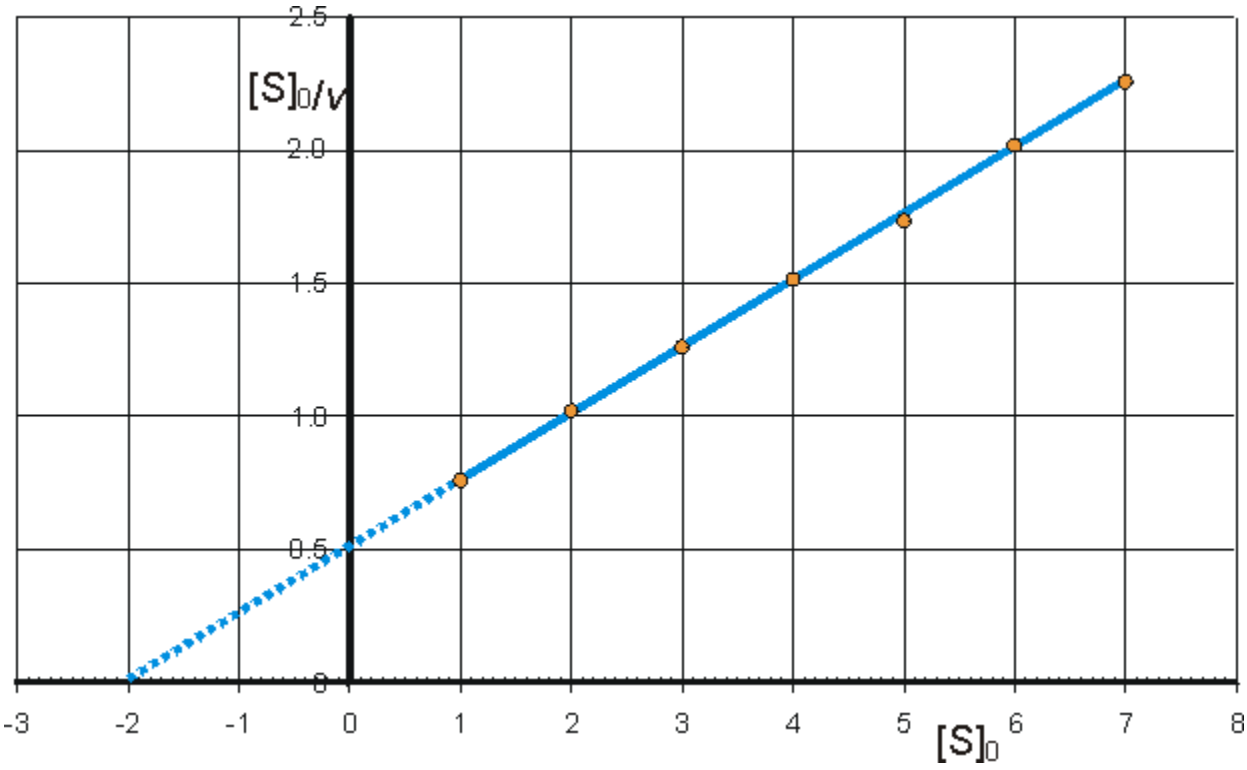
(b) Eadie-Hofstee plot of v against $v/[S]_0$ giving intercepts at V_{max} and V_{max}/K_m

$$v = -K_m x \frac{v}{[S]_0} + V_{max} \qquad (1.104)$$



c) Hanes-Woolf (half-reciprocal) plot of $[S]_0/v$ against $[S]_0$ giving intercepts at K_m/V_{max} and K_m.

$$\frac{[S]_0}{v} = \frac{1}{V_{max}} x[S]_0 + \frac{K_m}{V_{max}} \qquad (1.105)$$



The progress curve of the reaction (Figure 1.12) can be used to determine the specificity constant (k_{cat}/K_m) by making use of the relationship between time of reaction and fractional conversion (see equation (1.102). This has the advantage over the use of the initial rates (above) in that fewer determinations need to be made, possibly only one progress curve is necessary, and sometimes the initial rate of reaction is rather difficult to determine due to its rapid decline. If only the early part of the progress curve, or its derivative, is utilised in the analysis, this procedure may even be used in cases where there is competitive inhibition by the product, or where the reaction is reversible.

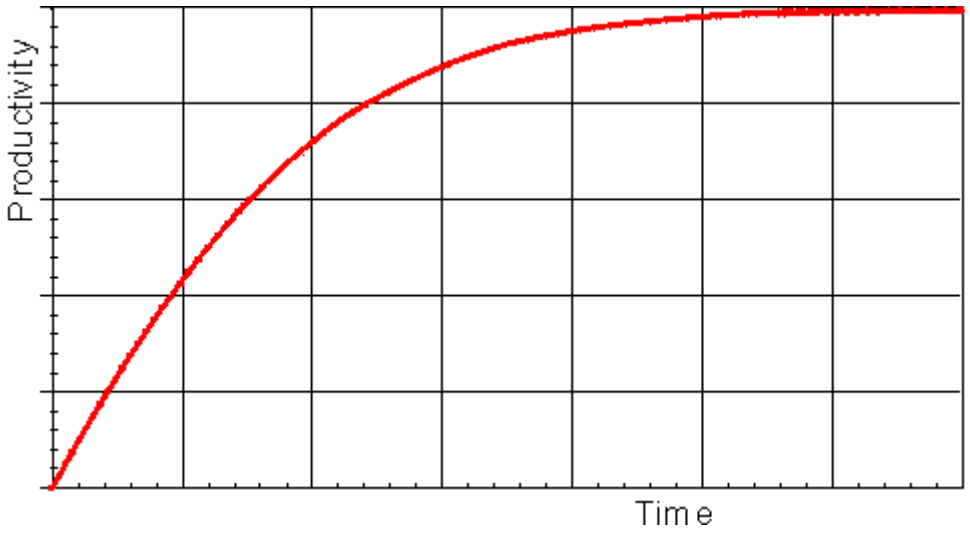


Figure 1.12. A schematic plot showing the amount of product formed (productivity) against the time of reaction, in a closed system. The specificity constant may be determined by a weighted least-squared fit of the data to the relationship given by equation (1.102).

The type of inhibition and the inhibition constants may be determined from the effect of differing concentrations of inhibitor on the apparent K_m, V_{max} and k_{cat}/K_m, although some more specialised plots do exist (e.g. Cornish-Bowden, 1974).

Summary and Bibliography of Chapter 1

a. Enzymes are specific catalysts of vast range and utility.
b. Their activity is governed by their structure and physical environment.
c. Care should be taken over the interpretation of reported units of enzymic activity and the conditions necessary for maximum productivity.
d. Enzymes may lose their catalytic activity reversibly or irreversibly due to denaturation or inhibition, dependent upon the conditions
e. The values of the K_m, V_{max}, specificity constants, $pH_{optimum}$ and rate of thermal denaturation are all of relevance and utility to enzyme technology

Effect of pH and ionic strength

Enzymes are amphoteric molecules containing a large number of acid and basic

groups, mainly situated on their surface. The charges on these groups will vary, according to their acid dissociation constants, with the pH of their environment (Table 1.1). This will effect the total net charge of the enzymes and the distribution of charge on their exterior surfaces, in addition to the reactivity of the catalytically active groups. These effects are especially important in the neighbourhood of the active sites. Taken together, the changes in charges with pH affect the activity, structural stability and solubility of the enzyme.

Table 1.1. pK_as[a] and heats of ionisation[b] of the ionising groups commonly found in enzymes.

Group	**Usual pK_a range**	**Approximate charge at pH 7**	**Heats of ionisation (kJ $mole^{-1}$)**
Carboxyl (C-terminal, glutamic acid, aspartic acid)	3 - 6	-1.0	± 5
Ammonio (N-terminal)	7 - 9	+1.0	+45
(lysine)	9 - 11	+1.0	+45
Imidazolyl (histidine)	5 - 8	+0.5	+30
Guanidyl (arginine)	11 - 13	+1.0	+50
Phenolic (tyrosine)	9 - 12	0.0	+25
Thiol (cysteine)	8 - 11	0.0	+25

[a] The pK_a (defined as $-Log_{10}(K_a)$) is the pH at which half the groups are ionised. Note the similarity between the K_a of an acid and the K_m of an enzyme, which is the substrate concentration at which half the enzyme molecules have bound substrate. (Back)

[b] By convention, the heat (enthalpy) of ionisation is positive when heat is withdrawn from the surrounding solution (i.e. the reaction is endothermic) by the dissociation of the hydrogen ions. (Back)

There will be a pH, characteristic of each enzyme, at which the net charge on the molecule is zero. This is called the isoelectric point (pI), at which the enzyme generally has minimum solubility in aqueous solutions. In a similar manner to the effect on enzymes, the charge and charge distribution on the substrate(s), product(s) and coenzymes (where applicable) will also be affected by pH changes.

Increasing hydrogen ion concentration will, additionally, increase the successful competition of hydrogen ions for any metal cationic binding sites on the enzyme, reducing the bound metal cation concentration. Decreasing hydrogen ion concentration, on the other hand, leads to increasing hydroxyl ion concentration which compete against the enzymes' ligands for divalent and trivalent cations causing their conversion to hydroxides and, at high hydroxyl concentrations, their complete removal from the enzyme. The temperature also has a marked effect on ionisations, the extent of which depends on the heats of ionisation of the particular groups concerned (Table 1.1). The relationship between the change in the pK_a and the change in temperature is given by a derivative of the Gibbs-Helmholtz equation:

$$\frac{d(pK_a)}{dT} = \frac{-\Delta H}{2.303RT^2} \qquad (1.12)$$

where T is the absolute temperature (K), R is the gas law constant (8.314 J M^{-1} K^{-1}), ?H is the heat of ionisation and the numeric constant (2.303) is the natural logarithm of 10, as pK_a's are based on logarithms with base 10. This variation is sufficient to shift the pI of enzymes by up to one unit towards lower pH on increasing the temperature by 50°C.

These charge variations, plus any consequent structural alterations, may be reflected in changes in the binding of the substrate, the catalytic efficiency and the amount of active enzyme. Both V_{max} and K_m will be affected due to the resultant modifications to the kinetic rate constants k_{+1}, k_{-1} and k_{cat} (k_{+2} in the Michaelis-Menten mechanism), and the variation in the concentration of active enzyme. The effect of pH on the V_{max} of an enzyme catalysed reaction may be explained using the, generally true, assumption that only one charged form of the enzyme is optimally catalytic and therefore the maximum concentration of the enzyme-substrate intermediate cannot be greater than the concentration of this species. In simple terms, assume EH^- is the only active form of the enzyme,

$$EH^- + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} EH^-S \xrightarrow{k_{+2}} P \qquad [\mathbf{1.8}]$$

The concentration of EH^- is determined by the two dissociations

$$EH_2 \overset{K_{a1}}{\rightleftharpoons} EH^- + H^+ \qquad [\mathbf{1.9}]$$

$$EH^- \overset{K_{a2}}{\rightleftharpoons} E^{2-} + H^+ \qquad [\mathbf{1.10}]$$

with

$$K_{a1} = \frac{[EH^-]_0[H^+]}{[EH_2]_0} \qquad (1.13)$$

and

$$K_{a2} = \frac{[E^{2-}]_0[H^+]}{[EH^-]_0} \qquad (1.14)$$

However,

$$[E]_0 = [EH_2]_0 + [EH^-]_0 + [E^{2-}]_0 \qquad (1.15)$$

therefore:

$$[E]_0 = [EH^-]_0\left(\frac{[H^+]}{K_{a1}} + 1 + \frac{K_{a2}}{[H^+]}\right) \qquad (1.16)$$

As the rate of reaction is given by $k_{+2}[EH^-S]$ and this is maximal when $[EH^-S]$ is maximal (i.e.. when $[EH^-S] = [EH^-]_0$):

$$V_{max} = k_{+2}[EH^-] = \frac{k_{+2}[E]_0}{\left(\frac{[H^+]}{K_{a1}} + 1 + \frac{K_{a2}}{[H^+]}\right)} \qquad (1.17)$$

The V_{max} will be greatest when

$$\frac{[H^+]}{K_{a1}} = \frac{K_{a2}}{[H^+]} \qquad (1.18)$$

therefore:

$$pH_{optimum} = \frac{pK_{a1} + pK_{a2}}{2} \qquad (1.19)$$

This derivation has involved a number of simplifications on the real situation; it ignores the effect of the ionisation of substrates, products and enzyme-substrate complexes and it assumes EH^- is a single ionised species when it may contain a mixture of differently ionised groups but with identical overall charge, although the process of binding substrate will tend to fix the required ionic species. It does, however, produce a variation of maximum rate with pH which gives the commonly

encountered 'bell-shaped' curve (Figure 1.4). Where the actual reaction scheme is more complex, there may be a more complex relationship between V_{max} and pH. In particular, there may be a change in the rate determining step with pH. It should be recognised that K_m may change with pH in an independent manner to the V_{max} as it usually involves other, or additional, ionisable groups. It is clear that at lower non-saturating substrate concentrations the activity changes with pH may or may not reflect the changes in V_{max}. It should also be noted from the foregoing discussion that the variation of activity with pH depends on the reaction direction under consideration. The $pH_{optimum}$ may well be different in the forward direction from that shown by the reverse reaction. This is particularly noticeable when reactions which liberate or utilise protons are considered (e.g. dehydrogenases) where there may well be greater than 2 pH units difference between the $pH_{optimum}$ shown by the rates of forward and reverse reactions.

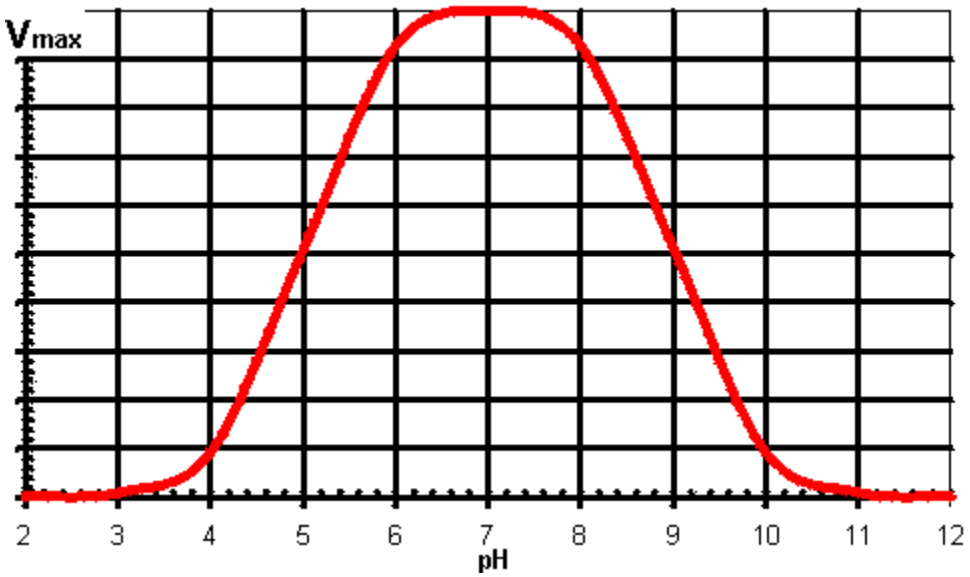


Figure 1.4. A generally applicable schematic diagram of the variation in the rate of an enzyme catalysed reaction (V_{max}) with the pH of the solution. The centre (optimum pH) and breadth of this 'bell-shaped' curve depend upon the acid dissociation constants of the relevant groups in the enzyme. It should be noted that some enzymes have pH-activity profiles that show little similarity to this diagram.

The variation of activity with pH, within a range of 2-3 units each side of the pI, is normally a reversible process. Extremes of pH will, however, cause a time- and temperature-dependent, essentially irreversible, denaturation. In alkaline solution (pH > 8), there may be partial destruction of cystine residues due to base catalysed □-elimination reactions whereas, in acid solutions (pH < 4), hydrolysis of the labile

peptide bonds, sometimes found next to aspartic acid residues, may occur. The importance of the knowledge concerning the variation of activity with pH cannot be over-emphasised. However, a number of other factors may mean that the optimum pH in the V_{max}-pH diagram may not be the pH of choice in a technological process involving enzymes. These include the variation of solubility of substrate(s) and product(s), changes in the position of equilibrium for a reaction, suppression of the ionisation of a product to facilitate its partition and recovery into an organic solvent, and the reduction in susceptibility to oxidation or microbial contamination. The major such factor is the effect of pH on enzyme stability. This relationship is further complicated by the variation in the effect of the pH with both the duration of the process and the temperature or temperature-time profile. The important parameter derived from these influences is the productivity of the enzyme (i.e. how much substrate it is capable of converting to product). The variation of productivity with pH may be similar to that of the V_{max}-pH relationship but changes in the substrate stream composition and contact time may also make some contribution. Generally, the variation must be determined under the industrial process conditions. It is possible to alter the pH-activity profiles of enzymes. The ionisation of the carboxylic acids involves the separation of the released groups of opposite charge. This process is encouraged within solutions of higher polarity and reduced by less polar solutions. Thus, reducing the dielectric constant of an aqueous solution by the addition of a co-solvent of low polarity (e.g. dioxan, ethanol), or by immobilisation (see Chapter 3), increases the pK_a of carboxylic acid groups. This method is sometimes useful but not generally applicable to enzyme catalysed reactions as it may cause a drastic change on an enzyme's productivity due to denaturation (but see Chapter 7). The pK_a of basic groups are not similarly affected as there is no separation of charges when basic groups ionise. However, protonated basic groups which are stabilised by neighbouring negatively charged groups will be stabilised (i.e. have lowered pK_a) by solutions of lower polarity. Changes in the *ionic strength* (□) of the solution may also have some effect. The ionic strength is defined as half of the total sum of the concentration (c_i) of every ionic species (i) in the solution times the square of its charge (z_i); i.e. . □□= $0.5\square(c_i z_i^2)$.

For example, the ionic strength of a 0.1 M solution of $CaCl_2$ is 0.5 x ($0.1 \times 2^2 + 0.2 \times 1^2$) = 0.3 M.

At higher solution ionic strength, charge separation is encouraged with a concomitant lowering of the carboxylic acid pK_as. These changes, extensive as they may be, have little effect on the overall charge on the enzyme molecule at neutral pH and are, therefore, only likely to exert a small influence on the enzyme's isoelectric point. Chemical derivatisation methods are available for converting surface charges from positive to negative and vice-versa. It is found that a single change in charge has little effect on the pH-activity profile, unless it is at the active site. However if all lysines are converted to carboxylates (e.g. by reaction with succinic anhydride) or if all the carboxylates are converted to amines (e.g. by coupling to ethylene diamine by means of a carbodiimide, see Chapter 3) the profile can be shifted about a pH unit towards higher or lower pH, respectively. The cause of these shifts is primarily the stabilisation or destabilisation of the charges at the active site during the reaction,

and the effects are most noticeable at low ionic strength. Some, more powerful, methods for shifting the pH-activity profile are specific to immobilised enzymes and described in Chapter 3.

The ionic strength of the solution is an important parameter affecting enzyme activity. This is especially noticeable where catalysis depends on the movement of charged molecules relative to each other. Thus both the binding of charged substrates to enzymes and the movement of charged groups within the catalytic 'active' site will be influenced by the ionic composition of the medium. If the rate of the reaction depends upon the approach of charged moieties the following approximate relationship may hold,

$$\log(k) = \log(k_0) + z_A z_B \sqrt{I} \qquad (1.20)$$

where k is the actual rate constant, k_0 is the rate constant at zero ionic strength, z_A and z_B are the electrostatic charges on the reacting species, and I is the ionic strength of the solution. If the charges are opposite then there is a decrease in the reaction rate with increasing ionic strength whereas if the charges are identical, an increase in the reaction rate will occur (e.g. the rate controlling step in the catalytic mechanism of chymotrypsin involves the approach of two positively charged groups, ^{57}histidine^{+} and ^{145}arginine^{+} causing a significant increase in k_{cat} on increasing the ionic strength of the solution). Even if a more complex relationship between the rate constants and the ionic strength holds, it is clearly important to control the ionic strength of solutions in parallel with the control of pH.

Effect of temperature and pressure

Rates of all reactions, including those catalysed by enzymes, rise with increase in temperature in accordance with the Arrhenius equation.

$$k = Ae^{-\Delta G^*/RT} \qquad (1.21)$$

where k is the kinetic rate constant for the reaction, A is the Arrhenius constant, also known as the frequency factor, ΔG^* is the standard free energy of activation (kJ M^{-1}) which depends on entropic and enthalpic factors, R is the gas law constant and T is the absolute temperature. Typical standard free energies of activation (15 - 70 kJ M^{-1}) give rise to increases in rate by factors between 1.2 and 2.5 for every 10°C rise in temperature. This factor for the increase in the rate of reaction for every 10°C rise in temperature is commonly denoted by the term $\mathbf{Q_{10}}$ (i.e. in this case, Q_{10} is within the range 1.2 - 2.5). All the rate constants contributing to the catalytic mechanism will vary independently, causing changes in both K_m and V_{max}. It follows that, in an exothermic reaction, the reverse reaction (having a higher activation energy)

increases more rapidly with temperature than the forward reaction. This, not only alters the equilibrium constant (see equation 1.12), but also reduces the optimum temperature for maximum conversion as the reaction progresses. The reverse holds for endothermic reactions such as that of glucose isomerase (see reaction [1.5]) where the ratio of fructose to glucose, at equilibrium, increases from 1.00 at 55°C to 1.17 at 80°C.

In general, it would be preferable to use enzymes at high temperatures in order to make use of this increased rate of reaction plus the protection it affords against microbial contamination. Enzymes, however, are proteins and undergo essentially irreversible **denaturation** (i.e.. conformational alteration entailing a loss of biological activity) at temperatures above those to which they are ordinarily exposed in their natural environment. These denaturing reactions have standard free energies of activation of about 200 - 300 kJ $mole^{-1}$ (Q_{10} in the range 6 - 36) which means that, above a critical temperature, there is a rapid rate of loss of activity (Figure 1.5). The actual loss of activity is the product of this rate and the duration of incubation (Figure 1.6). It may be due to covalent changes such as the deamination of asparagine residues or non-covalent changes such as the rearrangement of the protein chain. Inactivation by heat denaturation has a profound effect on the enzymes productivity (Figure 1.7).

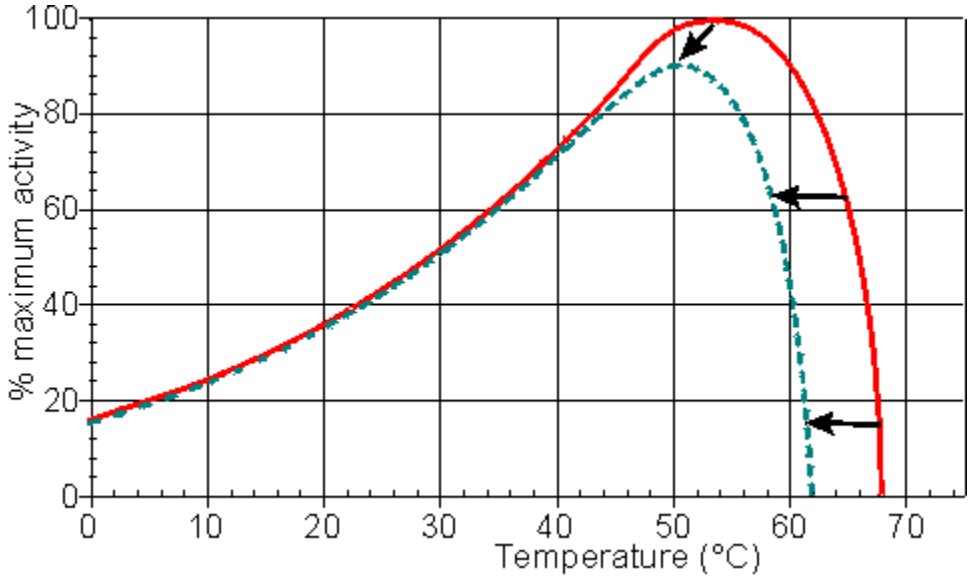


Figure 1.5. A schematic diagram showing the effect of the temperature on the activity of an enzyme catalysed reaction. —— short incubation period; ----- long incubation period. Note that the temperature at which there appears to be maximum activity varies with the incubation time.

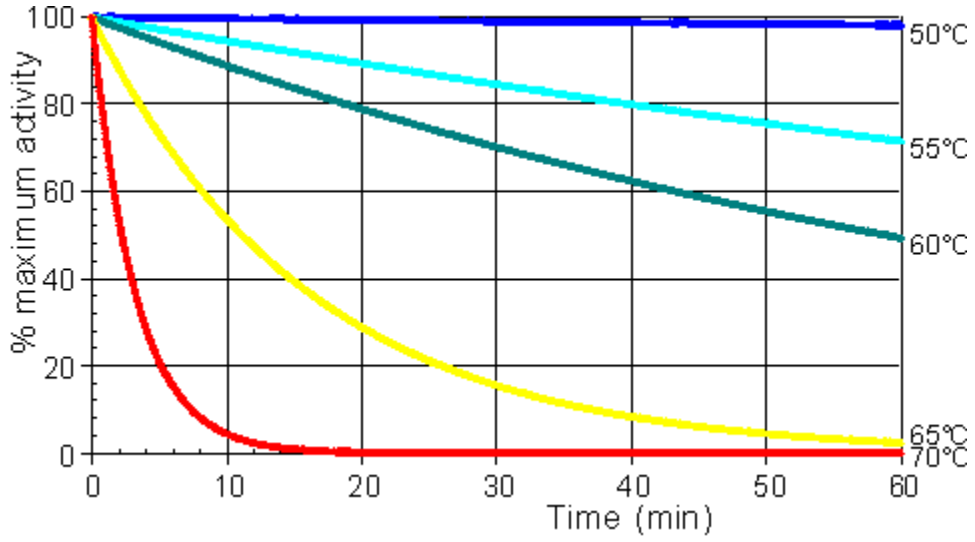


Figure 1.6. A schematic diagram showing the effect of the temperature on the stability of an enzyme catalysed reaction. The curves show the percentage activity remaining as the incubation period increases. From the top they represent equal increases in the incubation temperature (50°C, 55°C, 60°C, 65°C and 70°C).

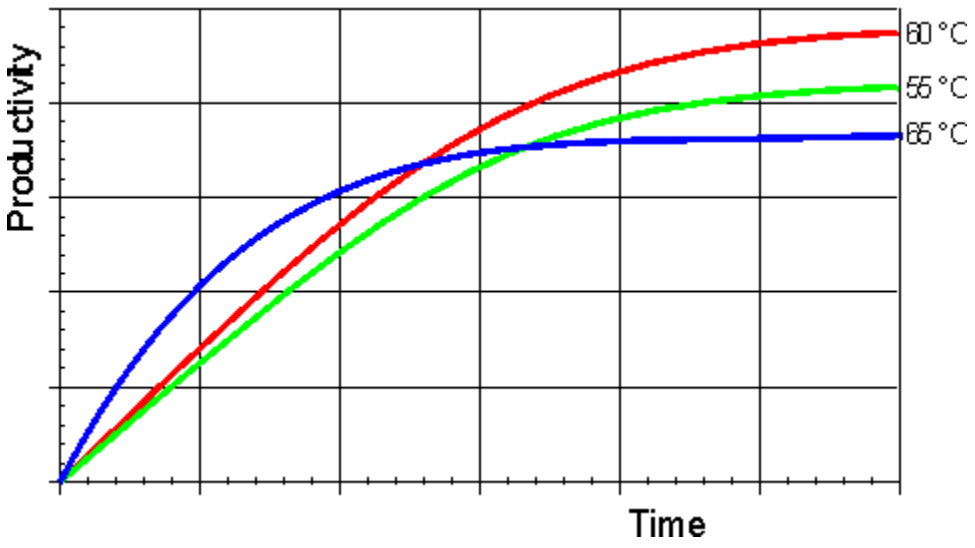


Figure 1.7. A schematic diagram showing the effect of the temperature on the productivity of an enzyme catalysed reaction. —— 55°C; —— 60°C; —— 65°C. The optimum productivity is seen to vary with the process time, which may be determined by other additional factors (e.g. overhead costs). It is often difficult to get precise control of the temperature of an enzyme catalysed process and, under these circumstances, it may be seen that it is prudent to err on the low temperature side.

The thermal denaturation of an enzyme may be modelled by the following serial deactivation scheme:

$$E \xrightarrow{k_{d1}} E_1 \xrightarrow{k_{d2}} E_2 \qquad \textbf{[1.11]}$$

where k_{d1} and k_{d2} are the first-order deactivation rate coefficients, E is the native enzyme which may, or may not, be an equilibrium mixture of a number of species, distinct in structure or activity, and E_1 and E_2 are enzyme molecules of average specific activity relative to E of A_1 and A_2. A_1 may be greater or less than unity (i.e. E_1 may have higher or lower activity than E) whereas A_2 is normally very small or zero. This model allows for the rare cases involving free enzyme (e.g. tyrosinase) and the somewhat commoner cases involving immobilised enzyme (see Chapter 3) where there is a small initial activation or period of grace involving negligible discernible loss of activity during short incubation periods but prior to later deactivation. Assuming, at the beginning of the reaction:

$$[E] = [E]_0 \qquad (1.22)$$

and:

$$[E_1] = [E_2] = 0 \qquad (1.23)$$

At time t,

$$[E] + [E_1] + [E_2] = [E]_0 \qquad (1.24)$$

It follows from the reaction scheme **[1.11]**,

$$-\frac{d[E]}{dt} = k_{d1}[E] \qquad (1.25)$$

Integrating equation 1.25 using the boundary condition in equation 1.22 gives:

$$[E] = [E]_0 e^{(-k_{d1}t)} \qquad (1.26)$$

From the reaction scheme [1.11],

$$-\frac{d[E_1]}{dt} = k_{d2}[E_1] - k_{d1}[E] \qquad (1.27)$$

Substituting for [E] from equation 1.26,

$$-\frac{d[E_1]}{dt} = k_{d2}[E_1] - k_{d1}[E]_0 e^{(-k_{d1}t)} \qquad (1.28)$$

Integrating equation 1.27 using the boundary condition in equation 1.23 gives:

$$[E_1] = \frac{k_{d1}[E]_0}{k_{d2} - k_{d1}}\left(e^{(-k_{d1}t)} - e^{(-k_{d2}t)}\right) \qquad (1.29)$$

If the term 'fractional activity' (A^f) is introduced where,

$$A^f = \frac{[E] + A_1[E_1] + A_2[E_2]}{[E]_0} \qquad (1.30)$$

then, substituting for $[E_2]$ from equation 1.24, gives:

$$A^f = \frac{[E] + A_1[E_1] + A_2([E]_0 - [E] - [E_1])}{[E]_0} \qquad (1.31)$$

therefore:

$$A^f = A_2 + \left(1 + \frac{A_1 k_{d1} - A_2 k_{d2}}{k_{d2} - k_{d1}}\right)e^{(-k_{d1}t)} - \frac{(A_1 - A_2)k_{d1}}{k_{d2} - k_{d1}} e^{(-k_{d2}t)} \qquad (1.32)$$

When both A_1 and A_2 are zero, the simple first order deactivation rate expression results

$$A^f = e^{(-k_{d1}t)} \qquad (1.33)$$

The **half-life** ($t_{1/2}$) of an enzyme is the time it takes for the activity to reduce to a half of the original activity (i,e. $A^f = 0.5$). If the enzyme inactivation obeys equation 1.33, the half-life may be simply derived,

$$Ln(1/2) = -k_{d1}t_{1/2} \qquad (1.34)$$

therefore:

$$t_{1/2} = \frac{0.693}{k_{d1}} \qquad (1.35)$$

In this simple case, the half-life of the enzyme is shown to be inversely proportional to the rate of denaturation.

Many enzyme preparations, both free and immobilised, appear to follow this series-type deactivation scheme. However because reliable and reproducible data is difficult to obtain, inactivation data should, in general, be assumed to be rather error-prone. It is not surprising that such data can be made to fit a model involving four determined parameters (A_1, A_2, k_{d1} and k_{d2}). Despite this possible reservation, equations 1.32 and 1.33 remain quite useful and the theory possesses the definite advantage of simplicity. In some cases the series-type deactivation may be due to structural microheterogeneity, where the enzyme preparation consists of a heterogeneous mixture of a large number of closely related structural forms. These may have been formed during the past history of the enzyme during preparation and storage due to a number of minor reactions, such as deamidation of one or two asparagine or glutamine residues, limited proteolysis or disulphide interchange. Alternatively it may be due to quaternary structure equilibria or the presence of distinct genetic variants. In any case, the larger the variability the more apparent will be the series-type inactivation kinetics. The practical effect of this is that usually k_{d1} is apparently much larger than k_{d2} and A_1 is less than unity.

In order to minimise loss of activity on storage, even moderate temperatures should be avoided. Most enzymes are stable for months if refrigerated (0 - 4°C). Cooling below 0°C, in the presence of additives (e.g. glycerol) which prevent freezing, can generally increase this storage stability even further. Freezing enzyme solutions is best avoided as it often causes denaturation due to the stress and pH variation caused by ice-crystal formation. The first order deactivation constants are often significantly lower in the case of enzyme-substrate, enzyme-inhibitor and enzyme-product complexes which helps to explain the substantial stabilising effects of suitable ligands, especially at concentrations where little free enzyme exists (e.g. $[S] >> K_m$). Other factors, such as the presence of thiol anti-oxidants, may improve the thermal stability in particular cases.

It has been found that the heat denaturation of enzymes is primarily due to the proteins' interactions with the aqueous environment. They are generally more stable in concentrated, rather than dilute, solutions. In a dry or predominantly dehydrated state, they remain active for considerable periods even at temperatures above 100°C. This property has great technological significance and is currently being exploited by the use of organic solvents (see Chapter 7).

Pressure changes will also affect enzyme catalysed reactions. Clearly any reaction involving dissolved gases (e.g. oxygenases and decarboxylases) will be particularly affected by the increased gas solubility at high pressures. The equilibrium position

of the reaction will also be shifted due to any difference in molar volumes between the reactants and products. However an additional, if rather small, influence is due to the volume changes which occur during enzymic binding and catalysis. Some enzyme-reactant mixtures may undergo reductions in volume amounting to up to 50 ml $mole^{-1}$ during reaction due to conformational restrictions and changes in their hydration. This, in turn, may lead to a doubling of the k_{cat}, and/or a halving in the K_m for a 1000 fold increase in pressure. The relative effects on k_{cat} and K_m depend upon the relative volume changes during binding and the formation of the reaction transition states.

Immobilization of Enzymes - Enzyme immobilization may be defined as confining the enzyme molecules to a distinct phase from the one in which the substrates and the products are present. The materials used for immobilization of enzymes, called carrier matrices, are usually inert polymers or inorganic materials.

The ideal carrier matrix has the following properties:

(i) low cost,

(ii) inertness,

(iii) physical strength,

(iv) stability,

(v) Regenerability after the useful lifetime of the immobilized enzyme,

(vi) enhancement of enzyme specificity,

(vii) Reduction in product inhibition,

(viii) a shift in the pH optimum for enzyme action to the desired value for the process, and

(ix) Reduction in microbial contamination and nonspecific adsorption.

The various methods used for immobilization of enzymes may be grouped into the following 5 types:

(i) Adsorption,

(ii) Covalent bonding,

(iii) Entrapment, and

(iv) Membrane confinement.

Entrapping by microencapsulation.

Immobilization of Enzymes by Adsorption - In case of adsorption, the enzyme molecules adhere to the surface of carrier matrix due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule.

Some of the commonly used matrices are ion exchange matrices, porous carbon, clays, hydrous metal oxides, glasses, and polymeric aromatic resins. Ion exchange matrices are costly, but they can be readily regenerated by a simple operation, e.g., washing off the adsorbed enzyme with a concentrated salt solution.

Adsorption of enzymes to the matrices is very easy and widely used. The enzyme is mixed with a suitable adsorbent under appropriate conditions of pH and ionic strength.
After incubation for a sufficient period of time, the carrier is washed to remove un adsorbed enzyme molecules, and the immobilized enzyme is ready for use. This method usually produces a high loading (about 1 g enzyme g matrix) of the enzyme.

Adsorption Method for Immobilization - The enzymes may be immobilized by adsorption to several types of materials (adsorbents with charged or neutral surfaces), that are used for the separation of proteins by adsorption chromatography. Since the activity of enzyme may be significantly reduced or lost during adsorption and subsequent release, the adsorbents should be chosen such that enzymes are bound firmly with minimum inactivation.
Following are some of the adsorbents often used for immobilization: alumina, amberlite CG-50, betonies, calcium phosphate gels, carbon, carboxymethyl cellulose, carboxymethyl Sephadex, collagen, DEAE-cellulose, DEAE-sephadex, glass, silica gel titania (ceramics).

The pH and ionic conditions of enzyme and adsorbent solution should be carefully controlled during immobilization. If adsorption involves predominantly ion interaction with very little hydrogen bonding, then a simple shift in pH or ionic strength could exchange the protein ion for another ion and thus desorption would occur.

In a typical adsorption method for immobilization, adsorbent is packed in a water jacketed column, which is washed with a preconditioning solution.

For instance, if we take titania as an adsorbent, it is washed with 05 $NaHCO_3$ and internal surfaces of the adsorbent are rendered air free. Enzyme solution is buffered fairly close to iso electric point of the enzyme with a low ionic strength (below 0.01 μM), and is then circulated through the column at a desired temperature for several hours.

After the enzyme solution is drained from column, the column is washed with water, then with O.5M NaCl and finally again with water. The immobilized enzyme column is now ready for the delivery of substrate solution and evaluation of performance.

Immobilization of Enzymes by Covalent Binding - In this system the enzyme molecules are attached to the carrier matrix by formation of covalent bonds. As a result, the strength of binding is very strong, and there is no enzyme loss during use.

The covalent bond formation occurs with the side chains of amino acids of the enzyme. Lysine residues are the most useful in covalent binding since they are usually exposed on the surface, are highly reactive and only very rarely occur at active sites of enzymes. Enzyme loading is quite low (Ca. 0.02g/g matrix) only in exceptional cases it may be 0.3 g/g matrix).

The most commonly employed matrices are agarose, celluloses and polyacrylamide. Sepharose, an agarose, is available commercially as beads, is highly hydrophilic and is generally inert to microbial attack. Sepharose is activated by treating it with chloroformates, carbodiimides, glutaraldehyde or other compounds.

Gluteraldehyde is a bifunctional reagent. It exists as an equilibrium mixture of monomer and oligomers. It can be used to covalently bind the enzyme molecules or, alternatively, it can be used to cross link enzyme molecules.

In cross linking, each bifunctional reagent molecule binds to two enzyme molecules; ultimately, a network of enzyme molecules linked together is produced. Glutaraldehyde is particularly useful for producing immobilized enzyme membranes for use in biosensors; this is achieved by cross linking the enzyme plus a non catalytic protein, used for dilution within a porous sheet, e.g., lens tissue paper or nylon net fabric.

Immobilization may lead to a loss in enzyme activity due to the involvement of active site in immobilization, or immobilization of the enzyme in an orientation, which either distorts the active site or renders it unavailable.
Covalent Binding Method for Immobilization - An enzyme can be covalently bound to support materials by different methods. The enzyme forms a covalent link with active groups of support material either

(i) through the reactive groups on side chains of its amino acids like lysine, arginine, histidine, tyrosine, cysteine, serine, aspartic acid and glutamic acid or

(ii) With terminal amino and carboxyl groups of the polypeptide chains. Some examples of covalent linkage between the support and the enzyme are as follows. (The reactants required to activate the support are indicated in corresponding figures).

Support with -OH group. Such supports can be activated for covalent linking by treating with either triazines or cyanogen bromide. The reaction with enzyme protein in each case involves the -NH2 group of the lysine.

(b) Support with -COOH group. Such supports like carboxymethyl cellulose can be activated via azide derivative or acyl isourea formation. The reaction involves -NH2 group of lysine, however other amino acids such as tyrosine, cysteine and serine are also implicated.

(c)Support with –NH2 group. Support containing amino group can be converted to a diazonium chloride by treating with NaNO2 + HCL.

The enzyme protein links with this derivative is forming an azo- linkage involving the tyrosine residue of the enzyme protein. Glutaraldehyde is also used sometime to activate the support containing-NH2 group. The reaction involves a Schiff's base formation between amino group of support and amino group of one of the amino acids of protein. The number of bonds between support and the enzyme molecule during convalent linkage is variable.

There are, for example, 17 bonds per papain molecule and 8 for subtilopetidase immobilized on semiadehyde starch derivative. Papain bound to porous glass is linked through three azo links per enzyme molecule.

Immobilization of Enzymes by Entrapment - In this approach, enzyme molecules are held or entrapped within suitable gels or fibres and there mayor may not be covalent bond formation between the enzyme molecules and the matrix.
A noncovalent entrapment may be viewed as putting the enzyme molecule in a molecular cage just as a caged bird/animal. When covalent binding is also to be generated, the enzyme molecules are usually treated with a suitable reagent. Enzyme loading is very high (1 g/g gel or fiber). However, diffusion of the substrate to the enzyme and of the product away from the enzyme creates difficulties.

Entrapping Method for Immobilization - Enzymes can be entrapped inside a cross linked gel matrix by allowing the gel to be formed in an aqueous solution containing one or more enzymes. The polymerization of the gel is carried out in the presence of enzyme(s). The enzyme is physically entrapped within the matrix and

cannot escape by permeation. However, substrate molecules, being smaller, can diffuse in, can be acted upon by enzyme and can then diffuse out of the matrix.

The important matrices for entrapping enzymes are polyacrylamide, starch and silicone rubber gel. However immobilization of enzymes by such an entrapment method is often associated with following three types of difficulties:

(1) There is continuous leakage of enzyme because of wide pore size distribution in the gel.

(2) There is reduced substrate accessibility to the enzyme.

(3) There is some loss of enzyme activity due to free radicals produced during polymerization of the gel

Entrapping by Microcapsulation Method for Immobilization - Microencapsulation is another approach in enzyme immobilization by entrapping method. In this approach enzyme can be immobilized within microcapsules prepared from organic polymers, so that the enzyme can not escape, although low molecular weight substrates and products can enter and leave the capsule by diffusion through the membrane. There are two general methods for preparing the membranous capsules for enzyme entrapping:

(1) The membranes are prepared by the process of phase separation, which is similar to homogenization of water in oil. One phase is not miscible with the other but forms a droplet or coacervate with the other phase, when mixed. The enzyme is entrapped within this droplet or coacervate.

(2) A water insoluble membrane is prepared by chemical polymerization and enzyme is entrapped during this polymerization.

Spheres made up of semipermeable nylon or collodion membranes are often used for microencapsulation of an enzyme. Enzymes are also entrapped inside fibres of cellulose triacetate. These fibres may be packed in columns or woven into fabric.

The choice of the method to be employed for immobilization would certainly vary according to the enzyme involved and its application. Sufficient variation has been reported in the level of retenton of enzyme activity on immobilization to different supports.

Reagents Used for Immobilize Enzymes by Cross Linking - 1. Bisdiazobenzidine 2, 2, -disulphonic acid

2, Diazobenzidine

3. Diazobenzidine 3, 3,-dianisidine

4. 4, 4' .Difluoro 3, 3' dinitrophenyl sulphone

5. Diphenyl 4. 4' - diisothiocyanate 2, 2' -disulphonic acid

6. Glutaraldehyde

7. N, N' - hexamethylene bisisodiacetamide

8. Hexamethylene di isocyanate

9. 3-methoxydiphenyl methane 4, 4' -di-isocyanate

10. Toluene 2, 4-di-isothiocyanate

11. Toluene 2-isocyanate 4-isothiocyana

Approaches Used in Immobilizing Enzymes by Cross Linking - Three types of basic approaches have been used in immobilizing enzymes by cross linking:

(1) Cross linking of enzyme with glutaraldehyde to form an insoluble aggregate; e.g. papain.

(2) Adsorption of enzyme onto a surface followed by cross linking; for instance, cross linking trypsin adsorbed to the surface of colloidal silica particles.

(3) Impregnation of porous material with the enzyme followed by cross linking of the enzyme in the pores; for instance papain in collodion membrane.

Immobilization of Aminoacylase -

Support	**Method**	**Approximate enzyme activity Immobilized (per cent)**
Polyacrylamide	Entrapment	53
Nylon	Encapsulation	36
DEAE cellulose	Ionic binding	55
DEAE sephadex A-50	Ionic binding	56
CM sephadex C.50	Ionic binding	0
lodoacetyl cellulose	Covalent binding	39

CNBr activated sephadex	Covalent binding	1.6
AE-cellulose	Cross linked with glutaraldehyde	0.6

Immobilization of Enzymes by Membrane Confinement - Enzyme molecules, usually in an aqueous solution, may be confined within a semipermeable membrane which, ideally, allows a free movement in either direction to the substrates and products but does not permit the enzyme molecules to escape. A number of strategies are employed for this purpose; the simplest strategy is as follows.

The reaction vessel is partitioned into two chambers by a semipermeable membrane; one chamber contains the enzyme while the other has the substrate and the product. Each immobilization strategy has some strong.

Advantage of Immobilization - 1. Enzymes are costly items. Immobilization permits their repeated use.

2. The product is readily freed from the enzyme.

3. Immobilized enzymes can be used in nonaqueous systems as well.

4. Continuous production systems can be used, which is not possible with free enzymes.

5. Thermostability of some enzymes may be increased.

6. Recovery of enzyme may also reduce effluent handling problems.

7. Enzymes can be used at much higher concentrations than free enzyme.

Comparison of the Various Features of the Different Immobilization Systems-

Feature	**Immobillization System**			
	Adsorption	**Covealent binding**	**Entrapment**	**Membrane confinement**
Matrices (examples)	Ion-exchange matrices,	Sepharose, cellulose,	Acrylamide, cellulose	Semipermeable membranes, e.g.

	clays, glasses, etc.	acrylamide, etc.	acetate, etc.	hollow-fibers, lipsomes, etc.
Preparation	Simple	Difficult	Difficult	Simple
Immobilization mechanism	Hydrophobic effects, salt links	Covalent bonds	Trapping in Gel/fibre; even covalent bonds	Confinement in a semipermeable membrane
Binding force	Variable	Strong	Weak	Strong
Enzyme loading	High (Ca. 19/9 matrix)	Small (Ca. 0.02 gig matrix)	-	-
Enzyme leakage during use	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Problems during operation	High	Low	High	High
Matrix effects on enzyme	Yes	Yes	Yes	No
Diffusional barriers *	Absent	Absent	Large	Large
Protection from microbial attack	No	No	Yes	Yes
Cost	Low	High	Moderate	High

Disadvantages of Immobilization - 1. Immobilization means additional cost.

2. Immobilization often adversely affects the stability and/or activity of the enzymes.

3. This approach can not be used when one of the substrates is insoluble.

4. Some immobilization strategies present large problems in diffusion of the substrate to reach the enzyme.

Enzyme	**Process**
A. Immobilized enzyme systems.	
1. L-Amino acid oxidase	D-amino acid production

2. Flavoprotein oxidase	N-oxidation of drugs containing hydrazine groups
3. Δ' -Steroid hydrogenase	Production of prednisolone
4. Ribonuclease	Nucleotide production from RNA
5. α Amylase	Com syrup production
6. Aminocyclase	Resolution of DL amino acids
7. Glucoamylase	Production of glucose from com syrup
8. Invertase	Conversion of sucrose into glucose and fructose

Uses of Enzymes in Solution - Enzymes have a wide variety of applications in industry, medicine research, etc. Some of the important applications are briefly discussed under the following headings:

(i) uses of enzymes in solution,

(ii) use of bi phasic systems,

(iii) uses of immobilized enzymes, and

(iv) biosensors.

Enzymes in Detergents - Detergents represent the largest industrial application of enzymes amounting to 25-30% of the total sales of enzymes. The enzymes used in detergents must be cost effective, safe to use and be able to perform the task in the presence of anionic and non ionic detergents, soaps, oxidants, etc. at pH between 8 and 10.5. Enzymes constitute only 0.4-0.8% crude enzyme by weight (about I % by cost) of detergents.

The chief enzymes used are proteases, a amylase and, sometimes, cellulase.

1. Proteases are used to digest away proteins present in blood stains, milk, grass, etc. and also in association with dirt; therefore, they help in removal of dirt as well. Only serine proteases are suitable for use in detergents.

These enzymes are produced by B. licheniformis and B. amyloliquefaciens. Proteases are packed inside dust free granules coated with wax materials made from Paraffin oil or PEG plus hydrophilic binders; the granules disperse in wash releasing the enzyme. This strategy protects users from hypersensitivity to the enzymes.

2. α-Amylase is used to digest away starch present in association with dirt and stains; they are produced by B. licheniformis.

3. Cellulases, produced by fungi, are used for washing cotton fabrics. The enzyme digests away the small fibers raised from the fabric without damaging the major fibers of the fabric. This restores the fabric to 'as new' condition, and also removes soil particles by digesting the associated cellulose.

4. Lipases suitable for detergent use have been identified, and are used for digestion of lipids present in stains and/or dirt.

Enzymes in Leather Industry- Alkaline proteases (0.1-1 % w/w) are used to remove hair from hides; this is safer and more pleasant than the traditional method using sodium sulphide. Dehaired hides are processed or bated often using pancreatic enzymes to increase their suppleness and softness in appearance. Bating is necessary for the production of soft leather clothing.

Enzymes in Production of Glucose Syrup- Glucose syrup is produced from liquefied starch of' 8-12 DE. DE (dextrose quivalent) value is obtained as follows.

DE = Amount of reducing sugar expressed as glucose / Total amount of carbohydrate x 100

Liquefied starch is obtained by heating to 105°C for 5 min a 30-40% granular starch slurry (containing 20-80 ppm Ca2+) to which a-amylase has been added at about 0.5-0.6 kg/ton (1500 U/kg). The starch becomes gelatinised due to heating; the temperature is lowered to between 90-100°C and incubation allowed for t -2 hr.

The enzyme a-amylase digests the starch to produce soluble dextrin molecules; this is called liquefaction. Small quantities of liquefied starch are spray-dried for use in baby foods and as bulking agents.

Liquefied starch is rapidly cooled to 60°C, pH adjusted to 4.0-4.5, and glucoamylase is added at 0.65-0.81/ton (200 U/kg). Generally, a debranching enzyme, e.g., pullulanase from Bacillus acidopullulyticus, is also added at 100 U/kg to digest branched oligosaccharides, which increases the DE of syrup to 98-99.

Glucoamylase is generally obtained from Aspergillus Niger. The mixture is incubated for 72 h, heated to 85°C to stop the reaction, cooled and filtered. Further purification may be done by using activated charcoal and ion exchange resins. The syrup composition is typically as follows: 9597% glucose, 1-2% maltose and 0.5-2% isomaltose.

Generally, starch liquefaction is a continuous process, while) saccharification is done in batch mode in large stirred tanks. Continuous saccharification is possible but at least 6 tanks will have to be used in a series.

Uses of Enzymes in Medicine- Enzyme applications in medicine are as extensive as in industry. Pancreatic enzymes have been used in digestive disorders since nineteenth century. Most enzymes are used extracellularly for

(i) topical applications, e.g., col1agenase,

(ii) removal of toxic substances, e.g., rhodonase, or in

(iii) disorders within blood circulation system, e.g., streptokinase, urokinase, etc. The enzyme preparations must be of high purity and free from unwanted contamination; therefore, they are generally from animal, sources and very costly.

For example, urokinase is isolated from human urine and costs nearly $ 200/mg; the annual market for this enzyme is nearly $150 million. Enzymes have a major potential application in treatment of cancer, e.g., asparagenase in the treatment of lymphocytic leukaemia.

Tumour cells are unable to synthesize L-asparagine due to an enzyme deficiency, and obtain this amino acid from body fluids. Asparaginase drastically reduces the levels of free L-asparagine in the blood stream, creating starvation in tumour cells for this amino acid; normal cells are not affected since they can synthesize L-asparagine.

Asparaginase is injected intravenously, shows half-life of about 1 day (in dog), and may lead to complete recovery in 60% of the cases. Enzyme applications in medicine are limited by and suffer from certain limitations.

1. Their large molecular size interferes with their distribution among body cells.

2. Enzymes are antigenic, and can elicit immune response in the patient, especially on prolonged use.

3. Most enzymes have short effective life in the circulatory system; e.g., of few minutes.

Immobilized Lactase- Lactase is used to remove lactose from milk and whey since many people are sensitive to lactose. Yeast (Kluyveromyces lactis) lactase is immobilized in cellulose triacetate fibers, which are cut up and used in batch STR (5°C, pH 6.4-6.8); it is used for processing of milk and sweet whey. But fungal lactase (from Aspergillus niger, optimum pH 3.0-4.0) is immobilized on porous silica and is used in a PBR for treatment of acid whey.

Antibiotic Production- Penicillin amidases (= penicillin acylases) obtained from E. coli are immobilized in a variety of ways, e.g., by covalent linkage to cyanogen bromide activated Sephadex 200. It is used in a batch or semi continuous mode STR, and may be used 100 times.

In a PBR, it has been used for 100 days yielding about 2 tonSj1tPf 6-aminopenicillonic acid/kg of immobilized enzyme. Penicillin G amidases may be used to synthesize penicillin and cephalosporins.

Preparation of Acrylamide- Acrylamide, used to produce several economically useful polymers, is obtained by addition of a water molecule to acrylo nitrile as follows.

CH_2=CHCN + H_2O -->CH_2=$CHCONH_2$

This reaction is achieved by nitrile hydratase (erroneously called a nitrilase) activity obtained from Rhodococcus. The bacterial cells are immobilized in a polyacrylamide gel, and are used at 10˚C and pH 8.0-8.5 in semibatchwise process keeping the substrate concentration below 3%. About 4000 tons of acrylamide is produced every year using this process.

Enzymes in Treatment of Diseases-

Enzyme	**Reaction catalyzed**	**Used for**
a-Amylase	Starch hydrolysis	Digestive disorders
Asparaginase	Asparagine --> aspartate	Leukaemia
Collagenase	Collagen hydrolysis	Skin ulcers
Ficin	Protein hydrolysis	Deworming
Glutaminase	L-Glutamine --> L-glutamate	Leukaemia
Lysozyme	Bacterial cell wall hydrolysis	Used as antibiotic
Lipase	Lipid hydrolysis	Digestive disorders
Papain	Protein hydrolysis	Deworming
Proteases	Protein hydrolysis	Digestive disorders
Rhodonase	Formation of cyanide sulphide	Cyanide poisoning
Ribonuclease	RNA digestion	As antiviral agent
β-Lactamase	Penicillin --> Penicilloate	Penicillin allergy
Streptokinase	Plasminogen --> plasmin	Blood clots

Uricase	Urate --> allantoin	Gout
Urokinase	Plasminogen --> plasmin	Blood clots

Aspartame Synthesis- Aspartame is a dipeptide containing one residue each of L-aspartic acid and methyl ester of L-phenylalanine. It is 180 times more sweet than sucrose, and is used as low calorie sweetener.

It is synthesized from an equal mixture of L-aspartic acid (the amino group is protected by a reaction with, usually, benzyl chloroformate) and methyl ester of L-phenylalanine by the protease thermolysin. D-phenylalanine methyl ester is also added in a quantity equal to that of the L-isomer; the D-isomer forms an addition complex with aspartame which forms a precipitate.

This removes aspartame from the reaction mixture and gives high yields at concentrations above 1 M. Later, aspartame is recovered from the precipitate by suitably changing the pH, and finally the benzyl chloroformate (attached to the amino group of L-aspartic acid) is removed by a simple hydrogenation process.

There are many examples of enzymic peptide synthesis using proteases, which may be immobilized for repeated use. For example, porcine insulin is converted into human insulin by replacing the C-terminal alanine (B30) residue by a threonine in a single step catalyzed by trypsin.

Similarly, lipases may be used to exchange the fatty acids in triglycerides to enhance their usefulness and value, e.g., conversion of palm oil to obtain a cocoa butter substitute for use in confectionery. Lipases are ordinarily used in aqueous-organic biphasic systems.

Important Applications of Immobilized Enzymes-

Enzyme	**Substrate**	**Enzyme reactor used***	**Product**
Aminoacylase (immobilized on anion exchange resins)	N-acyl-DL- amino acids	PBR	L-amino acids
Aspartate ammonialyase	Fumaric acid + $NH4^+$	PBR	L-aspartic acid
Cyanidase	Cyanide present in industrial waste, food or feed		Formic acid
Glucoamylase	Dextrins produced by α-amylase		D-glucose
Glucose isomerase	D-glucose in glucose syrup	PBR	High

(immobilized with glutara Idehyde by cross linking)			fructose corn syrup
Invertase	Sucrose	PBR	Invert sugar
Lactase (immobilized in cellulose triacetate fibers)	Milk and whey	STR	Lactose-free milk and whey
Lipase	Vegetable oils, e.g., palm oil		Cocoa butter substitute
Nitrile hydratase (immobilized cells)	Acrylonitrile		Acrylamide
Penicillin amylases	Penicillin G and penicillin V	STR, PBR	Penicillins
Raffinase (immobilized cells)	Raffinose in beet juice, soybean milk	STR	Raffinose-free solutions

*PBR, packed bed reactor; STR, stirred tank reactor.

High Fructose Corn Syrup- D-glucose is only 70% as sweet as sucrose and is comparatively less soluble in water. Fructose on the other hand is 30% more soluble than sucrose and is twice as soluble as glucose at lower temperatures.

Therefore, glucose syrup is treated with glucose isomerase to produce high fructose syrup; a 42% fructose syrup is good enough for many uses, but for soft drinks a 55% fructose syrup is required.

Glucose isomerase is obtained from Actinoplanes missouriensis, B. coagulans and Streptomyces spp. It is remarkably thermostable and acts at very high substrate concentrations. It is used in immobilized state obtained by cross linking with glutaraldehyde; in some cases, a protein diluent may be added. The process is continuous using a packed bed reactor.

Immobilized Invertase- Invertase was perhaps the first enzyme used during 1940s in an immobilized state; the enzyme obtained by autolysis of yeast cells was immobilized by adsorption onto bone char.

At present, the purified enzyme char mix is stabilized by cross linking; the enzyme has a half life of 90 days at 50°C, pH 5.5. The process is carried out in a continuous mode in a PBR, and 1 kg of granular enzyme produces 16 tons of inverted sugar.

Enzyme Carrier Systems - The choice of an immobilized enzyme system depends on the following two factors:

(a) Enzyme purification and stability

The manufacturers have to make a choice about the purity of enzyme the less purified enzyme requires a larger bulk of enzyme, while highly purified is more expensive. The higher purity may also avoid undesirable side reactions due to

(i) absence of other enzymes and due to the

(ii) low durations, for which the substrate has to be in contact with the enzyme.

Since long operating life or maximum yield of product per unit weight of immobilized enzyme is critical for its commercial success, stability of enzyme system is important. Highest total productivity is achieved at lowest temperature, although contamination and capital costs may be higher at low temperature.

The stability of the support material as well as the activity of enzyme are also influenced by pH, so that pH profile of enzyme system should also be known.

(b)Nature and composition of carrier system

The charge on the carrier also influences pH. For instance, if carrier is negatively charged, H+ ions will accumulate at its boundary leading to drop in pH and if positively charged, there will be increase in pH. Such changes in pH will reduce stability of the system and may be overcome by incorporation of the ions of interest into the carrier.

For example, magnesia incorporated into porous alumina support (developed by Corning) for glucose isomerase increased the observed activity of enzyme by more than 50%.

http://www.lsbu.ac.uk/biology/enztech/imeconom.html

Methods of immobilisation

There are four principal methods available for immobilising enzymes (Figure 3.1):

a. adsorption
b. covalent binding

c. entrapment
d. membrane confinement

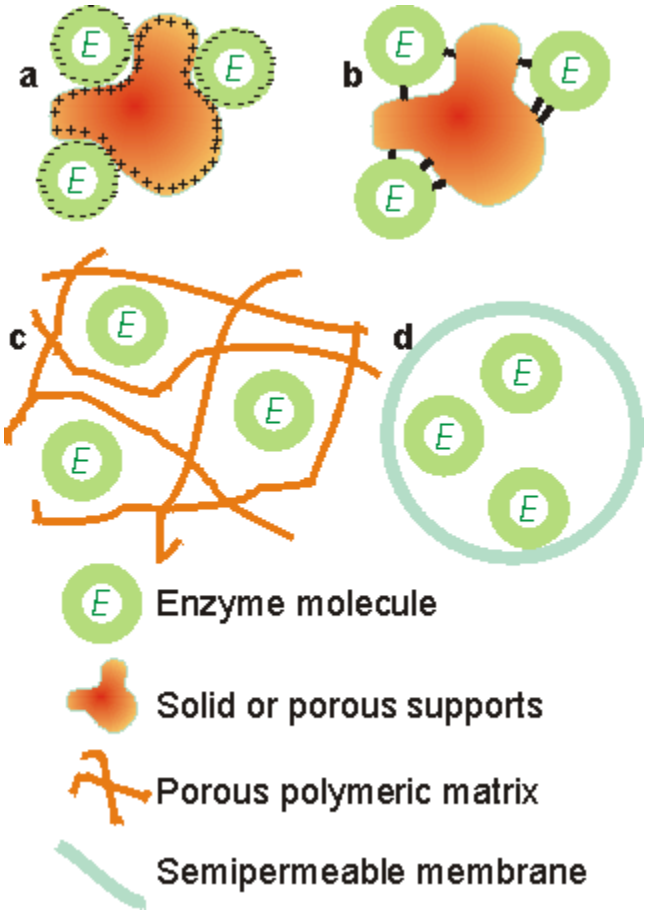


Figure 3.1. Immobilised enzyme systems. (a) enzyme non-covalently adsorbed to an insoluble particle; (b) enzyme covalently attached to an insoluble particle; (c) enzyme entrapped within an insoluble particle by a cross-linked polymer; (d) enzyme confined within a semipermeable membrane.

Carrier matrices for enzyme immobilisation by adsorption and covalent binding must be chosen with care. Of particular relevance to their use in industrial processes is their cost relative to the overall process costs; ideally they should be cheap enough to discard. The manufacture of high-valued products on a small scale may allow the use of relatively expensive supports and immobilisation techniques whereas these would not be economical in the large-scale production of low added-value materials. A substantial saving in costs occurs where the carrier may be regenerated after the useful lifetime of the immobilised enzyme. The surface density

of binding sites together with the volumetric surface area sterically available to the enzyme, determine the maximum binding capacity. The actual capacity will be affected by the number of potential coupling sites in the enzyme molecules and the electrostatic charge distribution and surface polarity (i.e. the hydrophobic-hydrophilic balance) on both the enzyme and support. The nature of the support will also have a considerable affect on an enzyme's expressed activity and apparent kinetics. The form, shape, density, porosity, pore size distribution, operational stability and particle size distribution of the supporting matrix will influence the reactor configuration in which the immobilised biocatalyst may be used. The ideal support is cheap, inert, physically strong and stable. It will increase the enzyme specificity (k_{cat}/K_m) whilst reducing product inhibition, shift the pH optimum to the desired value for the process, and discourage microbial growth and non-specific adsorption. Some matrices possess other properties which are useful for particular purposes such as ferromagnetism (e.g. magnetic iron oxide, enabling transfer of the biocatalyst by means of magnetic fields), a catalytic surface (e.g. manganese dioxide, which catalytically removes the inactivating hydrogen peroxide produced by most oxidases), or a reductive surface environment (e.g. titania, for enzymes inactivated by oxidation). Clearly most supports possess only some of these features, but a thorough understanding of the properties of immobilised enzymes does allow suitable engineering of the system to approach these optimal qualities.

Adsorption of enzymes onto insoluble supports is a very simple method of wide applicability and capable of high enzyme loading (about one gram per gram of matrix). Simply mixing the enzyme with a suitable adsorbent, under appropriate conditions of pH and ionic strength, followed, after a sufficient incubation period, by washing off loosely bound and unbound enzyme will produce the immobilised enzyme in a directly usable form (Figure 3.2). The driving force causing this binding is usually due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule. The particular choice of adsorbent depends principally upon minimising leakage of the enzyme during use. Although the physical links between the enzyme molecules and the support are often very strong, they may be reduced by many factors including the introduction of the substrate. Care must be taken that the binding forces are not weakened during use by inappropriate changes in pH or ionic strength. Examples of suitable adsorbents are ion-exchange matrices (Table 3.1), porous carbon, clays, hydrous metal oxides, glasses and polymeric aromatic resins. Ion-exchange matrices, although more expensive than these other supports, may be used economically due to the ease with which they may be regenerated when their bound enzyme has come to the end of its active life; a process which may simply involve washing off the used enzyme with concentrated salt solutions and re-suspending the ion exchanger in a solution of active enzyme.

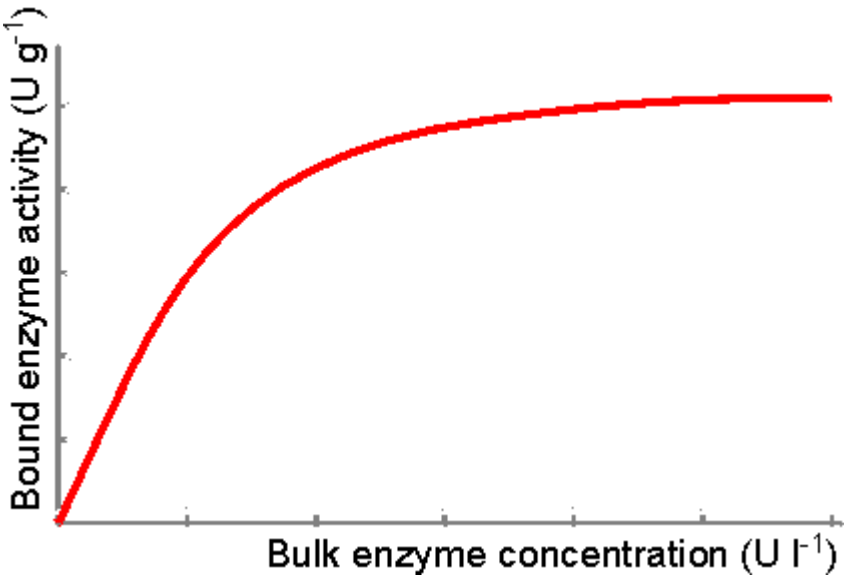


Figure 3.2. Schematic diagram showing the effect of soluble enzyme concentration on the activity of enzyme immobilised by adsorption to a suitable matrix. The amount adsorbed depends on the incubation time, pH, ionic strength, surface area, porosity, and the physical characteristics of both the enzyme and the support.

Table 3.1 Preparation of immobilised invertase by adsorption (Woodward 1985)

	Support type	
% bound at	**DEAE-Sephadex anion exchanger**	**CM-Sephadex cation exchanger**
pH 2.5	0	100
pH 4.7	100	75
pH 7.0	100	34

Immobilisation of enzymes by their covalent coupling to insoluble matrices is an extensively researched technique. Only small amounts of enzymes may be immobilised by this method (about 0.02 gram per gram of matrix) although in exceptional cases as much as 0.3 gram per gram of matrix has been reported. The strength of binding is very strong, however, and very little leakage of enzyme from the support occurs. The relative usefulness of various groups, found in enzymes, for covalent link formation depends upon their availability and reactivity (nucleophilicity), in addition to the stability of the covalent link, once formed (Table 3.2). The reactivity of the protein side-chain nucleophiles is determined by their state of protonation (i.e. charged status) and roughly follows the relationship $-S^-$ > -SH > $-O^-$ > $-NH_2$ > $-COO^-$ > -OH >> $-NH_3^+$where the charges may be estimated from a

knowledge of the pK_a values of the ionising groups (Table 1.1) and the pH of the solution. Lysine residues are found to be the most generally useful groups for covalent bonding of enzymes to insoluble supports due to their widespread surface exposure and high reactivity, especially in slightly alkaline solutions. They also appear to be only very rarely involved in the active sites of enzymes.

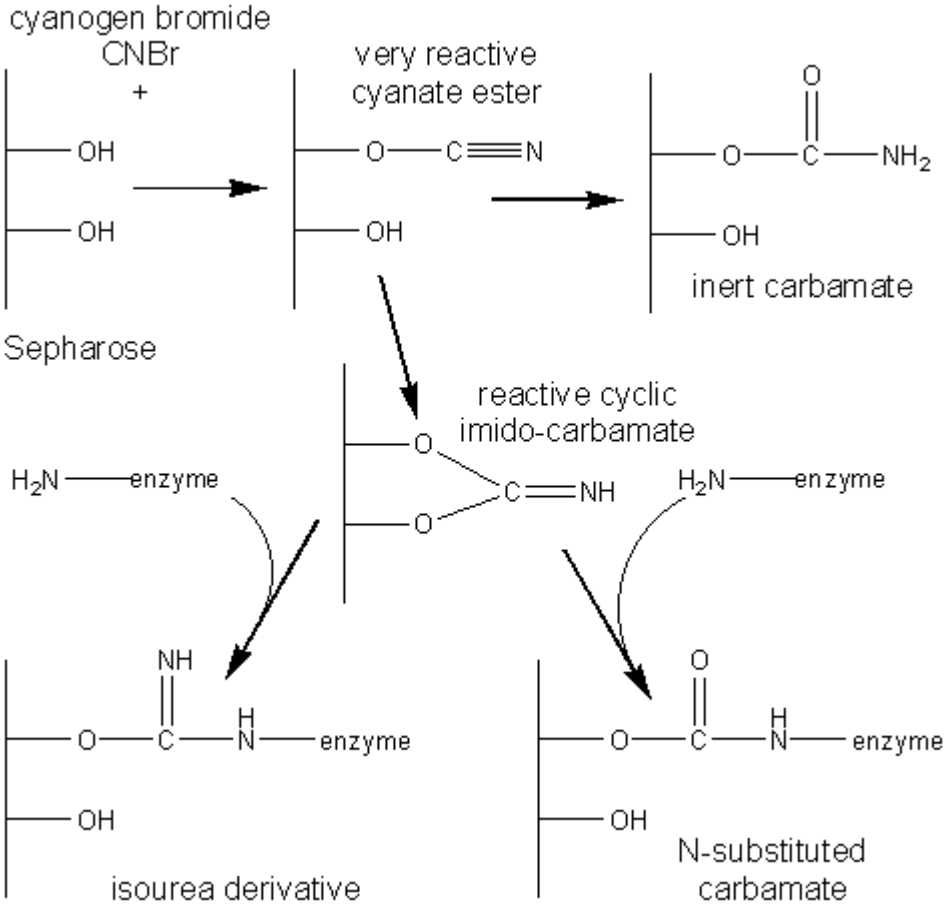
Table 3.2 Relative usefulness of enzyme residues for covalent coupling

Residue	Content	Exposure	Reactivity	Stability of couple	Use
Aspartate	+	++	+	+	+
Arginine	+	++	-	±	-
Cysteine	-	±	++	-	-
Cystine	+	-	±	±	-
Glutamate	+	++	+	+	+
Histidine	±	++	+	+	+
Lysine	++	++	++	++	++
Methionine	-	-	±	-	-
Serine	++	+	±	+	±
Threonine	++	±	±	+	±
Tryptophan	-	-	-	±	-
Tyrosine	+	-	+	_+	+
C terminus	-	++	+	+	+
N terminus	-	++	++	++	+
Carbohydrate	- ~ ++	++	+	+	±
Others	- ~ ++	-	-	- ~ ++	-

The most commonly used method for immobilising enzymes on the research scale (i.e. using less than a gram of enzyme) involves Sepharose, activated by cyanogen bromide. This is a simple, mild and often successful method of wide applicability. Sepharose is a commercially available beaded polymer which is highly hydrophilic and generally inert to microbiological attack. Chemically it is an agarose (poly-{□-1,3-D-galactose-□-1,4-(3,6-anhydro)-L-galactose}) gel. The hydroxyl groups of this polysaccharide combine with cyanogen bromide to give the reactive cyclic imido-carbonate. This reacts with primary amino groups (i.e. mainly lysine residues) on the enzyme under mildly basic conditions (pH 9 - 11.5, Figure 3.3a). The high toxicity of cyanogen bromide has led to the commercial, if rather expensive,

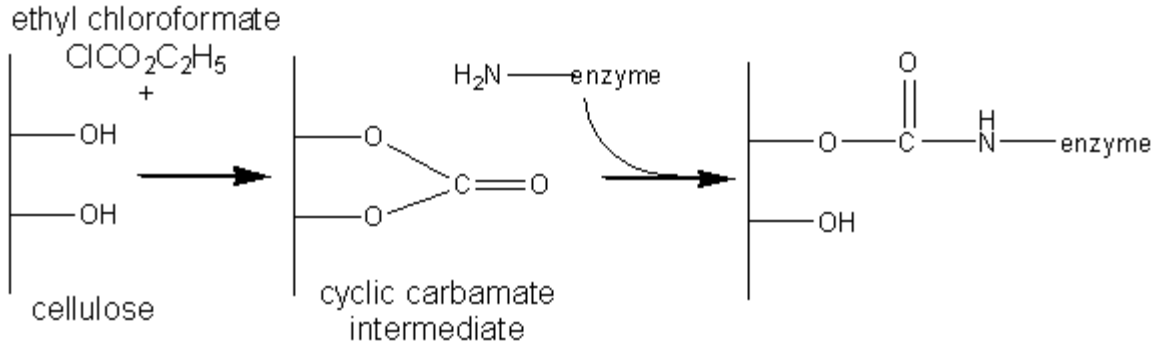
production of ready-activated Sepharose and the investigation of alternative methods, often involving chloroformates, to produce similar intermediates (Figure 3.3b). Carbodiimides (Figure 3.3c) are very useful bifunctional reagents as they allow the coupling of amines to carboxylic acids. Careful control of the reaction conditions and choice of carbodiimide allow a great degree of selectivity in this reaction. Glutaraldehyde is another bifunctional reagent which may be used to cross-link enzymes or link them to supports (Figure 3.3d). It is particularly useful for producing immobilised enzyme membranes, for use in biosensors, by cross-linking the enzyme plus a non-catalytic diluent protein within a porous sheet (e.g. lens tissue paper or nylon net fabric). The use of trialkoxysilanes allows even such apparently inert materials as glass to be coupled to enzymes (Figure 3.3e). There are numerous other methods available for the covalent attachment of enzymes (e.g. the attachment of tyrosine groups through diazo-linkages, and lysine groups through amide formation with acyl chlorides or anhydrides).

(a) cyanogen bromide



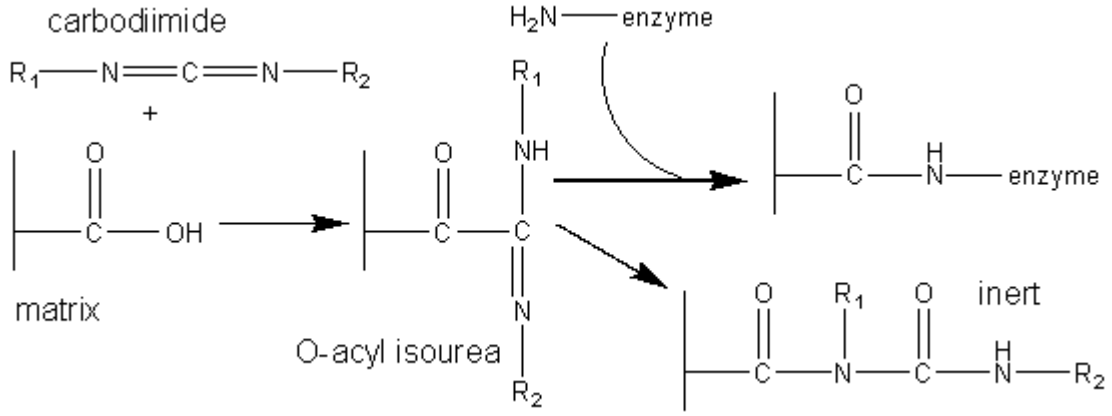
[3.1]

(b) ethyl chloroformate



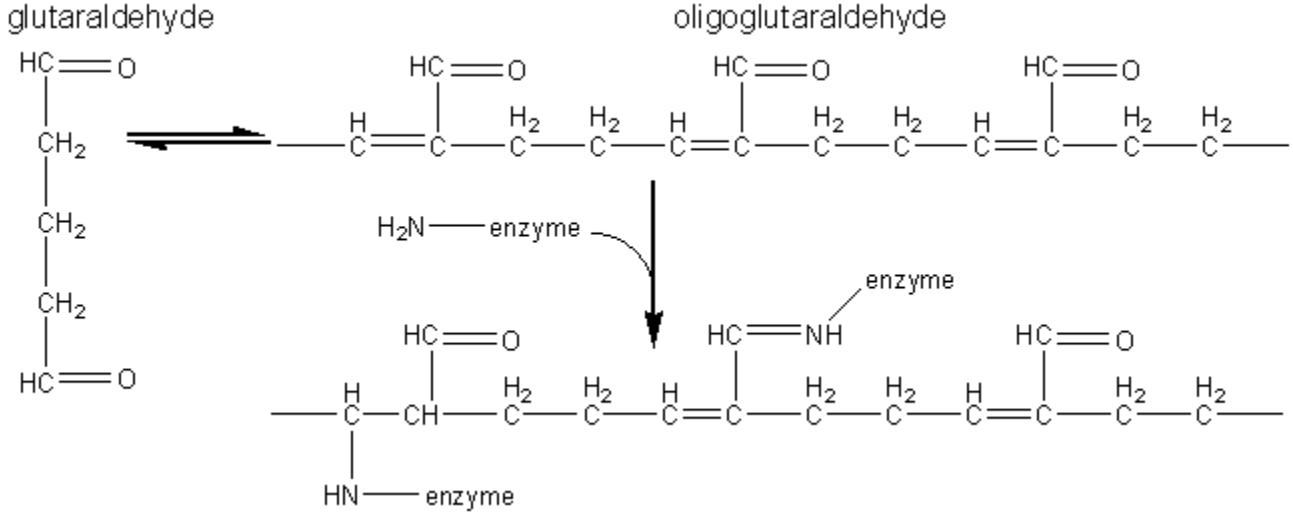
[3.2]

(c) carbodiimide



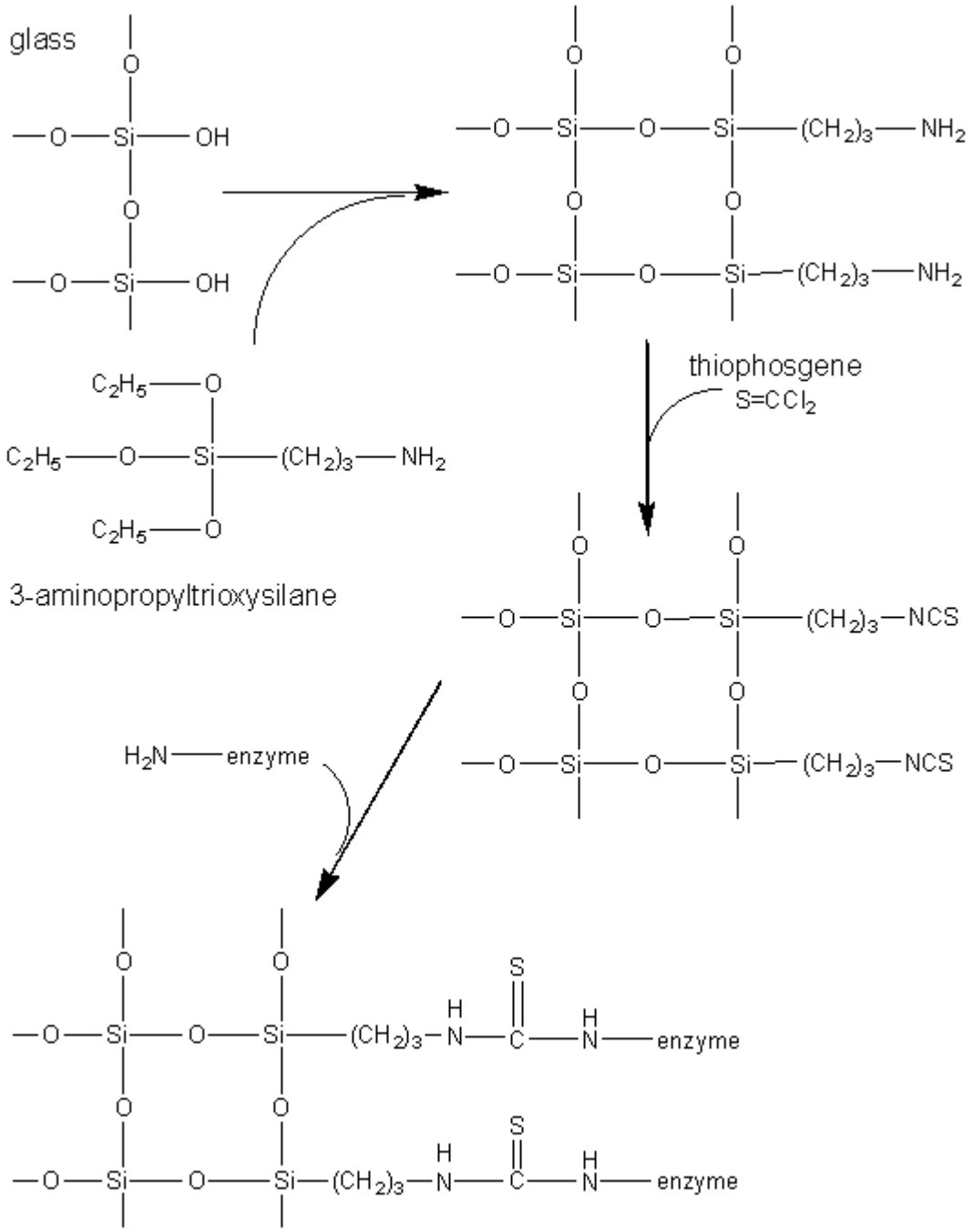
[3.3]

(d) glutaraldehyde



[3.4]

(e) 3-aminopropyltriethoxysilane



[3.5]

Figure 3.3. Commonly used methods for the covalent immobilisation of enzymes. (a) Activation of Sepharose by cyanogen bromide. Conditions are chosen to minimise the formation of the inert carbamate. (b) Chloroformates may be used to produce similar intermediates to those produced by cyanogen bromide but without its inherent toxicity. (c) Carbodiimides may be used to attach amino groups on the

enzyme to carboxylate groups on the support or carboxylate groups on the enzyme to amino groups on the support. Conditions are chosen to minimise the formation of the inert substituted urea. (d) Glutaraldehyde is used to cross-link enzymes or link them to supports. It usually consists of an equilibrium mixture of monomer and oligomers. The product of the condensation of enzyme and glutaraldehyde may be stabilised against dissociation by reduction with sodium borohydride. (e) The use of trialkoxysilane to derivatise glass. The reactive glass may be linked to enzymes by a number of methods including the use thiophosgene, as shown.

It is clearly important that the immobilised enzyme retains as much catalytic activity as possible after reaction. This can, in part, be ensured by reducing the amount of enzyme bound in non-catalytic conformations (Figure 3.4). Immobilisation of the enzyme in the presence of saturating concentrations of substrate, product or a competitive inhibitor ensures that the active site remains unreacted during the covalent coupling and reduces the occurrence of binding in unproductive conformations. The activity of the immobilised enzyme is then simply restored by washing the immobilised enzyme to remove these molecules.

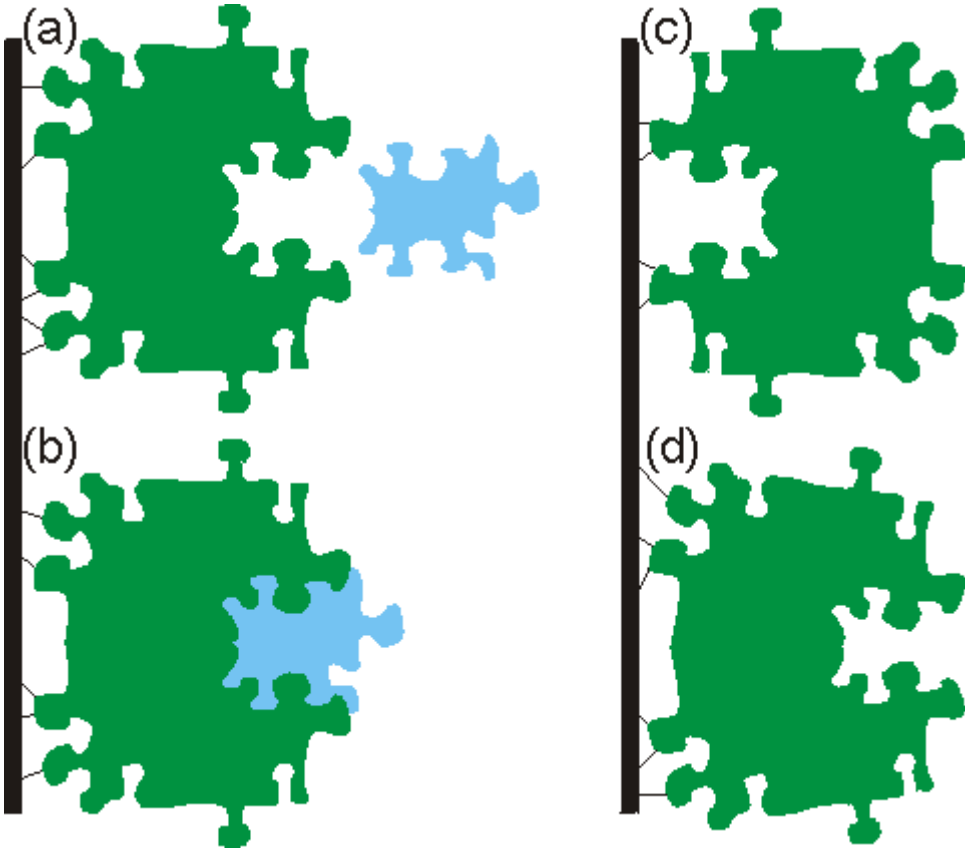


Figure 3.4. The effect of covalent coupling on the expressed activity of an immobilised enzyme. (a) Immobilised enzyme (E) with its active site unchanged and ready to accept the substrate molecule (S), as shown in (b). (c) Enzyme bound in a non-productive mode due to the inaccessibility of the active site. (d) Distortion of the active site produces an inactive immobilised enzyme. Non-productive modes are best prevented by the use of large molecules reversibly bound in or near the active site. Distortion can be prevented by use of molecules which can sit in the active site during the coupling process, or by the use of a freely reversible method for the coupling which encourages binding to the most energetically stable (i.e. native) form of the enzyme. Both (c) and (d) may be reduced by use of 'spacer' groups between the enzyme and support, effectively displacing the enzyme away from the steric influence of the surface.

Entrapment of enzymes within gels or fibres is a convenient method for use in processes involving low molecular weight substrates and products. Amounts in excess of 1 g of enzyme per gram of gel or fibre may be entrapped. However, the difficulty which large molecules have in approaching the catalytic sites of entrapped enzymes precludes the use of entrapped enzymes with high molecular weight substrates. The entrapment process may be a purely physical caging or involve covalent binding. As an example of this latter method, the enzymes' surface lysine residues may be derivatised by reaction with acryloyl chloride (CH_2=CH-CO-Cl) to give the acryloyl amides. This product may then be copolymerised and cross-linked with acrylamide (CH_2=CH-CO-NH_2) and bisacrylamide (H_2N-CO-CH=CH-CH=CH-CO-NH_2) to form a gel. Enzymes may be entrapped in cellulose acetate fibres by, for example, making up an emulsion of the enzyme plus cellulose acetate in methylene chloride, followed by extrusion through a spinneret into a solution of an aqueous precipitant. Entrapment is the method of choice for the immobilisation of microbial, animal and plant cells, where calcium alginate is widely used.

Membrane confinement of enzymes may be achieved by a number of quite different methods, all of which depend for their utility on the semipermeable nature of the membrane. This must confine the enzyme whilst allowing free passage for the reaction products and, in most configurations, the substrates. The simplest of these methods is achieved by placing the enzyme on one side of the semipermeable membrane whilst the reactant and product stream is present on the other side. Hollow fibre membrane units are available commercially with large surface areas relative to their contained volumes (> 20 $m^2\ l^{-1}$) and permeable only to substances of molecular weight substantially less than the enzymes. Although costly, these are very easy to use for a wide variety of enzymes (including regenerating coenzyme systems, see Chapter 8) without the additional research and development costs associated with other immobilisation methods. Enzymes, encapsulated within small membrane-bound droplets or liposomes (see Chapter 7), may also be used within such reactors. As an example of the former, the enzyme is dissolved in an aqueous

solution of 1,6-diaminohexane. This is then dispersed in a solution of hexanedioic acid in the immiscible solvent, chloroform. The resultant reaction forms a thin polymeric (Nylon-6,6) shell around the aqueous droplets which traps the enzyme. Liposomes are concentric spheres of lipid membranes, surrounding the soluble enzyme. They are formed by the addition of phospholipid to enzyme solutions. The micro-capsules and liposomes are washed free of non-confined enzyme and transferred back to aqueous solution before use.

Table 3.3 presents a comparison of the more important general characteristics of these methods.

Table 3.3 Generalised comparison of different enzyme immobilisation techniques.

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

Isolation and Purification - Isolation and purification is done immediately after termination of fermentation in a manner that retains the enzyme activity. If the cells are to be used for immobilization, the biomass is isolated and treated to make it ready for use.

The extracellular enzymes are recovered directly from broth, while enzymes localized within cells are isolated by rupturing the cells. Enzyme purification is based on various techniques whose efficacy and cost differ widely; the process used will mainly depend on the purity needed and the cost, which is acceptable.

α - Amylase - This enzyme is an endo hydrolase; it hydrolyzes starch into components, which have three or more linear α -1, 4-gulcan units. It stops hydrolysis when fragments with 2-6 glucose units remain; typically, such fragments contain an α -l,4-6 linked branch point residue.

The end products of starch hydrolysis are dextrins, which are used as adhesives and thickening agents in prepared foods. α -Amylase must be used at high temperatures. The enzyme from Bacillus licheniformis can be used for prolonged periods at 95°C and for a brief period at 105-110°C.

The bacteria are grown on complex media based on maize or potato starch supplemented with soybean meal or corn steep liquor (medium has 20% dry matter). The fermentation is carried out for about 5 days, then the broth is chilled and the cells and solids are removed by flocculation.

The enzyme is extracellular, and is recovered from the broth; it is always stabilized with Ca^{2+} ions. Its applications are: dextrin production, first stage in glucose manufacture, in brewing and bakery, for removal of starch in textile manufacture, etc.

Amyloglucosidase - Also called glucoamylase, this enzyme is an exohydrolase, and removes terminal glucose residues, one-by-one, from dextrins. This is produced by fungi, such as, Aspergillus or Rhizopus. The strains used for enzyme production are regulatory mutants (enzyme synthesis not repressed by free glucose), which are grown on α-amylase digested starch (20% w/v) medium.

The fermentation lasts for 4-5 days at pH 4.5, and is N limited. The enzyme is extracellular and is concentrated to about 5% active enzyme. The dextrins obtained by α-amylase digestion of starch are further digested to glucose by glucoamylase.

Glucose Isomerase - Commercial glucose isomerase is, in fact, D-xylose ketol-isomerase, and is produced by several bacteria (Table 10.10). Some improved strains produce the enzyme constitutively. The bacteria are grown in aerated batch cultures at 30°C and 7.0 pH for 2-3 days.

The enzyme is intracellular, and is best used in the form of immobilized cells. The cells are suitably treated to increase their stability and catalytic activity.

Purified glucose syrup is heated to remove dissolved O2 and increase glucose concentration to 40%. pH is adjusted between 7 and 8. The syrup is passed through a column containing immobilized bacterial cells with glucose isomerase activity; the temperature is kept at 60°C.

The enzyme longevity, under practical conditions, is 2,000-4,000 hr optimally; 20 tons or more product can be processed per kg of the catalyst. The end product of glucose isomerase action is a nearly 1 : 1 mixture of glucose and fructose; this has increased sweetness as compared to glucose and is virtually identical with 'invert sugar obtained from beet or cane sugar (sucrose).

Enzyme Biotechnology - Enzymes are biological catalysts, which initiate and accelerate thousands of biochemical reactions in living cells.

They process reactions which are otherwise not possible under normal conditions found in the cell. For instance, although, hydrolysis of starch in a test tube requires strong acidic medium and high temperature (boiling), in the alimentary canal it is hydrolysed and digested under normal conditions of acidity and temperature.

This is made possible by starch hydrolysing enzymes available in the stomach. In fact, almost all biochemical reactions require one or more enzymes for their completion. Non enzymatic conversions, though are known, but are very few.

Enzymes are proteinaceous in nature; they can be extracted from living tissues, purified and even crystallized. Under controlled conditions of isolation, they retain their original level of activity and in some cases even exhibit an increased activity. Thus, a purified enzyme can be used to carry on a specific biochemical reaction outside the cell.

Isolation and Purification of Enzymes - Enzymes are unstable molecules with a definite physico chemical organization. Even a slight change in this organization reduces the activity of enzyme and sometimes the enzyme is totally inactivated.

Therefore, the enzymes have to be isolated under controlled conditions of pH, ionic strength and temperature.

Since they are proteinaceous in nature, standard extraction and purification procedures for enzymes are the same as those used for proteins except that the activity of the enzyme is assayed at each of the following four steps of extraction and purification.

Preparation of Crude Enzymes - Centrifugation

The enzyme extract is centrifuged to remove cell debris, cell organelles and sometimes other molecular aggregates, leading to partial purification of enzymes.

It also helps in characterization of an enzyme, since, depending upon its mass and shape the enzyme will move through a solution at a definite speed and occupy a characteristic position in the centrifuge tube.

For most cytosolic enzymes, centrifugation at about 30,000 g for 30 minutes is good enough to obtain a fair amount of activity in the supernatant.

However, if the enzyme is located in a specific cell organelle, an extract rich in that organelle is prepared through 'preparative centrifugation'. (Centrifugation for different durations at different velocities allows the cell organelles to sediment according to their sizes. All centrifugation operations are conducted in cold (O-4°C).

Precipitation

Enzymes and other proteins are highly charged molecules, and can be precipitated with appropriate charge neutralizing chemicals. Once their charges are broken, they form aggregates and settle down as precipitate.

When, an acid or base is added, the enzyme protein can be brought to its isoelectric pH. At this pH, there is no net charge on enzyme molecules and electrostatic repulsion between them is low so that they tend to aggregate. Therefore, adjusting the pH to the isoelectric point of a protein causes its precipitation.

Acids and bases, however, often inactivate the enzyme, so that their use for precipitation is not recommended in most cases. Instead ammonium sulphate and other salts are used for precipitation in a process called 'salting out'. Salts can change the structure of the solvent, which can lead to large changes in protein conformation by altering the electrostatic interactions between charged groups on the protein surface.

The salt also competes with the protein for solvent molecules and thereby lowers its solvation. In large scale enzyme precipitation, use of many other neutral salts is preferred over ammonium sulphate, which is corrosive and releases NH3 at higher temperatures.

Some organic solvents like acetone, methanol and ethanol are also used for enzyme precipitation, since water miscible solvents decrease the solubility of proteins, leading to precipitation.

They are cooled upto 40 60°C before their use, and precipitation is carried out at O°C, because precipitation at room temperature causes denaturation of the enzyme, in most cases. Organic solvents are added drop by drop to avoid local concentration.

Water soluble non ionic polymers such as polyethylene glycol, alginate, pectate, carboxymethyl cellulose, polyacrylic and polymeta acrylic acids, etc. also cause enzyme precipitation.

Polyethyleneimine is also widely used as protein precipitant at large scale. They primarily act through the removal of solvent sphere of the enzyme protein.

Extraction of Enzymes - Fresh tissue is crushed into a paste with an extraction medium (often a buffer) in a mortar and pestle, or in a tissue homogenizer, or in a blender or by ultrasonic vibrations (sonication).

The molarity and pH of the buffer is suitably adjusted (which may vary for different enzymes) to achieve maximum solubility and activity of the enzyme. EDTA (ethylene diamine tetra acetic acid) is often included in the extraction medium to remove heavy metals (which otherwise inhibit enzyme activity), and for disrupting the membranes of cells and cell organelles. Detergents such as Triton-X are also used sometimes to solubilise the membranes.

Many enzyme proteins contain disulfide (S-S) bonds due to the presence of cysteine residues, which are easily broken during enzyme extraction leading to loss of enzyme activity. To overcome this problem are added, thiols such as mercaptoethanol whose sulfhydryl (-SH) group is able to maintain the S-S linkage in enzymes.

If the extract is not homogeneous, the homogenate (extract) is filtered to remove cell derbis, fibres etc., otherwise filtration may be avoided. All operations of extraction and purification are generally carried out in cold (O-4°C), since most of the enzymes get inactivated at higher temperatures.

Purification of Enzymes - Enzyme purification involves three steps, electrophoresis. These three techniques described in the following text
1. Dialysis

2. Chromatography.

Dialysis of Enzymes - Dialysis is the process that is used to remove small molecules from enzyme. For this, enzyme precipitate obtained in previous step is dissolved in a small quantity of buffer solution in which the enzyme was originally extracted. The solution is taken in a dialysis bag (may be a cellophane tube) and after sealing securely, the bag is suspended in either distilled water or a buffer of known molarity and ionic composition.

Some other salts or chemicals may have to be added sometimes in the outer solution, to prevent the loss of enzyme activity during dialysis. The dialysis is carried out for a few hours with regular change of the outer solution or distilled water. At large scale enzyme purification 'dialafitration' instead of dialysis is used.

The enzyme solution is filtered .for small molecules through a membrane generally mounted on a fibrous support, by pressure dirven operations.

Chromatography for Enzyme Purification - Chromatographic separation of proteins is the most common method of enzyme purification.

Following four types of chromatography are available for this purpose:

(i) adsorption or column chromatography;

(ii) ion exchange chromatography;

(iii) gel filtration chromatography and

(iv)affinity chromatography.

Adsorption Chromatography for Enzyme Purification - In adsorption chromatography, the protein or enzyme solution suspected to contain other proteinaceous impurities is passed through a column of inert material packed in a glass or steel tube. Most commonly used column materials include finely divided solids such as charcoal, silica, alumina, calcium phosphate, hydroxyapatite, etc.

The effluent solution is continuously collected in small fractions of 1.0 to 2.0 mi. The protein in each fraction is estimated by measuring the absorption at 280 nm using a UV spectrophotometer. The enzyme is also assayed in each fraction. Various spleen enzymes such as basic RNAase, acidic RNAase, acidic DNAse, phosphodiesterase, phos phomonoesterase, etc. are often separated from each other using adsorption chromatography.

For large scale chromatographic separation of enzymes, the process is accelerated by using motors and other mechanical devices for packing the column, for loading the enzyme on the column and for eluting the enzyme.

Ion Exchange Chromatography for Enzyme Purification - In ion exchange chromatography, generally a cellulosic ion exchange is taken in the column. The proteins are separated according to their charges.

The resolution is quite high and the technique can facilitate large scale protein purification. This has been successfully employed for insulin purification, plasma fractionation and for purification of many other enzymes.

Gel Filtration Chromatography for Enzyme Purification - In this chromatography, various proteins are separated on the basis of differences in their molecular sizes. This type of chromatography is also known as molecular exclusion chromatography or molecular sieve chromatography.

The basic arrangement for gel filtration chromatography is similar to that for adsorption chromatography. A column made up of glass or steel is taken and packed with a gel.

The most commonly used gel is 'sephadex' which is a cross linked dextran produced by certain strains of bacteria. Several types of sephadex, namely G-10, G-30, G-50, G-100, G-150, G-200, etc. are available, which differ according to their pore sizes.

When a mixture of enzymes or proteins is poured on top of the column, different proteins move downwards according to their molecular sizes and come out from the column in order of decreasing sizes; the, larger molecules are eluted first. The elution volume is logarithmically proportional to the molecular size.

Gel filtration chromatography can also be used for determining the molecular weight of the protein by calibrating the column with proteins of known molecular weights.

Affinity Chromatography for Enzyme Purification - In this method, enzymes are purified according to their specificity for a particular substrate or cofactor.

One component of the mixture containing enzyme binds covalently to the solid support of the column, and the other components percolate down through the column. The basic requirements for affinity chromatography are the same as in adsorption or gel filtration chromatography but the packing gel must have some component which can bind with one component of the mixture.

Many commercial gels available for affinity chromatography contain functional groups attached to the 'spacer arms' of the gel. The "spacer arm" is a chemical linkage between the functional group and the gel or matrix proper, so that the binding between functional group and the enzyme is kept away from the gel.

Thus the steric hindrance will be unlikely to prevent binding of the specific enzyme to the column.

Electrophoesis for Enzyme Purification -Electrophoresis is a technique in which molecules (enzymes, proteins, amino acids, nucleotides and nucleic acids) are

separated by differences in their net charge in the presence of an externally applied electric field.

This technique is routinely used in enzyme purification and isozymes separation in the laboratories, although it has found only limited application at large scale, since the technique is time consuming and is a bit expensive.

Various types of instrumental approaches have been used to separate and purify charged molecules using electrophoresis. However, the most common method for purifying enzymes is through electrophoresis on polyacrylamide gel.

Polyacrylamide is a polymer of acrylamide and methylene bisacarylamide and when prepared as a gel it is transparent, thermostable, non-ionic and extremely regular in structure.

The gel may be taken either in the form of a column or a slab, although the latter is preferred over the former. The protein mixture is loaded in the gel and the components are separated under a direct current of constant voltage. The migration rate of the various components of the mixture is dependent upon their charge and molecular weight.

A variation of the above polyacrylamide gel electrophoresis is the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which is used to determine the molecular weight of proteins. In this method, the separation is caused by the seiving action of the gel.

The proteins migrate through the gel depending on their shapes and mass to charge ratio. Gel electrophoresis is also used to separate various isozymes of a given enzyme. Isozymes perform the same catalytic function but differ in their regulatory and some kinetic aspects.

Final Step in Processing Enzymes - Most of the commercially available enzyme preparations, purified as above, are concentrated and sterile filtered, after purification. This is done to reduce both, the volume and the microbial contamination of the sample.

Often, before storage and transport, the sample is freeze dried with additives such as sugar substrates and dextrans.

http://www.lsbu.ac.uk/biology/enztech/sources.html

Sources of enzymes

Biologically active enzymes may be extracted from any living organism. A very wide range of sources are used for commercial enzyme production from *Actinoplanes* to *Zymomonas*, from spinach to snake venom. Of the hundred or so enzymes being used industrially, over a half are from fungi and yeast and over a third are from bacteria with the remainder divided between animal (8%) and plant (4%) sources (Table 2.1). A very much larger number of enzymes find use in chemical analysis and clinical diagnosis. Non-microbial sources provide a larger proportion of these, at the present time. Microbes are preferred to plants and animals as sources of enzymes because:

1. they are generally cheaper to produce.
2. their enzyme contents are more predictable and controllable,
3. reliable supplies of raw material of constant composition are more easily arranged, and
4. plant and animal tissues contain more potentially harmful materials than microbes, including phenolic compounds (from plants), endogenous enzyme inhibitors and proteases.

Attempts are being made to overcome some of these difficulties by the use of animal and plant cell culture.

Table 2.1. Some important industrial enzymes and their sources.

Enzyme[a]	EC number[b]	Source	Intra/extra-cellular[c]	Scale of production[d]	Industrial use
Animal enzymes					
Catalase	1.11.1.6	Liver	I	-	Food
Chymotrypsin	3.4.21.1	Pancreas	E	-	Leather
Lipase[e]	3.1.1.3	Pancreas	E	-	Food

Rennet[f]	3.4.23.4	Abomasum	E	+	Cheese
Trypsin	3.4.21.4	Pancreas	E	-	Leather
Plant enzymes					
Actinidin	3.4.22.14	Kiwi fruit	E	-	Food
□-Amylase	3.2.1.1	Malted barley	E	+++	Brewing
□-Amylase	3.2.1.2	Malted barley	E	+++	Brewing
Bromelain	3.4.22.4	Pineapple latex	E	-	Brewing
□-Glucanase[g]	3.2.1.6	Malted barley	E	++	Brewing
Ficin	3.4.22.3	Fig latex	E	-	Food
Lipoxygenase	1.13.11.12	Soybeans	I	-	Food
Papain	3.4.22.2	Pawpaw latex	E	++	Meat
Bacterial enzymes					
□-Amylase	3.2.1.1	*Bacillus*	E	+++	Starch
□-Amylase	3.2.1.2	*Bacillus*	E	+	Starch
Asparaginase	3.5.1.1	*Escherichia coli*	I	-	Health
Glucose isomerase[h]	5.3.1.5	*Bacillus*	I	++	Fructose syrup
Penicillin amidase	3.5.1.11	*Bacillus*	I	-	Pharmaceutical
Protease[i]	3.4.21.14	*Bacillus*	E	+++	Detergent
Pullulanase[i]	3.2.1.41	*Klebsiella*	E	-	Starch
Fungal enzymes					
□-Amylase	3.2.1.1	*Aspergillus*	E	++	Baking
Aminoacylase	3.5.1.14	*Aspergillus*	I	-	Pharmaceutical
Glucoamylase[k]	3.2.1.3	*Aspergillus*	E	+++	Starch
Catalase	1.11.1.6	*Aspergillus*	I	-	Food
Cellulase	3.2.1.4	*Trichoderma*	E	-	Waste
Dextranase	3.2.1.11	*Penicillium*	E	-	Food
Glucose oxidase	1.1.3.4	*Aspergillus*	I	-	Food
Lactase[l]	3.2.1.23	*Aspergillus*	E	-	Dairy
Lipase[e]	3.1.1.3	*Rhizopus*	E	-	Food
Rennet[m]	3.4.23.6	*Mucor miehei*	E	++	Cheese
Pectinase[n]	3.2.1.15	*Aspergillus*	E	++	Drinks
Pectin lyase	4.2.2.10	*Aspergillus*	E	-	Drinks

Protease[m]	3.4.23.6	*Aspergillus*	E	+	Baking
Raffinase[o]	3.2.1.22	*Mortierella*	I	-	Food
Yeast enzymes					
Invertase[p]	3.2.1.26	*Saccharomyces*	I/E	-	Confectionery
Lactase[l]	3.2.1.23	*Kluyveromyces*	I/E	-	Dairy
Lipase[e]	3.1.1.3	*Candida*	E	-	Food
Raffinase[o]	3.2.1.22	*Saccharomyces*	I	-	Food

[a] The names in common usage are given. As most industrial enzymes consist of mixtures of enzymes, these names may vary from the recommended names of their principal component. Where appropriate, the recommended names of this principal component is given below.
[b] The EC number of the principal component.
[c] I - intracellular enzyme; E - extracellular enzyme.
[d] +++ > 100 ton year^{-1}; ++ > 10 ton year^{-1}; + > 1 ton year^{-1}; - < 1 ton year^{-1}.
[e] triacylglycerol lipase;
[f] chymosin;
[g] Endo-1,3(4)-β-glucanase;
[h] xylose isomerase;
[i] subtilisin;
[j] α-dextrin endo-1,6-α-glucosidase;
[k] glucan 1,4-α-glucosidase;
[l] β-galactosidase;
[m] microbial aspartic proteinase;
[n] polygalacturonase;
[o] α-galactosidase;
[p] β-fructofuranosidase.

In practice, the great majority of microbial enzymes come from a very limited number of genera, of which *Aspergillus* species, *Bacillus* species and *Kluyveromyces* (also called *Saccharomyces*) species predominate. Most of the strains used have either been employed by the food industry for many years or have been derived from such strains by mutation and selection. There are very few examples of the industrial use of enzymes having been developed for one task. Shining examples of such developments are the production of high fructose syrup using glucose isomerase and the use of pullulanase in starch hydrolysis.

Producers of industrial enzymes and their customers will share the common aims of economy, effectiveness and safety. They will wish to have high-yielding strains of microbes which make the enzyme constitutively and secrete it into their growth medium (extracellular enzymes). If the enzyme is not produced constitutively, induction must be rapid and inexpensive. Producers will aim to use strains of microbe that are known to be generally safe. Users will pay little regard to the way

in which the enzyme is produced but will insist on having preparations that have a known activity and keep that activity for extended periods, stored at room temperature or with routine refrigeration. They will pay little attention to the purity of the enzyme preparation provided that it does not contain materials (enzymes or not) that interfere with their process. Both producers and users will wish to have the enzymes in forms that present minimal hazard to those handling them or consuming their product.

The development of commercial enzymes is a specialized business which is usually undertaken by a handful of companies which have high skills in

1. screening for new and improved enzymes,
2. fermentation for enzyme production,
3. large scale enzyme purifications,
4. formulation of enzymes for sale,
5. customer liaison, and
6. dealing with the regulatory authorities.

Screening for novel enzymes

If a reaction is thermodynamically possible, it is likely that an enzyme exists which is capable of catalysing it. One of the major skills of enzyme companies and suitably funded academic laboratories is the rapid and cost-effective screening of microbial cultures for enzyme activities. Natural samples, usually soil or compost material found near high concentrations of likely substrates, are used as sources of cultures. It is not unusual at international congresses of enzyme technologists to see representatives of enzyme companies collecting samples of soil to be screened later when they return to their laboratories.

The first stage of the screening procedure for commercial enzymes is to screen ideas, i.e. to determine the potential commercial need for a new enzyme, to estimate the size of the market and to decide, approximately, how much potential users of the enzyme will be able to afford to pay for it. In some cases, the determination of the potential value of an enzyme is not easy, for instance when it might be used to produce an entirely novel substance. In others, for instance when the novel enzyme would be used to improve an existing process, its potential value can be costed very accurately. In either case, a cumulative cash flow must be estimated, balancing the initial screening and investment capital costs including interest, tax liability and depreciation, against the expected long term profits. Full account must be taken of inflation, projected variation in feedstock price and source, publicity and other costs. In addition, the probability of potential market competition and changes in political or legal factors must be considered. Usually the sensitivity of the project to changes in all of these factors must be estimated, by informed guesswork, in order

to assess the risk factor involved. Financial re-appraisal must be frequently carried out during the development process to check that it still constitutes an efficient use of resources.

If agreement is reached, probably after discussions with potential users, that experimental work would be commercially justifiable, the next stage involves the location of a source of the required enzyme. Laboratory work is expensive in manpower so clearly it is worthwhile using all available databases to search for mention of the enzyme in the academic and patents literature. Cultures may then be sought from any sources so revealed. Some preparations of commercial enzymes are quite rich sources of enzymes other than the enzyme which is being offered for sale, revealing such preparations as potential inexpensive sources which are worth investigating.

If these first searches are unsuccessful, it is probably necessary to screen for new microbial strains capable of performing the transformation required. This should not be a 'blind' screen: there will usually be some source of microbes that could have been exposed for countless generations to the conditions that the new enzyme should withstand or to chemicals which it is required to modify. Hence, thermopiles are sought in hot springs, osmophiles in sugar factories, organisms capable of metabolizing wood preservatives in timber yards and so on. A classic example of the detection of an enzyme by intelligent screening was the discovery of a commercially useful cyanide-degrading enzyme in the microbial pathogens of plants that contain cyanogenic glycosides.

The identification of a microbial source of an enzyme is by no means the end of the story. The properties of the enzyme must be determined; i.e. temperature for optimum productivity, temperature stability profile, pH optimum and stability, kinetic constants (K_m, V_{max}), whether there is substrate or product inhibition, and the ability to withstand components of the expected feedstock other than substrate. A team of scientists, engineers and accountants must then consider the next steps. If any of these parameters is unsatisfactory, the screen must continue until improved enzymes are located. Now that protein engineering (see Chapter 8) can be seriously contemplated, an enzyme with sufficient potential value could be improved 'by design' to overcome one or two shortcomings. However, this would take a long time, at the present level of knowledge and skill, so further screening of microbes from selected sources would probably be considered more worthwhile.

Once an enzyme with suitable properties has been located, various decisions must be made concerning the acceptability of the organism to the regulatory authorities, the productivity of the organism, and the way in which the enzyme is to be isolated, utilised (free or immobilised) and, if necessary, purified. If the organism is unacceptable from a regulatory viewpoint two options exist; to eliminate that organism altogether and continue the screening operation, or to clone the enzyme into an acceptable organism. The latter approach is becoming increasingly attractive

especially as cloning could also be used to increase the productivity of the fermentation process. Cloning may also be attractive when the organism originally producing the enzyme is acceptable from the health and safety point of view but whose productivity is unacceptable (see Chapter 8). However, cloning is not yet routine and invariably successful so there is still an excellent case to be made for applying conventional mutation and isolation techniques for the selection of improved strains. It should be noted that although the technology for cloning glucose isomerase into 'routine' organisms is known, it has not yet been applied. Several of the glucose isomerase preparations used commercially consist of whole cells, or cell fragments, of the selected strains of species originally detected by screening.

The use of immobilised enzymes (see Chapter 3) is now familiar to industry and their advantages are well recognised so the practicality of using the new enzymes in an immobilised form will be determined early in the screening procedure. If the enzyme is produced intracellularly, the feasibility of using it without isolation and purification will be considered very seriously and strains selected for their amenability to use in this way.

It should be emphasised that there will be a constant dialogue between laboratory scientists and biochemical process engineers from the earliest stages of the screening process. Once the biochemical engineers are satisfied that their initial criteria of productivity, activity and stability can be met, the selected strain(s) of microbe will be grown in pilot plant conditions. It is only by applying the type of equipment used in full scale plants that accurate costing of processes can be achieved. Pilot studies will probably reveal imperfections, or at least areas of ignorance, that must be corrected at the laboratory scale. If this proves possible, the pilot plant will produce samples of the enzyme preparation to be used by customers who may well also be at the pilot plant stage in the development of the enzyme-utilizing process. The enzyme pilot plant also produces samples for safety and toxicological studies provided that the pilot process is exactly similar to the full scale operation.

Screening for new enzymes is expensive so that the intellectual property generated must be protected against copying by competitors. This is usually done by patenting the enzyme or its production method or, most usefully, the process in which it is to be used. Patenting will be initiated as soon as there is evidence that an innovative discovery has been made.

Media for enzyme production

Detailed description of the development and use of fermenters for the large-scale cultivation of microorganisms for enzyme production is outside the scope of this

volume but mention of media use is appropriate because this has a bearing on the cost of the enzyme and because media components often find their way into commercial enzyme preparations. Details of components used in industrial scale fermentation broths for enzyme production are not readily obtained. This is not unexpected as manufacturers have no wish to reveal information that may be of technical or commercial value to their competitors. Also some components of media may be changed from batch to batch as availability and cost of, for instance, carbohydrate feedstocks change. Such changes reveal themselves in often quite profound differences in appearance from batch to batch of a single enzyme from a single producer. The effects of changing feedstocks must be considered in relation to downstream processing. If such variability is likely to significantly reduce the efficiency of the standard methodology, it may be economical to use a more expensive defined medium of easily reproducible composition.

Clearly defined media are usually out of the question for large scale use on cost grounds but may be perfectly acceptable when enzymes are to be produced for high value uses, such as analysis or medical therapy where very pure preparations are essential. Less-defined complex media are composed of ingredients selected on the basis of cost and availability as well as composition. Waste materials and by-products from the food and agricultural industries are often major ingredients. Thus molasses, corn steep liquor, distillers solubles and wheat bran are important components of fermentation media providing carbohydrate, minerals, nitrogen and some vitamins. Extra carbohydrate is usually supplied as starch, sometimes refined but often simply as ground cereal grains. Soybean meal and ammonium salts are frequently used sources of additional nitrogen. Most of these materials will vary in quality and composition from batch to batch causing changes in enzyme productivity.

Preparation of enzymes

Readers of papers dealing with the preparation of enzymes for research purposes will be familiar with tables detailing the stages of purification. Often the enzyme may be purified several hundred-fold but the yield of the enzyme may be very poor, frequently below 10% of the activity of the original material (Table 2.2). In contrast, industrial enzymes will be purified as little as possible, only other enzymes and material likely to interfere with the process which the enzyme is to catalyse, will be removed. Unnecessary purification will be avoided as each additional stage is costly in terms of equipment, manpower and loss of enzyme activity. As a result, some commercial enzyme preparations consist essentially of concentrated fermentation broth, plus additives to stabilise the enzyme's activity.

Table 2.2. The effect of number of steps on the yield and costs in a typical enzyme purification process. The realistic assumptions are made that step yields are 75%, step purifications are three-fold and step costs are 10% of the initial costs (later purification steps are usually intrinsically more expensive but are necessarily of smaller scale).

Step	Relative weight	Yield (%)	Specific activity	Total cost	Cost per weight	Cost per activity
	1.000	100	1	1.00	1	1.00
1	0.250	75	3	1.10	4	1.47
2	0.063	56	9	1.20	19	2.13
3	0.016	42	27	1.30	83	3.08
4	0.004	32	81	1.40	358	4.92
5	0.001	24	243	1.50	1536	6.32

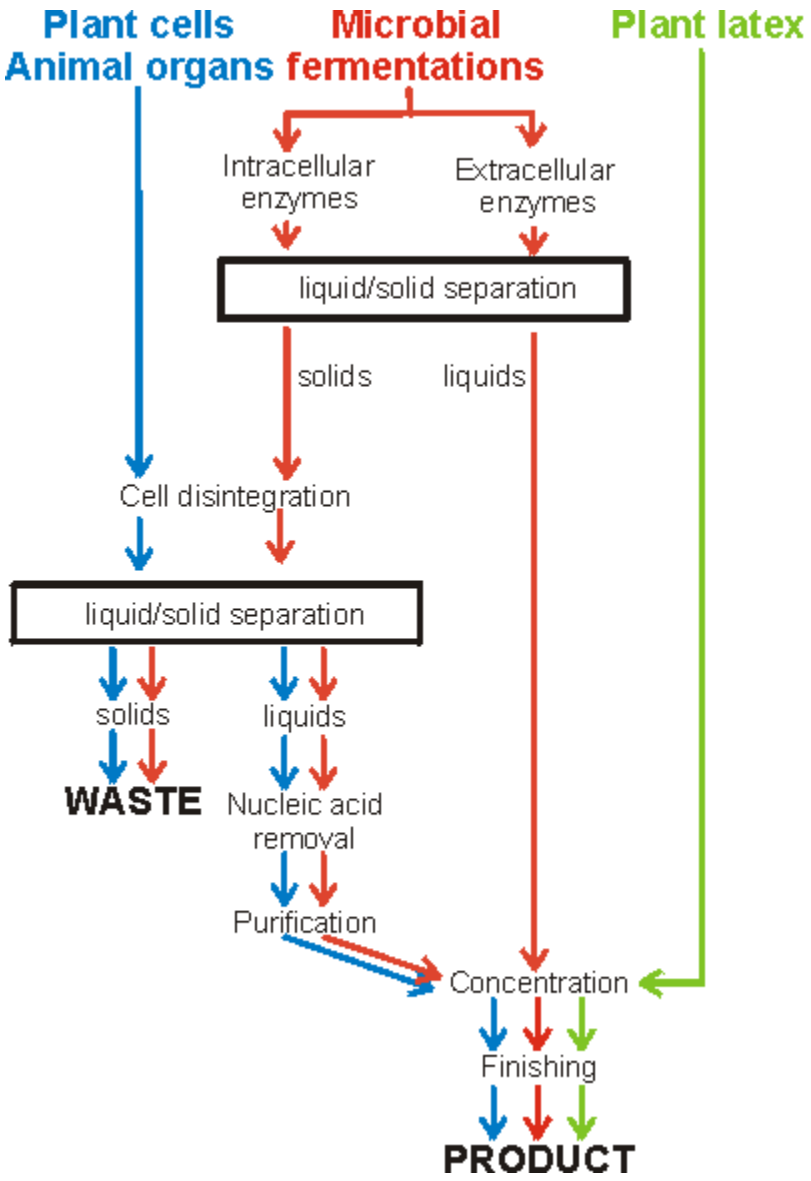
The content of the required enzyme should be as high as possible (e.g. 10% w/w of the protein) in order to ease the downstream processing task. This may be achieved by developing the fermentation conditions or, often more dramatically, by genetic engineering. It may well be economically viable to spend some time cloning extra copies of the required gene together with a powerful promoter back into the producing organism in order to get 'over-producers' (see Chapter 8).

It is important that the maximum activity is retained during the preparation of enzymes. Enzyme inactivation can be caused by heat, proteolysis, sub-optimal pH, oxidation, denaturants, irreversible inhibitors and loss of cofactors or coenzymes. Of these heat inactivation, which together with associated pH effects, is probably the most significant. It is likely to occur during enzyme extraction and purification if insufficient cooling is available (see Chapter 1), but the problem is less when preparing thermophilic enzymes. Proteolysis is most likely to occur in the early stages of extraction and purification when the proteases responsible for protein turnover in living cells are still present. It is also the major reason for enzyme inactivation by microbial contamination. In their native conformations, enzymes have highly structured domains which are resistant to attack by proteases because many of the peptide bonds are mechanically inaccessible and because many proteases are highly specific. The chances of a susceptible peptide bond in a structured domain being available for protease attack are low. Single 'nicks' by proteases in these circumstances may have little immediate effect on protein conformation and, therefore, activity. The effect, however, may severely reduce the conformational stability of the enzyme to heat or pH variation so greatly reducing its operational stability. If the domain is unfolded under these changed conditions, the whole polypeptide chain may be available for proteolysis and the same, specific, protease may destroy it. Clearly the best way of preventing proteolysis is to rapidly remove, or inhibit, protease activity. Before this can be achieved it is important to

keep enzyme preparations cold to maintain their native conformation and slow any protease action that may occur.

Some intracellular enzymes are used commercially without isolation and purification but the majority of commercial enzymes are either produced extracellularly by the microbe or plant or must be released from the cells into solution and further processed (Figure 2.1). Solid/liquid separation is generally required for the initial separation of cell mass, the removal of cell debris after cell breakage and the collection of precipitates. This can be achieved by filtration, centrifugation or aqueous biphasic partition. In general, filtration or aqueous biphasic systems are used to remove unwanted cells or cell debris whereas centrifugation is the preferred method for the collection of required solid material.

Figure 2.1. Flow diagram for the preparation of enzymes.


Plant cells
Animal organs
Microbial
fermentations
Plant latex
Intracellular
enzymes
Extracellular
enzymes
liquid/solid separation
solids
liquids
Cell disintegration
liquid/solid separation
solids
liquids
WASTE
Nucleic acid
removal
Purification
Concentration
Finishing
PRODUCT

http://www.molecular-plant-biotechnology.info/enzyme-technology/biosensors.htm

Biosensors- A biosensor is an analytical device, which employs a biological material to specifically interact with an analyte; this interaction produces some detectable physical change, which is measured and converted into an electrical signal by a transducer. Finally, the electrical signal is amplified, interpreted and displayed as analyte concentration in the solution/preparation.

An analyte is a compound whose concentration is to be determined, in this case, by the biosensor. The biological materials used are usually enzymes, but nucleic acids, antibodies, lectins; whole cells, entire organs or tissue slices are also used.

The nature of interaction between the analyte and the biological material used in the biosensor may be of two types:

(i) the analyte may be converted into a new chemical molecule (by enzymes; such biosensors are called catalytic biosensors), and

(ii) the analyte may simply bind to the biological material present on the biosensor (e.g., to antibodies, nucleic acids; these biosensors are known as affinity biosensors).

A successful biosensor must have at least some of the following features:

(i) it should be highly specific for the analyte,

(ii) the reaction used should be as independent of factors like stirring, pH, temperature, etc. as is manageable,

(iii) the response should be linear over a useful range of analyte concentrations,

(iv) the device should be tiny and biocompatible, in case it is to be used for analyses within the body,

(v) the device should be cheap, small and easy to use, and

(vi) it should be durable, i.e., should be capable of repeated use.

Features of Biosensors- A biosensor has two distinct types of components:

(i) biological, e.g., enzyme antibody; etc., and

(ii) physical, e.g., transducer, amplifier, etc.

The biological component of biosensor performs two key functions:

(i) it specifically recognises the analyte, and

(ii) interacts with it in such a manner, which produces some physical change detectable by the transducer. These properties of the biological component impart on the biosensor its specificity, sensitivity and the ability to detect and measure the analyte.The biological component is suitably immobilized on to the transducer.

Enzymes are usually immobilized by glutaraldehyde on a porous sheet like lens tissue paper or nylon net fabric; the enzyme membrane so produced is affixed to the transducer. Generally, correct immobilization of enzymes enhances their stability, which may be rather dramatic in some cases. As a result, many enzyme-immobilized systems can be used more than 10,000, times over a period of several months.

The biological component interacts specifically with the analyte, which produces a physical change close to the transducer surface. This physical change may be

(i) heat released or absorbed by the reaction (measured by calorimetric biosensors),

(ii) production of an electrical potential due to changed distribution of electrons (potentiometric biosensors),

(iii) movement of electrons due to redox reaction (amperometric biosensors),

(iv) light produced or absorbed during the reaction (optical biosensors), or

(v) change in mass of the biological component as a result of the reaction (acoustic wave biosensors).

The transducer detects and measures this change and converts it into an electrical signal. This signal is necessarily very small, and is amplified by an amplifier before it is fed into the microprocessor. The signal is then processed and interpreted, and is displayed in suitable units.

Thus biosensors convert a chemical information flow into an electrical information flow, which involves the following steps.

1. The analyte diffuses from the solution to the surface of the biosensor.

2. The analyte reacts specifically and efficiently with the biological component of the biosensor.

3. This reaction changes the physicochemical properties of the transducer surface.

4. This leads to a change in the optical or electronic properties of the transducer surface.

5. The change in optical/electronic properties is measured, converted into electrical signal, which is amplified, processed and displayed.

Biological Materials and Optically OR Electronically Active Devices Commonly Used in Biosensors -

Biological Material	**Defection device (or transducer)**	**Example**
Enzymes	1. Potentiometric electrodes	Enzyme electrode for urea (based on urease)
	2. Amperometric electrodes	Glucose biosensor (based on glucose oxidase)
Nucleic acids	Amperometric electrodes	
Antibodies	Wave guides (optical biosensors)	
Lectins	Grating couplers (optical biosensors)	
Cells	Acoustic wave sensors	
Organs	Conductimetric sensors	
Tissue slices	Thermometric sensors	

Types of Biosensors- As mentioned, the biosensors are of 5 types:

(i) calorimetric,

(ii) potentiometric,

(iii) amperometric,

(iv) optical and acoustic wave biosensors.

The chief features of calorimetric biosensors are briefly described here.Many enzyme catalyzed reactions produce heat (exothermic). Calorimetric biosensors measure the change in temperature of the solution containing the analyte following enzyme action and interpret it in terms of the analyte concentration in the solution.

The analyte solution is passed through a small (Ca. 1 ml) packed bed column containing immobilized enzyme; the temperature of the solution is determined just before entry of the solution into the column and just as it is leaving the column using separate thermistors.

This is the most generally applicable type of biosensor, and it can be used for turbid and strongly coloured solutions. The greatest disadvantage is to maintain the temperature of the sample stream at a constant, say+- 0.01˚C, temperature. The sensitivity (10-4 M) and tbe range (10-4 - 10-2 M) of such biosensors is quite low for most applications.

The sensitivity can be increased by using two or more enzymes of the pathway in the biosensor to link several reactions to increase the heat output. Alternatively, multifunctional enzymes may be used .An example is the use of glucose oxidase for determination of glucose.

Colorimetric Test Stripss- These are the simplest forms of biosensors. They are single use strips of cellulose coated with the appropriate enzyme and suitable reagents. They are dipped into the sample solution, and the presence of the substrate or analyte in the sample is detected by a change in the colour of the strips, which can be compared with a colour chart to estimate the approximate amount of the analyte.

An example of such test strips is the one used to detect glucose in blood/urine of diabetes patients. The strip contains glucose oxidase, horse radish peroxidase and a weakly coloured, chromogen, which is converted into a highly coloured dye on oxidation.

The H2O2 produced during aerobic oxidation of glucose by glucose oxidise is used to oxidise the chromogen as follows.

Chromogen(2H)+H_2O_2-->Dye + H_2O

Commercial Potential of Biosensors- Biosensors represent a rapidly expanding economic activity with a vast untapped potential. The estimated world market is approximately $ 25 billion! yr of which 30% is in health care. At present only 0.1 % of this market is using biosensors. Biosensor market is likely to expand rapidly with the development of low cost, easy to use and durable biosensors.

Bi and Poly Functional Enzymes- These enzymes are produced by genes obtained by fusion of the coding regions of two or more genes encoding different enzymes. In simple terms, the strategy for production of such enzymes is as follows. Suppose gene a produces enzyme A and gene b produces enzyme B, and for some reason it is desirable to bring the two enzyme activities together.

The stop codon, e.g., TAG, of gene a is deleted, and this modified gene a is then joined with the A TG or initiation codon of gene b. If needed, a linker sequence encoding 2-10 amino acids may be introduced between the genes a and b.

It is essential that the fusion of the two genes does not change the reading frame of gene b, i.e., the two genes are fused in the correct reading frame. The fused gene will produce a single polypeptide, which will possess/exhibit the activities of both the enzymes A and B.

Several bi- and multifunctional enzymes have been produced; some of the examples are, β-galactosidase-galactokinase, β-galactosidase-galactose dehydrogenase, β-galactokinase-galactose dehydrogenase- galactokinase, galactose dehydrogenase-luciferase.

Such enzymes provide the advantage that two or more enzyme activities required in sequence in a metabolic pathway are located in close proximity, i.e., in the same molecule, which may 'considerably enhance the efficiency of the pathway.

These enzymes are useful in preparation of biosensors (when two or more enzymes need to be immobilized together for enhanced efficiency in detection) and in metabolic engineering, and are easy to separate and purify.

The use of enzymes in analysis

Enzymes make excellent analytical reagents due to their specificity, selectivity and efficiency. They are often used to determine the concentration of their substrates (as analytes) by means of the resultant initial reaction rates. If the reaction conditions and enzyme concentrations are kept constant, these rates of reaction (v) are proportional to the substrate concentrations ([S]) at low substrate concentrations. When [S] < 0.1 K_m, equation 1.8 simplifies to give

$$v = (V_{max}/K_m)[S] \qquad (6.1)$$

The rates of reaction are commonly determined from the difference in optical absorbance between the reactants and products. An example of this is the □-D-galactose dehydrogenase (EC 1.1.1.48) assay for galactose which involves the oxidation of galactose by the redox coenzyme, nicotine-adenine dinucleotide (NAD^+).

□-D-galactose + NAD^+ ⟶ D-galactono-1,4-lactone + NADH + H^+ **[6.1]**

A 0.1 mM solution of NADH has an absorbance at 340nm, in a 1 cm path-length cuvette, of 0.622, whereas the NAD^+ from which it is derived has effectively zero absorbance at this wavelength. The conversion (NAD^+ ⟶ NADH) is, therefore, accompanied by a large increase in absorption of light at this wavelength. For the reaction to be linear with respect to the galactose concentration, the galactose is

kept within a concentration range well below the K_m of the enzyme for galactose. In contrast, the NAD^+ concentration is kept within a concentration range well above the K_m of the enzyme for NAD^+, in order to avoid limiting the reaction rate. Such assays are commonly used in analytical laboratories and are, indeed, excellent where a wide variety of analyses need to be undertaken on a relatively small number of samples. The drawbacks to this type of analysis become apparent when a large number of repetitive assays need to be performed. Then, they are seen to be costly in terms of expensive enzyme and coenzyme usage, time consuming, labour intensive and in need of skilled and reproducible operation within properly equipped analytical laboratories. For routine or on-site operation, these disadvantages must be overcome. This is being achieved by the production of biosensors which exploit biological systems in association with advances in micro-electronic technology.

What are biosensors?

A biosensor is an analytical device which converts a biological response into an electrical signal (Figure 6.1). The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilise a biological system directly. This very broad definition is used by some scientific journals (e.g. Biosensors, Elsevier Applied Science) but will not be applied to the coverage here. The emphasis of this Chapter concerns enzymes as the biologically responsive material, but it should be recognised that other biological systems may be utilised by biosensors, for example, whole cell metabolism, ligand binding and the antibody-antigen reaction. Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry (e.g. 6% of the western world are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring. The estimated world analytical market is about £12,000,000,000 $year^{-1}$ of which 30% is in the health care area. There is clearly a vast market expansion potential as less than 0.1% of this market is currently using biosensors. Research and development in this field is wide and multidisciplinary, spanning biochemistry, bioreactor science, physical chemistry, electrochemistry, electronics and software engineering. Most of this current endeavour concerns potentiometric and amperometric biosensors and colorimetric paper enzyme strips. However, all the main transducer types are likely to be thoroughly examined, for use in biosensors, over the next few years.

A successful biosensor must possess at least some of the following beneficial features:

1. The biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks (see later), show good stability over a large number of assays (i.e. much greater than 100).
2. The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilised with the enzyme (see Chapter 8).
3. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case, the biosensor should not be prone to fouling or proteolysis.
5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
6. There should be a market for the biosensor. There is clearly little purpose developing a biosensor if other factors (e.g. government subsidies, the continued employment of skilled analysts, or poor customer perception) encourage the use of traditional methods and discourage the decentralisation of laboratory testing.

The biological response of the biosensor is determined by the biocatalytic membrane which accomplishes the conversion of reactant to product. Immobilised enzymes possess a number of advantageous features which makes them particularly applicable for use in such systems. They may be re-used, which ensures that the same catalytic activity is present for a series of analyses. This is an important factor in securing reproducible results and avoids the pitfalls associated with the replicate pipetting of free enzyme otherwise necessary in analytical protocols. Many enzymes are intrinsically stabilised by the immobilisation process (see Chapter 3), but even where this does not occur there is usually considerable apparent stabilisation. It is normal to use an excess of the enzyme within the immobilised sensor system. This gives a catalytic redundancy (i.e. $\phi \ll 1$) which is sufficient to ensure an increase in the apparent stabilisation of the immobilised enzyme (see, for example, Figures 3.11, 3.19 and 5.8). Even where there is some inactivation of the immobilised enzyme over a period of time, this inactivation is usually steady and predictable. Any activity decay is easily incorporated into an analytical scheme by regularly interpolating standards between the analyses of unknown samples. For these reasons, many such immobilised enzyme systems are re-usable up to 10,000 times over a period of several months. Clearly, this results in a considerable saving in terms of the enzymes' cost relative to the analytical usage of free soluble enzymes.

When the reaction, occurring at the immobilised enzyme membrane of a biosensor, is limited by the rate of external diffusion, the reaction process will possess a number of valuable analytical assets. In particular, it will obey the relationship shown in equation 3.27. It follows that the biocatalyst gives a proportional change in reaction rate in response to the reactant (substrate) concentration over a substantial linear range, several times the intrinsic K_m (see Figure 3.12 line e). This is very useful as analyte concentrations are often approximately equal to the K_ms of their appropriate enzymes which is roughly 10 times more concentrated than can be normally determined, without dilution, by use of the free enzyme in solution. Also following from equation 3.27 is the independence of the reaction rate with respect to pH, ionic strength, temperature and inhibitors. This simply avoids the tricky problems often encountered due to the variability of real analytical samples (e.g, fermentation broth, blood and urine) and external conditions. Control of biosensor response by the external diffusion of the analyte can be encouraged by the use of permeable membranes between the enzyme and the bulk solution. The thickness of these can be varied with associated effects on the proportionality constant between the substrate concentration and the rate of reaction (i.e. increasing membrane thickness increases the unstirred layer (□) which, in turn, decreases the proportionality constant, k_L, in equation 3.27). Even if total dependence on the external diffusional rate is not achieved (or achievable), any increase in the dependence of the reaction rate on external or internal diffusion will cause a reduction in the dependence on the pH, ionic strength, temperature and inhibitor concentrations.

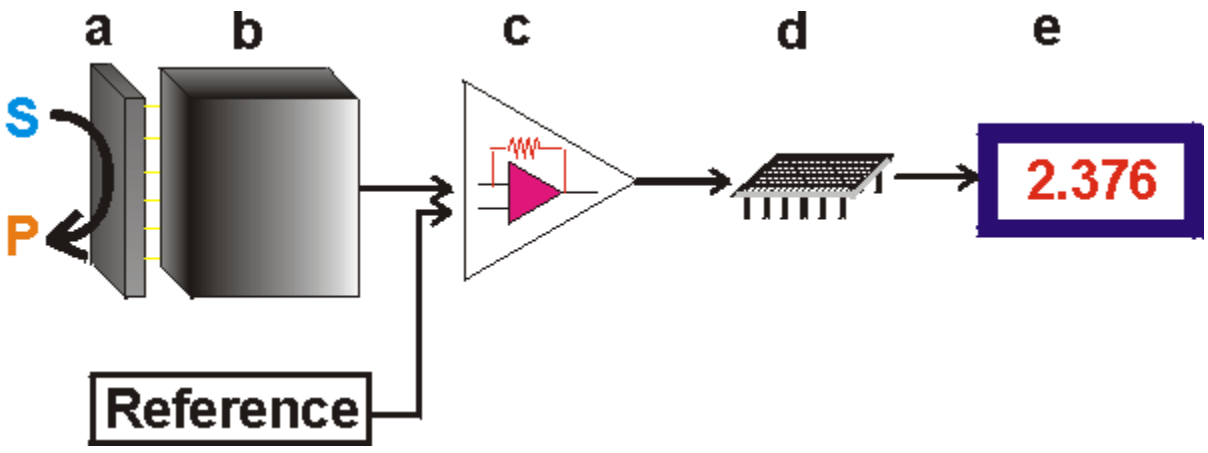


Figure 6.1. Schematic diagram showing the main components of a biosensor. The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e).

The key part of a biosensor is the transducer (shown as the 'black box' in Figure 6.1) which makes use of a physical change accompanying the reaction. This may be

1. the heat output (or absorbed) by the reaction (calorimetric biosensors),
2. changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors),
3. movement of electrons produced in a redox reaction (amperometric biosensors),
4. light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors), or
5. effects due to the mass of the reactants or products (piezo-electric biosensors).

There are three so-called 'generations' of biosensors; First generation biosensors where the normal product of the reaction diffuses to the transducer and causes the electrical response, second generation biosensors which involve specific 'mediators' between the reaction and the transducer in order to generate improved response, and third generation biosensors where the reaction itself causes the response and no product or mediator diffusion is directly involved.

The electrical signal from the transducer is often low and superimposed upon a relatively high and noisy (i.e. containing a high frequency signal component of an apparently random nature, due to electrical interference or generated within the electronic components of the transducer) baseline. The signal processing normally involves subtracting a 'reference' baseline signal, derived from a similar transducer without any biocatalytic membrane, from the sample signal, amplifying the resultant signal difference and electronically filtering (smoothing) out the unwanted signal noise. The relatively slow nature of the biosensor response considerably eases the problem of electrical noise filtration. The analogue signal produced at this stage may be output directly but is usually converted to a digital signal and passed to a microprocessor stage where the data is processed, converted to concentration units and output to a display device or data store.

Calorimetric biosensors

Many enzyme catalysed reactions are exothermic, generating heat (Table 6.1) which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration. This represents the most generally applicable type of biosensor. The temperature changes are usually determined by means of thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment (Figure 6.2). Under such closely controlled conditions, up to 80% of the heat generated in the reaction may be registered as a temperature change in the sample stream. This may be simply

calculated from the enthalpy change and the amount reacted. If a 1 mM reactant is completely converted to product in a reaction generating 100 kJ $mole^{-1}$ then each ml of solution generates 0.1 J of heat. At 80% efficiency, this will cause a change in temperature of the solution amounting to approximately 0.02°C. This is about the temperature change commonly encountered and necessitates a temperature resolution of 0.0001°C for the biosensor to be generally useful.

Table 6.1. Heat output (molar enthalpies) of enzyme catalysed reactions.

Reactant	**Enzyme**	**Heat output -ΔH (kJ $mole^{-1}$)**
Cholesterol	Cholesterol oxidase	53
Esters	Chymotrypsin	4 - 16
Glucose	Glucose oxidase	80
Hydrogen peroxide	Catalase	100
Penicillin G	Penicillinase	67
Peptides	Trypsin	10 - 30
Starch	Amylase	8
Sucrose	Invertase	20
Urea	Urease	61
Uric acid	Uricase	49

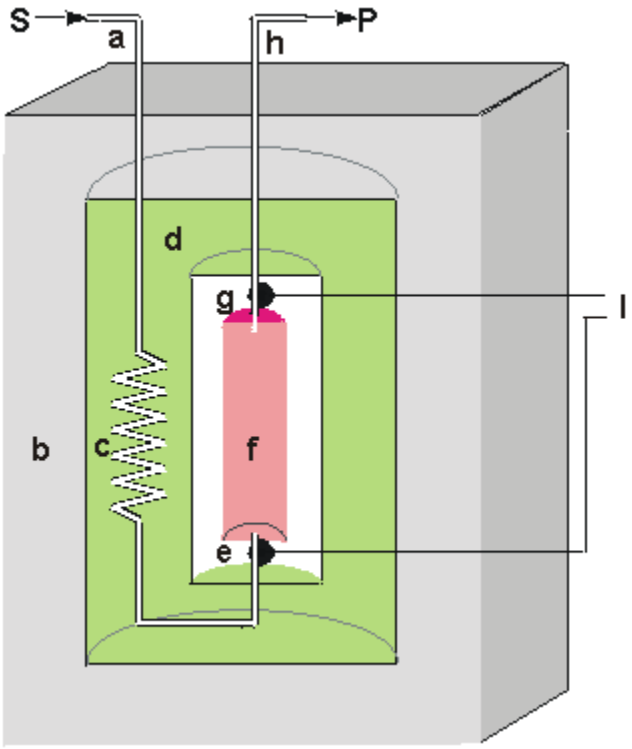


Figure 6.2. Schematic diagram of a calorimetric biosensor. The sample stream (a) passes through the outer insulated box (b) to the heat exchanger (c) within an aluminium block (d). From there, it flows past the reference thermistor (e) and into the packed bed bioreactor (f, 1ml volume), containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor (g) and the solution passed to waste (h). External electronics (l) determines the difference in the resistance, and hence temperature, between the thermistors.

The thermistors, used to detect the temperature change, function by changing their electrical resistance with the temperature, obeying the relationship

$$Ln\left(\frac{R_1}{R_2}\right) = B\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad (6.2)$$

therefore:

$$\frac{R_1}{R_2} = e^{B\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \qquad (6.2b)$$

where R_1 and R_2 are the resistances of the thermistors at absolute temperatures T_1 and T_2 respectively and B is a characteristic temperature constant for the thermistor. When the temperature change is very small, as in the present case, $B(1/T_1) - (1/T_2)$ is very much smaller than one and this relationship may be substantially simplified using the approximation when $x<<1$ that $e^x \approx 1 + x$ (x here being $B(1/T_1) - (1/T_2)$),

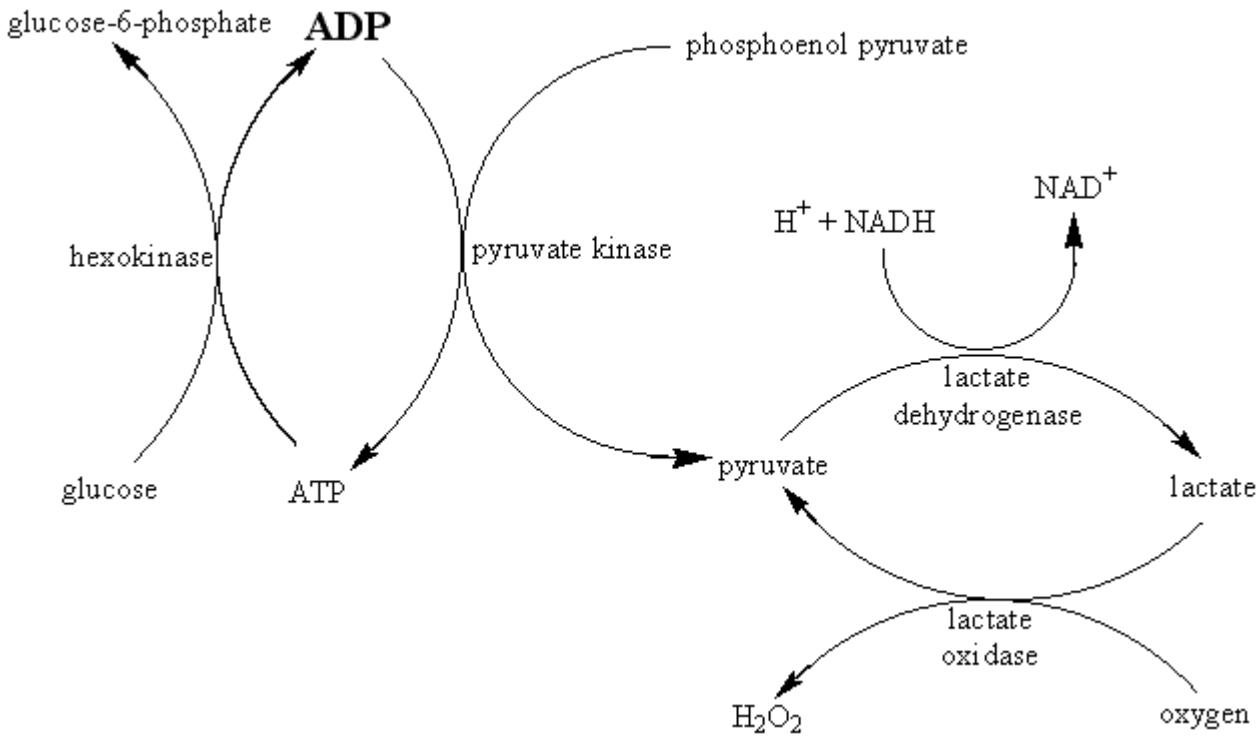
$$R_1 = R_2\left\{1+B\left(\frac{T_2-T_1}{T_1T_2}\right)\right\} \qquad (6.3)$$

As $T_1 \approx T_2$, they both may be replaced in the denominator by T_1.

$$\frac{\Delta R}{R} = -\left(\frac{B}{T_1^2}\right)\Delta T \qquad (6.4)$$

The relative decrease in the electrical resistance ($\Delta R/R$) of the thermistor is proportional to the increase in temperature (ΔT). A typical proportionality constant ($-B/T_1^2$) is $-4\%°C^{-1}$. The resistance change is converted to a proportional voltage change, using a balanced Wheatstone bridge incorporating precision wire-wound resistors, before amplification. The expectation that there will be a linear correlation between the response and the enzyme activity has been found to be borne out in practice. A major problem with this biosensor is the difficulty encountered in closely matching the characteristic temperature constants of the measurement and reference thermistors. An equal movement of only 1°C in the background temperature of both thermistors commonly causes an apparent change in the relative resistances of the thermistors equivalent to 0.01°C and equal to the full-scale change due to the reaction. It is clearly of great importance that such environmental temperature changes are avoided, which accounts for inclusion of the well-insulated aluminium block in the biosensor design (see Figure 6.2).

The sensitivity (10^{-4} M) and range (10^{-4} - 10^{-2} M) of thermistor biosensors are both quite low for the majority of applications although greater sensitivity is possible using the more exothermic reactions (e.g. catalase). The low sensitivity of the system can be increased substantially by increasing the heat output by the reaction. In the simplest case this can be achieved by linking together several reactions in a reaction pathway, all of which contribute to the heat output. Thus the sensitivity of the glucose analysis using glucose oxidase can be more than doubled by the co-immobilisation of catalase within the column reactor in order to disproportionate the hydrogen peroxide produced. An extreme case of this amplification is shown in the following recycle scheme for the detection of ADP.



[6.2]

ADP is the added analyte and excess glucose, phosphoenol pyruvate, NADH and oxygen are present to ensure maximum reaction. Four enzymes (hexokinase, pyruvate kinase, lactate dehydrogenase and lactate oxidase) are co-immobilised within the packed bed reactor. In spite of the positive enthalpy of the pyruvate kinase reaction, the overall process results in a 1000 fold increase in sensitivity, primarily due to the recycling between pyruvate and lactate. Reaction limitation due to low oxygen solubility may be overcome by replacing it with benzoquinone, which is reduced to hydroquinone by flavo-enzymes. Such reaction systems do, however, have the serious disadvantage in that they increase the probability of the occurrence of interference in the determination of the analyte of interest. Reactions involving the generation of hydrogen ions can be made more sensitive by the inclusion of a base having a high heat of protonation. For example, the heat output by the penicillinase reaction may be almost doubled by the use of Tris (tris-(hydroxymethyl)aminomethane) as the buffer.In conclusion, the main advantages of the thermistor biosensor are its general applicability and the possibility for its use on turbid or strongly coloured solutions. The most important disadvantage is the difficulty in ensuring that the temperature of the sample stream remains constant (± 0.01°C).

Potentiometric biosensors

Potentiometric biosensors make use of ion-selective electrodes in order to transduce the biological reaction into an electrical signal. In the simplest terms this consists of an immobilised enzyme membrane surrounding the probe from a pH-meter (Figure 6.3), where the catalysed reaction generates or absorbs hydrogen ions (Table 6.2). The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly from the pH-meter's display. Typical of the use of such electrodes is that the electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction.

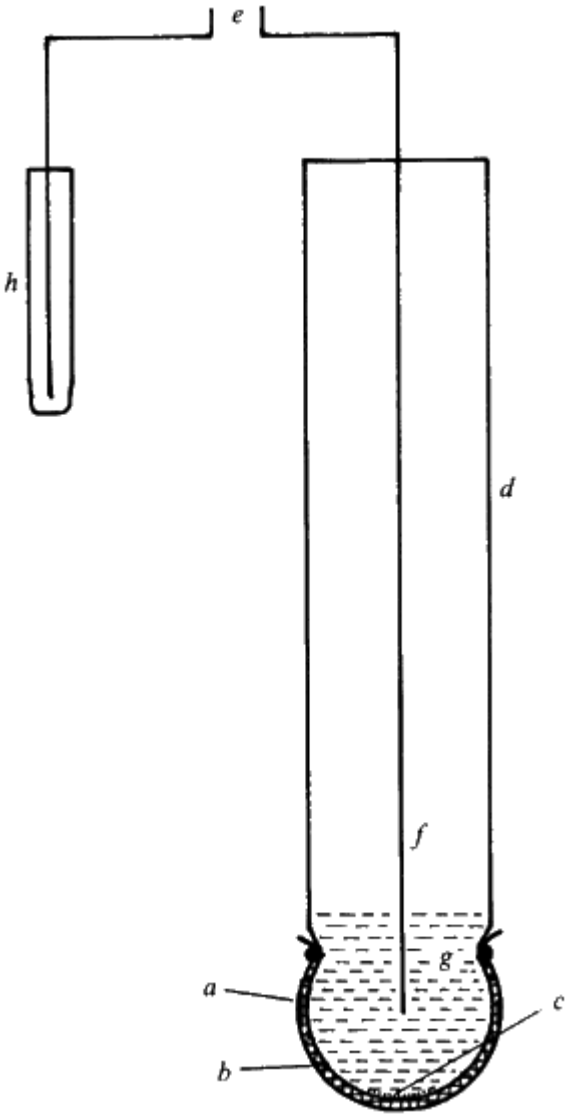


Figure 6.3. A simple potentiometric biosensor. A semi-permeable membrane (a) surrounds the biocatalyst (b) entrapped next to the active glass membrane (c) of a pH probe (d). The electrical potential (e) is generated between the internal Ag/AgCl electrode (f) bathed in dilute HCl (g) and an external reference electrode (h).

There are three types of ion-selective electrodes which are of use in biosensors:

1. Glass electrodes for cations (e.g. normal pH electrodes) in which the sensing element is a very thin hydrated glass membrane which generates a transverse electrical potential due to the concentration-dependent competition between the cations for specific binding sites. The selectivity of this membrane is determined by the composition of the glass. The sensitivity to H^+ is greater than that achievable for NH_4^+,
2. Glass pH electrodes coated with a gas-permeable membrane selective for CO_2, NH_3 or H_2S. The diffusion of the gas through this membrane causes a change in pH of a sensing solution between the membrane and the electrode which is then determined.
3. Solid-state electrodes where the glass membrane is replaced by a thin membrane of a specific ion conductor made from a mixture of silver sulphide and a silver halide. The iodide electrode is useful for the determination of I^- in the peroxidase reaction (Table 6.2c) and also responds to cyanide ions.

Table 6.2. Reactions involving the release or absorption of ions that may be utilised by potentiometric biosensors.

(a) H^+ cation,

$$\text{D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-1,5-lactone} + H_2O_2 \xrightarrow{H_2O} \text{D-gluconate} + H^+ \quad \textbf{[6.3]}$$

$$\text{penicillin} \xrightarrow{\text{penicillinase}} \text{penicilloic acid} + H^+ \quad \textbf{[6.4]}$$

$$H_2NCONH_2 + H_2O + 2H^+ \xrightarrow{\text{urease (pH 6.0)}^a} 2NH_4^+ + CO_2 \quad \textbf{[6.5]}$$

$$H_2NCONH_2 + 2H_2O \xrightarrow{\text{urease (pH 9.5)}^b} 2NH_3 + HCO_3^- + H^+ \quad \textbf{[6.6]}$$

$$\text{neutral lipids} + H_2O \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acids} + H^+ \quad \textbf{[6.7]}$$

(b) NH_4^+ cation,

$$\text{L-amino acid} + O_2 + H_2O \xrightarrow{\text{L-amino acid oxidase}} \text{keto acid} + NH_4^+ + H_2O_2 \quad \textbf{[6.8]}$$

$$\text{L-asparagine} + H_2O \xrightarrow{\text{asparaginase}} \text{L-aspartate} + NH_4^+ \quad \textbf{[6.9]}$$

$$H_2NCONH_2 + 2H_2O + H^+ \xrightarrow{\text{urease (pH 7.5)}} 2NH_4^+ + HCO_3^- \quad \textbf{[6.10]}$$

(c) I^- anion,

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{\text{peroxidase}} I_2 + 2H_2O \quad \textbf{[6.11]}$$

(d) CN^-anion,

$$\text{amygdalin} + 2H_2O \xrightarrow{\beta\text{-glucosidase}} 2\text{glucose} + \text{benzaldehyde} + H^+ + CN^- \quad \textbf{[6.12]}$$

[a] Can also be used in NH_4^+ and CO_2 (gas) potentiometric biosensors.

[b] Can also be used in an NH_3 (gas) potentiometric biosensor.es80ll66bp

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The response of an ion-selective electrode is given by

$$E = E_0 + \frac{RT}{zF} Ln([i]) \qquad (6.5)$$

where E is the measured potential (in volts), E_0 is a characteristic constant for the ion-selective/external electrode system, R is the gas constant, T is the absolute temperature (K), z is the signed ionic charge, F is the Faraday, and [i] is the concentration of the free uncomplexed ionic species (strictly, [i] should be the activity of the ion but at the concentrations normally encountered in biosensors, this is effectively equal to the concentration). This means, for example, that there is an increase in the electrical potential of 59 mv for every decade increase in the concentration of H^+ at 25°C. The logarithmic dependence of the potential on the ionic concentration is responsible both for the wide analytical range and the low accuracy and precision of these sensors. Their normal range of detection is 10^{-4} - 10^{-2} M, although a minority are ten-fold more sensitive. Typical response time are between one and five minutes allowing up to 30 analyses every hour.

Biosensors which involve H^+ release or utilisation necessitate the use of very weakly buffered solutions (i.e. < 5 mM) if a significant change in potential is to be determined. The relationship between pH change and substrate concentration is complex, including other such non-linear effects as pH-activity variation and protein buffering. However, conditions can often be found where there is a linear relationship between the apparent change in pH and the substrate concentration. A recent development from ion-selective electrodes is the production of ion-selective field effect transistors (**ISFET**s) and their biosensor use as enzyme-linked field effect transistors (**ENFET**s, Figure 6.4). Enzyme membranes are coated on the ion-selective gates of these electronic devices, the biosensor responding to the electrical potential change via the current output. Thus, these are potentiometric devices although they directly produce changes in the electric current. The main advantage of such devices is their extremely small size (<< 0.1 mm^2) which allows cheap mass-produced fabrication using integrated circuit technology. As an example, a urea-sensitive FET (ENFET containing bound urease with a reference electrode containing bound glycine) has been shown to show only a 15% variation in response to urea (0.05 - 10.0 mg ml^{-1}) during its active lifetime of a month. Several analytes may be determined by miniaturised biosensors containing arrays of ISFETs and ENFETs. The sensitivity of FETs, however, may be affected by the composition, ionic strength and concentrations of the solutions analysed.

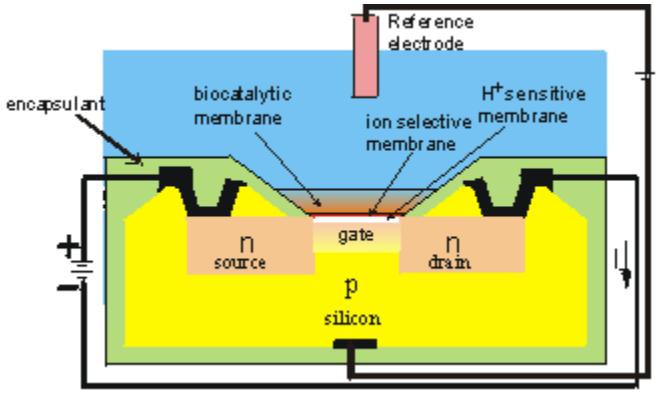


Figure 6.4. Schematic diagram of the section across the width of an ENFET. The actual dimensions of the active area is about 500 □m long by 50 □m wide by 300 □m thick. The main body of the biosensor is a p-type silicon chip with two n-type silicon areas; the negative source and the positive drain. The chip is insulated by a thin layer (0.1 □m thick) of silica (SiO_2) which forms the gate of the FET. Above this gate is an equally thin layer of H^+-sensitive material (e.g. tantalum oxide), a protective ion selective membrane, the biocatalyst and the analyte solution, which is separated from sensitive parts of the FET by an inert encapsulating polyimide photopolymer. When a potential is applied between the electrodes, a current flows through the FET dependent upon the positive potential detected at the ion-selective gate and its consequent attraction of electrons into the depletion layer. This current

(I) is compared with that from a similar, but non-catalytic ISFET immersed in the same solution. (Note that the electric current is, by convention, in the opposite direction to the flow of electrons).

Amperometric biosensors

Amperometric biosensors function by the production of a current when a potential is applied between two electrodes. They generally have response times, dynamic ranges and sensitivities similar to the potentiometric biosensors. The simplest amperometric biosensors in common usage involve the Clark oxygen electrode (Figure 6.5). This consists of a platinum cathode at which oxygen is reduced and a silver/silver chloride reference electrode. When a potential of -0.6 V, relative to the Ag/AgCl electrode is applied to the platinum cathode, a current proportional to the oxygen concentration is produced. Normally both electrodes are bathed in a solution of saturated potassium chloride and separated from the bulk solution by an oxygen-permeable plastic membrane (e.g. Teflon, polytetrafluoroethylene). The following reactions occur:

Ag anode $4Ag^0 + 4Cl^- \longrightarrow 4AgCl + 4e^-$ **[6.13]**

Pt cathode $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$ **[6.14]**

The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be effectively zero. The rate of this electrochemical reduction therefore depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration (see, for example, equation 3.13). It is clear that a small, but significant, proportion of the oxygen present in the bulk is consumed by this process; the oxygen electrode measuring the rate of a process which is far from equilibrium, whereas ion-selective electrodes are used close to equilibrium conditions. This causes the oxygen electrode to be much more sensitive to changes in the temperature than potentiometric sensors. A typical application for this simple type of biosensor is the determination of glucose concentrations by the use of an immobilised glucose oxidase membrane. The reaction (see reaction scheme **[1.1]**) results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes (Figure 6.6). Other oxidases may be used in a similar manner for the analysis of their substrates (e.g. alcohol oxidase, D- and L-amino acid oxidases, cholesterol oxidase, galactose oxidase, and urate oxidase)

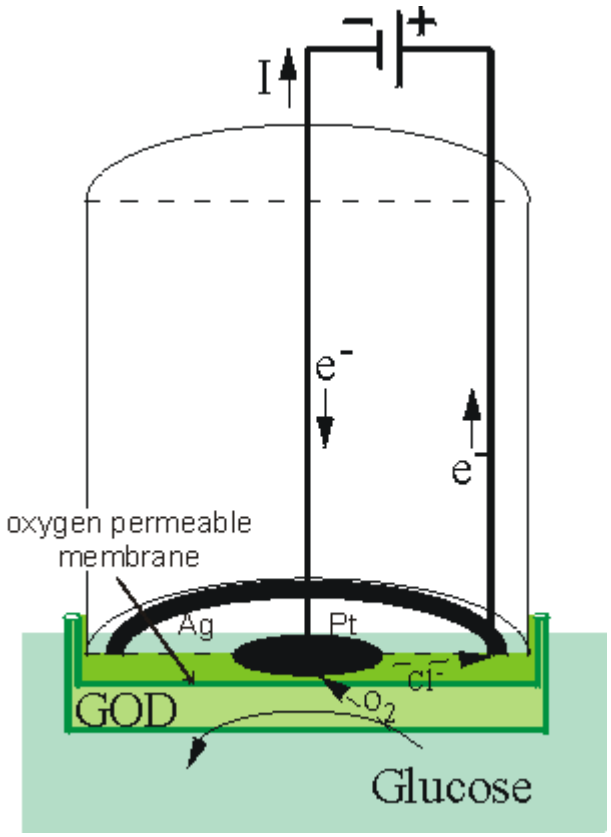


Figure 6.5. Schematic diagram of a simple amperometric biosensor. A potential is applied between the central platinum cathode and the annular silver anode. This generates a current (I) which is carried between the electrodes by means of a saturated solution of KCl. This electrode compartment is separated from the biocatalyst (here shown glucose oxidase, GOD) by a thin plastic membrane, permeable only to oxygen. The analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate(s) and product(s). This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilising dip-coated membranes.

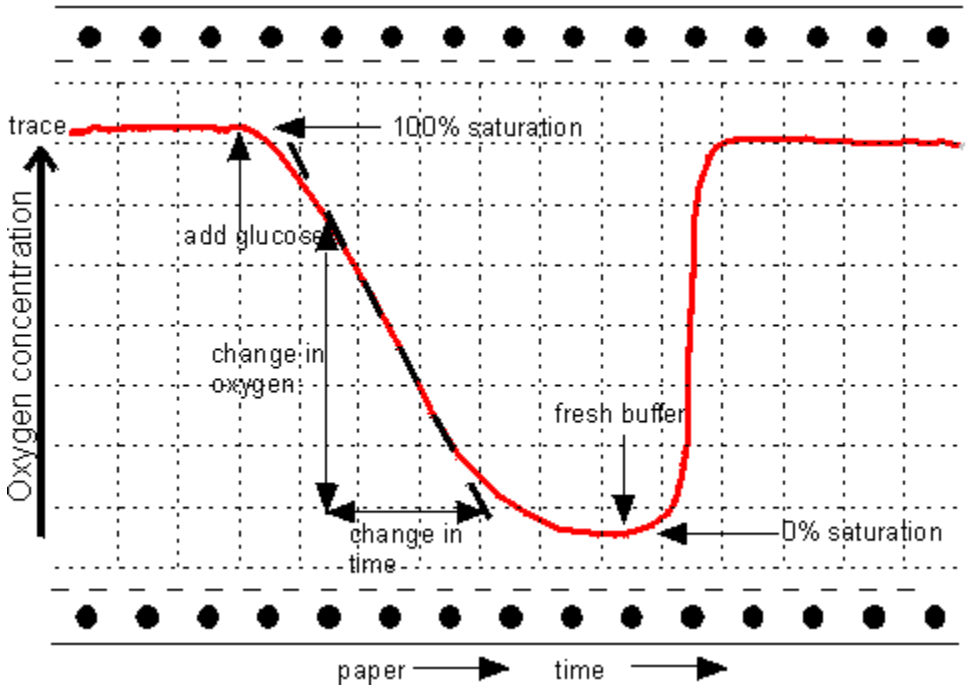


Figure 6.6. The response of an amperometric biosensor utilising glucose oxidase to the presence of glucose solutions. Between analyses the biosensor is placed in oxygenated buffer devoid of glucose. The steady rates of oxygen depletion may be used to generate standard response curves and determine unknown samples. The time required for an assay can be considerably reduced if only the initial transient (curved) part of the response need be used, via a suitable model and software. The wash-out time, which roughly equals the time the electrode spends in the sample solution, is also reduced significantly by this process.

An alternative method for determining the rate of this reaction is to measure the production of hydrogen peroxide directly by applying a potential of +0.68 V to the platinum electrode, relative to the Ag/AgCl electrode, and causing the reactions:

Pt anode $H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$ **[6.15]**

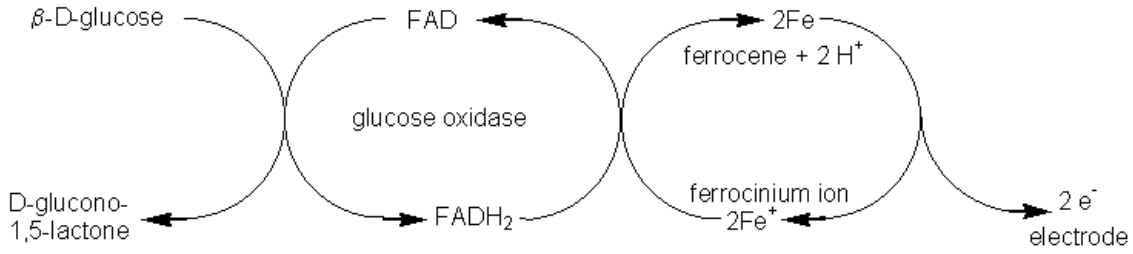
Ag cathode $2AgCl + 2e^- \longrightarrow 2Ag^0 + 2Cl^-$ **[6.16]**

The major problem with these biosensors is their dependence on the dissolved oxygen concentration. This may be overcome by the use of 'mediators' which transfer the electrons directly to the electrode bypassing the reduction of the oxygen co-substrate. In order to be generally applicable these mediators must possess a number of useful properties.

1. They must react rapidly with the reduced form of the enzyme.

2. They must be sufficiently soluble, in both the oxidised and reduced forms, to be able to rapidly diffuse between the active site of the enzyme and the electrode surface. This solubility should, however, not be so great as to cause significant loss of the mediator from the biosensor's microenvironment to the bulk of the solution. However soluble, the mediator should generally be non-toxic.
3. The overpotential for the regeneration of the oxidised mediator, at the electrode, should be low and independent of pH.
4. The reduced form of the mediator should not readily react with oxygen.

The ferrocenes represent a commonly used family of mediators (Figure 6.7a). Their reactions may be represented as follows,



[6.17]

Electrodes have now been developed which can remove the electrons directly from the reduced enzymes, without the necessity for such mediators. They utilise a coating of electrically conducting organic salts, such as N-methylphenazinium cation (NMP^+, Figure 6.7b) with tetracyanoquinodimethane radical anion ($TCNQ^-$ Figure 6.7c). Many flavo-enzymes are strongly adsorbed by such organic conductors due to the formation of salt links, utilising the alternate positive and negative charges, within their hydrophobic environment. Such enzyme electrodes can be prepared by simply dipping the electrode into a solution of the enzyme and they may remain stable for several months. These electrodes can also be used for reactions involving $NAD(P)^+$-dependent dehydrogenases as they also allow the electrochemical oxidation of the reduced forms of these coenzymes. The three types of amperometric biosensor utilising product, mediator or organic conductors represent the three generations in biosensor development (Figure 6.8). The reduction in oxidation potential, found when mediators are used, greatly reduces the problem of interference by extraneous material.

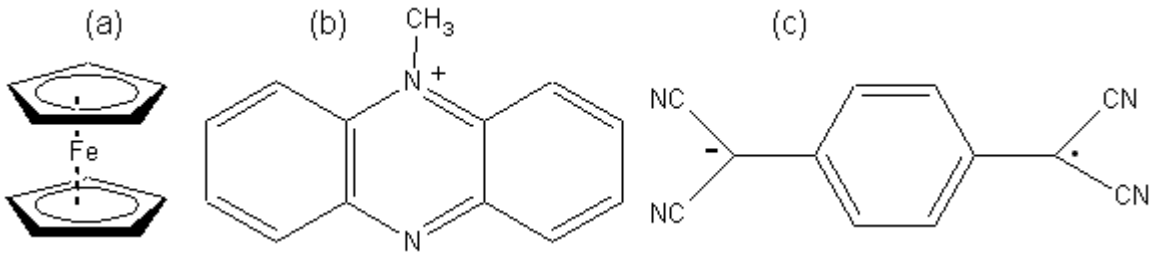


Figure 6.7. (a) Ferrocene (□5-bis-cyclopentadienyl iron), the parent compound of a number of mediators. (b) TMP^+, the cationic part of conducting organic crystals. (c) $TCNQ^{\cdot -}$, the anionic part of conducting organic crystals. It is a resonance-stabilised radical formed by the one-electron oxidation of $TCNQH_2$.

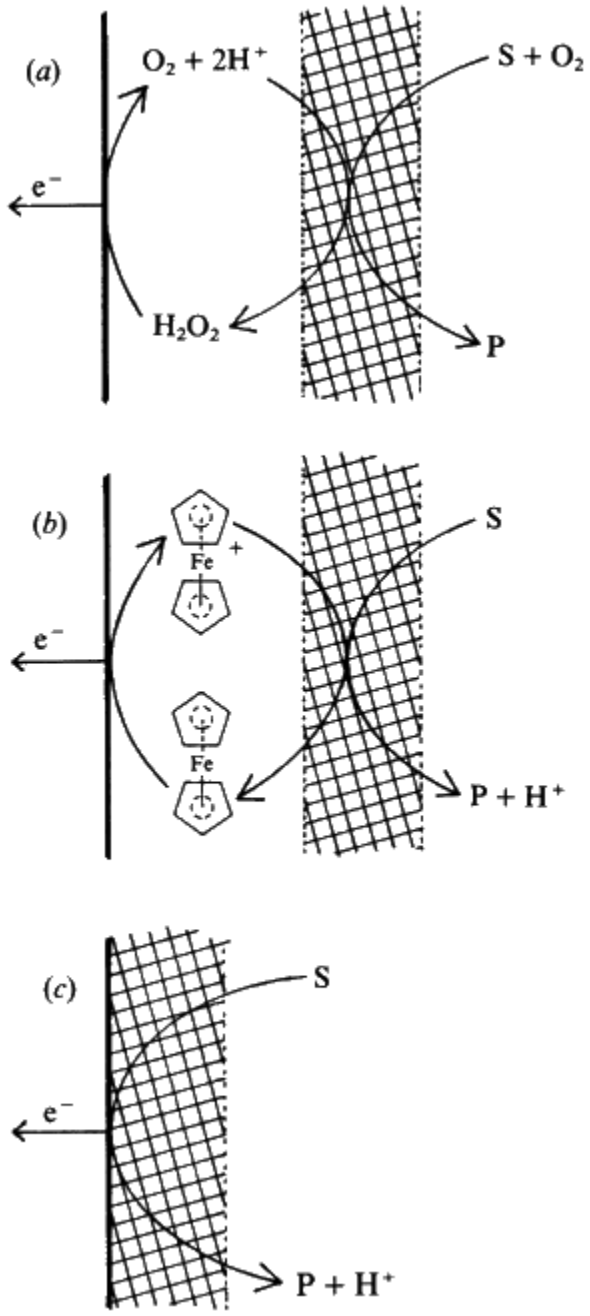


Figure 6.8. Amperometric biosensors for flavo-oxidase enzymes illustrating the three generations in the development of a biosensor. The biocatalyst is shown schematically by the cross-hatching. (a) First generation electrode utilising the H_2O_2 produced by the reaction. (E_0 = +0.68 V). (b) Second generation electrode utilising a mediator (ferrocene) to transfer the electrons, produced by the reaction, to the electrode. (E_0 = +0.19 V). (c) Third generation electrode directly utilising the electrons produced by the reaction. (E_0 = +0.10 V). All electrode potentials (E_0) are relative to the $Cl^-/AgCl,Ag^0$ electrode. The following reaction occurs at the enzyme in all three biosensors:

$$\text{Substrate(2H)} + \text{FAD-oxidase} \longrightarrow \text{Product} + \text{FADH}_2\text{-oxidasefi} \quad \textbf{[6.18]}$$

This is followed by the processes:

(a)
biocatalyst

$$\text{FADH}_2\text{-oxidase} + \text{O}_2 \longrightarrow \text{FAD-oxidase} + \text{H}_2\text{O}_2 \quad \textbf{[6.19]}$$

electrode

$$\text{H}_2\text{O}_2 \longrightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \quad \textbf{[6.20]}$$

(b)
biocatalyst

$$\text{FADH}_2\text{-oxidase} + 2\ \text{Ferricinium}^+ \longrightarrow \text{FAD-oxidase} + 2\ \text{Ferrocene} + 2\text{H}^+ \quad \textbf{[6.21]}$$

electrode

$$2\ \text{Ferrocene} \longrightarrow 2\ \text{Ferricinium}^+ + 2\text{e}^- \quad \textbf{[6.22]}$$

(c)
biocatalyst/electrode

$$\text{FADH}_2\text{-oxidase} \longrightarrow \text{FAD-oxidase} + 2\text{H}^+ + 2\text{e}^- \quad \textbf{[6.23]}$$

The current (i) produced by such amperometric biosensors is related to the rate of reaction (v_A) by the expression:

$$i = nFAv_A \qquad (6.6)$$

where n represents the number of electrons transferred, A is the electrode area, and F is the Faraday. Usually the rate of reaction is made diffusionally controlled (see equation 3.27) by use of external membranes. Under these circumstances the electric current produced is proportional to the analyte concentration and independent both of the enzyme and electrochemical kinetics.

Optical biosensors

There are two main areas of development in optical biosensors. These involve determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process. The former usually involve the widely established, if rather low technology, use of colorimetric test strips. These are disposable single-use cellulose pads impregnated with enzyme and reagents. The most common use of this technology is for whole-blood monitoring in diabetes control. In this case, the strips include glucose oxidase, horseradish peroxidase (EC 1.11.1.7) and a chromogen (e.g. *o*-toluidine or 3,3',5,5'-tetramethylbenzidine). The hydrogen peroxide, produced by the aerobic oxidation of glucose (see reaction scheme **[1.1]**), oxidising the weakly coloured chromogen to a highly coloured dye.

$$\text{chromogen}(2H) + H_2O_2 \xrightarrow{\text{peroxidase}} \text{dye} + 2H_2O \qquad \textbf{[6.24]}$$

The evaluation of the dyed strips is best achieved by the use of portable reflectance meters, although direct visual comparison with a coloured chart is often used. A wide variety of test strips involving other enzymes are commercially available at the present time.A most promising biosensor involving luminescence uses firefly luciferase (*Photinus*-luciferin 4-monooxygenase (ATP-hydrolysing), EC 1.13.12.7) to detect the presence of bacteria in food or clinical samples. Bacteria are specifically lysed and the ATP released (roughly proportional to the number of bacteria present) reacted with D-luciferin and oxygen in a reaction which produces yellow light in high quantum yield.

$$\text{ATP} + \text{D-luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{oxyluciferin} + \text{AMP} + \text{pyrophosphate} + CO_2 + \text{light (562 nm)} \qquad \textbf{[6.25]}$$

The light produced may be detected photometrically by use of high-voltage, and expensive, photomultiplier tubes or low-voltage cheap photodiode systems. The sensitivity of the photomultiplier-containing systems is, at present, somewhat greater ($< 10^4$ cells ml^{-1}, $< 10^{-12}$ M ATP) than the simpler photon detectors which use photodiodes. Firefly luciferase is a very expensive enzyme, only obtainable from the tails of wild fireflies. Use of immobilised luciferase greatly reduces the cost of these analyses.

Piezo-electric biosensors

Piezo-electric crystals (e.g. quartz) vibrate under the influence of an electric field. The frequency of this oscillation (f) depends on their thickness and cut, each crystal

having a characteristic resonant frequency. This resonant frequency changes as molecules adsorb or desorb from the surface of the crystal, obeying the relationshipes

$$\Delta f = \frac{Kf^2 \Delta m}{A} \qquad (6.7)$$

where ?f is the change in resonant frequency (Hz), ?m is the change in mass of adsorbed material (g), K is a constant for the particular crystal dependent on such factors as its density and cut, and A is the adsorbing surface area (cm^2). For any piezo-electric crystal, the change in frequency is proportional to the mass of absorbed material, up to about a 2% change. This frequency change is easily detected by relatively unsophisticated electronic circuits. A simple use of such a transducer is a formaldehyde biosensor, utilising a formaldehyde dehydrogenase coating immobilised to a quartz crystal and sensitive to gaseous formaldehyde. The major drawback of these devices is the interference from atmospheric humidity and the difficulty in using them for the determination of material in solution. They are, however, inexpensive, small and robust, and capable of giving a rapid response.

Immunosensors

Biosensors may be used in conjunction with enzyme-linked immunosorbent assays (**ELISA**). The principles behind the ELISA technique is shown in Figure 6.9. ELISA is used to detect and amplify an antigen-antibody reaction; the amount of enzyme-linked antigen bound to the immobilised antibody being determined by the relative concentration of the free and conjugated antigen and quantified by the rate of enzymic reaction. Enzymes with high turnover numbers are used in order to achieve rapid response. The sensitivity of such assays may be further enhanced by utilising enzyme-catalysed reactions which give intrinsically greater response; for instance, those giving rise to highly coloured, fluorescent or bioluminescent products. Assay kits using this technique are now available for a vast range of analyses.

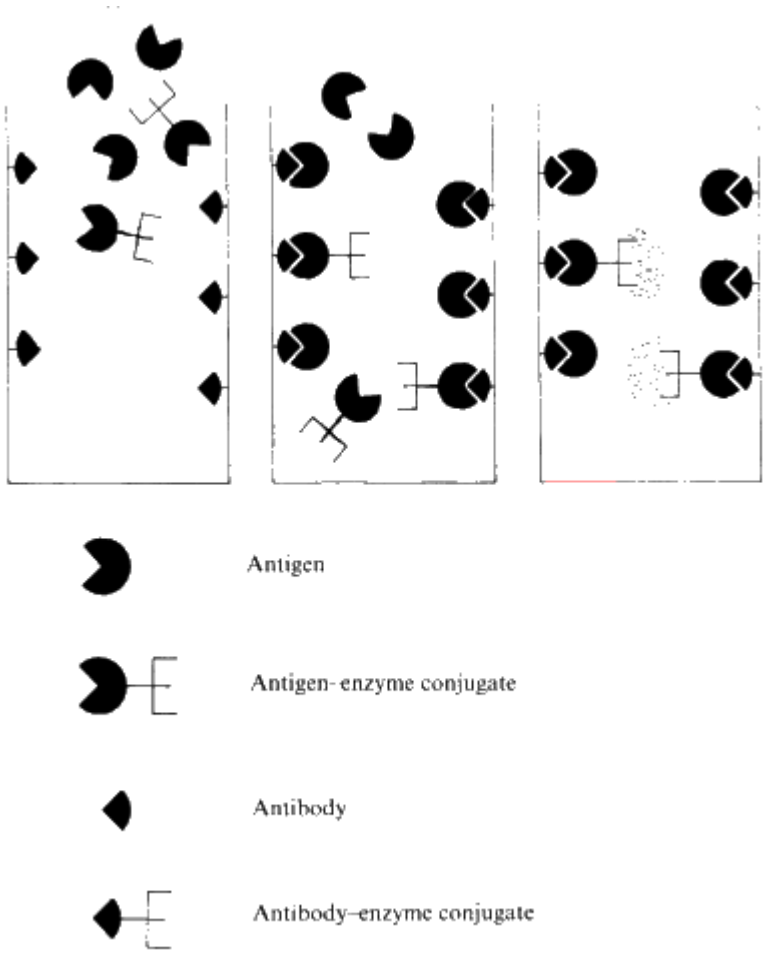


Figure 6.9. Principles of a direct competitive ELISA. (i) Antibody, specific for the antigen of interest is immobilised on the surface of a tube. A mixture of a known amount of antigen-enzyme conjugate plus unknown concentration of sample antigen is placed in the tube and allowed to equilibrate. (ii) After a suitable period the antigen and antigen-enzyme conjugate will be distributed between the bound and free states dependent upon their relative concentrations. (iii) Unbound material is washed off and discarded. The amount of antigen-enzyme conjugate that is bound may be determined by the rate of the subsequent enzymic reaction.

Recently ELISA techniques have been combined with biosensors, to form **immunosensors**, in order to increase their range, speed and sensitivity. A simple immunosensor configuration is shown in Figure 6.10 (a), where the biosensor merely replaces the traditional colorimetric detection system. However more advanced immunosensors are being developed (Figure 6.10 (b)) which rely on the direct detection of antigen bound to the antibody-coated surface of the biosensor. Piezoelectric and FET-based biosensors are particularly suited to such applications.

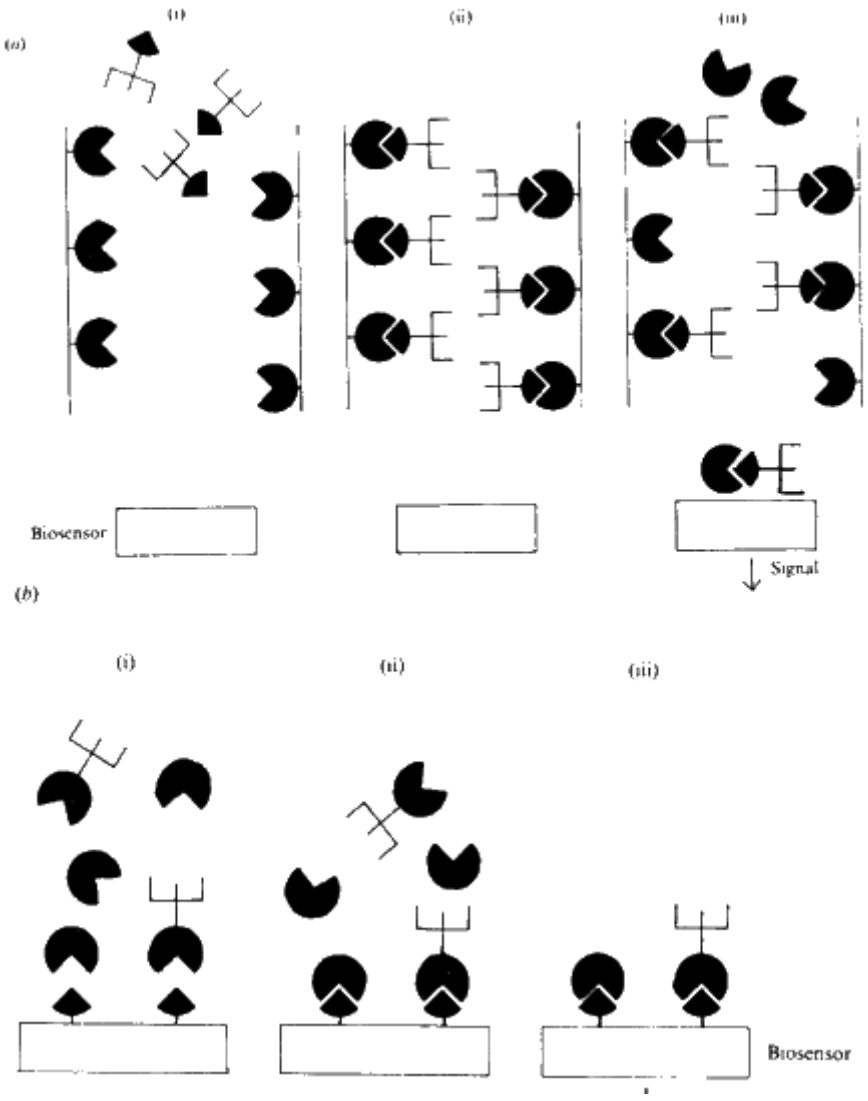


Figure 6.10. Principles of immunosensors. (a)(i) A tube is coated with (immobilised) antigen. An excess of specific antibody-enzyme conjugate is placed in the tube and allowed to bind. (a)(ii) After a suitable period any unbound material is washed off. (a)(iii) The analyte antigen solution is passed into the tube, binding and releasing some of the antibody-enzyme conjugate dependent upon the antigen's concentration. The amount of antibody-enzyme conjugate released is determined by the response from the biosensor. (b)(i) A transducer is coated with (immobilised) antibody, specific for the antigen of interest. The transducer is immersed in a solution containing a mixture of a known amount of antigen-enzyme conjugate plus unknown concentration of sample antigen. (b)(ii) After a suitable period the antigen and antigen-enzyme conjugate will be distributed between the bound and free states dependent upon their relative concentrations. (b)(iii) Unbound material

is washed off and discarded. The amount of antigen-enzyme conjugate bound is determined directly from the transduced signal.

Applications of biosensors

There are many potential application of biosensors of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based techniques in some situations. Some examples are given below:

- Glucose monitoring in diabetes patients <-- historical market driver
- Other medical health related targets
- Environmental applications e.g. the detection of pesticides and river water contaminants
- Remote sensing of airborne bacteria e.g. in counter-bioterrorist activities
- Detection of pathogens[3]
- Determining levels of toxic substances before and after bioremediation
- Detection and determining of organophosphate
- Routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay
- Determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey.
- Drug discovery and evaluation of biological activity of new compounds.
- Detection of toxic metabolites such as mycotoxins [4]

Biosensors

A **biosensor** is a device for the detection of an analyte that combines a biological component with a physicochemical detector component.[1]

It consists of 3 parts:

- the *sensitive biological element* (biological material (eg. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc), a biologically derived material or biomimic) The sensitive elements can be created by biological engineering.
- the *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified;
- associated electronics or signal processors that is primarily responsible for the display of the results in a user-friendly way.[2]

The most widespread example of a commercial biosensor is the blood glucose biosensor, which uses the enzyme glucose oxidase to break blood glucose down. In doing so it first oxidizes glucose and uses two electrons to reduce the FAD (a component of the enzyme) to FADH2. This in turn is oxidized by the electrode (accepting two electrons from the electrode) in a number of steps. The resulting current is a measure of the concentration of glucose. In this case, the electrode is the transducer and the enzyme is the biologically active component.

Recently, arrays of many different detector molecules have been applied in so called electronic nose devices, where the pattern of response from the detectors is used to fingerprint a substance. Current commercial electronic noses, however, do not use biological elements.

A canary in a cage, as used by miners to warn of gas could be considered a biosensor. Many of today's biosensor applications are similar, in that they use organisms which respond to toxic substances at a much lower level than us to warn

us of their presence. Such devices can be used both in environmental monitoring and in water treatment facilities.

Principles of Detection

Electrochemical

Electrochemical biosensors are normally based on enzymatic catalysis of a reaction that produces or consumes electrons (such enzymes are rightly called redox enzymes). The sensor substrate usually contains three electrodes, a reference electrode, an active electrode and a sink electrode. A counter electrode may also be present as an ion source. The target analyte is involved in the reaction that takes place on the active electrode surface, and the ions produced create a potential which is subtracted from that of the reference electrode to give a signal. We can either measure the current (rate of flow of electrons is now proportional to the analyte concentration) at a fixed potential or the the potential can be measured at zero current (this gives a logarithmic response). Note that potential of the working or active electrode is space charge sensitive and this is often used.

Another example, the potentiometric biosensor, works contrary to the current understanding of its ability. Such biosensors are screenprinted, conducting polymer coated, open circuit potential biosensors based on conjugated polymers immunoassays. They have only two electrodes and are extremely sensitive, robust and accurate. They enable the detection of analytes at levels previously only achievable by HPLC and LC/MS and without rigorous sample preparation. The signal is produced by electrochemical and physical changes in the conducting polymer layer due to changes occurring at the surface of the sensor. Such changes can be attributed to ionic strength, pH, hydration and redox reactions, the latter due to the enzyme label turning over a substrate([1]).

Medical and Environmental Probes

Medical Telesensors

This "medical telesensor" chip on a fingertip can measure and transmit body temperature.

A chip on your fingertip may someday measure and transmit data on your body temperature. An array of chips attached to your body may provide additional information on blood pressure, oxygen level, and pulse rate. This type of medical telesensor, which is being developed at ORNL for military troops in combat zones, will report measurements of vital functions to remote recorders. The goal is to develop an array of chips to collectively monitor bodily functions. These chips may be attached at various points on a soldier using a nonirritating adhesive like that

used in waterproof band-aids. These medical telesensors would send physiological data by wireless transmission to an intelligent monitor on another soldier's helmet. The monitor could alert medics if the data showed that the soldier's condition fit one of five levels of trauma. The monitor also would receive and transmit global satellite positioning data to help medics locate the wounded soldier.

Detecting Cancer and Health Abnormalities

Another type of biosensor uses sophisticated technology to detect a specific trait or abnormality in a living organism. ORNL researchers have invented several biosensors of this type.

Tuan Vo-Dinh of ORNL (left) and Bergein Overholt and Masoud Panjehpour, both of Thompson Cancer Survival Center of Knoxville, have developed a new laser technique for nonsurgically determining whether tumors in the esophagus are cancerous or benign.

Of these biosensors, the most publicized is the optical biopsy sensor developed by Tuan Vo-Dinh in collaboration with medical researchers at Thompson Cancer Survival Center in Knoxville. This sensor can tell whether a tumor in the esophagus is cancerou s or benign. In the past, determining accurately whether a patient has cancer of the esophagus has required surgical biopsy. However, our laser-based fluorescence method has eliminated the need for biopsy, reducing pain and recovery time for patients.

Biosensor/Probe	Principal Investigator(s)
BaP detector using antibodies	Tuan Vo-Dinh
Biological threat detector using optical spectra	Bill Whitten
Medical telesensor ASIC	Tom Ferrell
Pressure sensor using silicone fiber	Jeff Muhs,

	Steve Allison
Calcium ion detector	Tuan Vo-Dinh
Microcantilevers using mass and vibrational frequency	Thomas Thundat, Bruce Warmack, Eric Wachter
Optical biopsy sensor for cancer detection	Tuan Vo-Dinh
Diabetes monitor using protein fluorescence	Tuan Vo-Dinh
Bacteria with luciferase that eat toluene	Robert Burlage, Larry Simpson, Tuan Vo-Dinh
Bacteria with green fluorescent protein that eat toluene	Robert Burlage, Larry Simpson, Tuan Vo-Dinh
Platinized chloroplasts	Eli Greenbaum
Microspectrometer	Slo Rajic, Chuck Egert
Lab on a chip	Mike Ramsey, Steve Jacobson
DNA analysis by mass spectrometry	Michelle Buchanan, Winston Chen, Mitch Doktycz, Greg Hurst, Scott McLuckey
Protein analysis by mass spectrometry	Michelle Buchanan, Greg Hurst,

	Scott McLuckey
Lipid signatures of bacteria	David White
Mass spectral signatures of bacteria	Bill Whitten
Lipid fingerprints of children	Michelle Buchanan
DNA analysis: in situ synthesis	Bob Foote, Mitch Doktycz Ken Beattie
DNA analysis: labeling with tin isotopes	Bruce Jacobson
DNA analysis: surface-enhanced Raman labels	Tuan Vo-Dinh, Kelly Houck, David Stokes
DNA analysis: CCD pixels and fluorescence	Tuan Vo-Dinh
Genome mapping with atomic force microscopy	Dave Allison, Mitch Doktycz, Bruce Warmack
Anthropometry	Judson Jones

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GE3451-ENVIRONMENTAL SCIENCES & SUSTAINABILITY

LECTURE NOTES

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II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

Unit – I
Environment, Ecosystems and Biodiversity

Environment:

INTRODUCTION

The word environment is coined form the French word "Environ" meaning" surround or surroundings" i.e., each and everything surrounding us. **E.g.** Lion in a forest surrounded by living and non-living things like air, water, trees, other animals etc.

DEFINITIONS

1. Environment

Environment is defined as "the sum of total of all the living and the non-living things around us influencing one another." **E.g.** Lion in a jungle surrounded by living and non-living things like air, water, trees, other animals etc.

2. Environmental Science

The study of the environment, its biotic (living) and abiotic (non-living) components and their interrelationship is called environmental science. It includes the basic concepts of physics, chemistry, geography, geology etc., which is used in understanding the structure, function and physical characteristics of environment.

3. Environmental Engineering

Environmental engineering is the application of engineering principles, science, education, ethics and law in the protection and enhancement of the quality of environment, public health and welfare.

4. Environmental Studies

Environmental studies are the process of educating the people for preserving quality environment. It is the multidisciplinary studies of science, engineering, technology and management which shows the impact of human activities on the environment.

TYPES OF ENVIRONMENT

Environment is divided into 2 types:

1. Natural Environment: Natural environment consists of natural components including all biotic (biological) and abiotic (physical) components created through a natural process without any human support.
E.g. Soil, water, air, trees, radiations, noise etc.

2. Man- made environment: Man is most powerful agent who modifies the environment using modern technologies, according to his needs for survival and well-being.
E.g. Houses, parks, hospitals, schools, roads etc.

SCOPE OF ENVIRONMENTAL STUDIES

Environmental Studies is the tool for educating people to preserve environment. Main scope includes:

1. To get awareness and sensitivity of environment and its related problems.
2. To motivate the active participation of individuals in the protection and improvement of environment.
3. To develop skills for identifying and solving environmental problems.
4. To know the necessity of conservation of natural resources.
5. To conduct environmental programmes in terms of social, economic, ecological and aesthetic factors.

IMPORTANCE/ SIGNIFICANCE/ NEED OF ENVIRONEMNTAL STUDIES

The air we breathe, water we drink, food we eat and the land we live are all polluted. There is no zero pollution.

To solve the above problems, knowledge of environment and its studies are very important.

1. To understand the concept of "need of development without destruction of environment".
2. To gain knowledge of different types of environment their various resources and the effects of different environmental hazards.
3. To inform people about their effective role in protecting the environment by demanding changes in laws and enforcement systems.
4. To develop a concern and respect for the environment.

NEED FOR PUBLIC AWARENESS

1. Necessity to maintain a natural balance, sensible planning of development in order to save humanity from extinction.
2. To check nominal use of natural resources as watch dogs informing government about the degradation of environment.
3. To educate and create awareness through mass media like tv, radio, short films, internet, mobile phones, etc.,
4. To motivate and active participation of individuals in protecting the environment from various types of pollution.

ECOSYSTEM

Definition:

Ecology

Ecology is the study of various ecosystems which is the relationship between organisms and their surroundings (living and non-living).

Ecosystem

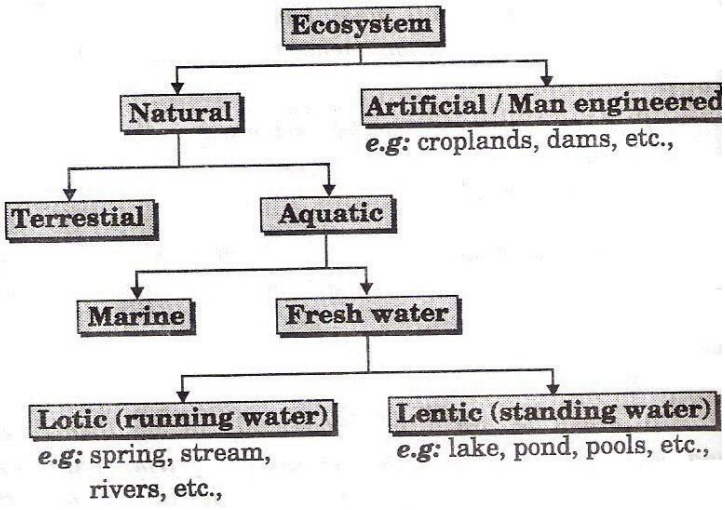
Ecosystem is the basic functional unit of ecology. It is derived from Greek word "study of home". Ecosystem is a group of organisms interacting among themselves and with the environment exchanging its energy and matter.

E.g, forest, desert etc.

Biome

Biome is a small ecosystem within another ecosystem having dominant species with similar lifestyle, climatic conditions and physical structure etc.,

TYPES OF ECOSYSTEM



STRUCTURE OR COMPONENTS OF AN ECOSYSTEM

The ecosystem has two major components (a) abiotic and (b) biotic.

(a) Abiotic Components

Non-living components of ecosystem (physical and chemical) form the abiotic community.

(i) Physical components: It includes energy, climate, raw materials and living space.

E.g. Air, water, soil, sunlight

(ii) Chemical components: They are the sources of nutrients.

(a) Organic substances E.g. proteins, lipids, carbohydrates

(b) Inorganic substances E.g. (C, N, O, P, K, H) and (Al, Co, Cu, Zn).

(b) Biotic Components

Living members in a community form the biotic community.

(i) **Autotrophic/Producers/Self-feeders**: They prepare their own food with the help of chlorophyll, sunlight, water and carbondioxide. E.g. plants, trees.

(ii)**Heterotrophic / Consumers/ other-feeders:** They lack chlorophyll and do not prepare their own food but depends on the producers for their food.

(a) Macro consumers: Herbivores, carnivores and omnivores.

(b) Micro consumers/ Saprotrophs: Decomposers (bacteria, fungi)

Classification/members of biotic components

Based on their source of food

1. **Autotrophs/ Producers**: Prepare their food through photosynthesis using chlorophyll, CO_2 and sunlight.

$6CO_2 + 12H_2O \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$

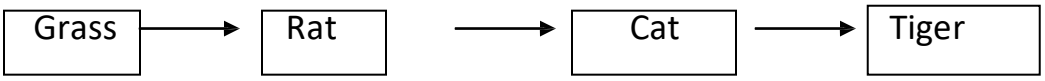
2. **Heterotrophs/Consumers**: They do not make their food but depend on producers for their food.

(A) Primary consumers/Herbivores. Depend on plants for their food. E.g. Insects, rats.

(B) Secondary consumers/Primary carnivores: They feed on primary consumers.

E.g. Frogs, cat, snakes.

(C) Tertiary consumers/Secondary carnivores: They feed on secondary consumers e.g. lions, tigers



3. Decomposers: They feed on dead plants and animals and decompose them into simpler compounds releasing inorganic nutrients. These are again utilized by plants with other organic substances for the synthesis of food.

e.g. Bacteria, fungi.

FUNCTION OF AN ECOSYSTEM

Its main function is to allow the flow of energy and nutrients.

Types of function

1. Primary function/ production: It is manufacturing of starch by photosynthesis.
2. Secondary function/ production: It is the distribution of energy to all consumers in the form of food which is stored by them.
3. Tertiary function: The dead systems (plants and animals) are decomposed by decomposers thereby initiating the third function called "cycling".

ENERGY FLOW IN THE ECOSYSTEM

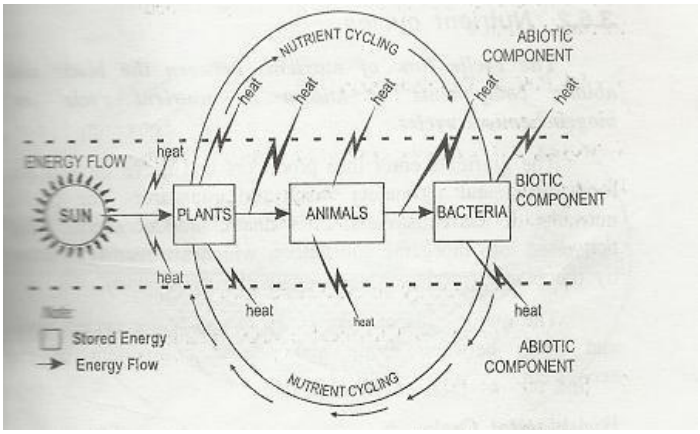
Solar energy is the main energy on earth's surface. About 1% of this is used by plants for photosynthesis. They convert this into chemical energy part of which is used for their growth and remaining is passed onto consumers.

Thus, energy enters ecosystem through photosynthesis and travels through different feeding or trophic levels at the rate of 10% and the rest 90% is lost in the form of heat. This indicates that the energy flow is greatly reduced at each trophic levels from producers to carnivores. The energy flow is unidirectional i.e energy from sun never return back to sun.

Energy flow through an atmosphere in an ecosystem is governed by laws of thermodynamics

I law of thermodynamics - Energy can neither be created nor destroyed, but it can only be converted from one form to another.

II law of thermodynamics - Whenever there is transformation of energy, there is loss of energy in the form of heat. The loss of energy takes place through respiration, running, hunting etc. Relationship between structure and function (flow model)



ECOLOGICAL SUCCESSION

The progressive replacement of one community by another till the development of a stable community in a particular area is called ecological succession.

Stages of Ecological succession

1. **Pioneer Community**:

The first group of organisms which establish their community in an area is called pioneer Community.

2. **Seres or Seral Stage:**

The various developmental stages of a community is called **'seres'**.

Community: Group of plants or animals living in an area.

Types of ecological succession

1. Primary succession: Involves gradual establishment of biotic communities on a lifeless ground.
2. (a) **Hydrarch**: Establishment starts in watery area (lake, pond)
3. (b) **Xerarch**: Establishment starts in dry land (desert, rocks)
4. Secondary succession: Involves the establishment of biotic communities in an area, where some type of biotic community is already present.

Process of ecological succession

Ecological succession takes place in the following steps:

1. **NUDATION**: It is the development of bare land without any life form.
2. **INVASION**: It is the establishment of one/more species on a bare land through migration followed by establishment.

(a) MIGRATION: Migration of seeds by wind, water and birds.

Types of food chain

1. **Grazing food chain**:

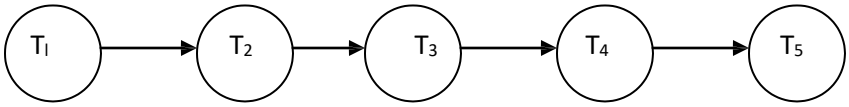
Found in grass land and pond ecosystems. It starts with green plants and goes to the decomposer/detritus food chain through herbivores and carnivores.

2. **Detritus food chain**:

Found in grassland and forest ecosystems. It starts with dead organic matter and goes to decomposer food chain through herbivores and carnivores.

Trophic levels

The various steps through which food energy passes in an ecosystem is called trophic levels.



T_1 – Green plants/producer
T_2 – Herbivores/primary consumers
T_3 – Carnivores/secondary consumers
T_4 – Tertiary consumers
T_5 — Decomposers

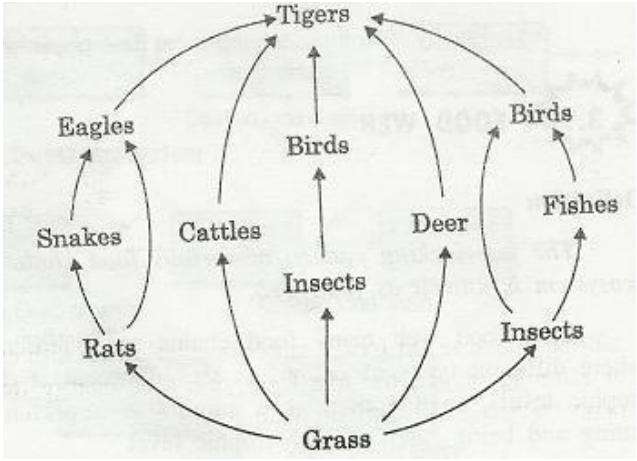
FOOD WEB

Definition: The interlocking pattern of various food chains which are linked together in an ecosystem is called food web. Different types of organisms are connected at different trophic levels so that there are number of opportunities of eating and being eaten at any trophic level.

Functions:

- Maintains the stability of ecosystems.
- Maintains the nutritional balance in an ecosystem.
- Control the population size of species in an ecosystem.
- Provide alternate food source.

Energy flow in a food web



Significance of food chains and food webs

1. Food webs and food chains play an important role in ecosystem as energy and nutrient flow takes place through them.
2. They maintain and regulate the population size of different trophic levels thereby maintaining ecological balance.
3. They have property of biomagnification. The passing of non-biodegradable material from one trophic level to another causing its concentration to increase and this is called biomagnification.

E.g., **Biomagnification of DDT**

The concentration of DDT sprayed on plants increases along the food chain through phytoplankton to zooplanktons and then goes to fish, animals and human beings. Thus concentration of DDT is magnified in birds, animals and humans damaging the egg shells in birds and cell tissues in humans. As DDT is fat soluble its accumulation in human body is easier and cannot be removed easily.

ECOLOGICAL PYRAMIDS

Definition: The graphical representation of structure and function of trophic levels of an ecosystem is called ecological pyramid.

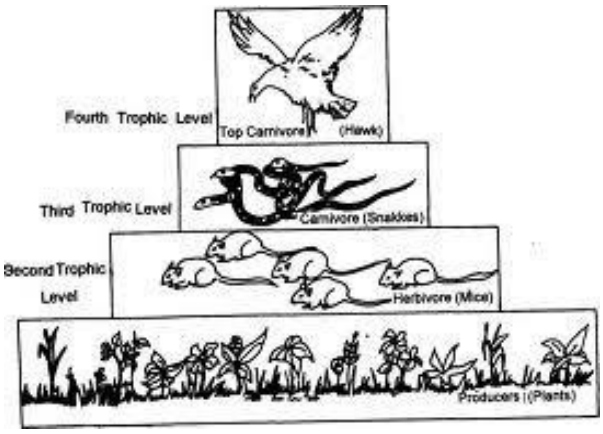
In an ecological pyramid the producers forms the base level and the tertiary consumer occupies the apex level.

Types of Ecological Pyramids

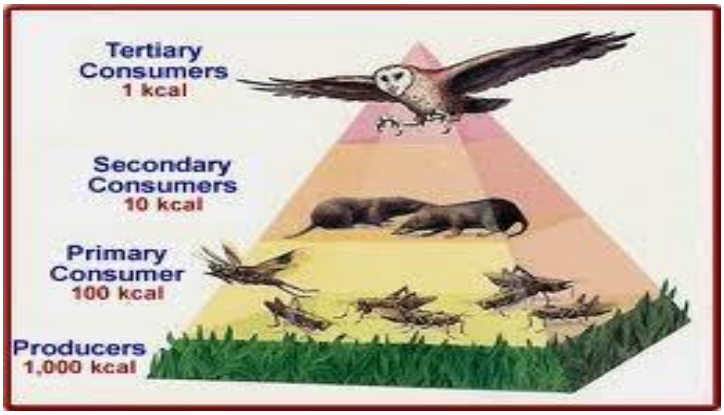
1.**Pyramids of Numbers**:

It represents the number of individual organisms present in each trophic level. e.g., Grassland ecosystem

Here, the producers are grasses which are small in size and large in numbers. So, they occupy the lower most level of the pyramid. The primary consumers (rats) occupy the second trophic level as its number is lower compared to that of grass. The secondary consumers (snakes) which are even larger in size and smaller in number form the third level. The tertiary consumers (eagles) occupy the top layer as the numbers of it is the least.

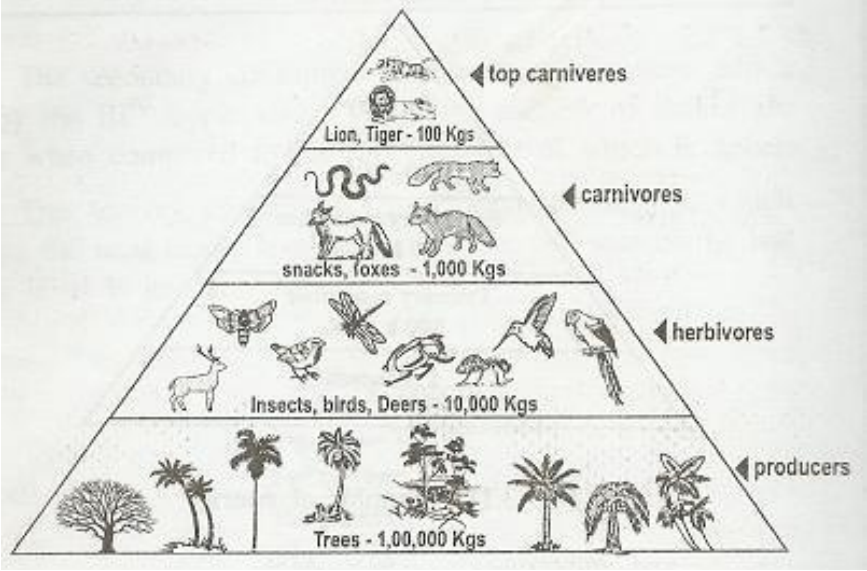


2. **Pyramids of energy**



It represents the amount of energy present in each trophic level. At every successive tropic level there is a heavy loss of energy (about 90%) in the form of heat. Thus at each next higher tropic level only 10% of energy is transferred.

3. Pyramids of energy



It represents the total amount of biomass (mass or weight of biological material or organism) present in each trophic level.

e.g., Forest ecosystem

The trees in forest ecosystem are the producers and they are maximum in number contributing to a huge biomass. The next tropic levels are the herbivores (insects, birds) and then carnivores (snakes, foxes). The topmost level is the tertiary consumers (tiger, lion) which are few and hence having low biomass.

FOREST ECOSYTEM

Forest consists of densely growing trees which cover 40% of world's land and 19% of Indian land.

Types of forests and their features

Depending on the climatic conditions forests are classified into the following types:

(a) **Tropical rain forest**: They are found near the equator. They are characterized by high temperature. Trees like teak and sandal and animals like lion and tiger are found in these forests.

(b) **Tropical deciduous forest**: They are found a little away from equator. They have warm climate and rain only during monsoon. Trees like maple, oak and animals like deer and fox are found in these forests.

(c) **Tropical shrub forest**: They have dry climate for long time. Have small deciduous trees and shrubs and animals like deer, fox, etc.

(d) **Temperate rain forest**: They are found in temperate areas with adequate rain.Coniferous trees like fir, pines and animals like squirrels, fox are found here.

(e) **Temperate deciduous forest**: These are found in areas with moderate temperatures. Trees like oak, hickory and animals like deer, fox are found in these forests.

Characteristics of forests ecosystem

1. They have warm climate and adequate rainfall, which generates number of ponds, lakes etc.
2. Forests maintain rainfall and climate.
3. Forests support many wild animals and protect biodiversity.

4. Soil is rich in nutrients and organic matter which support the growth of trees.
5. As sunlight penetration is poor, conversion of organic matter into nutrients is very fast.

Structure and function of forest ecosystem

1. **Abiotic components**: e.g., Temperature, light, rain and minerals.

They are the inorganic and organic substances found in soil and atmosphere.

2. **Biotic components**:

(a) **Producers:** Trees, shrubs

(b) **Consumers:**

(i) Primary consumers: Insects

(ii) Secondary consumers: Birds, snakes

(iii) Tertiary consumers: Tiger, lion

(c) **Decomposers**: Bacteria, fungi. Rate of decomposition of dead matter in tropical and subtropical forest is more than in temperate forest.

GRASSLAND ECOSYSTEM

Grasslands are large areas of grass with scattered trees which occupies about 20% of world's land.

1.19.1 Types and features of grasslands

1. **Tropical Grassland**: they are found near borders of tropical rain forests. They have high temperature and moderate rainfall (40 to 100cm). They are also called Savanna-type. They have tall grasses and shrubs and animals like zebra, giraffe.

2. **Temperate grassland**: They are found in centres of continents, on flat, sloped hills. They have cold winters and hot summers. Intense grazing and summer fires do not allow shrubs or trees to grow in this grassland.

3. **Polar grassland**: They are found in arctic polar region and have severe cold and strong wind with snow and ice. In summers several annual plants grow and animals like arctic wolf, arctic fox is found here.

Characteristics of grassland ecosystem

1. Grassland ecosystem is plain land occupied by grasses.
2. Soil is rich in nutrients and organic matter.
3. Since there are tall grasses, it is an ideal place for grazing animals.
4. It is characterized by low or even rainfall.

Structure and function of grassland ecosystem

1. **Abiotic components**: e.g., Nutrients (C, H, O ,N ,P , S) supplied by CO_2, H_2O, nitrates, phosphates and sulphates.

2**. Biotic components**:

(a) **Producers**: Grasses, shrubs

(b) **Consumers**:

(i) Primary consumers: Cows, deer

(ii) Secondary consumers: Snakes, lizards

(iii) Tertiary consumers: Eagles

(c) **Decomposers:** Fungi and bacteria.

DESERT ECOSYSTEM

Deserts occupy about 35% of world's land. The atmosphere is dry and hence a poor insulator.

Types and features of desert

1. **Tropical desert**: They are found in

(i) Africa: Sahara desert

(ii) India: Thar desert

They have few species and wind-blown sand dunes are common.

2. **Temperate desert**: They are found in

South California: Majave desert

They have very hot summer and very cool winter.

3. **Cold desert:** They are found in China: Gobi desert

They have cold winters and warm summers.

Characteristic features of desert ecosystem

1. The desert air is dry and climate is hot.
2. Annual rainfall is less than 25cm.
3. The soil is poor in nutrients and organic matter.
4. Vegetation is poor.

Structure and function of desert ecosystem

1. **Abiotic components**: Temperature, rainfall, sunlight

The temperature is very high and rainfall and nutrient cycling are very low.

2. **Biotic components:**

(a) **Producers**: Shrubs, bushes, some grass

In desert there are succulent plants like cacti which have water inside them and waxy outer coating to protect form sun.

(b) **Consumers**: Squirrels, mice, reptiles.

These animals dig holes in the ground to live and come out at night for food. Most of the desert animals can extract water from seeds.

(c) **Decomposers**: Fungi and bacteria

Desert has poor vegetation with low amount of dead organic matter. They are decomposed by few bacteria and fungi.

AQUATIC ECOSYSTEM

This deals with water bodies. The major types of organisms found in aquatic environments are determined by water salinity.

Types of aquatic ecosystems: Based on the salinity it is classified into 2 types as

(i) Fresh water ecosystem: Ponds, lakes, rivers, streams

(ii) Marine/ salt water ecosystem: Oceans, estuaries

1. Fresh water ecosystem:

POND ECOSYSTEM

Characteristic features of pond ecosystem

1. Pond is temporary, only seasonal.
2. It is stagnant fresh water body.
3. Pond gets polluted easily due to limited amount of water.

Structure and Function of Pond ecosystem

1. **Abiotic components**: Temperature, light, water, organic and inorganic compounds.

2. **Biotic components**:

(a) **Producers**: They are of 2 types

(i) ***Phytoplankton***: These are microscopic aquatic plants, which freely float on the water surface. e.g., Algae, pandorina.

(ii) ***Microphytes:*** These are large floating plants and submerged plants. e.g., Hydrilla, wolfia.

(b) **Consumers**: (i) **Primary consumers** (Zooplanktons): These are microscopic animals which float freely on the water surface. e.g., Protozoa, very small fish, ciliates.

Zooplanktons are found along with phytoplankton sans they feed on them.

(ii) **Secondary consumers** (Carnivores): Insects like water beetles and small fish.

(iii) **Tertiary consumers:** Large fish like game fish.

(c) **Decomposers**: Fungi, bacteria, flagellates.

LAKE ECOSYSTEM

Lakes are supplied water by rainfall, melting snow and streams.

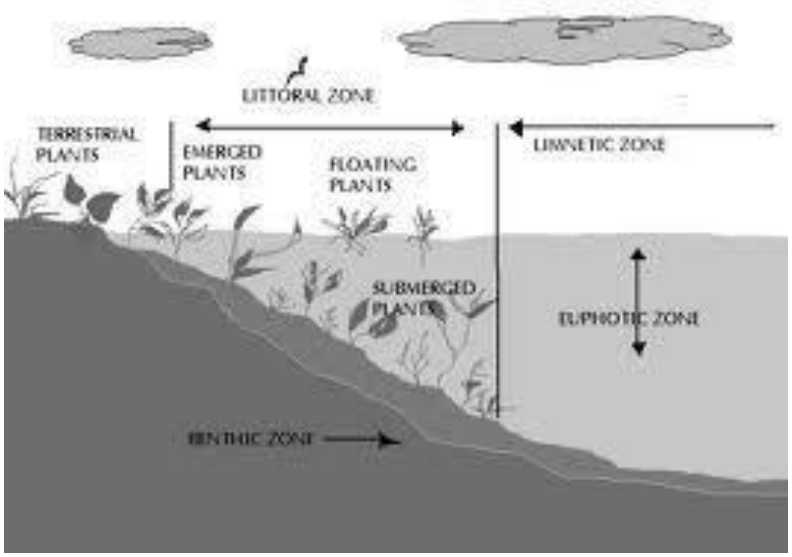
Types of lakes

1. Oligotrophic lakes: They have low nutrient concentrations.
2. Eutrophic lakes: They are over nourished by nutrients like N and P.
3. Dystrophic lakes: They have low pH, high humic content and brown waters.
4. Volcanic lakes: They receive water from magma after volcanic eruptions.
5. Meromictic lakes: They are rich in salts.
6. Artificial lakes: They are created due to construction of dams.

Zones of lake

Depending upon their distance from the shore, a lake consists of 4 distinct zones.

1. **Littoral zone**: It is the top layer of the lake. It has shallow water.
2. **Limnetic zone**: It lies below the littoral zone, where effective penetration of sunlight takes place.
3. **Profundal zone**: This is the deep open water, where it is too dark.
4. **Benthic zone**: This layer is the bottommost layer of the lake.



Characteristic feature of lake ecosystem

1. Lake is shallow fresh water body.
2. It is a permanent water body with large water resources.
3. It is useful for irrigation and drinking purpose.

Structure and function of Lake Ecosystem

1. **Abiotic components**: Temperature, light, proteins and lipids.
2. **Biotic components:**

(a) **Producers**: These are the green plants which may be submerged, free floating and amphibious plants. e.g., Phytoplankton, algae

(b) **Consumers:**

(i) Primary consumers (Zooplankton): Ciliates, protozoans.

(ii) Secondary consumers (Carnivores): Insects and small fishes.

(iii) Tertiary consumers: Large fish like game fish.

(c) **Decomposers:** Bacteria and fungi

RIVER/STREAM ECOSYSTEM

The running water of a river or stream is well oxygenated, because it absorbs oxygen from air. The numbers of animals are low in river or stream.

Characteristic features of river or stream ecosystem

1. It is fresh water and free flowing water system.
2. Due to mixing of water, dissolved oxygen content is more.
3. River deposits large amount of nutrients.

Structure and function of river ecosystem

1. **Abiotic components**: Temperature, light, pH, nutrients.
2. **Biotic components**:

(a) **Producers**: Phytoplankton, algae, water grasses.

(b) **Consumers**:

(i) Primary consumers: Water insects, snails

(ii) Secondary consumers: Birds and mammals

(c) **Decomposers**: Bacteria, fungi

MARINE/OCEAN ECOSYSTEM

Oceans cover more than two thirds of earth's surface. It supplies a vast variety of sea products and drugs. It has high concentration of salts and minerals.

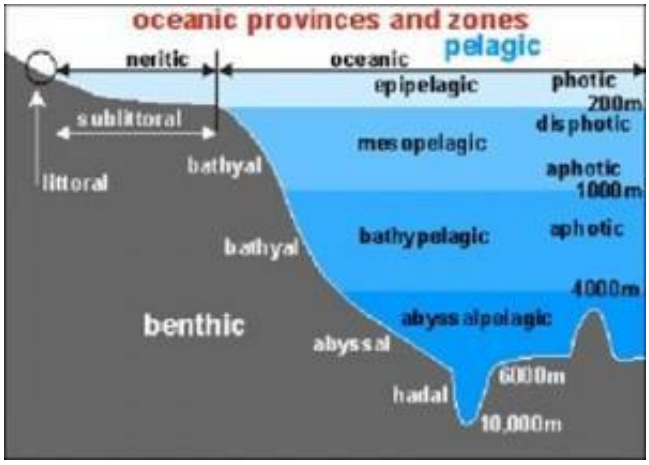
Zones of Oceans

The oceans have two major life zones.

(i) **Coastal zone**: It is relatively nutrient rich, shallow water and has high productivity because of high nutrients and sunlight.

(ii) **Open sea**: It is the deeper part of the ocean and is vertically divided into 3 regions. (a) *Euphotic zone*: It receives abundant light and shows high photosynthetic activity. (b) *Bathyal zone*: It receives dim light and is geologically active.

(c) *Abyssal zone*: It is the dark zone and is very deep (2000 to 5000 m).



Characteristic features of marine ecosystem

1. It occupies a large surface area with saline water.
2. Since, ships, submarines can sail in ocean a large number of commercial activities are carried out.
3. It is rich in biodiversity.
4. It moderates the temperature of earth.

Structure and function of marine ecosystem

1. **Abiotic components**: Temperature, light, NaCl, KCl.
2. **Biotic components**:

(a) **Producers**: Phytoplankton and marine plants

(b) **Consumers**:

(i) Primary consumers (Herbivores): Crustaceans, molluscs (ii) Secondary consumers (Carnivores): Herring, mackerel (iii) Tertiary consumers: Cod

(c) **Decomposers**: Bacteria, fungi.

ESTUARINE ECOSYSTEM

"***An estuary is a partially enclosed coastal area at the mouth of a river which joins the sea***".

They are rich in nutrients and have high food potential.

Characteristics of estuarine ecosystem

1. Estuaries are transition zones, which are strongly affected by tides of sea.
2. Water characteristics are periodically changed.
3. The living organisms here have wide tolerance.
4. Salinity in estuaries are highest in summer and lowest in winter.

Structure and function of estuarine ecosystem

1. **Abiotic components**: Temperature, pH, sodium and potassium salts.
2. **Biotic components**:

(a) **Producers**: Marsh grasses, sea weeds, sea grasses.

(b) **Consumers**: Oysters, Crabs, small fishes.

(c) **Decomposers**: Bacteria, fungi.

KEYSTONE SPECIES

Within a habitat each species connects to and depends on other species. But, while each species contribute to habitat functioning, some species do more than others in the overall scheme of things. Without the work of these key species, the habitat changes significantly. These species are called “keystone species". When a keystone species disappears from its habitat, that habitat

changes dramatically.

Illustration – 1

Elephants as keystone species in Grasslands

Elephants are keystone species in African grasslands. When elephants are taken away from grasslands, it is converted into forest or shrub areas by overgrowth of woody plants. As keystone species, elephants prevent this conversion.

Illustration – 2

Forest elephants hold keystone status in some woodland (forest) in western Africa

In the above forest elephants are the only species large enough to eat and disperse the seeds of some plant species whose shells are very hard. Thus only elephants can feed on them and disperse the seeds through their dung thereby maintains the forest.

Introduction to biodiversity definition: genetic, species and ecosystem diversity:

BIODIVERSITY DEFINITION*:* Bio means 'life' and diversity means 'variety', hence Biodiversity refers to variety of life on the earth. Planet earth (biosphere) contains more than 20 million species of organisms. They differ widely from one another. Diversification in the species is influenced by various physical and climatic factors, resulting in the production of new sub-species. Biodiversity is defined as, "the variety and variability among all groups of living organisms and the ecosystem in which they occur".

LEVELS OF BIODIVERSITY:

A. GENETIC BIODIVERSITY

The genes found in organisms can form enormous number of combinations each of which gives rise to some variability. When the genes within the same species show different version due to new combinations, it is called genetic variability. For example rice belongs to the species Oryzasativa which has many varieties that differ in size, shape, aroma etc.

B. SPECIES BIODIVERSITY

This is the variability found within the population of a species or between different species of a community. It broadly represents the species richness and their abundance in a community. Shannon Wiener index and Simpson index are two popular indices of measuring species diversity.

C. ECOSYSTEM BIODIVERSITY

This is the diversity of ecological complexity showing variations in ecological niche, trophic structure, food webs, nutrient cycling etc. The ecosystem also shows variations with respect to physical parameters like moisture, temperature, altitude, precipitation etc.

BIOGEOGRAPHICAL CLASSIFICATION OF INDIA:

S. No.	Biogeographic zone	Biotic province	Important Flora & Fauna
1	Trans-Himalayan	Upper region	Pine,deodar- Wild sheep, yak, leopard, wolf
2	Himalayan	North west, west, central and East Himalayas	Pine, cork tree, sal, dhaak- Wild bear, sambar, leopard, Sikkim stag, musk deer

3	**Desert**	**Kutch, Thar and Ladkh**	**Acacia, zizyphus, khejri, date palm- Camel, bastard, wild ass, desert cat, fox, rat**
4	**Semi-arid**	**Central India, Gujarat**	**Acacia, date palm, peepal -Gir lion, tiger, sariska and Ranthampore tiger**
5	**Western ghats**	**Malabar coast Western ghats mountain**	**Sheeshan, peepal, tuna, bahera- Tortoise, frog, lizards, snakes**
6	**Deccan peninsula**	**Deccan plateau**	**Acacia, palaash, tuna, pine, castor- Sambar, sloth bear, tiger, cheetal, four horned stag, wild elephant, wild buffalo**
7	**Gangetic plain**	**Upper and lower Gangetic plain**	**Sal, acacia, jamun, mango, bael- black chinkara, stag, rhinoceros, gazzel, Aligator, turtle**
8	**North-east India**	**Brahmaputra valley**	**Bamboo, sal, jack fruit, tuna, Chestnut cator- Elephnat, Rhinocers, yak, deer, porcupine**
9	**Islands**	**Andaman islands, Nicobar islands & Lakshadeep islands**	**Bahera, Harar, jack fruit, cardamom, coconut, cloves- Dolphin, alligator, Molluscs**
10	**Coasts**	**West coast East coast**	**Coconut, Banana, cashew Nut – Dugong, Dolphin, Turtle, Alligator, Molluscs**

VALUE OF BIODIVERSITY: (Consumptive use, Productive use, Social, Ethical, Aesthetic and Option values)

1. CONSUMPTIVE USE VALUE

- **Food:** A large number of wild plants are consumed by human beings as food. About 80,000 plants are from wild. About 90% of crops are domesticated from tropical forest.
- **Drugs and medicine**: About 75% of population depends upon plant or plant extracts for medicine. Penicillin antibiotic drug is derived from the fungus penicillium.
- **Fuel:** The fossil fuels coal, petroleum and natural gas are products of fossilized biodiversity.

2. PRODUCTIVE USE VALUE

These are the commercially usable values where the product is marketed and sold. It may include lumber or wild gene resources that can be traded for use by scientist for introducing desirable traits in the crops and domesticated animals. It includes animal products like tusk of elephants, musk deer, silk from silk worm, wool from sheep, fur of many animals etc. Many industries like paper and pulp. Silk, textile, ivory works industry depend on them.

3. SOCIAL VALUE

It is associated with social life, customs, and religion and psycho-spiritual aspects of the people. Many plants are considered holy and sacred in our country like tulsi, peepal, Mango, Lotus,

Bael etc. many animals like cow, snake, peacock, bull, owl etc also have significant place in social importance. The tribal people are very closely linked with the wildlife in the forest.

4. ETHICAL VALUE

It is otherwise called existence value. It involves ethical issues like "all life must be preserved" and "live and let live" concept. For the survival of human race, all biodiversity has to be protected because biodiversity is valuable.

5. AESTHETIC VALUE

People from far and wide spend a lot of time and money to visit wilderness areas where they can enjoy the aesthetic value of biodiversity and this type of tourism is known as eco –tourism. The willingness to pay concept annually generates 12 billion revenue.

6. OPTION VALUE

It is the value of knowing that there are biological resources existing on the biosphere that may one day prove to be an effective option for something important in the future it suggests that any species may prove to be miracle species someday.

BIODIVERSITY AT GLOBAL, NATIONAL AND LOCAL LEVEL:

BIODIVERSITY AT GLOBAL LEVEL:

It is estimated that there are about 20 million species of plants and animals in earth of which only 1.6 million species have been formally identified with 34 hotspot regions at the global level. There are 12 megadiversity nations which are highly rich in biodiversity which includes India. Most of the world's biodiversity are near the equator especially tropical rain forests and coral reefs. South America also has unique species and biodiversity.

BIODIVERSITY AT NATIONAL LEVEL:

India is rich in biodiversity due to its varying climate and topographical features. It occupies only 2.5% of global land of which about 40% is under cultivation. There are 96 national parks, 572 wildlife sanctuaries 14 biosphere reserves and 2 hotspots with 46,000 plant species and 91,000 animal species, 50,000 varieties of rice, 1000 varities of mango, etc.,

1. India ranks 10^{th} among the plant rich countries of the world
2. 11^{th} in terms of Endemic species.
3. 6^{th} among origin of agricultural crops.
4. 12^{th} mega biodiversity country in the world.

BIODIVERSITY AT REGIONAL OR LOCAL LEVEL:

Tamilnadu is rich in biodiversity with natural habitat constituting 4% of country's total area which shares the Western Ghats with Kerala, Karnataka, Maharashtra, Goa and, Eastern Ghats with Andhra Pradesh and Odisha accounting for nearly about one third of the total flora of India.

1. **Point Richness**: Refers to number of species at a single point.
2. **Alpha Richness**: Refers to the number of species found in a small homogeneous area.
3. **Beta Richness**: Refers to rate of change in species composition across different habitats.
4. **Gamma Richness**: Refers to the rate of change across large landscape gradients.

INDIA AS A MEGA BIODIVERSITY NATION:

India is one of the 12 mega biodiversity countries in the world. The Ministry of environmental and forests, Government of India (2000) records 47,000 species of plants and 81,000 species of animals which is about 7% and 6.5% respectively of global flora and fauna.

1. **Endemism**: Species which are restricted to only to a particular area are known as endemic. India shows a good number of endemic species. About 62% of amphibians and 50% of lizards are endemic.
2. **Centre of origin**: A large number of species have known to originate in India. Nearly 5000 flowering species, 166 species of crop plants and 320 species of wild relatives of cultivated crops origin in India.
3. **Marine diversity**: Along 7500 km long coastline of our country in the mangroves, estuaries, coral reefs, back waters etc. there exist a rich biodiversity. More than 340 species of corals of the world are found here.

HOTSPOTS OF BIODIVERSITY

A **biodiversity hotspot** is a biogeographic region with a significant reservoir of biodiversity that is under threat from humans. To qualify as a biodiversity hotspot on Myers 2000 edition of the hotspot- map, a region must meet two strict criteria:

1. It must contain at least 0.5% or 1,500 species of vascular plants as endemics.
2. It must have lost at least 70% of its primary vegetation.

Around the world, at least 25 areas qualify under this definition, with nine others possible candidates. These sites support nearly 60% of the world's plant, bird, mammal, reptile, and amphibian species, with a very high share of endemic species.

The importance of biodiversity: Biodiversity is often used to draw attention to issues related to the environment. It can be closely related to:

- The health of ecosystems.

For example, the loss of just one species can have different effects ranging from the disappearance of the species to complete collapse of the ecosystem itself. This is due to every species having a certain role within an ecosystem and being interlinked with other species.

- The health of mankind.

Experiencing nature is of great importance to humans and teaches us different values. It is good to take a walk in the forest, to smell flowers and breathe fresh air. More specifically, natural food and medicine can be linked to biodiversity.

Hot spots of Biodiversity in India:

The hot spots of biodiversity are the geographic areas which possess the high endemic species. At the global level these are the areas of high conservation priority, if these species are lost they can never be replaced or regenerated.

Criteria for recognizing Hotspots: The richness of the endemic species is the primary criterion; they should have a significant percentage of specialized species; the site should be under threat and should contain important gene pools of plants of potential use.

Two hot spots in India are:

1. Eastern Himalayas (Indo-Burma region) and
2. Western Ghats (Srilanka region).

Eastern Himalayas: Comprises of Nepal, Bhutan and neighboring states of Northern India- 35,000 plant species are found here and 30 % are endemic – also rich in wild plants of economic value eg. Rice, banana, citrus, ginger, chilli, jute and sugarcane – Taxal yielding plant also scarcely distributed – 63% mammals are from this region- 60% of Indian Birds- huge wealth of fungi, insects, mammals and birds found in this region

Western Ghats: Comprises of parts of Maharashtra, Karnataka, Tamilnadu and Kerala – nearly 1500 endemic, dicotyledones 62% amphibians and 50% lizards are endemic here- Ternstroemia, Japonica, Rhododendron and Hypericum common plants- Blue Bird and Lizard hawk are common animals.

Biodiversity is the richness & varied species of different organisms contained in a particular ecosystem – Indian biodiversity is highly diverse and rich such that there are various hot spots. However there are numerous threats to our Biodiversity.

THREATS TO BIODIVERSITY :(Habitat loss, Poaching of wildlife & Man-wildlife conflicts)

In 2006 many species were formally classified as rare or endangered or threatened; moreover, scientists have estimated that millions more species are at risk which has not been formally recognized. About 40 percent of the 40,177 species assessed using the IUCN Red List criteria are now listed as threatened with extinction.

LOSS OF HABITAT:

***Habitat destruction*:**

Habitat destruction has played a key role in extinctions, especially related to tropical forest destruction. Factors contributing to habitat loss are: overpopulation, deforestation, pollution (air pollution, water pollution, soil contamination) and global warming or climate change. Habitat size and numbers of species are systematically related. Physically larger species and those living at lower latitudes or in forests or oceans are more sensitive to reduction in habitat area.

Climate change:

Global warming is also considered to be a major potential threat to global biodiversity in the future. Climate change has seen many claims about potential to affect biodiversity but evidence supporting the statement is tenuous. Increasing atmospheric carbon dioxide certainly affects plant morphology and is acidifying oceans, and temperature affects species ranges, phenology, and weather, but the major impacts that have been predicted are still just *potential* impacts. We have not documented major extinctions yet, even as climate change drastically alters the biology of many species.

Poaching: Illegal trade of wildlife products by killing prohibited endangered animals i.e. poaching is another threat to wildlife. Despite international ban on trade in products from endangered species, smuggling of wildlife items like furs, hides, horns, tusks, live specimens and herbal products worth millions of dollars per year continues. The developing nations in Asia, Latin America and Africa are the richest source of biodiversity and have enormous wealth in wildlife.

Overexploitation:

Overexploitation occurs when a resource is consumed at an unsustainable rate. This occurs on land in the form of overhunting, excessive logging, poor soil conservation in agriculture and the illegal wildlife trade Joe Walston, director of the Wildlife Conservation Society's Asian programs, called the latter the "single largest threat" to biodiversity in Asia. The international trade of endangered species is second in size only to drug trafficking.

MAN-WILDLIFE CONFLICTS:

CAUSES OF MAN WILDLIFE CONFLICT:

1. Dwindling habitats of elephants, Tigers, rhinos and bears due to forest shrinkage compels them to move outside forest.
2. Usually ill, weak, and injured animals have a tendency to attack the humans.
3. Earlier Forest department used to cultivate paddy, sugarcane within the sanctuaries, due to lack of such practices the animals move out of forest food.
4. Villagers put Electric Wiring around their crop field which injures the elephants and turn them violent.
5. Wildlife corridors have been disrupted which makes the animals attack human beings during their migration.

REMEDIAL MEASURES TO CURB THE CONFLICT:

1. Tiger conservation Project (TCP) has made provisions for making available vehicles, tranquillizer guns, binoculars and radio sets etc., to tactfully deal with any imminent danger.
2. Adequate crop compensation and cattle compensation scheme must be started.
3. Solar powered fencing should be provided to prevent animals from straying into fields.
4. Cropping pattern should be changed near the border.
5. Wildlife corridors should be provided.

Introduced and invasive species:

Barriers such as large rivers, seas, oceans, mountains and deserts encourage diversity by enabling independent evolution on either side of the barrier, via the process of allopatric speciation. The term invasive species is applied to species that breach the natural barriers that would normally keep them constrained. Without barriers, such species occupy new territory, often supplanting native species by occupying their niches, or by using resources that would normally sustain native species.

Genetic pollution:

Endemic species can be threatened with extinction through the process of genetic pollution, i.e. uncontrolled hybridization, introgression and genetic swamping. Genetic pollution leads to homogenization or replacement of local genomes as a result of either a numerical and/or fitness advantage of an introduced species. Hybridization and introgression are side-effects of introduction and invasion.

Hybridization, genetic pollution/Erosion and food security

In agriculture and animal husbandry, the Green Revolution popularized the use of conventional hybridization to increase yield. Often hybridized breeds originated in developed countries and were further hybridized with local varieties in the developing world to create high yield strains resistant to local climate and diseases. Local governments and industry have been pushing hybridization. Formerly huge gene pools of various wild and indigenous breeds have collapsed causing widespread genetic erosion and genetic pollution. This has resulted in loss of genetic diversity and biodiversity as a whole.

ENDANGERED AND ENDEMIC SPECIES OF INDIA:

1. ENDANGERED SPECIES OFINDIA

The international Union for conservation of Nature and Natural Resources (IUCN) publishes the red Data book which includes the list of endangered species of plants and animals.

S.No.	Species	Names
1	Reptiles	Gharial, green sea turtle, tortoise,python
2	Birds	Great Indian bustard, Peacock, Pelican, Great Indian hornbill, Siberian White crane
3	Carnivors Mammals	Indian Wolf, red fox, sloth bear, red panda, tiger, leopard, Stripped Hyena, Indian lion, Golden cat, desert cat, Dugong
4	Primates	Hoolock Gibbon, lion tailed Macaque, Nilgiri languor, capped monkey, Golden monkey
5	Plants	A large number of species of Orchids, Rhododendrons, Medicinal Plants like Rauvolfia serpentine, the sandal wood tree santalum, Cycasbeddonei

2. ENDEMIC SPECIES OFINDIA:

India has two biodiversity hotspots and thus possesses a large number of endemic species. Out of about 47,000 species of plants in our country 7000 species are endemic. Thus, Indian subcontinent has about 62% endemic flora, restricted mainly to Himalayas, Khasi Hills and WesternGhats. Some of the endemic flora includes orchids and species like *Sapria Himalaya*, *Uvarialurdia*

A large number out of total 81,000 species of animals in our country is endemic. The Western Ghats are particularly rich in amphibians and reptiles. About 62% Amphibians and 50% lizards are endemic to Western Ghats. Different species of Monitor lizards, reticulated python and Indian salamander and viviparous toad are some important endemic species of our country.

CONSERVATION OF BIODIVERSITY (In-situ conservation & Ex-situ conservation)

In-situ and ex-situ conservation along with their merits and limitations:

Conservation of Biodiversity: Biodiversity faces threat of extinction – due human activities – to salvage situation – conservation of biodiversity need of the hour- to preserve biodiversity to prevent their extinction and future flourishing – conservation of Biodiversity required

In-situ conservation: Involves allocating large areas of the land mass for wild life development- such areas can be closed to the public for tourism – wild life can be allowed to flourish in their own environment- promotes genetic diversity- does not stagnate the gene pool

Advantages: cheap and convenient method Species gets adjusted the natural disasters like drought, floods, forest fires.

Limitations: Large surface area of the earth required – shortage of staff and pollution may lead to improper maintenance of the habitat.

Ex-situ conservation: Involves conservation of wild life in zoos, botanical gardens-human supervision- wildlife can grow under controlled conditions - animals would be properly taken care- food, shelter and water- help in the flourishing of endangered species- possible the gene pool could stagnate and result in no genetic diversity taking place.

Advantages: Special care and attention lead to survival of endangered species– In captive breeding, animals are assured food, water, shelter and security - hence longer life span- it is carried out for the endangered species, which do not have any chances of survival in the wild.

Limitations: Expensive method- freedom of wild life is lost – animals cannot survive in such confined places.

UNIT II NATURAL RESOURCES

Forest resources, Water resources, Mineral resources, Food resources, Energy resources, Land resources, equitable use of resources, resource conservation, Sustainable development.

Natural resources can be defined as the resources that exist (on the planet) independent of human actions. These are the resources that are found in the environment and are developed without the intervention of humans. Common examples of natural resources include air, sunlight, water, soil, stone, plants, animals, and fossil fuels. The natural resources are naturally occurring materials that are useful to man or could be useful under conceivable technological, economic or social circumstances or supplies drawn from the earth supplies such as food, building and clothing materials, fertilizers, metals, water, and geothermal power. For a long time, natural resources were the domain of the natural sciences. There are various methods of categorizing natural resources. These include the source of origin, stage of development, and by their renewability. On the basis of origin, natural resources may be divided into two types:

- **Biotic** — Biotic resources are obtained from the biosphere (living and organic material), such as forests and animals, and the materials that can be obtained from them. Fossil fuels such as coal and petroleum are also included in this category because they are formed from decayed organic matter.
- **Abiotic** – Abiotic resources are those that come from non-living, non-organic material. Examples of abiotic resources include land, fresh water, air, rare-earth elements, and heavy metals including ores, such as gold, iron, copper, silver, etc.

Considering their stage of development, natural resources may be referred to in the following ways:

- **Potential resources** — Potential resources are those that may be used in the future—for example, petroleum in sedimentary rocks that, until drilled out and put to use remains a potential resource
- **Actual resources** — Those resources that have been surveyed, quantified and qualified, and are currently used in development, such as wood processing, and are typically dependent on technology
- **Reserve resources** — The part of an actual resource that can be developed profitably in the future
- **Stock resources** — Those that have been surveyed, but cannot be used due to lack of technology—for example, hydrogen

On the basis of recovery rate, natural resources can be categorized as follows:

Renewable resources — Renewable resources can be replenished naturally. Some of these resources, like sunlight, air, wind, water, etc. are continuously available and their quantities are not noticeably affected by human consumption. Though many renewable resources do not have such a rapid recovery rate, these resources are susceptible to depletion by over-use. Resources from a human use perspective are classified as renewable so long as the rate of replenishment/recovery exceeds that of the rate of consumption. They replenish easily compared to non-renewable resources.

Non-renewable resources – Non-renewable resources either form slowly or do not naturally form in the environment. Minerals are the most common resource included in this category. From the human perspective, resources are non-renewable when their rate of consumption exceeds the rate of replenishment/recovery; a good example of this are fossil fuels, which are in this category because their rate of formation is extremely slow (potentially millions of years), meaning they are considered non-renewable. Some resources naturally deplete in amount without human interference, the most notable of these being radio-active elements such as uranium, which naturally decay into heavy metals. Of these, the metallic minerals can be re-used by recycling them, but coal and petroleum cannot be recycled. Once they are completely used they take millions of years to replenish.

FOREST RESOURCES

Forests are one of the most important natural resources on this earth. Covering the earth like a green blanket these forests not only produce innumerable material goods, but also provide several environmental services which are essential for life. About 1/3rd of the world's land area is forested which includes closed as well as open forests. Former USSR accounts for about a 5^{th} of the world's forests, Brazil for about a 7th and Canada and USA each for 6-7%. But it is a matter of concern that almost everywhere the cover of the natural forests has declined over the years. The greatest loss occurred in tropical Asia where one third of the forest resources have been destroyed.

Commercial uses: Forests provide us a large number of commercial goods which include timber, firewood, pulpwood, food items, gum, resins, non-edible oils, rubber, fibers, lac, bamboo canes, fodder, medicine, drugs and many more items, the total worth of which is estimated to be more than $ 300 billion per year. Half of the timber cut each year is used as fuel for heating and cooking. One third of the wood harvest is used for building materials as lumber, plywood and hardwood, particle board and chipboard. One sixth of the wood harvest is converted into pulp and used for paper industry. Many forest lands are used for mining, agriculture, grazing, and recreation and for development of dams.

Ecological uses: While a typical tree produces commercial goods worth about $590 it provides environmental services worth nearly $196, 250. The ecological services provided by our forests may be summed up as follows:

- **Production of oxygen:** The trees produce oxygen by photosynthesis which is so vital for life on this earth. They are rightly called as earth's lungs.
- **Reducing global warming:** The main greenhouse gas carbon dioxide (CO2) is absorbed by the forests as a raw material for photosynthesis. Thus forest canopy acts as a sink for CO2 thereby reducing the problem of global warming caused by greenhouse gas CO2.
- **Wild life habitat:** Forests are the homes of millions of wild animals and plants. About 7 million species are found in the tropical forests alone.
- **Regulation of hydrological cycle:** Forested watersheds act like giant sponges, absorbing the rainfall, slowing down the runoff and slowly releasing the water for recharge of springs. About 50-80 %of the moisture in the air above tropical forests comes from their transpiration which helps in bringing rains.

- **Soil Conservation:** Forests bind the soil particles tightly in their roots and prevent soil erosion. They also act as wind-breaks.
- **Pollution moderators:** Forests can absorb many toxic gases and can help in keeping the air pure. They have also been reported to absorb noise and thus help in preventing air and noise pollution.

Over Exploitation of Forests

Since time immemorial, humans have depended heavily on forests for food, medicine, shelter, wood and fuel. With growing civilization the demands for raw material like timber, pulp, minerals, fuel wood etc., shot up resulting in large scale logging, mining, road-building and clearing of forests. Our forests contribute substantially to the national economy. The international timber trade alone is worth over US $ 40 billion per year. Excessive use of fuel wood and charcoal, expansion of urban, agricultural and industrial areas and overgrazinghave together led to over-exploitation of our forests leading to their rapid degradation.

Major Causes of Deforestation

- **Shifting cultivation:** There are an estimated 300 million people living as shifting cultivators who practice slash and burn agriculture and are supposed to clear more than 5 lakh ha of forests for shifting cultivation annually. In India, we have this practice in North- East and to some extent in Andhra Pradesh, Bihar and M.P which contribute to nearly half of the forest clearing annually.
- **Fuel requirements:** Increasing demands for fuel wood by the growing population in India alone has shot up to 300-500 million tons in 2001 as compared to just 65 million tons during independence, thereby increasing the pressure on forests.
- **Raw materials for industrial use:** Wood for making boxes, furniture, railway-sleepers, plywood, match-boxes, pulp for paper industry etc. have exerted tremendous pressure on forests. Plywood is in great demand for packing tea for Tea industry of Assam while fir tree wood is exploited greatly for packing apples in J&K.
- **Development projects:** Massive destruction of forests occur for various development projects like hydroelectric projects, big dams, road construction, mining etc.
- **Growing food needs:** In developing countries this is the main reason for deforestation. To meet the demands of rapidly growing population, agricultural lands and settlements are created permanently by clearing forests.
- **Overgrazing:** The poor in the tropics mainly rely on wood as a source of fuel leading to loss of tree cover and the cleared lands are turned into the grazing lands. Overgrazing by the cattle leads to further degradation of these lands.

Major Consequences of Deforestation: Deforestation has far reaching consequences, which may be outlined as follows:

- It threatens the existence of many wild life species due to destruction of their natural habitat.
- Biodiversity is lost and along with that genetic diversity is eroded.
- Hydrological cycle gets affected, thereby influencing rainfall.

- Problems of soil erosion and loss of soil fertility increase.
- In hilly areas it often leads to landslides.

Major Activities in Forests

- **Timber Extraction**: Logging for valuable timber, such as teak and Mahogany not only involves a few large trees per hectare but about a dozen more trees since they are strongly interlocked with each other by vines etc. Also road construction for making approach to the trees causes further damage to the forests.

- **Mining**: Mining operations for extracting minerals and fossil fuels like coal often involves vast forest areas. Mining from shallow deposits is done by surface mining while that from deep deposits is done by sub-surface mining. More than 80,000 ha of land of the country is presently under the stress of mining activities. Mining and its associated activities require removal of vegetation along with underlying soil mantle and overlying rock masses. This results in defacing the topography and destruction of the landscape in the area.

Large scale deforestation has been reported in Mussorie and Dehradun valley due to indiscriminate mining of various minerals over a length of about 40 Km. The forested area has declined at an average rate of 33% and the increase in non-forest area due to mining activities has resulted in relatively unstable zones leading to landslides. Indiscriminate mining in forests of Goa since 1961 has destroyed more than 50,000 ha of forest land. Coal mining in Jharia, Raniganj and Singrauli areas have caused extensive deforestation in Jharkhand. Mining of magnesite and soap- stones have destroyed 14 ha of forest in the hill slopes at Khirakot, Kosi valley, Almora. Mining of radioactive minerals in Kerala, Tamilnadu and Karnataka are posing similar threats of deforestation. The rich forests of Western Ghats are also facing the same threat due to mining projects for excavation of copper, chromite, bauxite and magnetite.

Dams and their effects on Forests and People

Big dams and river valley projects have multi-purpose uses and have been referred to as Temples of modern India. However, these dams are also responsible for the destruction of vast areas of forests. India has more than 1550 large dams, the maximum being in the state of Maharashtra (more then 600), followed by Gujarat (more then 250) and Madhya Pradesh (130). The highest one is Tehri dam, on river Bhagirathi in Uttaranchal and the largest in terms of capacity is Bhakra dam on river Satluj in H.P. Big dams have been in sharp focus of various environmental groups all over the world which is mainly because of several ecological problems including deforestation and socio-economic problems related to tribal or native people associated with them. Silent Valley hydroelectric project was one of the first such projects situated in the tropical rain forest area of Western Ghats which attracted much concern of the people. Floods, droughts and landslides become more prevalent in such areas. Forests are the repositories of invaluable gifts of nature in the form of biodiversity and by destroying them (particularly, the tropical rain forests) we are going to lose these species even before knowing them. These species could be having marvelous economic or medicinal value and deforestation results in loss of this storehouse of species which have evolved over millions of years in a single stroke.

WATER RESOURCES

Water is an indispensable natural resource on this earth on which all life depends. About 97% of the earth's surface is covered by water and most of the animals and plants have 60-65% water in their body. Water is characterized by certain unique features which make it a marvellous resource:

(i) It exists as a liquid over a wide range of temperature i.e. from 0° to100°C.

(ii) It has the highest specific heat, due to which it warms up and cools down very slowly without causing shocks of temperature jerks to the aquatic life.

(iii) It has a high latent heat of vaporization. Hence, it takes a huge amount of energy for getting vaporized. That's why it produces a cooling effect as it evaporates.

(iv) It is an excellent solvent for several nutrients. Thus, it can serve as a very good carrier of nutrients, including oxygen, which are essential for life. But, it can also easily dissolve various pollutants and become a carrier of pathogenic microorganisms.

(v) Due to high surface tension and cohesion it can easily rise through great heights through the trunk even in the tallest of the trees like Sequoia.

(vi) It has an anomalous expansion behaviour i.e. as it freezes, it expands instead of contracting and thus becomes lighter. It is because of this property that even in extreme cold, the lakes freeze only on the surface. Being lighter the ice keeps floating, whereas the bottom waters remain at a higher temperature and therefore, can sustain aquatic organisms even in extreme cold.

The water we use keeps on cycling endlessly through the environment, which we call as Hydrological Cycle. We have enormous resources of water on the earth amounting to about 1404 million Km^3. The water from various moist surfaces evaporates and falls again on the earth in the form of rain or snow and passes through living organisms and ultimately returns to the oceans. Every year about 1.4 inch thick layer of water evaporates from the oceans, more than 90% of which returns to the oceans through the hydrological cycle. Solar energy drives the water cycle by evaporating it from various water bodies, which subsequently return through rainfall or snow. Plants play a very important role by absorbing the groundwater from the soil and releasing it into the atmosphere by the process of transpiration. Global distribution of water resources is quite uneven depending upon several geographic factors. Tropical rain forest areas receive maximum rainfall while the major world deserts occur in zones of dry, descending air (20-40° N and S) and receive very little rainfall.

Water use and over-exploitation

Due to its unique properties water is of multiple uses for all living organisms. Water is absolutely essential for life. Most of the life processes take place in water contained in the body. Uptake of nutrients, their distribution in the body, regulation of temperature, and removal of wastes are all mediated through water. Human beings depend on water for almost every developmental activity. Water is used for drinking, irrigation, transportation, washing and waste disposal for industries and used as a coolant for thermal power plants. Water shapes the earth's surface and regulates our climate. Water use by humans is of two types: **water withdrawal**: taking water from groundwater or surface water resource and **water consumption**: the water which is taken up but not returned for reuse. Globally, only about 60 percent of the water withdrawn is consumed due to loss through evaporation. With increasing

human population and rapid development, the world water withdrawal demands have increased many folds and a large proportion of the water withdrawn is polluted due to anthropogenic activities. On a global average 70 percent of the water withdrawn is used for agriculture. In India, we use 93% of water in agricultural sector while in a country like Kuwait, which is water-poor, only 4% is used for watering the crops. About 25% of water on global average is used in industry, which again varies from a high of 70% in European countries to as low as 5% in less developed countries. In USA, an average family of 4 consumes more than 1000 m^3 of water per year, which is many times more than that in most developing countries.

Water: A Precious Natural Resource

Although water is very abundant on this earth, yet it is very precious. Out of the total water reserves of the world, about 97% is salty water (marine) and only 3% is fresh water. Even this small fraction of fresh water is not available to us as most of it is locked up in polar ice caps and just 0.003% is readily available to us in the form of groundwater and surface water. Overuse of groundwater for drinking, irrigation and domestic purposes has resulted in rapid depletion of groundwater in various regions leading to lowering of water table and drying of wells. Pollution of many of the groundwater aquifers has made many of these wells unfit for consumption. Rivers and streams have long been used for discharging the wastes. Most of the civilizations have grown and flourished on the banks of rivers, but unfortunately, growth in turn, has been responsible for pollution of the rivers. As per the United Nations estimates (2002), at least 101 billion people do not even have access to safe drinking water and 2.4 billion do not have adequate sanitation facilities. Increasing population and expanding development would further increase the demands for wastes. It is estimated that by 2024, two-thirds of the world population would be suffering from acute water shortage.

Groundwater

About 9.86% of the total fresh water resources is in the form of groundwater and it is about 35-50 times that of surface water supplies. Till some time back groundwater was considered to be very pure. However, of late, even groundwater aquifers have been found to be contaminated by leachates from sanitary landfills etc. A layer of sediment or rock that is highly permeable and contains water is called an aquifer. Layers of sand and gravel are good aquifers whileclay and crystalline rocks (like granite) are not since they have low permeability. Aquifers may be of two types: Unconfined aquifers which are overlaid by permeable earth materials and they are recharged by water seeping down from above in the form of rainfall and snow melt. Confined aquifers which are sandwiched between two impermeable layers of rock or sediments and are recharged only in those areas where the aquifer intersects the land surface. Sometimes the recharged area is hundreds of kilometers away from the location of the well. Groundwater is not static, it moves, though at a very slow rate of about a meter or so in a year.

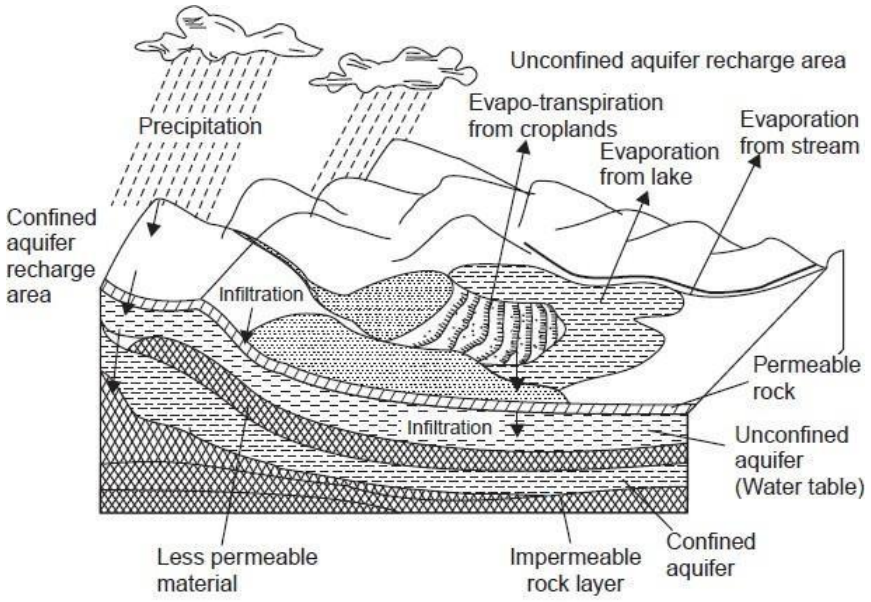


Figure 1: Groundwater system

Effects of Groundwater Usage

(i) Subsidence: When groundwater withdrawal is more than its recharge rate, the sediments in the aquifer get compacted, a phenomenon known as ground subsidence. Huge economic losses may occur due to this phenomenon because it results in the sinking of overlying land surface. The common problems associated with it include structural damage in buildings, fracture in pipes, reversing the flow of sewers and canals and tidal flooding.

(ii) Lowering of water table: Mining of groundwater is done extensively in arid and semi-arid regions for irrigating crop fields. However, it is not advisable to do excessive mining as it would cause a sharp decline in future agricultural production, due to lowering of water table.

(iii) Water logging: When excessive irrigation is done with brackish water it raises the water table gradually leading to water-logging and salinity problems.

Surface Water

The water coming through precipitation (rainfall, snow) when does not percolate down into the ground or does not return to the atmosphere as evaporation or transpiration loss, assumes the form of streams, lakes, ponds, wetlands or artificial reservoirs known as surface water. The surface water is largely used for irrigation, industrial use, public water supply, navigation etc. A country's economy is largely dependent upon its rivers.

Floods

In some countries like India and Bangladesh rainfall does not occur throughout the year, rather, 90% of it is concentrated into a few months (June-September). Heavy rainfall often causes floods in the low-lying coastal areas. Prolonged downpour can also cause the over-flowing of

lakes and rivers resulting into floods. Deforestation, overgrazing, mining, rapid industrialization, global warming etc. have also contributed largely to a sharp rise in the incidence of floods, which otherwise is a natural disaster. Floods have been regular features of some parts of India and Bangladesh causing huge economic loss as well as loss of life. People of Bangladesh are accustomed to moderate flooding during monsoon and they utilize the flood water for raising paddy. But, severe floods like that in 1970, 1988 and 1991 resulting from excessive Himalayan runoff and storms, had very disastrous consequences causing massive deaths and damages. In 1970, about one million people were drowned while 1,40,000 people died in 1991. Networking of rivers is being proposed at national level to deal with the problems of floods.

Droughts

There are about 80 countries in the world, lying in the arid and semi-arid regions that experience frequent spells of droughts, very often extending up to year long duration. When annual rainfall is below normal and less than evaporation, drought conditions are created. Ironically, these drought- hit areas are often having a high population growth which leads to poor land use and makes the situation worse.

Anthropogenic causes: Drought is a meteorological phenomenon, but due to several anthropogenic causes like over grazing, deforestation, mining etc. there is spreading of the deserts tending to convert more areas to drought affected areas. In the last twenty years, India has experienced more and more desertification, thereby increasing the vulnerability of larger parts of the country to droughts. Erroneous and intensive cropping pattern and increased exploitation of scarce water resources through well or canal irrigation to get high productivity has converted drought - prone areas into desertified ones. In Maharashtra there has been no recovery from drought for the last 30 years due to over-exploitation of water by sugarcane crop which has high water demands.

Remedial measures:

- Indigenous knowledge in control of drought and desertification can be very useful for dealing with the problem.
- Carefully selected mixed cropping help optimize production and minimize the risks of crop failures.
- Social Forestry and Wasteland development can prove quite effective to fight the problem, but it should be based on proper understanding of ecological requirements and natural process, otherwise it may even boomerang.
- The Kolar district of Karnataka is one of the leaders in Social Forestry with World Bank Aid, but all its 11 talukas suffer from drought. It is because the tree used for plantation here was Eucalyptus which is now known to lower the water table because of its very high transpiration rate.

Conflicts over Water:

Indispensability of water and its unequal distribution has often led to inter-state or international disputes. Issues related to sharing of river water have been largely affecting

our farmers and also shaking our governments. Some major water conflicts are discussed here.

Water conflict in the Middle East: Three river basins, namely the Jordan, the Tigris-Euphrates and the Nile are the shared water resources for Middle East countries. Ethiopia controls the head waters of 80% of Nile's flow and plans to increase it. Sudan too is trying to divert more water. This would badly affect Egypt, which is a desert, except for a thin strip of irrigated cropland along the river Nile and its delta. The population of Egypt is likely to double in the next 20 years, thereby increasing its water crisis. Likewise there is a fierce battle for water among Jordan, Syria and Israel for the Jordan River water share. Turkey has abundant water and plans to build 22 dams on Tigris-Euphrates for Hydroelectric power generation. But, it would drastically reduce the flow of water to Syria and Iraq, lying downstream. Turkey dreams to become the region's water Super power. It plans to transport and sell water to starved Saudi Arabia, Kuwait, Syria, Israel and Jordan. Probably, the next war in the Middle East would be fought over water and not oil.

The Indus Water Treaty: The Indus, one of the mightiest rivers is dying a slow death due to dams and barrages that have been built higher up on the river. The Sukkur barrage (1932), Ghulam Mohamad Barrage at Kotri (1958) and Tarbela and Chasma Dams on Jhelum, a tributary of Indus have resulted in severe shrinking of the Indus delta. In 1960, the Indus water treaty was established vide which Indus, the Jhelum and the Chenab were allocated to Pakistan and the Satluj, the Ravi and the Beas were allocated to India. Being the riparian state, India has pre-emptive right to construct barrages across all these rivers in Indian territory. However, the treaty requires that the three rivers allocated to Pakistan may be used for non-consumptive purposes by India i.e. without changing its flow and quality. With improving political relations between the two countries it is desirable to work out techno-economic details and go for an integrated development of the river basin in a sustainable manner.

The Cauvery water dispute: Out of India's 18 major rivers, 17 are shared between different states. In all these cases, there are intense conflicts over these resources which hardly seem to resolve. The Cauvery river water is a bone of contention between Tamilnadu and Karnataka and the fighting is almost hundred years old. Tamilnadu, occupying the downstream region of the river wants water-use regulated in the upstream. Whereas, the upstream state Karnataka refuses to do so and claims its primacy over the river as upstream user. The river water is almost fully utilized and both the states have increasing demands for agriculture and industry. The consumption is more in Tamilnadu than Karnataka where the catchment area is more rocky. On June 2, 1990, the Cauvery Water Dispute Tribunal was set up which through an interim award directed Karnataka to ensure that 205 TMCF of water was made available in Tamil Nadu's Mettur dam every year, till a settlement was reached. In 1991-92 due to good monsoon, there was no dispute due to good stock of water in Mettur, but in 1995, the situation turned into a crisis due to delayed rains and an expert Committee was set up to look into the matter which found that there was a complex cropping pattern in Cauvery basin. Sambra paddy in winter, Kurvai paddy in summer and some cash crops demanded intensive water, thus aggravating the water crisis. Proper selection of crop varieties, optimum use of water, better rationing, rational sharing patterns, and pricing of water are suggested as some measures to solve the problem.

The Satluj-Yamuna link (SYL) canal dispute: The issue of sharing the Ravi-Beas waters and SYL issue between Punjab and Haryana is being discussed time and again and the case is

in the Supreme Court. The Eradi Tribunal (1985) based the allocation of water on the basis of the time-inflow data of 20 years (1960-80), according to which 17.17 MAF (million acre feet) water was available. However, now it is argued by Punjab that in the last 17 years there has been consistent decline reducing the quantity to 14.34 MAF. The Supreme Court on January 15, 2002 directed Punjab to complete and commission the SYL within a year, failing which the Center was told to complete it. However, two years have passed, but neither the SYL has been completed nor the conflict over sharing of Ravi- Beas water is resolved. The conflict is that Punjab being the riparian state for Beas, Ravi and Satluj stakes its claim, Haryana has faced acute shortage of water after it became a state in 1966 and has been trying to help it out by signing an MOU (Memorandum of understanding) with UP, Rajasthan and Delhi for allocation of Yamuna waters. The Yamuna basin covers the state of Haryana while the Indus basin covers Punjab. The conflict revolving around sharing of river water needs to be tackled with greater understanding and objectivity.

Big Dams- Benefits

River valley projects with big dams have usually been considered to play a key role in the development process due to their multiple uses. India has the distinction of having the largest number of river-valley projects. These dams are often regarded as a symbol of national development. The tribals living in the area pin big hopes on these projects as they aim at providing employment and raising the standard and quality of life. The dams have tremendous potential for economic upliftment and growth. They can help in checking floods and famines, generate electricity and reduce water and power shortage, provide irrigation water to lower areas, provide drinking water in remote areas and promote navigation, fishery etc.

Environmental Problems

The environmental impacts of big-dams are also too many due to which very often the big dams become a subject of controversy. The impacts can be at the upstream as well as downstream levels.

(A) The upstream problems include the following:

(i) Displacement of tribal people
(ii) Loss of forests, flora and fauna
(iii) Changes in fisheries and the spawning grounds
(iv) Siltation and sedimentation of reservoirs
(v) Loss of non-forest land
(vi) Stagnation and waterlogging near reservoir
(vii) Breeding of vectors and spread of vector-borne diseases
(viii) Reservoir induced seismicity (RIS) causing earthquakes
(ix) Growth of aquatic weeds.
(x) Microclimatic changes.

(B) The downstream impacts include the following:

(i) Water logging and salinity due to over irrigation
(ii) Micro-climatic changes
(iii) Reduced water flow and silt deposition in river
(iv) Flash floods
(v) Salt water intrusion at river mouth

(vi) Loss of land fertility along the river since the sediments carrying nutrients get deposited in the reservoir
(vii) Outbreak of vector-borne diseases like malaria

Thus, although dams are built to serve the society with multiple uses, but it has several serious side-effects. That is why now there is shift towards construction of small dams or mini-hydel projects.

MINERAL RESOURCES

Minerals are naturally occurring, inorganic, crystalline solids having a definite chemical composition and characteristic physical properties. There are thousands of minerals occurring in different parts of the world. However, most of the rocks, we see everyday are just composed of a few common minerals like quartz, feldspar, biotite, dolomite, calcite, laterite etc. These minerals, in turn, are composed of some elements like silicon, oxygen, iron, magnesium, calcium, aluminium etc.

Uses and Exploitation- Minerals find use in a large number of ways in everyday use in domestic, agricultural, industrial and commercial sectors and thus form a very important part of any nation's economy. The main uses of minerals are as follows:

(i) Development of industrial plants and machinery.
(ii) Generation of energy e.g. coal, lignite, uranium.
(iii) Construction, housing, settlements.
(iv) Defence equipments - weapons, armaments.
(v) Transportation means.
(vi) Communication- telephone wires, cables, electronic devices.
(vii) Medicinal system- particularly in Ayurvedic System.
(viii) Formation of alloys for various purposes (e.g. phosphorite).
(ix) Agriculture.as fertilizers, seed dressings and fungicides (e.g. zineb containing zinc, Maneb - containing manganese etc.).
(x) Jewellery. e.g. Gold, silver, platinum, diamond.

Based on their properties, minerals are basically of two types:

(i) Non-metallic minerals e.g. graphite, diamond, quartz, feldspar.
(ii) Metallic minerals e.g. Bauxite, laterite, haematite etc.

Use of metals by human beings has been so extensive since the very beginning of human civilization that two of the major epochs of human history are named after them as Bronze Age and Iron Age. The reserves of metals and the technical know-how to extract them have been the key elements in determining the economy and political power of nations. Out of the various metals, the one used in maximum quantity is Iron and steel (740 million metric tons annually) followed by manganese, copper, chromium, aluminium and Nickel. It is evident from the Tables that the CIS countries (The Commonwealth of Independent States i.e. 12 republics of former USSR), the United States of America, Canada, South Africa and Australia are having the major world reserves of most of the metallic minerals. Due to huge mineral and energy resources, the USA became the richest and the most powerful nation in the world in even less than 200 years. Japan too needs a mention here, as there are virtually no metal reserves, coal, oil and timber resources in Japan and it is totally dependent on other countries for its resources.

But, it has developed energy efficient technologies to upgrade these resources to high quality finished products to sustain its economy.Minerals are sometimes classified as Critical and Strategic. Critical minerals are essential for the economy of a nation e.g. iron, aluminium, copper, gold etc. Strategic minerals are those required for the defence of a country e.g. Manganese, cobalt, platinum, chromium etc.

Some Major Minerals of India

(i) Energy generating minerals - Coal and lignite: West Bengal, Jharkhand, Orissa, M.P., A.P., Uranium (Pitchblende or Uranite ore): Jharkhand, Andhra Pradesh (Nellore, Nalgonda), Meghalaya, Rajasthan (Ajmer).

(ii) Other commercially used minerals - Aluminium (Bauxite ore): Jharkhand, West Bengal, Maharashtra, M.P., Tamilnadu, Iron (haematite and magnetite ore): Jharkhand, Orissa, M.P., A.P., Tamilnadu, Karnataka, Maharashtra and Goa, Copper (Copper Pyrites): Rajasthan (Khetri), Bihar, Jharkhand, Karnataka, M.P., West Bengal, Andhra Pradesh and Uttaranchal.

Environmental Impacts of Mineral Extraction and Use

The issue related to the limits of the mineral resources in our earth's crust or in the ocean is not so significant. More important environmental concern arises from the impacts of extraction and processing of these minerals during mining, smelting etc.

Indian Scenario: India is the producer of 84 minerals the annual value of which is about Rs. 50,000 crore. At least six major mines need a mention here which are known for causing severe problems:

(i) Jaduguda Uranium Mine, Jharkhand, exposing local people to radioactive hazards.

(ii) Jharia coal mines, Jharkhand, underground fire leading to land subsidence and forced displacement of people.

(iii) Sukinda chromite mines, Orissa, seeping of hexavalent chromium into river posing serious health hazard, Cr^{6+} being highly toxic and carcinogenic.

(iv) Kudremukh iron ore mine, Karnataka, causing river pollution and threat to biodiversity.

(v) East coast Bauxite mine, Orissa, Land encroachment and issue of rehabilitation unsettled.

(vi) North-Eastern Coal Fields, Assam, Very high sulphur contamination of groundwater.

Impacts of mining: Mining is done to extract minerals (or fossil fuels) from deep deposits in soil by using sub-surface mining or from shallow deposits by surface mining.

- The former method is more destructive, dangerous and expensive including risks of occupational hazards and accidents.
- Surface mining can make use of any of the following three types:

(a) Open-pit mining in which machines dig holes and remove the ores (e.g. copper, iron, gravel, limestone, sandstone, marble, granite).

(b) Dredging in which chained buckets and draglines are used which scrap up the minerals from under-water mineral deposits.

(c) Strip mining in which the ore is stripped off by using bulldozers, power shovels and stripping wheels (e.g. phosphate rocks).

The environmental damage caused by mining activities are as follows:

(i) **Devegetation and defacing of landscape:** The topsoil as well as the vegetation are removed from the mining area to get access to the deposit. While large scale deforestation or devegetation leads to several ecological losses as already discussed in the previous section, the landscape also gets badly affected. The huge quantities of debris and tailings along with big scars and disruptions spoil the aesthetic value of the region and make it prone to soil erosion.

(ii) **Subsidence of land:** This is mainly associated with underground mining. Subsidence of mining areas often results in tilting of buildings, cracks in houses, buckling of roads, bending of rail tracks and leaking of gas from cracked pipelines leading to serious disasters.

(iii) **Groundwater contamination:** Mining disturbs the natural hydrological processes and also pollutes the groundwater. Sulphur, usually present as an impurity in many ores is known to get converted into sulphuric acid through microbial action, thereby making the water acidic. Some heavy metals also get leached into the groundwater and contaminate it posing health hazards.

(iv) **Surface water pollution:** The acid mine drainage often contaminates the nearby streams and lakes. The acidic water is detrimental to many forms of aquatic life. Sometimes radioactive substances like uranium also contaminate the water bodies through mine wastes and kill aquatic animals. Heavy metal pollution of water bodies near the mining areas is a common feature creating health hazards.

(v) **Air pollution:** In order to separate and purify the metal from other impurities in the ore, smelting is done which emits enormous quantities of air pollutants damaging the vegetation nearby and has serious environmental health impacts. The suspended particulate matter (SPM), SOx, soot, arsenic particles, cadmium, lead etc. shoot up in the atmosphere near the smelters and the public suffers from several health problems.

(vi) **Occupational Health Hazards:** Most of the miners suffer from various respiratory and skin diseases due to constant exposure to the suspended particulate matter and toxic substances. Miners working in different types of mines suffer from asbestosis, silicosis, black lung disease etc.

Remedial measures: Safety of mine workers is usually not a priority subject of industry. Statistical data show that, on an average, there are 30 non-fatal but disabling accidents per ton of mineral produced and one death per 2.5 tons of mineral produced. In order to minimize the adverse impacts of mining it is desirable to adopt eco-friendly mining technology. The low-grade ores can be better utilized by using microbial-leaching technique. The bacterium Thiobacillus ferroxidans has been successfully and economically used for extracting gold embedded in iron sulphide ore. The ores are inoculated with the desired strains of bacteria, which remove the impurities (like sulphur) and leave the pure mineral. This biological method is helpful from economic as well as environmental point of view. Restoration of mined areas by re-vegetating them with appropriate plant species, stabilization of the mined lands, gradual restoration of flora, prevention of toxic drainage discharge and conforming to the standards of air emissions are essential for minimizing environmental impacts of mining.

FOOD RESOURCES

We have thousands of edible plants and animals over the world out of which only about three dozen types constitute the major food of humans. The main food resources include wheat, rice, maize, potato, barley, oats, cassava, sweet potato, sugarcane, pulses, sorghum, millet, about twenty or so common fruits and vegetables, milk, meat, fish and seafood. Amongst these rice, wheat and maize are the major grains, about 1500 million metric tons of which are grown each year, which is about half of all the agricultural crops. About 4 billion people in the developing countries have wheat and rice as their staple food. Meat and milk are mainly consumed by more developed nations of North America, Europe and Japan who consume about 80% of the total. Fish and sea-food contribute about 70 million metric tons of high quality protein to the world's diet. But there are indications that we have already surpassed sustainable harvests of fish from most of the world's oceans.The Food and Agriculture Organization (FAO) of United Nations estimated that on an average the minimum caloric intake on a global scale is 2,500 calories/day. People receiving less than 90% of these minimum dietary calories are called undernourished and if it is less than 80% they are said to be seriously undernourished. Besides the minimum caloric intake we also need proteins, minerals etc. During the last 50 years world grain production has increased almost three times, thereby increasing per capita production by about 50%. But, at the same time population growth increased at such a rate in LDCs (Less developed countries) that it outstripped food production. Every year 40 million people (fifty percent of which are young children between 1 to 5 years) die of undernourishment and malnutrition. This means that every year our food problem is killing as many people as were killed by the atomic bomb dropped on Hiroshima during World War II. These startling statistical figures more than emphasize the need to increase our food production, equitably distribute it and also to control population growth.

Indian Scenario: Although India is the third largest producer of staple crops, an estimated 300 million Indians are still undernourished. India has only half as much 1and as USA, but it has nearly three times population to feed. Our food problems are directly related to population. The World Food Summit, 1996, has set the target to reduce the number of undernourished to just half by 2015, which still means 410 million undernourished people on the earth.

Impacts of Overgrazing and Agriculture

Overgrazing: Livestock wealth plays a crucial role in the rural life of our country. India leads in livestock population in the world. The huge population of livestock needs to be fed and the grazing lands or pasture areas are not adequate. Very often we find that the livestock grazing on a particular piece of grassland or pasture surpass the carrying capacity. Carrying capacity of any system is the maximum population that can be supported by it on a sustainable basis. However, most often, the grazing pressure is so high that its carrying capacity is crossed and the sustainability of the grazing lands fails.

Impacts of Overgrazing:

Land Degradation: Overgrazing removes the vegetal cover over the soil and the exposed soil gets compacted due to which the operative soil depth declines. So the roots cannot go much deep into the soil and adequate soil moisture is not available. Organic recycling also declines in the ecosystem because not enough detritus or litter remains on the soil to be decomposed. The humus content of the soil decreases and overgrazing leads to organically poor, dry,

compacted soil. Due to trampling by cattle the soil loses infiltration capacity, which reduces percolation of water into the soil and as a result of this more water gets lost from the ecosystem along with surface run off. Thus over grazing leads to multiple actions resulting in loss of soil structure, hydraulic conductivity and soil fertility.

Soil Erosion: Due to overgrazing by cattle, the cover of vegetation almost gets removed from the land. The soil becomes exposed and gets eroded by the action of strong wind, rainfall etc. The grass roots are very good binders of soil. When the grasses are removed, the soilbecomes loose and susceptible to the action of wind and water.

Loss of useful species: Overgrazing adversely affects the composition of plant population and their regeneration capacity. The original grassland consists of good quality grasses and forbs with high nutritive value. When the livestock graze upon them heavily, even the root stocks which carry the reserve food for regeneration get destroyed. Now some other species appear in their place. These secondary species are hardier and are less nutritive in nature. As a result of overgrazing vast areas in Arunachal Pradesh and Meghalaya are getting invaded by thorny bushes, weeds etc. of low fodder value. Thus overgrazing makes the grazing land lose its regenerating capacity and once good quality pasture land gets converted into an ecosystem with poor quality thorny vegetation. Some livestock keep on overgrazing on these species also. Ultimately the nutritious, juicy fodder giving species like Cenchrus, Dichanthium, Panicum and Heteropogon etc. are replaced by unpalatable and sometimes thorny plants like Parthenium, Lantana, Xanthium etc. These species do not have a good capacity of binding the soil particles and, therefore, the soil becomes more prone to soil erosion.

Agriculture: In the early years of human existence on this earth, man was just a hunter gatherer and was quite like other animal species. Some 10,000 to 12,000 years ago he took to agriculture by cultivating plants of his own choice. He used the practice of Slash and burn cultivation or shifting cultivation, which is still prevalent in many tribal areas, as in the North East Hills of India. The type of agriculture practiced these days is very different from the traditional ones and their outputs in terms of yield as well as their impacts on the environment show lots of differences.

Traditional agriculture and its impacts: It usually involves a small plot, simple tools, naturally available water, organic fertilizer and a mix of crops. It is more near to natural conditions and usually it results in low production. It is still practiced by about half the global population. The main impacts of this type of agriculture are as follows:

(i) **Deforestation:** The slash and burn of trees in forests to clear the land for cultivation and frequent shifting result in loss of forest cover.

(ii) **Soil erosion:** Clearing of forest cover exposes the soil to wind, rain and storms, thereby resulting in loss of top fertile layer of soil.

(iii) **Depletion of nutrients:** During slash and burn the organic matter in the soil gets destroyed and most of the nutrients are taken up by the crops within a short period, thus making the soil nutrient poor which makes the cultivators shift to another area.

Modern Agriculture and its impacts: It makes use of hybrid seeds of selected and single crop variety, high-tech equipments and lots of energy subsidies in the form of fertilizers, pesticides

and irrigation water. The food production has increased tremendously, evidenced by green revolution. However, it also gave rise to several problematic off-shoots as discussed below:

(i) **Impacts related to high yielding varieties (HYV):** The uses of HYVs encourage monoculture i.e. the same genotype is grown over vast areas. In case of an attack by some pathogen, there is total devastation of the crop by the disease due to exactly uniform conditions, which help in rapid spread of the disease.

(ii) Fertilizer related problems:

(a) Micronutrient imbalance: Most of the chemical fertilizers used in modern agriculture have nitrogen, phosphorus and potassium (N, P, K) which are essential macronutrients. Farmers usually use these fertilizers indiscriminately to boost up crop growth. Excessive use of fertilizers cause micronutrient imbalance. For example, excessive fertilizer use in Punjab and Haryana has caused deficiency of the micronutrient zinc in the soils, which is affecting productivity of the soil.

(b) Nitrate Pollution: Nitrogenous fertilizers applied in the fields often leach deep into the soil and ultimately contaminate the ground water. The nitrates get concentrated in the water and when their concentration exceeds 25 mg/L, they become the cause of a serious health hazard called Blue Baby Syndrome. or methaemoglobinemia. This disease affects the infants to the maximum extent causing even death. In Denmark, England, France, Germany and Netherlands this problem is quite prevalent. In India also, problem of nitrate pollution exists in many areas.

(c) Eutrophication: Excessive use of N and P fertilizers in the agricultural fields leads to another problem, which is not related to the soil, but relates to water bodies like lakes. A large proportion of nitrogen and phosphorus used in crop fields is washed off and along with runoff water reach the water bodies causing over nourishment of the lakes, a process known as Eutrophication (eu=more, trophic=nutrition). Due to eutrophication the lakes get invaded by algal blooms. These algal species grow very fast by rapidly using up the nutrients. They are often toxic and badly affect the food chain. The algal species quickly complete their life cycle and die thereby adding a lot of dead organic matter. The fishes are also killed and there is a lot of dead matter that starts getting decomposed. Oxygen is consumed in the process of decomposition and very soon the water gets depleted of dissolved oxygen. This further affects aquatic fauna and ultimately anaerobic conditions are created where only pathogenic anaerobic bacteria can survive. Thus, due to excessive use of fertilizers in the agricultural fields the lake ecosystem gets degraded. This shows how an unmindful action can have far reaching impacts.

(iii) **Pesticide related problems:** Thousands of types of pesticides are used in agriculture. The first generation pesticides include chemicals like sulphur, arsenic, lead or mercury to kill the pests. DDT (Dichlorodiphenyl trichloroethane) whose insecticidal properties were discovered by Paul Mueller in 1939 belongs to the second generation pesticides. After 1940, a large number of synthetic pesticides came into use. Although these pesticides have gone a long way in protecting our crops from huge losses occurring due to pests, yet they have a number of side-effects, as discussed below:

(a) Creating resistance in pests and producing new pests: Some individuals of the pest species usually survive even after pesticide spray. The survivors give

rise to highly resistant generations. About 20 species of pests are now known which have become immune to all types of pesticides and are known as Super pests.

(b) Death of non-target organisms: Many insecticides are broad spectrum poisons which not only kill the target species but also several non-target species that are useful to us.

(c) Biological magnification: Many of the pesticides are non-biodegradable and keep on accumulating in the food chain, a process called biological magnification. Since human beings occupy a high trophic level in the food chain, hence they get the pesticides in a bio-magnified form which is very harmful.

(d) Water Logging: Over irrigation of croplands by farmers for good growth of their crop usually leads to waterlogging. Inadequate drainage causes excess water to accumulate underground and gradually forms a continuous column with the water table. Under water-logged conditions, pore-spaces in the soil get fully drenched with water and the soil-air gets depleted. The water table rises while the roots of plants do not get adequate air for respiration. Mechanical strength of the soil declines, the crop plants get lodged and crop yield falls. In Punjab and Haryana, extensive areas have become water-logged where adequate canal water supply or tube-well water encouraged the farmers to use it over- enthusiastically leading to water-logging problem. Preventing excessive irrigation, sub-surface drainage technology and bio-drainage with trees like Eucalyptus are some of the remedial measures to prevent water-logging.

(e) Salinity problem: At present one third of the total cultivable land area of the world is affected by salts. In India about seven million hectares of land are estimated to be salt-affected which may be saline or sodic. Saline soils are characterized by the accumulation of soluble salts like sodium chloride, sodium sulphate, calcium chloride, magnesium chloride etc. in the soil profile. Their electrical conductivity is more than 4 dS/m. Sodic soils have carbonates and bicarbonates of sodium, the pH usually exceeds 8.0 and the exchangeable sodium percentage (ESP) is more than 15%.

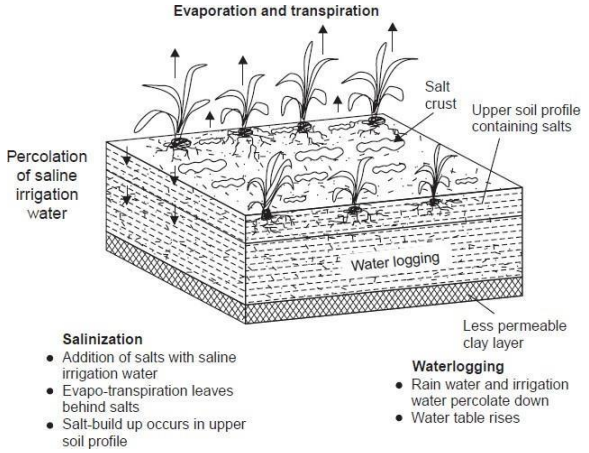


Figure 2: Salinization and Water logging

Causes: A Major cause of salinization of soil is excessive irrigation. About 20% of the world's croplands receive irrigation with canal water or ground water which unlike rainwater often contains dissolved salts. Under dry climates, the water evaporates leaving behind salts in the upper soil profile. Thousands of hectares of land area in Haryana and Punjab are affected by soil salinity and alkalinity. Salinity causes stunted plant growth and lowers crop yield. Most of the crops cannot tolerate high salinity.

Remedy: The most common method for getting rid of salts is to flush them out by applying more good quality water to such soils. Another method is laying underground network of perforated drainage pipes for flushing out the salts slowly. This sub-surface drainage system has been tried in the experimental station of CSSRI at Sampla, Haryana. The Central Soil Salinity Research Institute (CSSRI) located in Karnal, Haryana has to its achievement the success story of converting Zarifa Viran village to Zarifa Abad i.e. from the barren land to productive land through its research applications.

ENERGY RESOURCES

Energy consumption of a nation is usually considered as an index of its development. This is because almost all the developmental activities are directly or indirectly dependent upon energy. We find wide disparities in per capita energy use between the developed and the developing nations. The first form of energy technology probably was the fire, which produced heat and the early man used it for cooking and heating purposes. Wind and hydropower have also been in use for the last 10,000 years. The invention of steam engines replaced the burning of wood by coal and coal was later replaced to a great extent by oil. In 1970s due to Iranian revolution and Arab oil embargo the prices of oil shot up. This ultimately led to exploration and use of several alternate sources of energy.

Growing Energy Needs

Development in different sectors relies largely upon energy. Agriculture, industry, mining, transportation, lighting, cooling and heating in buildings all need energy. With the demands of growing population the world is facing further energy deficit. The fossil fuels like coal, oil and natural gas which at present are supplying 95% of the commercial energy of the world resources and are not going to last for many more years. Our life style is changing very fast and from a simple way of life we are shifting to a luxurious life style. If you just look at the number of electric gadgets in your homes and the number of private cars and scooters in your locality you will realize that in the last few years they have multiplied many folds and all of them consume energy. Developed countries like U.S.A. and Canada constitute about 5% of the world's population but consume one fourth of global energy resources. An average person there consumes 300 GJ (Giga Joules, equal to 60 barrels of oils) per year. By contrast, an average man in a poor country like Bhutan, Nepal or Ethiopia consumes less than 1 GJ in a year. So a person in a rich country consumes almost as much energy in a single day as one person does in a whole year in a poor country. This clearly shows that our life-style and standard of living are closely related to energy needs. Fig. shows the strong correlation between per capita energy use and GNP (Gross National product). U.S.A., Norway, Switzerland etc. with high GNP show high energy use while India, China, etc., have low GNP and low energy use. Bahrain and Qatar are oil-rich states (UAE) and hence their energy consumption and GNP are more, although their development is not that high.

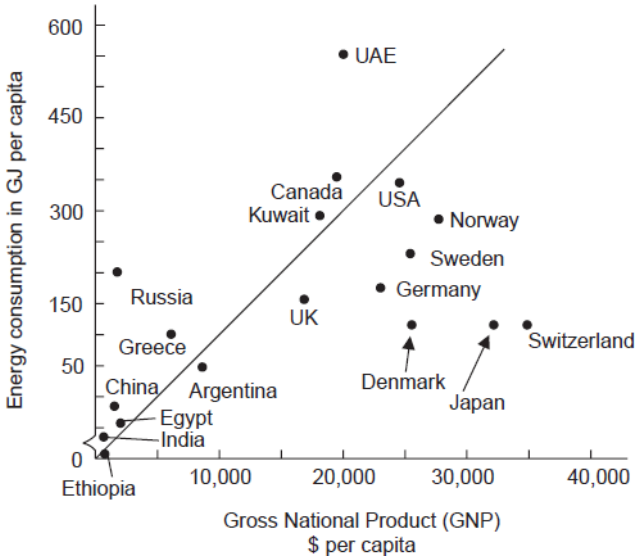


Figure 3: Per capita energy use and GNP

A source of energy is one that can provide adequate amount of energy in a usable form over a long period of time. These sources can be of two types:

(1) Renewable Resources which can be generated continuously in nature and are inexhaustible e.g. wood, solar energy, wind energy, tidal energy, hydropower, biomass energy, bio-fuels, geo-thermal energy and hydrogen. They are also known as non-conventional sources of energy and they can be used again and again in an endless manner.

(2) Non-renewable Resources which have accumulated in nature over a long span of time and cannot be quickly replenished when exhausted e.g. coal, petroleum, natural gas and nuclear fuels like uranium and thorium. Wood is a renewable resource as we can get new wood by growing a sapling into a tree within 15-20 years but it has taken millions of years for the formation of coal from trees and cannot be regenerated in our life time, hence coal is not renewable.

(a) Renewable Energy Resources

Solar energy:

Sun is the ultimate source of energy, directly or indirectly for all other forms of energy. The nuclear fusion reactions occurring inside the sun release enormous quantities of energy in the form of heat and light. The solar energy received by the near earth space is approximately 1.4 kilojoules/second/m2 known as solar constant. Traditionally, we have been using solar energy for drying clothes and food-grains, preservation of eatables and for obtaining salt from sea-water. Now we have several techniques for harnessing solar energy. Some important solar energy harvesting devices are discussed here.

Solar heat collectors: These can be passive or active in nature. Passive solar heat collectors are natural materials like stones, bricks etc. or material like glass which absorb heat during the day time and release it slowly at night. Active solar collectors pump a heat absorbing medium (air or water) through a small collector which is normally placed on the top of the building.

Solar cells: They are also known as photovoltaic cells or PV cells. Solar cells are made of thin wafers of semiconductor materials like silicon and gallium. When solar radiations fall on them, a potential difference is produced which causes flow of electrons and produce electricity. Silicon can be obtained from silica or sand, which is abundantly available and inexpensive. By using gallium arsenide cadmium sulphide or boron, efficiency of the PV cells can be improved. The potential difference produced by a single PV cell of 4 cm2 size is about 0.4-0.5 volts and produces a current of 60 milliamperes.

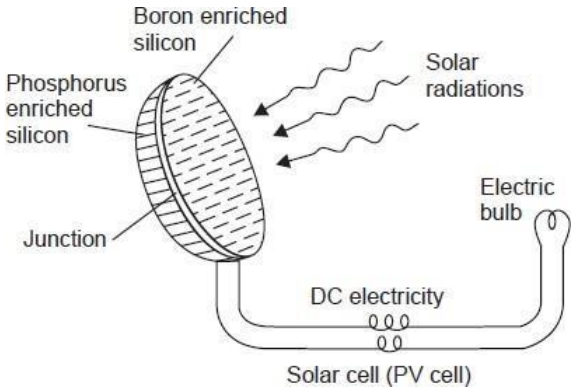


Figure 4: Solar cell

A group of solar cells joined together in a definite pattern form a solar panel which can harness a large amount of solar energy and can produce electricity enough to run street-light, irrigation water pump etc. Solar cells are widely used in calculators, electronic watches, street lighting, traffic signals, water pumps etc. They are also used in artificial satellites for electricity generation. Solar cells are used for running radio and television also. They are more in use in remote areas where conventional electricity supply is a problem.

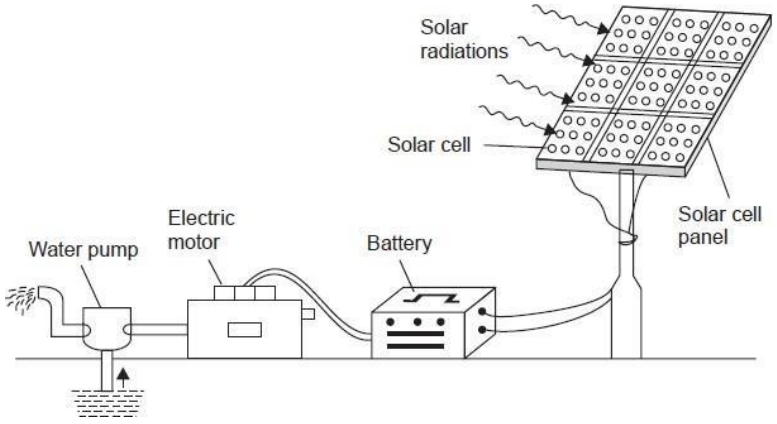


Figure 5: A solar pump run by electricity produced by solar cells

Solar cooker: Solar cookers make use of solar heat by reflecting the solar radiations using a mirror directly on to a glass sheet which covers the black insulated box within which the raw food is kept. A new design of solar cooker is now available which involves a spherical reflector (concave or parabolic reflector) instead of plane mirror that has more heating effect and hence greater efficiency. The food cooked in solar cookers is more nutritious due to slow heating. However it has the limitation that it cannot be used at night or on cloudy days. Moreover, the direction of the cooker has to be adjusted according to the direction of the sun rays.

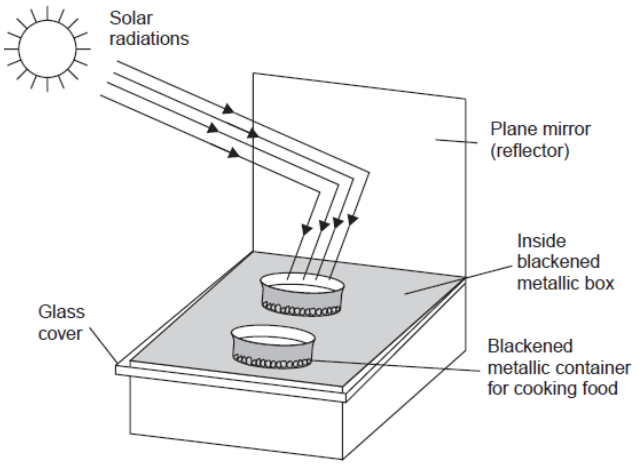


Figure 6: Simple box-type solar cooker

Solar water heater: It consists of an insulated box painted black from inside and having a glass lid to receive and store solar heat. Inside the box it has black painted copper coilthrough which cold water is made to flow in, which gets heated and flows out into a storage tank. The hot water from the storage tank fitted on roof top is then supplied through pipes into buildings like hotels and hospitals.

Solar furnace: Here thousands of small plane mirrors are arranged in concave reflectors, all of which collect the solar heat and produce as high a temperature as 3000°C.

Solar power plant: Solar energy is harnessed on a large scale by using concave reflectors which cause boiling of water to produce steam. The steam turbine drives a generator to produce electricity. A solar power plant (50 K Watt capacity) has been installed at Gurgaon, Haryana.

Wind Energy

The high speed winds have a lot of energy in them as kinetic energy due to their motion. The driving force of the winds is the sun. The wind energy is harnessed by making use of wind mills. The blades of the wind mill keep on rotating continuously due to the force of the striking wind. The rotational motion of the blades drives a number of machines like water pumps, flour mills and electric generators. A large number of wind mills are installed in clusters called wind farms, which feed power to the utility grid and produce a large amount of electricity. These farms are ideally located in coastal regions, open grasslands or hilly regions, particularly mountain passes and ridges where the winds are strong and steady. The minimum wind speed required for satisfactory working of a wind generator is 15 km/hr. The wind power potential of our country is estimated to be about 20,000 MW, while at present we are generating about 1020 MW. The largest wind farm of our country is near Kanyakumari in Tamil Nadu generating 380 MW electricity. Wind energy is very useful as it does not cause any air pollution. After the initial installation cost, the wind energy is very cheap. It is believed that by the middle of the century wind power would supply more than 10% of world's electricity.

HYDROPOWER

The water flowing in a river is collected by constructing a big dam where the water is stored and allowed to fall from a height. The blades of the turbine located at the bottom of the dam move with the fast moving water which in turn rotate the generator and produces electricity.

We can also construct mini or micro hydel power plants on the rivers in hilly regions for harnessing the hydro energy on a small scale, but the minimum height of the water falls should be 10 metres. The hydropower potential of India is estimated to be about 4 × 1011 KW-hours. Till now we have utilized only a little more than 11% of this potential. Hydropower does not cause any pollution, it is renewable and normally the hydro power projects are multi-purpose projects helping in controlling floods, used for irrigation, navigation, etc. However, big dams are often associated with a number of environmental impacts.

Tidal Energy

Ocean tides produced by gravitational forces of sun and moon contain enormous amounts of energy. The high tide and low tide refer to the rise and fall of water in the oceans. A difference of several meters is required between the height of high and low tide to spin the turbines. The tidal energy can be harnessed by constructing a tidal barrage. During high tide, the sea-water flows into the reservoir of the barrage and turns the turbine, which in turn produces electricity by rotating the generators. During low tide, when the sea-level is low, the sea water stored in the barrage reservoir flows out into the sea and again turns the turbines. There are only a few sites in the world where tidal energy can be suitably harnessed. The bay of Fundy Canada having 17-18 m high tides has a potential of 5,000 MW of power generation. The tidal mill at La Rance, France is one of the first modern tidal power mill. In India Gulf of Cambay, Gulf of Kutch and the Sunderbans deltas are the tidal power sites.

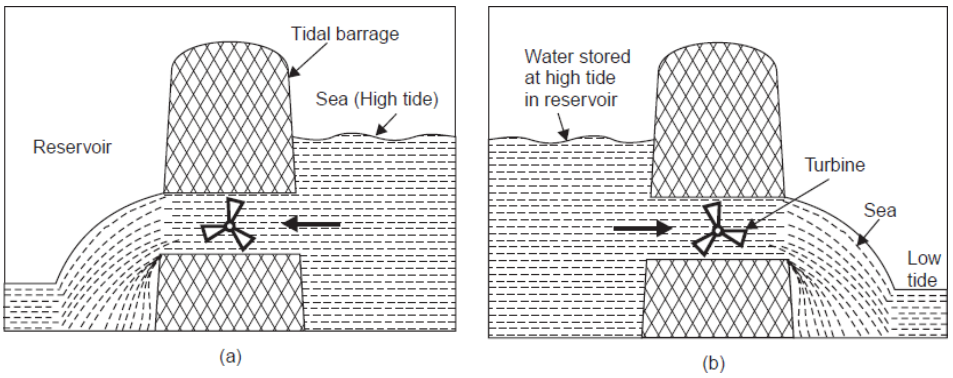


Figure 7: Water flows into the reservoir to turn the turbine at high tide (a), and flows out from the reservoir to the sea, again turning the turbine at low tide (b).

Ocean Thermal Energy (OTE)

The energy available due to the difference in temperature of water at the surface of the tropical oceans and at deeper levels is called Ocean Thermal Energy. A difference of 20°C or more is required between surface water and deeper water of ocean for operating OTEC (Ocean Thermal Energy Conversion) power plants. The warm surface water of ocean is used to boil a liquid like ammonia. The high pressure vapours of the liquid formed by boiling are then used to turn the turbine of a generator and produce electricity. The colder water from the deeper oceans is pumped to cool and condense the vapours into liquid. Thus the process keeps on going continuously for 24 hours a day.

Geothermal Energy

The energy harnessed from the hot rocks present inside the earth is called geothermal energy. High temperature, high pressure steam fields exist below the earth's surface in many places. This heat comes from the fission of radioactive material naturally present in the rocks. In some places, the steam or the hot water comes out of the ground naturally through cracks in the form of natural geysers as in Manikaran, Kullu and Sohana, Haryana. Sometimes the steam or boiling water underneath the earth do not find any place to come out. We can artificially drill a hole up to the hot rocks and by putting a pipe in it make the steam or hot water gush out through the pipe at high pressure which turns the turbine of a generator to produce electricity. In USA and New Zealand, there are several geothermal plants working successfully.

Biomass Energy - Biomass is the organic matter produced by the plants or animals which include wood, crop residues, cattle dung, manure, sewage, agricultural wastes etc. Biomass energy is of the following types:

(a) **Energy Plantations:** Solar energy is trapped by green plants through photosynthesis and converted into biomass energy. Fast growing trees like cottonwood, poplar and Leucaena, non-woody herbaceous grasses, crop plants like sugarcane, sweet sorghum and sugar beet, aquatic weeds like water hyacinth and sea-weeds and carbohydrate rich potato, cereal etc. are some of the important energy plantations. They may produce energy either by burning directly or by getting converted into burnable gas or may be converted into fuels by fermentation.

(b) **Petro-crops:** Certain latex-containing plants like Euphorbias and oil palms are rich in hydrocarbons and can yield an oil like substance under high temperature and pressure. This oily material may be burned in diesel engines directly or may be refined to form gasoline. These plants are popularly known as petro-crops.

(c) **Agricultural and Urban Waste biomass:** Crop residues, bagasse (sugarcane residues), coconut shells, peanut hulls, cotton stalks etc. are some of the common agricultural wastes which produce energy by burning. Animal dung, fishery and poultry waste and even human refuse are examples of biomass energy. In Brazil 30 % of electricity is obtained from burning bagasse. In rural India, animal dung cakes are burnt to produce heat. About 80 % of rural heat energy requirements are met by burning agricultural wastes, wood and animal dung cakes. In rural areas these forms of waste biomass are burned in open furnaces called Chulhas, which usually produce smoke and are not so efficient (efficiency is <8 %). Now improved Chulhas with tall chimney have been designed which have high efficiency and are smokeless. The burning of plant residues or animal wastes cause air pollution and produce a lot of ash as waste residue. The burning of dung destroys essential nutrients like N and P. It is therefore, more useful to convert the biomass into biogas or bio fuels.

Biogas - Biogas is a mixture of methane, carbon dioxide, hydrogen and hydrogen sulphide, the major constituent being methane. Biogas is produced by anaerobic degradation of animal wastes (sometimes plant wastes) in the presence of water. Anaerobic degradation means break down of organic matter by bacteria in the absence of oxygen. Biogas is a non-polluting, clean and low cost fuel which is very useful for rural areas where a lot of animal waste and agricultural waste are available. India has the largest cattle population in the world (240 million) and has tremendous potential for biogas production. From cattle dung alone, we can produce biogas of a magnitude of 22,500 Mm^3 annually. A sixty cubic feet gobar gas plant can serve the needs of one average family. Biogas has the following main advantages :

- It is clean, nonpolluting and cheap. There is direct supply of gas from the plant and there is no storage problem.
- The sludge left over is a rich fertilizer containing bacterial biomass with most of the nutrients preserved as such.
- Air-tight digestion/degradation of the animal wastes is safe as it eliminates health hazards which normally occur in case of direct use of dung due to direct exposure to faecal pathogens and parasites.

Biogas plants used in our country are basically of two types:

1. Floating gas-holder type and 2. Fixed-dome type.

1. **Floating gas holder type biogas plant:** This type has a well-shaped digester tank which is placed under the ground and made up of bricks. In the digester tank, over the dung slurry an inverted steel drum floats to hold the bio-gas produced. The gas holder can move which is controlled by a pipe and the gas outlet is regulated by a valve. The digester tank has a partition wall and one side of it receives the dung water mixture through inlet pipe while the other side discharges the spent slurry through outlet pipe. Sometimes corrosion of steel gas-holder leads to leakage of biogas. The tank has to be painted time and again for maintenance which increases the cost.

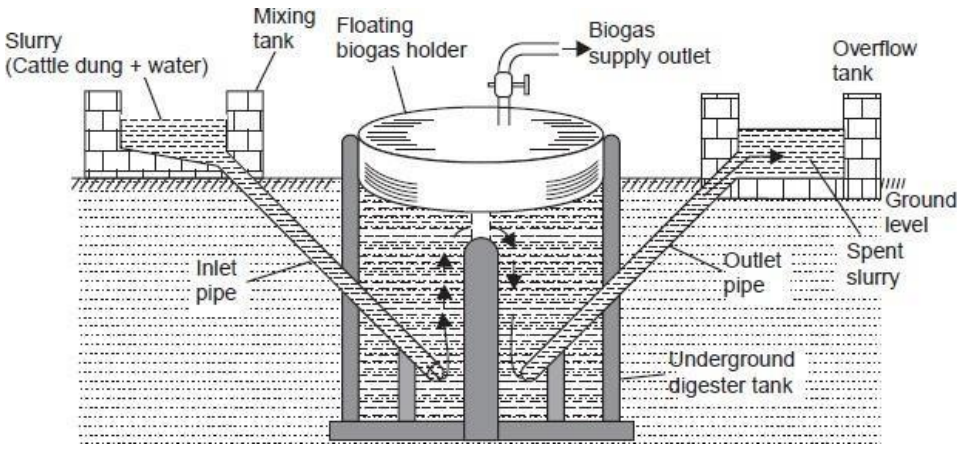


Figure 8: Floating gas holder type biogas plant

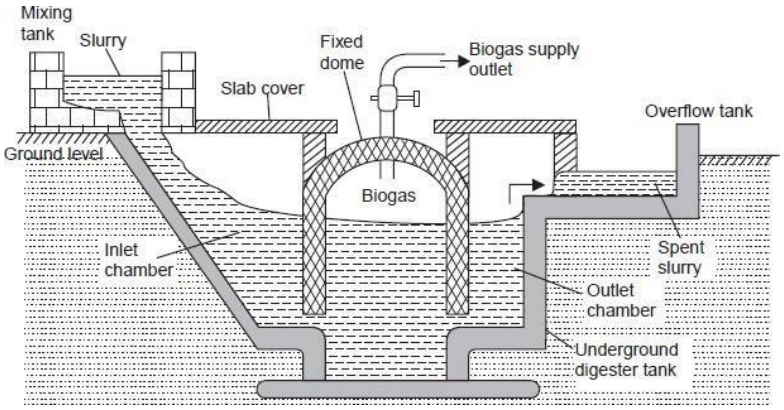


Figure 9: Fixed dome type biogas plant

2. **Fixed dome type biogas plant:** The structure is almost similar to that of the previous type. However, instead of a steel gas-holder there is dome shaped roof made of cement and bricks. Instead of partitioning, here there is a single unit in the main digester but it has inlet and outlet chambers as shown. The Ministry of Non-Conventional Energy Sources (MNES) has been promoting the Biogas Programme in India. Out of the various models, the important ones used in rural set-up are KVIC Model (Floating drum type), Janta Model (Fixed dome type), Deenbandhu Model (Fixed dome type), Pragati Model (floating drum type), Ganesh Model (KVIC type but made of bamboo and polythene sheet) and Ferro-cement digester Model (KVIC type with ferro-cement digester).

Biofuels

Biomass can be fermented to alcohols like ethanol and methanol which can be used as fuels. Ethanol can be easily produced from carbohydrate rich substances like sugarcane. It burns clean and is non-polluting. However, as compared to petrol its calorific value is less and therefore, produces much less heat than petrol. **Gasohol** is a common fuel used in Brazil and Zimbabwe for running cars and buses. In India too gasohol is planned to be used on trial basis in some parts of the country, to start with in Kanpur. Gasohol is a mixture of ethanol and gasoline. **Methanol** is very useful since it burns at a lower temperature than gasoline or diesel. Thus the bulky radiator may be substituted by sleek designs in our cars. Methanol too is a clean, non-polluting fuel. Methanol can be easily obtained from woody plants and ethanol from grain-based or sugar-containing plants.

Hydrogen as a Fuel

As hydrogen burns in air, it combines with oxygen to form water and a large amount of energy (150 kilojoules per gram) is released. Due to its high, rather the highest calorific value, hydrogen can serve as an excellent fuel. Moreover, it is non-polluting and can be easily produced. Production of Hydrogen is possible by thermal dissociation, photolysis or electrolysis of water:

(i) By thermal dissociation of water (at 3000°K or above) hydrogen (H_2) is produced.

(ii) Thermochemically, hydrogen is produced by chemical reaction of water with some other chemicals in 2-3 cycles so that we do not need the high temperatures as in direct thermal method and ultimately H_2 is produced.

(iii) Electrolytic method dissociates water into hydrogen (H_2) and oxygen by making acurrent flow through it.

(iv) Photolysis of water involves breakdown of water in the presence of sun light to release hydrogen. Green plants also have photolysis of water during photosynthesis. Efforts are underway to trap hydrogen molecule which is produced during photosynthesis.

However, hydrogen is highly inflammable and explosive in nature. Hence, safe handling is required for using H_2 as a fuel. Also, it is difficult to store and transport. And, being very light, it would have to be stored in bulk. Presently, H_2 is used in the form of liquid hydrogen as a fuel in spaceships.

(b) Non-renewable Energy Resources

These are the fossil fuels like coal, petroleum, natural gas and nuclear fuels. These were formed by the decomposition of the remains of plants and animals buried under the earth millions of years ago. The fuels are very precious because they have taken such a long time to be formed and if we exhaust their reserves at such a fast rate as we have been doing, ever since we discovered them, then very soon we will lose these resources forever.

Coal

Coal was formed 255-350 million years ago in the hot, damp regions of the earth during the carboniferous age. The ancient plants along the banks of rivers and swamps were buried after death into the soil and due to the heat and pressure gradually got converted into peat and coal over millions of years of time. There are mainly three types of coal, namely anthracite (hard coal), bituminous (Soft coal) and lignite (brown coal). Anthracite coal has maximum carbon (90%) and calorific value (8700 kcal/kg.) Bituminous, lignite and peat contain 80, 70 and 60% carbon, respectively. Coal is the most abundant fossil fuel in the world. At the present rate of usage, the coal reserves are likely to last for about 200 years and if its use increases by 2% per year, then it will last for another 65 years. India has about 5% of world's coal and Indian coal is not very good in terms of heat capacity. Major coal fields in India are Raniganj, Jharia, Bokaro, Singrauli, and Godavari valley. The coal states of India are Jharkhand, Orissa, West Bengal, Madhya Pradesh, Andhra Pradesh and Maharashtra. Anthracite coal occurs only in J & K. When coal is burnt it produces carbon dioxide, which is a greenhouse gas responsible for causing enhanced global warming. Coal also contains impurities like sulphur and therefore as it burns the smoke contains toxic gases like oxides of sulphur and nitrogen.

Petroleum

It is the lifeline of global economy. There are 13 countries in the world having 67% of the petroleum reserves which together form the OPEC (Organization of Petroleum exporting countries). About $1/4^{th}$ of the oil reserves are in Saudi Arabia. At the present rate of usage, the world's crude oil reserves are estimated to get exhausted in just 40 years. Some optimists, however, believe that there are some yet undiscovered reserves. Even then the crude oil reserves will last for another 40 years or so. Crude petroleum is a complex mixture of alkane hydrocarbons. Hence it has to be purified and refined by the process of fractional distillation, during which process different constituents separate out at different temperatures. We get a large variety of products from this, namely, petroleum gas, kerosene, petrol, diesel, fuel oil, lubricating oil, paraffin wax, asphalt, plastic etc. Petroleum is a cleaner fuel as compared to coal as it burns completely and leaves no residue. It is also easier to transport and use. That is the reason why petroleum is preferred amongst all the fossil fuels.

Liquefied petroleum gas (LPG): The main component of petroleum is butane, the other being propane and ethane. The petroleum gas is easily converted to liquid form under pressure as LPG. It is odourless, but the LPG in our domestic gas cylinders gives a foul smell. This is, in fact, due to ethyl mercaptan, a foul smelling gas, added to LPG so that any leakage of LPG from the cylinder can be detected instantaneously. Oil fields in India are located at Digboi (Assam), Gujarat Plains and Bombay High, offshore areas in deltaic coasts of Godavari, Krishna, Kaveri and Mahanadi.

Natural Gas

It is mainly composed of methane (95%) with small amounts of propane and ethane. It is a fossil fuel. Natural gas deposits mostly accompany oil deposits because it has been formed by decomposing remains of dead animals and plants buried under the earth. Natural gas is the cleanest fossil fuel. It can be easily transported through pipelines. It has a high calorific value of about 50KJ/G and burns without any smoke. Currently, the amount of natural gas deposits in the world are of the order of 80,450 gm^3. Russia has maximum reserves (40%), followed by Iran (14%) and USA (7%). Natural gas reserves are found in association with all the oil fields in India. Some new gas fields have been found in Tripura, Jaisalmer, Off-shore area of Mumbai and the Krishna Godavari Delta. Natural gas is used as a domestic and industrial fuel. It is used as a fuel in thermal power plants for generating electricity. It is used as a source of hydrogen gas in fertilizer industry and as a source of carbon in tyre industry.

Compressed natural gas (CNG): It is being used as an alternative to petrol and diesel for transport of vehicles. Delhi has totally switched over to CNG where buses and auto rickshaws run on this new fuel. CNG use has greatly reduced vehicular pollution in the city.

Synthetic natural gas (SNG): It is a mixture of carbon monoxide and hydrogen. It is a connecting link between a fossil fuel and substituted natural gas. Low grade coal is initially transformed into synthetic gas by gasification followed by catalytic conversion to methane.

Nuclear Energy

Nuclear energy is known for its high destructive power as evidenced from nuclear weapons. The nuclear energy can also be harnessed for providing commercial energy. Nuclear energy can be generated by two types of reactions:

(i) **Nuclear Fission:** It is the nuclear change in which nucleus of certain isotopes with large mass numbers are split into lighter nuclei on bombardment by neutrons and a large amount of energy is released through a chain reaction as shown. Nuclear Reactors make use of nuclear chain reaction. In order to control the rate of fission, only 1 neutron released is allowed to strike for splitting another nucleus. Uranium-235 nuclei are most commonly used in nuclear reactors.

$$_{92}U^{235} + {}_{0}n^{1} \rightarrow {}_{36}Kr^{92} + {}_{56}Ba^{141} + 3_{0}\,n^{1} + Energy$$

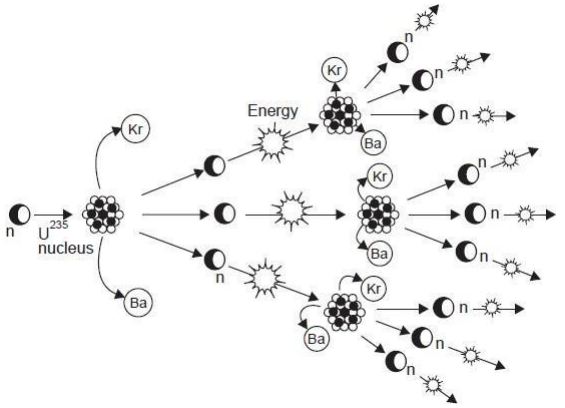


Figure 10: Nuclear fission – a chain reaction initiated by one neutron that bombards a Uranium (U^{235}) nucleus, releasing a huge quantity of energy, two smaller nuclei (Ba, Kr) and 3 neutrons

(i) **Nuclear fusion:** Here two isotopes of a light element are forced together at extremely high temperatures (1 billion °C) until they fuse to form a heavier nucleus releasing enormous energy in the process. It is difficult to initiate the process but it releases more energy than nuclear fission. Two hydrogen-2 (Deuterium) atoms may fuse to form the nucleus of Helium at 1 billion °C and release a huge amount of energy. Nuclear fusion reaction can also take place between one Hydrogen-2 (Deuterium) and one Hydrogen-3 (Tritium) nucleus at 100 million °C forming Helium-4 nucleus, one neutron and a huge amount of energy. Nuclear energy has tremendous potential but any leakage from the reactor may cause devastating nuclear pollution. Disposal of the nuclear waste is also a big problem. Nuclear power in India is still not very well developed. There are four nuclear power stations with an installed capacity of 2005 MW. These are located at Tarapur (Maharashtra), Rana Pratap Sagar near Kota (Rajasthan), Kalpakkam (Tamil Nadu) and Narora (U.P.).

$$_1H^{\ 2} +_1H^{\ 2} \rightarrow_3 He^2 +_0n^1 + Energy$$

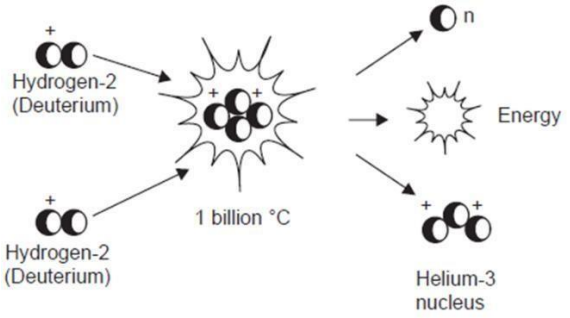


Figure 11: Nuclear fusion reaction between two hydrogen – 2 nuclei, which take place at a very high temperature of 1 billion °C; one neutron and one fusion nucleus of helium-3 is formed along with a huge amount of energy

LAND RESOURCES

Land as a Resource

Land is a finite and valuable resource upon which we depend for our food, fibre and fuel wood, the basic amenities of life. Soil, especially the top soil, is classified as a renewable resource because it is continuously regenerated by natural process though at a very slow rate. About 200-1000 years are needed for the formation of one inch or 2.5 cm soil, depending upon the climate and the soil type. But, when rate of erosion is faster than rate of renewal, then the soil becomes a non-renewable resource.

Land Degradation

With increasing population growth the demands for arable land for producing food, fibre and fuel wood is also increasing. Hence there is more and more pressure on the limited land resources which are getting degraded due to over-exploitation. Soil degradation is a real cause of alarm because soil formation is an extremely slow process as discussed above and the average annual erosion rate is 20-100 times more than the renewal rate.Soil erosion, water-logging, salinization and contamination of the soil with industrial wastes like fly-ash, press-mud or heavy metals all cause degradation of land.

Soil Erosion

The literal meaning of soil erosion is wearing away of soil. Soil erosion is defined as the movement of soil components, especially surface-litter and top soil from one place to another. Soil erosion results in the loss of fertility because it is the top soil layer which is fertile. If we look at the world situation, we find that one third of the world's cropland is getting eroded. Two thirds of the seriously degraded lands lie in Asia and Africa. Soil erosion is basically of two types based upon the cause of erosion:

(i) **Normal erosion or geologic erosion:** caused by the gradual removal of top soil by natural processes which bring an equilibrium between physical, biological and hydrological activities and maintain a natural balance between erosion and renewal.

(i) **Accelerated erosion:** This is mainly caused by anthropogenic (man-made) activities and the rate of erosion is much faster than the rate of formation of soil. Overgrazing, deforestation and mining are some important activities causing accelerated erosion.

There are two types of agents which cause soil erosion:

(i) **Climatic agents:** water and wind are the climatic agents of soil erosion. Water affects soil erosion in the form of torrential rains, rapid flow of water along slopes, run-off, wave action and melting and movement of snow. Water induced soil erosion is of the following types:

- **Sheet erosion:** when there is uniform removal of a thin layer of soil from a large surface area, it is called sheet erosion. This is usually due to run-off water.
- **Rill erosion:** When there is rainfall and rapidly running water produces finger-shaped grooves or rills over the area, it is called rill erosion.
- **Gully erosion:** It is a more prominent type of soil erosion. When the rainfall is very heavy, deeper cavities or gullies are formed, which may be U or V shaped.
- **Slip erosion:** This occurs due to heavy rainfall on slopes of hills and mountains.
- **Stream bank erosion:** During the rainy season, when fast running streams take a turn in some other direction, they cut the soil and make caves in the banks.

Wind erosion is responsible for the following three types of soil movements:

- **Saltation:** This occurs under the influence of direct pressure of stormy wind and the soil particles of 1-1.5 mm diameter move up in vertical direction.
- **Suspension:** Here fine soil particles (less than 1 mm dia) which are suspended in the air are kicked up and taken away to distant places.
- **Surface creep:** Here larger particles (5-10 mm diameter) creep over the soil surface along with wind.

(ii) **Biotic agents:** Excessive grazing, mining and deforestation are the major biotic agents responsible for soil erosion. Due to these processes the top soil is disturbed or rendered devoid of vegetation cover. So the land is directly exposed to the action of various physical forces facilitating erosion. Overgrazing accounts for 35% of the world's soil erosion while deforestation is responsible for 30% of the earth's seriously eroded lands. Unsustainable

methods of farming cause 28% of soil erosion. Deforestation without reforestation, overgrazing by cattle, surface mining without land reclamation, irrigation techniques that lead to salt build-up, water-logged soil, farming on land with unsuitable terrain, soil compaction by agricultural machinery, action of cattle trampling etc., make the top soil vulnerable to erosion.

Soil Conservation Practices: In order to prevent soil erosion and conserve the soil the following conservation practices are employed:

(i) **Conservational till farming:** In traditional method the land is ploughed and the soil is broken up and smoothed to make a planting surface. However, this disturbs the soil and makes it susceptible to erosion when fallow (i.e. without crop cover). Conservational till farming, popularly known as no-till-farming causes minimum disturbance to the top soil. Here special tillers break up and loosen the subsurface soil without turning over the topsoil. The tilling machines make slits in the unploughed soil and inject seeds, fertilizers, herbicides and a little water in the slit, so that the seed germinates and the crop grows successfully without competition with weeds.

(i) **Contour farming:** On gentle slopes, crops are grown in rows across, rather than up and down, a practice known as contour farming. Each row planted horizontally along the slope of the land acts as a small dam to help hold soil and slow down loss of soil through run-off water.

(ii) **Terracing:** It is used on still steeper slopes are converted into a series of broad terraces which run across the contour. Terracing retains water for crops at all levels and cuts down soil erosion by controlling run off. In high rainfall areas, ditches are also provided behind the terrace to permit adequate drainage.

(iv) **Strip cropping:** Here strips of crops are alternated with strips of soil saving covercrops like grasses or grass-legume mixture. Whatever run-off comes from the cropped soil is retained by the strip of cover crop and this reduces soil erosion. Nitrogen fixing legumes also help in restoring soil fertility.

(v) **Alley cropping:** It is a form of inter-cropping in which crops are planted between rows of trees or shrubs. This is also called Agro forestry. Even when the crop is harvested, the soil is not fallow because trees and shrubs still remain on the soil holding the soil particles and prevent soil erosion.

(vi) **Wind breaks or shelterbelts:** They help in reducing erosion caused by strong winds. The trees are planted in long rows along the cultivated land boundary so that wind is blocked. The wind speed is substantially reduced which helps in preventing wind erosion of soil. Thus, soil erosion is one of the world's most critical problems and, if not slowed, will seriously reduce agricultural and forestry production, and degrade the quality of aquatic ecosystems as well due to increased siltation. Soil erosion, is in fact, a gradual process and very often the cumulative effects becomes visible only when the damage has already become irreversible. The best way to control soil erosion is to maintain adequate vegetational cover over the soil.

Water Logging - In order to provide congenial moisture to the growing crops, farmers usually apply heavy irrigation to their farmland. Also, in order to leach down the salts deeper into the soil, the farmer provides more irrigation water. However, due to inadequate drainage andpoor quality irrigation water there is accumulation of water underground and gradually it forms a continuous column with the water table. We call these soils as waterlogged soils which affect

crop growth due to inhibition of exchange of gases. The pore-spaces between the soil particles get fully drenched with water through the roots. Water logging is most often associated with salinity because the water used for irrigation contains salts and the soils get badly degraded due to erroneous irrigation practices. An estimated loss of Rs.10,000 million per annum occurs due to water-logging and salinity in India. It is a startling fact because the cost of development of the irrigation projects is very high and in the long run they cause problems like water logging and salinity thereby sharply reducing soil fertility.

Landslides - Various anthropogenic activities like hydroelectric projects, large dams, reservoirs, construction of roads and railway lines, construction of buildings, mining etc are responsible for clearing of large forested areas. Earlier there were few reports of landslides between Rishikesh and Byasi on Badrinath Highway area. But, after the highway was constructed, 15 landslides occurred in a single year. During construction of roads, mining activities etc. huge portions of fragile mountainous areas are cut or destroyed by dynamite and thrown into adjacent valleys and streams. These land masses weaken the already fragile mountain slopes and lead to landslides. They also increase the turbidity of various nearby streams, thereby reducing their productivity.

Desertification - Desertification is a process whereby the productive potential of arid or semiarid lands falls by ten percent or more. Moderate desertification is 10-25% drop in productivity, severe desertification causes 25-50% drop while very severe desertification results in more than 50% drop in productivity and usually creates huge gullies and sand dunes. Desertification leads to the conversion of rangelands or irrigated croplands to desert like conditions in which agricultural productivity falls. Desertification is characterized by devegetation and loss of vegetal cover, depletion of groundwater, salinization and severe soil erosion. Desertification is not the literal invasion of desert into a non-desert area. It includes degradation of the ecosystems within as well as outside the natural deserts. The Sonoran and Chihuahuan deserts are about a million years old, yet they have become more barren during the last 100 years. So, further desertification has taken place within the desert.

Causes of Desertification: Formation of deserts may take place due to natural phenomena like climate change or may be due to abusive use of land. Even the climate change is linked in many ways to human activities. The major anthropogenic activities responsible for desertification are as follows:

(a) Deforestation: The process of denuding and degrading a forested land initiates a desert producing cycle that feeds on itself. Since there is no vegetation to hold back the surface run-off, water drains off quickly before it can soak into the soil to nourish the plants or to replenish the groundwater. This increases soil erosion, loss of fertility and loss of water.

(b) Overgrazing: The regions most seriously affected by desertification are the cattle producing areas of the world. This is because the increasing cattle population heavily graze in grasslands or forests and as a result denude the land area. When the earth is denuded, the microclimate near the ground becomes inhospitable to seed germination. The dry barren land becomes loose and more prone to soil erosion. The top fertile layer is also lost and thus plant growth is badly hampered in such soils. The dry barren land reflects more of the sun's heat, changing wind patterns, driving away moisture laden clouds leading to further desertification.

(c) Mining and quarrying: These activities are also responsible for loss of vegetal cover and denudation of extensive land areas leading to desertification. Deserts are found to occur in the arid and semi-arid areas of all the continents. During the last 50 years about 900 million hectares of land have undergone desertification over the world. This problem is especially severe in Sahel region, just south of the Sahara in Africa. It is further estimated that if desertification continues at the present rate, then by 2010, it will affect such lands which are presently occupied by 20% of the human population. Amongst the most badly affected areas are the sub Saharan Africa, the Middle East, Western Asia, parts of Central and South America, Australia and the Western half of the United States. It is estimated that in the last 50 years, human activities have been responsible for desertification of land area equal to the size of Brazil. The UNEP estimates suggest that if we do not make sincere efforts now then very soon 63% of rangelands, 60% of rain-fed croplands and 30% of irrigated croplands will suffer from desertification on a worldwide scale, adding 60,000 Km^2 of deserts every year.

EQUITABLE USE OF RESOURCES

There is a big divide in the world as North and South, the more developed countries (MDCs) and less developed countries (LDCs), the haves and the have nots. The less developed does not mean that they are backward as such, they are culturally very rich or even much more developed, but economically they are less developed. The gap between the two is mainly because of population and resources. The MDCs have only 22% of world's population, but they use 88% of its natural resources, 73% of its energy and command 85% of its income. In turn, they contribute a very big proportion to its pollution. These countries include USA, Canada, Japan, the CIS, Australia, New Zealand and Western European Countries. The LDCs, on the other hand, have very low or moderate industrial growth, have 78% of the World's population and use about 12% of natural resources and 27% of energy. Their income is merely 15% of global income. The gap between the two is increasing with time due to sharp increase in population in the LDCs. The rich have grown richer while the poor have stayed poor or gone even poorer. As the rich nations are developing more, they are also leading to more pollution and sustainability of the earth's life support system is under threat. The poor nations, on the other hand, are still struggling hard with their large population and poverty problems. Their share of resources is too little leading to unsustainability. As the rich nations continue to grow, they will reach a limit. If they have a growth rate of 10 % every year, they will show 1024 times increase in the next 70 years. Will this much of growth be sustainable? The answer is No, because many of our earth's resources are limited and even the renewable resources will become unsustainable if their use exceeds their regeneration. Thus, the solution to this problem is to have more equitable distribution of resources and wealth. We cannot expect the poor countries to stop growth in order to check pollution because development brings employment and the main problem of these countries is to tackle poverty. A global consensus has to be reached for more balanced distribution of the basic resources like safe drinking water, food, fuel etc., so that the poor in the LDCs are at least able to sustain their life. Unless they are provided with such basic resources, we cannot think of rooting out the problems related to dirty, unhygienic, polluted, disease infested settlements of these people-which contribute to unsustainability. Thus, the two basic causes of unsustainability are over population in poor countries who have under consumption of resources and over consumption of resources by the rich countries, which generate wastes. In order to achieve sustainable life

styles it is desirable to achieve a more balanced and equitable distribution of global resources and income to meet everyone's basic needs. The rich countries will have to lower down their consumption levels while the bare minimum needs of the poor have to be fulfilled by providing them resources. A fairer sharing of resources will narrow down the gap between the rich and the poor and will lead to sustainable development for all and not just for a privileged group.

Role of an Individual

Different natural resources like forests, water, soil, food, mineral and energy resources play a vital role in the development of a nation. However, overuse of these resources in our modern society is resulting in fast depletion of these resources and several related problems. If we want our mankind to flourish there is a strong need to conserve these natural resources. While conservation efforts are underway at National as well as International level, the individual efforts for conservation of natural resources can go a long way. Environment belongs to each one of us and all of us have a responsibility to contribute towards its conservation and protection. .Small droplets of water together form a big ocean. Similarly, with our small individual efforts we can together help in conserving our natural resources to a large extent.

Conserve Water

- Don't keep water taps running while brushing, shaving, washing or bathing.
- In washing machines fill the machine only to the level required for your clothes.
- Install water-saving toilets that use not more than 6 liters per flush.
- Check for water leaks in pipes and toilets and repair them promptly.
- A small pin-hole sized leak will lead to the wastage of 640 liters of water in a month.
- Reuse the soapy water of washings from clothes for washing off the courtyards, driveways etc.
- Water the plants in your kitchen-garden and the lawns in the evening when evaporation losses are minimum. Never water the plants in mid-day.
- Use drip irrigation and sprinkling irrigation to improve irrigation efficiency and reduce evaporation.
- Install a small system to capture rain water and collect normally wasted used water from sinks, cloth-washers, bath- tubs etc., which can be used for watering the plants.
- Build rain water harvesting system in your house.

Conserve Energy

- Turn off lights, fans and other appliances when not in use.
- Obtain as much heat as possible from natural sources. Dry the clothes in sun insteadof drier if it is a sunny day.
- Use solar cooker for cooking your food on sunny days which will be more nutritious and will cut down on your LPG expenses.
- Build your house with provision for sunspace which will keep your house warmer and will provide more light.
- Grow deciduous trees and climbers at proper places outside your home to cut off intense heat of summers and get a cool breeze and shade. This will cut off your electricity charges on coolers and air-conditioners. A big tree is estimated to have a cooling effect equivalent to five air conditioners. The deciduous trees shed their leaves in winter. Therefore they do not put any hindrance to the sunlight and heat.

- Drive less, make fewer trips and use public transportations whenever possible. You can share by joining a carpool if you regularly have to go to the same place.
- Add more insulation to your house. During winter close the windows at night. During summer close the windows during days if using an A.C. Otherwise loss of heat would be more, consuming more electricity.
- Instead of using the heat convector more often wear adequate woollens.
- Recycle and reuse glass, metals and paper.
- Try riding bicycle or just walk down small distances instead of using your car or scooter.
- Lower the cooling load on an air conditioner by increasing the thermostat setting as 3-5 % electricity is saved for every one degree rise in temperature setting.

Protect the Soil

- While constructing your house, don.t uproot the trees as far as possible. Plant the disturbed areas with a fast growing native ground cover.
- Grow different types of ornamental plants, herbs and trees in your garden. Grow grass in the open areas which will bind the soil and prevent its erosion.
- Make compost from your kitchen waste and use it for your kitchen-garden or flower-pots.
- Do not irrigate the plants using a strong flow of water, as it would wash off the soil.
- Better use sprinkling irrigation.
- Use green manure and mulch in the garden and kitchen-garden which will protect the soil.
- If you own agricultural fields, do not over-irrigate your fields without proper drainage to prevent water logging and salinisation.
- Use mixed cropping so that some specific soil nutrients do not get depleted.

Promote Sustainable Agriculture

- Do not waste food. Take as much as you can eat.
- Reduce the use of pesticides.
- Fertilize your crop primarily with organic fertilizers.
- Use drip irrigation to water the crops.
- Eat local and seasonal vegetables. This saves lot of energy on transport, storage and preservation.
- Control pests by a combination of cultivation and biological control methods.

SUSTAINABLE DEVELOPMENT

Sustainable development is defined as meeting the needs of the present without compromising the ability of future generations to meet their own needs. This definition was given by the Norwegian Prime Minister, G.H. Brundtland, who was also the Director of World Health Organisation (WHO). Today sustainable development has become a buzz word and hundreds of programmes have been initiated in the name of sustainable development. If you want to test whether or not a proposal will achieve the goals of sustainability just try to find out the following.

- ❖ Does it protect our biodiversity?

- ❖ Does it prevent soil erosion?
- ❖ Does it slow down population growth?
- ❖ Does it increase forest cover?
- ❖ Does it cut off the emissions of CFC, SOx, NOx and CO2?
- ❖ Does it reduce waste generation and does it bring benefits to all?

These are only a few parameters for achieving sustainable growth. Until now development has been human-oriented, that too mainly, for a few rich nations. They have touched the greatest heights of scientific and technological development, but at what cost? The air we breathe, the water we drink and the food we eat have all been badly polluted. Our natural resources are just dwindling due to over exploitation. If growth continues in the same way, very soon we will be facing a doomsday - as suggested by Meadows et al (1972) in their world famous academic report **"The Limits to Growth".** This is unsustainable development which will lead to a collapse of the interrelated systems of this earth.Although the fears about such unsustainable growth and development started in 1970s, yet a clear discussion on sustainable development emerged on an international level in 1992, in the UN Conference on Environment and Development (UNCED), popularly known as The Earth Summit, held at Rio de Janeiro, Brazil. The Rio Declaration aims at a **"new and equitable global partnership through the creation of new levels of cooperation among states ..."** Out of its five significant agreements Agenda-21 proposes a global programme of action on sustainable development in social, economic and political context for the 21st Century.

These are the key aspects for sustainable development:

(a) Inter-generational equity: This emphasizes that we should minimize any adverse impacts on resources and environment for future generations i.e. we should hand over a safe, healthy and resourceful environment to our future generations. This can be possible only if we stop over-exploitation of resources, reduce waste discharge and emissions and maintain ecological balance.

(b) Intra-generational equity: This emphasizes that the development processes should seek to minimize the wealth gaps within and between nations. The Human Development Report of United Nations (2001) emphasizes that the benefits of technology should seek to achieve the goals of intra-generational equity. The technology should address to the problems of the developing countries, producing drought tolerant varieties for uncertain climates, vaccines for infectious diseases, clean fuels for domestic and industrial use. This type of technological development will support the economic growth of the poor countries and help in narrowing the wealth gap and lead to sustainability.

Measures- Some of the important measures for sustainable development are as follows:

- Using appropriate technology is one which is locally adaptable, eco-friendly, resource-efficient and culturally suitable.
- It mostly involves local resources and local labour.
- Indigenous technologies are more useful, cost-effective and sustainable.
- Nature is often taken as a model, using the natural conditions of that region as its components. This concept is known as design with nature.

- The Technology should use less of resources and should produce minimum waste.
- **Reduce, Reuse, Recycle approach:** The 3-R approach advocating minimization of resource use, using them again and again instead of passing it on to the waste stream and recycling the materials goes a long way in achieving the goals of sustainability. It reduces pressure on our resources as well as reduces waste generation and pollution.

Prompting environmental education and awareness:

- Makin environmental education the centre of all learning process will greatly help in changing the thinking and attitude of people towards our earth and the environment.
- Introducing the subject right from the school stage will inculcate a feeling of belongingness to earth in the small children.
- Earth thinking will gradually get incorporated in our thinking and action which will greatly help in transforming our life styles to sustainable ones.

Resource utilization as per carrying capacity:

- Any system can sustain a limited number of organisms on a long-term basis which is known as its carrying capacity.
- In case of human beings, the carrying capacity concept becomes all the more complex.
- It is because unlike other animals, human beings, not only need food to live, but need so many other things to maintain the quality of life.

Sustainability of a system depends largely upon the carrying capacity of the system. If the carrying capacity of a system is crossed (say, by over exploitation of a resource), environmental degradation starts and continues till it reaches a point of no return. Carrying capacity has two basic components:

- **Supporting capacity i.e. the capacity to regenerate**
- **Assimilative capacity i.e. the capacity to tolerate different stresses.**

In order to attain sustainability it is very important to utilize the resources based upon the above two properties of the system. Consumption should not exceed regeneration and changes should not be allowed to occur beyond the tolerance capacity of the system.

The Indian Context

India has still to go a long way in implementing the concept of sustainable development. We have to lay emphasis on framing a well-planned strategy for our developmental activity while increasing our economic growth. We have tremendous natural diversity as well as a huge population which makes planning for sustainable growth all the more important and complex. The National Council of Environmental Planning and Coordination (NCPC) set up in 1972 was the focal agency in this regard. The Ministry of Environment & Forests, set up in 1985 has formulated guidelines for various developmental activities keeping in view the sustainability principles.

UNIT III ENVIRONMENTAL POLLUTION AND DISASTER MANAGEMENT

Definition – causes, effects and control measures of: (a) Air pollution (b) Water pollution (c) Soil pollution (d) Marine pollution (e) Noise pollution (f) Thermal pollution (g) Nuclear hazards – soil waste management: causes, effects and control measures of municipal solid wastes, climate change, acid rain, disaster management: floods, earthquake, cyclone and landslides.

Pollution is the introduction of contaminants into the environment that cause harm or discomfort to humans or other living organisms, or that damage the environment, which can come in the form of chemical substances, or energy such as noise, heat or light. Pollutants can be naturally occurring substances or energies, but are considered contaminants when inexcess of natural levels. Environmental pollution takes place when the environment cannot process and neutralize harmful by-products of human activities (poisonous gas emissions) in due course without any structural or functional damage to its system. Pollution occurs, on the one hand, because the natural environment does not know how to decompose the unnaturally generated elements (i.e., anthropogenic pollutants), and, on the other, there is a lack of knowledge on the part of humans on how to decompose these pollutants artificially. It may last many years during which the nature will attempt to decompose the pollutants; in one of the worst cases – that of radioactive pollutants – it may take as long as thousands of years for the decomposition of such pollutants to be completed.

DEFINITION

Environmental pollution can, therefore, be defined as any undesirable change in the physical, chemical or biological characteristics of any component of the environment (air, water, soil), which can cause harmful effects on various forms of life or property.

Why does pollution matter?

It matters first and for most because it has negative impacts on crucial environmental services such as provision of clean air and clean water without which life on Earth as we know it would not exist. People are the reason we have pollution. Pollution affects our environment because the water pollution can affect the living conditions of people and plants. Pollution can cause our environment to start and fall. If we don’t have clean water for plants and trees how are we going to make paper and grow veggies for people to eat? This is why the big idea affects the environment and people are the main cause of pollution.

What are the types of pollution?

Environmental pollution may be of six types – air pollution, water pollution, soil pollution, noise pollution, pollution by radioactive substances and thermal pollution. Depending on the nature of pollutants, they can belong to three major categories – biological (pathogenic organisms, products of biological origin), chemical (toxic metals, agro chemicals, gaseous pollutants, particulates, hazards chemicals, carcinogenic substances, petroleum products, acidic or basic substances and radiation (ionizing and non-ionizing), radioactive substances, sound waves, foul odours. Due to rapid growth of population and industrialization environmental pollution is increasing at an alarming rate. Thus water is getting polluted by sewage and domestic effluents, industrial effluents, agricultural discharges, detergents, toxic

metals and radioactive materials. Even underground water has been polluted by leaching of various pollutants from the surface.

❖ AIR POLLUTION

Chemical Composition of Atmosphere:

Atmosphere is the cover of air that envelopes the earth. It extends up to 500 kms from the earth surface. The atmosphere is highly essential for all living organisms. The atmosphere contains many gases, most in small amounts, including some pollutants and greenhouse gases. The most abundant gas in the atmosphere is nitrogen, with oxygen second. Argon, an inert gas, is the third most abundant gas in the atmosphere.

The atmosphere is concentrated at the earth's surface and rapidly thins as you move upward, blending with space at roughly 100 miles above sea level. The atmosphere is actually very thin compared to the size of the earth, equivalent in thickness to a piece of paper laid over a beach ball. However, it is responsible for keeping our earth habitable and for producing weather.

The atmosphere is composed of a mix of several different gases in differing amounts. The permanent gases whose percentages do not change from day to day are nitrogen, oxygen and argon. Nitrogen accounts for 78% of the atmosphere, oxygen 21% and argon 0.9%. Gaseslike carbon dioxide, nitrous oxides, methane, and ozone are trace gases that account for about a tenth of one percent of the atmosphere. Water vapour is unique in that its concentration varies from 0-4% of the atmosphere depending on where you are and what time of the day it is. In the cold, dry artic regions water vapour usually accounts for less than 1% of the atmosphere, while in humid, tropical regions water vapour can account for almost 4% of the atmosphere. Water vapour content is very important in predicting weather. Greenhouse gases whose percentages vary daily, seasonally, and annually have physical and chemical properties which make them interact with solar radiation and infrared light (heat) given off from the earth to affect the energy balance of the globe. This is why scientists are watching the observed increase in greenhouse gases like carbon dioxide and methane carefully, because even though they are small in amount, they can strongly affect the global energy balance and temperature over time.

Troposphere: The troposphere starts at the Earth's surface and extends 8 to 14.5 kilometers high (5 to 9 miles). This part of the atmosphere is the most dense. Almost all weather is in this region.

Stratosphere: The stratosphere starts just above the troposphere and extends to 50 kilometers (31 miles) high. The ozone layer, which absorbs and scatters the solar ultraviolet radiation, is in this layer.

Mesosphere: The mesosphere starts just above the stratosphere and extends to 85 kilometers (53 miles) high. Meteors burn up in this layer.

Thermosphere: The thermosphere starts just above the mesosphere and extends to 600 kilometers (372 miles) high. Aurora and satellites occur in this layer.

Ionosphere: The ionosphere is an abundant layer of electrons and ionized atoms and molecules that stretches from about 48 kilometers (30 miles) above the surface to the edge of space at about 965 km (600 mi), overlapping into the mesosphere and thermosphere. This dynamic

region grows and shrinks based on solar conditions and divides further into the sub-regions: D, E and F; based on what wavelength of solar radiation is absorbed. The ionosphere is a critical link in the chain of Sun-Earth interactions. This region is what makes radio communications possible.

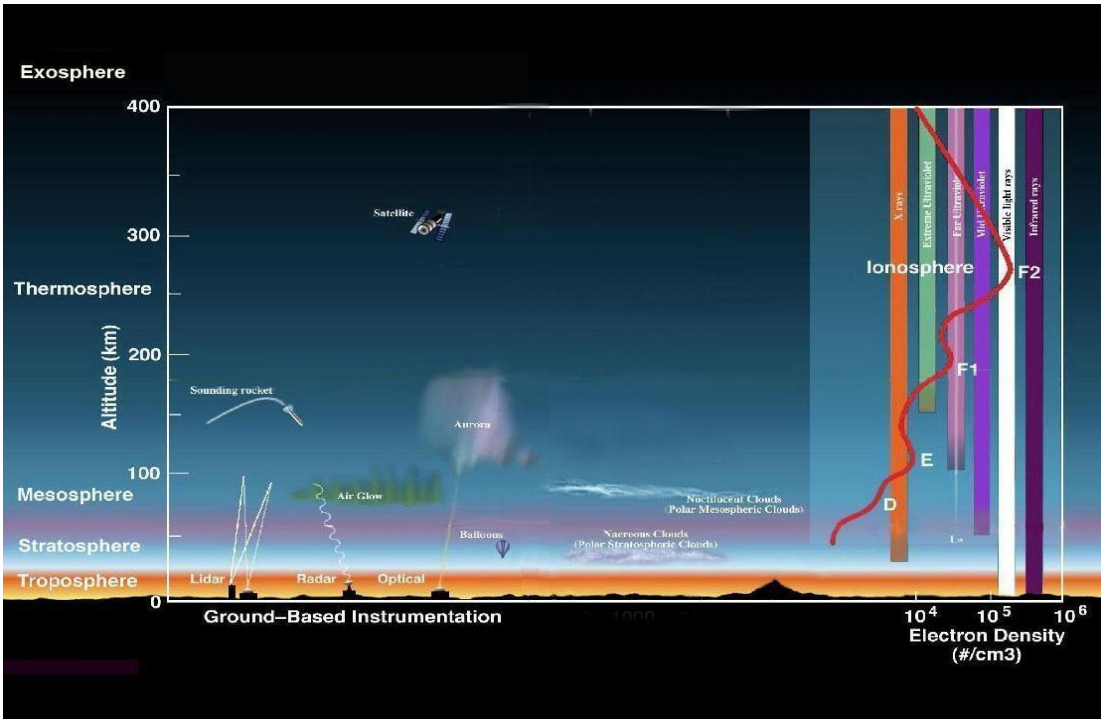


Figure 1: Levels of Atmosphere

Exosphere: This is the upper limit of our atmosphere. It extends from the top of the thermosphere up to 10,000 km (6,200 mi).

Gaseous pollutants include oxides of sulphur (mostly SO2, SO3 oxides of nitrogen (mostly NO and NO2 or NOx), carbon monoxide (CO), volatile organic compounds (mostly hydrocarbons) etc. Particulate pollutants include smoke, dust, soot, fumes, aerosols, liquid droplets, pollen grains etc. Radioactive pollutants include radon-222, iodine-131, strontium-90, plutonium-239 etc.

Classification of Air Pollutants:

Air pollutants can be classified depending upon the form in which they are present in the environment as:

1) Primary pollutants are those emitted directly into the atmosphere in the harmful form. Eg:. CO, NO, SO_2 etc.

2) Secondary pollutants: Some of the primary pollutants might react with one another or with the basic components of air to form new pollutants. These resultant new pollutants are called secondary pollutants.

Eg.: $NO / NO_2 \xrightarrow{Moisture} HNO_3 / NO_3$

Indoor Air Pollutants: These are primary air pollutants. Important example is Radon.

(i) Radon gas emitted from the building materials like bricks. Concrete, tiles etc. – derived from soil containing Radium.

(ii) Also present in natural gas, ground water and is emitted during their usage indoors.

(iii) Burning of fuels in the kitchen, smoking, - CO, SO_2, formaldehyde, BAP (Benzo-a-pyrene), etc.,

WHO (World Health Organization) has made it known, that according to the statistics available to it, more than 1.1 billion people live in urban areas where outdoor air is unhealthy to breathe. With the ever increasing rate of urbanization the number of such affected presently may be much larger. Some of the air pollutants are detailed below:

(1) Carbon monoxide (CO):

Description: It is a colourless, odourless gas (hence not perceived by the nose to get alerted about the lurking danger) that is poisonous to air breathing beings. It is formed during the incomplete combustion of carbon containing fuels:

$$2C + O_2 \rightarrow 2CO$$

Even in villages, people using fire wood and charcoal for their cooking purposes can cause this event. The general human sources for this dangerous pollutant are: cigarette smoking, incomplete burning of fossil fuels. About 77% comes from motor vehicle exhaust. (Dogs get asphyxiated in the mine walk ups due to the CO contamination.)

Health Effects: CO reacts with the Haemoglobin in red cells and reduces the ability of blood to bring Oxygen to body cells and tissues – causing headaches and anaemia. At high levels it causes coma, irreversible brain cell damage and death.

(2) Nitrogen Dioxide (NO_2):

It is a reddish brown irritating gas that gives photochemical smog. In the atmosphere it can get converted into Nitric Acid (HNO_3).

$$NO_2 + Moisture \rightarrow HNO_3$$

Human Sources: Fossil fuel burning motor vehicles (49%) and Industrial Power Plants (40%) are some of the sources of nitrogen dioxide.

Health Effects: Lung irritation and damage

Environmental effects: Acid deposition of HNO_3 could damage trees, soils and aquatic life in lakes. HNO_3 could corrode metals and eat away stone on the buildings, statues and monuments. NO_2 could damage fabrics.

(3) Sulphur Dioxide (SO_2):

It is again an irritating gas that is also colourless. It is mostly formed from the combustion of Sulphur containing Fossil fuels such as coal and oil. (Start up of the Sulphuric Acid plant). In the atmosphere it can be converted into Sulphurous and Sulphuric acids. These are the major components of acid deposition.

Human Sources: Coal combustion in Thermal Power Plants (88%) and other industrial processes (10%) are some of the sources of sulphur dioxide.

Health Effects: Breathing problems even for healthy people

Environmental effects: Reduces visibility, acid deposition of H_2SO_4. It can cause damage to trees, soils and aquatic life in lakes.

(4) Suspended Particulate Matter (SPM):

These include a variety of particles and droplets (aerosols). They can be suspended in atmospheric air for short to long periods. (Room deodorizers)

Human Sources*:* Burning of coal in power and industrial plants (40%), burning diesel and other fuels in vehicles(17%), agriculture, unpaved roads, construction work etc.

Health Effects: Nose and throat irritation, lung damage, bronchitis, asthma, reproductive problems and cancer.

Environmental Effects: Reduces visibility, causes acid deposition and H_2SO_4 droplets. These could damage trees, soils and aquatic life in lakes.

(5) Ozone:

This is a highly reactive gas which possess an unpleasant odour and is an irritant. It forms a major portion of the troposphere. It is the major component of photochemical smog.

Human Sources: Chemical reaction with volatile organic compounds (emitted mostly by cars and industries) and nitrogen oxides.

Environmental Effect: Moderates the climate.

(6) Photochemical Smog:

A photochemical reaction is any reaction activated by light. Air pollution known as photochemical smog is a mixture of more than 100 primary and secondary pollutants formed under the influence of sunlight. Its formation begins inside automobile engines and the boilers in coal-burning power and industrial plants.

Health Effects: Breathing problems, cough, eye, nose and throat irritation, heart ailments, reduces resistance to colds and pneumonia.

Environmental effects: Ozone can cause damage to plants and trees, smog can affect visibility

(7) Lead (Pb): This is a toxic solid metal. Even its compounds, emitted into the atmosphere as particulate matter is also a pollutant.

Human sources: Paint, smelters (Metal refineries), lead manufacture, storage batteries, leaded petrol.

Health effects: accumulates in the body, brain and nervous system damage and possible mental retardation (especially in children); digestive and other problems. Some lead containing chemicals caused cancer in test animals.

Environmental effects: Can harm wildlife

Causes / Sources of Air Pollution:

The sources of air pollution are natural and man-made (anthropogenic).

Natural Sources:

The natural sources of air pollution are volcanic eruptions, forest fires, sea salt sprays, biological decay, photochemical oxidation of terpenes, marshes, extra-terrestrial bodies, pollen grains of flowers, spores etc. Radioactive minerals present in the earth crust are the sources of radioactivity in the atmosphere.

Man-made Sources:

Man-made sources include thermal power plants, industrial units, vehicular emissions, fossil fuel burning, agricultural activities etc. Thermal power plants have become the major sources for generating electricity in India as the nuclear power plants could not be installed as planned. The main pollutants emitted are fly ash and SO_2. Metallurgical plants also consume coal and produce similar pollutants. Fertilizer plants, smelters, textile mills, tanneries, refineries, chemical industries, paper and pulp mills are other sources of air pollution.

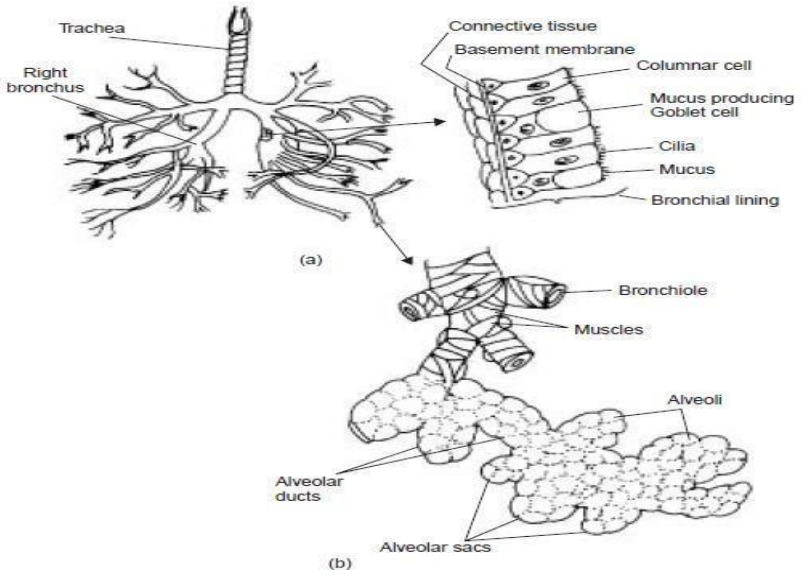
Indoor Air Pollution

The most important indoor air pollutant is radon gas. Radon gas and its radioactive daughters are responsible for a large number of lung cancer deaths each year. Radon can be emitted from building materials like bricks, concrete, tiles etc. which are derived from soil containing radium. Radon is also present in groundwater and natural gas and is emitted indoors while using them.

Effects of Air Pollution:

Air pollution has adverse effects on living organisms and materials.

Effects on Human Health:



Lower respiratory system of human beings (*a* and *b*) and cross section of bronchial lining showing cilia and goblet cells.

Figure 2: Air pollution effect on lungs

Human respiratory system has a number of mechanisms for protection from air pollution. Bigger particles (>10 μm) can be trapped by the hairs and sticky mucus in the lining of the nose. Smaller particles can reach tracheobronchial system and there get trapped in mucus. They are sent back to throat by beating of hair like cilia from where they can be removed by spitting or swallowing. Years of exposure to air pollutants (including cigarette smoke) adversely affect these natural defenses and can result in lung cancer, asthma, chronic bronchitis and emphysema (damage to air sacs leading to loss of lung elasticity and acute shortness of breath).

Oxides of nitrogen especially NO_2 can irritate the lungs and cause conditions like chronic bronchitis and emphysema. Carbon monoxide (CO) reaches lungs and combines with haemoglobin of blood to form carboxy-haemoglobin. CO has affinity for haemoglobin 210 times more than oxygen. Haemoglobin is, therefore, unable to transport oxygen to various parts of the body. This causes suffocation. Long exposure to CO may cause dizziness, unconsciousness and even death.

Effects on Plants:

Air pollutants affect plants by entering through stomata (leaf pores through which gases diffuse), destroy chlorophyll and affect photosynthesis. Pollutants also erode waxy coating of the leaves called cuticle. Cuticle prevents excessive water loss and damage from diseases, pests, drought and frost. Damage to leaf structure causes necrosis (dead areas of leaf), chlorosis (loss or reduction of chlorophyll causing yellowing of leaf) or epinasty (downward curling of leaf), and abscission (dropping of leaves). Particulates deposited on leaves can form encrustations and plug the stomata. The damage can result in death of the plant.

Effects on aquatic life:

Air pollutants mixing up with rain can cause high acidity (lower pH) in fresh water lakes. This affects aquatic life especially fish. Some of the freshwater lakes have experienced total fish death.

Effects on materials:

Because of their corrosiveness, particulates can cause damage to exposed surfaces. Presence of SO2 and moisture can accelerate corrosion of metallic surfaces. SO2 can affect fabric, leather, paint, paper, marble and limestone. Ozone in the atmosphere can cause cracking of rubber. Oxides of nitrogen can also cause fading of cotton and rayon fibres.

Ozone Depletion:

Ozone layer or shield is present in the stratosphere. It protects the earth from short-waveultra-violet rays (below 300 nm) by changing the same into infra-red rays. A large hole has appeared in ozone shield over Antarctica (first detected by Farman, 1982) and a smaller one over North pole. Size of the holes varies with the seasons.

Ozone hole: During the period 1956-1970 the spring time 03 layer thickness above Antarctica varied from 280-325 Dobson unit. Thickness was sharply reduced to 225 DU in 1979 and 136 DU in 1985. Antarctic air is completely isolated from rest of world by natural circulation of wind called as polar vertex. The decline in spring time, ozone layer thickness is called ozone hole. It was first noted in 1985 over Antarctica. Thinning of ozone shield has also been reported elsewhere (Eg.: 8% between 30°—50°NP).

Depletion of ozone layer allows harmful ultra-violet radiations to reach earth. It is the major cause of skin cancer, cataract, dimming of eye sight, decrease in immune system and increased susceptibility to herpes. Thinning of ozone shield is being caused by a number of pollutants like chlorofluorocarbons (14% of total depletion), nitrogen oxides (3.5% depletion), sulphur dioxide, halon, carbon tetrachloride, methyl chloroform, chlorine, etc. Many of these are being released by jets flying in the stratosphere and rockets being fired into space. Others are persistent in the troposphere and gradually pass into stratosphere.

Ozone layer as protective layer: The ozone layer in the stratosphere is very useful to human beings because it absorbs the major part of harmful ultraviolet radiation coming from the sun. Therefore, it is called protective layer. However, it has been observed that the ozone layer is getting depleted. One of the reasons for depletion of ozone layer is action of aerosols spray propellants. These are the chemicals such as fluorocarbons and chlorofluorocarbons. These compounds react with ozone gas in the atmosphere thereby depleting it. Scientists all over the world are worried at the destruction of ozone layer. If the ozone layer in the atmosphere is significantly decreased, these harmful radiations would reach the earth and would cause many damages such as skin cancer & genetic disorders in man and other living forms. Efforts are being made to find substitutes of these chemicals which do not react with ozone.

Effects of UV radiations on human:

1. In humans, the increased UV radiation increases the incidence in cancer (including melanoma).
2. Reduces the functioning of immune system.
3. Cornea absorbs UV-B radiations, and a high dose of UV-B causes inflammation of cornea called snow blindness, cataract etc. Exposure may permanently damage cornea and cause cataract.

Measures to prevent ozone layer depletion

- Cut down the use of CFCs.
- Do not use polystyrene cups that have chlorofluorocarbon molecules in them which destroy ozone layer.
- Use CFC free refrigerators.
- Use the chemicals derived from peaches and plums to clean computer chips and circuit boards instead of CFCs.

Acid rain:

"Acid rain" is a broad term used to describe several ways that acids fall out of the atmosphere. A more precise term is acid deposition, which has two parts: wet and dry. Wet deposition refers to acidic rain, fog, and snow. As this acidic water flows over and through the ground, it affects a variety of plants and animals. The strength of the effects depend on many factors, including how acidic the water is, the chemistry and buffering capacity of the soils involved, and the types of fish, trees, and other living things that rely on the water. Dry deposition refers to acidic gases and particles. About half of the acidity in the atmosphere falls back to earth through dry deposition. The wind blows these acidic particles and gases onto buildings, cars, homes, and trees. Dry deposited gases and particles can also be washed from trees and other surfaces by rainstorms. When that happens, the runoff water adds those acids to the acid rain, making the combination more acidic than the falling rain alone. Prevailing winds blow the compounds that cause both wet and dry acid deposition across state and national borders, and sometimes over

hundreds of miles. Scientists discovered, and have confirmed, that sulphur dioxide (SO_2) and nitrogen oxides (NOx) are the primarycauses of acid rain. In the US, About 2/3 of all SO_2 and 1/4 of all NOx comes from electric power generation that relies on burning fossil fuels like coal. Acid rain occurs when these gases react in the atmosphere with water, oxygen, and other chemicals to form various acidic compounds. Sunlight increases the rate of most of these reactions. The result is a mild solution of sulfuric acid and nitric acid.

Effects of Acid Rain:

Acid rain causes acidification of lakes and streams and contributes to damage of trees at high elevations (for example, red spruce trees above 2,000 feet) and many sensitive forest soils. In addition, acid rain accelerates the decay of building materials and paints, including irreplaceable buildings, statues, and sculptures that are part of our nation's cultural heritage. Prior to falling to the earth, SO_2 and NOx gases and their particulate matter derivatives, sulphates and nitrates, contribute to visibility degradation and harm public health.

Control of Air Pollution

Air pollution can be minimized by the following methods:

- Siting of industries after proper Environmental Impact Assessment studies.
- Using low sulphur coal in industries
- Removing sulphur from coal (by washing or with the help of bacteria)
- Removing NOx during the combustion process.
- Removing particulate from stack exhaust gases by employing electrostatic precipitators, bag-house filters, cyclone separators, scrubbers etc.
- Vehicular pollution can be checked by regular tune-up of engines ; replacement of more polluting old vehicles; installing catalytic converters; by engine modification to have fuel efficient (lean) mixtures to reduce CO and hydrocarbon emissions; and slow and cooler burning of fuels to reduce NOx emission (Honda Technology).
    - Using mass transport system, bicycles etc.
    - Shifting to less polluting fuels (hydrogen gas).
    - Using non-conventional sources of energy.
    - Using biological filters and bio-scrubbers
- Reduction of Air Pollution at Source
- Control of particulate pollutants

Cyclones Separator: Cyclones provide a low-cost, low-maintenance method of removing larger particulates from a gas stream. The general principle of inertia separation is that the particulate-laden gas is forced to change direction. As gas changes direction, the inertia of the particles causes them to continue in the original direction and be separated from the gas stream. The walls of the cyclone narrow toward the bottom of the unit, allowing the particles to be collected in a hopper. The cleaner air leaves the cyclone through the top of the chamber, flowing upward in a spiral vortex, formed within a downward moving spiral. Cyclones are efficient in removing large particles but are not as efficient with smaller particles. For this reason, they are used with other particulate control devices. Because the particulate control devices discussed above capture the pollutants but don't destroy them, proper disposal of the collected material is needed. Collected solid particles are most often disposed of in a landfill.

Wastewater generated by scrubber must be sent to a wastewater treatment facility. When possible, collected particle matter is recycled and reused.

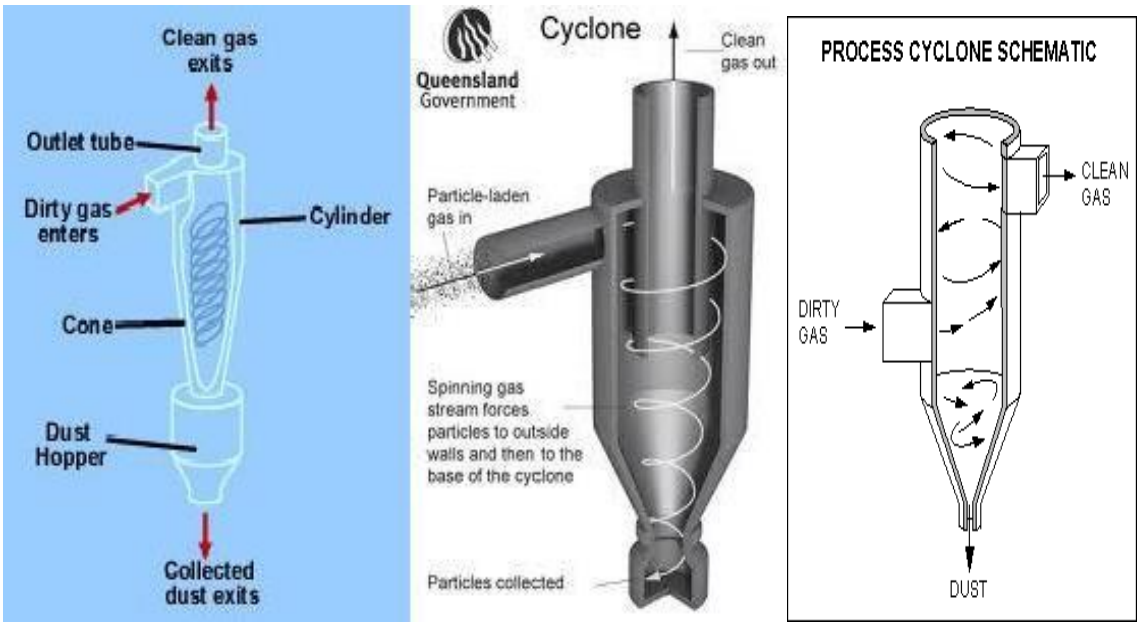


Figure 3: Cyclone separator

Electrostatic precipitator: The electrostatic precipitator works by removing particles and smoke from a gas stream using an induced electrostatic charge. Dust particles pass by wires that have a high DC voltage applied, which ionises the surrounding gas. This is a known as a 'corona discharge' from which the particles pick up a small electrostatic charge. These particles are then attracted to an oppositely charged plate where the charge is neutralised and the particles retained. Particles are physically removed by regular tapping (rapping) of the plates. In some situations, the particles may be pre-conditioned by introducing a water spray to reduce the electrical resistivity of the particles allowing them to accept the charge more easily. Electrostatic precipitators are an efficient way of removing particles and do not cause a significant pressure drop across the unit.

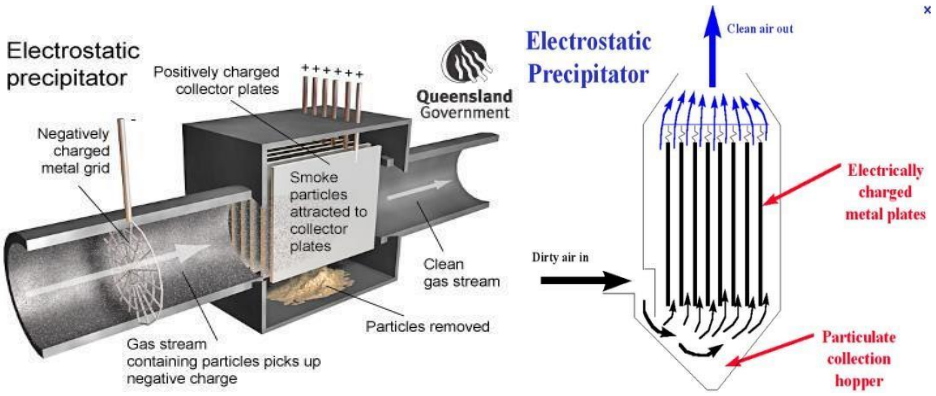


Figure 4: Electrostatic Precipitator

Wet scrubber: A wet scrubber is a device that removes gaseous and/or particle contaminants from a gas stream. It operates by bringing the gas stream into contact with a scrubbing liquid (usually water). To increase the contact between the gas and scrubbing liquid and thus increase

the removal efficiency some form of packing is often used. These are known as packed bed wet scrubbers. Gaseous pollutants are removed by absorption into the scrubbing liquid (absorbers) while particles are removed by physical capture of the particles in the droplets. While packed bed scrubbers can remove both gaseous pollutants and particles they areusually engineered to be more efficient at one or the other depending on the conditions. Gaseous pollutant removal can also be enhanced by using specific scrubbing liquids. An example would be the removal of acidic pollutants using an alkaline liquid. Some gas streams may require pre-conditioning, either to reduce high temperatures or to remove very high dust loadings that would otherwise clog the packing material. Other designs may use an entrainment separator to remove any scrubbing liquid droplets that may be carried along in the cleaned gas stream, or to recycle the scrubbing liquid either directly or after suitable treatment.

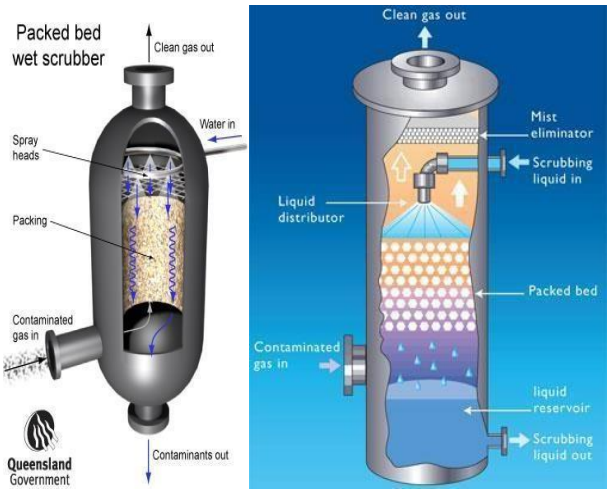


Figure 5: Wet Scrubber

Bag House filters: Bag filters, alternatively known as fabric filters or baghouses, use fabric filter bags to remove particles from dust-laden gas. These filters can achieve high efficiencies for very fine particles due to the build-up of particles on the surface of the bag.

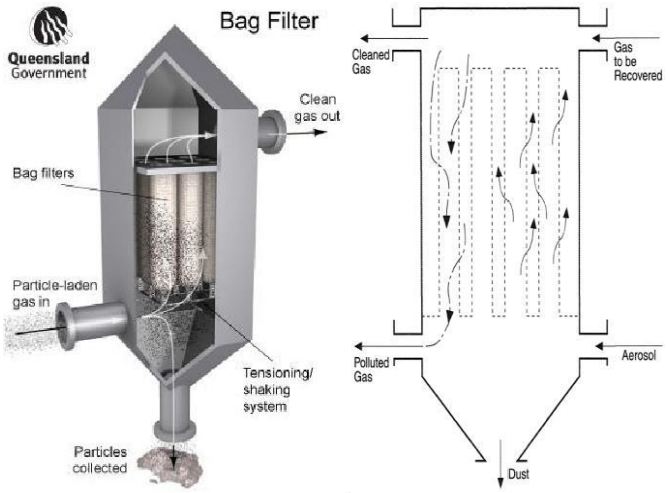


Figure 5: Wet Scrubber

Collection can occur on either the inside or outside of the bag depending on the design. As particles build up, the pressure drop across the bag increases, so the filters must undergo a regular cleaning schedule, usually by having the filters running in tandem—while one is

operating the other is undergoing cleaning. Cleaning schedules are normally automated and the methods employed are mechanical shaking, the use of a reversed air flow, or a pulse of compressed air. Each method is used to dislodge the cake of dust from the fabric surface, which then falls by gravity into a collection system.

❖ NOISE POLLUTION

We hear various types of sounds every day. Sound is mechanical Energy from a vibrating source. A type of sound may be pleasant to someone and at the same time unpleasant to others. The unpleasant and unwanted sound is called noise.

Sound can propagate through a medium like air, liquid or solid. Sound wave is a pressure perturbation in the medium through which sound travels. Sound pressure alternately causes compression and rarefaction. The number of compressions and rarefactions of the molecules of the medium (for example air) in a unit time is described as frequency. It is expressed in Hertz (Hz) and is equal to the number of cycles per second.

Sources of Noise Pollution:

The main sources of noise are various modes of transportation (like air, road, rail-transportation), industrial operations, construction activities and celebrations (social/religious functions, elections etc) electric home appliances. High levels of noise have been recorded in some of the cities of the world. In Nanjing (China) noise level of 105 dB has been recorded, while in some other cities of the world these levels are: Rome 90 dB, New York 88 dB, Calcutta 85 dB, Mumbai 82 dB, Delhi 80 dB, Kathmandu 75 dB.

Effects of Noise:

Noise causes the following effects.

(i) Interferes with man's communication: In a noisy area communication is severely affected.

(ii) Hearing damage:

Noise can cause temporary or permanent hearing loss. It depends on intensity and duration of sound level. Auditory sensitivity is reduced with noise level of over 90 dB in the mid high frequency for more than a few minutes.

(iii) Physiological and Psychological changes:

Continuous exposure to noise affects the functioning of various systems of the body. It may result in hypertension, insomnia (sleeplessness), gastro-intestinal and digestive disorders, peptic ulcers, blood pressure changes, behavioural changes, emotional changes etc.

Noise Pollution during Diwali

Diwali is the Indian festival of lights. Noise generated by various firecrackers is beyond the permissible noise levels of 125 decibels as per the Environmental (Protection) (Second Amendment) Rules, 1999.

1. The manufacture, sale or use of fire-crackers generating noise level exceeding 125 dB (AI) or 145 dB (C) at 4 meters distance from the point of bursting shall be prohibited.

2. The use of fireworks or fire crackers shall not be permitted except between 6:00pm and 10:00pm. No fireworks or fire crackers shall be used between 10:00pm and 6:00am.

3. Fire crackers shall not be used at any time in silence zones. Silence Zone in an area comprising not less than 100 meters around hospitals, educational institutions, courts, religious places or any other area which is declared as such by the competent authority.

Control of Noise Pollution

1. Reduction in sources of noise: Sources of noise pollution like heavy vehicles and old vehicles may not be allowed to ply in the populated areas.

2. Noise making machines should be kept in containers with sound absorbing media.

3. Proper oiling will reduce the noise from the machinery.

4. Use of sound absorbing silencers: Silencers can reduce noise by absorbing sound.

5. Planting more trees having broad leaves.

❖ WATER POLLUTION

Water pollution can be defined as alteration in physical, chemical or biological characteristics of water making it unsuitable for designated use in its natural state.

Sources of Water Pollution:

Water has the property to dissolve many substances in it, therefore, it can easily get polluted. Pollution of water can be caused by point sources or non-point sources.

Point sources

Point sources are specific sites near water which directly discharge effluents into them. Major point sources of water pollution are

- industries,
- power plants,
- underground coal mines,
- offshore oil wells

Non Point Sources

The non-point sources are

- Surface run-off from agricultural fields,
- overflowing small drains,
- rain water sweeping roads and fields, atmospheric deposition

Ground water pollution

Ground water forms about 6.2% of the total water available on planet earth and is about 30 times more than surface water (streams, lakes) there are a number of potential sources of ground water pollution. Septic tanks, industry (textile, chemical, tanneries), deep well injection, mining

etc. are mainly responsible for ground water pollution, Ground water pollution with arsenic, fluoride and nitrate are posing serious health hazards.

Surface water pollution

1. Sewage
2. Industrial effluents
3. Synthetic detergents
4. Agrochemicals
5. Oil
6. Waste Heat

Effects of Water Pollution

Oxygen demanding wastes:

Organic matter which reaches water bodies is decomposed by micro-organisms present in water. Dissolved oxygen (DO) is the amount of oxygen dissolved in a given quantity of water at a particular temperature and atmospheric pressure. Amount of dissolved oxygen depends on aeration, photosynthetic activity in water, respiration of animals and plants and ambient temperature.

Nitrogen and Phosphorus Compounds (Nutrients):

Addition of compounds containing nitrogen and phosphorus helps in the growth of algae and other plants which when die and decay consume oxygen of water.

Pathogens:

Many wastewaters especially sewage contain many pathogenic (disease causing) and non-pathogenic micro-organisms and many viruses. Water borne diseases like cholera, dysentery, typhoid, jaundice etc.

Toxic Compounds:

Pollutants such as heavy metals, pesticides, cyanides and many other organic and inorganic compounds are harmful to aquatic organisms.

Control of Water Pollution

(i) Judicious use of agrochemicals like pesticides and fertilizers which will reduce their surface run-off and leaching. Avoid use of these on sloped lands.

(ii) Use of nitrogen fixing plants to supplement the use of fertilizers.

(iii) Adopting integrated pest management to reduce relying on pesticides.

(iv) Prevent run-off of manure. Divert such run-off to basin for settlement. The nutrient rich water can be used as fertilizer in the fields.

(v) Separate drainage of sewage and rain water should be provided to prevent overflow of sewage with rainwater.

(vi) Planting trees would reduce pollution by sediments and will also prevent soil erosion.

Waste Water Treatment:

The size and capacity of wastewater treatment systems are determined by the estimated volume of sewage generated from residences, businesses, and industries connected to sewer systems as well as the anticipated inflows and infiltration (I&I). The selection of specific on-lot, clustered, or centralized treatment plant configurations depends upon factors such as the number of customers being served, the geographical scenario, site constraints, sewer connections, average and peak flows, influent wastewater characteristics, regulatory effluent limits, technological feasibility, energy consumption, and the operations and maintenance costs involved. The predominant method of wastewater disposal in large cities and towns is discharge into a body of surface water. Suburban and rural areas rely more on subsurface disposal. In either case, wastewater must be purified or treated to some degree in order to protect both public health and water quality. Suspended particulates and biodegradable organics must be removed to varying extents. Pathogenic bacteria must be destroyed. It may also be necessary to remove nitrates and phosphates (plant nutrients) and to neutralize or remove industrial wastes and toxic chemicals. The degree to which wastewater must be treated varies, depending on local environmental conditions and governmental standards. Two pertinent types of standards are stream standards and effluent standards. Stream standards, designed to prevent the deterioration of existing water quality, set limits on the amounts of specific pollutants allowed in streams, rivers, and lakes. The limits depend on a classification of the "maximum beneficial use" of the water. Water quality parameters that are regulated by stream standards include dissolved oxygen, coliforms, turbidity, acidity, and toxic substances. Effluent standards, on the other hand, pertain directly to the quality of the treated wastewater discharged from a sewage treatment plant. The factors controlled under these standards usually include biochemical oxygen demand (BOD), suspended solids, acidity, and coliforms. There are three levels of wastewater treatment: primary, secondary, and tertiary (or advanced). Primary treatment removes about 60 percent of total suspended solids and about 35 percent of BOD; dissolved impurities are not removed. It is usually used as a first step before secondary treatment. Secondary treatment removes more than 85 percent of both suspended solids and BOD. A minimum level of secondary treatment is usually required in the United States and other developed countries. When more than 85 percent of total solids and BOD must be removed, or when dissolved nitrate and phosphate levels must be reduced, tertiary treatment methods are used. Tertiary processes can remove more than 99 percent of all the impurities from sewage, producing an effluent of almost drinking-water quality. Tertiary treatment can be very expensive, often doubling the cost of secondary treatment. It is used only under special circumstances. For all levels of wastewater treatment, the last step prior to discharge of the sewage effluent into a body of surface water is disinfection, which destroys any remaining pathogens in the effluent and protects public health. Disinfection is usually accomplished by mixing the effluent with chlorine gas or with liquid solutions of hypochlorite chemicals in a contact tank for at least 15 minutes. Because chlorine residuals in the effluent may have adverse effects on aquatic life, an additional chemical may be added to dechlorinate the effluent. Ultraviolet radiation, which can disinfect without leaving any residual in the effluent, is becoming more competitive with chlorine as a wastewater disinfectant.

Primary treatment:

Primary treatment removes material that will either float or readily settle out by gravity. It includes the physical processes of screening, comminution, grit removal, and sedimentation.

Screens are made of long, closely spaced, narrow metal bars. They block floating debris such as wood, rags, and other bulky objects that could clog pipes or pumps. In modern plants the screens are cleaned mechanically, and the material is promptly disposed of by burial on the plant grounds. A comminutor may be used to grind and shred debris that passes through the screens. The shredded material is removed later by sedimentation or floatation processes.

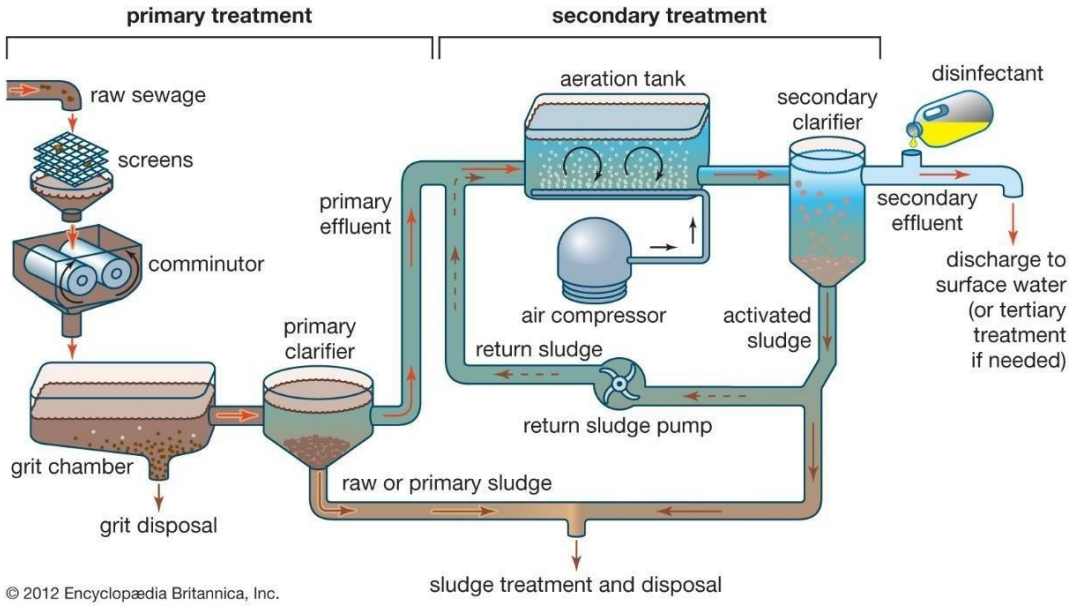


Figure 6: Primary and secondary treatment of sewage, using the activated sludge process.

Grit chambers are long narrow tanks that are designed to slow down the flow so that solids such as sand, coffee grounds, and eggshells will settle out of the water. Grit causes excessive wear and tear on pumps and other plant equipment. Its removal is particularly important in cities with combined sewer systems, which carry a good deal of silt, sand, and gravel that wash off streets or land during a storm. Suspended solids that pass through screens and grit chambers are removed from the sewage in sedimentation tanks. These tanks, also called primary clarifiers, provide about two hours of detention time for gravity settling to take place. As the sewage flows through them slowly, the solids gradually sink to the bottom. The settled solids—known as raw or primary sludge—are moved along the tank bottom by mechanical scrapers. Sludge is collected in a hopper, where it is pumped out for removal. Mechanical surface-skimming devices remove grease and other floating materials.

Secondary treatment

Secondary treatment removes the soluble organic matter that escapes primary treatment. It also removes more of the suspended solids. Removal is usually accomplished by biological processes in which microbes consume the organic impurities as food, converting them into carbon dioxide, water, and energy for their own growth and reproduction. The sewage treatment plant provides a suitable environment, albeit of steel and concrete, for this natural biological process. Removal of soluble organic matter at the treatment plant helps to protect the dissolved oxygen balance of a receiving stream, river, or lake.

There are three basic biological treatment methods: the trickling filter, the activated sludge process, and the oxidation pond. A fourth, less common method is the rotating biological contacter.

Trickling Filter

A trickling filter is simply a tank filled with a deep bed of stones. Settled sewage is sprayed continuously over the top of the stones and trickles to the bottom, where it is collected for further treatment. As the wastewater trickles down, bacteria gather and multiply on the stones. The steady flow of sewage over these growths allows the microbes to absorb the dissolved organics, thus lowering the biochemical oxygen demand (BOD) of the sewage. Aircirculating upward through the spaces among the stones provides sufficient oxygen for the metabolic processes. Settling tanks, called secondary clarifiers, follow the trickling filters. These clarifiers remove microbes that are washed off the rocks by the flow of wastewater. Two or more trickling filters may be connected in series, and sewage can be recirculated in order to increase treatment efficiencies.

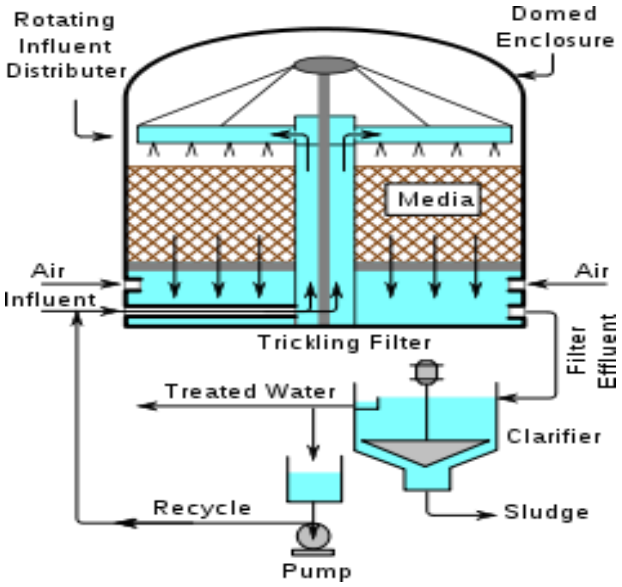


Figure 7: Trickling filter

Activated sludge

The activated sludge treatment system consists of an aeration tank followed by a secondary clarifier. Settled sewage, mixed with fresh sludge that is recirculated from the secondary clarifier, is introduced into the aeration tank. Compressed air is then injected into the mixture through porous diffusers located at the bottom of the tank. As it bubbles to the surface, the diffused air provides oxygen and a rapid mixing action. Air can also be added by the churning action of mechanical propeller-like mixers located at the tank surface. Under such oxygenated conditions, microorganisms thrive, forming an active, healthy suspension of biological solids—mostly bacteria—called activated sludge. About six hours of detention is provided in the aeration tank. This gives the microbes enough time to absorb dissolved organics from the sewage, reducing the BOD. The mixture then flows from the aeration tank into the secondary clarifier, where activated sludge settles out by gravity. Clear water is skimmed from the surface of the clarifier, disinfected, and discharged as secondary effluent. The sludge is pumped out

from a hopper at the bottom of the tank. About 30 percent of the sludge is recirculated back into the aeration tank, where it is mixed with the primary effluent. This recirculation is a key feature of the activated sludge process. The recycled microbes are well acclimated to the sewage environment and readily metabolize the organic materials in the primary effluent. The remaining 70 percent of the secondary sludge must be treated and disposed of in an acceptable manner (see Sludge treatment and disposal). Variations of the activated sludge process include extended aeration, contact stabilization, and high-purity oxygen aeration. Extended aeration and contact stabilization systems omit the primary settling step. They are efficient for treating small sewage flows from motels, schools, and other relatively isolated wastewater sources. Both of these treatments are usually provided in prefabricated steel tanks called package plants. Oxygen aeration systems mix pure oxygen with activated sludge. A richer concentration of oxygen allows the aeration time to be shortened from six to two hours, reducing the required tank volume.

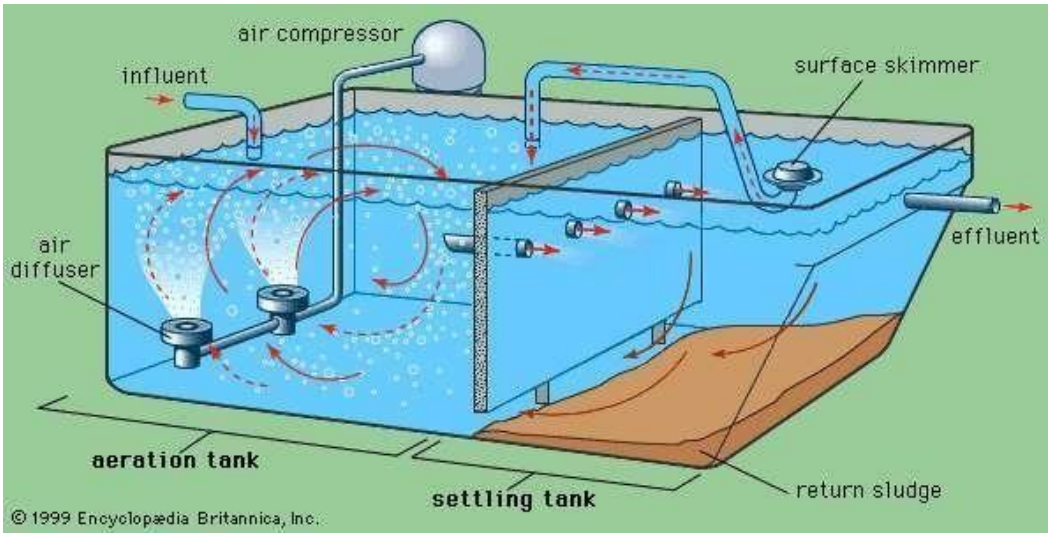


Figure 8: Schematic diagram of a prefabricated package plant for the aeration treatment of small sewage flows.

Oxidation pond

Oxidation ponds, also called lagoons or stabilization ponds, are large, shallow ponds designed to treat wastewater through the interaction of sunlight, bacteria, and algae. Algae grow using energy from the sun and carbon dioxide and inorganic compounds released by bacteria in water. During the process of photosynthesis, the algae release oxygen needed by aerobic bacteria. Mechanical aerators are sometimes installed to supply yet more oxygen, thereby reducing the required size of the pond. Sludge deposits in the pond must eventually be removed by dredging. Algae remaining in the pond effluent can be removed by filtration or by a combination of chemical treatment and settling.

Rotating biological contacter

In this treatment system a series of large plastic disks mounted on a horizontal shaft are partially submerged in primary effluent. As the shaft rotates, the disks are exposed alternately to air and wastewater, allowing a layer of bacteria to grow on the disks and to metabolize the organics in the wastewater.

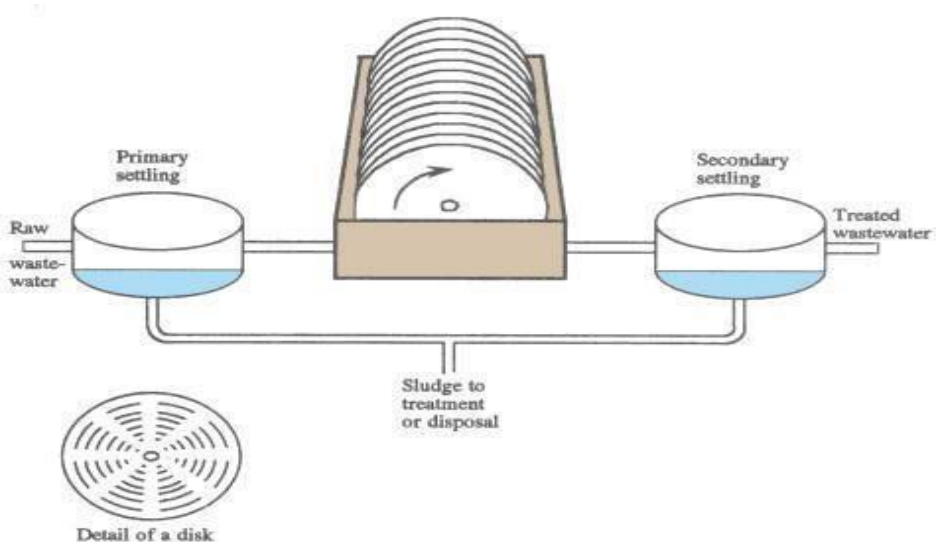


Figure 9: Rotating biological contacter

❖ THERMAL POLLUTION

Thermal pollution can be defined as presence of waste heat in the water which can cause undesirable changes in the natural environment.

Causes of Thermal Pollution

Heat producing industries i.e., thermal power plants, nuclear power plants, refineries, steel mills etc. are the major sources of thermal pollution. Excess of heat reaching water bodies causes thermal pollution of water.

Effects of Thermal Pollution

(i) The dissolved oxygen content of water is decreased as the solubility of oxygen in water is decreased at high temperature.

(ii) High temperature becomes a barrier for oxygen penetration into deep cold waters.

(iii) Toxicityof pesticides, detergents and chemicals in the effluents increases with increase in temperature.

(iv) Discharge of heated water near the shores can disturb spawning and can even kill young fishes.

(v) Fish migration is affected due to formation of various thermal zones.

CONTROL OF THERMAL POLLUTION:

The following methods can be employed for control of thermal pollution:

- Cooling ponds,
- Spray Ponds,
- Cooling towers

(i) Cooling Ponds:

A cooling pond is a man-made body of water primarily formed for the purpose of cooling heated water and/or to store and supply cooling water to a nearby power plant or industrial facility such as a petroleum refinery, pulp and paper mill, chemical plant, steel mill or smelter. Water from condensers is stored in ponds where natural evaporation cools the water which can then be recirculated or discharged in nearby water body

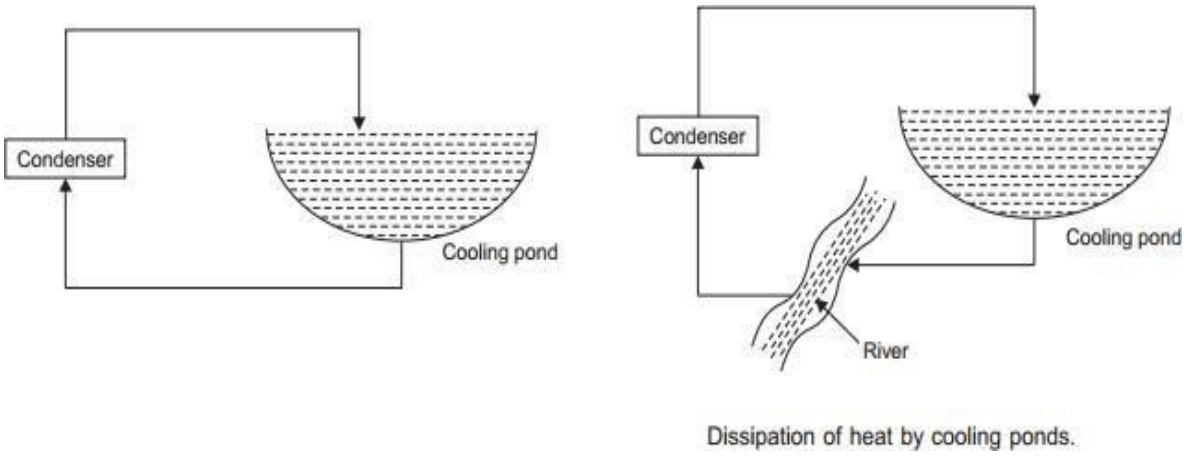


Figure 10: Cooling ponds

(ii) Spray Ponds:

A spray pond is a reservoir in which warmed water (e.g. from a power plant) is cooled before reuse by spraying the warm water with nozzles into the cooler air. Cooling takes place by exchange of heat with the ambient air, involving both conductive heat transfer between the water droplets and the surrounding air and evaporative cooling (which provides by far the greatest portion, typically 85 to 90%, of the total cooling). The primary purpose of spray pond design is thus to ensure an adequate degree of contacting between the hot injection water and the ambient air, so as to facilitate the process of heat transfer. The spray pond is the predecessor to the natural draft cooling tower, which is much more efficient and takes up less space but has a much higher construction cost. A spray pond requires between 25 and 50 times the area of a cooling tower. However, some spray ponds are still in use today. The water from condensers is received in spray ponds. Here the water is sprayed through nozzles where fine droplets are formed. Heat from these fine droplets is dissipated to the atmosphere.

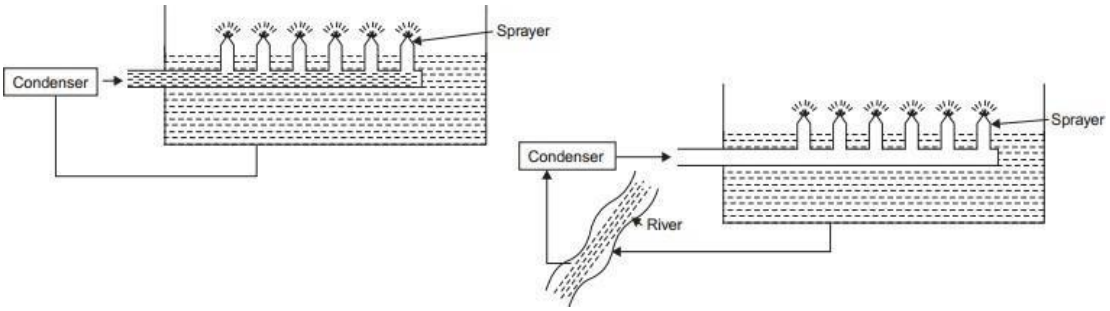


Figure 11: Spray ponds

(iii) Cooling Towers:

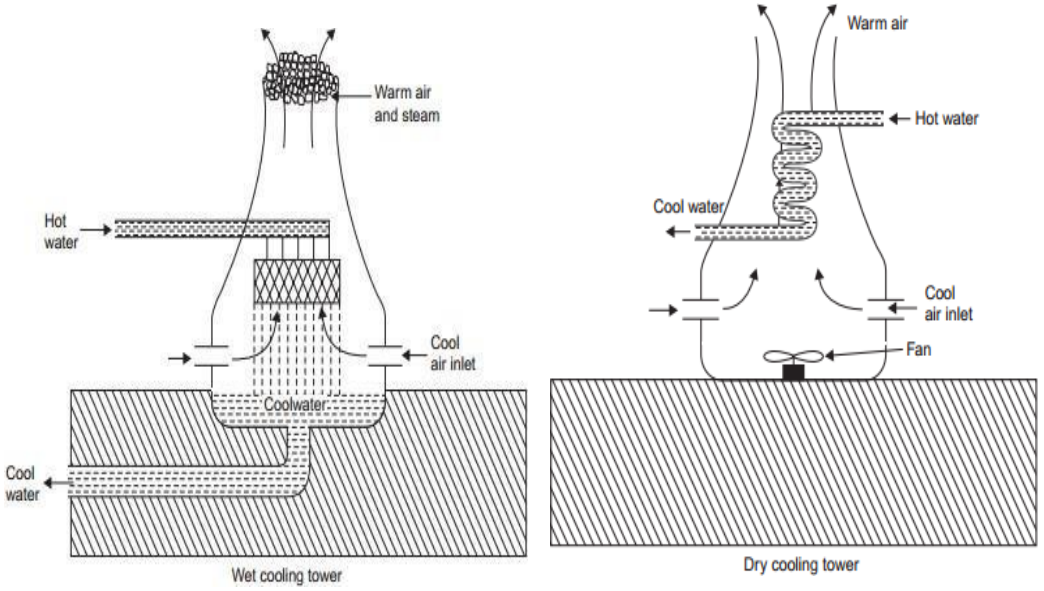


Figure 12: Cooling towers a) Wet cooling tower, b) Dry cooling tower

A cooling tower is a heat rejection device that rejects waste heat to the atmosphere through the cooling of a water stream to a lower temperature. Cooling towers may either use the evaporation of water to remove process heat and cool the working fluid to near the wet-bulb air temperature or, in the case of closed circuit dry cooling towers, rely solely on air to cool the working fluid to near the dry-bulb air temperature. Common applications include cooling the circulating water used in oil refineries, petrochemical and other chemical plants, thermal power stations, nuclear power stations and HVAC systems for cooling buildings. With respect to the heat transfer mechanism employed, the main types are:

- wet cooling towers (or open circuit cooling towers) operate on the principle of evaporative cooling. The working fluid and the evaporated fluid (usually water) are one and the same.
- closed circuit cooling towers (or fluid coolers) pass the working fluid through a tube bundle, upon which clean water is sprayed and a fan-induced draft applied. The resulting heat transfer performance is close to that of a wet cooling tower, with the advantage of protecting the working fluid from environmental exposure and contamination.
- dry cooling towers are closed circuit cooling towers which operate by heat transfer through a surface that separates the working fluid from ambient air, such as in a tube to air heat exchanger, utilizing convective heat transfer. They do not use evaporation.
- hybrid cooling towers are closed circuit cooling towers that can switch between wet and dry operation. This helps balance water and energy savings across a variety of weather conditions.

❖ MARINE POLLUTION

Marine pollution is defined as the introduction of substances to the marine environment directly or indirectly by man resulting in adverse effects such as hazardous to human health, obstruction of marine activities and lowering the quality of sea water.

Sources of Marine Pollution:

a. Municipal waste and sewage from residences and hotels in coastal towns are directly discharged into sea.

b. Pesticides and fertilizers from agriculture which are washed off by rain enter water courses and finally to sea. India is estimated to use 55,000 tons of pesticides annually and about 25 percent of it is carried to-ocean.

c. Petroleum and oil washed off from roads normally enter sewage system and finally into seas.

d. Ship accidents and accidental spillage at sea can therefore be very damaging to the marine environment.

e. Off shore oil exploration also pollute the sea water to a large extent,

f. Dry docking: All ships periodic dry docking servicing; cleaning the hulls etc. during this period when cargo compartments are emptied, residual oil goes into sea.

g. Pollution due to organic wastes: When O2 concentration falls 1.5 mg/L, the rate of aerobic oxidants reduced and replaced by the anaerobic bacteria that can oxidize the organic molecules without the use of oxygen.

h. Pollution due to oil: Crude oil is transported by sea after a tanker has unloaded its cargo of oil; it has to take on sea water ballast for return journey. This ballast water is stored in cargo compartments that previously contained oil. During unloading of cargo certain amount of oil remains clinging to the walls of container and this may amount to 800t in a 200,000t tankers. The ballast water thus contaminated with oil. When fresh crag of oil is to be loaded these compartments are clean with water which discharges the dirty ballast along with oil into sea.

i. Tanker accidents: In the natural process, a large no. of oil tanker accidents happens every year. Sometimes this can results in major disasters.

j. Volcanic eruptions in the sea.

k. Deep sea mining is a relatively new mineral retrieval process that takes place on the ocean floor. Ocean mining sites are usually done at about 1,400 – 3,700 meters below the ocean's surface. The vents create sulphide deposits, which contain precious metals such as silver, gold, copper, manganese, cobalt, and zinc. These raise questions about environment damage to surrounding areas. Removal of parts of the sea floor will result in disturbances to the benthic layer, and habitat of benthic organisms. Beside from direct impact of mining the area, leakage, spills and corrosion would alter the mining area's chemical makeup.

Effects of Marine Pollution:

a. Apart from causing Eutrophication, a large amount of organic wastes can also result in the development of 'red tides'. These are phytoplankton blooms because of which the whole area is discoloured.

b. Commercially important marine species are also killed due to clogging of gills and other structures.

c. When oil is spilled on the sea, it spreads over the surface of the water to form a thin film called as oil slick. This damages marine life to a large extent. Commercial damage to fish by tainting which gives unpleasant flavor to fish and sea food reduces market values of sea food and causes death of birds through its effect on feathers. Birds often clean their plumage by pruning and in the process consume oil which can lead to intestinal, renal and liver failure.

d. For salt marshy plants oil slick can affect the flowering, fruiting and germination.

e. Organic waste addition results in end products such as hydrogen sulphide, ammonia and methane which are toxic to many organisms. This process results in the formation of an anoxic zone which is low in its oxygen content; from which most life disappears except for anaerobic microorganisms and renders the water foul-smelling.

f. The coral reefs are the productive ecosystems offer many benefits to people. These coral reefs are threatened by (a) the sediments from deforestation carried by the runoffs, (b) the agricultural and industrial chemicals reaching through river discharges. To mention an example. River Ganga is estimated to carry 1.5 billion tons of sediments due to deforestation and intensive farming in India, Bangladesh and Nepal through which it flows to Bay of Bengal.

g. Drill cuttings dumped on the seabed result in the production of toxic sulphides in the bottom sediment thus eliminating the benthic fauna.

Control Measures of Marine Pollution:

a. Introduction of sewage treatment plants to reduce BOD of final product before discharging into sea.

b. Cleaning oil from surface waters and contaminated beaches can be accelerated through the use of chemical dispersants which can be sprayed on the oil.

c. Load on top system reduce oil pollution cleaned with high pressures jets of water.

d. Crude oil washing: The clingage is removed by jets of crude oil while the cargo is being unloaded.

e. Skimming off the oil surface with a section device.

f. Spreading a high density powder over the oil spill, so that oil can I be sunk to the bottom.

g. Developmental activities on coastal areas should be minimized.

h. Ecologically sensitive coastal areas should be protected by not allowing drilling.

❖ SOIL POLLUTION

Soil is the upper layer of the earth crust which is formed by weathering of rocks. Organic matter in the soil makes it suitable for living organisms. Dumping of various types of materials especially domestic and industrial wastes causes soil pollution. Domestic wastes include garbage, rubbish material like glass, plastics, metallic cans, paper, fibres, cloth rags, containers, paints, varnishes etc. Leachates from dumping sites and sewage tanks are harmful and toxic, which pollute the soil. Some of the persistent toxic chemicals inhibit the non-target organisms, soil flora and fauna and reduce soil productivity. These chemicals accumulate in food chain and ultimately affect human health. Indiscriminate use of pesticides specially is a matter of concern. It is important to understand that all soils contain compounds that are harmful/toxic to human beings and other living organisms. However, the concentration of such substances in unpolluted soil is low enough that they do not pose any threat to the surrounding ecosystem. When the concentration of one or more such toxic substances is high enough to cause damage to living organisms, the soil is said to be contaminated.

The root cause of soil pollution is often one of the following:

- Agriculture (excessive/improper use of pesticides)
- Excessive industrial activity
- Poor management or inefficient disposal of waste

The challenges faced in soil remediation (decontamination of soil) are closely related to the extent of soil pollution. The greater the contamination, the greater the requirement of resources for remediation.

Effects of Soil Pollution

Soil pollution harbours a broad spectrum of negative consequences that affect plants, animals, humans, and the ecosystem as a whole. Since children are more susceptible to diseases, polluted soil poses a greater threat to them. Some important effects of soil pollution are detailed in this subsection.

Effects on Human Beings

Soil contaminants can exist in all three phases (solid, liquid, and gaseous). Therefore, these contaminants can find their way into the human body via several channels such as direct contact with the skin or through the inhalation of contaminated soil dust. The short term effects of human exposure to polluted soil include:

- Headaches, nausea, and vomiting.
- Coughing, pain in the chest, and wheezing.
- Irritation of the skin and the eyes.
- Fatigue and weakness.

A variety of long-term ailments have been linked to soil pollution. Some such diseases are listed below.

- Exposure to high levels of lead can result in permanent damage to the nervous system. Children are particularly vulnerable to lead.
- Depression of the CNS (Central Nervous System).
- Damage to vital organs such as the kidney and the liver.
- Higher risk of developing cancer.
- It can be noted that many soil pollutants such as petroleum hydrocarbons and industrial solvents have been linked to congenital disorders in humans. Thus, soil pollution can have several negative effects on human health.

Effects on Plants and Animals

Since soil pollution is often accompanied by a decrease in the availability of nutrients, plant life ceases to thrive in such soils. Soils contaminated with inorganic aluminium can prove toxic to plants. Also, this type of pollution often increases the salinity of the soil, making it inhospitable for the growth of plant life. Plants that are grown in polluted soil may accumulate high concentrations of soil pollutants through a process known as bioaccumulation. When these plants are consumed by herbivores, all the accumulated pollutants are passed up the food chain. This can result in the loss/extinction of many desirable animal species. Also, these pollutants can eventually make their way to the top of the food chain and manifest as diseases in human beings.

Effects on the Ecosystem

Since the volatile contaminants in the soil can be carried away into the atmosphere by winds or can seep into underground water reserves, soil pollution can be a direct contributor to air and water pollution. It can also contribute towards acid rain (by releasing huge quantities of ammonia into the atmosphere). Acidic soils are inhospitable to several microorganisms that improve soil texture and help in the decomposition of organic matter. Thus, the negative effects of soil pollution also impact soil quality and texture.

Crop yield is greatly affected by this form of pollution. In China, over 12 million tons of grain (worth approximately 2.6 billion USD) is found to be unfit for human consumption due to contamination with heavy metals (as per studies conducted by the China Dialogue).

Control of Soil Pollution

Several technologies have been developed to tackle soil remediation. Some important strategies followed for the decontamination of polluted soil are listed below.

- Excavation and subsequent transportation of polluted soils to remote, uninhabited locations.
- Extraction of pollutants via thermal remediation – the temperature is raised in order to force the contaminants into the vapour phase, after which they can be collected through vapour extraction.
- Bioremediation or phytoremediation involves the use of micro-organisms and plants for the decontamination of soil.
- Mycoremediation involves the use of fungi for the accumulation of heavy metal contaminants.
- Effluents should be properly treated before discharging them on the soil.
- Solid wastes should be properly collected and disposed off by appropriate method.
- From the wastes, recovery of useful products should be done.
- Biodegradable organic waste should be used for generation of biogas.
- Cattle dung should be used formethane generation. Night soil (human faeces) can also be used in the biogas plant to produce inflammable methane gas.
- Microbial degradation of biodegradable substances is also one of the scientific approaches for reducing soil pollution.

❖ NUCLEAR POLLUTION / RADIOACTIVE POLLUTION

Radiation is a term given for waves caused by electromagnetism and high energy particles. Well known electromagnetic waves are radio waves, light, infrared rays, UV rays, X-rays, Gamma rays. These are well known and are used extensively in the fields of communications, industry, medicine and research. Radioactive substances have High energy particles which are tiny bits of matter that are made to move at high speeds releasing nuclear radiation. There are

about 50 naturally occurring radioactive substances and More than 2000 man-made ones. There are three kinds of radioactive radiation – alpha particles, beta particles and gamma radiation. Contamination of the atmosphere by radiation and radioactive particles is called nuclear pollution. Alpha particles can be interrupted by a sheet of paper while beta particles can be blocked by a piece of wood or a few millimeters of aluminum sheet. The gamma rays can pass through paper and wood but can be stopped by concrete wall, lead slabs or water.

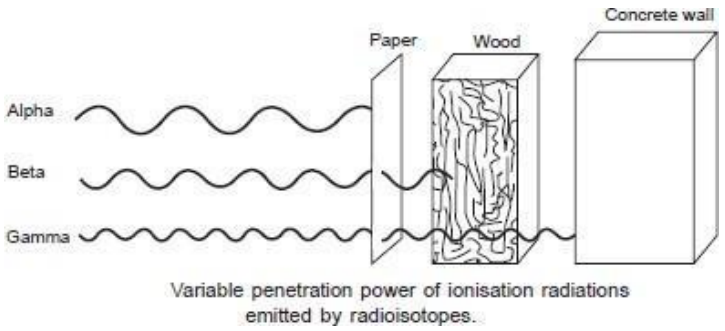


Figure 13: Penetration of Radiation

Causes of Nuclear Pollution

Most activities that involve radioactive substances have potential to contaminate the environment. These include;

Nuclear weapons testing- Beginning with the Second World War when Japan was subdued after the use of the nuclear bombs on the cities of Hiroshima and Nagasaki, countries have been in the race to develop their own nuclear arms, in the name of defence, but more to threaten rival nations. These were led by US, Russia, Britain, France and China. Nowadays N. Korea, Iran and many of the developing countries are equipped to build these weapons as well. Testing the weapons involves explosions is the atmospheric layer called stratosphere. The exploded debris emitting radiation then falls back to the earth. Some of the radiation is absorbed by our atmosphere. But some of it reaches the earth falling on areas that are far away from the site where the weapon was released initially. This is called Fallout. When these particles settle on the vegetation and are consumed by animals they enter into the food chain. When fallout settles over the sea, the ecosystem of the sea gets affected and again entering the food chain.

Nuclear Power Plants- Intense Nuclear energy from radioactive fuel is used to heat water to steam. The steam is then used to turn the turbines that in turn work the generators to produce electricity. Small amounts of radiation are released during this process into the water which may then dispose off indiscriminately causing nuclear pollution.

Improper disposal of spent nuclear fuel - Spent nuclear fuel contains very active radioactive atoms that remain so sometimes almost for 600yrs or more. These must be disposed of in a very careful manner, with strict regulations in well designated spaces. But the fact is many governments tend to approve of dumping nuclear fuel as far from their country as possible. The favourite dumping ground of many countries was the Pacific Ocean. Greenpeace an organisation dedicated to preserving the environment and saving the earth from pollution has brought attention to this activity and opposes it with fervour. Some plants store spent fuel in

underground water pools as these release a high amount of heat and need to be cooled down. There is always the danger of seepage into the land nearby, contaminating ground water and surrounding lands. On a smaller scale is the radioactive waste that is produced in diagnostic Imaging in Health sector.

Accident/Damage to Nuclear power plants - This most famous of these was the Chernobyl Nuclear Disaster in Russia in 1986. The fallout of this accident was felt over three countries- Russia, Ukraine and Belarus. The area surrounding the reactor is still polluted and not suitable for inhabitation or farming.

Effects of Nuclear Pollution

The effect of nuclear pollution is seen on every organism in the environment from the bacteria to plants to human beings. Nothing is spared.

- The immediate and closest to the source, experience Radiation Sickness. In small doses of 75-200 rems. One experiences vomiting, fatigue and loss of appetite. At higher exposures of 300 rem and more changes in the blood cells and bleeding occurs. Above 600 rems there is loss of hair, loss of immunity usuallyresulting in death in a few days to weeks. Radiation causes changes in the cell and gene structure of rapidly multiplying cells of the body, such as bone marrow, skin, intestines, lymphoid tissue and embryo.
- Those exposed from a distance may not show any immediate symptom. But the tendency to develop various forms of cancers and have a shortened life span is seen. Radiation also causes cell mutations which can be transferred to the next generation.
- Foetuses are affected with birth defects and cancers. They may also have a shorter life span.
- Plants die and some show genetic changes and stunted growth. Animals are also affected and do not survive for too long.
- The radiation in the atmosphere will not dissipate quickly. Every water source will also be affected. In fact it may take years or centuries to reach a point where such a space may become habitable.
- An average person will be exposed to about 180 milli rem of radiation in a year from exposure to natural radiation, medical and dental X rays, Colour TVs, airport baggage X rays etc.

Control of Nuclear Pollution

- Whilst undergoing procedures for X rays or radiation therapy, correct protection gear such as lead aprons must be worn. This includes pregnant women. Using lead sheathed walls in imaging facilities is also mandatory.
- As a lay person one must be aware of the dangers of nuclear pollution. If living in the vicinity of a nuclear plant or hearing of one being planned, one should use one's right to make sure the governing bodies are planning thoroughly on the building, implementing and disposal of the wastes. Make certain that the authorities are prepared in case of a disaster, to handle all the situations such as containing the contamination to arranging an evacuation.

- While working at a radiation facility or in nuclear plant workers are always monitored for the amount of radiation they have been exposed to.
- Radioactive wastes are actually recyclable to a good extent because usable fuel is still being created in the wasted material which can then be reprocessed.
- Governments are authorising research on developing better means for disposal of radioactive wastes. The most feasible method now appears to be deep underground storage of wastes.
- Power plants must ensure that the radioactive fuel and wastes are being transported and disposed of in safe containers which are long lasting and unbreakable.
- Governing agencies need to make sure that radioactive material does not fall into wrong hands that will, for a profit sell these to people who are in the business of war mongering.

Nuclear energy is a clean source of energy, inexpensive and extensive too. With a small amount of fuel a large amount of energy can be generated. Though there have been mishaps in the past and wrongful use of this energy, there is still great potential for it. Any well intentioned effort must be backed by good research, a well-designed plan and proper back up plans for any setbacks. The safety of the environment and the people must always come first.

SOLID WASTE MANAGEMENT

Solid waste management is a term that is used to refer to the process of collecting and treating solid wastes. It also offers solutions for recycling items that do not belong to garbage or trash. As long as people have been living in settlements and residential areas, garbage or solid waste has been an issue. Waste management is all about how solid waste can be changed and used as a valuable resource. Solid waste management should be embraced by each and every household, including the business owners across the world. The industrialization has brought a lot of good things and bad things as well. One of the adverse effects of industrialization is the creation of solid waste.

Categories of Waste

- Organic waste: Kitchen waste, waste from food preparation, vegetables, flowers, leaves, fruits, and market places.
- Combustibles: Paper, wood, dried leaves, packaging for relief items etc. that are highly organic and having low moisture content.
- Non-combustibles: Metal, Tins, Cans, bottles, stones, etc.
- Toxic waste: Old medicines, paints, chemicals, bulbs, spray cans, fertilizer and pesticide containers, batteries, shoe polish.
- Recyclables: Paper, glass, metals, plastics.
- Ashes or Dust: Residue from fires that are used for cooking.
- Construction waste: Rubble, roofing, broken concrete etc.
- Hazardous waste: Oil, battery acid, medical waste, industrial waste, hospital waste.
- Dead animals: Carcasses of dead livestock or other animals.
- Bulky waste: Tree branches, tires etc.
- Soiled waste: Hospital waste such as cloth soiled with blood and other body fluids.

Table 1: The types of litter and their approximate degeneration time

Type of litter	**Approximate time taken to degenerate the litter**
Organic waste such as vegetable and fruit peels, leftover foodstuff, etc.	A week or two.
Paper	10–30 days
Cotton cloth	2–5 months
Wood	10–15 years
Woollen items	1 year
Tin, aluminium, and metal cans	100–500 years
Plastic bags	One million years
Glass bottles	Undetermined

Various Sources of Solid Waste

Every day, tonnes of solid waste are disposed of at various landfill sites. This waste comes from homes, offices, industries and various other agricultural related activities. These landfill sites produce foul smell if waste is not stored and treated properly. It can pollute the surrounding air and can seriously affect the health of humans, wildlife and our environment. The following are major sources of solid waste:

1. Residential

Residences and homes where people live are some of the major sources of solid waste. The garbage from these places includes food wastes, plastics, paper, glass, leather, cardboard, metals, yard wastes, ashes and special wastes like bulky household items such as electronics, tires, batteries, old mattresses and used oil. Most homes have garbage bins where they can throw away their solid wastes in and later, the bin is emptied by a garbage collecting firm or person for treatment.

2. Industrial

Industries are known to be one of the biggest contributors to solid waste. They include light and heavy manufacturing industries, construction sites, fabrication plants, canning plants, power and chemical plants. These industries produce solid waste in the form of housekeeping wastes, food wastes, packaging wastes, ashes, construction and demolition materials, special wastes, medical wastes as well as other hazardous wastes.

3. Commercial

Commercial facilities and buildings are yet another source of solid waste today. Commercial buildings and facilities, in this case, refer to hotels, markets, restaurants, godowns, stores and office buildings. Some of the solid wastes generated from these places include plastics, food wastes, metals, paper, glass, wood, cardboard materials, special wastes and other hazardous wastes.

4. Institutional

The institutional centers like schools, colleges, prisons, military barracks and other government centers also produce solid waste. Some of the common solid wastes obtained from these places include glass, rubber waste, plastics, food wastes, wood, paper, metals, cardboard materials, electronics as well as various hazardous wastes.

5. Construction and Demolition Areas

Construction and demolition sites also contribute to the solid waste problem. Construction sites include new construction sites for buildings and roads, road repair sites, building renovation sites and building demolition sites. Some of the solid wastes produced in these places include steel materials, concrete, wood, plastics, rubber, copper wires, dirt and glass.

6. Municipal Services

The urban centers also contribute immensely to the solid waste crisis in most countries today. Some of the solid waste brought about by the municipal services include street cleaning, wastes from parks and beaches, wastewater treatment plants, landscaping wastes and wastes from recreational areas, including sludge.

7. Treatment Plants and Sites

Heavy and light manufacturing plants also produce solid waste. They include refineries, power plants, processing plants, mineral extraction plants and chemical plants. Among the wastes produced by these plants, there are industrial process wastes, unwanted specification products, plastics, metal parts, just to mention a few.

8. Agriculture

Crop farms, orchards, dairies, vineyards and feedlots are also sources of solid wastes. Among the wastes they produce are agricultural wastes, spoiled food, pesticide containers and other hazardous materials.

9. Biomedical

This refers to hospitals and biomedical equipment and chemical manufacturing firms. In hospitals, there are different types of solid wastes produced. Some of these solid wastes include syringes, bandages, used gloves, drugs, paper, plastics, food wastes and chemicals. All these require proper disposal or else they will cause a huge problem for the environment and the people in these facilities.

Effects of Poor Solid Waste Management

1. Litter Surroundings

Due to improper waste disposal systems, particularly by municipal waste management teams, wastes heap up and become a menace. While people clean their homes and places of work, they litter their surroundings, which affect the environment and the community.

2. Impact on Human Health

Improper waste disposal can affect the health of the population living nearby the polluted area or landfills. The health of waste disposal workers and other employees involved with these landfill facilities are also at a greater risk. Exposure to wastes that handled improperly can cause skin irritations, respiratory problems, blood infections, growth problems, and even reproductive issues.

3. Disease-causing Pests

This type of dumping of waste materials forces biodegradable materials to rot and decompose under improper, unhygienic and uncontrolled conditions. After a few days of decomposition, a foul smell is produced, and it becomes a breeding ground for different types of disease-causing insects as well as infectious organisms. On top of that, it also spoils the aesthetic value of the area.

4. Environmental Problems

Solid wastes from industries are a source of toxic metals, hazardous wastes, and chemicals. When released to the environment, the solid wastes can cause biological and physicochemical problems to the environment that may affect or alter the productivity of the soils in that particular area.

5. Soil and Groundwater Pollution

Toxic materials and chemicals may seep into the soil and pollute the groundwater. During the process of collecting solid waste, hazardous wastes usually mix with ordinary garbage and other flammable wastes making the disposal process even harder and risky.

6. Emission of Toxic Gases

When hazardous wastes like pesticides, batteries containing lead, mercury or zinc, cleaning solvents, radioactive materials, e-waste and plastics mixed up with paper and other non-toxic scraps are burned they produce dioxins, furans, polychlorinated biphenyls, and other gases. These toxic gases have the potential of causing various diseases, including cancer.

7. Impact on Land and Aquatic Animals

Our carelessness with our waste and garbage also affects animals, and they suffer the effects of pollution caused by improperly disposed of wastes and rubbish. Consuming styrofoam and cigarette butts have been known to cause deaths in marine animals. Animals are also at risk of poisoning while consuming grasses near contaminated areas or landfills as the toxins seep into the soil.

Methods of Solid Waste Management

There are different methods of solid waste management. The following are some of the recognized methods:

1. Sanitary Landfill

This is the most popular solid waste disposal method used today. Garbage is basically spread out in thin layers, compressed and covered with soil or plastic foam. Modern landfills are

designed in such a way that the bottom of the landfill is covered with an impervious liner, which is usually made of several layers of thick plastic and sand. This liner protects the groundwater from being contaminated because of leaching or percolation. When the landfill is full, it is covered with layers of sand, clay, topsoil and gravel to prevent seepage of water.

Advantage: If landfills are managed efficiently, it is an ensured sanitary waste disposal method.

Constraint: It requires a reasonably large area.

2. Incineration

This method involves the burning of solid wastes at high temperatures until the wastes are turned into ashes. Incinerators are made in such a way that they do not give off extreme amounts of heat when burning solid wastes. Incinerators that recycle heat energy through furnace and boiler are called waste-to-energy plants. These waste-to-energy systems are more expensive to set up and operate compared to plain incinerators because they require special equipment and controls, highly skilled technical personnel, and auxiliary fuel systems. This method of solid waste management can be done by individuals, municipalities and even institutions. The good thing about this method is the fact that it reduces the volume of waste up to 20 or 30% of the original volume.

Advantage: The volume of combustible waste is reduced considerably by burning waste. In the case of off-site pits, it is an appropriate method to minimize scavenging.

Constraint: It can cause smoke or fire hazard and also emits gaseous pollutants.

3. Recovery and Recycling

Recycling or recovery of resources is the process of taking useful but discarded items for the next use. Plastic bags, tins, glass and containers are often recycled automatically since, in many situations, they are likely to be scarce commodities. Traditionally, these items are processed and cleaned before they are recycled. The process aims at reducing energy loss, consumption of new material and reduction of landfills. The most developed countries follow a strong tradition of recycling to lower volumes of waste.

Advantage: Recycling is environmentally friendly.

Constraint: It is expensive to set up, and in most emergencies, there is limited potential.

4. Composting

Due to a lack of adequate space for landfills, biodegradable yard waste is allowed to decompose in a medium designed for the purpose. Only biodegradable waste materials are used in composting. It is a biological process in which micro-organisms, specifically fungi and bacteria, convert degradable organic waste into substances like humus. This finished product, which looks like soil, is high in carbon and nitrogen. Good quality environmentally friendly manure is formed from the compost that is an excellent medium for growing plants and can be used for agricultural purposes.

Advantage: Composting is environmentally friendly as well as beneficial for crops.

Constraint: It requires intensive management and experienced personnel for large scale operation.

5. Pyrolysis

This is a method of solid waste management whereby solid wastes are chemically decomposed by heat without the presence of oxygen. It usually occurs under pressure and at temperatures of up to 430 degrees Celsius. The solid wastes are changed into gasses, solid residue of carbon and ash and small quantities of liquid.

Advantage: This will keep the environment clean and reduce health and settlement problems.

Constraint: The systems that destroy chlorinated organic molecules by heat may create incomplete combustion products, including dioxins and furans. These compounds are highly toxic in the parts per trillion ranges. The residue it generates may be hazardous wastes, requiring proper treatment, storage, and disposal. To summarize, proper solid waste management is an integral part of environmental conservation that should be observed by both individuals and companies globally.

CLIMATE CHANGE

While global warming focuses on the rising average temperature of the planet, climate change usually refers to the shifts in things like precipitation, wind patterns, and temperatures over a given period. Measured changes in climate could last a few years, decades, or even millions of years. Climate change has occurred for as long as the earth has existed. It happens anytime earth's climate patterns change and remain in place for a measurable amount of time. This has notably manifested itself in natural cycles of cooling and warming. Before human causes started to shift the global climate, five main factors interacted with one another as climate changes occurred. These five factors include:

- Atmosphere (air)
- Biosphere (living things)
- Cryosphere (ice and permafrost)
- Hydrosphere (water)
- Lithosphere (earth's crust and upper mantle)

Causes of Climate Change:

- **Greenhouse Gases**

Greenhouse gases play a vital role in the earth's climate cycles. As the planet gets hit with the sun's rays, some of the energy is absorbed, and the rest of that energy and heat gets reflected into space. Greenhouse gases in the atmosphere trap the reflected energy, redirecting it back down to the earth and eventually contributing to global warming. Various gases play this role, including:

- Water vapour
- Carbon dioxide (CO_2)
- Methane

- Nitrous oxide
- Chlorofluorocarbons (CFCs)

While some of these greenhouse gases, such as water vapour, are naturally occurring, others, such as CFCs, are synthetic. CO_2 is released into the atmosphere from both natural and human-made causes and is one of the leading contributors to climate change. CO_2 has been increasing at an alarming rate and has the potential to stay in the earth's atmosphere for thousands of years unless it gets absorbed by the ocean, land, trees, and other sources. However, as CO_2 production has steadily risen, the earth's natural resources to absorb it has also been diminished. This is already occurring in many ways as earth's resources are disappearing from things like deforestation. Some studies even predict that plants and soil will be able to absorb less CO_2 as the earth continues to warm—possibly accelerating climate change even further.

- **Solar Activity**

Solar activity, as mentioned above, does play a role in the earth's climate. While the sun does go through natural cycles, increasing and decreasing the amount of energy that it emits to the earth, it is unlikely that solar activity is a major contributor to global warming or climate change. Since scientists began to measure the sun's energy hitting our atmosphere, there has not been a measurable upward trend.

- **Agriculture**

There are many significant ways in which agriculture impacts climate change. From deforestation in places like the Amazon to the transportation and livestock that it takes to support agricultural efforts around the world, agriculture is responsible for a significant portion of the world's greenhouse gas emissions. However, agriculture is also an area that is making tremendous strides to become more sustainable. As productivity increases, less carbon is being emitted to produce more food. Agriculture also has the potential to act as a carbon sink, and could eventually absorb nearly the same amount of CO_2 it emits.

- **Deforestation**

Deforestation and climate change often go hand in hand. Not only does climate change increase deforestation by way of wildfires and other extreme weather, but deforestation is also a major contributor to global warming. According to the Earth Day Network, deforestation is the second leading contributor to global greenhouse gasses. Many people and organizations fighting against climate change point to reducing deforestation as one of, if not the most, important issues that must be addressed to slow or prevent climate change.

- **Human Activity**

According to the Environmental Protection Agency, the most significant contributor to climate change in the United States is the burning of fossil fuels for electricity, heat, and transportation. Of these factors, transportation in the form of cars, trucks, ships, trains, and planes emits the largest percentage of CO_2–speeding up global warming and remaining a significant cause of climate change.

- **Livestock**

While interconnected to many of the agricultural and deforestation issues we have already touched on, livestock in the form of cattle, sheep, pigs, and poultry play a significant role in climate change. According to one study, "Livestock and Climate Change," livestock around the world is responsible for 51% of annual global greenhouse gas emissions.

Effects of Climate change

From melting glaciers to more extreme weather patterns, people everywhere are beginning to take notice of the real impacts of climate change. While some nations around the world are taking action with initiatives such as the Paris Climate Agreement, others are continuing business as usual–pumping millions of tons of carbon into the atmosphere year after year. While the long-term consequences are still to be seen, for now, climate change continues to cause extreme weather as well as safety and economic challenges on a global scale.

- **Extreme Weather**

Changes to weather are perhaps the most noticeable effect of climate change for the average person. One reason for this is the financial impact severe weather events can have. So far in the U.S. during 2019, there have been six climate disasters, most costing more than $1 billion. According to the National Climate Assessment, extreme weather events will continue to increase in frequency and intensity as climate change continues to happen. Extreme weather influenced by climate change includes:

- Stronger storms & hurricanes
- Heatwaves
- Wildfires
- More flooding
- Heavier droughts

- **Safety & Economic Challenges**

Climate change posed a severe and immediate threat to national security. According to former Secretary of Defense, Chuck Hagel, "Rising global temperatures, changing precipitation patterns, climbing sea levels, and more extreme weather events will intensify the challenges of global instability, hunger, poverty, and conflict." Furthermore, climate change is likely to cause economic challenges in many parts of the world. Some estimates have the U.S. already spending around $240 billion annually due to human-caused climate change, and future costs are projected to be even higher. However, putting an exact number on the real costs of climate change is difficult once you consider the staggering costs of losing natural resources like clean air and water.

Long term impact of Climate change

The long term impact of climate change could be absolutely devastating to the planet and everyone and everything living on it. If the world continues on its current trajectory, then we will likely continue to see increasing effects on everyday life.

- **Health**

There are many ways in which climate change could impact people's health. Depending on age, location, and economic status, climate change is already affecting the health of many and has the potential to impact millions more. According to the Center for Disease Control and Prevention, climate change-related health risks may include:

- Heat-related illness
- Injuries and fatalities from severe weather
- Asthma & cardiovascular disease from air pollution
- Respiratory problems from increased allergens
- Diseases from poor water quality
- Water & food supply insecurities

- **Negative Impact in Ecosystems**

Ecosystems are interconnected webs of living organisms that help support all kinds of plant and biological life. Climate change is already changing seasonal weather patterns and disrupting food distribution for plants and animals throughout the world, potentially causing mass extinction events. Some studies estimate that nearly 30% of plant and animal species are at risk of extinction if global temperatures continue to rise.

- **Water & Food Resources**

Climate change could have a significant impact on food and water supplies. Severe weather and increased temperatures will continue to limit crop productivity and increase the demand for water. With food demand expected to increase by nearly 70% by 2050, the problem will likely only get worse.

- **Sea Levels Rising**

Rising sea levels could have far-reaching effects on coastal cities and habitats. Increasing ocean temperatures and melting ice sheets have steadily contributed to the rise of sea levels on a global scale. At current rates the National Oceanic and Atmospheric Administration estimate sea levels to rise by at least 8 inches by 2100, potentially causing increased flooding and decrease in ocean and wetland habitats.

- **Shrinking Ice Sheets**

While contributing to rising sea levels, shrinking ice sheets present their own set of unique problems, including increased global temperatures and greenhouse gas emissions. Climate change has driven summer melt of the ice sheets covering Greenland and Antarctica to increase by nearly 30% since 1979.

- **Ocean Acidification**

The ocean is one of the main ways in which CO_2 gets absorbed. While at first glance that may sound like a net positive, the increasingly human-caused CO_2 is pushing the world's oceans to their limits and causing increased acidity. As pH levels in the ocean decrease, shellfish have difficulty reproducing, and much of the oceans' food cycle becomes disrupted.

Solutions for Climate Change

While the effects of climate change can seem bleak, there is still hope. By taking immediate action to curb climate change, we may never see the worst consequences. Likewise, as the world adopts cleaner, more sustainable energy solutions, there may be millions of new jobs created and billions of dollars of economic benefits. Below are some practical ways you can battle climate change, including:

- Purchase Renewable Energy Certificate for your home power needs
- Make our home energy efficient
- Buy carbon offsets
- Adopt a plant-based diet
- Reduce food waste
- Recycle
- Stop using fossil fuels
- Stop deforestation

ACID RAIN

Acid Rain as the name suggests can be said to be the precipitation of acid in the form of rain in the simplest manner. When atmospheric pollutants like oxides of nitrogen and sulphur react with rainwater and come down with the rain, then this results in Acid Rain. Acid rain is made up of highly acidic water droplets due to air emissions, most specifically the disproportionate levels of sulphur and nitrogen emitted by vehicles and manufacturing processes. Often called acid rain as this concept contains many types of acidic precipitation. The acidic deposition takes place in two ways: wet, and dry. Wet deposition is any form of precipitation which removes acids from the atmosphere and places them on the surface of the earth. In the absence of precipitation, dry deposition of polluting particles and gases sticks to the ground through dust and smoke.

Causes of Acid rain:

The causes of acid rain are Sulphur and Nitrogen particles which get mixed with the wet components of rain. Sulphur and Nitrogen particles which get mixed with water are found in two ways either man-made i.e as the emissions are given out from industries or by natural causes like how a lightning strike in the atmosphere releases nitrogen ions and sulphur is released from volcanic eruptions. According to the Royal Society of Chemistry, which considers him the "father of acid rain," the word acid rain was invented in 1852 by Scottish chemist Robert Angus Smith. Smith decided on the word while studying rainwater chemistry near industrial towns in England and Scotland. The regular clean rain we experience, even

though it is not clean i.e water and carbon dioxide react together to form weak carbonic acid which essentially by itself is not extremely harmful. The reaction occurring is :

$$H_2O\ (l) + CO_2\ (g) \rightleftharpoons H_2CO_3\ (aq)$$

The pH value of regular rainwater is around 5.7, giving it an acidic nature. The oxides of nitrogen and sulphur are blown away by the wind along with the dust particles. They settle on the earth's surface after coming down in the form of precipitation. Acid rain is essentially a by-product of human activities which emit oxides of nitrogen and sulphur in the atmosphere. Example – the burning of fossil fuels, unethical waste emission disposal techniques.

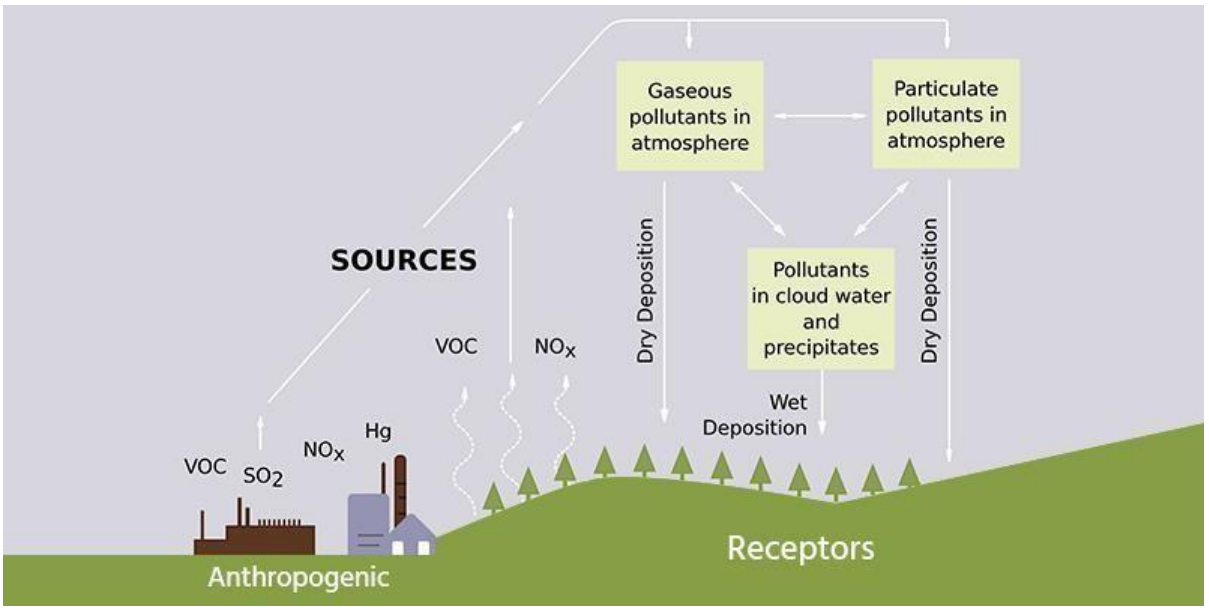


Figure 13: Acid Rain cycle

Sulphur dioxide and nitrogen dioxide undergo oxidation and then they react with water resulting in the formation of sulphuric acid and nitric acid respectively. The following reaction will clarify the acid formation reaction:

$$2SO_2\ (g) + O_2\ (g) + 2H_2O\ (l) \rightarrow 2H_2SO_4\ (aq)$$
$$4NO_2\ (g) + O_2\ (g) + 2H_2O\ (l) \rightarrow 4HNO_3\ (aq)$$

Effects of Acid Rain

- Acid rain is very harmful to agriculture, plants, and animals. It washes away all nutrients which are required for the growth and survival of plants. Acid rain affects agriculture by the way how it alters the composition of the soil.
- It causes respiratory issues in animals and humans.
- When acid rain falls down and flows into the rivers and ponds it affects the aquatic ecosystem. As it alters the chemical composition of the water, to a form which is actually harmful to the aquatic ecosystem to survive and causes water pollution.
- Acid rain also causes the corrosion of water pipes. Which further results in leaching of heavy metals such as iron, lead and copper into drinking water.
- It damages the buildings and monuments made up of stones and metals.

Taj Mahal, one of the 7 wonders of the world, is largely affected by acid rain. The city of Agra has many industries which emit the oxides of sulphur and nitrogen in the atmosphere. People continue to use low-quality coal and firewood as a domestic fuel, adding to this problem. Acid rain has the following reaction with the marble (calcium carbonate):

$$CaCO_3 + H_2SO_4 \rightarrow CaSO_4 + H_2O + CO_2$$

The formation of calcium sulphate results in the corrosion of this beautiful monument. Statue of Liberty which is made of copper has also been damaged by the cumulative action of acid rain & oxidation for over 30 years and is, therefore, becoming green in colour.

Prevention of Acid Rain

- The only precaution that we can take against acid rain is having a check at the emission of oxides of nitrogen and sulphur.
- We have so far seen the details of acid rain and its harmful effect on animals, plants and the monuments.
- Being responsible citizens, one should be aware of the harmful effects they cause and of the industries which give out nitrogen and sulphur compound wastes unethically.

DISASTER MANAGEMENT

Geological processes like earthquakes, volcanoes, floods and landslides are normal natural events which have resulted in the formation of the earth that we have today. They are, however, disastrous in their impacts when they affect human settlements. Human societies have witnessed a large number of such natural hazards in different parts of the world and have tried to learn to control these processes, to some extent.

Earthquakes: Earthquakes occur due to sudden movements of Earth's crust. The earth's crust has several tectonic plates of solid rock which slowly move along their boundaries. When friction prevents these plates from slipping, stress builds up and results in sudden fractures which can occur along the boundaries of the plates or fault lines (planes of weakness) within the plates. This causes earthquakes, the violent, short-term vibrations in the earth. The point on a fault at which the first movement occurs during an earthquake is called the epicenter. The largest earthquake ever recorded occurred on May 22, 1960 in Chile with the estimated magnitude of 9.5 on Richter Scale, affecting 90,000 square miles and killing 6,000 people. The devastating earthquake which hit Bhuj Town in Gujarat had caused massive damage, killing 20,000-30,000 people and leaving many injured. It had an energy equivalent to a 5.3 megaton hydrogen bomb. Earthquake-generated water waves called tsunamis can severely affect coastal areas. These giant sea swells can move at a speed upto 1000 Km/hr or even faster. While approaching the sea shore they may often reach 15 m or sometimes upto 65 m in height and cause massive devastation in coastal areas. In China such waves killed 8,30,000 people in 1556 and 50,000 in 1976. Anthropogenic activities can also cause or enhance the frequency of earthquakes. Three such activities identified are:

- Impoundment of huge quantities of water in the lake behind a big dam.
- Underground nuclear testing.

- Deep well disposal of liquid waste.

Damage to property and life can be prevented by constructing earthquake-resistant buildings in the earthquake prone zones or seismic areas. For this, the structures are heavily reinforced, weak spots are strategically placed in the building that can absorb vibrations from the rest of the building, pads or floats are placed beneath the building on which it can shift harmlessly during ground motion. Wooden houses are preferred in earthquake prone areas as in Japan.

Floods

Generally the stream channels accommodate some maximum stream flow. However, due to heavy rains or sudden snow melt the quantity of water in streams exceeds their capacity and water overflows the banks and causes inundation of the surrounding land. This situation is called flood. A flood generally does not damage property or cause casualties to an extent as done by other natural disasters. However, it causes a great economic loss and health related problems due to widespread contamination. Virtually anything the flood water touches gets contaminated, posing serious threat to health due to outbreak of epidemics. Human activities have been the main causes for increasing the severity and frequency of floods. Construction of roads, parking space and buildings that cover the earth's surface hardly allows infiltration of water into the soil and speeds up the runoff. Clearing of forests for agriculture has also increased the severity of floods. In India, Uttar Pradesh is considered to be amongst the worst flood hit states of the country. It has nearly 20%of the total 40 million hectares of flood prone zone of the country. Flood plains, the low lying areas which get inundated during floods help to reduce floods. Building up of flood control structures like flood walls or deepening of river channels have only transferred the problems downstream. Building walls prevents spilling out the flood water over flood plains, but it increases the velocity of water to affect the areas downstream with greater force. On an average, every year one major disaster hits India, causing huge economic losses and loss of human life. There is a need for systematic studies and strategies to evolve a Disaster Management Plan for our country. To check the floods, efforts need to be made to restore wetlands, replace ground cover on water-courses, build check-dams on small streams, move buildings off the flood plains etc. Instead of raising buildings on flood plains, it is suggested that floodplains should be used for wildlife habitat, parks, recreational areas and other uses, which are not susceptible to flood damage. River-networking in the country is also being proposed to deal with the flood problem.

Landslides

Landslide occurs when coherent rock of soil masses move downslope due to gravitational pull. Slow landslips do not cause much worry but sudden rockslides and mudslides are dangerous. Water and vegetation influence landslides. Chemical action of water gradually cause chemical weathering of rocks making them prone to landslides. Vegetation consolidates the slope material, provides cohesion by its root system and also retards the flow of water and its erosion capacity. However, this can be masked by many other exerting factors like:

- Earthquakes, vibrations etc.
- Disturbances in resistant rock overlying rock of low resistance.
- Saturation of the unconsolidated sediments with water.

- Unconsolidated sediments exposed due to logging, road or house building.

Landslides are governed by the forces which tend to pull the earth material down slope (move in case of slopes with steeper slip plane) and resisting forces which tend to resist such movements. It is difficult to control landslides. However, these can be minimized by stabilizing the slope by:

1. Draining the surface and subsurface water.
2. Providing slope support like gabions (wired stone blocks)
3. Concrete support at the base of a slope.

Cyclones

Cyclones are recurring phenomena in the tropical coastal regions. Tropical cyclones in the warm oceans are formed because of heat and moisture. One of the requirements for formation of tropical cyclones is that the sea surface temperature (SST) should be above 26°C. Tropical cyclones move like a spinning top at the speed of 10-30 Km per hour. They can last for a week or so and have a diameter varying between 100 to 1500 Km. Since in the western parts of the main ocean no cold currents exist, tropical cyclones originate there. Tropical cyclones are called hurricanes in the Atlantic, Caribbean and north eastern Pacific, typhoons in the western Pacific; and cyclones in the Indian Ocean and willy willies in the sea around Australia. More storms occur in the Bay of Bengal than in the Arabian Sea. Of 5-6 storms that form in the year about half of them are severe. Hurricane winds (74 miles per hour or more), rains and storm surge (often 50-100 miles wide dome of water) often devastate the area where it strikes on land. The devastation is more when storm surge and normal astronomical tide coincide. Sea water with combined force rushes inlands and inundates the low lying areas.

Management: It is difficult to stop the recurrence of cyclones. Some long term defence measures can help to protect us from devastation. Such measures include, planting more trees on the coastal belt, construction of dams, dykes, embankments, storm shelter, wind breaks, proper drainage and wide roads for quick evacuation.

UNIT IV

SOCIAL ISSUES AND THE ENVIRONMENT

4.1 From unsustainable to sustainable development

Sustainable development is defined as meeting the needs of the present generation without compromising the requirement of future generation.

Sustainable development aims at:

- Optimum use of natural resources
- High degree of reusability
- Minimum wastage
- Least generation of toxic by products
- Maximum productivity

4.1.1 Significance of sustainable development

- A symbiotic relationship between consumer and producer.
- Suitability between ecology and economics
  The goals of sustainable development.
- Promoting equality
- Economic efficiency
- Ecological harmony
- Sustaining our natural resources
- Improving the quality of life

The above goals can be achieved through the following equality.

(a) Inter generational equity

It states that we should handover a healthy environment to our future generation.

(b) Intra generational equity

It approaches that the technical development of rich countries should support the economic growth of the poor countries and help to improve their wealth.

4.1.2 Approches for sustainable development

- ✓ Conserving all non-renewable sources by recycling and reuse
- ✓ Controlling and avoiding pollution

- ✓ Degradation of resources should be avoided by educating the people
- ✓ Developing appropriate technologies with minimum environmental hazards
- ✓ The stock and supply of natural resources are to be known by the people
- ✓ Providing environmental education and awareness, the attitude of the people towards our earth and resources can be changed

4.2 Urban problems related to energy

4.2.1 Definition

Movement of human population from rural area to urban area for the search of better economics, education, employment, communication, health and daily needs is known as urbanization.

4.2.2 Causes of urbanization:

- ✓ Poor availability of energy
- ✓ Lack of job opportunities
- ✓ Lack of modernization of agricultural section
- ✓ Poor life style, health, education and transportation facilities

4.2.3 Energy demands

- ✓ Urban growth is very fast in developing countries
- ✓ Unplanned growth makes the pollution uncontrollable
- ✓ The energy requirement of urban people are much higher than rural people because of their high standard of life
- ✓ Urban people generate a lot of wastes and pollute the environment

Energy demanding activities include-

- ✓ Residential and commercial lighting
- ✓ Transportation
- ✓ Modern life style using a large number of electrical appliances
- ✓ Industries
- ✓ Control and prevention of pollution require more energy depending technologies
- ✓ Disposal of large amount of wastes also require more energy and money

4.2.4 Solution for urban energy problem

- ✓ Minimise energy use
- ✓ Increase production capacity
- ✓ Use energy efficient technologies
- ✓ Use public transport instead of individual vehicles
- ✓ Impose strict laws, penalties and energy audit
- ✓ Use solar, wind and tidal energy

4.3 Water conservation

4.3.1 Definition

The process of storing water for future use is called water conservation

4.3.2 Need for water conservation

- ✓ Better life styles require more water
- ✓ With increase in population, the requirement for water also increases
- ✓ The annual rainfall decreases due to deforestation
- ✓ Over exploitation of ground water leads to drought
- ✓ Agricultural and industrial activities require more fresh water

4.3.3 Measures of water conservation

- Decreasing run-off losses:
  This can be achieved by using contour cultivation or terrace farming
- Reducing irrigation losses:
  - Drip irrigation or sprinking irrigation can conserve water up to 50%
  - Irrigation in early morning or late evening reduces evaporation loss
  - Growing hybrid crop barieties which require less water and also conserve water
- Reuse of water
  - Treated water can be used for irrigation
  - The grey water from washings can be used for washing cars, watering gardens
- Preventing waste of water

- Closing the taps when not inuse
- Repairing any leakage in pipes
- Using small capacity flush in toilets

- Discharge of sewage
  The discharge of sewage into natural water resource should be prevented as much as possible
- Reducing evaporation losses
  Evaporation of water in humid regions can be reduced by placing horizontal barriers of asphalt below the soil surface

4.3.4 Methods of water conservation

The following two methods are important in water conservation

Rain water harvesting

Watershed management

4.3.4.1 Rain water harvesting

4.3.4.1 (a) Definition

The process of collection of rain water directly

4.3.4.1 (b)Objectives of rain water harvesting

- ✓ To reduce run off loss
- ✓ To avoid flooding roads
- ✓ To minimize water conflicts
- ✓ To reduce ground water contamination
- ✓ To avoid the intrusion of saline water at ground level
- ✓ To meet the increasing demands of water
- ✓ To reduce storm, water run-off and soil erosion

4.3.4.1 (c) Methods of rain water harvesting

Before adopting the rain water harvesting

- ✓ Soil properties
- ✓ Topography

- ✓ Rainfall pattern
- ✓ Climate conditions

Should be analysed.

The most common method of rain water harvesting is the roof top rain water harvesting method.

4.3.4.1(d) Roof top rainwater harvesting method

It is the process of collecting rain water from the roof of the building and storing in the underground for further use.

4.3.4.1 (e)Method

The rain water from the top of the roofs and other areas is diverted into the surface tank or recharge pits through a delivery system.

The surface tank or recharge pit is filled with stones, gravels and sand which serve as a sand filter.

4.3.4.1 (f) Advantages of rain water harvesting

- ✓ It raises the ground water levels
- ✓ It increases the availability of water from wells
- ✓ It reduces flood and soil erosion
- ✓ It reduces the effects of droughts
- ✓ It upgrade the social and environmental status

4.3.4.1 (g)Disadvantages

- ✓ The water supply is very limited
- ✓ Uncertainty of rainfall
- ✓ It is a seasonal method

4.3.4.2 Watershed management

4.3.4.2(a) Definition

Watershed is defined as the land areas from which water drains under the influence of gravity into stream, lake, reservoir, estuary or other water body of surface water.

4.3.4.2(b) Definition

The management of rainfall and resultant runoff is called watershed management.

4.3.4.2(c) Factors affecting watershed

- ✓ Climate changes affect the watershed
- ✓ Overgrazing, deforestation, mining, forest fire, soil erosion, construction activities, industrialization, modern agricultural methods etc. affect and degrade various watersheds.
- ✓ The watersheds are found to be degraded due to uncontrolled, unplanned and unscientific land use activities.

4.3.4.2(d) Objectives of watershed management

- ✓ To raise the ground water level
- ✓ To protect the forestry activities
- ✓ To protect the soil from erosion by runoff
- ✓ To improve the good productivity of the land
- ✓ To minimize the risks of flood, droughts and landslides
- ✓ To develop riral areas and providing economy to them
- ✓ To provide domestic water supply, irrigation, hydropower generation

4.3.4.2(e) Watershed management techniques

- Pits or Trenches:
  Pits were dug at equal intervals to improve ground water level.

- Earthen dam or stone embankment:
  It is constructed in the catchment area to check the runoff water.
- Farm pond:
  It is built to improve water storage capacity of the catchment area.
- Underground barriers:
  It is built along the voids to raise the water table.

4.3.4.2(f) Maintenanace of watershed

- ✓ Protect the vegetation along stream banks which prevents stream bank erosion.
- ✓ Forestations are used to prevent soil erosion and retention of moisture in watershed areas.
- ✓ Use animal wastes on farms to prevent water contamination in water in watershed area.
- ✓ Terrecing, bunding, contour cropping, strip cropping etc are to be used to minimize soil erosion and runoff on the slopes of watersheds.

4.4 Resettlement and rehabilitation of people

4.4.1 Definition

Resettlement is the simple relocation or displacement of human population.

4.4.2 Definition

Rehabilitation is the process of replacing the lost economic assets, safeguard employment, public safe land for building, restore services, repair damaged infrastructures etc.

4.4.3 Causes of displacement of people

- Due to developmental activities
  Construction of dams, roads, airports, power plants, urban expansion, industrial and mining activities.
- Disasters like earthquake, floods, droughts, landslides, tsunami, volcanic eruptions, industrial accidents, nuclear accidents, dam burst etc.

- Due to conservation initiatives:
  These include national park, sanctuary, forest reserve, biosphere reserve etc.

4.4.4.Important issues of displacement

- ✓ Tribals are most affected among the displaced who are already poor.
- ✓ Breakup of families is an important social issue in which the women are the worst affected.
- ✓ The tribals are not familiar with the market policies and trends.
- ✓ The land acquisition law ignores the communal ownership property.
- ✓ Marriages, social and cultural functions their folk songs, dances and activities vanish with their displacement.
- ✓ Loss of identity and loss of the intimate link between the people and environment is one of the biggest loss.

4.4.5 Rehabilitation policy

- The extent of damage and suffering that the proposed project would cause should be studied and ascertained before starting the project.
- The rehabilitation and resettlement work should be a part of the project.
- The people should be rehabilitated on minimum dislocation basis by choosing adjacent areas.
- The extent of rehabilitation should meet the ends of social justice and balanced development.

4.5 Role of non government organization in environmental awareness

Non-government organization is a people's organization. It can be used to protect the environment by creating awareness and consciousness of the people.

4.5.1 The following are some NGO functions in India

- The NGO's help the government in local environmental issues.
- **Mahila mandals** creates awareness amongst the women of remote villages. They educate them and help them to become healthy and economically self-dependent.

- **Chipko movement** works for the conservation of trees by **Darholi Gram Swarajya Mandal** is Gopeshwar, uttarkhand.
- **Narmad Bachan Andolan (NBA)** was organized by kalpavriksh.
- **Centre for science and Environment (CSE)** works as a think tank an environment development issues in India.
- **Kerala Sastra Sahiya Parishad (KSSP)** is claimed to be a people's science movement.
- The NGO's act as an action or pressure group.
- The NGO's act as an effective and viable link between people and government.

4.6.Environmental ethics

4.6.1 Definition

Environmental ethics is the discipline that deals with the moral relationship between human beings and environment.

4.6.2 Functions of environment

- ✓ It moderates the climate conditions of the soil.
- ✓ It is the life supporting medium for all organism.
- ✓ It provides food, air, water and other important natural resources to the human beings.
- ✓ It disintegrates all waste materials discharged by the modern society.

4.6.3 Environmental problem

Human beings have totally devasted the nature through the following activities:

- ✓ Pollution
- ✓ Deforestation
- ✓ Water scarcity
- ✓ Land degradation
- ✓ Population
- ✓ Urbanisation

4.6.4 Solutions to environmental problems through environmental ethics

- ✓ Soil degradation must be stopped.
- ✓ Over exploitation of biodiversity and resources should be stopped immediately.
- ✓ Reduction, recycling and reuse (3R) should be followed for pollution free energy generation and waste disposal.
- ✓ Reduce population and increase the economic growth of the country.
- ✓ The technology used in developed countries can also be tried.

4.7 Climate change

4.7.1 Definition

The average weather condition over a long period is called climate.

It is dynamic

It changes always through a natural cycle.

4.7.2 Causes of climate change

- ✓ Volcanic eruptions
- ✓ Ocean currents
- ✓ Rotation of earth on its axis
- ✓ Seasonal changes
- ✓ Industrialization
- ✓ Transportation
- ✓ Use of CFC
- ✓ Combustion of fossil fuels
- ✓ Global warming
- ✓ Depletion of ozone layer
- ✓ Variation in solar energy

4.7.3 Effect of climate change

- ✓ It disturb the agricultural activities
- ✓ Migration of animals and human beings
- ✓ It totally upsets the hydrological cycle which leads to flood and drought.

4.8 Global warming (or) Greenhouse effect

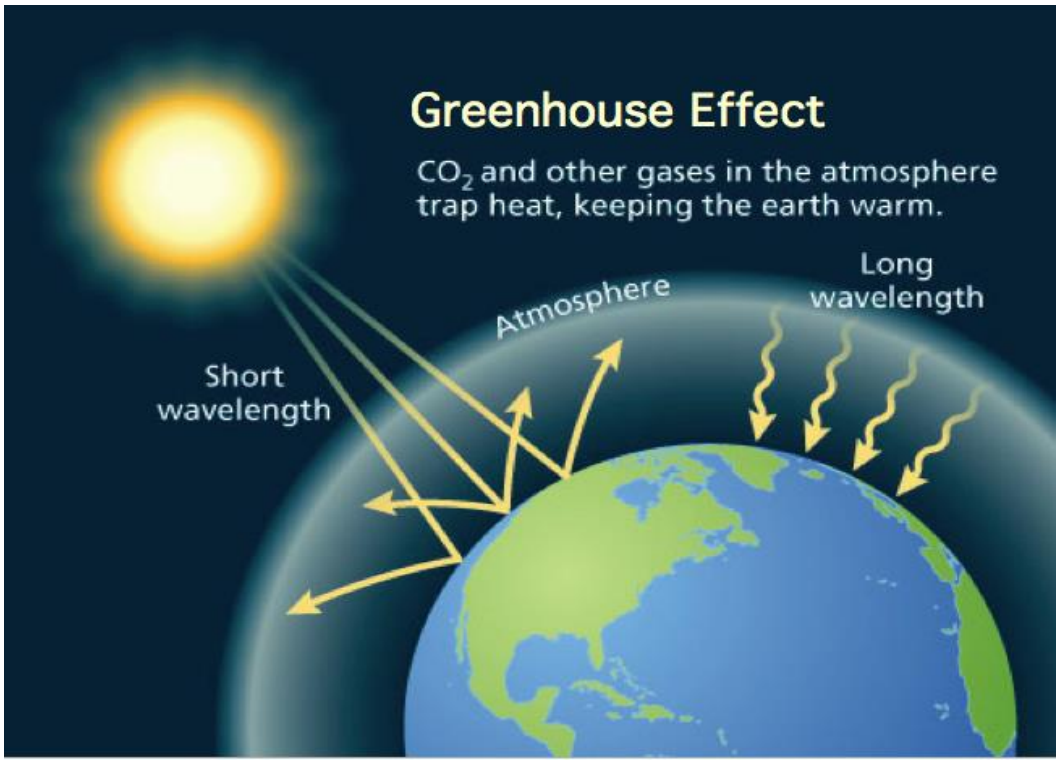
4.8.1 Definition

The progressive increase in earth's temperature due to the effect of increase in composition of man-made CO_2 in the atmosphere is called global warming.

The major greenhouse gases are:

CO_2, CH_4, N_2O, CFC etc.,

O_3 and SO_2 also act as serious pollutants for global warming.



4.8.2 Causes of global warming

- ✓ Deforestation
- ✓ Combustion of fossil fuels
- ✓ Industrialization
- ✓ Transportation

4.8.3 Effects of global warming

- ✓ The temperature of the earth will increase from the range 1.5°C to 5.5°C by 2050.
- ✓ Increase in global temperature leads to increase in sea water level (20cm rise by 2030) due to the melting of polar ice and glaciers.
- ✓ The global warming is completely changing the rainfall pattern.
- ✓ There would be an increase in water borne diseases and infectious disease carried by mosquitoes and other vectors.

4.8.4 Measures to reduce global warming

- ✓ Reduce the usage of CFC and fossil fuels.
- ✓ Use energy more efficiently.
- ✓ Use nuclear power plants for production of electricity.
- ✓ Plant more trees.
- ✓ Adopt sustainable agriculture.
- ✓ Stabilise population growth.
- ✓ Utilise solar, wind and hydropower.

4.9Acid rain (or) Acid precipitation

4.9.1 Definition

Acid rain means the presence of excessive acids in rain.

If the pH of rain water is low (i.e., below 6) due to the presence of CO_2, SO_2 and NO_2 gases in the atmosphere, then the rain is called acid rain.

4.9.2 Formation of acid rain

CO_2, SO_2 and NO_2 gases are dissolved by rain water and produce carbonic acid, sulphuric acid and nitric acid respectively. This process is called acid precipitation or acid deposition.

$CO_2 + H_2O \rightarrow H_2CO_3$

$SO_2 + H_2O \rightarrow H_2SO_3$

$2SO_2 + O_2 \rightarrow 2SO_3$

$2SO_3 + H_2O \rightarrow H_2SO_4$

$4NO_2 + 2H_2O + O_2 \rightarrow 4HNO_3$

The other names of acid rain are (i) acid fog (ii) acid snow

4.9.3 Effects of acid rain

- Acid rain decays buildings, made up of marble
  Eg: Tajmahal
- It damages stone statues
  Eg: British parliament building
- It damages metals by corrosion
- In terrestrial vegetation, acid rain reduces the rate of photosynthesis and growth.
- It retards the growth of beans, radish, potato etc.,
- Many insects and fungi are more tolerant to acidic conditions. Hence, they can attack trees and cause diseases.
- It affects the human nervous system, respiratory system and digestive system.

4.9.4 Control measures of acid rain

- Avoid the unnecessary usage of motor vehicles.
- Use the natural gas instead of coal.
- Coal with lower sulphur content must be used in thermal power plants.
- Emission of CO_2, SO_2 and NO_2 from industries and power plant should be reduced using pollution control equipments.

4.10 Ozone layer depletion

4.10.1Definition

The process of creation of hole in the ozone layer in the stratosphere is called ozone layer depletion.

4.10.2 Formation of ozone

Ozone (O_3) is formed in the stratosphere by photochemical reaction from oxgen as follows.

$O_2 \rightarrow O + O$

O = Oxygen free radical

The atomic oxygen rapidly reacts with molecular oxygen (O_2) to form ozone (O_3).

$O + O_2 + M \rightarrow O3 + M$

4.10.3 Mechanism of ozone layer depletion

In 1970, it was found that the ozone layer was attacked by chlorofluorocarbon.

CFC release chlorine atom which breaks ozone to oxygen.

$CCl_2F_2 \rightarrow CClF_2 + Cl$

$Cl + O_3 \rightarrow ClO + O_2$

$CClF_2 + O_2 \rightarrow CF_2O + ClO$

$ClO + O_3 \rightarrow ClO_2 + O_2$

$ClO + O \rightarrow Cl + O_2$

Each chlorine atom is capable of attacking several ozone molecules. Thus, chain reaction occurs.

4.10.4 Ozone depleting substances

No	Substances	Source
1	CFC	Refrigerants,
2	HCFC	Refrigerants,
3	BFC	Fire extiguishers
4	SO_2	Atmosphere
5	NO	Air crafts

4.10.5 Effects of ozone layer depletion

- The effects of ozone layer depletion are penetration of more amount of uv light which produces
  (i) skin burns (ii) skin cancer (iii) slow blindness
- It reduces human resistivity leading to a number of diseases such as cancer, allergies and infectious diseases.
- It affect the aquatic life, fish, crabs etc.,
- It kills lower flora and fauna
- Yield of vital crops like corn, rice, soya bean, bean, cotton and wheat will decrease.

4.10.6 Measurement of amount of ozone

The amount of ozone in the atmosphere is measured by Dobson spectrometer. It is expressed in Dobson unit (DU)

4.10.7 Control measures

- Manufacturing of CFC must be avoided.
- The release of CFC must be controlled.
- The use of methyl bromide, a crop fumigant, should be controlled.

4.11 Nuclear Accidents

Nuclear reactors produce energy either by nuclear fission or nuclear fusion. It is very clean and safe energy. But there is any leakage of radioactive materials into the atmosphere, it causes harmful effect.

4.11.1Types of nuclear accidents

- **Nuclear test**

  Nuclear testing carried out in underground leads to emission of radioactive rays in the atmosphere.

  Eg. Nuclear bomb test in May, 1998 by India.

- **Nuclear power plant accidents**
  The nuclear power plant located in the seismic, vulnerable areas can cause nuclear accident.
- **Improper disposal of radioactive wastes**
  This serves as another source of nuclear accidents. Drums stored underground can rust and leak radioactive rays into air, land and water.
- **During tranporatation**
  Trucks carrying radioactive wastes or fuels are involved in frequent accidents in developing countries.
- **Core melt down**
  The major nuclear accident at a nuclear power plant is core melt down.

4.11.2 Effect of nuclear radiation

- Radiations break the DNA cells which cause prolonged effects to human being. It is also carried to future generation.
- Low dose of radiation(up to 250 rads) causing vomiting, hair loss, fatigue.
- High dose of radiations (up to 500 rads) affect bone marrow, natural resistance, blood cells and clotting.
- Very high dose of radiations (up to 10000 rads) completely destroy the organisms.

4.12 Nuclear holocaust

4.12.1 Definition

Nuclear holocaust is the destruction of biodiversity by nuclear equipment and nuclear bombs.

4.12.2 Effects of nuclear holocausts

- When nuclear accidents occurs, large level destructions can happen due to the release of enormous amount of heat and light. These include:
  - ✓ Immediate death
  - ✓ Blindness
  - ✓ Retinal damage
  - ✓ Ignition of buildings

- ✓ Metal crushing's
- ✓ Rupture of ear drums
- ✓ Destruction of homes, offices.

- Nuclear war would cause very high destruction of environment also.
- Nuclear winter:
  Nuclear accidents will cause combustion of wood, petroleum, plastics; forest etc., large quantity of black soot will be carried to the atmosphere due to above combustion. Black soot will absorb all uv radiations and will not allow the uv rays to reach the earth. This resulting in cooling of earth.it reduces the evaporation of water. So in stratosphere there is no significant moisture to rainout the thick soot. Thus the process known as opposite to global warming occurs due to nuclear explosion. This is called nuclear winter,

4.12.3 Control measures

- Emission of radiations must be monitored continuously.
- Nuclear materials must be handled by properly trained persons
- Nuclear power plants are constantly monitored and controlled by the authorities.
- Nuclear weapons should be safe guarded by the officials and prevent from terrorists.

4.13 Wasteland reclamation

4.13.1 Definition

The lands which are unfit for cultivation, grazing and other economic uses due to environmental pollution is called wasteland.

- Eg. salt affected lands
- Sandy areas
- Snow covered lands
- Rocky areas
- Marsh land

4.13.2 Causes for wasteland formation

- Deforestation
- Overgrazing
- Mining
- Soil erosion
- Salinity
- Waterlogging
- Construction of dams
- Hydropower projects
- Disposal of wastes
- Improper agricultural practices
- Over utilization of natural resources
- Excessive use of pesticides
- Loss of soil productivity

4.13.3 Definition

The process of conservation of wasteland into useful land make it fit for various purposes is called wasteland reclamation.

4.13.4 Objectives of wasteland reclamation

- ✓ To conserve the natural ecosystem.
- ✓ To prevent soil erosion, flooding and landslides.
- ✓ To avoid over exploitation of natural resources.
- ✓ To conserve the biological resources of the land for sustainable use.
- ✓ To improve physical structure and quality of the soil.

4.13.5 Methods of waste land reclamation

- ✓ **Drainage**

  Excess water is removed by artificial drainage. This adopted for water logged soil.

- ✓ **Leaching**
  It is the process of salt removing from the soil by applying excess amount of water.
- ✓ **Reclamation using chemicals**
  Sodium carbonate in the soil is removed by spreading gypsum on the land. Dilute sulphuric acid is applied to the land to neutralize the alkaline present in the soil.

4.14 Consumerism and waste products

4.14.1 Definition

Consumerism means consumption of resources by the people.

4.14.2 Definition

The materials discharged as unwanted matter after the consumption of resources is called the waste products.

4.14.3 Objectives of consumerism

- ✓ It improves the rights and power of the user.
- ✓ It forces the producer to reuse and recycle the waste.
- ✓ It involves making the producer liable for the entire cycle of a product.
- ✓ Active consumerism improves human health and happiness and saves resources.
- ✓ The reusable packing materials like bottles, metal cans etc., can be returned to the manufacturer which makes products cheaper.

4.14.4 Sources of wastes

- ✓ Agriculture mining, industries, municipality and commercial sectors.
- ✓ The wastes generated from these are – food waste, plastic cans, metals, paper, glass, debris and dead animals.
- ✓ The materials like computers, printers, mouse, key board, Xerox machines, and calculators are called e-wastes.

4.14.5 Effects of wastes

- ✓ The wastes released from chemical industries and from explosives are dangerous to human life.
- ✓ Waste materials degrade the quality of the soil and ground water and make unfit for irrigation.
- ✓ Non-biodegradable plastic materials produce several toxic gases during combustion.

4.15 Environmental legislation

4.15.1 Wildlife protection act 1972 amended in 1983,1986 and 1991.

The major objectives of this act are –

- To preserve biodiversity
- To maintain essential ecological processes and life supporting systems.
- To ensure a continuous use of species.

The important future of this act are-

- It covers the rights and non-rights of forest dwellers.
- It defines the wildlife related terminology
- It provides the appointments of wildlife advisory board.
- Listing of endangered wildlife and prohibition of hunting them.
- It provides for setting up of national parks, wildlife sanctuaries and central zoo authority.
- It provides legal powers to officers and punishment to offenders.
- It provides for captive breeding programme for endangered species.
- Any person found guilty under this act will be imprisoned for 2 years with penalty of Rs 2000/-.

4.15.2 Water (prevention and control of pollution) act, 1974\

The objectives of the act are-

- Prevention and control of water pollution
- Maintaining the wholesomeness of water

- Establishment of boards for the prevention and control of water pollution.

The important features of these act are-

- This act aims at to protect the water from all kinds of pollution and to reserve the quality of the water.
- It provides for the establishment of central board and state boards to prevent water pollution.
- The states are empowered to restrain any person from discharging a pollutant into any water bodies without the consent of the boards.
- Any violation of the guidelines would attract penal action including imprisonment ranging from 1.5 years to 6 years with fine.

4.15.3 Forest (conservation) act, 1980

The major objectives of this act are-

- Protection and conservation of forest
- To ensure judicious use of forest products.
- To arrest deforestation.

The important features of this act are-

- This act has the main aim to protect all types of forest.
- This act helps to maintain the ecosystem and biodiversity.
- The reserved forests cannot be converted to unreserved forest without the prior permission of the central govt.
- The central government has the authority to maintain ecological balance in the forest extending from tropical to temperate regions.

4.15.4 The air (prevention and control of pollution)act ,1981

The objectives of this act are-

- Prevention, control and abatement of air pollution.
- To maintain the quality of air.
- To establish the central and state boards for the prevention and control of air pollution.

The important features of this act are-

- The act defines the following terms-

Air pollutant, Air pollution, Approved fuel, Chimney emission and Control equipment.

- It deals with the formation of central board and state boards, their terms and conditions, delegation of powers to various officers.
- The central board may lay down the standards for quality of air
- The central board coordinates, provides technical assistance and guidance to state boards.
- The various boards can collect compile and publish the data regarding air pollution.
- The boards can guide the industries for the effective prevention and control of air pollution.
- The boards are empowered to give directions to industries.
- The boards are authorized to declare the air pollution control areas, instruct the emission standards from automobiles and restrict the activities of certain industries.

- The state boards can examine the manufacturing processes and the control equipment's to verify whether they meet the prescribed standards.

  The operation of an industrial unit is prohibited in a heavily polluted area without the consent of the central board.

  If the industry or person fails to follow the standards, they will be punished.

  i.e) imprisonment for not less than 15 years, it may be extended to 6 years and with fine.

4.15.5 The environment (Protection) Act, 1986.

The objectives of this Act are:

To protect and improvement of the environment.
To prevent hazards to all living organisms and property.

To maintain harmonious relationship between humans and their environment.

The important features of this act are:

- The act defines the terms-

  Environment

  Environmental pollution

- The act empowers the central govt to take measures to protect and improve the environment.
- The act further empowers the govt to lay down procedures and safeguards for the prevention of accident which cause pollution and remedial measures if accident occurs.
- The government has the power to close or prohibit or regulate any industry or its operations, if the act is violated.
- The act fixes the liability of the offence punishable under act on the person who is directly in charge.
- The act empowers the central govt officers to inspect the site or the machinery to prevent pollution.
- They can collect samples of air, water, soil and other materials from any factory for testing.
- Thus, this act is the most comprehensive legislation with powers for the central government to act directly.

4.16 Environmental audits

4.16.1 Definition

Environmental audits are the periodical measurement damages due to developmental activities.

4.16.2 ISO 14000

- ISO 14000 is the environmental standards which helps the organization to minimize the environmental Damages and comply with applicable laws and regulations.
- The major purpose of this standard is to promote the effective and efficient environment management in organization.
- It provides useful tools which improves the environmental qualities.

4.16.3 Issues involved in enforcement of laws

- Wildlife protection act does not include any locally evolved conservation measures.
- Jammu and Kashmir has its own wildlife protection act which paves the way for illegal hunting and trading of endangered species in that state.
- The offenders of this act are given only minimum punishment.
- The ownership certificate for some animal articles serves as a tool for illegal trading.
- As the forest conservation act transforms the powers from state to center, the local communities have been completely neglected to decide the nature of the forest area.
- This act prevents the tribal from using forest resources.
- The role and contribution of tribal are not acknowledged with forest conservation act.
- All pollution control acts are not effective as the power and authority are given only to central government.
- The penalties suggested by the pollution control acts is very small when compared to the damage caused by the big industries.
- The court is burdened with numerous cases pending at a time.
- Public interest litigation (PIL) has in way been successful.
- Political and administrative interventions may result in anti-environmental judgments.

4.17 Public awareness

- Environmental education must be taught in schools and colleges.
- Mass media like radio, TV, cinema, cable networks can educate the people through cartoons, documentaries and art films on environment issues.
- All newspapers and magazine must publish periodically the environmental problems.
- Special audio visual and slide shows can be arranged in public places during any important festivals to educate environmental science.

- Mass rally, chain rally and street plays can be organized to popularize the need for environmental protection.
- In order to attract the rural people attention towards environmental protection, folk plays and dramas can be performed in villages.
- The non-government organization like Leo club, lions club, rotary club, NCC, NSS and scouts can be used to spread the environmental awareness by organizing public movements.
- People must be educating to live in simple and ecofriendly manner.
- The government must plan to eliminate poverty by providing employment that will overcome the basic environmental issues.
- Top political leaders, cine actors, popular sportsmen, leading personalities and social workers can make an appeal to the public regarding the importance of environmental protection.
- Painting, storytelling, essay writing, group discussion and elocation competition on environmental issues should be arranged for students as well as for the public.

4.18 Function of pollution control boards

4.18.1 Central pollution control board (CPCB)

According to section 16-A the following are the functions of CPCB

- It advises the central government regarding the pollution prevention.
- It plans for the prevention and control of pollution.
- It lays down standards for the well water and air.
- It identifies areas or industries causing air pollution.
- It encourages industries to adopt 3R for wastes.
- It advices the industries to treat effluents with modern technology.
- It establishes laboratories for the analysis of air and water samples.
- It provides technical assistance and guidance to state boards.
- It sponsors research regarding water and air pollution.
- It emphasizes clean technology by the industries to reduce pollution.

4.18.2 State pollution control boards (SPCB)

Under section 7-B the following are the functions of the SPCB

- Planning a comprehensive programme regarding for prevention, control and abatement of pollution.
- Advise government regarding the pollution control or location of industries.
- Conducting and encouraging investigation and research related to different aspects of pollution.

- Inspecting effluent and water treatment plants.
- Prescribing effluent standards.
- Establishing laboratories for analyses of samples.
- Performing any functions assigned by the CPCB or state government.
- Evolving economical and reliable methods of disposal, treatment and reuse of wastes.
- Organizing educational programme in collaboration with CPCB.

4.19 Environmental impact assessment (EIA)

4.19.1 Definition

EIA is the study of predicting environmental effects of any proposed developmental projects.

4.19.2 Objectives of EIA

- To recover by products
- To conserve the natural resources
- To ensure the utilization of efficient equipment's.
- To identify the issues and problems of the proposed projects.

4.19.3 Benefits of EIA

- Biodiversity is maintained.
- Human health is improved.
- Usages of resources are reduced.
- Waste disposal cost is also minimized.
- Performance of the project is improved.
- Cost and time of the projects will be reduced.

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UNIT-5 HUMAN POPULATION AND THE ENVIRONMENT

Population:

The group of individuals belonging to the same species living in an area at a given time.

Population Density

It is expressed as the number of individuals of the population per unit area. This varies in response to changes in the environment and introduction with other living organism.

Parameters affecting population size

1. Birth rate or natality

It is the number of live birth per 1000 people in a population in a given year.

2. Death rate or Mortality

It is the number of death per 1000 people in a population in a given year.

3. Immigration

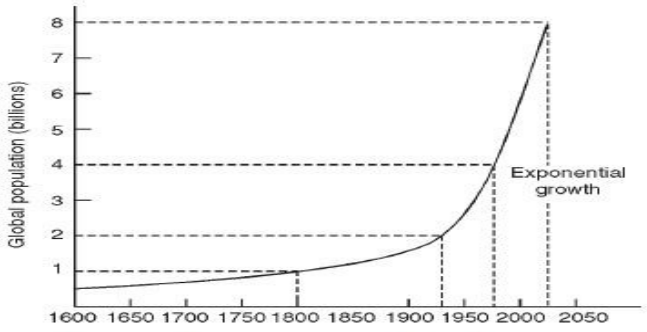
It denotes the arrival of individuals from neighboring population.

4. Emigration

It denotes the dispersal of individuals from native area to a new area.

Population Growth

- In 1800, the earth was home to about 1 billion people.
- It took about 39,000 years of human history to reach 1 billion, 130 years to reach the second billion, 45 years to reach 4 billion and the next doubling is likely within a span of a few decades.
- Population had already crossed 6 billion and may reach 10 billion by 2050 as per the World Bank estimates. The dramatic way in which global human population grew thereafter is shown in Figure



Global population growth trends in the last four centuries.

Reasons of this trend of human population growth:

During the Stone Age, population was quite stable. Environmental conditions were hostile and humans had not yet developed adequate artificial means for adaptations to these stresses.

Droughts and outbreak of diseases used to be quite common leading to mass deaths. 14th century A.D. experienced large scale mortality due to bubonic plague when about 50% of people in Asia and Europe died due to the disease.

With scientific and technological advancement, life expectancy of humans improved. People started living in definite settlements leading a more stable life with better sanitation, food and medical facilities. Victory over famine-related deaths and infant mortality became instrumental for a rapid increase in population size.

Effects:

The rapid increase of human population is putting an incredible strain on our environment. While developed countries continue to pollute the environment and deplete its resources, developing countries are under increasing pressure to compete economically and their industrial advancements are damaging as well.

The demands that this growth places on our global environment are threatening the future of sustainable life on earth. As the world's population grows, improving living standards without destroying the environment is a global challenge.

Many basic resources that are strained by our current population are given below:

1. **Water Scarcity:**

It is the lack of sufficient available water resources to meet the demands of water usage (water for consumption, agriculture and sanitation) within a region.

Aquifers are being depleted faster than they can be replenished. Melting glaciers threaten the water supply for billions. More than 1.2 billion people lack access to clean drinking water.

The supply of freshwater is finite, but demand is soaring as population grows and uses per capita rises. Depending on future rates of population growth, between 2.6 billion and 3.1 billion people may be living in either water-scarce or water-stressed conditions by 2025.

2. **Food supply:**

In 64 of 105 developing countries studied by the UN Food and Agriculture Organization,

the population has been growing faster than food supplies.

Population pressures have degraded some 2 billion hectares of arable land, one billion people, one out of every seven people alive, go to bed hungry. Every day 25,000 people die of malnutrition and hunger related diseases.

Almost 18,000 of them are children under 5 years old. The number of people living in countries where cultivated land is critically scarce is projected to increase to between 600 million and 986 million in 2025.

Food production and distribution could catch up if our population stopped growing and dropped to a sustainable level.

3. **Coastlines and oceans:**

   Half of all coastal ecosystems are pressured by high population densities and urban development. A tide of pollution is rising in the world's seas. Ocean fisheries are being overexploited, and fishery is down. Most of the world's ocean fisheries are already being fished to their maximum capacities and are in decline**.**

4. **Forests**:

   Over 1.8 billion people live in 36 countries with less than 0.1 hectare of forested land per capita, an indicator of critically low levels of forest cover.Based on the medium population projection and current deforestation trends, by 2025 the number of people living in forest-scarce countries could nearly double to 3 billion.Nearly half of the world's original forest cover has been lost, and each year another 16 million hectares are cut, bulldozed, or burned.Forests provide over US$400 billion to the world economy annually and are vital to maintaining healthy ecosystems. Yet, current demand for forest products may exceed the limit of sustainable consumption by 25%.

5. **Global Warming:**

The earth's surface is warming due to greenhouse gas emissions, largely from burning fossil fuels. If the global temperature rises as projected, sea levels would rise by several meters, causing widespread flooding. Globalwarming also could cause droughts and disrupt agriculture.

In 1998, global data are available for both population and heat-trapping carbon dioxide emissions, per capita emissions of CO_2 continued the upward trend that dominated the middle 1990s. When combined with growing world population, these increased per capita emissions accelerated the accumulation of greenhouse gases in the global atmosphere and, thus, future

global warming.

6. **Species Extinction:**

More than 1.1 billion people live in areas that conservationists consider the richest in non-human species and the most threatened by human activities. While these areas comprise about 12 percent of the planet's land surface, they hold nearly 20 percent of its human population.

The population in these biodiversity hotspots is growing at a collective rate of 1.8 percent annually, compared to the world's population's annual growth rate of 1.3 percent.

Human population growth is the number one threat to the world's environment. Each person requires energy, space and resources to survive, which results in environmental losses. If the human population were maintained at sustainable levels, it would be possible to balance these environmental losses with renewable resources and regeneration. But our population is rapidly rising beyond the earth's ability to regenerate and sustain us with a reasonable quality of life. We are exceeding the carrying capacity of our planet. We need to limit our growth voluntarily, and promote contraceptive use, before Nature controls our population for us with famines, drought and plagues.

Population characteristics:

1. **Exponential growth:**

When a quantity increases by a constant amount per unit time e.g. 1, 3, 5, 7 etc. it is called **linear growth.** And when it increases by a fixed percentage it is known as exponential growth e.g. 10, 102, 103, 104, or 2, 4, 8, 16, 32 etc.

2. **Doubling time:**

The time needed for a population to double its size at a constant annual rate is known as doubling time. It is calculated as follow-

$$Td = 70/r$$

Td = Doubling time in years r = annual growth rate

3. **Total Fertility rates (TFR) :**

The average number of children that would be born to a woman in her lifetime if the age specific birth rates remain constant. The value of TFR varies from 1.9 in developed nations to 4.7

in developing nations.

4. Infant mortality rate (IMR) :

It is the percentage of infants died out of those born in a year. Although this rate has declined in the last 50 years, but the pattern differs widely in developed and developing countries.

5. Replacement level:

This is an important concept in population dynamics or demography. Two parents bearing two children will be replaced by their offspring. But, due to infant mortality this replacement level is usually changed.

For developing nations, where infant mortality is high and life expectancy is low, the replacement level is approx 2.7, whereas in developed nations it is 2.1.

6. Age Structure:

Based upon people belonging to different age classes like pre-reproductive (0-14 years), reproductive (15-44 years) and post reproductive (45 years and above) Age structure of population of a nation can be represented by age pyramids,

a. Pyramid shaped:

The very young population is more, making a broad base and old people are less. This indicates growing population such as India, Bangladesh, Ethiopia, and Nigeria.

The large number of individuals in very young age will soon enter into reproductive age, thus causing an increase in population, whereas less number of people in old age indicates less loss of population due to death.

b. Bell shaped:

In Countries like France, USA and Canada where birth rates have in the past one or two decades declined resulting in people of almost equal number in age group 0-35 years.

So in the next 10 years, the people entering into reproductive age group is not going to change much and such age-pyramids indicate stable populations.

c. Urn shaped:

In Countries like Germany, Italy, Hungary, Sweden and Japan number of individuals in very young class is smaller than the middle reproductive age class.

In the next 10 years the number in reproductive age class will thus become less than before resulting in a decline of population growth.

The TFR, age structure, infant mortality and replacement level are all important parameters determining population growth. But population will not stop growing even when all couples have only 2 children.

7. Zero population growth (ZPG):

When birth plus immigration in a population are just equal to deaths plus emigration, it is said to be zero population growth.

8. Male-Female ratio:

The ratio of boys and girls should be fairly balanced in a society to flourish. However, due to female infanticides and gender-based abortions, the ratio has been upset in many countries including India.

In China, the ratio of boys to girls became 140:100 in many regions which led to scarcity of brides.

9. Life expectancy:

It is the average age that a newborn infant is expected to attain in a given country. The average life expectancy, over the globe, has risen from 40 to 65.5 years over the past century.

In India, life expectancy of males and females was only 22.6 years and 23.3 years, respectively in 1900. In the last 100 years improved medical facilities and technological advancement has increased the life expectancy to 60.3 years and 60.5 years, respectively for the Indian males and females.

10. Demographic transition:

Population growth is usually related to economic development. There occurs a typical fall in death rates and birth rates due to improved living conditions leading to low population growth, a phenomenon called demographic transition. It is associated with urbanization and growth and occurs in four phases:

i. **Pre industrial phase** - high growth and death rates and net population growth is low.

ii. **Transitional phase** - advent of industrialization providing better hygiene and medical facilities andadequate food, thereby reducing deaths.

Birth rates, however, remain high and the population shows 2.5-3% growth rate.

iii. **Industrial phase** - fall in birth rates thereby lowering growth rate.

iv. **Post industrial phase** - zero population growth is achieved.

Demographic transition is already observed in most developing nations. As a result of demographic transition the developed nations are now growing at a rate of about 0.5% with a doubling time of 118 years.

However more than 90% of the global population is concentrated in developing nations which have a growth rate a little more than 2%, and a doubling time of less than 35 years.

POPULATION EXPLOSION

The enormous increase in population due to low death rate (mortality) and high birth rate (natality) is termed as population explosion.

The population clock:

Every second, on an average 4-5 child are born and 2 people die, thus resulting in net gain of nearly 2.5 people every second. This means that every hour we are growing by about 9000 and everyday by about 2, 14,000.

The Indian Scenario:

a. For the developing countries like India, population explosion is a curse and is damaging to the development of the country and its society. The developing countries already facing a lack in their resources, and with the rapidly increasing population, the resources available per person are reduced further, leading to increased poverty, malnutrition, and other large population-related problems.

b. India is the second most populous country of the world with 1.27 billion people. Currently, there are about 51 births in India in a minute. India represents almost 17.31% of the world's population, which means one out of six people on this planet live in India.

c. Although, China leads in population for decades, India is all set to take the number one position by 2030. With the population growth rate at 1.58%, India is predicted to have more than 1.53 billion people by the end of 2030.

Causes:

1. Decline in the Death Rate:

Until recently, birth rates and death rates were about the same, keeping the population stable. The success in reducing death rates was attributable to several factors like

1. increase in food production and distribution

2. improvement in public health (water and sanitation)
3. medical technology (vaccines and antibiotics)
4. awareness education and standards of living

The fall in death rates that is decline in mortality rate is one fundamental causes of overpopulation. This has resulted in an increase in the life expectancy of individuals. Mortality rate has declined leading to an increase in population. Thus the overall death rate has gone down same time as brought with it, the curse of overpopulation.

2. Rise in the Birth Rate:

With the new discoveries in nutritional science, we are able to bring in increase in the fertility rates of human beings.

Medicines of today can boost the reproductive rate in human beings.

There are medicines and treatments, which can help in conception. Thus, science has led to an increase in birth rate.

3. Migration:

The inhabitants of various countries migrate to a particular part of the world and settle over there, the area is bound to suffer from the ill effects of overpopulation.

If the rates of emigration from a certain nation do not match the rates of immigration to that country, overpopulation makes its way.

Crowding of immigrants in certain parts of the world, results in an imbalance in the density of population.

4. Lack of Education:

Illiteracy is another important cause of overpopulation. Those lacking education fail to understand the need to prevent excessive growth of population. They are unable to understand the harmful effects that overpopulation has.

They are unaware of the ways to control population. Lack of family planning is commonly seen in the illiterate lot of the world. This is one of the major factors leading to overpopulation.

Consequences:

Population grows fastest in the world's poorest countries. Overpopulation and poverty have long been associated with increased death and disease.

The world's current and projected population growth calls for an increase in efforts to meet the needs for food, water, health care, technology and education.

In the poorest countries, massive efforts are needed to keep social and economic conditions from deteriorating further; any real advances in well-being and the quality of life are negated by further population growth.

Many countries lack adequate supplies of basic materials needed to support their current population. Not every nation is capable of providing its people with the adequate amount of resources.

The ever-increasing population will eventually leave no nation capable of providing its people with the resources they need to thrive. When the environment fails to accommodate the living beings that inhabit it, overpopulation becomes a disaster.

Measures:

Alarmed by its swelling population, India started taking measures to stem the growth rate quite early. India launched the National Family Planning program in 1952 and became the first country in the world to have a population policy.

The family planning program yielded some noticeable results, bringing down significantly the country's fertility rate. In 1965-2009 the contraceptive usage more than tripled and the fertility rate more than halved.

The efforts did produce positive results, however, failed to achieve the ultimate goal and the population of India since getting independence from Britain in 1947 increased almost three times.

Whereas India has missed almost all its targets to bring the rate of population growth under control, China's 'One Child Policy' in 1978, has brought tremendous results for the latter. The policy claims to have prevented between 250 and 300 million births from 1978 to 2000 and 400 million births from 1979 to 2010.

Problem with implementing measures:

Population explosion is causing severe resource depletion and environmental degradation. Our resources like land, water, fossil fuels, minerals etc. are limited and due to over exploitation these resources are getting exhausted.

Even many of the renewable resources like forests, grasslands etc. are under tremendous pressure. Industrial and economic growth are raising our quality of life but adding toxic

pollutants into the air, water and soil. As a result, the ecological life-support systems are getting jeopardized.The two very important views on population growth are-

1. Malthusian Theory:

According to Malthus, human populations tend to grow at an exponential or compound rate whereas food production increases very slowly or remains stable. Therefore, starvation, poverty, disease, crime and misery are invariably associated with population explosion.

He believes positive checks like famines, disease outbreak and violence as well as preventive checks like birth control need to stabilize population growth.

2. Marxian Theory:

According to Karl Marx, population growth is a symptom rather than the cause of poverty, resource depletion, pollution and other social ills.

He believed that social exploitation and oppression of the less privileged people leads to poverty, overcrowding, unemployment, environmental degradation that in turn, causes over population.

FAMILY WELFARE PROGRAMS

It was implemented by the government of india as a voluntary programme. It is an integral part of overall national policy of growth covering human health, family welfare child care and womens rights.

Family planning:

Family planning allows couples to decide their family size and also the time spacing of their offspring. Almost every culture in the past used to practice some traditional fertility control methods through some traditions, taboos and folk medicine.

Modern science has provided several birth control techniques including mechanical barriers, surgical methods, chemical pills and physical barriers to implantation. More than a hundred contraceptive methods are on trial.

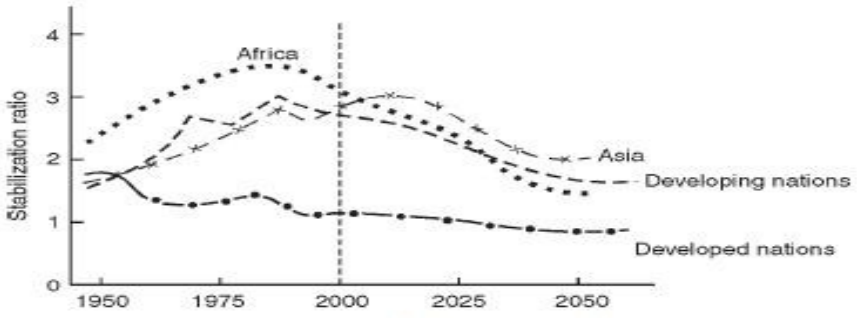
The United Nations Family Planning Agency provides funds to 135 countries. Many of these countries include abortion as a part of the population control programme which very often encourages female infanticide thereby disturbing the optimal male and female ratio in a society.

The birth control programmes have often faced strong opposition from religious groups. Nonetheless, World Health Organization (WHO) estimates that today about 50 percent of the

worlds married couples adopt some family planning measures as compared to just 10% about 30 years back. Still some 300 million couples do not have access to family planning.

India started the family planning programme in 1951 with the objective of "reducing the birthrate to the extent necessary to stabilize the population at a level consistent with the requirements of national economy."

Evolution:



Stabilization ratio of developing & developed nations, Africa and Asia. A ratio of 1 achieved in developed nations around 2000 indicates zero population growth in developed nations while Africa is presently having the highest ratio.

The United Nations projections about population stabilization of developed and developing nations and that of Asia are shown in above Figure.

The ratio is derived by dividing crude birth rate by crude death rate. As evident, developed nations have already achieved a stabilization ratio of 1 around the year 2000, which is more or less stabilized indicating zero population growth.

Developing nations including Asia, on the other hand, is yet having a high stabilization ratio nearing 3, which is however, on a decline and is expected to lower down substantially by 025.

Stabilization in developing nations is possible only through various family welfare programmes.

ENVIRONMENT AND HUMAN HEALTH

A physically fit person not suffering from any disease is called a healthy person. According to World Health Organization (WHO) health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

Human health is influenced by many factors like nutritional, biological, chemical or

psychological. These factors may cause harmful changes in the body's conditions called disease.

Infectious organisms:

Disease causing organisms pose greater environmental threats to health, more severely in the developing countries especially the tropical ones.

High temperature and moisture along with malnutrition help many diseases to spread in these countries. Microbes especially bacteria can cause food poisoning by producing toxins in the contaminated food.

Some moulds grow on food and produce poisonous toxins. Infectious organisms can also cause respiratory diseases (pneumonia, tuberculosis, influenza etc.) and gastrointestinal diseases (diarrhoea, dysentery, cholera etc.) There are various types of parasites that cause diseases like malaria, schistosomiasis, filariasis etc. Most of these infections take place when the environmental conditions are unclean and unhygienic.

Chemicals:

A large number of chemicals are introduced in the environment by anthropogenic activities. Industrial effluents containing various chemicals are of major concern. Chemicals can be divided into two categories i.e. hazardous and toxic chemicals. Hazardous are the dangerous chemicals like explosives, inflammable chemicals etc and toxic chemicals (toxins) are poisonous chemicals which kill cells and can cause death.

Many other chemicals can cause cancer (carcinogenic), affect genetic material (DNA) in cells (mutagenic) or cause abnormalities during embryonic growth and development (teratogenic), while there are others that affect nervous system (neurotoxins) and the reproductive system.

Some of the pesticides and other industrial pollutants may act as hormone analogs in humans and other species. These environmental hormones affect reproduction, development and cause various types of ailments including tumors.

Many chemicals like DDT and other chlorinated pesticides bio-accumulate in food-chain and show deleterious effects at the top of the food chain. Many chemical substances present in wastewaters like heavy metals (mercury, cadmium, lead etc.) fluoride and nitrate can affect human health.

Metals can contaminate food while cooking in various types of utensils including alloys like steel. Containers for canned food, especially which are acidic in nature, contaminate the

food with lead. Lead also comes in water from the water-pipes where it is added for plumbing purposes.

Various alcoholic beverages contain lead while tobacco contains cadmium that goes in the body and affects human health. Various chemicals, gases and particulates laden with chemicals, spewed into the environment from various industries cause air pollution and affect human health.

Noise:

Although human ear is capable of tolerating a range of sound levels, yet if sound levels beyond the permissible level exist for certain duration, it becomes painful and sometimes irreparable damage occurs.

Radiations:

Radiations are known to cause short-term and long term changes in various organs. Cosmic rays and ultra-violet rays cause harmful effects on human health which may include cancer.

Diet:

Diet has a very important role in maintaining health. Malnutrition makes humans prone to other diseases. There is a strong correlation between cardiovascular diseases and the amount of salt and fat in one's diet. Food contamination can cause various ill effects. There had been cases of Dropsy in India, a disease which occurred due to contamination of mustard oil with the poisonous seeds of Argemonemexicana.

Settlement:

Proper environment, availability of basic necessities of life like, water, sanitation etc. are essential for healthy living. Improper settlement and poor physical environment may cause various psychological problems which affect various vital physiological processes in the body.

HUMAN RIGHTS:

Human beings are born equal in dignity and rights. These are moral claims which are inalienable and inherent in all individuals by virtue of their humanity alone, irrespective of caste, colour, creed, and place of birth, sex, cultural difference or any other consideration.

These claims are articulated and formulated in what is today known as human rights. Human rights are sometimes referred to as fundamental rights, basic rights, inherent rights, natural rights

and birth rights.

Definition:

The Universal Declaration of Human Rights (UDHR) 1948 defines human rights as "Rights derived from the inherent dignity of the human person." Human rights when they are guaranteed by a written constitution are known as "Fundamental Rights" because a written constitution is the fundamental law of the state.

Human rights are essential for the overall development of individuals. The Constitution of India makes provisions for basic rights also known as Fundamental Rights for its citizens as well as for aliens. A distinction is made between Specific Fundamental Rights and Unspecified Fundamental Rights.

Draft Declaration of Human Rights and Environment:

The draft declaration describes the rights as well as duties that apply to individuals, governments, international organizations and transnational corporations.

The preamble envisages a deep concern regarding the consequences of environmental harm caused by poverty, debt programmes and international trade. Environmental damages are often irreversible.

Human rights violations may lead to further environmental degradation on a long-term basis and the environmental degradation, in turn would lead to further human rights violation.

The principles of the draft declaration are divided into five parts.

- **Part I:** It deals with human rights for an ecologically sound environment, sustainable development andpeace for all. It also emphasizes the present generation's rights to fulfill its needs to lead a dignified and good quality life. But, at the same time it lays stress on the fact that it should be without impairing the rights of the future generations to meet their needs.
- **Part II:** It mainly deals with human rights related to an environment free from pollution and degradation. Italso emphasizes the rights to enjoyment of natural ecosystems with their rich biodiversity. It defines right to own native land or home. No one can be evicted from one.s native place except in emergency or due to a compelling purpose benefitting the society as a whole which is not attainable by other means. All persons have the right to timely assistance in the event of any natural or technological disaster.

- **Part III:** It deals with right of every person to environmental information, education, awareness and alsopublic participation in environmental decision making.
- **Part IV**: It deals with the duties to protect and preserve the environment and prevent environmental harm.It includes all remedies for environmental degradation and measures to be taken for sustainable resource use. It emphasizes that states shall avoid using environment as a means of war and shall respect international law for protection of environment.
- **Part V:** This lays stress on social justice and equity with respect to use of natural resources and sustainabledevelopment. Till now, however, it has not been defined in practical terms the threshold, below which level of environmental quality must fall before a breach of individual human right will said to have occurred or above which the level of environmental quality must rise. Right to development has to be linked to .right to safe and clean environment Which has to be considered not only at the level of individual but at community, national and global level.

- **VALUE EDUCATION:**

Education:

It is one of the most important tools in bringing about socioeconomic and cultural progress of a country. However, the objective should not merely be imparting coaching to the students that they get through the examinations with good results and get some good job.

Education does not simply mean acquiring a lot of information but also its righteousness and use within the framework of a spectrum of ethical values.

Current scenario:

The rapid strides of scientific and technological advancements have no doubt, brought revolutionary changes in our everyday life and information technology has shrunk the whole world into a global village with access to very information sitting in one corner over the internet.

But, in this frenzy for development and mad race for progress perhaps man has become too materialistic, self-centered and over ambitious and the desired ideals of a real good life have been pushed to the background.

Objective of value education:

1. It has a very significant role in providing proper direction to inculcate a positive attitude and to teach the distinction between right and wrong.

2. It teaches one to be compassionate, helpful, peace loving, generous and tolerant so that they can move towards a more harmonious, peaceful, enjoyable and sustainable future.
3. It helps in arriving at value-based judgments in life based on practical understanding of various natural principles rather than acquiring certain prejudices.
4. It encompasses human values, social values, professional values, religious values, national values, aesthetic values and environmental values.
5. It increases awareness about our national history, our cultural heritage, national pride, constitutional rights and duties, national integration, community development and environment.

Different phases:

Phases include value awareness, value orientation, value appraisal, value selection, value commitment and value action. The basic aim is to create and develop awareness about the values, their significance and role.

After knowing them mindset of students would get oriented towards those values and they will try to critically analyze the same and then select the values which really appeal to him.

Value-based Environmental Education:

Following the Supreme Court directives (in M.C. Mehta's Union of India, 1988) environmental education has been included in the curriculum right from the school stage to college/university level. The prime objective of it is to make everyone environment literate.

Environmental education can be made value based by,

1. Preparing text-books and resource materials about environmental education that play an important role in building positive attitudes about environment. The basic human value is man in nature rather than nature for man needs to be infused through the same.
2. Including Social values like love, compassion, tolerance and justice which are the basic teachings of most of our religions into environmental education. These are the values to be nurtured so that all forms of life and the biodiversity on this earth are protected.
3. Cultural and religious values enshrined in Vedas emphasize that man should not exploit nature without nurturing her. Our cultural customs and rituals in many ways teach us to perform such functions as would protect and nurture nature and respect every aspect of nature, treating them as sacred, are it rivers, earth, mountains or forests.

4. Encompassing the Environmental education with the ethical values of earth-centric rather than human-centric world-view. The educational system should promote the earth-citizenship thinking. Instead of considering human being as supreme we have to think of the welfare of the earth.
5. Global values stress upon the concept that the human civilization is a part of the planet as a whole and similarly nature and various natural phenomena over the earth are interconnected and inter-linked with special bonds of harmony. Disturbing this harmony anywhere will be an ecological imbalance leading to catastrophic results.
6. Spiritual values highlight the principles of self-restraint, self discipline, contentment, reduction of wants, freedom from greed and austerity. All these values promote conservationism and transform our consumerist approach.

Value-based environmental education can bring in a total transformation of our mindset, our attitudes and our lifestyles. The above mentioned human values will go a long way in attaining the goals of sustainable development and environmental conservation. The value elements in environmental education alone can succeed in achieving the real goals of environmental literacy.

HIV/AIDS

Dr.Robert Gallo at National Institute of Health, USA and Luc Montagnier at Pasteur Institute, Paris isolated the virus, HIV which causes AIDS.

HIV stands for **H**-Human: This particular virus can only infect human beings. **I**-Immunodeficiency: HIV weakens your immune system by destroying important cells that fight disease and infection. **V**-Virus: A virus can only reproduce itself by taking over a cell in the body of its host.

AIDS stands for **A**-Acquired: AIDS is not something you inherit from your parents. You acquire AIDS after birth. **I**-Immuno: our body's immune system includes all the organs and cells that work to fight off infection or disease. **D**-Deficiency: one get AIDS when your immune system is deficient. **S**-Syndrome: A syndrome is a collection of symptoms and signs of disease.

The terms “HIV” refers to the virus itself and “AIDS” refers to the late stage of HIV infection.

Function of HIV inside human body:

HIV is like other viruses that cause the flu or the common cold. But an important difference over time is that our immune system can clear most viruses out of our body. That is not the same in case of HIV, the human immune system can't seem to get rid of it i.e., once you have HIV, you have it for life.

HIV can hide for long periods of time in the cells of your body and that it attacks a key part of immune system. White Blood Cells are responsible in the formation of antibodies are called T-helper cells.

Our body has to have these cells to fight infections and disease, but HIV invades them, uses them to make more copies of it, and then destroys them. Over time, HIV can destroy so many of your CD4 cells that your body can't fight infections and diseases anymore.

When that happens, HIV infection can lead to AIDS, the final infection. No safe and effective cure currently exists, but scientists are working hard to find one. But with proper medical care, HIV can be controlled.

Treatment for HIV is often called antiretroviral therapy or ART. This can dramatically prolong the lives of many people infected with HIV and lower their chance of infecting others.

Origin:

Though sufficient knowledge about the disease has been gained, yet a definite source of this virus could not be identified. But it is believed that it is transferred to humans from African monkey, through contaminated polio vaccine prepared from monkey's kidney, Through Hepatitis-B viral vaccine, Through small pox vaccine programme of Africa.

Transmission:

1. It is transmitted through certain body fluids from an HIV-infected person-Blood, Semen, Rectal fluids, Vaginal fluids, Breast milk. These body fluids when come into contact with a mucous membrane or damaged tissue or when directly injected into bloodstream (by a needle or syringe) the transmission is possible.
2. Having unprotected sex with someone who has HIV.
3. Sharing needles, syringes, rinse water, or other equipment used to prepare injection drugs with someone who is infected with HIV.
4. Being born to an infected mother. (HIV can be passed from mother to child during

pregnancy, birth, or breastfeeding).

5. Receiving blood transfusions, blood products, or organ/tissue transplants that are contaminated with HIV.
6. Contact between broken skin, wounds, or mucous membranes and HIV-infected blood or blood-contaminated body fluids.

HIV is NOT spread by:

- Air or water
- Insects, including mosquitoes or ticks
- Saliva, tears, or sweat
- Casual contact, like shaking hands, hugging or sharing dishes/drinking glasses

Symptoms:

1. Early Stage of HIV

Within 2-4 weeks after HIV infection, many people experience flu-like symptoms often described as the

'worst flu ever'.

This is called "acute retroviral syndrome" (ARS) or "primary HIV infection," and it is the body's natural response to the HIV infection.

Symptoms include Fever, Swollen glands, Sore throat, Rash, Fatigue, Headache, Muscle and joint aches.

2. The Clinical Latency Stage

Latency means a period where a virus is living or developing in a person without producing symptoms. During the clinical latency stage, people who are infected with HIV experience no HIV-related symptoms, or only mild ones. This stage is sometimes called "asymptomatic HIV infection" or "chronic HIV infection."

During the clinical latency stage, the HIV virus reproduces at very low levels, although it is still active. If one take antiretroviral therapy (ART), they may live with clinical latency for several decades because treatment helps keep the virus in check.

It is important to remember that people in this symptom-free period are still able to transmit HIV to others even if they are on ART, although ART greatly reduces the risk of transmission.

3. Progression to AIDS

If one have HIV and you are not taking HIV medication (antiretroviral therapy), eventually the HIV virus will weaken your body's immune system. The onset of symptoms signals the transition from the clinical latency stage to AIDS.

During this late stage of HIV infection, people infected with HIV may have the following symptoms:

- Rapid weight loss
- Recurring fever or profuse night sweats
- Extreme and unexplained tiredness
- Prolonged swelling of the lymph glands in the armpits, groin, or neck
- Diarrhea that lasts for more than a week
- Sores of the mouth, anus, or genitals
- Pneumonia
- Red, brown, pink, or purplish blotches on or under the skin or inside the mouth, nose, or eyelids
- Memory loss, depression, and other neurologic disorders.

Many of the severe symptoms and illnesses of HIV disease come from the opportunistic infections that occur because your body's immune system has been damaged.

Diagnosis:

1. ELISA test (Enzyme Linked Immuno Sorbent Assay) is a sensitive preliminary blood test used to detect HIV antibodies.
2. Western Blot is the confirmatory test, which is highly specific and based on specific antibodies to viral core proteins

Control and Management:

- Education to people about protected sexual behavior and practices, the do's and don'ts in AIDS contraction and bringing more awareness among the public.
- Protected sexual behavior.
- Screening of blood and blood products before blood transfusion.
- Usage of disposable syringes in the hospitals.

- Not sharing the razors / blades in the saloon.
- Avoid tattooing using common needle.
- Making the antiretroviral drugs such as AZTs (Azidothymidine/Zidovudin) and saquinovir available to patients.

WOMEN WELFARE

Women and children are usually the soft targets, who suffer in a number of ways mainly because they are weaker, helpless and economically dependent. The main aim is to improve the status of women by providing oppurtunities in education, employment and economic independence.

Need:

Women usually suffer gender discrimination and devaluation at home, at workplace, in matrimony, in inheritance, in public life and power, particularly in developing countries. The gender violence, victimization and harassment take many forms across culture, race or nation.

The exceptionally high number of cases of abduction, dowry deaths, rape, domestic violence, criminal offences and mental torture to women is something that needs immediate attention and reforms in the interest of the women.

Women are often the worst victims of communal enmities. The human rights of women are violated too often in a male dominated patriarchal society. Thus, there is an urgent need for policy reforms and more stringent legislation as well as educational and legal awareness amongst women for checking the atrocities and injustice towards her.

There is a full-fledged Ministry for Women and Child development whose sole aim is to work for the welfare and upliftment of women encompassing family planning, health care, education and awareness.

Environmental degradation:

Women are also the victims of capitalism, development and environment. The exploitative nature of capitalist development not only affects the natural environment but the traditional, social, cultural and family life of women.

After losing the forests and getting rehabilitated from their native places, men folk usually migrate to towns in search of some job while the women are left behind to look after the family and household with little resources.

Development projects like mining very often play havoc with the life of women. Men can

still work in the mines or migrate to towns after getting compensation from the government. The National Network for Women and Mining (NNWM) with about 20 groups in different mining states of India is rightly fighting for a .gender audit of India's mining companies.

The displaced women are the worst affected as they do not get any compensation and are totally dependent upon the males for wages. The displaced women driven out from their land-based work are forced to take up marginalized work which is highly un-organised and often socially humiliating. Issues related to their dignity and honour has not yet received any attention.

The NNWM is now working for rights of women over natural resources, resettlement and compensation issues. Besides the government initiatives there are now a number of nongovernment organizations mostly as MahilaMandals.

To create awareness amongst women of remote villages even to empower them, train them, educate them and help them to become economically self-dependent On an international level, the United Nations Decade for Women (1975-85) witnessed inclusion of several women welfare related issues on international agenda.

The CEDAW (International Convention on the Elimination of all forms of Discrimination Against Women, 1979) has been a landmark outcome of the decade to be accepted as an international standard for the protection and promotion of women's human rights and socio-economic upliftment. It is, however, most important for all women in the mainstream, tribal's, refugees and the down-trodden to be educated about these issues.

National commission for women:

It has been created by government of India and its objectives are

- To examine constitutional and legal rights for women
- To review existing legislations
- To sensitize the enforcement and administrative machinery to women's causes.

CHILD WELFARE

Children are considered to be the assets of a society. They nearly occupy 40% of total population. But the statistical figures tell us that about a million babies, out of 21 million born every year in India are abandoned soon after their birth due to different socio-economic reasons.

Around 20 million children in our country are estimated to be working as child labours, some of them in various hazardous industries like the match industry, firework industry,

brassware industry and pottery industry.

Child labours:

Poverty is the main reason to drive these children into long hours of work in miserable, unhealthy conditions and yet they do not get the minimum nutritive food, what to talk of educational and recreational facilities, which are their childhood rights.

Various organizations towards child welfare:

The UN General Assembly in 1959 adopted the Declaration of the Rights of a child. After the UN convention on Rights of Child, it became International Law in the year 1990, consisting of 54 articles and a set of international standards and measures to promote and protect the well being of children in a society.

The law defines right of the child to survival, protection, development and participation.

The right to survival emphasizes on adequately good standards of living, good nutrition and health.

The right to protection means freedom from exploitation, abuse, inhuman treatment and neglect.

The right to development ensures access to education, early childhood care and support, social security and right to leisure and recreation.

The right to participation means freedom of thought, conscience and religion and appropriate information to the child.

The World Summit on Children, held on September 30, 1990 had a focused agenda for the well being of the children targeted to be achieved in the beginning of the new millennium.

India is also a signatory to the World Declaration on Survival, protection and development of children.

A national plan of action for children has been formulated by the Ministry of Human Resource Development (MHRD), Government of India in which a strategic plan has been formulated for children's welfare in the priority areas of health, education, nutrition, clean and safe drinking water, sanitation and environment.

Universalisation of effective access to at least primary level schooling, special emphasis on girl child's education including health and nutrition, up gradation of home-based skills, mid-day meals scheme, expansion of early childhood development activities including low-cost family based involvements are some of the important actions envisaged.

Children are also the most affected due to environmental pollution. .They consume more

water, food and air than adults, hence more susceptible to any environmental contamination.- says one of the scientific reports of Center for Science and Environment (CSE), New Delhi. It is high time to work together for a secure and cleaner environment so as to give our children a cleaner and safer world to live in.

EIA

EIA is an evaluation procedure that helps planners and decision-makers to understand the environmental impacts of a proposed project or activity.

EIA is used according to two principal functions-

• As a planning tool to minimise adverse impacts caused by a development activity emphasis is on the methodologies and techniques for identifying, predicting and evaluating the environmental impacts of a proposed project or programme. Increasingly, EIA is also being viewed as a key mechanism for involving the public in the planning
process through stakeholder analysis.

• As a decision-making instrument to decide upon the acceptability of a project based on its environmental costs. It is designed to:

1. anticipate and prevent environmental problems
2. identify ways to increase environmental benefits
3. support informed decisions on project options and trade-offs
4. integrate environmental considerations into the planning, design and construction of projects at all scales

If used properly, EIA can help to achieve the following benefits:

- Avoiding mistakes that can be expensive and damaging in ecological, social and economic terms
- Avoiding conflicts and increasing project acceptance
- Integrating short-term needs with long-term goals
- Addressing transboundary issues
- Improving project design and reducing capital and operating costs
- Improving institutional co-ordination
- Considering alternative projects and designs
- Improving accountability and transparency in planning and decision-making.

It is a tool used for decision making regarding developmental projects and programmes and it may be defines as a formal process used to predict the environmental consequences of any developmental project. Thus it ensures that the potential problems are foreseen and addressed at an early stage in the project planning and design.

This is often carried out in order to produce an environmental statement/report. This statement must include-

- Description of the project
- Description of significant effects
- Mitigating measures
- A non technical summary

ROLE OF IT IN HUMAN HEALTH AND ENVIRONMENT

Information technology has tremendous potential in the field of environmental education and health as in any other field like business, economics, politics or culture. Development of internet facilities, worldwide web, geographical information system (GIS) and information through satellites has generated a wealth of up-to-date information on various aspects of environment and health.

A number of soft-wares have been developed for environment and health studies which are user friendly and can help an early learner in knowing and understanding the subject.

a. **Database:**

Database is the collection of inter-related data on various subjects. It is usually in computerized form and can be retrieved whenever required. In the computer the information of database is arranged in a systematic manner that is easily manageable and can be very quickly retrieved.

Application includes

- **The Ministry of Environment and Forests**, Government of India has taken up the task of compilinga database on various biotic communities. The comprehensive database includes wildlife database, conservation database, forest cover database etc. Database is also available for diseases like HIV/AIDS, Malaria, Fluorosis, etc.
- **National Management Information System (NMIS)** of the Department of Science

and Technologyhas compiled a database on Research and Development Projects along with information about research scientists and personnel involved.

- **Environmental Information System (ENVIS)** The Ministry of Environment and Forests,Government of India has created an Information System called Environmental Information System (ENVIS).

  Headquarters: Delhi, it functions in 25 different centers all over the country.

  The ENVIS centers work for generating a network of database in areas like pollution control, clean technologies, remote sensing, coastal ecology, biodiversity, western Ghats and eastern Ghats, environmental management, media related to environment, renewable energy, desertification, mangroves, wildlife, Himalayan ecology, mining, etc.

- **The National Institute of Occupational Health** provides computerized information on occupationalhealth i.e. the health aspects of people working in various hazardous and nonhazardous industries, safety measures etc.

b. Remote sensing:

Remote sensing is the acquisition of information about an object or phenomenon without making physical contact with the object and thus in contrast to in situ observation.

In modern usage, the term generally refers to the use of aerial sensor technologies to detect and classify objects on Earth (surface, in the atmosphere and oceans) by means of propagated signals (electromagnetic radiation).

It may be split into active remote sensing

or passive. It is applied in following fields-

In agriculture: provide valuable information about land and water management.

In forestry: provide valuable information for sustainable forest management.

In land cover: spatial information on land use is required at different scale depending on usage.

In water resources: for surface water mapping, ground water targeting, wetland, flood monitoring runoff modeling…etc

Satellite imageries provide us actual information about various physical and biological resources and also to some extent about their state of degradation in a digital form through remote sensing. We are able to gather digital information on environmental aspects like water logging, desertification, deforestation, urban sprawl, river and canal network, mineral and energy reserves

and so on.

c. Geographical Information System (GIS):

It has proved to be a very effective tool in environmental management.

GIS is a technique of superimposing various thematic maps using digital data on a large number of inter related or interdependent aspects.

Its applications include

1. Different thematic maps containing digital information on a number of aspects like water resources, industrial growth, human settlements, road network, soil type, forest land, crop land or grassland etc. are superimposed in a layered form in computer using softwares. Such information is very useful for future land-use planning.
2. Even interpretations of polluted zones, degraded lands or diseased cropland etc. can be made based on GIS. Planning for locating suitable areas for industrial growth is now being done using GIS by preparing Zoning Atlas.
3. GIS serves to check unplanned growth and related environmental problems.

d. Satellite data:

It helps in providing correct, reliable and verifiable information about forest cover, success of conservation efforts etc.

They also provide information of atmospheric phenomena like approach of monsoon, ozone layer depletion, inversion phenomena, smog etc. We are able to discover many new reserves of oil, minerals etc. with the help of information generated by remote sensing satellites.

e. World Wide Web:

A vast quantum of current data is available on World Wide Web.

Important on-line learning center:

1. www.mhhe.com/environmental science
2. Multimedia Digital Content Manager (DCM) in the form of CD-ROM

Provides the most current and relevant information on principles of environmental science, various problems, queries, applications and solutions.

The World Wide Web with resource material on every aspect, class-room activities, digital files of photos, power-point lecture presentations, animations, web-exercises and quiz has

proved to be extremely useful both for the students and the teachers of environmental studies.

Features:

1. Student friendly features:

These include practice quiz, how-to study tips and hyperlinks on every chapter topics with detailed information, web exercises, case studies, environment maps, key-terms, career information, current articles, interactive encyclopedia and how to contact your elected officials.

2. Teacher-friendly features:

In addition to above it include supplement resource charts, additional case studies, answers to web exercises, solutions to critical thinking questions, editing facility to add or delete questions and create multiple versions of same test etc.

Thus remote sensing and GIS play a key role in resource mapping, environmental conservation, management, planning and environmental impact assessment. It also helps in identifying several disease infested areas which are prone to some vector-borne diseases like malaria, schistosomiasis etc. based upon mapping of such areas. There are several Distribution Information Centres (DICs) in our country that are linked with each other and with the central information network having access to international database.

Information technology is expanding rapidly with increasing applications and new avenues are being opened with effective role in education, management and planning in the field of environment and health.

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BT3491-CHEMICAL PROCESS CALCULATIONS

LECTURE NOTES

REGULATION 2021

II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

Process Calculations for Biotechnologist - 21BT32T

Unit - 1

Dr. M. Gopinath

Associate Professor

DEPARTMENT OF BIOTECHNOLOGY

VEL TECH HIGH TECH

Dr. RANGARAJAN Dr. SAKUNTHALA ENGINEERING COLLEGE

Avadi, Chennai

UNIT I BASIC CHEMICAL CALCULATIONS

Systems of units – fundamental and derived quantities, Dimension, Dimensional Homogeneity and Dimensional Analysis, limitations and significance, unit conversion from one system to the other – composition of mixtures and solutions – mass fraction, mass %, mole fraction, mole %, mass ratios, molarity, molality, normality, ppm, composition by density.

Total period required = 15 (9 + 6)

COURSE OUTCOMES

Solve the basic chemical and biochemical engineering problems

Biochemical Engineering

- Biochemical engineering is the profession in which a knowledge of mathematics, chemistry, biology and other natural sciences gained by study, experience, and practice is applied with judgment to develop economic ways of using materials and energy for the benefit of mankind.
- The profession encompasses the spectrum from products, to the processes and equipment for making them, and to their applications.

Major chemical process industries

Industry	*Products*	*End uses*
Inorganic chemicals	Sulphuric acid	Fertilizers, chemicals, petroleum refining, paint pigments, metal processing, explosives
	Nitric acid	Explosives, fertilizers
	Sodium hydroxide	Chemicals, rayon and film processing industries, petroleum refining, lye, cleansers, soap, metal processing
Organic chemicals	Acetic anhydride	Rayons, resins, plastics
	Ethylene glycol	Antifreeze, cellophane, dynamite, synthetic fibres
	Formaldehyde	Plastics
	Methanol	Formaldehyde manufacture, antifreeze, solvent
Petroleum and petrochemicals	Gasoline	Motor fuel
	Kerosene	Jet fuel, domestic fuel
	Oils	Lubricating, heating
	Ammonia	Fertilizers, chemicals
	Ethyl alcohol	Acetaldehyde, solvent, other chemical manufacture
	Alkyl aryl sulphonate	Detergents
	Styrene	Synthetic rubber and plastics
Pulp and paper	Paper, cardboard, fibre board, etc.	Books, records, news paper, boxes, building materials
Pigments and paint	Zinc oxide, titanium oxide, carbon black, lead chromate,	Pigments for paints, ink, plastic, rubber, ceramic, linoleum
	Linseed oil	Drying oil
	Phenolic, alkyd resins	Basic lacquers, varnishes and enamel paints
Rubber	Natural rubber, synthetic rubber (GR-S, Neoprene, Butyl)	Automobile tyres, moulded goods and sheeting, footwears, electrical insulation, etc.

Major chemical process industries

Industry	*Products*	*End uses*
Plastics	Phenol formaldehyde, polystyrene, polymethyl-methacrylate, PVC, polyethylene, polyesters	Various uses in all areas of everyday life
Synthetic fibres	Rayon, nylon, polyesters, acrylics	Cloth and clothing
Minerals	Glass, ceramic, cement	Windows, containers, bricks, pipes, concrete for construction of buildings, highways
Cleansing agents	Soaps, synthetic detergents, wetting agents	Household and industrial cleansing
Biochemicals	Pharmaceuticals and drugs	Health and medicinal applications
	Fermentation products: Penicillin, ethyl alcohol	Medicinal use, solvent, beverage
	Food products	Human sustenance
Metals	Steel, copper, aluminium, zirconium	Building material, machinery, etc.
	Uranium	Nuclear fuel

Different types of unit operations

Unit operations		*Unit processes*	
Fluid flow	Size reduction	Oxidation	Polymerization
Heat transfer	Sedimentation	Reduction	
Mass transfer	Filtration	Nitration	Pyrolysis
Drying	Mixing	Sulphonation	Hydrolysis
Distillation	Evaporation	Electrochemical reactions	
Crystallization	Sublimation	Industrial microbiological processes	
Extraction	Centrifugation	Halogenation	
Adsorption	Materials handling		
Gas absorption	Ion exchange		

Units

Define Units.

A given physical quantity can be measured using a variety of units.

Units is a way to assign a number (or) measurements to that dimension.

(or)

Units are used to establish the size (or) magnitude of a dimension.

Example.

- Length of an object may be given in metres
- Mass of an object may be given in Kilograms

Dimensions

Define Dimension.

Dimension is a measure of a physical variable. (or)
Dimensions are used to describe objects and actions.

Example.

- Length is a dimension.

Physical quantities

Physical quantities can be classified as

1. **Fundamental quantities**
2. **Derived quantities**

Fundamental and Derived Units

Define fundamental Quantities.

Length, mass, time and temperature, theses are called base units (or) dimensions (or) primary units and these units are represented by symbols L.M.θ.T.

Define Derived quantities.

Which is derived from fundamental quantities. Derived quantities are represented algebraically in terms of multiplication and division of fundamental quantities.

Example.: Area, Force, Pressure, Energy, etc.,

Systems of units

There are several systems of units, but two primary systems that engineers use are the International System of Units (SI system) and the American Engineering System of Units (AES). Other systems are the centimeter–gram–second (CGS), foot–pound–second (FPS), and the British System of Units (British).

SI Base Units

Quantity	Name	Symbol
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Electric current	Ampere	a
Temperature	Kelvin	k
Amount of substance	Mole	mol
Luminous intensity	Candela	cd

Various systems of units

System	Mass (M)	Length (L)	Time (t)	Temperature (T)
SI	Kilogram (kg)	Meter (m)	Second (s)	Kelvins (K)
AES	Pound mass (lb_m)	Foot (ft)	Second (s)	Degrees Fahrenheit (^oF)
CGS	Gram (g)	Centimeter (cm)	Second (s)	Kelvins (K)
FPS	Pound mass (lb_m)	Foot (ft)	Second (s)	Degrees Fahrenheit (^oF)
British	Slug	Foot (ft)	Second (s)	Degrees Celsius (^oC)

Derived quantity

Quantity	*Units (symbolic abbreviation in brackets)*			*Dimension (Based on SI)*
	SI	MKS/CGS	FPS	
Area	square metres (m^2)	square centimetre (cm^2)	square feet (ft^2)	L^2
Volume	cubic metres (m^3)	cubic centimetre (cm^3), litre (L)	cubic feet (ft^3)	L^3
Density	kilograms per cubic metre ($kg\ m^{-3}$)	grams per cubic centimetre ($g\ cm^{-3}$)	pounds per cubic foot ($lb\ ft^{-3}$)	ML^{-3}
Mass flow rate	kilogram per second ($kg\ s^{-1}$)	gram per second $g\ s^{-1}$	pounds per hour ($lb\ h^{-1}$)	MT^{-1}
Molar flow rate	moles per second ($mol\ s^{-1}$)	moles per second ($mol\ s^{-1}$)	pound moles per hour ($lb\text{-}mol\ h^{-1}$)	nT^{-1}
Volumetric flow rate	cubic metres per second ($m^3\ s^{-1}$)	cubic centimetre per second ($cm^3\ s^{-1}$), litre per second ($L\ s^{-1}$)	cubic feet per hour ($ft^3\ h^{-1}$)	L^3T^{-1}
Mass flux	moles per square metre per second ($mol\ s^{-1}\ m^{-2}$)	moles per square metre per second ($mol\ s^{-1}\ m^{-2}$)	pound moles per hour per square foot ($lb\text{-}mol\ h^{-1}\ ft^{-2}$)	$nT^{-1}L^{-2}$

(Contd.)

Derived quantity

Quantity	*Units (symbolic abbreviation in brackets)*			*Dimension (Based on SI)*
	SI	MKS/CGS	FPS	
Force	newton (N)	kilogram-force (kgf)	pound force (lbf)	MLT^{-2}
Pressure	newton per square metre or pascal (N m^{-2} or Pa)	kilogram force per square centimetre (kgf cm^{-2})	pound force per square foot (lbf ft^{-2})	$ML^{-1}T^{-2}$
Energy	joule (J)	metre kilogram force (m kgf)	foot pound force (ft lbf)	ML^2T^{-2}
Power	watts or joules per second (W or J s^{-1})	horse power (HP)	foot pound force per second (ft lbf s^{-1})	ML^2T^{-3}
Heat, Enthalpy	joule (J)	kilocalories (kcal)	British Thermal Unit (Btu)	ML^2T^{-2}
Heat capacity	joules per kilogram per degree kelvin (J kg^{-1} K^{-1})	kilocalories per kilogram per degree celsius, (kcal kg^{-1} $°C^{-1}$)	Btu per pound per degree Fahrenheit (Btu lb^{-1} $°F^{-1}$)	$L^2T^{-2}K^{-1}$
Molar heat capacity	joules per mole per degree kelvin (J mol^{-1} K^{-1})	kilocalories per mole per degree celsius, (kcal mol^{-1} $°C^{-1}$)	Btu per pound mole per degree Fahrenheit (Btu lb-mol^{-1} $°F^{-1}$)	$ML^2T^{-2}K^{-1}n^{-1}$
Heat flux	joules per square metre per second (J s^{-1} m^{-2}, W m^{-2})	kilocalories per hour per square metre (kcal h^{-1} m^{-2})	Btu per hour per square foot (Btu h^{-1} ft^{-2})	MT^{-3}

SI Prefix

Factor	*Prefix*	*Symbol*	*Factor*	*Prefix*	*Symbol*
10^{24}	yotta	Y	10^{-1}	deci	d
10^{21}	zeta	Z	10^{-2}	centi	c
10^{18}	exa	E	10^{-3}	milli	m
10^{15}	peta	P	10^{-6}	micro	μ
10^{12}	tera	T	10^{-9}	nano	n
10^{9}	giga	G	10^{-12}	pico	p
10^{6}	mega	M	10^{-15}	femto	f
10^{3}	kilo	k	10^{-18}	atto	a
10^{2}	hecto	h	10^{-21}	zepto	z
10^{1}	deka	da	10^{-24}	yocto	y

Guideline on the use of SI Units

Guideline	*Correct usage (examples)*	*Incorrect usage or usage not recommended (examples)*
Unit symbols are printed in lower-case letters. *Exception:* The symbol or the first letter of the symbol is an upper-case letter when the name of the unit is derived from the name of a person. Also, the recommended symbol for the litre is L.	m (metre) Pa (pascal) s (second) kW (kilowatts) V (volt)	M (Metre) pa (Pascal) S (Second) kw (Kilowatts) v (Volt)
Unit symbols are unaltered in the plural.	Length is 50 cm.	Length is 50 cms.
Unit symbols are not followed by a period. Unit symbols will be followed by a period at the end of a sentence.	Mass should be 25 kg or more. Mass should be 25 kg.	Mass is 25 kg. or more.
Symbols for units formed from other units by multiplication are indicated by means of either a centred dot or a space.	N·m or N m m·s^{-1} or m s^{-1}	As ms^{-1} means reciprocal milliseconds, it is incorrect to write ms^{-1} for indicating metre per second.

Guideline on the use of SI Units

Guideline	*Correct usage (examples)*	*Incorrect usage or usage not recommended (examples)*
Symbols for units formed from other units by division are indicated by means of a solidus (oblique stroke, /), a horizontal line, or negative exponents. However, to avoid ambiguity, the solidus must not be repeated on the same line without using parentheses.	m/s, $\frac{\text{m}}{\text{s}}$, or $\text{m}\cdot\text{s}^{-1}$ m/s^2 or $\text{m}\cdot\text{s}^{-2}$ $\text{m}\cdot\text{kg}/(\text{s}^3\cdot\text{A})$ or $\text{m}\cdot\text{kg}\cdot\text{s}^{-3}\cdot\text{A}^{-1}$	m/s/s $\text{m}\cdot\text{kg}/\text{s}^3/\text{A}$
Unit symbols and unit names are not used together.	W/m^2 or watts per square metre	watts/m^2 or W/square metre
Because acceptable units generally have internationally recognized symbols and names, it is not permissible to use abbreviations for their unit symbols or names, such as sec (for either s or second), mins, (for either min or minutes), hrs (for either h or hours), lit (for either L or litre), amps (for either A or amperes), AMU (for either u or unified atomic mass unit), or mps (for either m/s or metre per second).	s or second mm^2 or square millimetre cm^3 or cubic centimetre min (minutes) h (hours) L or litre m/s or metre per second	sec or Sec sq. mm cc or ml mins hrs lit mps
Prefix symbols are printed in roman (upright) type regardless of the type used in the surrounding text, and are attached to unit symbols without a space between the prefix symbol and the unit symbol. This last rule also applies to prefixes attached to unit names.	mL (millilitre) pm (picometre) GΩ (gigaohm) THz (terahertz)	m L (milli litre) p m (pico metre) G Ω (giga ohm)

Guideline on the use of SI Units

Guideline	*Correct usage (examples)*	*Incorrect usage or usage not recommended (examples)*
The prefix symbols Y (yotta), Z (zetta), E (exa), P (peta), T (tera), G (giga), and M (mega) are printed in upper-case letters while all other prefix symbols are printed in lower-case letters. Prefixes are normally printed in lower-case letters.	THz GΩ	tHz gΩ
The grouping formed by a prefix symbol attached to a unit symbol constitutes a new inseparable symbol (forming a multiple or submultiple of the unit concerned) which can be raised to a positive or negative power and which can be combined with other unit symbols to form compound unit symbols. Prefixes are also inseparable from the unit names to which they are attached.	millimetre (mm) 5 cm^3 = 5.0 $(cm)^3$ millimetre, micropascal, meganewton, are single words	milli metre (m m)
Compound prefix symbols, that is, prefix symbols formed by the juxtaposition of two or more prefix symbols, are not permitted. This rule also applies to compound prefixes.	nm (nanometer)	mμm (millimicrometer)

Guideline on the use of SI Units

Guideline	*Correct usage (examples)*	*Incorrect usage or usage not recommended (examples)*
In a derived unit formed by division, the use of a prefix symbol (or a prefix) in both the numerator *and* the denominator may cause confusion. In a derived unit formed by multiplication, the use of more than one prefix symbol (or more than one prefix) may also cause confusion.	10 MV/m (preferred because it contains only one prefix symbol and it is in the numerator) 10 kV·s (preferred)	10 kV/mm (is acceptable but not preferred) 10 MV·ms (not preferred)
For historical reasons, the name *kilogram* for the SI base unit of mass contains the name *kilo*, the SI prefix for 10^3. Thus, because compound prefixes are unacceptable, symbols for decimal multiples and submultiples of the unit of mass are formed by attaching SI prefix symbols to g, the unit symbol for gram, and the names of such multiples and submultiples are formed by attaching SI prefixes to the name *gram*. *Example*: 1 mg *but not* 1 microkg.	10^{-6} kg = 1 mg (1 milligram)	10^{-6} kg = 1 µkg (1 microkilogram)
When writing numbers, digits should be separated into groups of three, counting from the decimal marker towards the left and right, by the use of a thin, fixed space. Comma should not be used for the purpose. However, this practice is not usually followed for numbers having only four digits on either side of the decimal marker except when uniformity in a table is desired.	83 254 346 56 235.175 73 0.562 345 5 8765 or 8 765	83,254,522 56,235.175 73 0.5623455 8,765

Guideline on the use of SI Units

Guideline	*Correct usage (examples)*	*Incorrect usage or usage not recommended (examples)*

The SI unit of volume is the cubic metre (m^3) and may be used to express the volume of any substance, whether solid, liquid, or gas. The litre (L) is a special name for the cubic decimetre (dm^3) but the CGPM recommends that the litre not be used to give the results of high accuracy measurements of volumes.

Celsius temperature (t) is defined in terms of thermodynamic temperature (T) by the equation $t = T - T_0$, where $T_0 = 273.15$ K by definition. This implies that the numerical value of a given temperature interval or temperature difference whose value is expressed in the unit degree celsius (°C) is equal to the numerical value of the same interval or difference when its value is expressed in the unit kelvin (K); or $\{\Delta t\}_{°C} = \{\Delta T\}_K$. Thus temperature intervals or temperature differences may be expressed in either the degree celsius or the kelvin using the same numerical value. *Example*: The difference in temperature between the freezing point of gallium and the triple point of water is $\Delta t = 29.7546°C = \Delta T = 29.7546$ K.

Conversion of Units

To convert 10 inch into metre

LHS

Hint:
1 m = 100 cm
1 m = 1000 mm
1 inch = 2.54 X 10-2 m

$$10\ \text{in} =$$

RHS

$$= 10\ \text{in} \times \left(\frac{2.54 \times 10^{-2}\ \text{m}}{\text{in}} \right) \qquad = 0.254\ \text{m}$$

Force

The definition of force follows from Newton's second law of motion, which states that force is proportional to the product of mass and acceleration.

$$F \propto m \times a$$

Introducing a proportionality constant K,

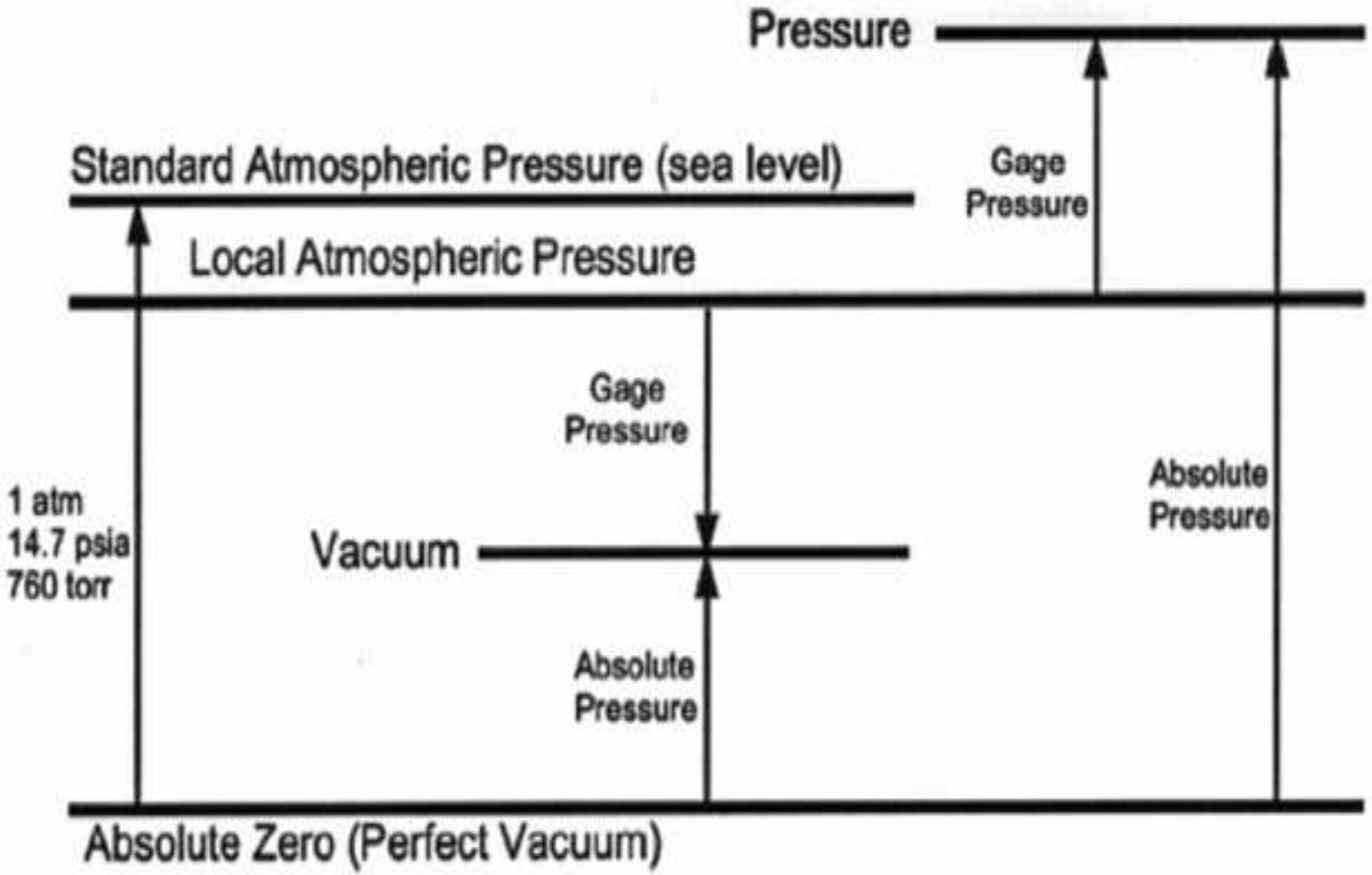
$$F = K\, m\, a$$

Pressure

Pressure is defined as the force acting on unit area exposed to the pressure

$$p = \frac{F}{A} \qquad p = \frac{F \times \text{distance}}{A \times \text{distance}} = \frac{\text{work}}{\text{volume}} = \frac{\text{energy (J)}}{\text{volume (m}^3\text{)}}.$$

The common units of pressure in SI, mks and fps units are N/m^2 (known as Pascal, Pa), kgf/cm^2 and $1bf/in^2$ (commonly known as psi), respectively.


Pressure
Standard Atmospheric Pressure (sea level)
Gage Pressure
Local Atmospheric Pressure
Gage Pressure
Absolute Pressure
1 atm
14.7 psia
760 torr
Vacuum
Absolute Pressure
Absolute Zero (Perfect Vacuum)

Atmospheric pressure

- Atmospheric pressure, also known as barometric pressure (after the barometer), is the pressure within the atmosphere of Earth.
- The standard atmosphere (symbol: atm) is a unit of pressure defined as 101,325 Pa (1,013.25 hPa; 1,013.25 mbar), which is equivalent to 760 mm Hg, 29.9212 inches Hg, or 14.696 psi.
- The atm unit is roughly equivalent to the mean sea-level atmospheric pressure on Earth, that is, the Earth's atmospheric pressure at sea level is approximately 1 atm.

Absolute pressure = gauge pressure + atmospheric pressure

1 atm = 101 325 Pa (exact)

1 bar = 10^5 Pa = 1.019 716 kgf/cm^2 = 0.986 923 atm

Pressure head = absolute pressure/density

1 atm = 760 torr (or mm Hg) = 10.33 m H_2O

Vacuum refers to sub-atmospheric pressure.

Absolute pressure = atmospheric pressure – vacuum

Vacuum is usually expressed in torr (mm Hg) or Pa or mbar.

Work or Energy

Work (energy) is defined as the product of the force acting on a body and the distance travelled by the body.

$$W = \boldsymbol{F} \times d$$

The units of work (energy) in SI, mks, cgs and fps systems are joule, m·kgf, erg and ft · 1bf, respectively.

$$1 \text{ J} = 10^7 \text{ erg}$$

Power P is defined as the work W done per unit time.

$$\text{Power } P = \frac{W}{\theta}$$

$$1 \text{ Watt} = 1 \text{ J/s}$$

1 metric horsepower = 75 (m · kgf)/s = 0.7355 kW

= 0.986 32 hp

1 British horsepower = 550 (ft · lbf)/s = 0.7457 kW

= 1.013 87 metric hp

Horsepower units are not recommended for use with SI.

Heat

Heat is one form of energy that flows from higher temperature to lower temperature, i.e., enthalpy in transit. The units of heat in SI, mks, cgs and fps systems are the joule (J), kilocalorie (kcal), calorie (cal) and British thermal unit (Btu), respectively and are same as those for energy.

There are several definitions of Btu and cal. All are defined in terms of the joule. Each Btu and its corresponding cal are related by a heat capacity equation.

$$1 \text{ calorie (thermochemical)} = 4.184 \text{ J}$$

$$1 \text{ calorie (International Steam Tables, called IT)} = 4.1868 \text{ J}$$

$$1 \text{ Btu (International Steam Tables, called IT)} = 1055.056 \text{ J}$$

The Celsius Heat Unit (CHU) and Therm were also used in the fps system.

$$1 \text{ CHU} = 1.8 \text{ Btu}$$

$$1 \text{ Therm} = 10^5 \text{ Btu}$$

In SI system, heat flux (i.e., heat flow) rate, ϕ, is customarily expressed in unit of power, i.e. watts (W).

Derived electrical unit

Current is the undamental quantity in electricity. The volt V is the unit of electromotive force or of potential difference. Resistance (R in ohms) of the conductor is defined as

$$R = \frac{V}{I}$$

where R is the resistance in ohms, V is the potential difference in volts and I is the current in amperes.

Coulomb is the unit of quantity of electricity and is defined as the quantity of electricity carried in one second by a current of one amperes across any cross-section

1 Faraday (F) = 96 485.309 C/mol (based on carbon-12)

The quantity coulomb (C) an important quantity in electrochemistry.

Unit Conversion factor

Length:	1 m = 1.093 613 yd
	= 3.280 84 ft
	1 cm = 0.393 701 in
	1 km = 0.621 37 miles
Area:	1 m^2 = 10.763 91 ft^2
	= 1.195 99 yd^2
	1 cm^2 = 0.155 in^2
	1 km^2 = 0.386 102 $mile^2$
	1 ha = 100 00 m^2
	= 2.471 05 acre
	= 0.003 861 $mile^2$
Volume:	1 m^3 = 1000 dm^3 = 1000 L
	= 35.314 67 ft^3
	= 1.307 95 yd^3
	1 cm^3 = 0.061 024 in^3
Capacity:	1 L = 0.219 969 Imperial gal
	= 0.264 172 US gal
	= 0.035 3147 ft^3
	1 kL = 1000 L
	= 0.000 810 71 acre·ft

Mass:	1 kg	= 1000 g
		= 2.204 623 lb
Mass:	1 t	= 1000 kg
		= 0.984 21 T
		= 1.102 311 T (short, used in USA)
		= 2204.623 lb
	1 g	= 15.4324 grain
Density:	1 kg/dm^3	= 1 kg/L
		= 70 156.8 grain/Imperial gal
		= 58 417.82 grain/US gal
	1 g/cm^3	= 62.427 95 lb/ft^3
		= 10.0224 lb/Imperial gal
		= 8.345 405 lb/US gal
		= 0.036 127 lb/in^3

Specific volume:	1 m^3/kg	= 16.018 48 ft^3/lb
		= 99.7765 Imperial gal/lb
		= 119.8265 US gal/lb
Force:	1 N	= 0.101 972 kgf
		= 0.224 809 lbf
Pressure:	1 kPa	= 0.010 197 kgf/cm^2
		= 0.145 038 lbf/in^2 or psi
	1 bar	= 0.1 MPa
		= 1.019 716 kgf/cm^2
		= 14.503 77 lbf/in^2
	1 atm	= 101.325 kPa (defined)
		= 1.013 25 bar
		= 1.033 227 kgf/cm^2
		= 14.695 95 lbf/in^2
	1 torr (1 mm Hg)	= 133.3224 Pa
		= 1.333 224 mbar
		= 0.039 37 in Hg
	1 mbar	= 0.750 06 torr

Energy:	1 J	= 0.238 846 cal (IT)
		= $2.777\ 778 \times 10^{-7}$ kWh
		= $9.478\ 172 \times 10^{-4}$ Btu (IT)
		= 0.101 972 kgf·m
		= 0.737 562 lbf·ft
		= $9.869\ 233 \times 10^{-3}$ L·atm
	1 kWh	= 859.8452 kcal (IT)
		= 3412.142 Btu (IT)
	1 kcal (IT)	= 3.968 321 Btu (IT)
		= 4.1868 kJ
	1 kgf·m	= 7.233 lbf·ft
Power:	1 kW	= 1.359 62 metric hp
		= 1.341 02 hp (British)
		= 859.8452 kcal (IT)/h
		= 3412.142 Btu (IT)/h
	1 (m·kgf)/s	= 7.233 (ft.lbf)/s
Heat capacity:	1 J/(g·K)	= 0.238 846 kcal (IT)/(kg·°C)
		= 0.238 846 Btu (IT)/(lb·°F)
Temperature:		°C = 5/9 (F° – 32)
		°F = (9/5) °C + 32

Ex. 1.1

The volumetric flow rate of kerosene in 80 mm nominal diameter pipe is 75 Imperial gallons per minute. Taking the density of kerosene as 0.8 kg/dm^3, find the mass flow in kg/s.

$$\text{Volumetric flow rate } q_v = 75 \text{ (gallon/min)} \times \left(\frac{1}{60}\right) \text{(min/s)} \times \left(\frac{1}{0.129\,67}\right) \text{(dm}^3\text{/gallon)}$$

$$= 5.683 \text{ dm}^3\text{/s}$$

$$\text{Density, } \rho = 0.8 \text{ kg/dm}^3$$

$$\text{Mass flow rate, } q_m = q_v \times \rho$$

$$= 5.683 \times 0.8 = 4.546 \text{ kg/s}$$

Ex. 1.2

In a double effect evaporator plant, the second effect is maintained under vacuum of 475 torr (mm Hg). Find the absolute pressure in kPa, bar and psi.

[*Ans.* 38 kPa, 0.38 bar, 5.51 psi]

$$\text{Vacuum} = 475 \text{ Torr}$$

$$\begin{aligned}\text{Absolute pressure} &= 760 - 475 = 285 \text{ Torr} \\ &\equiv 285 \times 101.325/760 \\ &\equiv 38 \text{ kPa or } 0.38 \text{ bar}\end{aligned}$$

$$1 \text{ Pa} = 1.450\,377 \times 10^{-4} \text{ psi}$$

$$\begin{aligned}\text{Pressure} &= 38\,000 \times 1.450\,377 \times 10^{-4} \\ &= 5.511 \text{ psia}\end{aligned}$$

Ex.1.3.

A force equal to 192.6 kgf is applied on a piston with a diameter of 5 cm. Find the pressure exerted on the piston in kPa, bar and psi.

[*Ans.* 98.066 kPa, 0.981 bar, 14.227 psi]

$$\text{Cross sectional area of piston} = \frac{\pi}{4}(5)^2$$

$$= 19.635 \text{ cm}^2$$

$$\text{Pressure} = \text{Force/Area} = 192.6 \times 10^4/(19.635 \times 10^6)$$

$$= 0.0981 \text{ MPa} \equiv 0.981 \text{ bar}$$

$$\text{In FPS units, pressure} = 0.0981 \times 10^6 \times (1.450\ 377 \times 10^{-4})$$

$$= 14.228 \text{ psi}$$

Ex.1.4

Iron metal weighing 500 lb occupies a volume of 29.25 L. Calculate the density of Fe in kg/dm^3.

[*Ans.* 7.754 kg/dm^3]

$$\text{Weight} = 500 \text{ lb} \equiv 500 \times 0.4536 \equiv 226.8 \text{ kg}$$

$$\text{Volume} = 29.25 \text{ L} = 0.029\ 25 \text{ m}^3$$

$$\text{Density of Fe, } \rho = 226.8/0.029\ 25$$

$$= 7753.85 \text{ kg/m}^3 \equiv 7.754 \text{ t/m}^3$$

EX.1.5

The diameter and height of a vertical cylindrical tank are 5 ft and 6 ft 6 in respectively. It is full up to 75% height with carbon tetrachloride (CCl_4), the density of which is 1.6 kg/L. Find the mass in kilograms.

[*Ans.* 4336 kg]

Diameter = 5 ft = 1.524 m

Height of tank = 6 ft 6 inch = 1.981 m

Volume of tank = $(\pi/4)(\text{Dia})^2(\text{Height})$

$= (3.1416/4)(1.524)^2(1.981)$

$= 3.614\ m^3$

Volume of CCl_4 liquid in the tank

$= 3.614 \times 0.75$

$= 2.71\ m^3$

Density, $\rho = 1600\ kg/m^3$

Mass of CCl_4 in the tank = $2.71 \times 1600 = 4336$ kg

Ex.1.6

A bag filter of 5 micron rating is designed for a pressure drop of 0.05 lbf/in^2 per US gallon per minute of water solution in clean conditions. Calculate pressure drop in kPa from the filter for water flow rate of 10 m^3/h.

[*Ans.* 15.178 kPa]

$$\text{Pressure drop} = 0.05 \frac{\text{lbf} \cdot \text{min}}{(\text{in}^2 \cdot \text{US gal})} \times 6894.759 \frac{(\text{Pa. in}^2)}{\text{lbf}}$$

$$\times \frac{1}{3.785\ 412 \times 10^{-3}} \frac{\text{US gal}}{\text{m}^3} \times \frac{1}{60} \frac{\text{h}}{\text{min}} \times 10 \frac{\text{m}^3}{\text{h}}$$

$$\times \frac{1}{1000} \frac{\text{kPa}}{\text{Pa}}$$

$$= 15.178 \text{ kPa}$$

Ex.1.7

Corrosion rates are normally reported in mills per year (mpy) in the chemical process industry. For the measurement of the rates, a corrosion test coupon is inserted in the process stream for a definite period. The loss of weight is measured during the period of insertion.

In a particular test, a coupon of carbon steel was kept in a cooling water circuit. The dimensions of the coupon were measured to be 7.595 cm × 1.276 cm × 0.1535 cm. Mass of the coupon before insertion in the circuit and after exposure for 50 days were measured to be 14.9412 g and 14.6254 g, respectively. Calculate the rate of corrosion. Take the density of carbon steel to be the same as the one calculated in Exercise 1.4.

$$\text{Total exposed area} = 2 \times 7.595 \times 1.276 + 2 \times 7.595 \times 0.1535 + 2 \times 1.276 \times 0.1535$$

$$= 22.106 \text{ cm}^2$$

$$\text{Weight loss} = 14.9412 - 14.6254 = 0.3158 \text{ g}$$

$$\text{Density} = 7753.85 \text{ kg/m}^3$$

$$\text{Corrosion rate} = \frac{0.3158}{1000} \times \frac{1}{7753.85} \times \frac{10^6}{1} \times \frac{365}{50} \times \frac{1}{22.106}$$

$$= 0.0134 \text{ cm/a}$$

$$\equiv 0.0053 \text{ in/year} \equiv 5.3 \text{ mpy}$$

Note: 1 mil per year = 1/1000 in per year

Ex.1.8

Vapour pressure of benzene in the temperature range of 280.65 K (7.5°C) to 377.15 K (104°C) can be calculated using the following Antoine equation.

$$\log_{10} p = 6.9057 - \frac{1211.0}{(t + 220.8)}$$

where p **= Vapour pressure in torr (mm Hg), and**

t **= Temperature in °C**

Convert the above equation in SI units.

Let p' be the vapour pressure in kPa and T be the temperature in K

$$101\ 325 \text{kPa} = 760 \text{ Torr}$$

or
$$1 \text{ kPa} = 7.500\ 62 \text{ Torr}$$

$$p = 7.500\ 62\ p'$$

$$T = t + 273.15$$

or
$$t = T - 273.15$$

Substitute the values in the equation,

$$\log_{10} (7.500\ 62\ p') = 6.9057 - \frac{1211.0}{(T - 273.15 + 220.8)}$$

$$\log_{10} p' + 0.8751 = 6.9057 - \frac{1211.0}{(T - 52.35)}$$

$$\log_{10} p' = 6.0306 - \frac{1211.0}{(T - 52.35)}$$

Ex.1.9

Heat capacity of gaseous *n*-butane is given by

$$C^o_{mp} = 4.429 + 40.159 \times 10^{-3}\, T - 68.562 \times 10^{-7}\, T^2$$

where C^o_{mp} **= Heat capacity in Btu/(lb mole · °R) and**

T **= Temperature in °R**

Convert the equation in SI units.

Let $C^{o\prime}_{mp}$ be the heat capacity of *n*-butane in SI units.

$$1 \text{ Btu/(lb mole} \cdot \text{°R)} = 1 \text{ kcal/(kmol} \cdot \text{K)}$$
$$= 4.1868 \text{ kJ/(kmol} \cdot \text{K)}$$
$$C^{o\prime}_{mp}/4.1868 = C^o_{mp}$$
$$T = 1.8\ T'$$

Substituting the values in the equation,

$$(C^{o\prime}_{mp}/4.1868) = 4.429 + 40.159 \times 10^{-3} \times 1.8\ T' - 68.562 \times 10^{-7}\,(1.8\ T')^2$$

Simplifying,

$$C^{o\prime}_{mp} = 18.5433 + 302.6479 \times 10^{-3}\, T' - 93.006 \times 10^{-6}\, T'^2$$

Ex.1.10

Pressure drop across a venturi scrubber can be calculated using following Calvert equation[12].

$$\Delta p = (5 \times 10^{-5})\, v^2\, L$$

where Δp **= pressure drop, in WC**

L **= liquid flow rate, US gal/1000 ft^3 gas**

v **= gas velocity in the venturi throat, ft/s**

Convert the equation in SI units.

Let $\Delta p'$, v' and L' be pressure drop in cm WC, gas velocity is m/s and liquid flow rate in m^3/m^3 gas flow.

$$\Delta p' = \Delta p \times 2.54$$

$$v = v' \times 3.281$$

$$L' = (L/264.172)\ 35.314\ 67$$

$$= 0.133\ 68\ L$$

Substitute above values in the Calvert equation.

$$\frac{\Delta p'}{2.54} = 5 \times 10^{-5} \times (v' \times 3.281)^2 \times \frac{L'}{0.133\ 68}$$

$$\Delta p' = 1.0227 \times 10^{-2}\, v'^2\, L'$$

Ex.1.11

In the case of fluids, the local heat-transfer coefficient for long tubes and using bulk-temperature properties is expressed by the empirical equation[13].

$$h = 0.023\, G^{0.8} \times k^{0.67} \times C_p^{0.33}/(D^{0.2} \times \mu^{0.47})$$

where h = heat-transfer coefficient, Btu/(h · ft^2 · °F)

G = mass velocity of fluids, lb/(ft^2 · s)

C_p = heat capacity of fluid at constant pressure, Btu/(lb · °F)

k = thermal conductivity, Btu/(h · ft · °F)

D = diameter of tube, ft and

μ = viscosity of liquid, lb/(ft · s)

Convert the empirical equation into SI units.

Let h' = heat transfer coefficient, kW/(m^2 · K)

G' = mass velocity of fluid, kg/(m^2 · s)

C'_p = specific heat, kJ/(kg · K)

k' = thermal conductivity of fluid, kW/(m · K)

D' = diameter of tube, m

μ' = viscosity of fluid, kg/(m · s)

$$h = \frac{3412.142 \times 10^{-3}}{10.7639 \times 1.8} h' = 0.1761\ h'$$

$$G = \frac{2.204\ \ 62 \times 3600}{10.7639} G' = 737.28\ G'$$

$$C_p = \frac{9.478\ 172 \times 10^{-4}}{2.204\ \ 62 \times 1.8} = 2.2885 \times 10^{-4}\ C'_p$$

$$k = \frac{3412.142 \times 10^{-3}}{3.2\,808 \times 1.8}\ k' = 0.5778\ k'$$

$$D = 3.2808\ D'$$

$$\mu' = \frac{2.204\ 62 \times 3600}{32808}\ \mu' = 2419.11\ \mu'$$

Substituting the values,

$$0.1761\ h' = \frac{0.023(737.28G')^{0.8}(0.5778k')^{0.67}(2.3885 \times 10^{-4} C_p')^{0.33}}{(3.2808D')^{0.2}(2419.11\mu')^{0.47}}$$

or $\quad h' = 0.023\ G'^{0.8}\ k'^{0.67}\ C_1'^{0.33} / (D'^{0.2}\ \mu'^{0.47})$

Thus the equation does not change when consistent SI units are used. This is because the equation is a simplified form of Sieder-Tate equation which is made-up of three dimensionless numbers.

Mole

The mole (symbol: mol) is the unit of measurement for amount of substance in the International System of Units (SI).

A mole of a substance or a mole of particles is defined as exactly $6.02214076 \times 10^{23}$ particles, which may be atoms, molecules, ions, or electrons.

In short, for particles 1 mol = $6.02214076 \times 10^{23}$.

Molar mass

- The molar mass of a substance is the mass of a sample, in multiples of the gram, divided by the amount of substance in that sample.
- The terms molecular mass, molecular weight, and molar mass are often used interchangeably in areas of science where distinguishing between them is unhelpful.
- The amount of substance is the number of moles in the sample.

Molar fraction

- The molar fraction or mole fraction of a substance in a mixture (such as a solution) is the number of moles of the compound in one sample of the mixture, divided by the total number of moles of all components.
- For example, if 20 g of NaCl is dissolved in 100 g of water, the amounts of the two substances in the solution will be (20 g)/(58.443 g/mol) = 0.34221 mol and (100 g)/(18.015 g/mol) = 5.5509 mol, respectively; and the molar fraction of NaCl will be 0.34221/(0.34221 + 5.5509) = 0.05807.

Molecular formula

- A molecular formula consists of the chemical symbols for the constituent elements followed by numeric subscripts describing the number of atoms of each element present in the molecule.
- The empirical formula represents the simplest whole-integer ratio of atoms in a compound.

Example

How many grams of NH_4Cl are there in 5 mol?

Molar mass of $NH_4Cl = 14 + 4 + 35.5 = 53.5$ g

5 mol of $NH_4Cl = 5 \times 53.5 = 267.5$ g NH_4Cl

Example Convert 499 g $CuSO_4.5H_2O$ into mol. Find equivalent mol of $Cu\ SO_4$ in the crystals.

$$\text{Molar mass of } CuSO_4 = 159.5 \text{ g}$$

$$\text{Molar mass of } CuSO_4.5H_2O = 159.5 + 5\ (1 \times 2 + 16) = 249.5 \text{ g}$$

$$\text{Moles of } CuSO_4.5H_2O = \frac{499}{249.5} = 2 \text{ mol}$$

In the formula of $CuSO_4.5H_2O$, the moles of $CuSO_4$ are equal (one in each) and hence, the equivalent moles of $CuSO_4$ in the crystals are also 2.0 mol.

Example How many moles of K_2CO_3 will contain 117 kg K?

$$\text{Atomic mass of K, } m_K = 39$$

$$\text{Atoms of K} = \frac{117}{39} = 3 \text{ kg atom}$$

Each mole of K_2CO_3 contains 2 atoms 2 atoms of K.

$$2 \text{ atoms of K} \equiv 1 \text{ mole of } K_2CO_3$$

(The sign ≡ refers to 'equivalent to' and not 'equal to')

$$\text{Moles of } K_2CO_3 = \frac{3}{2} = 1.5 \text{ kmol}$$

Ex.2.1 Find the moles of oxygen present in 500 g.

$$\text{Molar mass of oxygen} = 2 \times 16 = 32 \text{ g/mol}$$
$$\text{Amount of oxygen} = 500/32$$
$$= 15.625 \text{ mol}$$

Ex.2.2 How many grams of carbon are present in 600 g $CaCO_3$?

1 mol C is present in 1 mol CO_2.

$$12 \text{ g C} \equiv 44 \text{ g } CO_2$$
$$\text{Carbon content in 264 g } CO_2 = (12 \times 264/44)$$
$$= 72 \text{ g}$$

Ex.2.3 Find the molar mass of $KMnO_4$.

$$\text{Molar mass of } KMnO_4 = 39 + 55 + 4 \times 16 = 158$$

Ex.2.4

A mass of 100 g each of HNO_3 and H_2SO_4 is filled in two separate bottles. Which bottle contains more atoms? How many more?

[*Ans.* Bottle containing HNO_3 will have 0.567 mol or 3.415×10^{23} atoms more than the other bottle.]

Molar mass of $HNO_3 = 63$

Molar mass of $H_2SO_4 = 98$

$100 \text{ g } HNO_3 = 100/63 = 1.5873 \text{ mol}$

$100 \text{ g } H_2SO_4 = 100/98 = 1.0204 \text{ mol}$

$\text{Excess} = 1.5873 - 1.0204$

$= 0.5669 \text{ mol } HNO_3$

$\text{Excess atoms} = 0.5669 \times 6.022\ 142 \times 10^{23}$

$= 3.414 \times 10^{23} \text{ atoms of } HNO_3$

Ex.2.5 What is the equivalent mass of $Al_2(SO_4)_3$?

$$\text{Molar mass of } Al_2(SO_4)_3 = 2 \times 27 + 3(32 + 4 \times 16) = 342$$

$$\text{Valence of } Al_2(SO_4)_3 = 6$$

$$\text{Equivalent mass of } Al_2(SO_4)_3 = 342/6 = 57$$

Ex.2.6 How many equivalents are there in 500 g $KMnO_4$?

$$\text{Molar mass of } KMnO_4 = 158$$

$$\text{Valence of } KMnO_4 = 5 \text{ (based on oxidation number)}$$

$$\text{Equivalent mass of } KMnO_4 = 158/5 = 31.6$$

$$500 \text{ g } KMnO_4 = 500/31.6 = 15.82 \text{ g eq}$$

Ex.2.7

The analysis magnesite ore obtained from Chalk Hill area, Salem district, yields 81% $MgCO_3$. 14% SiO_2 and 5% H_2O (by mass), Convert the analysis into mole %.

[*Ans.* 65.3% $MgCO_3$, 15.8% SiO_2, 18.9% H_2O (mole basis)]

Basis: 100 kg magnesite ore

Compound	Molar mass	kg	kmol	mole %
$MgCO_3$	84.3	81	0.961	65.28
SiO_2	60	14	0.233	15.83
H_2O	18	5	0.278	18.89
Total	—	100	1.472	100.00

Ex.2.8

The analysis of a sample of glass yields 7.8% Na_2O, 7.0% MgO, 9.7% ZnO, 2.0% Al_2O_3, 8.5% B_2O_3 and 65.0% SiO_2 (by mass). Convert this composition into mole%

[*Ans.* 7.65% Na_2O, 10.57% MgO, 7.25% ZnO, 1.19% Al_2O_3, 7.43% B_2O_3 and 65.91% SiO_2 (mole basis)]

Basis: 100 kg glass

Compound	Molar mass	kg	kmol	mole %
Na_2O	62	7.8	0.1258	7.65
MgO	40.3	7.0	0.1737	10.57
ZnO	81.4	9.7	0.1192	7.25
Al_2O_3	102.0	2.0	0.0196	1.19
B_2O_3	69.6	8.5	0.1221	7.43
SiO_2	60.0	65.0	1.0833	65.91
Total	—	100	1.6437	100.00

Ex.2.9 **A sample of sea water contains 35 000 ppm solids. Express the concentration of the solids as mass percentage.**

$$\text{Concentration of solids} = 35\,000 \text{ ppm or mg/L}$$
$$\equiv (35\,000 \times 100)/10^6 \equiv 3.5\%$$

For watery solutions, 10 000 ppm = 1% by mass

Ex.2.10 **A sample of milliolite limestone, obtained from Porbandar, Gujarat, is found to contain 54.5% CaO (by mass). If this CaO is present as $CaCO_3$ in the limestone, find the content of $CaCO_3$ in the limestone.**

Basis: 100 kg limestone

$$1 \text{ kmol CaO} = 1 \text{ kmol } CaCO_3$$

$$CaCO_3 \text{ in limestone} = \frac{\text{Molar mass of } CaCO_3 \times 54.5}{\text{Molar mass of CaO}}$$
$$= 100 \times 54.5/56$$
$$= 97.32\% \text{ (by mass)}$$

Ex.2.11

Calculate the available nitrogen in the following:

(a) Commercial ammonium sulphate (96% pure)

(b) Pure sodium nitrate (100%)

[*Ans.* (a) 20.36%; (b) 16.47% (mass basis)]

(a) In one mole of ammonium sulphate, two atoms (or one mole) nitrogen are present.

Nitrogen content of commercial ammonium sulphate =

$$\frac{\text{Molar mass of } N_2 \times 96}{\text{Molar mass of } (NH_4)_2SO_4} = \frac{28 \times 96}{132}$$

$$= 20.36\%$$

(b) One mole of sodium nitrate contains half mole of nitrogen.

Nitrogen content of pure sodium nitrate =

$$\frac{0.5 \times \text{Molar mass of } N_2 \times 100}{\text{Molar mass of } NaNO_3} = \frac{0.5 \times 28 \times 100}{85}$$

$$= 16.47\%$$

Normality (N) (gm/lit)

$$Normality = \frac{Number\ of\ gram\ equivalents}{volume\ of\ solution\ in\ litres}$$

It is defined as the number of gram equivalent dissolved in 1 litre of solution.

Uses of Normality

- Normality is used mostly in three common situations:
- In determining the concentrations in acid-base chemistry. For instance, normality is used to indicate hydronium ions (H^3O^+) or hydroxide ions (OH^-) concentrations in a solution.
- Normality is used in precipitation reactions to measure the number of ions which are likely to precipitate in a specific reaction.
- It is used in redox reactions to determine the number of electrons that a reducing or an oxidizing agent can donate or accept.

Limitations in Using Normality

- Many chemists use normality in acid-base chemistry to avoid the mole ratios in the calculations or simply to get more accurate results. While normality is used commonly in precipitation and redox reactions there are some limitations to it. These limitations are as follows:
- It is not a proper unit of concentration in situations apart from the ones that are mentioned above. It is an ambiguous measure and molarity or molality are better options for units.
- Normality requires a defined equivalence factor.
- It is not a specified value for a particular chemical solution. The value can significantly change depending on the chemical reaction. To elucidate further, one solution can actually contain different normalities for different reactions.

What is the normality of the following?

A. 0.1381 M NaOH

B. 0.0521 M H_3PO_4

Solution:

A. N = 0.1381 mol/L × (1 eq/1mol) = 0.1381 eq/L
= 0.1381 N

B. N = 0.0521 mol/L × (3 eq/1mol) = 0.156 eq/L
= 0.156 N

Molarity (M) (mol/lit)

It is define as the number of mol of solute dissolved in 1 litre of solution.

$$Molarity = \frac{Number\ of\ moles\ of\ solute}{volume\ of\ solution\ in\ litres}$$

Differences between normality and molarity

Here are some key differences between normality and molarity.

Normality	Molarity
Also known as equivalent concentration.	Known as molar concentration.
It is defined as the number of gram equivalent per litre of solution.	It is defined as the number of moles per litre of solution.
It is used in measuring the gram equivalent in relation to the total volume of the solution.	It is used in measuring the ratio between the number of moles in the total volume of the solution.
The units of normality are N or eq L^{-1}	The unit of molarity is M or Moles L^{-1}

Molality (m) (mol/kg)

It is defined as the number of mol of solute dissolved in 1 kg of solvent.

$$Molality = \frac{Number\ of\ moles\ of\ solute}{1\ Kg\ of\ solvent}$$

Ex.2.12

1000 pounds per minute of a gas (average molecular weight = 30.24) is being sent to an absorption column. What is the molar flow rate of the gas in kmol/h?

$$1000\ \text{lb} = 1000\ (\text{lb}) \times 0.4536 \left(\frac{\text{kg}}{\text{lb}}\right) = 453.6\ \text{kg}$$

$$\text{Mass flow rate} = 453.6\ \text{kg/min} = 453.6 \times 60 = 27216\ \text{kg/h} = \frac{27216}{30.24} = 900\ \text{kmol/h}$$

Ex.2.13

How many molecules are present in 691 g K_2CO_3?

The atomic weights of the elements are:
potassium = 39.1, carbon = 12.0, oxygen = 16.0
The molecular weight of K_2CO_3 is

$$39.1 \times 2 + 12.0 + 16 \times 3 = 138.2$$

$$691 \text{ g } K_2CO_3 = \frac{691}{138.2} = 5 \text{ mol } K_2CO_3$$

One mole of a substance contains 6.023×10^{23} molecules. Therefore, 5 mol K_2CO_3 contains $5 \times 6.023 \times 10^{23} = 30.115 \times 10^{23}$ molecules.

Ex.2.14

The solubility of sodium chloride in water at 290 K is 35.8 kg/100 kg of water. Express the solubility as the following:

(a) Mass fraction and mass percent of NaCl
(b) Mole fraction and mole percent of NaCl
(c) kmol NaCl per 1000 kg of water

Solution *Basis:* 100 kg of water and the salt dissolved in it in a saturated solution at 290 K.

(a) Mass of NaCl = 35.8 kg

Mass of solution = mass of water + mass of NaCl = 100 + 35.8 = 135.8 kg

$$\text{Mass fraction of NaCl} = \frac{35.8}{135.8} = 0.2636$$

$$\text{Mass percent of NaCl} = \text{mass fraction} \times 100$$
$$= 0.2636 \times 100 = 26.36\%$$

(b)
$$\text{kmol of NaCl} = \frac{\text{mass of NaCl in kg}}{\text{molecular weight}} = \frac{35.8}{58.45} = 0.6125 \text{ kmol}$$

$$\text{kmol water} = \frac{\text{mass of water in kilograms}}{\text{molecular weight}}$$
$$= \frac{100}{18.016} = 5.5494 \text{ kmol}$$

$$\text{Mole fraction} = \frac{\text{moles of NaCl}}{\text{moles of solution}}$$
$$= \frac{0.6125}{(5.5506 + 0.6125)} = 0.0994$$

$$\text{Mole percent} = \text{mole fraction} \times 100 = 9.94\%$$

(c) 100 kg of water dissolves 0.6125 kmol NaCl. Therefore, kmol NaCl per 1000 kg of water is 6.125 kmol.

Ex.2.15

Pure water and alcohol are mixed to get a 60% (weight) alcohol solution. The densities (kg/m^3) of water, alcohol and the solution may be taken to be 998, 798 and 895 respectively at 293 K. Calculate the following:

(a) The volume percent of ethanol in the solution at 293 K
(b) The molarity
(c) The molality

Basis: 100 kg of 60% (weight) alcohol–water solution

(a) Volume of 100 kg of solution is

$$\frac{\text{mass}}{\text{density}} = \frac{100}{895} = 0.1117\ \text{m}^3$$

The volume of pure alcohol mixed to get the above solution is

$$60\ \text{kg} = \frac{60}{798} = 0.0752\ \text{m}^3$$

Volume percent of ethanol in the solution is

$$\frac{0.0752}{0.1117} \times 100 = 67.3\%$$

(*Note:* The volume of the solution resulting on mixing two pure liquids need not be equal to the sum of the volumes of the liquids. Therefore, the volume percent of the individual constituents will not add to give 100.)

(b) $$\text{Molarity of the solution} = \frac{\text{moles of ethanol}}{\text{volume of solution in litres}}$$

$$\text{Moles of ethanol in 100 kg solution} = \frac{60}{46.048} = 1.303 \text{ kmol} = 1303 \text{ mol}$$

Volume of solution is $0.1117\ m^3 = 111.7$ L. Therefore,

$$\text{molarity} = \frac{1303}{111.7} = 11.67 \text{ mol/L}$$

(c) $$\text{Molality of the solution} = \frac{\text{moles of alcohol}}{\text{kilograms of water}}$$

Mass of water in 100 kg solution is 40 kg. Hence,

$$\text{molality} = \frac{1303}{40} = 32.575 \text{ mol/(kg of water)}$$

Ex.2.16

An aqueous solution of K_2CO_3 contains 50% salt and the specific gravity of the solution is 1.53. Determine the following:

(a) The mole percent of the salt in the solution
(b) The volume percent of water assuming density of water is 1000 kg/m^3 and there is no volume change on mixing
(c) The molality of the solution
(d) The molarity of the solution
(e) The normality of the solution

Basis: 100 g of solution

Molecular weight of K_2CO_3 = 138.20

(a) Mass of K_2CO_3 = 50 g = $\frac{50}{138.20} = 0.3618$ mol

Mass of water = 50 g = $\frac{50}{18.016} = 2.7753$ mol

Mole percent of salt = $\frac{0.3618}{0.3618 + 2.7753} \times 100 = 11.53\%$

(b) Volume of the solution = $\frac{\text{mass}}{\text{density}}$

$= \frac{100}{1.53} = 65.36$ mL

Volume of water in the solution = $\frac{\text{mass}}{\text{density}}$

$= \frac{50}{1000 \times 10^{-3}} = 50$ mL

Volume percent of water = $\frac{\text{volume of water}}{\text{solution volume}} \times 100$

$= \frac{50}{65.36} \times 100 = 76.50\%$.

(c) 50×10^{-3} kg water contains 0.3618 mol K_2CO_3.

$$\text{Molality} = \frac{\text{mol solute}}{\text{kg solvent}}$$

$$= \frac{0.3618}{50 \times 10^{-3}} = 7.236 \text{ mol/kg}$$

(d) 65.36 mL solution contains 0.3618 mol K_2CO_3.

$$\text{Molarity} = \frac{\text{mol solute}}{\text{litre solution}}$$

$$= \frac{0.3618}{65.36 \times 10^{-3}} = 5.536 \text{ mol/L}$$

(e) Equivalent weight of K_2CO_3 = molecular weight/2 = 138.20/2 = 69.10

$$\text{Number of gram equivalents of } K_2CO_3 = \frac{\text{mass in grams}}{\text{equivalent weight}}$$

$$= \frac{50}{69.10} = 0.7236 \text{ gram equivalents}$$

$$\text{Normality} = \frac{\text{gram equivalents}}{\text{litre solution}}$$

$$= \frac{0.7236}{65.36 \times 10^{-3}}$$

$$= 11.07 \text{ gram equivalents/L or } 11.07 \text{ N}$$

Ex.2.17

Nitric acid and water forms a maximum boiling azeotrope containing 62.2 mole % water [boiling temperature = 403.6 K (130.6°C).]. Find the composition of the azeotrope by mass.

[*Ans.* 68.02% HNO_3 (mass)]

Basis: 1 kmol azeotropic mixture

Compound	kmol	Molar mass	kg	% by mass
H_2O	0.622	18	11.196	31.98
HNO_3	0.378	63	23.814	68.02
Total	1.000	—	35.010	100.00

Ex.2.18

An aqueous solution of common salt (NaCl) contains 25% salt (by mass) at 298.15 K (25°C). Find the mole % of NaCl in the solution.

[*Ans.* 9.3 mol% NaCl]

Basis: 100 kg saline solution

Compound	kg	Molar mass	kmol	mole %
NaCl	25	58.5	0.4274	9.3
H_2O	75	18	4.1667	90.7
Total	100	—	4.5941	100.0

Ex.2.19

An aqueous solution contains 19.0% NH_3, 65.6% NH_4NO_3 and 6.0% urea (by mass). Calculate the available nitrogen content solution.

[*Ans.* 41.41% Nitrogen]

Basis: 100 kg solution

Compound	kg	Nitrogen content kg/kmol comp.	Molar mass	Nitrogen Content kg
NH_3	19	14	17	15.647
NH_4NO_3	65.6	28	80	22.960
NH_2CONH_2	6	28	60	2.800
Total	90.6	—	—	41.407

Nitrogen content of the solution = 41.41% *Ans.*

Ex.2.20

Make the following conversions:

(a) 294 g/L H_2SO_4 to normality (*N*)

(b) 4.8 mg/mL $CaCl_2$ to normality (*N*)

(c) 5 *N* H_3PO_4 to g/L

(d) 54.75 g/L HCl to molarity (M)

(e) 3 M K_2SO_4 to g/L

(a) Equivalent mass of H_2SO_4 = 98/2 = 49

Concn. of H_2SO_4 in solution = 294/49

= 6 mol/L

≡ 6 *N*

(b) Equivalent mass of $CaCl_2$ = 112/2 = 55.5

Concn. of $CaCl_2$ in solution = 4.8 × 1000/(1000 × 55.5)

= 0.0865 mol/L

≡ 0.0865 *N*

(c) Equivalent mass of H_3PO_4 = 98/3 = 32.67

Concn. of H_3PO_4 in solution = 5 *N*

≡ 5 × 32.67

≡ 163.35 g/L

(d) Equivalent mass of HCl = 36.5

Concn. of HCl in solution = 54.75/36.5 = 1.5 g/L

≡ 1.5 *N* ≡ 1.5 M

(e) Molar mass of K_2SO_4 = 174

Concn. of K_2SO_4 in solution = 174 × 3

= 522 g/L

Ex.2.21

An aqueous solution of acetic acid of 35% concentration (by mass) has density 1.04 kg/L at 298.15 K (25°C). Find the molarity, normality and molality of the solution.

[*Ans.* 6.066 M; 6.066 *N*; 8.974 Molality]

Basis: 100 kg aqueous acetic acid solution

Molar mass of acetic acid = 60

Acetic acid content = 35/60 = 0.5833 kmol

Volume of 100 kg solution = 100/1.04 = 96.15 L

$$\text{Molarity} = 0.5833 \times 1000/96.15$$

$$= 6.066 \text{ M}$$

Since acetic acid is monovalent.

Normality of the solution = 6.066 *N*

$$\text{Molality} = 0.5833 \times 1000/65 = 8.974$$

Ex.2.22

A gas mixture has the following composition by volume:

Ethylene	30.6%
Benzene	24.5%
Oxygen	1.3%
Methane	15.5%
Ethane	25.0%
Nitrogen	3.1%

Find (a) the average molar mass of the gas mixture, (b) the composition by mass and (c) the density of the mixture in kg/m^3 at NTP.

[*Ans.* (a) 38.94; (b) ethylene 22.0%, benzene 49.07%, oxygen 1.07%, methane 6.37%, ethane 19.26%, nitrogen 2.23% (by mass); (c) 1.737 kg/m^3]

Basis: 100 kmol gas

Gas	Formula	Molar mass	kmol	kg	mass %
Ethylene	C_2H_4	28	30.6	856.8	22.00
Benzene	C_6H_6	78	24.5	1911.0	49.07
Oxygen	O_2	32	1.3	41.6	1.07
Methane	CH_4	16	15.5	248.0	6.37
Ethane	C_2H_6	30	25.0	750.0	19.26
Nitrogen	N_2	28	3.1	86.8	2.23
Total	—	—	100.00	3894.2	100.00

Average molar mass = 3894.2/100 = 38.942

Ideal gas occupies 22.414 m^3/kmol at NTP.

Density of gas mixture = 38.942/22.414

= 1.737 kg/m^3 *Ans.*

Thank You All

Unit – 1 Completed

Process Calculations for Biotechnologist - 21BT32T

Unit - 2

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UNIT 2 IDEAL AND ACTUAL GAS EQUATIONS

Ideal and actual gas equations, Vander Walls, compressibility factor equations, Application to pure gas & gas mixtures – partial pressures, partial volumes – Air-water vapour systems, Humidification and dehumidification, Humidity, Molar Humidity, Relative Humidity, % Saturation, humid Volume – Humidity chart – wet, Dry bulb, Dew point temperatures, pH of solutions, Vapour pressure and applications of psychrometric chart.

Total period required = 15 (9 + 6)

COURSE OUTCOMES

Analyze the importance of ideal and actual gas equations in various industrial processes

Ideal and actual gas equations

- **Pure Substance**: A pure substance is one that has a homogeneous and invariable chemical composition. It may exist in more than one phase but chemical composition is the same in all phases.
- Sometimes the mixture of gases, such as air is considered a pure substance as long as there is no change of phase. Further our emphasis will be on simple compressible substances

- Early experiments on the **variables of state** (such as T, P, V, and n) showed that only two of these variables of state need to be known to know the state of a sample of matter.
- **Extensive variables**: depend on the amount of substance present. Examples include the volume, energy, enthalpy, and heat capacity.
- **Intensive variables**: do not depend on the amount of substance present. Examples include the temperature and pressure.

Gases

- The direct weighing of gases is ruled out in practice.
- The volume of a gas can be conveniently measured and converted into mass from the density of the gas.
- In order to know the density of a gas, both pressure and temperature should be known.
- Various equation of state (also known as pVT relations) can be employed for this purpose.

Ideal gas law

According to Boyle, for a given mass of an ideal gas the product of the pressure and volume is constant at a constant temperature, i.e.,

$$p \times V = \text{constant}$$

where, p is the absolute pressure and V is the volume occupied by 1 kmol gas.

Charles proposed the law that for a given mass of an ideal gas, the ratio of the volume to temperature is constant at a given pressure.

$$\frac{V}{T} = \text{constant}$$

where, T is the absolute temperature.

Combining the above two law, an ideal gas law can be formulated as

$$\frac{(p \times V)}{T} = \text{constant}$$

ideal gas law

"The law is usually expressed as

$$PV = nRT$$

Where, P = Pressure

V = Volume

n = number of moles of gas

R = universal gas constant

T = absolute temperature

The ideal gas law expresses three facts.

- The volume of a gas is directly proportional to the number of moles
- The volume is directly proportional to the absolute temperature.
- The volume is inversely proportions to the pressure.
- The deviation of a gas from ideal behaviour depends on the nature of the gas, its temperature and pressure.

Gas Constant Value

(a) Find the value of the gas constant R in $\frac{\text{m}^3\ \text{mmHg}}{\text{mol K}}$ (b) In the MKS system energy is usually expressed as calories or kilocalories. What will be the value of R when it is expressed in $\frac{\text{cal}}{\text{mol K}}$?

(a) For one mole of ideal gas at the standard conditions, $R = \frac{P_0 V_0}{T_0}$. The standard conditions are P_0 = 760 mm Hg, T_0 = 273.15 K and $V_0 = 22.4143 \times 10^{-3}$ m^3/mol. Substituting these values in the above equation, we get

$$R = \frac{760 \times 22.4143 \times 10^{-3}}{273.15} = 6.2364 \times 10^{-2}\ \frac{\text{m}^3\ \text{mm Hg}}{\text{mol K}}$$

(b) When $P_0 = 1.01325 \times 10^5$ N/m^2, T_0 = 273.15 K and $V_0 = 22.4143 \times 10^{-3}$ m^3/mol, we get

$$R = \frac{1.01325 \times 10^5\ (22.4143 \times 10^{-3})}{273.15} = 8.314\ \frac{\text{N.m}}{\text{mol K}} = 8.314\ \frac{\text{J}}{\text{mol K}}$$

Since 1 cal (thermo-chemical calorie[1]) = 4.184 J, $R = \frac{8.314}{4.184} = 1.987\ \frac{\text{cal}}{\text{mol K}}$

Gas Constant Value

Value of Universal Gas Constant (R) in Different Units

Numerical Value of R	Units
0.083 14	$m^3 \cdot bar/(kmol \cdot K)$
8.314 51	$m^3 \cdot kPa/(kmol \cdot K)$
8.314 51	$J/(mol \cdot K)$
1.987 216	kcal/(kmol·K) or Btu/(lb mol·°R)
0.082 058	L·atm/(mol·K) or $m^3 \cdot atm/kmol \cdot K$)
62 363.95	$cm^3 \cdot torr/(mol \cdot K)$
10.731 64	$ft^3 \cdot psia/(lb\ mol \cdot °R)$

Raoult's law

It states that; when the gas mixture in equilibrium with an ideal liquid solution also follows the ideal gas law, "The partial pressure of a solute gas A equals the product of its vapour pressure P at the same temperature and its mole fraction in the low solution x". This is Raoult's law.

$$\bar{P} = P * X$$

Henry's law

Henry's law: At low concentration of gas in the liquid, Raoult's law does not hold gold. For such non ideal behaviour. Henry's law is found to be useful.

It expresses the relationship between "The concentration of gas dissolved in a liquid and the equilibrium partial pressure of the gas above the liquid surface it is given by

$$P = H * x$$

Where,

P = equilibrium P. Pr of gas in contact with liquid

X = mole fraction of gas dissolved in liquid.

H = Henry's constant

The factor H is a function of the specific nature of gas liquid

Dalton's law: The total pressure exerted by a gaseous mixture is equal to the sum of the partial pressure's (i.e)

where C=number of components

$$P = \sum_{i=1}^{C} P_i$$

Amagat's law: The total volume occupied by a gaseous mixture is equal to the sum of the partial volumes (i.e).

$$V = \sum_{i=1}^{C} V_i$$

mole fraction = volume fraction = pressure fraction

Dalton's Law

$$P = p_A + p_B + p_C + \ldots = \sum_i p_i$$

$$p_A V = n_A RT$$
$$p_B V = n_B RT$$
$$p_C V = n_C RT$$

$$(p_A + p_B + p_C + \ldots)V = (n_A + n_B + n_C + \ldots)RT$$

$$\left(\sum_i p_i\right)V = \left(\sum_i n_i\right)RT = nRT$$

Amagat's Law

$$V = V_A + V_B + V_C + \ldots = \sum_i V_i$$

$$PV_A = n_A RT$$
$$PV_B = n_B RT$$
$$PV_C = n_C RT$$

$$P(V_A + V_B + V_C + \ldots) = (n_A + n_B + n_C + \ldots)RT$$

$$P\left(\sum_i V_i\right) = \left(\sum_i n_i\right)RT = nRT$$

Average Molecular Weight

Through the use of average molecular weight, a complex gas mixture can be treated as though it is a pure gas. If m is the mass of a mixture of gases and M_{av} its average molecular weight, then the number of moles of the mixture n is given by

$$m = n\, M_{av}$$

We can calculate the average molecular weight of a mixture of gases knowing its molal composition. Assume a suitable basis, say 100 moles of the mixture, and evaluate the number of moles of each constituent in the mixture. The mass of each constituent in the mixture is obtained by multiplying the number of moles by the respective molecular weight.

$$m_i = n_i M_i$$

where m_i is the mass and n_i is the number of moles of constituent i in the mixture and M_i its molecular weight. The total mass of the mixture is the sum of the individual masses.

$$m = \Sigma\, m_i = \Sigma\, n_i M_i$$

The average molecular weight is calculated as

$$M_{av} = \frac{m}{n}$$

Density and Specific Gravity

The density of a mixture of gases is the mass per unit volume of the mixture at the specified conditions of temperature and pressure. It is expressed in kg/m^3, g/L, lb/ft^3, etc. From the molal composition of the mixture, the mass of n moles of the mixture can be evaluated by the method outlined above.

Let M_{av} be the average molecular weight of the gas mixture. Then, the mass of n moles of the gas is $m = nM_{av}$. Volume is obtained from the ideal gas equation $V = \frac{nRT}{P}$. Therefore,

$$\text{density} = \frac{m}{V} = \frac{PM_{av}}{RT}$$

As volume of gas depends strongly on temperature and pressure, the density is very much dependent on the temperature and pressure of the gas. When the standard temperature and pressure are used in the computation, the value so calculated is the density of the gas at STP.

The specific gravity of a gas is the ratio of the density of the gas to the density of dry air at the same temperature and pressure.

$$\text{Specific gravity} = \frac{1}{Density}$$

Humidity

Humidification

The operation that is carried to increase the amount of vapour in a gas stream is known as humidification.

Dehumidification

The operation that is carried to reduce the vapour content of gas stream is known as dehumidification.

Absolute humidity

It is defined as the weight of vapor, present in a unit weight of dry (non-condensable) gas.

$$H(s) = \frac{\text{Kg vapour}}{\text{Kg dry (non-condensable) gas}}$$

$$\text{Unit: } \frac{\text{Kg water vapour}}{\textit{Kg dry air}}$$

Molar Humidity

It is defined as the ratio of moles of vapor (condensable) to the moles of dry (non-condensable) gas.

$$H_m(S_m) = \frac{Kmol\ vapour}{\text{Kmol dry gas}}$$

Relative Humidity

It is defined as the ratio of the partial pressure of condensable vapor in gas phase to the vapor pressure of liquid at DB.

$$\therefore \%\ RH\ (RS) = \frac{P_A}{P_S} \times 100$$

Where

P_A = Partial pressure of vapor in gas phase

P_S = Vapor pressure of pure liquid at DB

Saturation humidity

It is defined as the absolute humidity of vapour – gas mixture at 100% saturation.

$$H_S = \left(\frac{P_s}{P - P_s} \right) \frac{M_A}{M_B}$$

Where M_A & M_B – Molecular weights of vapour dry gas.

percentage Humidity (or) percentage absolute humidity

It is defined as the percentage ratio of the existing weight of vapour per unit weight of vapour free gas (dry gas) to the weight of vapour that would exist per unit weight of vapour free gas (dry gas) if the mixture were saturated at the existing temperature and pressure.

$$\% \text{ Humidity} = \frac{H}{H_s} \times 100$$

Humid heat

It is defined as the heat capacity of 1 kg dry air and moisture contained in it.

For air – water system, the humid heat is given by:

$C_S = 1.006 + 1.84\ H$ KJ / Kg dry air K

Humid Volume (V_H)

Humid volume is the volume of a mixture of air and water vapor per kg of dry air. This is also known as psychometric volume.

$$V_H = \left[\frac{H}{M_A} - \frac{1}{M_B} \right] 22.4136 \times \left[\frac{D_B}{273} \right] \times \left[\frac{101.325}{p} \right]$$

$$VHS = RT/PS \cdot M_A$$

WBT

If a thermometer having bulb covered with a wet wick is kept in air water vapor mixture it will read a steady value after a few seconds. This is called wet bulb temperature.

DBT

It is a temperature of vapor gas mixture recorded by immersion of a thermometer in the mixture.

Dew point temperatures

It is the temperature at which condensation will first occur (i.e., vapor begins to condense) when the vapor – gas mixture is cooled at constant pressure. At this temperature the gas is saturated with vapor.

Adiabatic saturation temperature (AST)

It is temperature that the vapor – gas mixture would reach if it were saturated through an adiabatic process.

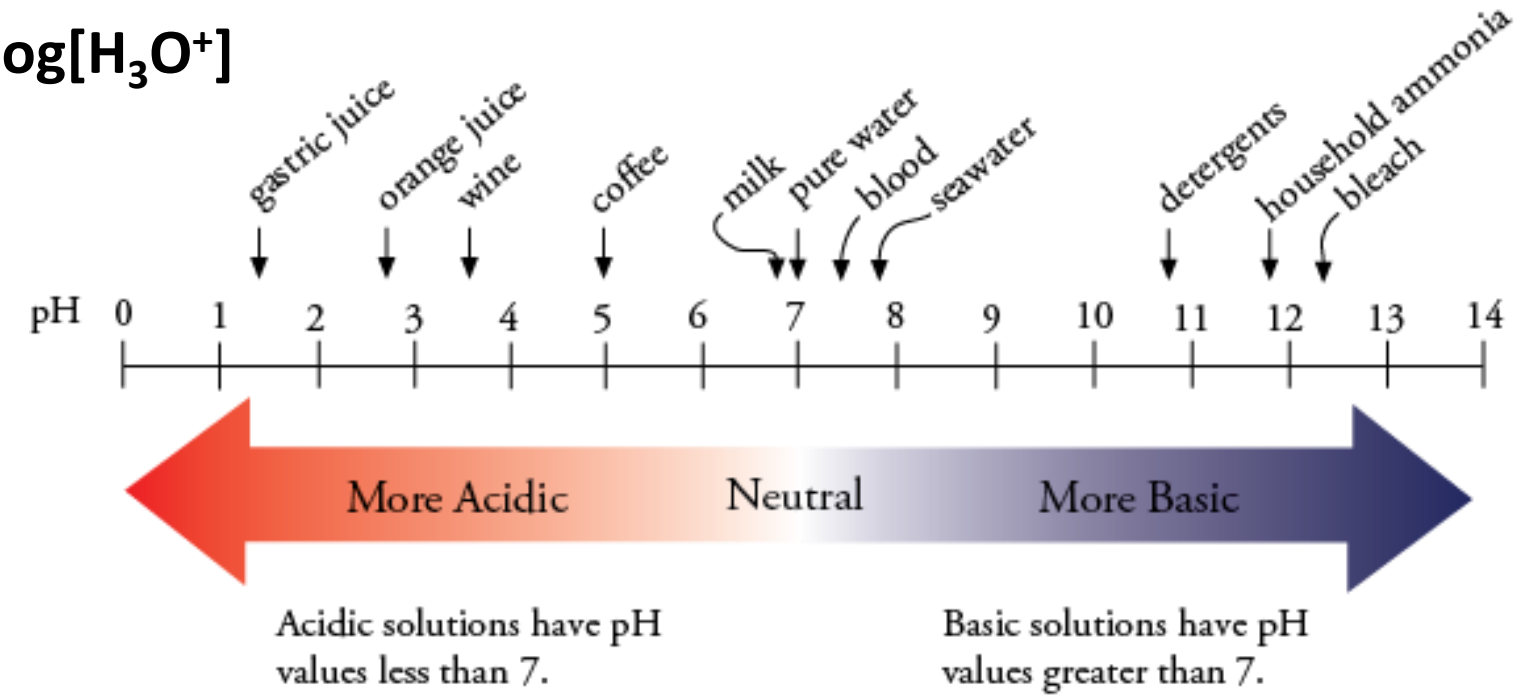
pH of solutions

Quantitatively, the acidity **of a solution is measured by and is equal to the concentration of hydronium ion in that solution**.

The acidity of a solution was originally viewed as the molar concentration of H_3O^+ present in it.

This idea was used by the Swedish chemist S. P. L. Sorenson when in 1909 he defined the acidity of a solution expressed in logarithmic notation as:

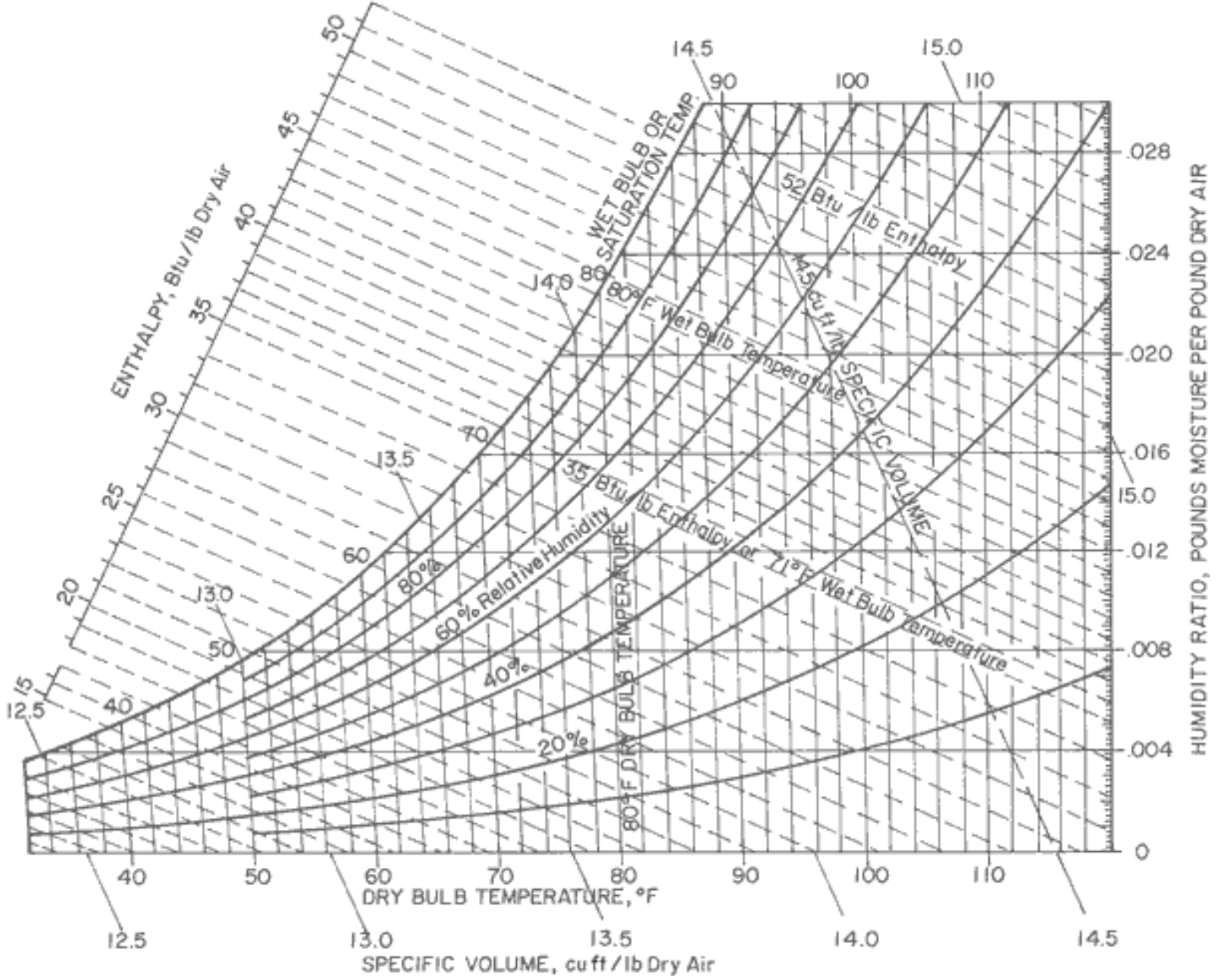
$pH = -\log[H_3O^+]$

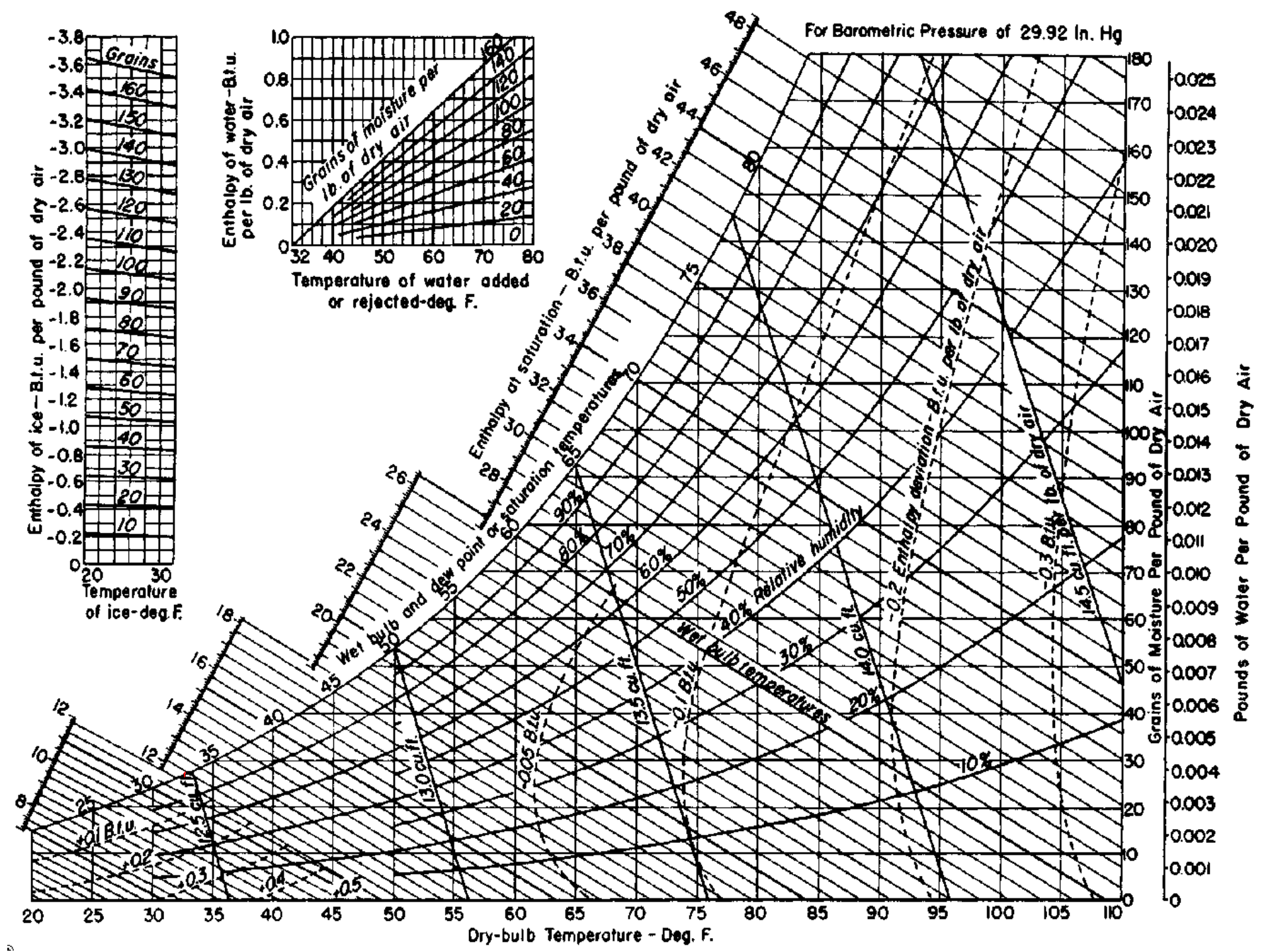


Vapor pressure

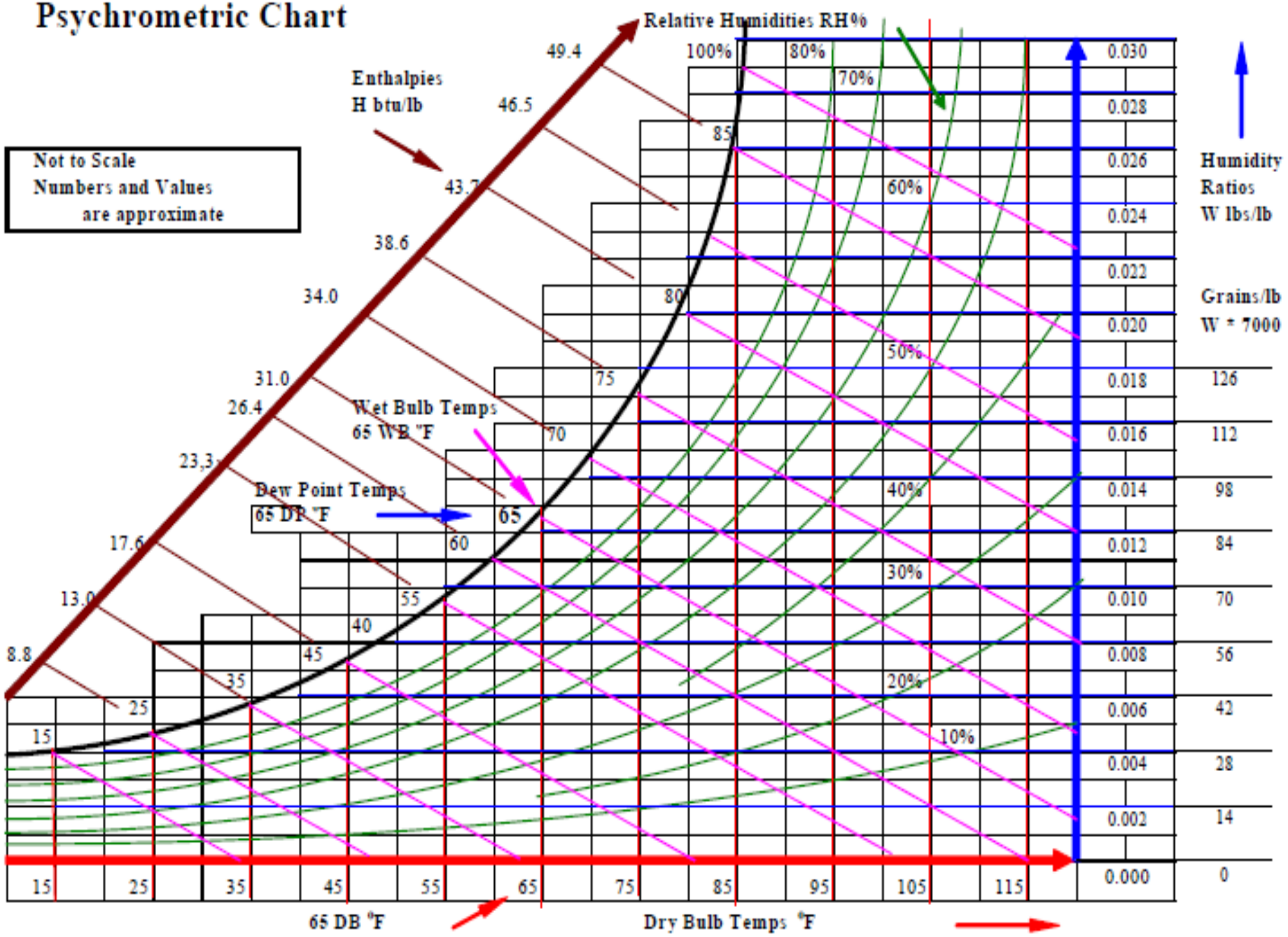
Vapor pressure is defined as the pressure exerted by a vapor in thermodynamic equilibrium with its condensed phases (solid or liquid) at a given temperature in a closed system.

Humidity chart



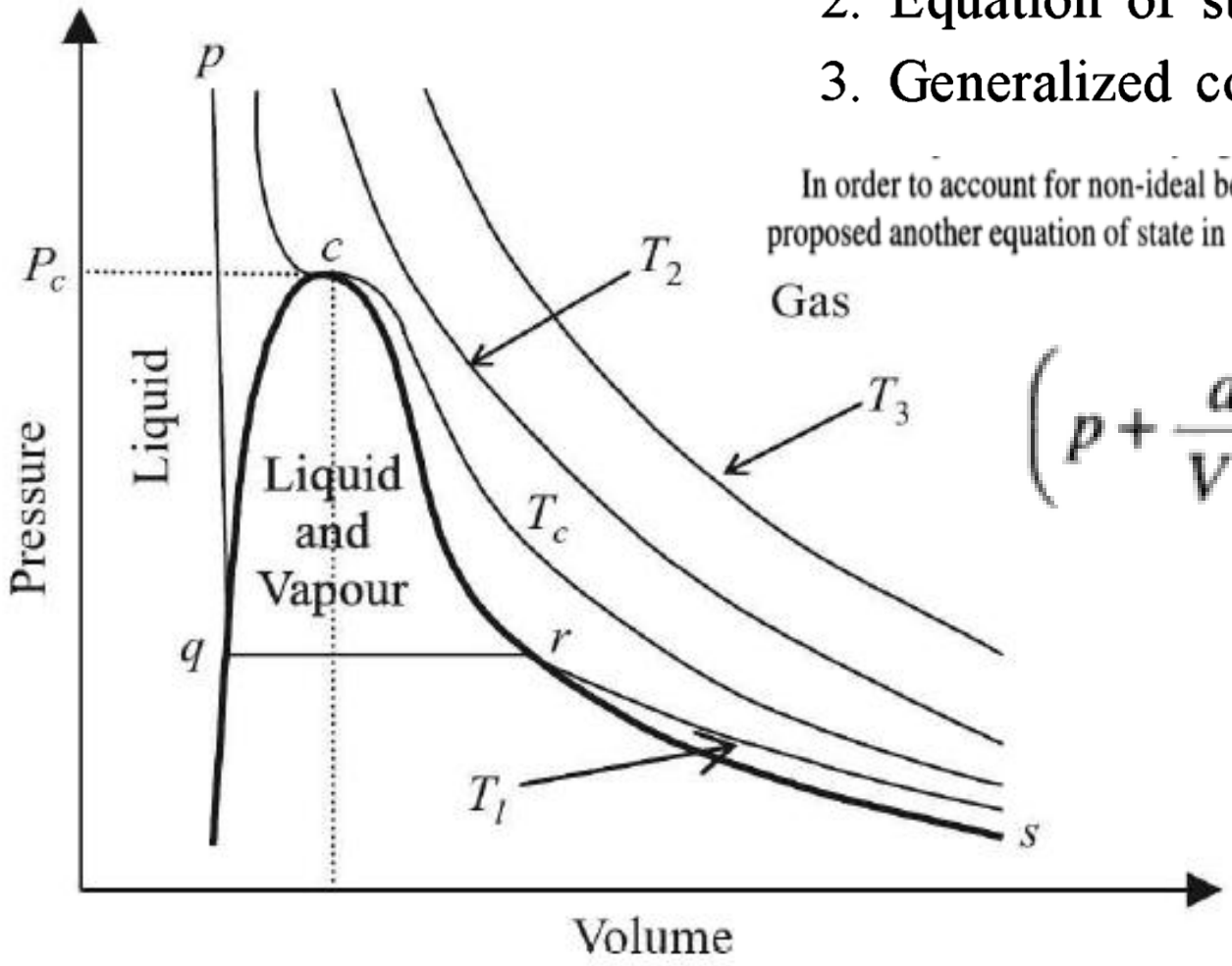

For Barometric Pressure of 29.92 In. Hg
Enthalpy of ice – B.t.u. per pound of dry air
Grains
Temperature of ice-deg. F.
Enthalpy of water – B.t.u. per lb. of dry air
Grains of moisture per lb. of dry air
Temperature of water added or rejected-deg. F.
Enthalpy at saturation – B.t.u. per pound of dry air
Wet bulb and dew point or saturation temperatures
Relative humidity
Wet bulb temperatures
Enthalpy deviation – B.t.u. per lb. of dry air
13.0 cu. ft.
13.5 cu. ft.
14.0 cu. ft.
14.5 cu. ft. per lb. of dry air
Grains of Moisture Per Pound of Dry Air
Pounds of Water Per Pound of Dry Air
Dry-bulb Temperature - Deg. F.

Psychrometric Chart



Real Gases

1. Experimental *P-V-T* data
2. Equation of state
3. Generalized compressibility charts.



In order to account for non-ideal behaviour of the gases, J.D. van der Waals proposed another equation of state in 1873 as follows:

$$\left(p + \frac{a}{V^2}\right)(V - b) = RT$$

$T_c = 647.3$ K, $P_c = 221.2$ bar and $V_c = 57.1 \times 10^{-6}$ m^3/mol

Real Gases - Derive vander waals constant

The van der Waals equation for n moles is

$$\left(P + \frac{a\,n^2}{V^2}\right)(V - nb) = nRT$$

For 1 mole

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

$$PV + \frac{a}{V} - Pb - \frac{ab}{V^2} - RT = 0$$

Multiply equation by V^2 / P

$$\frac{V^2}{P}\left(PV + \frac{a}{V} - Pb - \frac{ab}{V^2} - RT\right) = 0$$

$$V^3 + \frac{aV}{P} + - bV^2 - \frac{ab}{P} - \frac{RTV^2}{P} = 0$$

When the above equation is rearranged in powers of V

$$V^3 - \left[\frac{RT}{P} + b\right]V^2 + \left[\frac{a}{P}\right]V - \left[\frac{ab}{P}\right] = 0 \text{ --- } (6.26)$$

$$V = V_C$$

$$V - V_C = 0$$

$$(V - V_C)^3 = 0$$

$$V^3 - 3V_C V^3 + 3V_C{}^2 V - V_C{}^3 = 0$$

$$-3V_C V^2 = -\left[\frac{RT_C}{P_C} + b\right]V^2$$

$$3V_C = \frac{RT_C}{P_C} + b$$

Use this equation to find a and b value

$$3V_C^2 = \frac{a}{P_C}$$

$$V_C^3 = \frac{ab}{P_C}$$

$$\frac{V_C^3}{3V_C^2} = \frac{ab / P_C}{a / P_C}$$

$$\frac{V_C}{3} = b$$

i.e. $V_C = 3b$

i.e. $V_C = 3b$

$$3V_C^2 = \frac{a}{P_C}$$

$$P_C = \frac{a}{3V_C^2} = \frac{a}{3(3b^2)} = \frac{a}{3 \times 9b^2} = \frac{a}{27\,b^2}$$

$$P_C = \frac{a}{27b^2}$$

$$3V_C = b + \frac{R\,T_C}{P}$$

$$3(3b) = b + \frac{R\,T_C}{\left(a/27\,b^2\right)}$$

$$9b - b = \left(\frac{R\,T_C}{a}\right) 27\,b^2$$

$$8b = \frac{T_C\,R\,27b^2}{a}$$

$$\therefore \quad T_C = \frac{8\,a\,b}{27\,R\,b^2} = \frac{8\,a}{27\,R\,b}$$

$$T_C = \frac{8\,a}{27\,R\,b}$$

Find a and b Value

$$V_c = 3b$$

$$T_c = \frac{8a}{27Rb}$$

$$P_c = \frac{RT_c}{2b} - \frac{a}{9b^2}$$

$$a = \frac{27R^2T_c^{\,2}}{64P_c}$$

$$b = \frac{RT_c}{8P_c}$$

$V_c = 3b$

$3V_c = b + \frac{RT_c}{P_c}$

$3V_c^2 = \frac{a}{P_c}$

$b = \frac{V_c}{3}$

$3V_c = b + \frac{RT_c}{P_c}$

$3V_c = \frac{V_c}{3} + \frac{RT_c}{P_c}$

$3V_c - \frac{V_c}{3} = \frac{RT_c}{P_c}$

$\frac{9V_c - V_c}{3} = \frac{RT_c}{P_c}$

$\frac{8V_c}{3} = \frac{RT_c}{P_c}$

Taking square on both side

$\frac{8^2 V_c^2}{3^2} = \frac{R^2 T_c^2}{P_c^2}$

$\frac{64 V_c^2}{9} = \frac{R^2 T_c^2}{P_c^2}$

$V_c^2 = \frac{9R^2 T_c^2}{64 P_c^2}$

we know that

$3V_e^2 = \frac{a}{P_c}$

$3 \times \frac{9R^2 T_c^2}{64 P_c^2} = \frac{a}{P_c}$

$\frac{27 R^2 T_c^2}{64 P_c} = \frac{a}{P_c}$

$$\boxed{\frac{27 R^2 T_c^2}{64 P_c} = a}$$

Put the value of V_c in eq.

$$3V_c = b + \frac{RT_c}{P_c}$$

$$\therefore V_c = 3b$$

$$3 \times 3b = b + \frac{RT_c}{P_c}$$

$$9b = b + \frac{RT_c}{P_c}$$

$$9b - b = \frac{RT_c}{P_c}$$

$$8b = \frac{RT_c}{P_c}$$

$$\boxed{b = \frac{1}{8}\frac{RT_c}{P_c}}$$

$$a = \frac{27R^2T_c^{\,2}}{64P_c}$$

$$b = \frac{RT_c}{8P_c}$$

Compressibility factor, Density & Specific gravity

$$Z = \frac{pV}{RT}$$

$$\text{Density of gas} = \frac{\text{molar mass } (M)}{\text{molar volume}}$$

$$\text{Specific gravity of a gas} = \frac{\text{molar mass of the gas}}{\text{molar mass of the air}}$$

Ex. 2.1

Calculate the pressure developed by one kmol gaseous ammonia contained in a vessel of 0.6 m^3 capacity at a constant temperature of 473 K by the following methods:

(a) Using the ideal gas equation
(b) Using the van der Waals equation given that $a = 0.4233$ N m^4/mol^2; $b = 3.73 \times 10^{-5}$ m^3/mol

(a) Use the ideal gas equation, $P = \frac{RT}{V}$, where V is the molar volume of the gas. Here, $V = 0.6 \times 10^{-3}$ m^3/mol. Therefore, the pressure given by the ideal gas equation is

$$P = \frac{8.314 \times 473}{0.6 \times 10^{-3}} = 6.554 \times 10^6 \text{ N/m}^2 = 65.54 \text{ bar}$$

(b) The van der Waals equation may be rearranged as

$$P = \frac{RT}{V - b} - \frac{a}{V^2}$$

Substituting of the values gives

$$P = \frac{8.314\,(473)}{(0.6 \times 10^{-3}) - (3.73 \times 10^{-5})} - \frac{0.4233}{(0.6 \times 10^{-3})^2} = 5.8128 \times 10^6 \text{ N/m}^2 = 58.13 \text{ bar}$$

Ex.2.2

Estimate the molar volume of CO_2 at 500 K and 100 bar using the (a) ideal gas equation and (b) the van der Waals equation. The van der Waals constants are 0.364 N m^4/mol^2 and 4.267×10^{-5} m^3/mol.

$$V = 0.4157 \times 10^{-3} \text{ m}^3$$

Ideal gas

$$V = 3.717 \times 10^{-4} \text{ m}^3/\text{mol}.$$

Vander Waals

Ex.2.3

Calculate the average molar mass and composition by mass of air.

Composition of Air at Mean Seal Level

Gas	Mole%
Nitrogen	78.084
Oxygen	20.946
Argon	0.934
Carbon dioxide	0.033
Neon	18×10^{-4}
Helium	5.2×10^{-4}
Krypton	1.1×10^{-4}
Hydrogen	0.5×10^{-4}
Xenon	0.08×10^{-4}

Basis: 100 kmol air

Composition of Air Without Trace/Noble Gases

Gas	Formula	Molar mass	kmol	Mass kg	Mass %
Oxygen	O_2	31.9988	21	671.786	23.19
Nitrogen	N_2	28.0135	78	2185.051	75.43
Argon	Ar	39.948	1	39.948	1.38
Total			100	2896.785	100.00

$$\text{Average molar mass of air} = \frac{2897}{100} = 28.97$$

Ex.2.4

Cracked gas from a petroleum refinery has the following composition by volume: methane 45%, ethane 10%, ethylene 25%, propane 7%, propylene 8%, *n*-butane 5%.

Find (a) the average molar mass of the gas mixture, (b) the composition by mass, and (c) specific gravity of the gas mixture.

Composition of Refinery Gas

Gas	Formula	Molar mass	kmol	Mass kg	Mass %
Methane	CH_4	16	45	720	27.13
Ethane	C_2H_6	30	10	300	11.30
Ethylene	C_2H_4	28	25	700	26.37
Propane	C_3H_8	44	7	308	11.61
Propylene	C_3H_6	42	8	336	12.66
n-Butane	C_4H_{10}	58	5	290	10.93
Total	–	–	100	2654	100.00

$$\text{Average molar mass of gas mixture} = \frac{2654}{100} = 26.54$$

$$\text{Specific gravity of gas mixture} = \frac{26.54}{28.97} = 0.9161$$

Ex.2.5

Calculate the specific volume of superheated steam at 100 bar a and 623.15 K (350°C) using (a) the ideal gas law, and (b) the van der Waals equation.

If the actual specific volume* of steam at the above conditions is 0.022 42 m^3/kg, find the percentage error in the above cases.

Molar mass of steam (water) = 18.0153

Ideal gas law states

$$pV = RT$$

$p = 100$ bar a, $T = 623.15$ K

$$\text{Molar volume } V = \frac{RT}{p}$$

$$= \frac{0.083\,14 \times 623.15}{100} = 0.5180 \text{ m}^3/\text{kmol}$$

$$\text{Specific volume } v = \frac{V}{M}$$

$$= 0.518/18.0153 \text{ (m}^3/\text{kmol) (kmol/kg)}$$

$$= 0.0288 \text{ m}^3/\text{kg}$$

Evaluation of van der Waals constants:

$$\left(p + \frac{a}{V^2}\right)(V - b) = RT$$

where $$a = \frac{27\,R^2\,T_c^2}{64\,p_c}\ (\mathrm{m^3})^2.\ \mathrm{bar/(kmol)^2}$$

and $$b = \frac{RT_c}{8p_c}\ \mathrm{m^3/kmol}$$

$p_c = 220.76$ bar, $T_c = 647.11$ K for water,

$$a = \frac{\left[27 \times (0.083\,14)^2 \times (647.11)^2\right]}{(64 \times 220.76)}$$

$$a = 5.5315\ \mathrm{m^6.\ bar/(kmol)^2}$$

$$b = \frac{(0.083\,14 \times 647.11)}{(8 \times 220.76)}$$

$$= 0.030\,46\ \mathrm{m^3/kmol}$$

Substituting the value of a and b in van der Waals equation.

$$\left(\frac{100 + 5.5315}{V^2}\right)(V - 0.030\,46) = 0.083\,14 \times 623$$

Simplifying

$$10\,V^3 - 5.4836\,V^2 + 0.5526\,V - 0.0168 = 0$$

Such equations can be solved by using a numerical method such as Newton-Raphson method. According to this method, if $F(V) = 0$ then

$$V_{n+1} = V_n - \frac{F(V_n)}{F'(V_n)}$$

$$V = 0.4285 \text{ m}^3/\text{kmol}$$

$$\text{Specific volume, } v = \frac{0.4285}{18.0153} = 0.0238 \text{ m}^3/\text{kg}$$

Such equations can be readily solved by specialised mathematical software like Mathcad®.

$$\text{Define } f(V) := 100V^3 - 54.8422V^2 + 5.5315V - 0.168$$

or

$$f(V) := \left[100 + \frac{5.5315}{V^2}\right](V - 0.03046) - 51.7962$$

Guess $V := 0.518$

$\text{soln} := \text{root}(f(V), V)$

$\text{soln} := 0.429 \text{ m}^3/\text{kmol}$

or

$$v = \frac{0.429}{18.0153} = 0.2381 \text{ m}^3/\text{kg}$$

$$\text{Correct value} = 0.022\,42 \text{ m}^3/\text{kg}$$

$$\% \text{ Error by using ideal gas law} = \left[\frac{(0.0288 - 0.022\,42)}{0.022\,42}\right] \times 100 = 28.01$$

$$\% \text{ Error by using van der Waals equation} = \left[\frac{(0.0238 - 0.022\,42)}{0.022\,42}\right] \times 100 = 6.16$$

EX.2.6

Calculate the volume occupied by 20 kg of chlorine gas at a pressure of 100 kPa and 298 K. (Nov/Dec, 2018)

Sol. : Basis : 20 kg Cl_2 gas.

$$\text{Moles of } Cl_2 \text{ gas} = \frac{20}{71} = 0.2817 \text{ kmol}$$

$$PV = nRT$$

$$\therefore \quad V = \frac{nRT}{P}$$

where, $n = 0.2817$ kmol, $T = 298$ K, $P = 100$ kPa, $R = 8.31451$ m^3·kPa/(kmol·K)

$$\text{Volume, } V = \frac{0.2817 \times 8.31451 \times 298}{100} = 6.98 \text{ m}^3$$

Ex.2.7

15 kg of Carbon dioxide is compressed at a temperature of 303 K to a volume of 0.5 m^3. Calculate the pressure required for given duty. Assume ideal gas law is applicable. (Nov/Dec, 2018)

(ii) 15 kg of carbondioxide is compressed atb a temperature of 303 K (30 °C) to a volume of 0.5 m^3. Calculate the pressure required for given duty. Assume ideal gas law is applicable. (7)

Sol. : Basis : 15 kg of carbon dioxide gas

Molecular weight $CO_2 = 44$

$$\therefore \quad \text{Moles of } CO_2 = \frac{15}{44} = 0.341 \text{ kmol}$$

$$PV = nRT$$

$$\therefore \quad P = \frac{nRT}{V}$$

where, $n = 0.341$ kmol, $T = 303$ K, $V = 0.5\ m^3$, $R = 8.31451\ m^3 \cdot kPa/(kmol \cdot K)$

$$\text{Pressure, } P = \frac{0.341 \times 8.31451 \times 303}{0.5} = 1718.16 \text{ kPa}$$

Ex.2.8

Carbon dioxide weighing 1.10 kg occupies a volume of 33 liters of 300 K. Calculate the pressure using the Vander Waals equation of state. a = 3063 $(m^3)^2$ kPa/$k(mol)^2$ and b = 403 x 10^{-2} m^3/kmol for CO_2 (Nov/Dec, 2018)

Solution : Basis : 1.10 kg of CO_2 gas at 300 K.

The Van der Waals equation is

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

Amount of CO_2 gas = 1.10 kg = 0.025 kmol

Volume occupied by this gas = 33 l = 0.033 m^3

$$\therefore \quad V = \frac{0.033}{0.025} = 1.32 \text{ m}^3/\text{kmol}$$

$a = 3.60 \text{ (m}^3)^2 \cdot \text{kPa/(kmol)}^2$

$b = 4.3 \times 10^{-2} \text{ m}^3/\text{kmol}$

$R = 8.31451 \text{ m}^3 \cdot \text{kPa/(kmol} \cdot \text{K)}$

$T = 300 \text{ K}$

$$\left[P + \frac{3.60}{(1.32)^2}\right][1.32 - 4.3 \times 10^{-2}] = 8.31451 \times 300$$

Solving we get,

$P = 1951 \text{ kPa}$

$= 1.951 \text{ MPa}$ ... **Ans.**

The pressure and volume corrections to the ideal gas law result in Van der Waals equation as follows :

$$\left(P + \frac{n^2a}{V^2}\right)(V - nb) = nRT$$

where V is the volume in m^3 of n kmol of gas.

a and b are the Van der Waals constant.

For volume in m^3 per kmol of gas, it reduces to

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

V is in m^3/kmol.

Ex.2.9

Calculate the weight of 1 m^3 of chlorine gas at a temperature of 298 K and a pressure of 101.325 kPa. (CO2/K3) (Nov/Dec 2018)

Sol. : Basis : 1 m^3 Cl_2 gas

$$PV = nRT$$

$$\therefore \quad \text{Moles of } Cl_2 \text{ gas, } n = \frac{PV}{RT}$$

where, P = 101.325 kPa, V = 1 m^3, T = 298 K, R = 8.31451 $m^3 \cdot kPa/(kmol \cdot K)$

$$\therefore \quad \text{Moles of } Cl_2 \text{ gas} = \frac{101.325 \times 1}{8.31451 \times 298} = 0.0409 \text{ kmol}$$

$$\text{Weight of } Cl_2 \text{ gas} = 0.0409 \times 71 = 2.904 \text{ kg}$$

Ex.2.10

Moist air contains 0.0109 kg water vapour per cubic metre of the mixture at 300 K and 101.3 kPa. Calculate the following

a. **The partial pressure of water vapour**
b. **The relative saturation**
c. **The absolute humidity of the air**
d. **% saturation**
e. **The temperature to which the mixture be heated so that its percent saturation becomes 10%.**

The vapour pressure of water (in kPa) is approximated by the Antoine equation as

$$\ln P^s = 16.26205 - \frac{3799.887}{T - 46.854}$$

Solution:

The number of moles of the mixture in one cubic metre at 300 K and 101.3 kPa is

$$\frac{V}{22.414} \times \frac{P}{P_0} \times \frac{T_0}{T} = \frac{1}{22.414} \times \frac{101.3}{101.3} \times \frac{273.15}{300} = 0.0406 \text{ kmol}$$

$$0.0109 \text{ kg of water} = 0.0109/18.016 = 6.05 \times 10^{-4} \text{ kmol}$$

(a) The mole fraction of water vapour is

$$\frac{n_w}{n} = \frac{6.05 \times 10^{-4}}{0.0406} = 0.0149$$

The partial pressure of water vapour is

$$\text{mole fraction} \times \text{total pressure} = 0.0149 \times 101.3 = 1.51 \text{ kPa}$$

(b) The vapour pressure at 300 K is calculated using the Antoine equation:

$$\ln P^S = 16.26205 - \frac{3799.887}{300 - 46.854} \Rightarrow P^S = 3.5 \text{ kPa}$$

Relative saturation is the ratio of partial pressure to vapour pressure, i.e.

$$\text{RS} = \frac{1.51}{3.5} \times 100 = 43.14\%$$

(c) Absolute humidity can be evaluated by Eq. (8.7):

$$Y' = \frac{p_A}{P - p_A} \times \frac{18}{29} = \frac{1.51}{101.3 - 1.51} \times \frac{18}{29}$$

$$= 9.39 \times 10^{-3} \text{ kg water/kg dry air}$$

(d) Saturation absolute humidity is

$$Y_s' = \frac{P_A^S}{P - P_A^S} \times \frac{18}{29} = 0.0222 \text{ kg water/kg dry air}$$

$$\text{Percent saturation (PS)} = \frac{Y'}{Y_S'} \times 100 = 42.27\%$$

(e) When the mixture is heated at constant pressure, its humidity (Y') will not change. However, saturation humidity at the new temperature is different from the earlier value. Since the percent saturation at the new temperature is given to be 10%, we have

$$\text{PS} = \frac{Y'}{Y_S'} \times 100 = 10.0\%$$

Ex.2.11

The dry bulb temperature and dew point of ambient air were found to be 302 K (29c) and 291 k (18 c) respectively. Barometer reads 100 Kpa. Calculate.

(a) The absolute molar humidity,
(b) The absolute humidity
(c) % RH
(d) % saturation
(e) Humid Heat and
(f) Humid volume

Data:
Vapour pressure of water at 291 K = 2.0624 Kpa
Vapour pressure of water at 302 K = 4 .004 kpa

Table 6.8 VAPOUR PRESSURE OF WATER

Temperature t °C	Temperature T K	Vapour Pressure, kPa									
		Temperature interval, K or °C									
		0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	273	0.6108	0.615	0.6195	0.6241	0.6286	0.6333	0.6379	0.6426	0.6473	0.6519
1	274	0.6566	0.6615	0.6663	0.6711	0.6759	0.6809	0.6858	0.6907	0.6958	0.7007
2	275	0.7055	0.7109	0.7159	0.721	0.7262	0.7314	0.7366	0.7419	0.7473	0.7526
3	276	0.7575	0.7633	0.7687	0.7742	0.7797	0.7851	0.7907	0.7963	0.8019	0.8077
4	277	0.8129	0.8191	0.8249	0.8306	0.8365	0.8423	0.8483	0.8543	0.8603	0.8663
5	278	0.8718	0.8785	0.8846	0.8907	0.897	0.9033	0.9095	0.9158	0.9222	0.9286
6	279	0.9345	0.9415	0.9481	0.9546	0.9611	0.9678	0.9745	0.9813	0.9881	0.9949
7	280	1.0012	1.0086	1.0155	1.0224	1.0295	1.0366	1.0436	1.0508	1.058	1.0652
8	281	1.072	1.0799	1.0872	1.0947	1.1022	1.1096	1.1172	1.1248	1.1324	1.14
9	282	1.1472	1.1556	1.1635	1.1714	1.1792	1.1872	1.1952	1.2032	1.2114	1.2195
10	283	1.227	1.2294	1.2443	1.2526	1.261	1.2694	1.2779	1.2864	1.2951	1.3038
11	284	1.3116	1.3212	1.33	1.3388	1.3478	1.3567	1.3658	1.3748	1.3839	1.3931
12	285	1.4014	1.4116	1.421	1.4303	1.4397	1.4492	1.4587	1.4683	1.4779	1.4876
13	286	1.4965	1.5072	1.5171	1.5269	1.5369	1.5471	1.5572	1.5673	1.5776	1.5879
14	287	1.5973	1.6085	1.6191	1.6296	1.6401	1.6508	1.6615	1.6723	1.6831	1.694
15	288	1.7039	1.7159	1.7269	1.7381	1.7493	1.7605	1.7719	1.7832	1.7947	1.8061
16	289	1.8168	1.8293	1.841	1.8529	1.8648	1.8766	1.8886	1.9006	1.9128	1.9249
17	290	1.9362	1.9494	1.9618	1.9744	1.9869	1.9994	2.0121	2.0249	2.0377	2.0505
18	291	2.0624	2.0765	2.0896	2.1028	2.116	2.1293	2.1426	2.156	2.1694	2.183
19	292	2.1957	2.2106	2.2245	2.2383	2.2523	2.2663	2.2805	2.2947	2.309	2.3234
20	293	2.3366	2.3523	2.3668	2.3815	2.3963	2.4111	2.4261	2.441	2.4561	2.4713
21	294	2.4853	2.5018	2.5171	2.5326	2.5482	2.5639	2.5797	2.5955	2.6114	2.6274
22	295	2.6422	2.6595	2.6758	2.6922	2.7086	2.7251	2.7418	2.7584	2.7751	2.7919
23	296	2.8076	2.8259	2.843	2.8602	2.8775	2.895	2.9124	2.93	2.9478	2.9655
24	297	2.9821	3.0014	3.0195	3.0378	3.056	3.0744	3.0928	3.1113	3.1299	3.1485
25	298	3.166	3.186	3.205	3.224	3.2432	3.2625	3.282	3.3016	3.3213	3.3411
26	299	3.3597	3.3809	3.4009	3.4211	3.4413	3.4616	3.482	3.5025	3.5232	3.544
27	300	3.5636	3.586	3.607	3.6282	3.6496	3.671	3.6925	3.7141	3.7358	3.7577
28	301	3.7782	3.8016	3.8237	3.846	3.8683	3.8909	3.9135	3.9363	3.9593	3.9823
29	302	4.004	4.0286	4.0519	4.0754	4.099	4.1227	4.1466	4.1705	4.1945	4.2186
30	303	4.2415	4.2673	4.2918	4.3164	4.3411	4.3659	4.3908	4.4159	4.4412	4.4667
31	304	4.4911	4.518	4.5439	4.5698	4.5958	4.6219	4.6482	4.6746	4.7011	4.7279
32	305	4.7534	4.7816	4.8087	4.8359	4.8632	4.8907	4.9184	4.9461	4.974	5.002
33	306	5.0288	5.0585	5.0869	5.1154	5.1441	5.173	5.202	5.2312	5.2605	5.2898

Table 6.8 (Continued)

Temperature		Vapour Pressure, kPa									
t	T	Temperature interval, K or °C									
°C	K	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
34	307	5.318	5.349	5.3788	5.4088	5.439	5.4693	5.4997	5.5302	5.5609	5.5918
35	308	5.6216	5.6541	5.6854	5.7169	5.7485	5.7802	5.8122	5.8443	5.8766	5.9088
36	309	5.94	5.9739	6.0067	6.0396	6.0727	6.106	6.1395	6.1731	6.207	6.241
37	310	6.2739	6.3093	6.3437	6.3783	6.4131	6.448	6.4831	6.5183	6.5537	6.5893
38	311	6.624	6.6609	6.6969	6.733	6.7693	6.8058	6.8425	6.8794	6.9166	6.9541
39	312	6.9908	7.0294	7.0673	7.1053	7.1434	7.1817	7.2202	7.2589	7.2977	7.3367
40	313	7.375	7.414	7.454	7.494	7.534	7.574	7.614	7.654	7.695	7.737
41	314	7.777	7.819	7.861	7.902	7.943	7.986	8.029	8.071	8.114	8.157
42	315	8.199	8.242	8.285	8.329	7.373	8.417	8.461	8.505	8.549	8.594
43	316	8.639	8.685	8.73	8.775	8.821	8.867	8.914	8.961	9.007	9.054
44	317	9.1	9.147	9.195	9.243	9.291	9.339	9.387	9.435	9.485	9.534
45	318	9.582	9.633	9.682	9.731	9.781	9.831	9.882	9.933	9.983	10.034
46	319	10.086	10.138	10.19	10.242	10.294	10.346	10.399	10.452	10.506	10.559
47	320	10.612	10.666	10.72	10.775	10.83	10.884	10.939	10.994	11.048	11.104
48	321	11.162	11.216	11.274	11.331	11.388	11.446	11.503	11.56	11.618	11.676
49	322	11.736	11.794	11.852	11.911	11.971	12.031	12.091	12.151	12.211	12.272

Temperature		Vapour Pressure, kPa									
t	T	Temperature interval, K or °C									
°C	K	0	1	2	3	4	5	6	7	8	9
50	323	12.335	12.961	13.613	14.293	15.002	15.741	16.511	17.313	18.147	19.016
60	333	19.92	20.861	21.838	22.855	23.912	25.009	26.15	27.334	28.563	29.838
70	343	31.162	32.535	33.958	35.434	36.964	38.549	40.191	41.891	43.652	45.474
80	353	47.36	49.311	51.329	53.416	55.573	57.803	60.108	62.489	64.948	67.487

Table 6.8 (Continued)

Temperature		Vapour Pressure, kPa									
		Temperature interval, K or °C									
t	T										
°C	K	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
90	363	70.109	70.362	70.63	70.898	71.167	71.437	71.709	71.981	72.254	72.527
91	364	72.815	73.075	73.351	73.629	73.907	74.186	74.465	74.746	75.027	75.31
92	365	75.608	75.876	76.162	76.447	76.734	77.022	77.31	77.599	77.89	78.182
93	366	78.489	78.767	79.06	79.355	79.651	79.948	80.245	80.544	80.844	81.145
94	367	81.461	81.749	82.052	82.356	82.661	82.968	83.275	83.583	83.892	84.202
95	368	84.526	84.825	85.138	85.452	85.766	86.082	86.4	86.717	87.036	87.356
96	369	87.686	87.997	88.319	88.643	88.967	89.293	89.619	89.947	90.275	90.618
97	370	90.944	91.266	91.598	91.931	92.266	92.602	92.939	93.276	93.615	93.954
98	371	94.301	94.636	94.979	95.323	95.667	96.012	96.359	96.707	97.056	97.407
99	372	97.761	98.109	98.463	98.816	99.171	99.528	99.885	100.244	100.602	100.964
100	373	101.325	101.688	102.052	102.417	102.782	103.15	103.517	103.887	104.258	104.629
101	374	105.001	105.374	105.749	106.125	106.501	106.879	107.258	107.638	108.019	108.402

(Reproduced from: (i) Perry's Chemical Engineers' Handbook, 6th Edition, McGraw-Hill, USA, 1984 and (ii) SP:26-1983, Bureau of Indian Standards, New Delhi with permissions).

Solution:

Air – water vapour mixture with DB = 302 k and DP = 291 k

At DP, partial pressure of water in air is equal to vapour pressure of water.

P_A= partial pressure of water vapour in air= 2.0624 kpa.

P= total pressure = 100 k Pa

$$\text{Absolute moral humidity} = H_m = \frac{P_A}{P - P_A}$$

$$= \frac{2.0624}{100 - 2.0624}$$

$$= 0.02106 \frac{\text{Kmol water vapour}}{\text{Kmol dry air}} \quad \text{(a)}$$

Mol.wt.H_2O = 18

Mol.wt. air = 29

$$\text{Absolute Humidity}=H=H_m \times \frac{\text{Mol.wt.}H_2O}{\text{Mol.wt.air}}$$

$$=0.02106 \times \frac{18}{19}$$

$$=0.0131 \frac{\text{Kg water vapour}}{\text{kg dry air}} \text{ – –(b)}$$

At saturation,

DB=WB=DP=302 K

Vapour pressure at saturation (i.e)

302 k =P_s=4.004 kpa

$$\%RH = \frac{P_A}{P_s} \times 100$$

$$= \frac{2.0624}{4.004} \times 100$$

=51.51 ---(c)

$$\text{Saturation Humidity}=H_s = \left[\frac{P_s}{P - P_s}\right]\frac{\text{Mol.wt.}H_2O}{\text{Mol.wt.air}}$$

$$H_s = \left[\frac{4.004}{100 - 4.004}\right]\times\frac{18}{29}$$

$$=0.02589\,\frac{\text{kg water vapour}}{\text{Kg dry air}}$$

$$\%\ \text{saturation} = \frac{H}{HS}\times 100$$

$$= \frac{0.0131}{0.02589}\times 100$$

$$=50.60.....(d)$$

Humid heat =Cs=1.006+1.84H

=1.006+1.884 x 0.0131

=1.03kJ/Kg dry air k-.....(e)

$$\text{Humidityvolume} = V_H = \left[\left(\frac{H}{M_A}\right)+\left(\frac{1}{M_B}\right)\right]22.4136$$

$$\times\left[\frac{DB}{273}\right]\times\frac{101.325}{P}$$

$$=\left[\frac{0.131}{18}+\frac{1}{19}\right]22.4136\times\left[\frac{302}{273}\right]\times\frac{101.325}{100}$$

$=0.8846 m^3/kg$ dry air ....(f)

Ex.2.12

A mixture of acetone vapour and nitrogen contains 14.8% acetone by volume. Calculate the following at 293k (20°C) and a pressure of 99.33 Kpa.

(a) Partial pressure of acetone

(b) Moles of acetone per mole of nitrogen

(c) Relative saturation of mixture at 293k (20°C) and

(d) Percentage saturation of mixture at 293k

Data: vapour pressure of acetone at 293 k = 24.638 kpa

Solution:-

Basis: 1 kmol of mixture of acetone vapour and nitrogen at 293 k

Mole % acetone in mixture = volume % acetone

= 14.8%

P = total pressure = 99.33 Kpa

P_A = partial pressure of acetone vapour

y= Molefraction of acetone in mixture

$P_A = Y_A X P$

= 0.1488 x 99.33

= 14.7 kpa -------- (a)

Acetone in mixture = $\frac{14.8}{100} \times 1$

= 0.148 kmol

Nitrogen in gas mixture = 1 - 0.148

= 0.852 kmol

H_m = *Kmols* acetone per kmol nitrogen = $\frac{0.148}{0.852}$

= 0.174 ------ (b)

Vapour pressure of acetone at 293 K = 24.638 kpa

P_s = 24.638

% relative saturation = $\frac{P_A}{P_s} \times 100$

= $\frac{14.7}{24.638} \times 100$

= 59. 66 ------ (c)

consider one kmol of saturated mixture 293 k and 99.33 kpa

Volume % acetone = Pressure % acetone

$$= \frac{P_s}{P} \times 100$$

$$= \frac{24.638}{99.33} \times 100$$

$$= 24.80$$

Mole % acetone = Volume % acetone = 24.8

Acetone in saturated mixture = 0.248 x 1 = 0.248 kmol

Nitrogen in saturated mixture = 1 – 0.248 = 0.752

H_{ms} = Moles acetone per mole of nitrogen in saturated mixture = $\frac{0.248}{0.752}$

$$\% \text{ Saturation} = \frac{H_m}{H_{ms}} \times 100$$

$$= \frac{0.174}{0.329} \times 100$$

$$= 52.9 \quad \text{------} \quad \text{(d)}$$

Ex.2.13

an air – water vapour sample at 101.3 kPa has a DBT of 328 K and is 10% saturated with water vapour. Using the psychrometric chart determine the following:

a) The absolute humidity, kg water vapour per Kg dry air.

(b) The partial pressure of water vapour

(c) The absolute saturation humidity at 328 K

(d) The vapour pressure of water at 328 K

(e) The present relative saturation

(f) The dew point of the system

Solution

(a) Refer to Figure 8.4. Point *P* in the psychrometric chart corresponds to 328 K and 10% saturation. The *y*-coordinate of this point is read from the chart. This is the humidity at this condition. This is equal to 0.012 kg water vapour/kg dry air. Y = 0.012 kg water per kg dry air.

(b) Partial pressure is calculated using Eq. (8.7):

$$Y' = \frac{p_A}{P - p_A} \times \frac{18}{29} = 0.012$$

$0.0193(P - p_A) = p_A$ where P is 101.3 kPa. Solving this, we get p_A = 1.921 kPa.

(c) The saturation humidity at 328 K is obtained directly from the psychrometric chart by reading the y-coordinate of point Q where point Q is the point on the 100% saturation curve corresponding to temperature 328 K. This is found to be 0.115 kg water per kg dry air. Y_S = 0.115 kg water per kg dry air.

(d) Equation (8.8) can be used to calculate vapour pressure:

$$Y_s' = \frac{P_A^S}{P - P_A^S} \times \frac{M_A}{M_B} = \frac{P_A^S}{P - P_A^S} \times \frac{18}{29}$$

$$0.115 = \frac{P_A^S}{101.3 - P_A^S} \times 0.6207$$

Solving this, we get P_A^S = 15.8 kPa.

(*The vapour pressure at* 328 K *is found to be* 15.7 kPa *from steam tables, which compares well with the value calculated above.*)

(e) The percent relative saturation is obtained using Eq. (8.9):

$$\text{RS} = \frac{p_A}{P_A^S} \times 100\% = \frac{1.921}{15.8} \times 100\% = 12.16\%$$

(f) The dew point is obtained by moving from point P horizontally to the saturation curve and noting the temperature coordinate of point S. The dew point is found to be 290 K.

(*The vapour pressure at the dew point should be equal to the partial pressure of water in the air, which is obtained as* 1.921 *in part (b). Using steam tables, the vapour pressure at* 290 K *is found to be* 1.93 kPa.)

Ex.2.14

Estimate the molar volume of CO_2 at 500 K and 100 bar using the (a) Using the ideal gas equation and (b) Using the van der waals equation given that a = 0.364 N m^4/mol^2; b = $4.267X10^{-5}$ m^3/mol

Solution

(a) Using the ideal gas equation,

$$V = \frac{RT}{P} = \frac{8.314\,(500)}{(1\times10^{7})} = 0.4157\times10^{-3}\ \text{m}^3$$

(b) Volume can be determined using the van der Waals equation by trial and error. Here, $a = 0.364\ \text{N m}^4/(\text{mol})^2$ and $b = 4.267\times10^{-5}\ \text{m}^3/\text{mol}$. The van der Waals equation gives

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

Substituting the values, we get

$$\left(100\times10^{5} + \frac{0.364}{V^2}\right)(V - 4.267\times10^{-5}) = 8.314\times500$$

This can be simplified as

$$V^3 - 4.5837\times10^{-4}\,V^2 + 3.64\times10^{-8}V - 1.5582\times10^{-12} = 0$$

Solving this, the molar volume of CO_2 in the present case is found to be

$$V = 3.717\times10^{-4}\ \text{m}^3/\text{mol}.$$

Ex.2.15

- Natural gas is piped from the wall at 300K and 400 kPa. The gas is found to contain 93% methane, 4.5% ethane and rest the nitrogen. Calculate the following: **(Nov/Dec, 2016)**
    a. The partial pressure of nitrogen
    b. The pure component volume of ethane in 10 m^3 of the gas
    c. Density at standard conditions in kg/m^3
    d. Average molecular weight of the gas
    e. Composition in weight percent.

Solution

(a) Mole percent of nitrogen = 100 – 93.0 – 4.5 = 2.5. Therefore,

$$\text{mole fraction of nitrogen} = 2.5/100 = 0.025$$

Using Eq. (4.18),

$$\text{partial pressure of nitrogen} = \text{mole fraction of nitrogen} \times \text{total pressure}$$
$$= 0.025 \times 400 = 10 \text{ kPa}$$

(b) Using Eq. (4.23),

$$\text{pure-component volume of ethane} = \text{mole fraction of ethane} \times \text{total volume}$$
$$= (4.5/100) \times 10 = 0.45 \text{ m}^3$$

(c) *Basis:* 100 mol natural gas

The calculation are presented as follows:

Component	*Molecular weight*	*No. of moles*	*Weight* (g)	*Weight fraction*
Methane	16.032	93.0	16.032 × 93.0 = 1490.98	1490.98/1696.24 = 0.8790
Ethane	30.048	4.5	30.048 × 4.5 = 135.22	135.22/1696.24 = 0.0797
Nitrogen	28.014	2.5	28.014 × 2.5 = 70.04	(1 – 0.8790 – 0.0797) = 0.0413
Total		100.0	1696.24	1.00

$$\text{density} = \frac{\text{mass}}{\text{volume}}$$

The mass of 100 moles = 1696.24 g = 1.696 kg.
The volume of 100 moles at STP is

$$100(22.414 \times 10^{-3}) = 2.2414 \text{ m}^3$$

Therefore,

$$\text{density} = \frac{1.696}{2.2414} = 0.7567 \text{ kg/m}^3$$

(d) The volume at 300 K and 400 kPa is

$$V_0 \times \frac{P_0}{P} \times \frac{T}{T_0} = 2.2414 \left(\frac{101.325}{400}\right)\left(\frac{300}{273.15}\right) = 0.6236 \text{ m}^3$$

Therefore, the density at 300 K and 400 kPa is

$$\frac{1.696}{0.6236} = 2.7197 \text{ kg/m}^3$$

(e) The mass of 100 moles of the gas = 1696 g. Therefore,

average molecular weight = 1696.0/100 =16.96

(f) Composition in weight percent is obtained by multiplying the weight fraction by 100. Thus

Methane = 87.90%, Ethane = 7.97%, and Nitrogen = 4.13%

Ex.2.16

Estimate the density of chlorine gas at temperature of 503 K and 15.2 MPa pressure by using

(i) Ideal gas equation and

(ii) Van der Waals equation. Compare and analyse the results. **(April/May, 2015)**

Solution : Basis : 1 kmol of chlorine gas

(i) Using ideal gas law,

$$PV = nRT$$

$$PV = \frac{m}{M} RT$$

but

$$\rho = \frac{m}{V}$$

Molecular weight $= M = 71$ of chlorine

$P = 15.2$ MPa, $M = 71$

$R = 0.008314\ m^3 \cdot MPa/(kmol \cdot K)$

$T = 503$ K

$$\rho = \frac{15.2 \times 71}{0.008314 \times 503}$$

$$= 258.1\ kg/m^3$$

(ii) Using the van der Waals equation,

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

$a = 0.6354\ (m^3)^2 \cdot MPa/(kmol)^2$

$b = 0.0543\ m^3/kmol$

$$\left(15.2 + \frac{0.6354}{V^2}\right)(V - 0.0543) = 0.008314 \times 503$$

$$\left(15.2 + \frac{0.6354}{V^2}\right)(V - 0.0543) = 4.182$$

$$(15.2\,V^2 + 0.6354)(V - 0.0543) = 4.182\,V^2$$

$$15.2\,V^3 - 5.007\,V^2 + 0.6354\,V - 0.0345 = 0$$

Now, we will make use of the Newton-Raphson method to evaluate V. According to this method, if f(V) = 0 then

$$V_{n+1} = V_n - \frac{f(V_n)}{f'(V_n)}$$

where V_n is the starting root and V_{n+1} is the corrected root.

To start with V_0 may be taken as the volume obtained by using the ideal gas law. Determine V_1 using V_0. Compare V_0 and V_1. If they are close enough then V_1 is the final root. If there is large difference between V_0 and V_1, evaluate V_2 and so on. We will get the exact root within four–five iterations.

$$V_0 = \frac{nRT}{P}$$

$$= \frac{1 \times 0.008314 \times 503}{15.2}$$

$$= 0.275 \text{ m}^3$$

$$f(V) = 15.2\,V^3 - 5.007\,V^2 + 0.6354\,V - 0.0345$$

$$f'(V) = 45.6\,V^2 - 10.014\,V + 0.6354$$

1st iteration : Put the value of V_0 in the above two equations to evaluate $f(V_0)$ and $f'(V_0)$.

$$f(V_0) = 15.2\,(0.275)^3 - 5.007\,(0.275)^2 + 0.6354\,(0.275) - 0.0345$$

$$= 0.0777$$

$$f'(V_0) = 45.6\,(0.275)^2 - 10.014\,(0.275) + 0.6354$$

$$= 1.3301$$

$$V_1 = V_0 - \frac{f(V_0)}{f'(V_0)}$$

$$= 0.275 - \frac{0.0777}{1.3301}$$

$$= 0.2166$$

As there is large difference between V_1 and V_0, evaluate V_2.

2nd iteration :

$$f(V_1) = 15.2\,(0.2166)^3 - 5.007\,(0.2166)^2 + 0.6354\,(0.2166) - 0.0345$$
$$= 0.02273$$
$$f'(V_1) = 45.6\,(0.2166)^2 - 10.014\,(0.2166) + 0.6354$$
$$= 0.6057$$
$$V_2 = V_1 - \frac{f(V_1)}{f'(V_1)}$$
$$= 0.2166 - \frac{0.02273}{0.6057}$$
$$= 0.1791$$

3rd iteration :

$$f(V_2) = 15.2\,(0.1791)^3 - 5.007\,(0.1791)^2 + 0.6354\,(0.1791) - 0.0345$$
$$= 0.00652$$
$$f'(V_2) = 45.6\,(0.1791)^2 - 10.014\,(0.1791) + 0.6354$$
$$= 0.3046$$

$\therefore$
$$V_3 = V_2 - \frac{f(V_2)}{f'(V_2)}$$
$$= 0.1791 - \frac{0.00652}{0.3046}$$
$$= 0.1577$$

4th iteration : Evaluate V_4

$$f(V_3) = 15.2\,(0.1577)^3 - 5.007\,(0.1577)^2 + 0.6354\,(0.1577) - 0.0345$$
$$= 8.1258 \times 10^{-4}$$
$$f'(V_3) = 45.6\,(0.1577)^2 - 10.014\,(0.1577) + 0.6354$$
$$= 0.1902$$

$$\therefore \quad V_4 = V_3 - \frac{f(V_3)}{f'(V_3)}$$

$$= 0.1577 - \frac{8.1258 \times 10^{-4}}{0.1902}$$

$$= 0.1534$$

$V_3 = 0.1577$ and $V_4 = 0.1534$.

As V_3 and V_4 are close enough, we stop the iteration and hence V_4 is the final root.

$\therefore$ Volume of Cl_2 gas $= V = V_4 = 0.1534\ m^3$.

This is the volume of 1 kmol of Cl_2, it means it is a volume of 71 kg of Cl_2.

$\therefore$ Density as per the Van der Waals equation is

$$\rho = \frac{m}{V}$$

$$= \frac{71}{0.1534}$$

$$= 462.84\ kg/m^3 \qquad \text{... Ans. (ii)}$$

Ex.2.17

Calculate the pressure developed by one kmol gaseousammonia contained in a vessel of 0.6 m3 capacity at a constant temperature of 473 K by the following methods:

- **Using the ideal gas equation**
- **Using the van der waals equation given that a = 0.4233 N m^4/mol^2; b = 3.73X10^{-5} m^3/mol**

Solution

(a) Use the ideal gas equation, $P = \frac{RT}{V}$, where V is the molar volume of the gas. Here, $V = 0.6 \times 10^{-3}$ m^3/mol. Therefore, the pressure given by the ideal gas equation is

$$P = \frac{8.314 \times 473}{0.6 \times 10^{-3}} = 6.554 \times 10^6 \text{ N/m}^2 = 65.54 \text{ bar}$$

(b) The van der Waals equation may be rearranged as

$$P = \frac{RT}{V - b} - \frac{a}{V^2}$$

Substituting of the values gives

$$P = \frac{8.314\,(473)}{(0.6 \times 10^{-3}) - (3.73 \times 10^{-5})} - \frac{0.4233}{(0.6 \times 10^{-3})^2} = 5.8128 \times 10^6 \text{ N/m}^2 = 58.13 \text{ bar}$$

Ex.2.18

Carburetted water gas has the following composition by volume: Hydrogen 35.2%, Methane 14.8% Ethylene 12.8%, Carbon dioxide 1.5%, Carbon monoxide 33.9% and Nitrogen 1.8%.

The gas is available at 773.15 K (500°C) and 4 bar a. Find the molar volume of the mixture using (a) the ideal gas law, and (b) the van der Waals equation.

Ideal gas law: $pV = RT$

$$p = 4 \text{ bar } a$$

$$T = 773.15 \text{ K}$$

$$V = \frac{RT}{p}$$

$$= 0.08314 \times \frac{773}{4} = 16.067 \text{ L/mol}$$

Composition of Carburetted Water Gas

Component	Formula	Mole Fraction	Critical temp., K		Critical pressure, bar a	
		y_i	T_c	$y_i \cdot T_c$	p_c	$y \cdot p_c$
Hydrogen	H_2	0.352	32.20	11.334	12.97	4.57
Methane	CH_4	0.148	190.50	28.194	46.04	6.81
Ethylene	C_2H_4	0.128	282.34	36.140	50.39	6.45
Carbon monoxide	CO	0.339	132.85	45.036	34.94	11.84
Carbon dioxide	CO_2	0.015	304.12	4.562	73.74	1.11
Nitrogen	N_2	0.018	126.09	2.270	33.94	0.61
Total		1.000		127.536		31.39

$$a = \frac{27R^2T_c^2}{64p_c}$$

$$= \frac{\left[27\,(0.083\,14)^2 \times (127.536)^2\right]}{(64 \times 31.39)} = 1.511\ \text{L}^2 \cdot \text{bar/(mol)}^2$$

$$b = \frac{RT_c}{8p_c}$$

$$= 0.083\ 14 \times \frac{127.536}{(8 \times 31.390)} = 0.042\ 32\ \text{L/mol}$$

substituting these values,

$$\left(4 + \frac{1.511}{V^2}\right)(V - 0.042\ 32) = 0.083\ 14 \times 773.15$$

$$= 64.267\ 22$$

Solving the equation by the Newton-Raphson method,

$$V = 15.74\ \text{L/mol}$$

Mathcad solution:

Define $f(V) := \left[4 + \frac{1.511}{V^2}\right](V - 0.04232) - 64.267\ 22$

Guess $V := 16.067$

soln := root $(f(V), V)$

soln = 16.086 L/mol

Ex.2.19

Ambient air on a particular day in Ahmedabad has the following condition

Total pressure = 100 kPa (750 torr)

Dry bulb temperature = 308.15 K (35°C)

Dew point = 294.45 K (21.3°C)

Find the absolute humidity of the air

Data: Vapour pressure of water at 294.45 K = 2.5326 kPa = 19 torr

Solution

Partial pressure of water

vapour in the air, p_w = vapour pressure for water at dew point

= 2.5326 kPa

Now, according to Dalton's law),

$$\frac{(\text{Moles of water vapour})}{(\text{Moles of dry air})} = \frac{(\text{Partial pressure of water vapour})}{(\text{Partial pressure of air})}$$

$$= \frac{2.5326}{100 - 2.5326} = \frac{2.5326}{97.4674}$$

$$\frac{(\text{Mass of water vapour})}{(\text{Mass of dry air})} = \frac{2.5326}{97.4674} \times \frac{(\text{Molar mass of water})}{(\text{Molar mass of air})}$$

$$= \frac{2.5326}{97.4674} \times \frac{18.0153}{28.9679}$$

$$= 0.016\ 16 \text{ kg/kg}$$

$$\equiv 16.16 \frac{\text{g water vapour}}{\text{kg dry air}}$$

Ex.2.20

The analysis of a sewage gas sample from a municipal sewage treatment plant is given below on a volume basis:

Methane	68%
Carbon dioxide	30%
Ammonia	2%
H_2S, SO_2, etc.	Traces

Find (a) the average molar mass of the gas; and (b) the density of the gas at NTP.

[*Ans.* (a) 24.42; (b) 1.09 kg/m^3]

Basis: 100 kmol sewage gas

Gas	Formula	Molar mass	kmol	kg	mass %
Methane	CH_4	16	68	1088	44.55
Carbon dioxide	CO_2	44	30	1320	54.05
Ammonia	NH_3	17	2	34	1.40
Total	—	—	100	2442	100.00

Average molar mass = 2442/100 = 24.42

Density of sewage gas = 24.42/22.414

= 1.09 kg/m^3

Ex.2.21

A weight of 1.10 kg of carbon dioxide occupies a volume of 33 L at 300.15 K(27°C). Using the van der Waals equation of state, calculate the pressure.

Data: For CO_2, take $a = 3.60$ $[(m^3)^2.kPa]/(kmol)^2$ and $b = 4.3 \times 10^{-2}$ $m^3/kmol$

[*Ans.* 19.52 bar a]

Basis: 1.10 kg CO_2

$$\text{Moles of } CO_2 = 1.10/44 = 0.025 \text{ kmol}$$

$$\text{Volume occupied} = 33 \text{ L} = 0.033 \text{ m}^3$$

$$V = 0.033/0.025 = 1.32 \text{ m}^3/\text{kmol}$$

$$T = 300 \text{ K}$$

van der Waals equation:

$$\left(p + \frac{a}{V^2}\right)(V - b) = RT$$

$$\left(p + \frac{3.6}{1.32^2}\right)(1.32 - 0.043) = 0.008\,314 \times 300 \times 1000$$

Solving the equation, $p = 1951.104$ kPa

$\equiv 19.51$ bar

Ex.2.22

Calculate the density of chlorine gas 503.15 K(230°C) and 152 bar a using (a) the ideal gas law, and (b) the van der Waals eauation.

[*Ans.* (a) 258.1 kg/m^3; (b) 464.05 kg/m^3]

For chlorine gas:

(a) Ideal gas law:

$$p = 15.2 \text{ MPa a}, \quad T = 503.15 \text{ K } (230°\text{C})$$

$$V = RT/p = 0.008\ 314 \times 503.15/15.2$$

$$= 0.2752 \text{ m}^3/\text{kmol}$$

$$\text{Density} = \frac{\text{Molar mass}}{V} = \frac{71}{0.2752} = 258.0 \text{ kg/m}^3$$

(b) van der Waals equation: For chlorine,

$$p_c = 79.77 \text{ bar a} \quad \text{and} \quad T_c = 416.90 \text{ K}$$

$$a = 27\ R^2\ \text{T}^2/64\ p_c$$

$$= 27(0.083\ 14)^2(416.90)^2/(64 \times 79.77)$$
$$= 6.353\ 72\ (\mathrm{m}^3)^2 \cdot \mathrm{bar}/(\mathrm{kmol})^2$$
$$b = RT_c/8p_c = 0.083\ 14 \times 416.9/(8 \times 79.77)$$
$$= 0.054\ 314\ \mathrm{m}^3/\mathrm{kmol}$$

Substituting in the van der waals equation

$$\left(152 + \frac{6.353\ 72}{V^2}\right)(V - 0.054\ 314) = 0.083\ 14 \times 503.15 = 41.831\ 89$$

Simplifying,

$$152\ V^3 - 5.0075\ V^2 + 0.6354\ V - 0.03451 = 0$$

Solving the equation by Newton-Raphson method.

$$V = 0.152\ \mathrm{m}^3/\mathrm{kmol}$$
$$\text{Density} = 71/0.152 = 467.1\ \mathrm{kg}/\mathrm{m}^3$$

Mathcad Solution:

$$V := 0.1$$

$$F(V) := \left(152 + \frac{6.35372}{V^2}\right) \cdot (V - 0.054314 - 41.8318$$

$$V := \mathrm{root}(F(V), V)$$

$$V = 0.1536\ \frac{\mathrm{m}^3}{\mathrm{kmol}}$$

$$\text{Density} = 71/0.1536 = 462.24\ \mathrm{kg}/\mathrm{m}^3$$

Ex.2.23

In the manufacture of nitric acid, initially ammonia and air are mixed at 7.09 bar g and 923 K (650°C). The composition of the gas mixture (by volume) is as follows:

Nitrogen	70.5%
Oxygen	18.8%
Water	1.2%
Ammonia	9.5%

Find (i) the density of the gas mixture using (a) ideal gas law, and (b) the van der Waals equation and (ii) the specific gravity of the gas mixture.

[*Ans.* (i) (a) 2.912 kg/m^3; (b) 2.907 kg/m^3; (ii) 0.952]

(a) Ideal gas law:

$$p = 7.09 + 1.013\ 25 = 8.103\ 25$$

$$T = 923.15\ \text{K}\ (650\ ^\circ\text{C})$$

$$V = \frac{RT}{p} = \frac{0.08314 \times 923.15}{8.103\ 25}$$

$$= 9.472\ \text{m}^3/\text{kmol}$$

$$\text{Density} = 27.587/9.472 = 2.912\ \text{kg/m}^3$$

(b) van der Waals equation:

$$a = \frac{27\ R^2 T_c^2}{64\ p_c} \quad \text{and} \quad b = RT_c/8p_c$$

where p_c and T_c are pseudo critical properties.

Gas	Mole fraction n_i	Molar mass M_i	Mass kg $n \cdot M_i$	Critical temp T_c, K		Critical pressure p_c, bar	
				T_c	$n_i \cdot T_c$	p_c	$n_i \cdot p_c$
N_2	0.705	28	19.740	126.09	88.893	33.94	23.93
O_2	0.188	32	6.016	154.58	29.061	50.42	9.48
H_2O	0.012	18	0.216	643.30	7.718	221.2	2.65
NH_3	0.095	17	1.615	405.50	38.523	113.50	10.78
Total	1.000	—	27.587		164.195		46.84

Pseudo critical temperature, $T_c = 164.195$ K

Pseudo critical pressure, $p_c = 46.84$ bar

$$a = [27(0.083\ 14)^2(164.195)^2]/(64 \times 46.84)$$
$$= 1.678\ (m^3)^2 \cdot bar/(kmol)^2$$
$$b = (0.083\ 14 \times 164.195)/(8 \times 46.84)$$
$$= 0.036\ 42\ m^3/kmol$$

Substituting the values in the equation,

$$\left(8.1 + \frac{1.679}{V^2}\right)(V - 0.036\ 43) = 0.083\ 14 \times 923.15 = 75.7507$$

Mathcad Solution:

$$V := 9.47$$

$$F(V) := \left(8.10325 + \frac{1.678}{V^2}\right) \cdot (V - 0.03642) - 75.750$$

$$V := \text{root}(F(V), V)$$

$$V = 9.3626$$

Average molar mass = 27.587

Density = 27.587/9.3626 = 2.9465 kg/m^3

Specific gravity of the gas mixture = 27.587/28.96 = 0.952 *Ans.*

Ex.2.24

The Orsat (dry) analysis of the flue gas from a boiler house is given as (volume basis); CO_2: 10.0%, O_2: 7.96%, N_2: 82.0% and SO_2: 0.04%. The temperature and pressure of flue gases are 463 K (190°C) and 100 kPa (750 torr), respectively. The dew point of the gas is found to be 320 K (20°C). Find the absolute humidity of the flue gases

Basis: 100 kmol flue gases

Gas	Molar mass	kmol	kg
CO_2	44	10.00	440.00
O_2	32	7.96	254.72
N_2	28	82.00	2296.00
SO_2	64	0.04	2.56
Total	—	100.00	2993.28

$$\text{Avg. molar mass} = 29.933 \text{ (of dry gas)}$$

$$\text{Partial pressure of water} = \text{Vapour pressure of water at } DP$$

$$= 10.612 \text{ kPa} \qquad \text{(ref. Chapter 6)}$$

$$\text{Absolute humidity} = [10.612 \times 18]/[(100 - 10.612)\ 29.933]$$

$$= 0.071\ 39 \text{ kg/kg} \equiv 71.39 \text{ g/kg}$$

Ex.2.25

A domestic liquefied petroleum gas (LPG) cylinder, conforming to IS:4576, is stored at 313.15 K (40°C). It is a mixture of 30% propane, 45% *n*-butane, and 25% *i*-butane by volume. Calculate (a) average molar mass of LPG, (b) specific gravity of LPG, and (c) pressure in the cylinder.

Date: Vapour pressures of propane, *n*-butane and *i*-butane are 1350, 383 and 535 kPa, respectively at 313.15 K(40°C). [Ref. : Table 5.4]

[*Ans.* : (a) 53.916, (b) 2.25 and (c) 7.11 bar a]

Basis: 1 kmol LPG mixture at 313.15 K (40 °C).

Gas	kmol	Molar mass	kg
C_3H_8	0.30	44.0956	13.229
n-C_4H_{10}	0.45	58.1222	26.155
i-C_4H_{10}	0.25	58.1222	14.531
Total	1.00		53.915

Average molar mass = 53.915

$$\text{Specific gravity} = \frac{53.915}{28.97} = 1.861$$

Gas	kmol	Vapour pressure bar	Partial pressure bar
C_3H_8	0.30	13.975	4.1925
n-C_4H_{10}	0.45	3.773	1.6979
i-C_4H_{10}	0.25	5.290	1.3225
Total	1.00		7.2129

Pressure in LPG cylinder = 7.2129 bar

2 litres of NH_3 at 303 K (30°C) and 20.265 kPa is neutralized by 135 ml of solution of H_2SO_4. Find the normality of the acid.

Solution:-

Basis : 2 litres of NH_3

PV = nRT

n = moles of NH_3 = PV/RT

where P = 20.265 kPa, T = 303 K, R = 8.31451 m^3. kPa/kmol K (Or) R = 8.31451 lit. kPa/mol K, V = 2 lit.

Moles of NH_3 = $\frac{20.265 \times 2}{8.31451 \times 303}$ =0.01609 mol.

$2NH_3 + H_{2}SO_4 \rightarrow (NH_4)_2 SO_4$

For neutralization of 2 moles of NH_3, 1 mole of H_2SO_4 is required.

Moles of H_2SO_4 required $= \frac{1}{2} \times 0.01609$ = 8.045×10^{-3} mol

Grams of H_2SO_4 required = $8.045 \times 10^{-3} \times 98 = 0.788$ gm.

Cm-equivalents of H_2SO_4 required = 0.788/49 = 0.01609

Volume of H_2SO_4 solution = 135 ml = 0.135 lit.

Normality of acid = $\frac{0.01609}{0.135}$ = 0.12 N.


Thank You

Process Calculations for Biotechnologist - 21BT32T

Unit - 3

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UNIT 3 MATERIAL BALANCE

Basics of unit operations and unit processes involved in biotechnology industries and its applications. Material balance concept – overall & component – material balance applications for evaporator, gas absorber without reaction, Distillation (Binary system), Liquid extraction, solid-liquid extraction, drying, crystallization, Humidification, Reverse Osmosis separation, Mixing and fermentation, Recycle, Purge and Bypass illustration.

Total period required = 15 (9 + 6)

COURSE OUTCOMES

Apply the material balance concepts in various industrial processes

Material balance.

The material balance calculations gives information regarding material entering, leaving accumulated or depleted during unit operation or unit process.

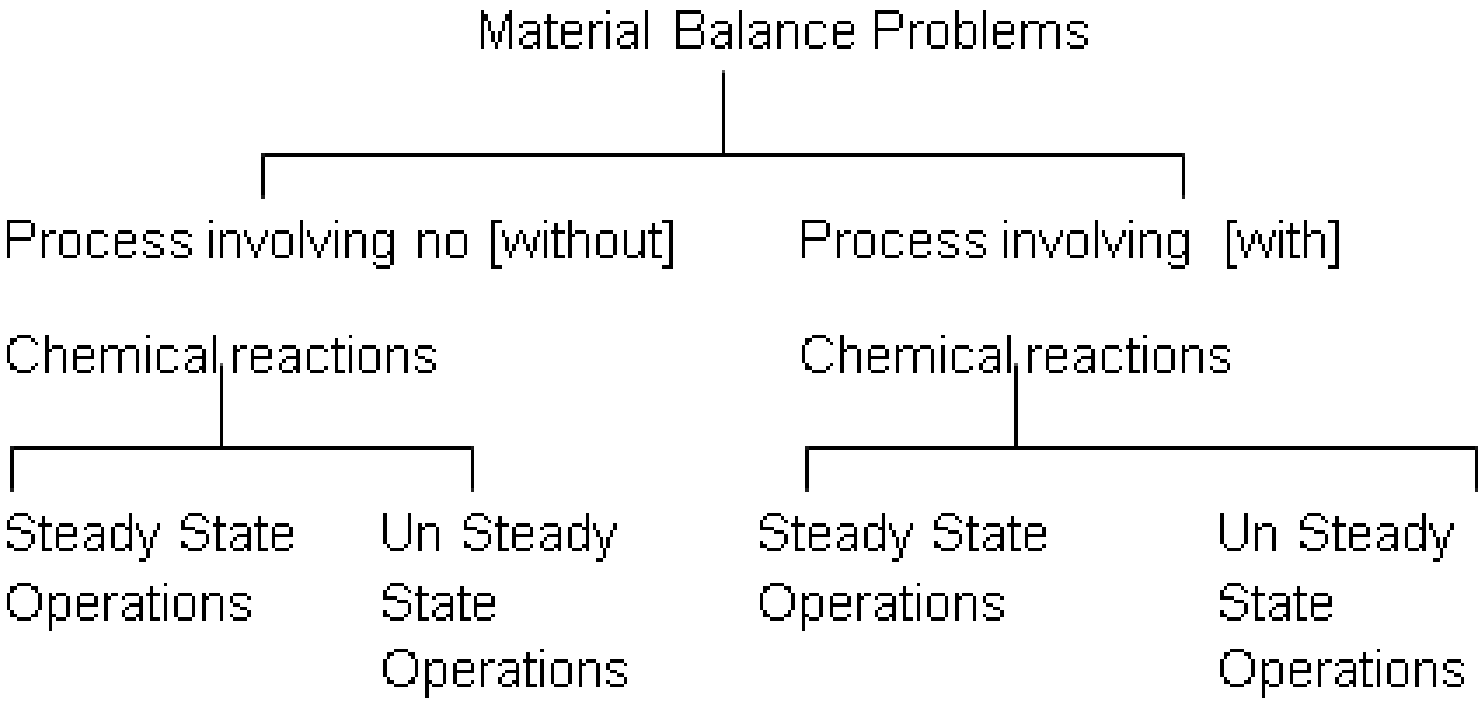
Law of conservation of mass.

It states that the total mass of various components involved remains constant during an unit operation or unit process.

Important points includes in a process flow sheet

- Flow rate or quantity of each stream.
- Operating conditions of each stream, such as pressure and temperature.
- Heat added / removed in particular equipment.
- Any specific information which is useful in understanding the process. For example symbolic presentation of a hazard, safety precautions. Sequence of flow if it is a batch process, corrosive nature of materials etc.

Classification of Material Balances



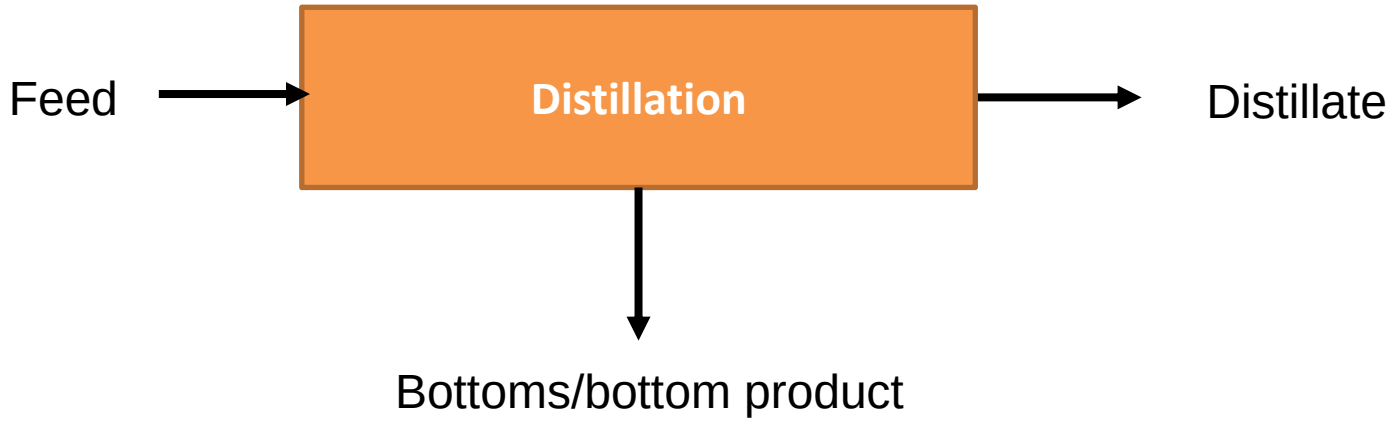
Input = Output + accumulation + disappeared.

Guidelines for solving material balance problems where in no chemical reaction is involved.

- The basis of calculations should be specified in weight units e.g. gm (or) Kg.
- When system involves a component material the quantity of which remains unchanged during a given operation, then for simplification of problem, make the material balance of such material [Tie – material].
- Whenever we are dealing with the system involving inert chemical species the calculations are simplified by making the material balance of inert chemical species.
- Whenever system involves two or more components and if all the components are undergoing change simultaneously during a particular operation.

Block diagram of Distillation process

Block diagram of distillation for binary system (A+B)



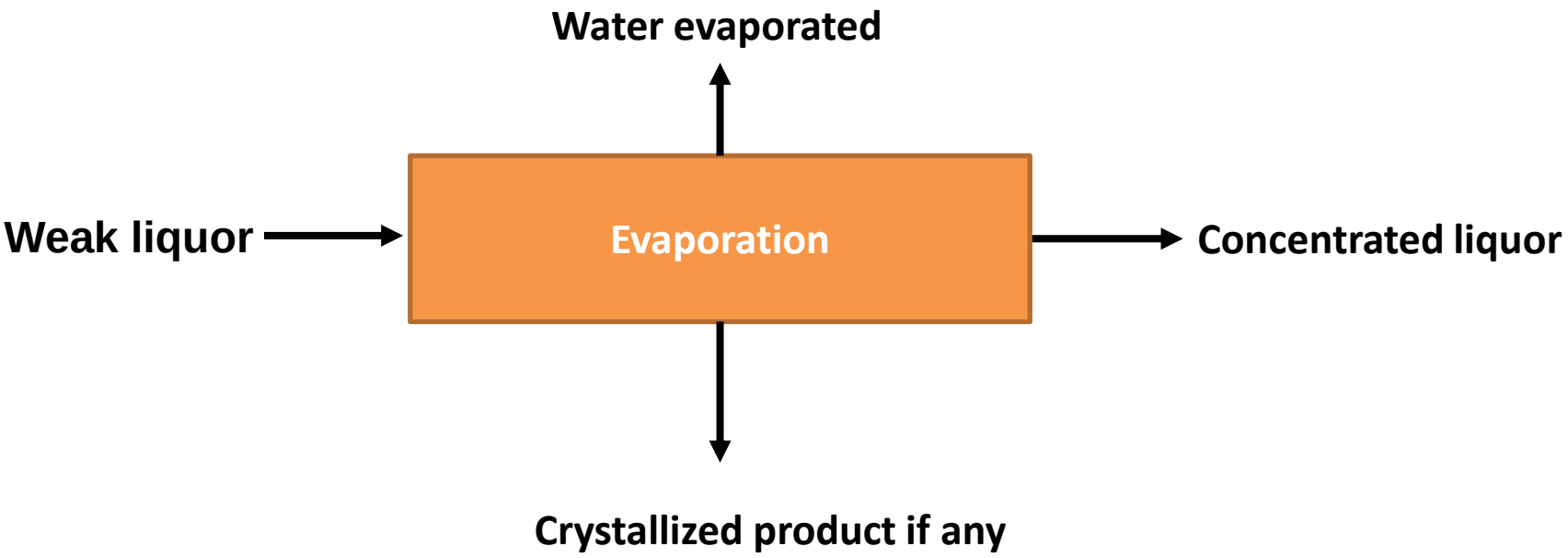
Overall Material Balance

Feed = Distillate + bottoms (on weight or mole basis)

Individual material balance of component ‘A’.

‘A’ in feed = ‘A’ in Distillate + ‘A’ in bottoms

Block Diagram of Evaporation



Overall Material Balance:

Weak liquor = Water evaporated + Crystallized – Product + thick liquor

If crystallized product is nil, then:

Weak liquor = water evaporated + thick liquor

Solid balance:

Solids in weak liquor = Solids in thick liquor

Water balance.

Water in weak liquor = Water evaporated + water in thick liquor

Wood containing 40% moisture is dried to 5% moisture. Calculate the loss of water by evaporation?

In a textile mill, a single – effect evaporated system concentrates weak liquor containing 4% solids (by weight) of caustic soda to produce a lye containing 25% solids (by weight). Calculate the evaporation of water per 1000 kg feed in the evaporator

The spent acid from a nitrating process contains 33% H_2SO_4 36% HNO_3 and 31% water by weight. This acid is to be strengthened by the addition of concentrated sulphuric acid containing 95% $H_2SO_4$and concentrated nitric acid containing 78% HNO_3. The strengthened mixed acid is to contain 40% H_2SO_4 and 43% HNO_3. Calculate the quantities spent and concentrated acids that should be mixed together to yield 1500 kg of the desired mixed acid.

- Basis: 1500 kg of the desired mixed acid.
- Let, x=wt. of waste acid (in kg) required
- Y=wt. of conc H_2SO_4 (in kg) required
- Z= wt. of conc. HNO_3 (in kg) required
- Overall material balance is,
- X+y+z=1500 ….(1)
- Sulphuric acid balance is,
- 0.33x+0.95y=0.4 $\times$1500=600 ……(2)
- Nitric acid balance is,
- 0.36x + 0.78z=0.43$\times$1500=645 …..(3)

- Putting (4) and (5) in Eq. (1)

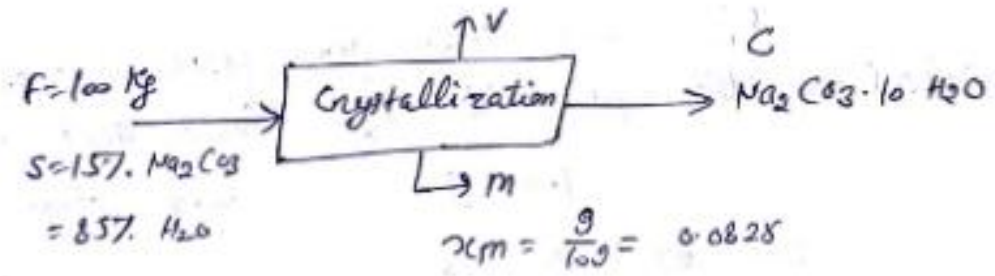
- From (4), y=556.1 kg
- From(5), z=726.6 kg.
-
- Weights of waste (spent) acid, conc. H_2SO_4 and co. HNO_3 required are 217.3, 556.1 and 726.6 kg respectively.

10000 kg of mixed acid of composition 40% H_2SO_4, 45% HNO_3 and 15% H_2O is to be produced by strengthening waste acid of composition 30% H_2SO_4, 36% HNO_3 and 34% H_2O by weight. Concentrated sulphuric acid of strength 95% and concentrated nitric acid containing 80% are available for this purpose. How many kilograms of spent acid and concentrated acids are to be mixed together?

An aqueous solution of Na_2CO_3 contains 15% carbonate by weight. 80% of the carbonate is recovered as Na_2CO_3 at 278 K is 9.0 % (wt.). on the basis of 100 kg of solution treated, determine the following:

a. The quality of crystals formed

b. The amount of water evaporated



F=C+M+V

$$F \times 0.15 = M \times 0.09 + C \times (\frac{106}{288})$$

Na_2CO_3 = $F \times 0.15 = 15kg$

80% Na_2CO_3 = $0.8 \times 15 = 12kg$

$$C \times \frac{106}{288} = 12$$

C=32.37 kg

M=33.36 kg

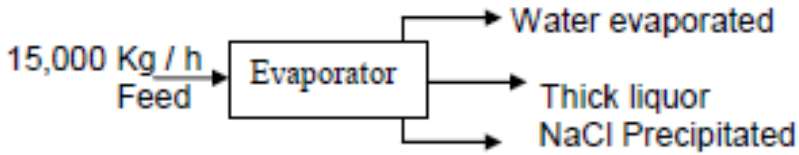
V=34.26 kg

An evaporator is fed with 15,000 kg/ hr of a solution containing 10% NaCl, 15% NaOH and rest water. In operation water is evaporated and NaCl is precipitated as crystals. The thick liquor leaving the evaporator contains 45% NaOH, 2% NaCl and rest water.

Calculate :

- **Kg / h water evaporated**
- **Kg / h salt precipitated**
- **Kg / h thick liquor.**

Basis: 15,000 Kg / h of solution fed to the evaporator



Let x, y and Z be the Kg/h of water evaporated, thick liquor and NaCl precipitated as crystals
overall Material Balance:
II

input = Output

15,000= x + y + z

Material Balance of NaOH :

$$\begin{Bmatrix} NaCl \text{ in feed} \\ \text{solution} \end{Bmatrix} = \begin{Bmatrix} NaCl \text{ in thick} \\ \text{liquor} \end{Bmatrix} + \begin{Bmatrix} NaCl \text{ precipitated} \\ \text{as crystals} \end{Bmatrix}$$

$0.1\,x\ 15{,}000 = 0.02 \times 5000 + Z$

$Z = 1400$ kg / hr

$x + y + z = 15{,}000$

$x+5000+1400 = 15000$

$x = 8600$ kg / hr

Check ⇒

Material Balance of water:

$$\begin{bmatrix} \textit{Water in} \\ \text{feed solution} \end{bmatrix} = \begin{bmatrix} \text{Water} \\ \text{evaporated} \end{bmatrix} + \begin{bmatrix} \textit{Water} \text{ in thick} \\ \textit{liquor} \end{bmatrix}$$

$$0.75 \times 15000 = 8600 + 0.53 \times 5000$$

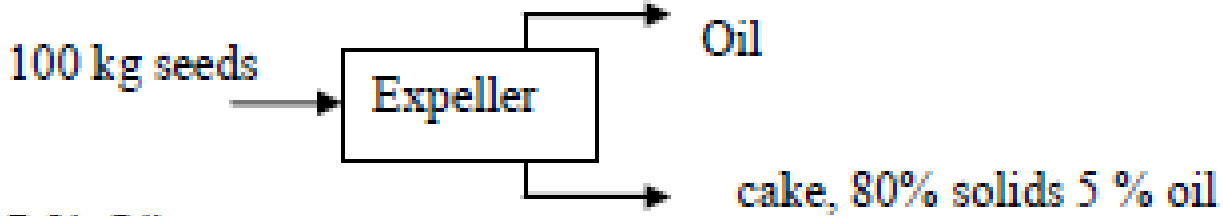
$$11250 = 8600 + 2650$$
$$11250 = 11250$$

$\therefore$ Water evaporated = 8600 kg/h

Thick liquor obtained = 5000 kg/h
NaCl precipitated as crystals = 1400 kg/h

The ground nut seeds containing 45% oil and 45% solids are fed to expeller, the cake coming out of expeller is found to contain 80% solids and 5% oil. Find the percentage recovery of oil.

Basis: 100 kg of ground nut seeds.



45 % Oil
45% Solids

Let x be the Kg of cake obtained

Material Balance of solids:

Solids in seeds = Solids in cake

0.45 x 100 = 0.8 x

$x = 56.25$ Kg

Material balance of oil:

Oil in seeds = Oil in cake + Oil recovered

0.45 x 100 = 0.05 x 56.25 + Oil recovered

∴ Oil recovered = 45 – 2.81

= 42. 19 Kg

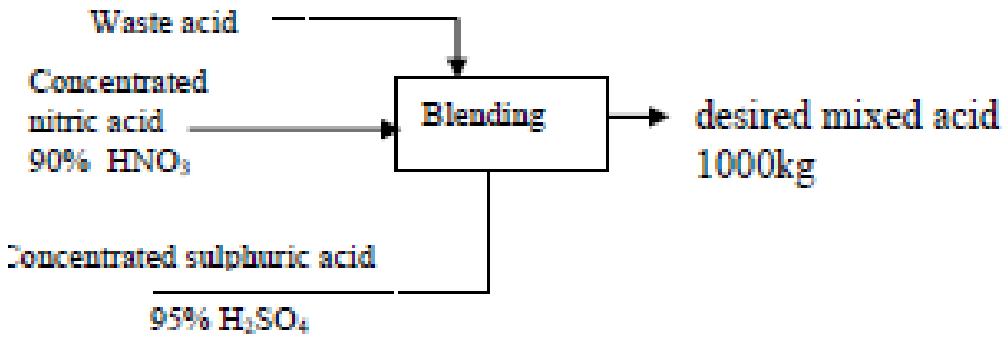
$$\%\text{Recovery of oil} = \frac{Kg \text{ oil recovered}}{Kg \text{ oil in seeds}} x100$$

$$= \frac{42.19}{45} x100$$

$$= 93.76$$

The waste acid from a nitrating process containing 20% HNO_3, 55% H_2SO_4 and 25% H_2O by weight is to be concentrated by addition of Concentrated sulphuric acid containing 95% H_2SO_4 and concentrated nitric acid containing 90% HNO_3 to get desired mixed acid containing 26% HNO_3 and 60% H_2SO_4. Calculate the quantities of waste and concentrated acids required for 1000 kg of desired mixed acid.

Basis: 1000 Kg of desired mixed acid



Let x, y, and Z be the kg of waste acid, concentrated sulphuric acid and concentrated nitric acid required to make 1000 kg desired acid.

Overall Material Balance:

$x + y + z = 1000$ ---------- (1)

Material Balance of H_2SO_4

$$0.55\,x + 0.95\,y = 0.6 \times 1000$$
$$0.55\,x + 0.95\,y = 600 \quad \text{---------- (2)}$$

$$y = 600 - \frac{0.55x}{0.95}$$

$$\therefore \quad y = 631.58 - 0.58\,x \quad \text{---------- (3)}$$

Material Balance of HNO_3:

$$0.2x + 0.90\,z = 0.26 \times 1000$$
$$0.2x + 0.9z = 260 \quad \text{---------- (4)}$$

$$z = 260 - \frac{0.2x}{0.9}$$

$$\therefore \quad z = 288.9 - 0.222x \text{ ---------- (5)}$$

Put values of y and z from equations (3) and (5) in equation (1) and solve for x.

$x + (631.58 - 0.58\,x) + (288.9 - 0.222x) = 1000$

$0.198\,x = 79.52$

$x = 401.6$ kg

We have, $y = 631.58 - 0.58\,x$

$y = 631.58 - 0.58 \times 401.6$

$y = 398.65$ kg

we have, $z = 288.9 - 0.222\,x$

$z = 288.9 - 0.222 \times 401.6$

$= 199.75$ kg

RESULT:

∴ Waste acid required = 401.6 kg.

Concentrated sulphuric acid = 398. 65 kg.
Concentrated nitric acid = 199.75 kg

Spent lye obtained from a soap manufacturing unit contains 9.6% glycerol and 10.3% salt. It is concentrated at a rate of 4000 kg/h is a double effect evaporator until the final solution contains 80% glycerol and 6% salt. Assume that 5% glycerol is lost in entrainment. Calculate the evaporation taking place in the system and the amount of salt crystallised in the evaporator

Basis: 4000 kg/h of spent lye sample.

Glycerol in sample = 0.096 x 4000

= 384 kg/h

salt in sample = 0.103x 4000

= 412 kg/h

Let x be the kg/h of solution in evaporator.

Glycerol in final solution = 0.80 x kg/h

Entrainment loss of glycerol = 0.05 x 0.80x

= 0.04 x kg/h

Material Balance of glycerol:

Glycerol in sample	=	glycerol in solution	+	glycerol lost as entrainment
384	=	0.8 x		+ 0.04 x
x	=	457 kg/h		

Salt in final solution in Evaporator = 0.06 x 457

= 27.42 kg/h

Salt material Balance:

Salt in glycerol = Salt in final solution + salt crystallised

Salt crystallised = 412 – 27.42
= 384.58 kg/h

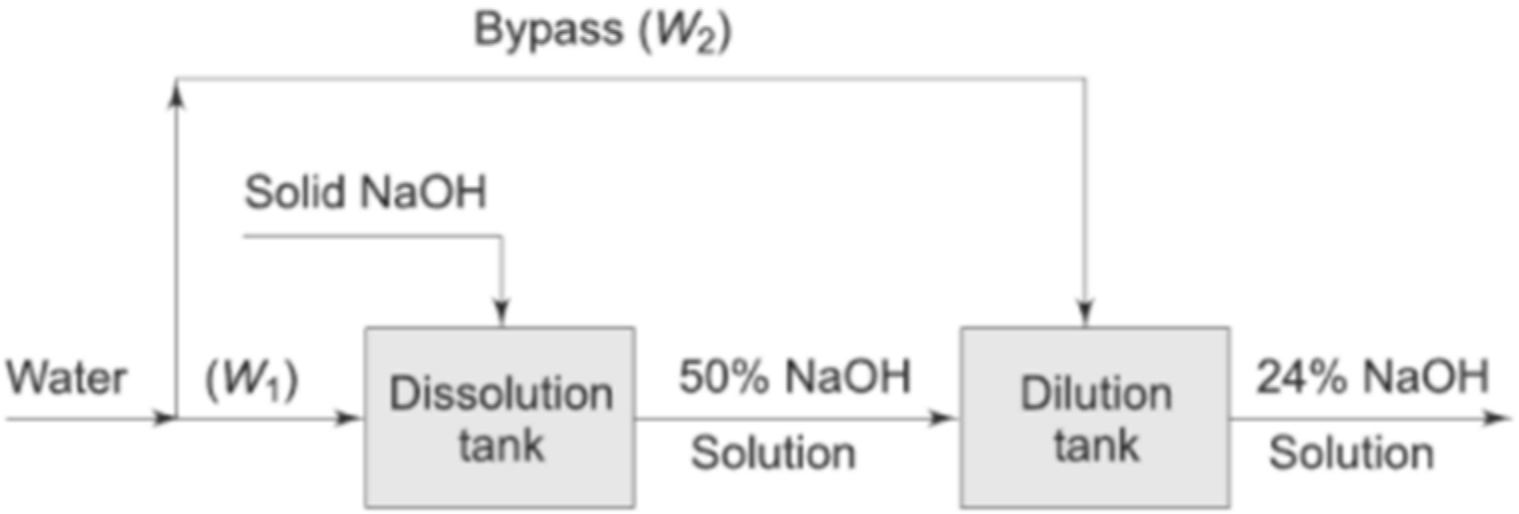
Evaporation

Taking place = kg/h of sample – kg/h of solution
- kg/h salt crystallised

= 4000 – (457 + 384. 58)
= 3158.41 kg/h

In a textile industry, it is desired to make a 24% solution (by mass) of caustic soda for a mercerisation process. Due to the very high heat of dissolution of caustic soda in water, the above solution is prepared by a two-step process.

First, in a dissolution tank, caustic soda is dissolved in the correct quantity of water to produce 50% (by mass) solution. After complete dissolution and cooling, the solution is taken to dilution tank where some more water is added to produce 24% solution. The two-step process is shown in Fig.



Let 'X' be kg of 50% solution

Material balance of caustic soda (solid):

Solid caustic soda charged to dissolution tank = solid caustic

Soda in 50% NaoH solution:

$$100 = 0.5\ x$$

$$\therefore \quad x = 200 \text{ kg}$$

Material Balance over Dissolution Tank:

$\therefore$ Kg of solution = Kg of solid NaoH + Kg water

$$200 = 100 + \text{kg water}$$

Kg water = Kg water added to dissolution tank = 100

$$\therefore W_1 = 100 \text{ kg}$$

Let W_2 = Kg of by pass water.

Let 'y' by kg 24% NaoH solution

Material balance of caustic soda over dilution tank

Solid NaOH in 50% solution = Solid NaoH in 24% solution

$$100 = 0.24\ y$$

$$y = 416.66 \text{ kg}$$

$$\text{Kg water in 24\% solution} = 416.66 - 100$$

$$= 316.66 \text{ kg}$$

Material Balance of water over Dilution Tank:

Kg water in 24% solution = Kg water in 50% solution
+ kg water by pass (w_2)

$$316.66 = 100 + w_2$$

$$w_2 = 316.66 - 100$$

$$w_2 = 216.66 \text{ kg}$$

$$\text{Weight ratio} = \frac{w_1}{w_2}$$

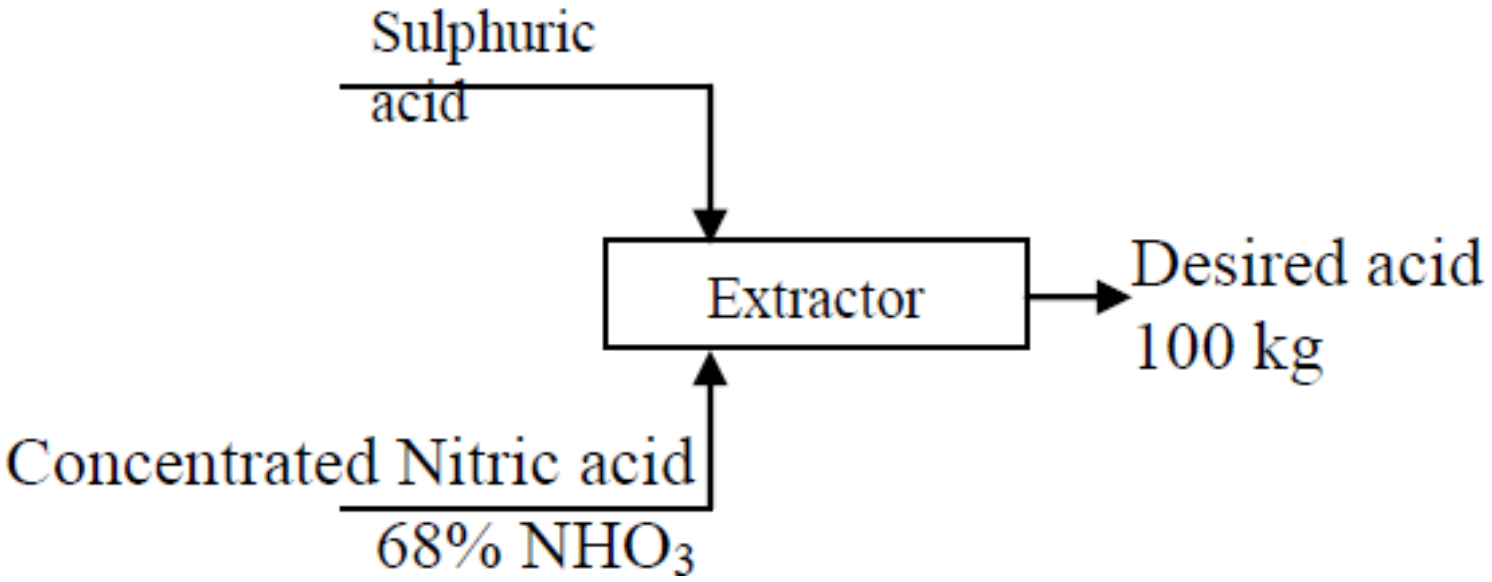
$$= \frac{100}{216.66}$$

$$0.4615$$

For carrying out Nitration reaction it is desired to have a mixed acid containing 39% HNO_3, 42% H_2So_4, and 19% H_2O (by wt) Nitric acid of 68.3% (by weight) is readily available, calculate:

a. **Required strength of sulphuric acid to obtain the above mixed acid.**
b. **The weight ratio of Nitric acid to sulphuric acid to be mixed.**

Basis: 100 kg of desired mixed acid.
It contains 39 kg HNO_3, 42 kg H_2So_4 and 19 kg water.



Let x be the kg of nitric acid and y be the kg of sulphuric acid of unknown strength required to be mixed.

Overall material Balance:

$x + y = 100$ _____ (1)

Material Balance of HNO_3:

$0.683\ x = 39$

$x = 57.1$ kg

Material Balance of H_2So_4 in mixed acid = 42 kg

$H_2\ So_4$ in sulphuric acid = H_2So_4 mixed acid = 42 kg

We have,

$x + y = 100$

$57.1 + y = 100$

$Y = 42.9$ kg.

Strength of sulphuric acid in weight % H_2So_4 $= \frac{\text{Kg } H_2So_4}{\text{Kg sulphuric acid}} \text{x}100$

$= \frac{42}{42.9} \times 100$

$= 97.9$

Weight ratio of nitric acid to sulphuric acid $= \frac{kg \text{ nitric acid}}{kg \text{ sulphuric acid}}$

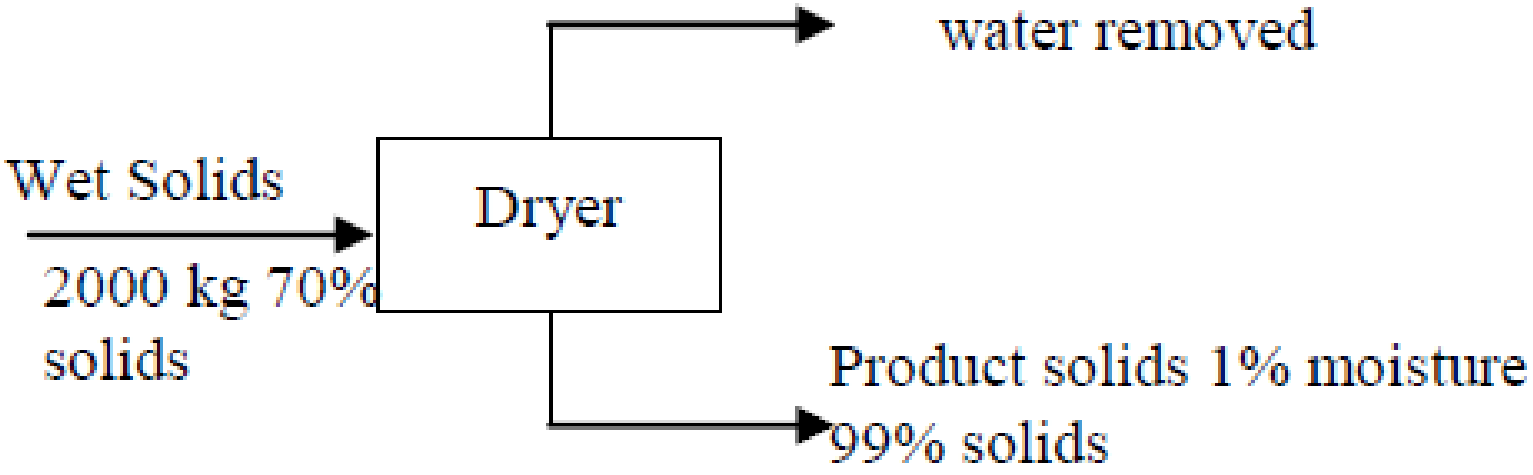
$= \frac{57.1}{42.9}$

$= 1.331$

2000 kg of wet solids containing 70% solids by weight are fed to tray dryer where it is dried by hot air. The product finally obtained is found to contain 1% moisture by weight, calculate;

1. **Kg of water removed from wet solids**
2. **Kg of product obtained.**

Basis: 2000 kg of wet solids fed to dryer.



Let x be the kg of product obtained and y be the kg of water removed.

Moisture in product = 1%
Solids in product = 100 -1 = 99%

Material Balance of solids:

Solids in wet solids = Solids in product

$$0.7 \times 2000 = 0.99\ x$$

$$X = 1414.14 \text{ kg}$$

Overall Material Balance:

$$2000 = x + y$$

$$y = 2000 - 1414.14$$

$$= 585.86 \text{ kg}$$

Check $\Rightarrow$

Material Balance of water:

Water in wet solids =Water in product + water removed

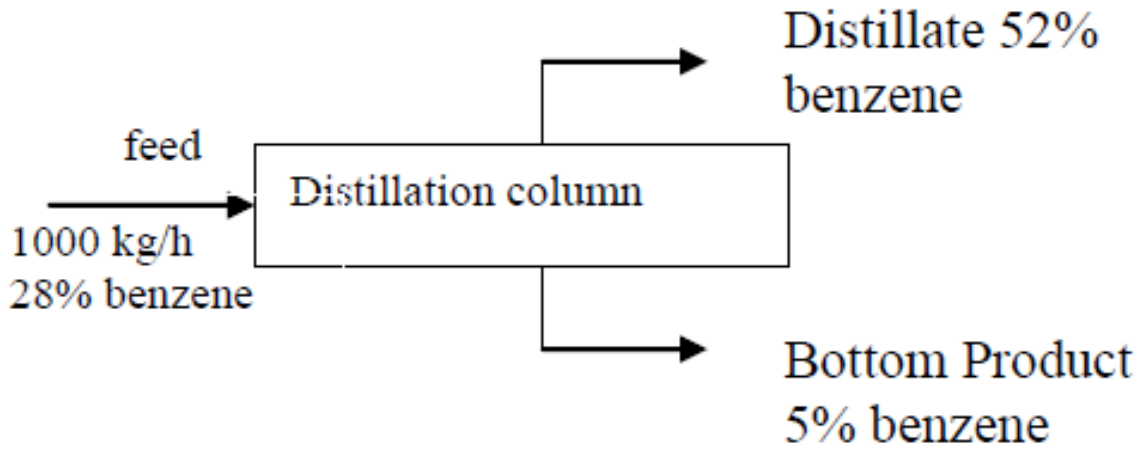
$$0.3 \times 2000 = 0.01 \times 1414.14 + 586.86$$
$$600 = 600$$
$$LHS = RHS$$

$\therefore$ Water removed in dryer = 585.86 kg

Product obtained = 1414.14 kg

The feed to a continuous fractionating column analysis by weight 28 percent benzene and 72 percent toluene. The analysis of the distillate shows 52 weight percent benzene and 5 weights percent benzene was found in the bottom product. Calculate the amount of distillate and bottom product per 1000 kg of feed per hour. Also calculate the percent recovery of benzene.

Basis: 1000 kg / h of feed to column.



Let x and y be the kg/h of distillate and bottom product respectively.

Overall Material Balance:

$x + y = 1000$ ---------- (1)

Material Balance of Benzene:

$$\begin{bmatrix}\text{Benzene in}\\ \text{feed}\end{bmatrix} = \begin{bmatrix}\text{Benezene in}\\ \text{distillate}\end{bmatrix} + \begin{bmatrix}\text{Benzene in}\\ \text{bottom product}\end{bmatrix}$$

$$0.28 \times 1000 = 0.52x + 0.05\,y$$

$$0.52x + 0.05y = 280 \quad \text{---------- (2)}$$

$$y = 280 - 0.52x \,/\, 0.05$$

$$y = 5600 - 10.4x \quad \text{---------- (3)}$$

Put value of y from equation (3) in Equation (1) and solve for x

$$x + (5600 - 10.4x) = 1000$$

$$x = 489.4 \text{ kg /h}$$

$$y = 1000 - 489.4$$

$$= 510.6 \text{ kg / h}$$

Distillate flow rate = 489.4 kg /h

Bottom product rate = 510. 6 kg /h

Benzence in feed = 0.28 x 1000

280 kg/h

Benzence in distillate = 0.52 x 489.4

= 254.49 kg /h

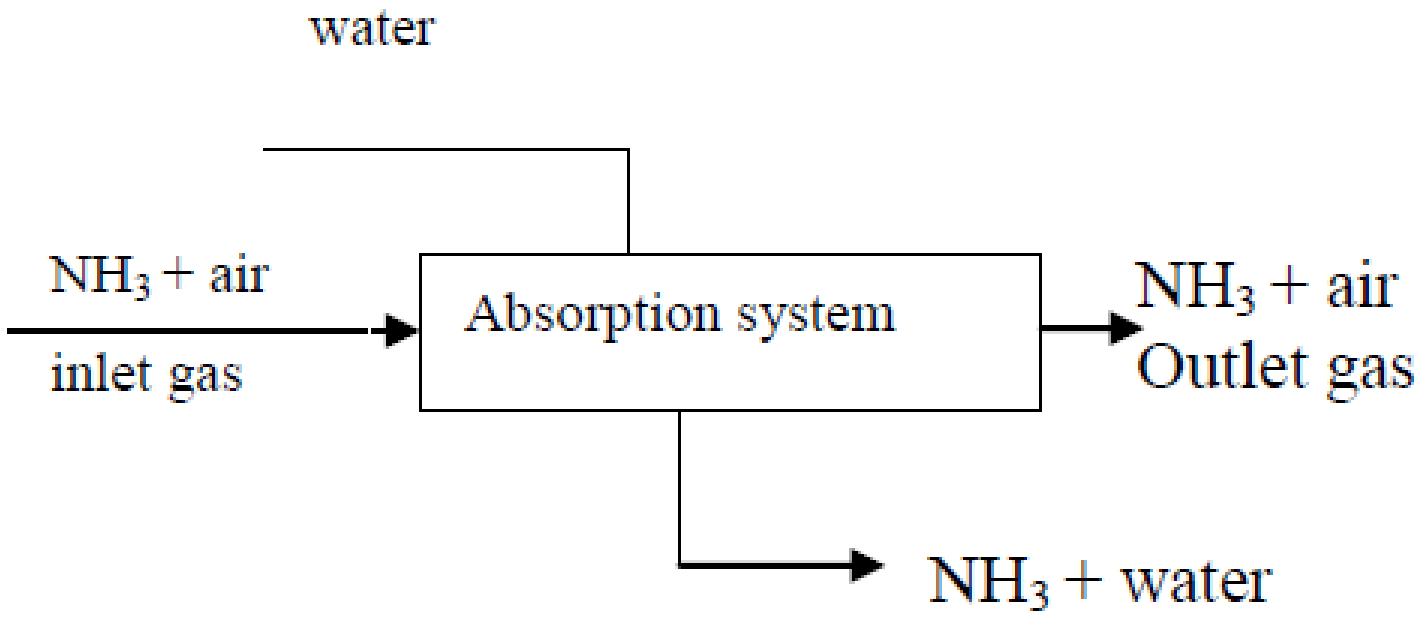
$$\% \text{ recovery of benzene} = \frac{\text{benzene in distillate}}{\textit{benzene}\,\text{inf}\,\textit{eed}} \times 100$$

$$= \frac{254.49}{280} x100$$

$$= 90.89$$

The NH_3 – air mixture containing 0.2 kg NH_3 per kg air enters into absorption system where ammonia is absorbed in water. The gas leaving the system is found to contain 0.004 kg NH_3 per kg of air. Find the percentage recover of ammonia.

Basis: 1 kg of air in gas inlet to absorption system.



Ammonia in inlet to absorption system $= \frac{0.2}{1} \times 1$

Air is inert as far as absorption operation is concerned. Therefore,

Air in gas leaving the system = Air in inlet gas
= 1 kg

NH_3 in gas leaving the system $= \frac{0.004}{1} \times 1$
= 0.004 kg

Material Balance of NH_3:

NH_3 in gas entering = NH_3 in gas leaving + NH_3 absorbed

0.2 = 0.004 + NH_3 absorbed

NH_3 absorbed = 0.2 – 0.004
= 0.196 kg.

$$\%\ recovery\ \text{of}\ NH_3 = \frac{NH_3 absorbed}{NH_3\ \text{in gas inlet}} \times 100$$

$$= \frac{0.196}{0.2} \times 100$$

$$= 98\%$$

It is required to make 1000 kg of mixed acid containing 60% H_2SO_4, 32% HNO_3 and 8% water by blending (i) the spent acid containing 11.3% HNO_3, 44.4% H_2SO_4 and 44.3% H_2O, (ii) aqueous 90% HNO_3, and (iii) aqueous 98% H_2SO_4. All percentages are by mass. Calculate the quantities of each of the three acids required for blending.

Solution *Basis* 1000 kg of mixed acid

It contains 600 kg of H_2SO_4, 320 kg of HNO_3 and 80 kg of water. Let x, y and z be the quantities of spent, aqueous nitric and aqueous sulphuric acids, respectively, required for blending

Overall material balance

$$x + y + z = 1000 \qquad \text{(i)}$$

Balance of sulphuric acid

$$0.444\,x + 0.98\,z = 600 \qquad \text{(ii)}$$

Balance of nitric acid

$$0.113\,x + 0.9\,y = 320 \qquad \text{(iii)}$$

Solving Eqs (i), (ii) and (iii), by elimanation method,

$$\mathbf{x = 76.3\ kg \quad y = 346.0\ kg \quad z = 577.7\ kg}$$

Dry neem leaves were subjected to extraction with supercritical carbon dioxide at 200 bar and 60°C (333 K). Dry leaves are analysed to contain 0.46% α-tocopherol and 0.01% β-carotene[5]. The extract is found to contain 15.5% α-tocopherol and 0.41% β-carotene. All percentages are by mass. If β-carotene content of the leached residue is nil, calculate (a) the mass of extract phase per kg of dry leaves, and (b) % recovery of α-tocopherol.

Solution

Basis 1 kg of dry neem leaves

β-carotene content of the leaves = 0.01/100 = 0.0001 kg

Extract contains 0.41% β-carotene.

$$\text{Extract quantity} = \frac{0.0001}{0.41} \times 100$$

$$= 0.0244 \text{ kg}$$

$$\alpha\text{-tocopherol in the extract} = 0.0244 \times 0.155$$

$$= 0.003\ 78 \text{ kg}$$

$$\alpha\text{-tocopherol in the neem leaves} = 0.46/100 = 0.0046 \text{ kg}$$

$$\text{Recovery of } \alpha\text{-tocopherol} = \frac{0.003\ 78}{0.0046} \times 100$$

$$= \mathbf{82.2\%}$$

In a textile mill, a double-effect evaporator system concentrates weak liquor containing 4% (by mass) caustic soda to produce a lye containing 25% solids (by mass). Calculate the evaporation of water per 100 kg feed in the evaporator.

Solution In this problem, the basis is defined.

Basis 100 kg of weak liquor (feed)

It contains 4 kg of caustic soda (the tie material).

Let the quantity of the lye be x kg.

Caustic soda in the lye = $0.25\,x$

However, the caustic soda does not take part in the evaporation.

$$0.25\,x = 4$$

$$x = 4/0.25 = 16 \text{ kg}$$

$$\text{Evaporation} = 100 - 16 = \mathbf{84\ kg}$$

Soybean seeds are extracted with *n*-hexane in batch extractors. The flaked seeds contain 18.6% oil, 69.0% solids and 12.4% moisture. At the end of the extraction process, de-oiled cake (DOC) is separated from the *n*-hexane-oil mixture. DOC analysis yields 0.8% oil, 87.7% solids and 11.5% moisture. Find the percentage recovery of oil. All percentage are by mass. **[96.6% recovery]**

Basis: 100 kg flaked soybean seeds

Solids (unextractable, i.e. inerts) = 69 kg

Final deoiled cake after extraction (meal) = 69/0.877 = 78.68 kg

Oil retained in DOC = $78.68 \times 0.008 = 0.63$ kg

Oil recovery = $(18.6 - 0.63)100/18.6 = 96.6\%$

Ans.

A multiple-effect evaporator system has a capacity of processing one tonne per day of solid caustic soda when it concentrates weak liquor from 4 to 25% (both on mass basis). When the plant is fed with 5% weak liquor and if it is concentrated to 50% (both on mass basis), find the capacity of the plant in terms of solid caustic soda, assuming the water-evaporating capacity to be the same in both cases. [1.167 t/d]

Basis: 1000 kg solid caustic soda to be processed/d.

When the evaporator system concentrates 4% solution to 25% concentration,

$$\text{evaporation} = 100 - (4/0.25)$$

$$= 84 \text{ kg/100 kg original solution}$$

However, the capacity of the evaporation system is 4 kg solid caustic soda per 100 kg original solution. Consider another case in which 5% solution is concentrated to 50% concentration the

$$\text{evaporation} = 100 - (5/0.5)$$

$$= 90 \text{ kg/100 kg original solution}$$

The plant capacity is thus 5 kg caustic soda. It may be noted that the plant capacity in terms of evaporation remains constant, i.e. the plant has actual capacity of 84 kg evaporation per 100 kg solution.

In second case, for 84 kg evaporation,

$$\text{Caustic soda processed} = 5 \times 84/90 = 4.667 \text{ kg.}$$

Plant Capacity is terms of solid caustic soda

$$= 4.667 \times 1000/4$$

$$= 1166.7 \text{ kg/d} \qquad \textit{Ans.}$$

Crystals of $MgCl_2.6H_2O$ have a solubility[18] of 190 g per 100 g ethanol at 25°C (298.15 K). It is desired to make 1000 kg of saturated solution. Calculate the quantities of the crystals and ethanol required to make the above solution. Also, find the composition of the saturated solution by mass.

[Crystals = 655.5 kg, ethanol = 344.5 kg, Composition: $MgCl_2$ = 30.73%, H_2O = 34.82%, C_2H_2OH = 34.45% (mass basis)]

Basis: 1000 kg saturated solution of magnesium chloride in ethanol at 298.15 K (25 °C)

Let x = mass of crystals of $MgCl_2$ $6H_2O$, kg

y = mass of ethanol, kg

$$x + y = 1000 \quad (1)$$

$$\text{Crystal/solvent} = 190/100 = 1.9 = x/y$$

or $$x = 1.9y \quad (2)$$

solving the equations, $x = 655.5$ kg and $y = 344.5$ kg

$$MgCl_2 \text{ present in solution} = 95.32 \times 655.5/203.32$$

$$= 307.3 \text{ kg}$$

$$\text{Water present in solution} = 655.5 - 307.3$$

$$= 348.2 \text{ kg}$$

Compound	Formula	molar mass	mass, kg	mass %	kmol	mole %
Magnesium chloride	$MgCl_2$	95.32	307.3	30.73	3.224	10.72
Water	H_2O	18	348.2	34.82	19.344	64.36
Ethanol	C_2H_5OH	46	344.5	34.45	7.490	24.92
Total	—	—	1000.0	100.00	30.057	100.00

Ans.

3. a. (i) a single effect evaporator is fed with 1000 kg/h of weak liquor containing 15 % caustic by weight and is concentrated to get thick liquor containing 40% by weight caustic (NaOH) . Calculate: (a) kg/h of water evaporated and (b) kg/h of thick liquor obtained.

(6)

Sol. : Basis : 10,000 kg/h of weak liquor.

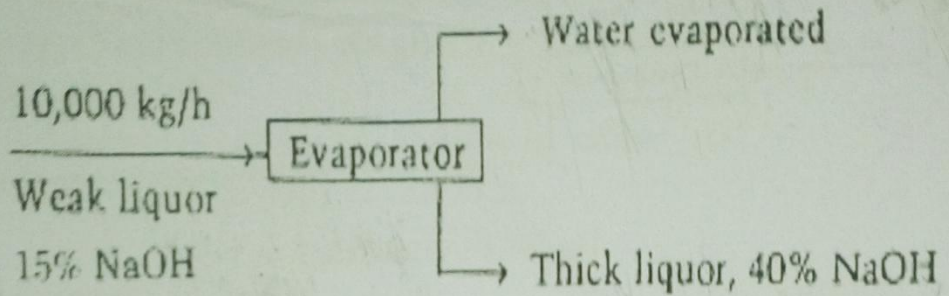


Fig. 3.11 : Block diagram of evaporation

Let x be the kg/h thick liquor obtained and y be the kg/h water evaporated.

Overall Material Balance :

$$\Sigma \text{ kg/h input stream} = \Sigma \text{ kg/h output stream}$$

$$\text{kg/h weak liquor} = \text{kg/h thick liquor} + \text{kg/h water evaporated}$$

$$\therefore \quad 1000 = x + y$$

Material Balance of NaOH :

NaOH in input stream = NaOH in output stream

NaOH in weak liquor = NaOH in thick liquor

$0.15 \times 10000 = 0.40\ x$

$\therefore \quad x = 3750$ kg/h

We have, $x + y = 10000$

$3750 + y = 10000$

$\therefore \quad y = 6250$ kg/h

$\therefore$ Water evaporated = 6250 kg/h

and Thick liquor obtained = 3750 kg/h

(ii) Illustrate the overall material balance and component balance for a binary system of distillation operation. (7)

Distillation : This operation is used for separation of components of liquid mixture by partial vaporization and condensation. Distillation with rectification or (fractional distillation) gives almost pure product. The product removed from top is called distillate or overhead product and that removed from bottom is called bottoms or bottom product. Material balance is generally based on more volatile component.

Block diagram of distillation operation for binary system of (A + B) :

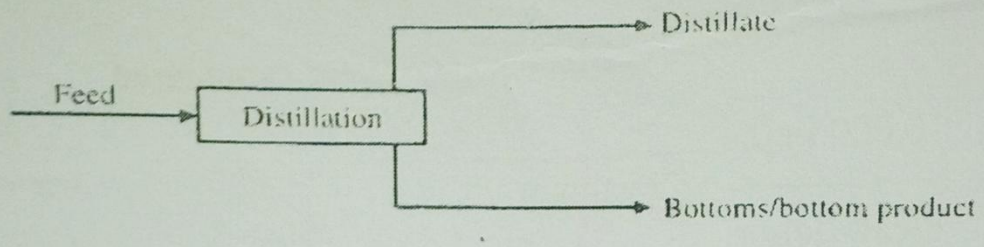


Fig. 3.1 : Distillation

Overall Material Balance :

$$\text{Feed} = \text{Distillate} + \text{Bottoms (on weight or mole basis)}$$

$$F = D + W$$

where F is feed to column in kg or kg/h, D is distillate in kg or kg/h and W is the bottom product in kg or kg/h.

Material Balance of Component A :

$$\text{A in feed} = \text{A in distillate} + \text{A in bottoms}$$

$$x_F \cdot F = x_D \cdot D + x_W \cdot W$$

where x_F, x_D and x_W are mole fractions of A in feed, distillate and bottoms.

13. b. (i) soyabean seeds are extracted with hexane in batch extractors. The flaked seeds are found to contain 18.6% oil, 69.6% solid and 12.4% moisture (by weight). At the end of the extraction process, cake (meal) is separated from hexane- oil mixture. The cake is analysed to contain 0.8% oil, 87.7% solids and 11.5% moisture (by weight). Find the percentage recovery of oil.

(6)

Sol. : Basis : 100 kg flaked seeds.

It contains 18.6 kg of oil, 69 kg of solids and 12.4 kg of moisture.

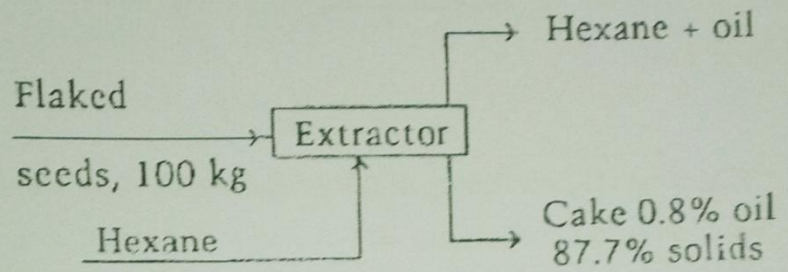


Fig. 3.26 : Block diagram for solvent extraction of oil

Let x be the kg of cake (meal) obtained.

Material Balance of Solids :

$$\text{Solids in seeds} = \text{Solids in cake}$$

$$0.69 \times 100 = 0.877\,x$$

$$\therefore \quad x = 78.68 \text{ kg}$$

Oil in cake is given as 0.8%

$$\therefore \quad \text{Oil in cake} = 0.008 \times 78.68 = 0.63 \text{ kg}$$

Material Balance of Oil :

$$\text{Oil in seeds} = \text{Oil in cake} + \text{Oil recovered}$$

$$18.6 = 0.63 + \text{Oil recovered}$$

$$\therefore \quad \text{Oil recovered} = 18.6 - 0.63 = 17.97 \text{ kg}$$

$$\% \text{ recovery of oil} = \frac{\text{Oil recovered}}{\text{Oil in seeds}} \times 100$$

$$= \frac{17.97}{18.6} \times 100 = 96.61$$

(ii) It is desired to makeup 1000 kg of a solution containing 35% by weight of a substance 'A'. two solutions are available, one containing 10 weight percent 'A' and other containing 50 weight percent of 'A'. How many kilograms of each solution will be required? (7)

Sol. : Basis : 1000 kg of desired solution.

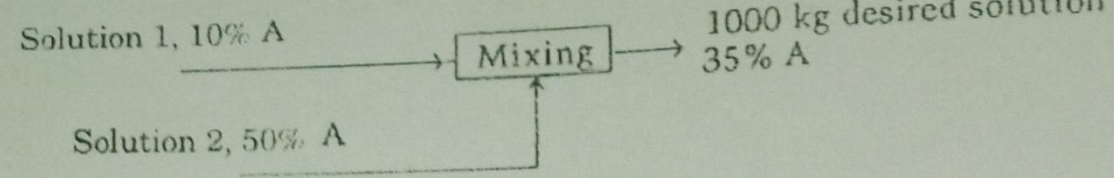


Fig. 3.16 : Block diagram for preparation of desired solution

Let x be the kg of solution containing 10% A and y be the kg of solution containing 50% A.

Overall Material Balance :

~~kg of solution Waste acid, 20% HNO_3, 55% H_2SO_4 26% HNO_3, 60% H_2SO_4~~

$$x + y = 1000 \quad \ldots (1)$$

Material Balance of Component A :

$$0.1\,x + 0.5\,y = 0.35 \times 1000$$

$$0.1\,x + 0.5\,y = 350 \quad \ldots (2)$$

$$y = \frac{350 - 0.1\,x}{0.5}$$

$$\therefore \quad y = 700 - 0.2\,x \quad \ldots (3)$$

Put the value of y from equation (3) in equation (1) and solve for x.

$$x + (700 - 0.2\,x) = 1000$$

$$\therefore \quad x = 375 \text{ kg}$$

$$y = 700 - 0.2\,x = 700 - 0.2\,(375)$$

$$\therefore \quad y = 625 \text{ kg}$$

Quantity of 10% A solution = 375 kg

Quantity of 50% A solution = 625 kg

b. The feed containing 60 mole % A, 30 mole % B and 10 mole % inerts enters a reactor. The product stream leaving the reactor is found to contain 2 mole % A. Reaction taking place is:

$2A + B \rightarrow C$

Find the percentage of original 'A' getting converted to C.

(15)

Sol. : Basis : 100 kmol of feed.

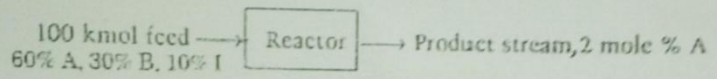


Fig. 4.6

Feed contains 60 kmol A, 30 kmol B and 10 kmol inerts

Let x be the kmol of A reacted by reaction

$$2A + B \rightarrow C$$

From reaction, 2 kmol A ≡ 1 kmol B ≡ 1 kmol C

$$\text{B reacted} = \frac{1}{2} \times x = 0.5x \text{ kmol}$$

$$\text{C formed} = \frac{1}{2} \times x = 0.5x \text{ kmol}$$

Material Balance of A gives,

$$\text{A unreacted} = (60 - x) \text{ kmol}$$

Material Balance of Inerts :

$$\text{Inerts in feed} = \text{Inert in product} = 10 \text{ kmol}$$

$$\text{C formed} = \frac{1}{2} \times x = 0.5x$$

$$\text{B unreacted} = (30 - 0.5x) \text{ kmol}$$

$$\therefore \text{Total moles of product stream} = (60 - x) + (30 - 0.5x) + 10 + 0.5x$$

$$= 100 - x \text{ kmol}$$

$$\text{Mole \% of A in product stream} = 2$$

$$\text{Mole \% of A} = \left(\frac{\text{kmol A in product stream}}{\text{total kmol of product stream}} \right) \times 100$$

$$2 = \frac{60 - x}{100 - x} \times 100$$

$\therefore$ Solving we get, $x = 59.184$ kmol

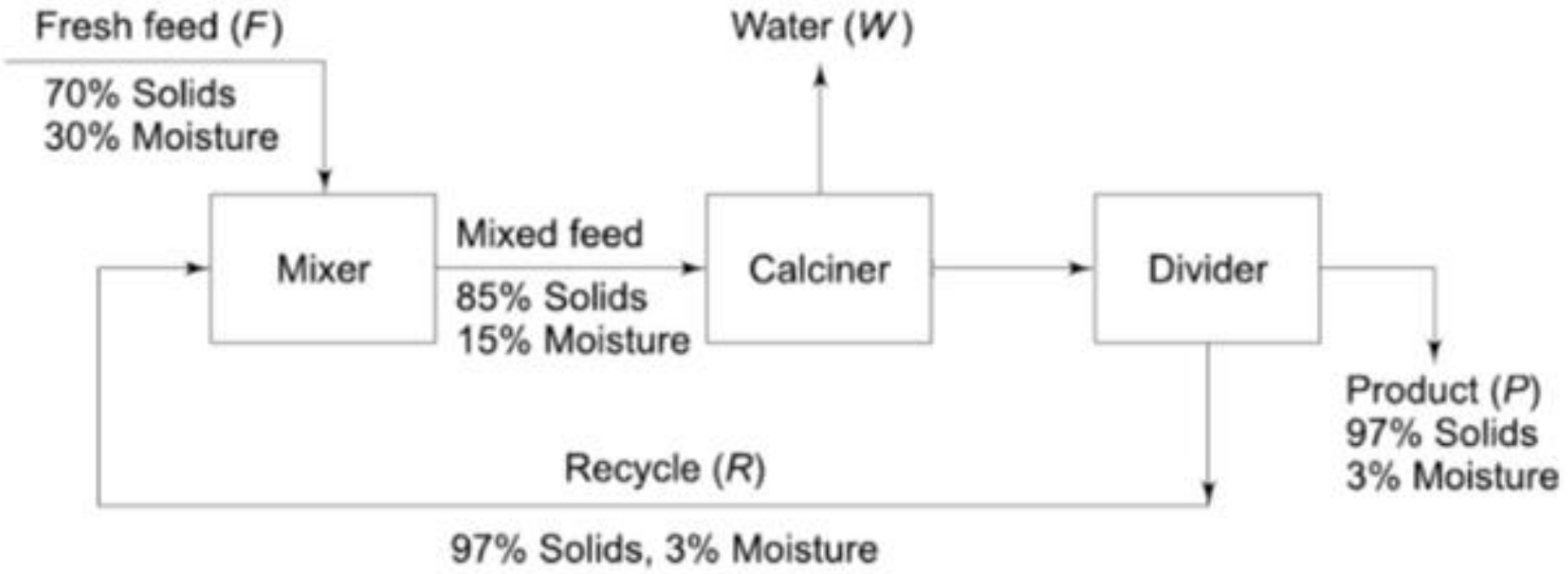
Amount of A reacted by reaction = 59.184 kmol

% of original A getting converted to C

$$= \frac{\text{kmol A reacted}}{\text{kmol A in feed}} \times 100$$

$$= \frac{59.184}{60} \times 100 = 98.64$$

In a particular drying operation, it is necessary to hold the moisture content of feed to a calciner to 15% (mass) to prevent lumping and sticking. This is accomplished by mixing the feed having 30% moisture (mass) with a recycle stream of dried material having 3% moisture (mass). The drying operation is shown in Fig. What fraction of the dried product must be recycled? **[63.4%]**



Basis: 1000 kg/h feed rate

Material balance across mixer:

Let recycle and mixed feed be R and M kg/h, respectively.

Overall balance:

$$M = 1000 + R \qquad (1)$$

Balance of solids:

$$0.15\ M = 300 + 0.03\ R$$

$$M = 2000 + 0.2\ R \qquad (2)$$

Equating Eq. (1) and (2);

$$1000 + R = 2000 + 0.2\ \mathrm{R}$$

$$0.8\ R = 1000$$

or $$R = 1250 \text{ kg/h} \quad \text{and} \quad M = 2250 \text{ kg/h}$$

Balance across calciner:

Let the evaporation in calciner be W kg/h and product from the divider by P kg/h.

$$M = W + P + R$$

But $$P = 700/0.97 = 721.65 \text{ kg/h}$$

$$W = M - P - R = 2250 - 721.65 - 1250$$

$$= 278.35 \text{ kg/h}$$

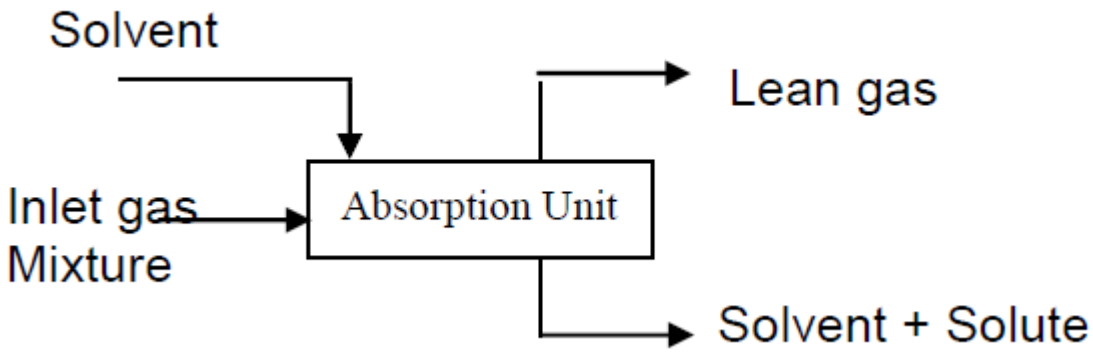
Total dry product from the calciner $= P + R$

$$= 1250 + 721.65$$

$$= 1971.65 \text{ kg/h}$$

$$\text{Recycle fraction} = 1250 \times 100/1971.65 = 63.4\% \qquad \textit{Ans.}$$

Block diagram of Absorption



Inert Gas Balance :

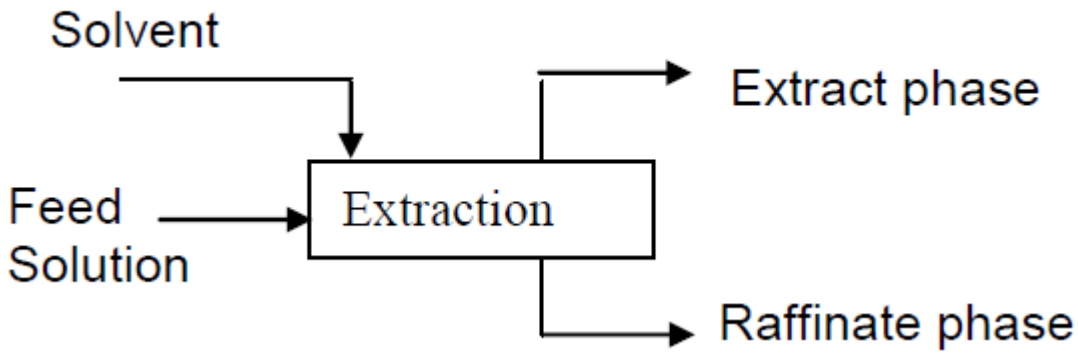
Inert gas in gas entering tower = inert gas leaving the tower
(i.e inert gas in lean gas)

Solute Gas Balance :

$$\begin{pmatrix}\text{Solute gas}\\ \text{in inlet gas}\end{pmatrix}-\begin{pmatrix}\text{Solute gas}\\ \text{in lean gas}\end{pmatrix}=\begin{pmatrix}\text{Solute gas in}\\ \text{Solvent at exist}\end{pmatrix}-\begin{pmatrix}\text{Solute gas in}\\ \text{solvent at inlet}\end{pmatrix}$$

= solute gas removed by absarption

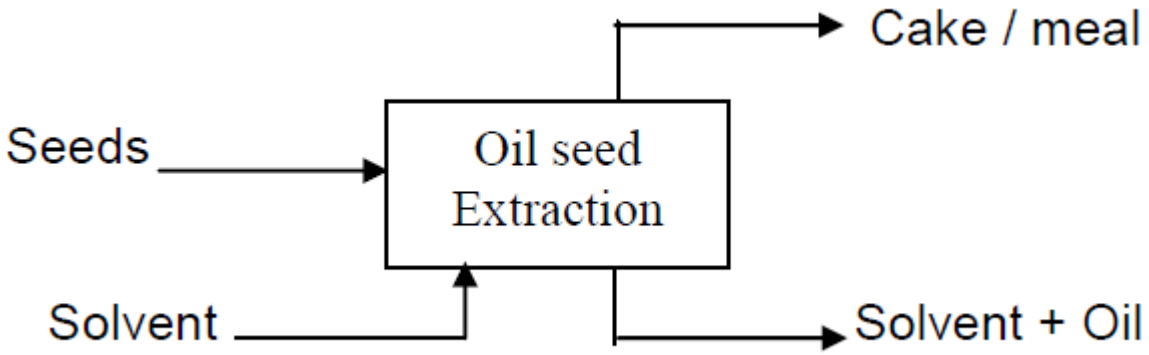
Block diagram of Extraction



Feed solution + solvent = Extract phase + Raffinate phase
If A is the solute to be extracted, then material balance of 'A' for fresh solvent:

A in feed solution = 'A' in extract phase + A in Raffinate phase

Oil seed extraction

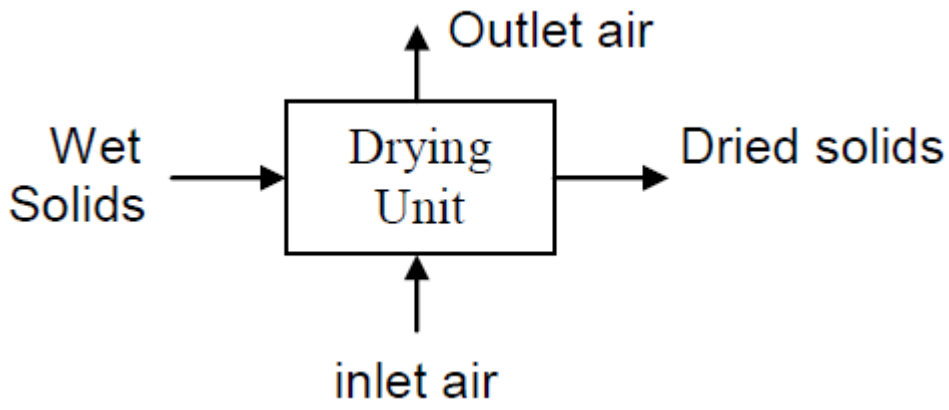


Solids in seeds= Solids in meal (if no solids in solvent)

Oil balance:

Oil in seeds = Oil in meal + oil in solvent (Extracted oil)

Block diagram of Drying

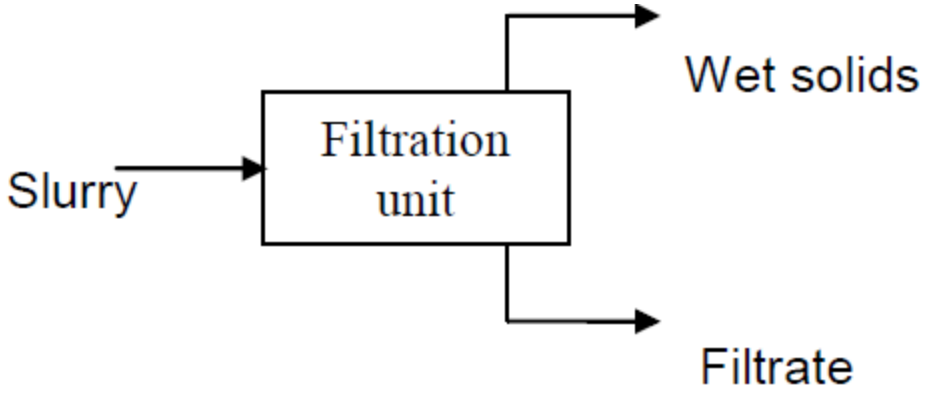


Material Balance of Moisture.

Moisture removed from solids = Moisture added in air.

$$\left.\begin{matrix}\text{Initial moisture}\\ \text{with wet solids}\end{matrix}\right\} = \left\{\begin{matrix}\text{Moisture in}\\ \text{dried solids}\end{matrix}\right\} = \left\{\begin{matrix}\text{Moisture in}\\ \text{outlet air}\end{matrix}\right\} - \left\{\begin{matrix}\text{Moisture}\\ \text{in inlet air}\end{matrix}\right\}$$

Block diagram of Filtration



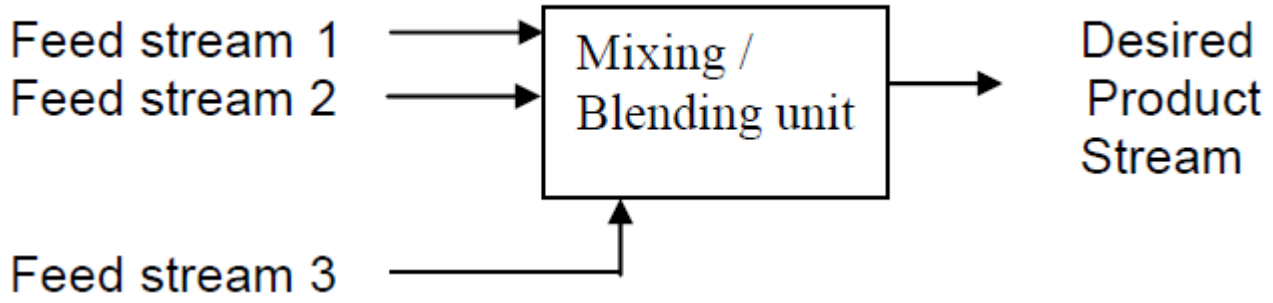
Overall Material Balance:

Feed slurry = Wet solids + filtrate

Material Balance of solids:

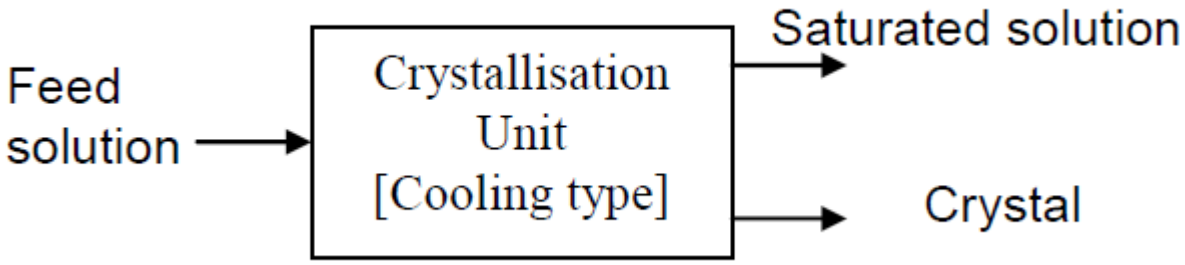
Solids in slurry = solids in wet solid product +Solids in filtrate (if any)

Block diagram of Mixing / Blending



Feed stream -1 Feed Stream -2
+ Feed Stream -3
} = *Desired* product Stream

Block diagram of crystallization



Overall Material Balance:

Feed solution = Saturated solution + crystals.

Crystal Balance:

Crystal in feed solution = Crystal obtained + *Crystal* in *saturated* solution

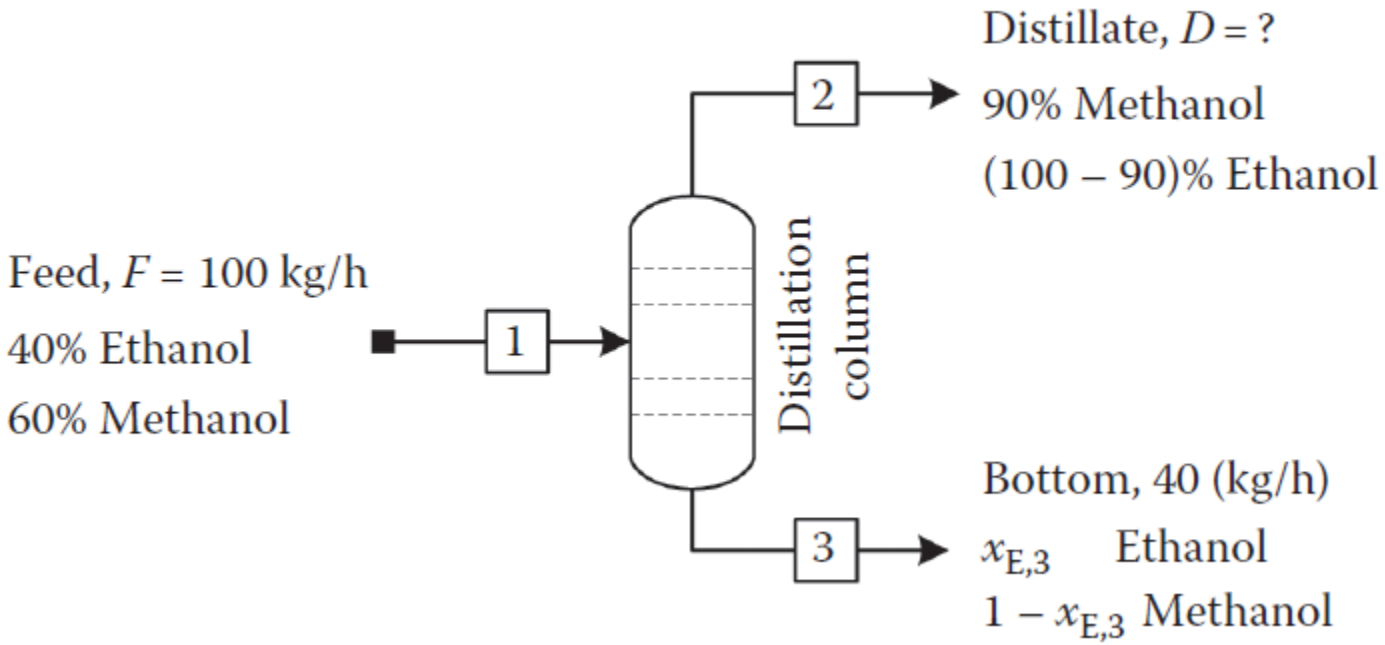
Degrees of freedom

This concept is useful in physical chemistry to define a system in equilibrium having more than one phase.

The same concept is also useful in stoichiometry in solving the problems of a multi-variable system.

The difference between the number of unknown variables and the number of equations is known as the number of degrees of freedom.

A 100 kg/h ethanol–methanol stream is to be separated in a distillation column. The feed has 40% ethanol and the distillate has 90% methanol. The flow rate of the bottom stream product is 40 kg/h. Determine the percentage of methanol in the bottom stream.


Distillate, D = ?
2
90% Methanol
(100 – 90)% Ethanol
Feed, F = 100 kg/h
40% Ethanol
60% Methanol
1
Distillation column
Bottom, 40 (kg/h)
3
$x_{E,3}$ Ethanol
$1 - x_{E,3}$ Methanol

Total mass balance:

$$100\ \text{kg/h} = D + 40\ \text{kg/h}$$

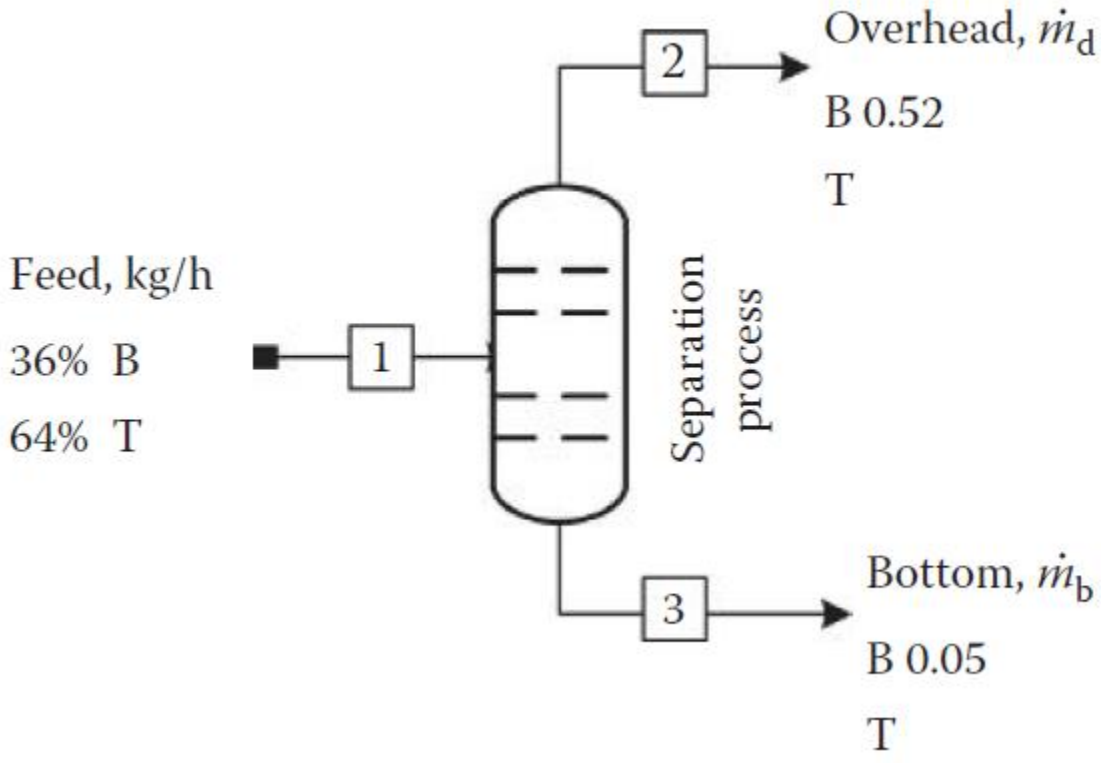
$$D = 60\ \text{kg/h}$$

Component balance (methanol):

$$0.6(100\ \text{kg/h}) = 0.9\ (60\ \text{kg/h}) + (1 - x)\ 40$$

$$x = 0.85$$

The feed to a distillation column contains 36% benzene (B) by weight, and the remainder toluene (T). The overhead distillate is to contain 52% benzene by weight, while the bottom is to contain 5% benzene by weight. Calculate the percentage of the benzene in the feed that is contained in the distillate, and the percentage of the total feed that leaves as distillate.


Overhead, $\dot{m}_d$
B 0.52
T
2
Feed, kg/h
36% B
64% T
1
Separation process
3
Bottom, $\dot{m}_b$
B 0.05
T

Substitute $\dot{m}_b$:

$$0.36(100\ \text{kg/h}) = 0.52\ \dot{m}_d + 0.05(100 - \dot{m}_d)$$

$$36\ \text{kg/h} = 0.52\ \dot{m}_d + 5 - 0.05\ \dot{m}_d \Rightarrow \dot{m}_d = 66\ \text{kg/h}$$

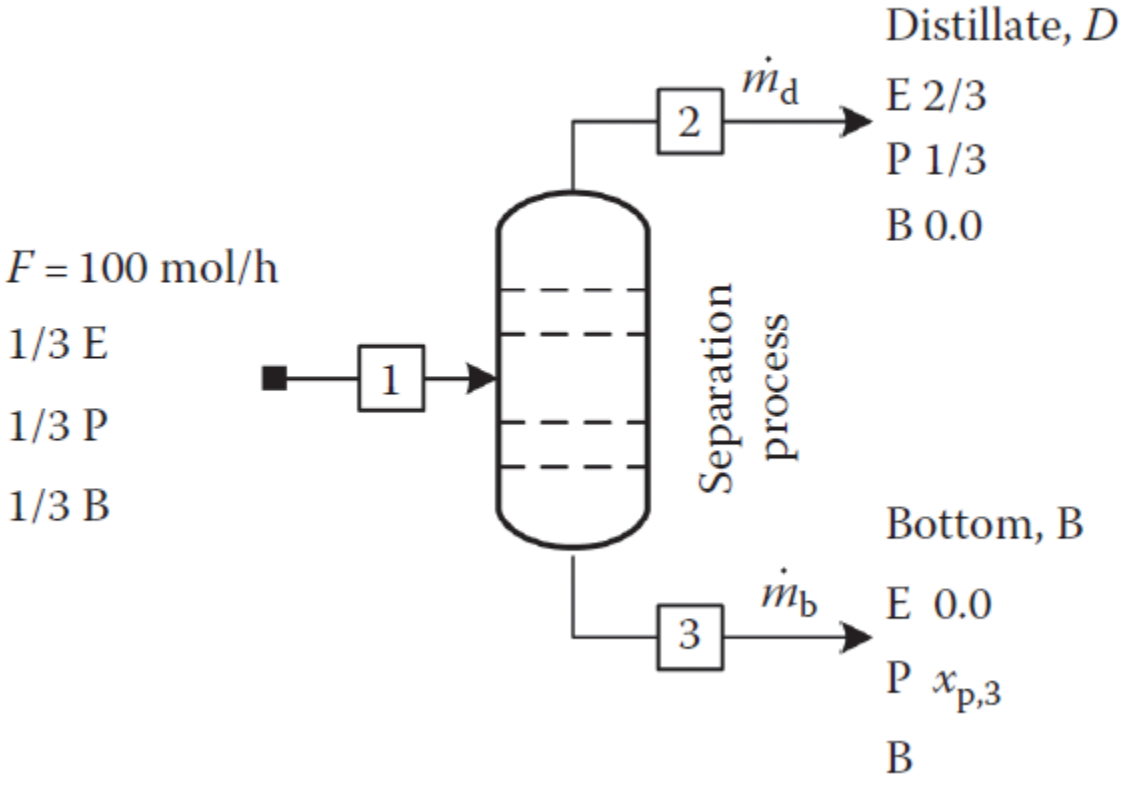
The bottom mass flow rate is obtained by substituting $\dot{m}_d$ in the total mass balance equation.

$$\text{Total balance}: 100\ \text{kg/h} = 66\ \frac{\text{kg}}{\text{h}} + \dot{m}_b$$

The bottom mass flow rate, $\dot{m}_b = 34\ \text{kg/h}$.

$$\text{The mass ratio of distillate to bottom mass flow rate} = \frac{\dot{m}_d}{\dot{m}_b} = \frac{66\ \text{kg/h}}{34\ \text{kg/h}}$$

A feed rate of 100 mol/h of an equimolar mixture of ethanol (E), propanol (P), and butanol (B) is separated in a distillation column into two streams. The overhead stream (distillate) contains 2/3 ethanol and no butanol, and the bottom stream is free of ethanol. Calculate the rates and compositions of the overhead and bottom streams.


Distillate, D
$\dot{m}_d$
2
E 2/3
P 1/3
B 0.0
$F = 100$ mol/h
1/3 E
1/3 P
1/3 B
1
Separation process
Bottom, B
$\dot{m}_b$
3
E 0.0
P $x_{p,3}$
B

Component balance (E):

$$\frac{1}{3}(100\ \text{mol/h}) = \left(\frac{2}{3}\right)\dot{m}_{\text{d}} + 0.0 \Rightarrow \dot{m}_{\text{d}} = 50\ \text{mol/h}$$

From the total material balance,

$$100\ \text{mol/h} = 50\ \text{mol/h} + \dot{m}_{\text{b}} \Rightarrow \dot{m}_{\text{b}} = 50\ \text{mol/h}$$

Component balance (P):

$$\frac{1}{3}(100\ \text{mol/h}) = \left(\frac{2}{3}\right)\dot{m}_{\text{d}} + x_{\text{P},3}\dot{m}_{\text{b}}$$

Substitute $\dot{m}_{\text{b}}$ and $\dot{m}_{\text{d}}$ in the P component balance:

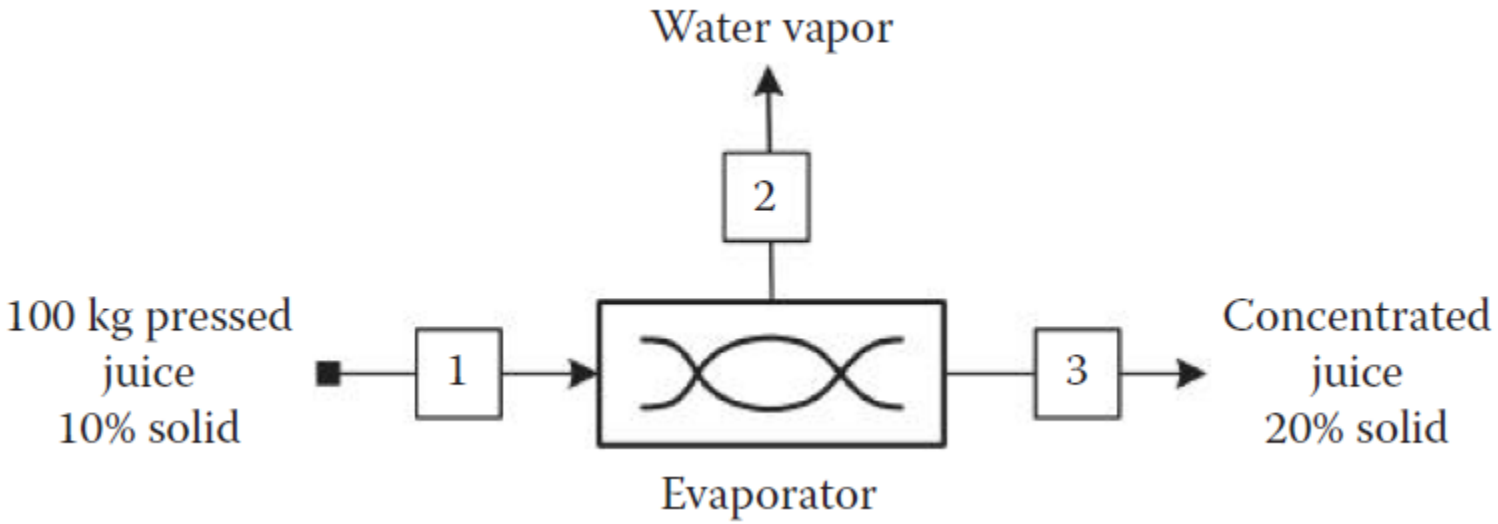
$$\frac{1}{3}(100\ \text{mol/h}) = \frac{1}{3}(50\ \text{mol/h}) + x_{\text{P},3}(50\ \text{mol/h})$$

Divide both sides of the equation by 50 to reduce to

$$\frac{1}{3}(2) = \frac{1}{3} + x_{\text{P},3}$$

$$x_{\text{P},3} = \frac{1}{3}$$

The initial amount of pressed lemon juice contains 10% of total solids. It is desired to increase the figure to 20% of total solids by evaporation. The resulting concentrated juice consists of 20% total solids. Calculate the quantity of water that must be removed.



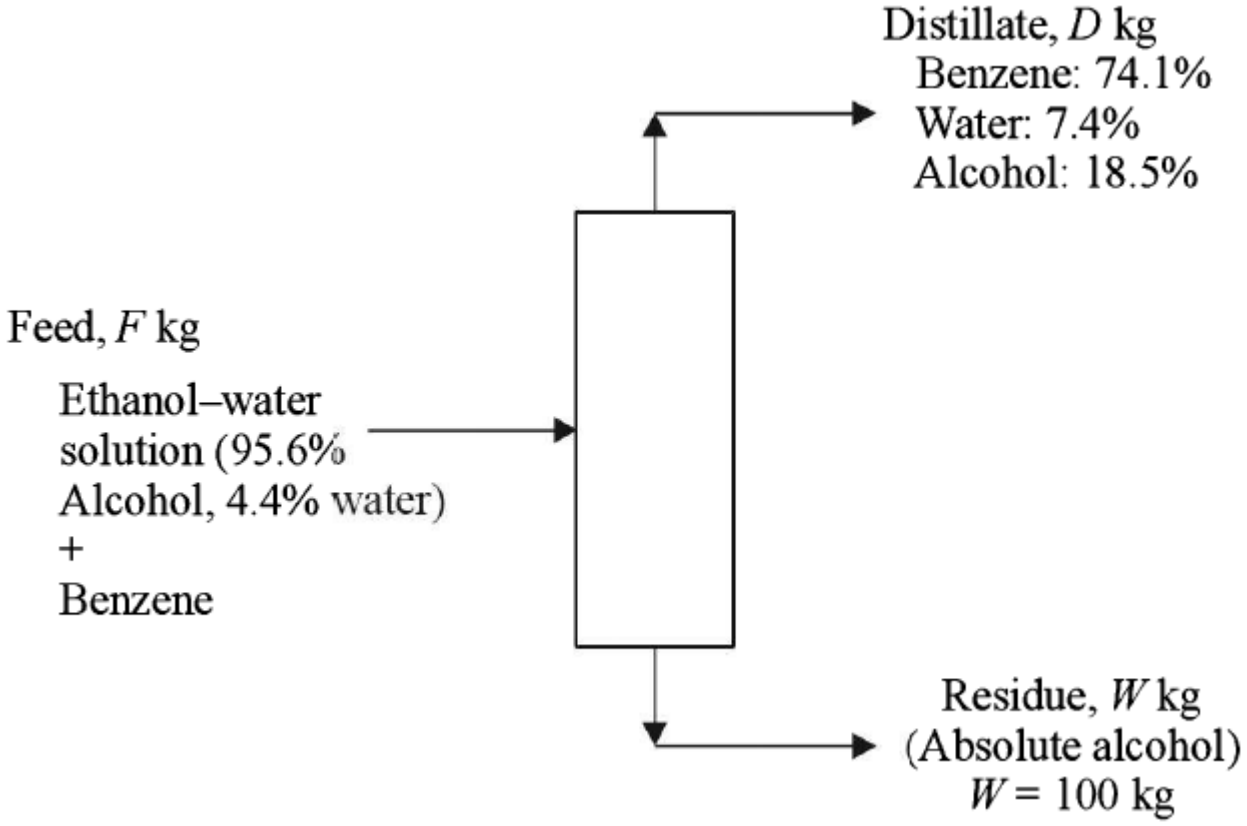
Basis: 100 kg of pressed juice.
Solid balance:

$$0.10 \times 100 \text{ kg} = 0.2 \times m_3$$

$$m_3 = 50 \text{ kg}$$

$$\text{Amount of water evaporated} = 100 - 50 = 50 \text{ kg}$$

In the azeotropic distillation of an ethanol–water solution, a feed mixture containing 95.6% alcohol is mixed with pure benzene and distilled. The benzene forms a ternary azeotrope with alcohol–water with a composition of 74.1% benzene, 7.4% water and 18.5% alcohol, which is distilled over as the overhead product. Absolute alcohol is obtained as the residue product. Determine the quantity of benzene required for producing 100 kg of absolute alcohol.



$$F = D + W \quad \Rightarrow \quad F = D + 100 \tag{A}$$

$$Fw_{F,a} = 0.185D + W \quad \Rightarrow \quad Fw_{F,a} = 0.185D + 100 \tag{B}$$

Water balance:

$$Fw_{F,w} = 0.074D \tag{C}$$

Benzene balance:

$$Fw_{F,b} = 0.741D \tag{D}$$

Since the sum of the weight fractions is unity, we have

$$w_{F,a} + w_{F,w} + w_{F,b} = 1 \tag{E}$$

$$\frac{w_{F,a}}{w_{F,w}} = \frac{95.6}{4.4} = 21.7273 \quad \Rightarrow \quad w_{F,a} = 21.7273 w_{F,w}$$

Basis: 100 kg of overhead product

D = 100 kg. Choose water as the key component. Let x be the weight of 95.6% alcohol in the feed. Then, water balance gives

$$\frac{4.4}{100}x = 7.4 \quad \Rightarrow \quad x = 168.1818 \text{ kg}$$

The amount of absolute alcohol is obtained from alcohol balance.

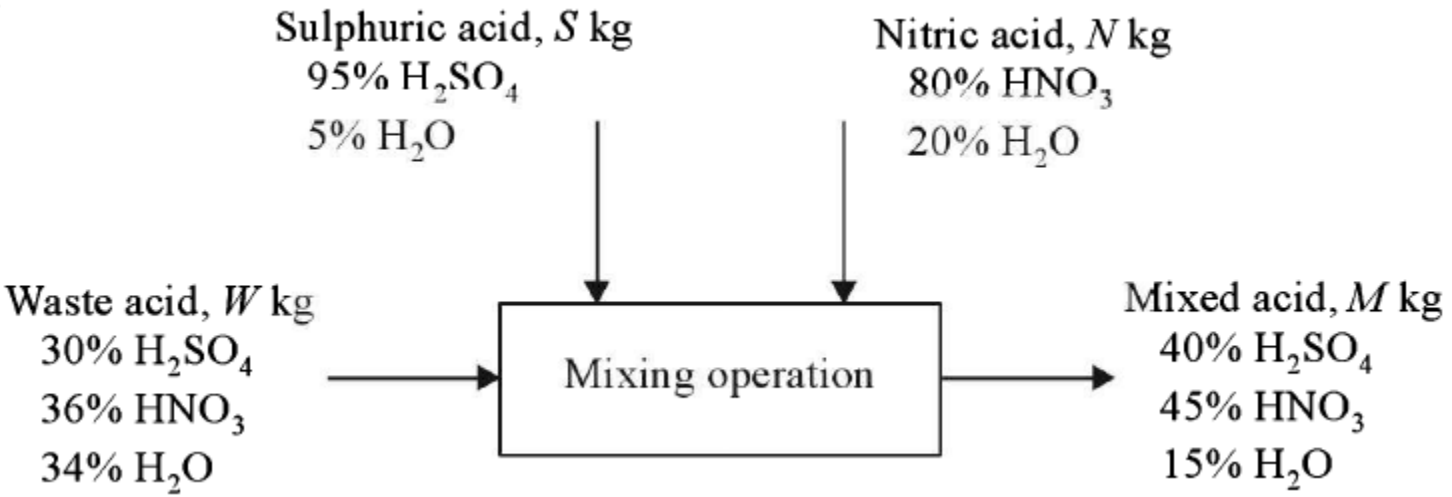
$$168.1818 \times 0.956 = 18.5 + W$$

$$W = 168.1818 \times 0.956 - 18.5 = 142.28 \text{ kg}$$

When 142.28 kg of absolute alcohol is produced, benzene used is 74.1 kg. Therefore, for 100 kg of absolute alcohol, benzene required is

$$\left(\frac{74.1}{142.28}\right)100 = 52.08 \text{ kg}$$

1000 kg of mixed acid of composition 40% H_2SO_4, 45% HNO_3 and 15% H_2O is to be produced by strengthening waste acid of composition 30% H_2SO_4, 36% HNO_3 and 34% H_2O by weight. Concentrated sulphuric acid of strength 95% and concentrated nitric acid containing 80% acid are available for this purpose. How many kilograms of spent acid and concentrated acids are to be mixed together?



Basis: 1000 kg of mixed acid. $M = 1000$ kg.

We can write four material balance equations—one total material balance, three component balances, one each for H_2SO_4, HNO_3 and H_2O.

Total material balance:

$$W + S + N = 1000 \tag{A}$$

H_2SO_4 balance:

$$0.3W + 0.95S = 0.4 \times 1000 \tag{B}$$

HNO_3 balance:

$$0.36W + 0.8N = 0.45 \times 1000 \quad \text{(C)}$$

H_2O balance:

$$0.34W = 0.05S + 0.20N = 0.15 \times 1000 \quad \text{(D)}$$

There are only three independent material balance equations as Eq. (D) can be obtained by adding together Eqs. (B) and (C) and subtracting the sum from Eq. (A). The number of unknown quantities is three. They are W, S and N. Equations (A), (B) and (C) are solved simultaneously to determine the quantities W, S and N. We get $W = 70.22$ kg, $S = 398.88$ kg, $N = 530.90$ kg.

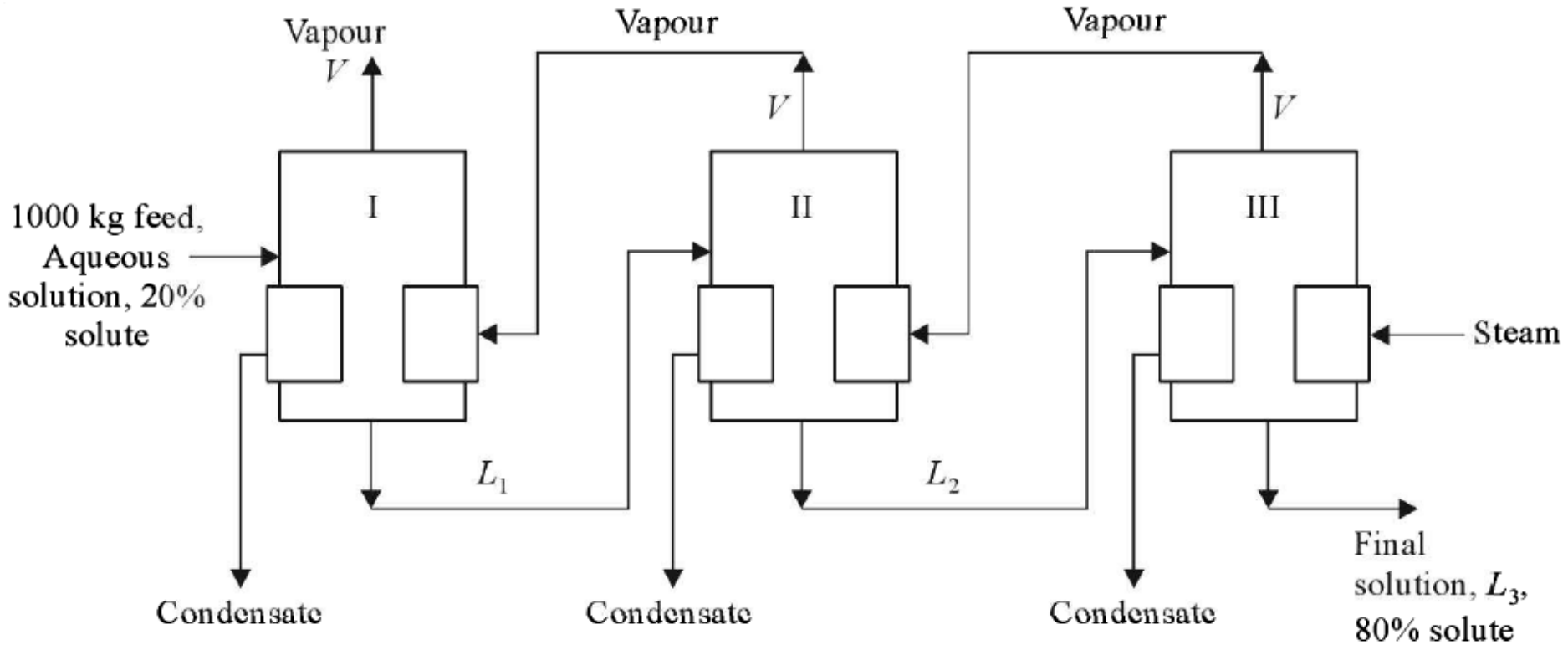
Quantities to be mixed are:

Waste acid = 70.22 kg

Concentrated H_2SO_4 = 398.88 kg

Concentrated HNO_3 = 530.90 kg

A triple effect evaporator is used to concentrate 1000 kg of aqueous solution from a concentration of 20% solute to 80% solute. Assuming an equal amount of vaporization in each effect, calculate the composition and weight of the solution entering the second and third effects.



Solution *Basis:* 1000 kg feed to the first effect

Let L_1, L_2 and L_3 be the rate at which the concentrated solution is leaving each effect, x_1, x_2 and x_3 respectively be the concentrations of solute in these effects. Let V be the amount of vapour produced in each effect. Consider the combined system consisting of all the three effects. The total material balance yields

$$1000 = 3V + L_3 \tag{A}$$

The solute balance is

$$1000 \times 0.2 = L_3 x_3$$

$$200 = 0.8 L_3 \tag{B}$$

Solving Eqs. (A) and (B), we get $L_3 = 250$ kg and $V = 250$ kg.
Now we can write the material balance equation for the first effect.
Total balance:

$$1000 = L_1 + V = L_1 + 250 \tag{C}$$

Solute balance:

$$1000 \times 0.2 = L_1 x_1 \tag{D}$$

From Eq. (C), $L_1 = 750$ kg. Substituting this in Eq. (D), we get $x_1 = 0.2667$.

For the second effect, the corresponding equations are

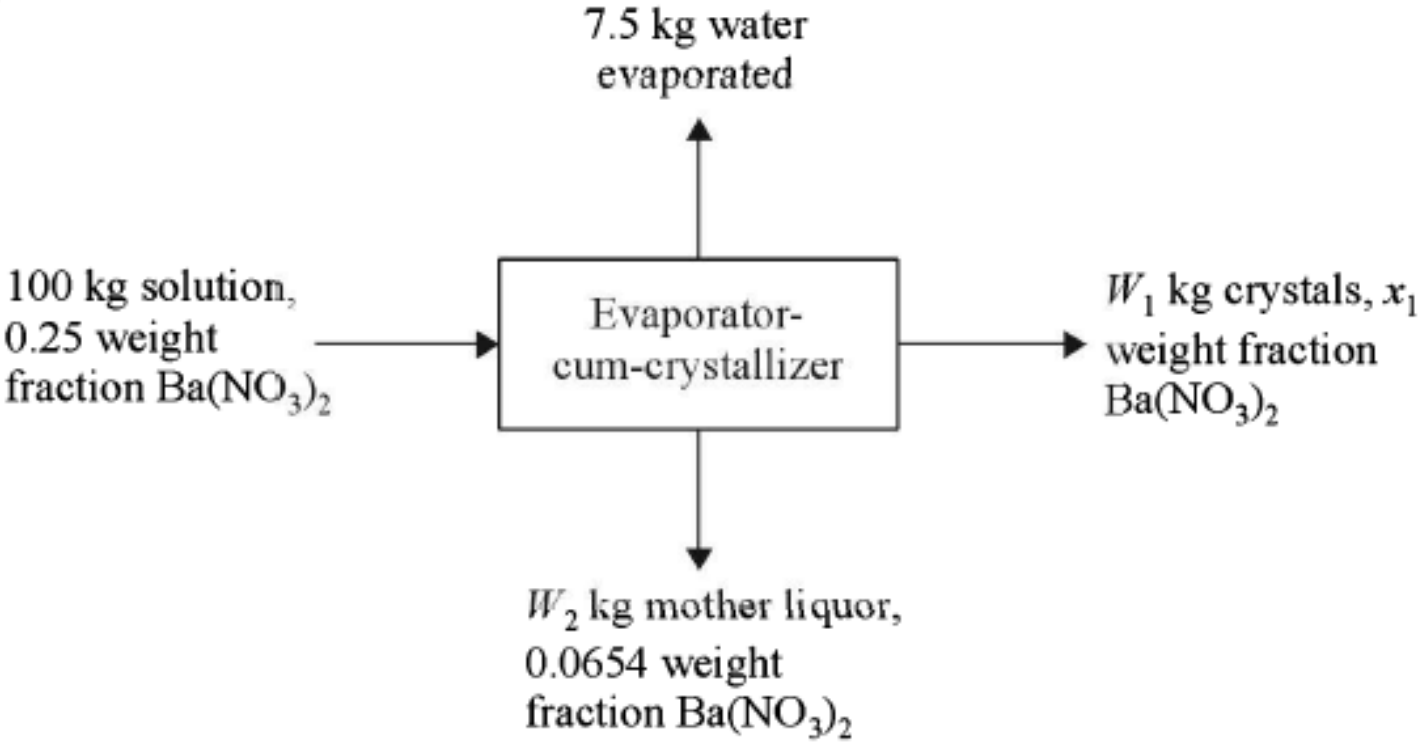
$$L_1 = L_2 + V \quad \Rightarrow \quad 750 = L_2 + 250 \tag{E}$$

$$L_1 x_1 = L_2 x_2 \quad \Rightarrow \quad 750 \times 0.2667 = L_2 x_2 \tag{F}$$

From Eqs. (E) and (F), we get $L_2 = 500$ kg and $x_2 = 0.4$.
The results of material balance calculation for the triple-effect evaporator system are summarized as follows:

Effect	*Feed* (kg)	*Products* (kg)		*Liquid concentration* (%)	
		Liquid	*Vapour*	*Feed*	*product*
I	1000	750	250	20	26.67
II	750	500	250	26.67	40
III	500	250	250	40	80

A crystallizer is charged with 100 kg of a solution containing 25% $Ba(NO_3)_2$ in water. On cooling 10% of the original water present evaporates. Calculate the yield of crystals when the solution is cooled to 283 K. The solubility at 283 K is 7.0 kg $Ba(NO_3)_2$/100 kg total water.



Solution *Basis:* 100 kg of feed solution

Let W_1 be the weight of crystals, F the weight of feed, and x_F weight fraction of solute in the feed. Let x_2 be the solubility of the solute in weight fraction of solute, and W_2 and W_3 be the weights of mother liquor remaining after crystallisation and the weight of water evaporated during crystallization operation

$$F = 100 \text{ kg}, \quad x_F = 0.25, \quad x_2 = 7/107 = 0.0654, \quad W_3 = 0.75 \times 100 \times 0.1 = 7.5 \text{ kg}$$

Total material balance

$$F = W_1 + W_2 + W_3$$

That is,
$$100 = W_1 + W_2 + 7.5 \quad \Rightarrow \quad W_1 + W_2 = 92.5 \tag{A}$$

Solute balance

$$Fx_F = W_1 x_1 + W_2 x_2$$

$$100 \times 0.25 = W_1 \times 1.0 + W_2 \times 0.0654 \quad \Rightarrow \quad W_1 + 0.0654 W_2 = 25 \tag{B}$$

Solving Eqs. (A) and (B) simultaneously, we get $W_1 = 20.28$ kg.

An aqueous solution of Na_2CO_3 contains 15% carbonate by weight. 80% of the carbonate is recovered as $Na_2CO_3 \cdot 10H_2O$ by evaporation of water and subsequent cooling to 278 K. The solubility of Na_2CO_3 at 278 K is 9.0% (weight). On the basis of 100 kg of solution treated, determine the following:

(a) The quantity of crystals formed
(b) The amount of water evaporated

Solution *Basis:* 100 kg solution treated

See Figure 9.13. Let W_1 be the weight of hydrated crystals, x_1 the weight fraction of Na_2CO_3 in the crystals, F the weight of feed and x_F the weight fraction of Na_2CO_3 in the feed. Let x_2 be the solubility of Na_2CO_3 in weight fraction Na_2CO_3, and W_2 and W_3 be the weights of mother liquor remaining after crystallization and the weight of water evaporated during crystallization operation.

Total material balance:

$$F = W_1 + W_2 + W_3 \tag{A}$$

Na_2CO_3 balance:

$$Fx_F = W_1x_1 + W_2x_2 \tag{B}$$

Here F = 100 kg, x_F = 0.15, the molecular weight of Na_2CO_3 = 106 and the molecular weight of $Na_2CO_3 \cdot 10H_2O$ = 286.

(a) The weight fraction of Na_2CO_3 in the crystals is

$$x_1 = \frac{106}{286} = 0.3706$$

The mass of Na_2CO_3 recovered in the crystals is

$$W_1x_1 = 0.8Fx_F = 0.8 \times 100 \times 0.15 = 12 \text{ kg}$$

Therefore, the quantity of crystals formed is

$$W_1 = \frac{12}{0.3706} = 32.28 \text{ kg}$$

(b) Substituting the values in Eq. (B), we get,

$$100 \times 0.15 = 12 + W_2x_2$$

The mass of Na_2CO_3 in the mother liquor is

$$W_2x_2 = 15 - 12 = 3 \text{ kg}$$

The weight fraction of Na_2CO_3 in the mother liquor = $x_2 = 0.09$ (from solubility)
The weight of mother liquor is

$$W_2 = \frac{W_2x_2}{x_2} = \frac{3}{x_2} = \frac{3}{0.09} = \frac{100}{3} \text{ kg}$$

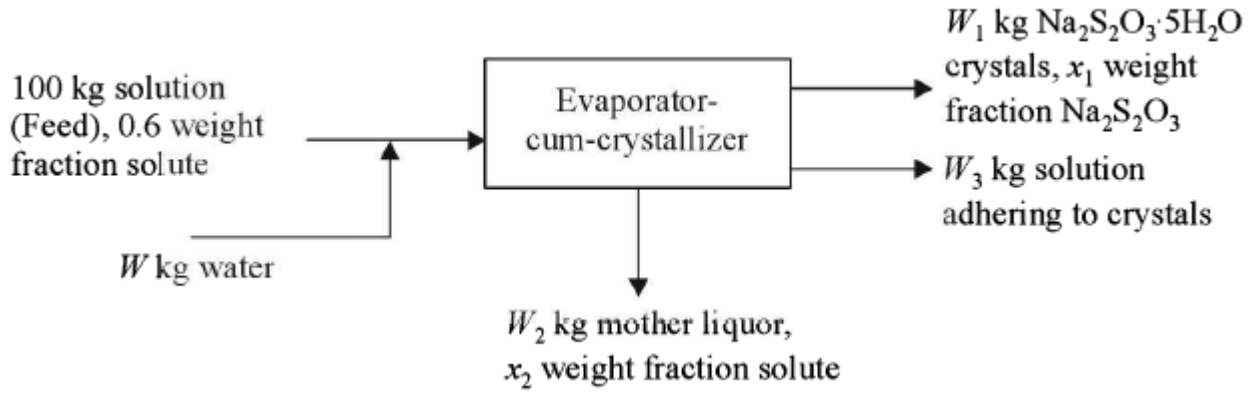
From Eq. (A), we get

$$W_3 = F - (W_1 + W_2)$$
$$= 100 - (32.38 + 33.33) = 34.29 \text{ kg}$$

Hence, the amount of water evaporated = 34.29 kg

An aqueous solution containing 60% $Na_2S_2O_3$ and 1% soluble impurities is diluted with water and fed to a crystallizer where it is cooled in order to crystallize $Na_2S_2O_3 \cdot 5H_2O$. The crystals carry 0.05 kg of solution (excluding impurities) per kg of crystals. The free water present in the adhering solution is removed on drying the crystals. The final dried product contains not more than 0.1% impurity. The solubility of the pentahydrate is 1.5 kg $Na_2S_2O_3 \cdot 5H_2O$/kg free water. On the basis of 100 kg of 60% solution, calculate the following:

(a) The amount of water in kilograms added before cooling
(b) The amount of crystals formed
(c) The percentage recovery of the $Na_2S_2O_3$ in the dried hydrated crystals



Solution *Basis:* 100 kg 60% solution

Refer to Figure 9.15. Let W kg pure water is added to the original solution. W_1, W_2 and W_3 are the weights of $Na_2S_2O_3 \cdot 5H_2O$ crystallized, the mother liquor obtained and the solution carried away by the crystals, respectively. The impurities present in the feed get dissolved in the free water and is present in the products in association with W_2 kg mother liquor and W_3 kg adhering solution. When the crystals are dried, the impurities in the adhering solution gets deposited on the crystals, the concentration of which in the final dried crystals being limited to 0.1%.

The molecular weight of $Na_2S_2O_3$ = 158 and the molecular weight of $Na_2S_2O_3 \cdot 5H_2O$ = 248. Since no water is lost by evaporation, all the free water present in the combined feed will be present in the mother liquor. 60 kg of anhydrous salt forms $60 \times \frac{248}{158} = 94.117$ kg of pentahydrate, the free water present in the system is $100 + W - 1 - 94.177 = W + 4.823$ kg. Therefore, the concentration of impurity in the mother liquor is $x_1 = \frac{1}{W + 4.823}$ kg of impurity/kg of free water. Now consider the material balance over the crystallizer.

Total (impurities-free) balance:

$$100 - 1 + W = W_1 + W_2 + W_3$$

That is,

$$W_1 + W_2 + W_3 - W = 99 \qquad \text{(A)}$$

$Na_2S_2O_3$ balance:

$$60 = \left(W_1 + W_2 \times \frac{1.5}{2.5} + W_3 \times \frac{1.5}{2.5} \right) \times \frac{158}{248}$$

which simplifies as

$$W_1 + 0.6W_2 + 0.6W_3 = 94.177 \qquad \text{(B)}$$

Since each kilogram of crystals carry 0.05 kg solution, $W_3 = 0.05W_1$. Substituting this in Eqs. (A) and (B), we get

$$1.05W_1 + W_2 - W = 99 \quad \text{(C)}$$

$$1.03W_1 + 0.6W_2 = 94.177 \quad \text{(D)}$$

Impurity in the adhering solution is

$$W_3 \times \frac{1}{2.5} \times \frac{1}{W + 4.823} \text{ kg}$$

Substituting $W_3 = 0.05W_1$, the amount of impurity in the adhering solution is

$$\frac{0.05W_1}{2.5(W + 4.823)} \text{ kg}$$

However, this should not be more than 0.1% of the final weight of crystals. The final weight of impurity-free crystals is

$$W_1 + W_3 \frac{1.5}{2.5} = W_1 + 0.05W_1 \frac{1.5}{2.5} = 1.03W_1$$

Therefore,

$$\frac{\text{weight of impurities in the crystals}}{\text{weight of pure crystals}} = 0.001$$

$$= \frac{0.05W_1/2.5(W + 4.823)}{1.03W_1} = \frac{0.0194}{W + 4.823}$$

Solving the above equation, we get W = 14.577 kg. Substituting this in Eq. (C), we get

$$1.05W_1 + W_2 = 113.577 \qquad \text{(E)}$$

Solving Eqs. (D) and (E), we get W_1 = 65.08 kg, W_2 = 45.25 kg and, $W_3 = 0.05W_1$ = 45.25 kg

(a) The amount of water added before cooling = 14.58 kg.

(b) The amount of $Na_2S_2O_3 \cdot 5H_2O$ crystals formed = 65.08 kg

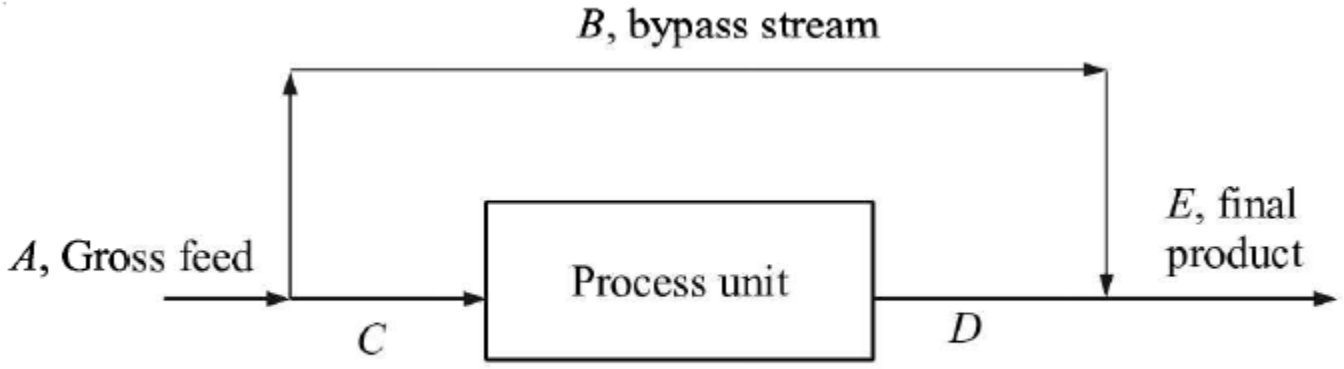
(c) $Na_2S_2O_3$ in the adhering solution gets deposited on the crystals on drying. Therefore, total anhydrous $Na_2S_2O_3$ produced

$$W_1 \times \frac{158}{248} + W_3 \times \frac{1.5}{2.5} \times \frac{158}{248} = 42.701\,\text{g}$$

The percentage recovery of $Na_2S_2O_3$ = $\frac{42.70}{60} \times 100 = 71.2\%$

Bypass

Bypass is employed mainly for effecting relatively small changes in a process stream. This is achieved by introducing large changes in a small portion of the original stream and diverting the other portion without passing it through the unit accomplishing this change. The portion so diverted is known as *bypass stream*.

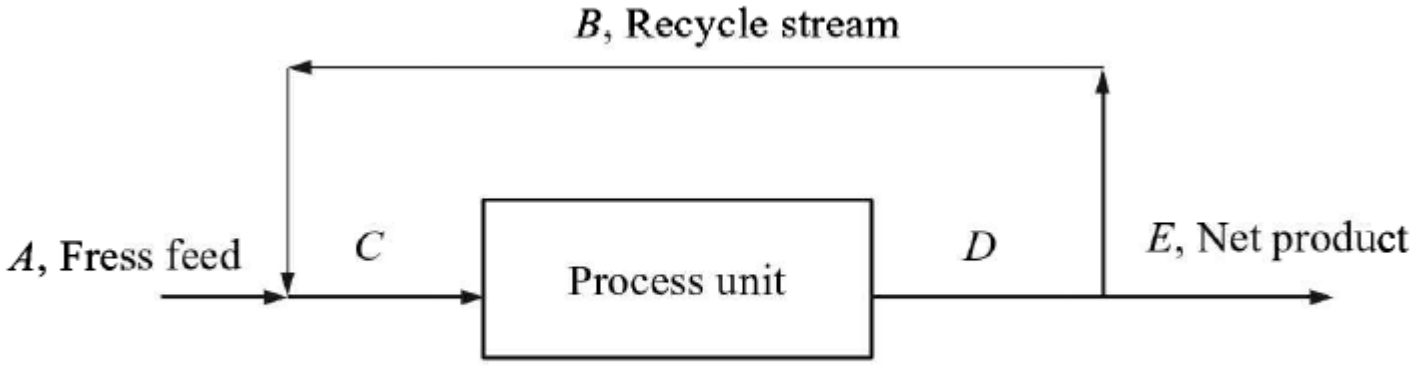


Bypass operation.

Recycle

A system with recycle is one in which a stream leaving a processing unit is partly returned to the same unit for reprocessing or reusing. The recycling of the fluid stream in chemical processing is a common practice to increase yields, to increase the purity, to enrich a product, to conserve heat, or to improve operations.

Recycling may also be used to recover an expensive catalyst, reuse a lubricant or refrigerant, maintain feed concentration below certain levels by diluting the process streams either to control flow or to control the rate of reaction. .



Recycle operation.

The ratio of the quantity of a substance in the recycled stream to the quantity of the same substance in the fresh feed is known as *recycle ratio*.

Recycle process necessitates the specification of two types of conversions—the *overall conversion* and the *single pass conversion*. The overall conversion is the fraction of the reactant in the fresh feed that is reacted considering the overall process.

$$\text{Overall conversion} = \frac{\begin{matrix}\text{moles of reactant} \\ \text{in the fresh feed}\end{matrix} - \begin{matrix}\text{moles of reactant in the product} \\ \text{from the overall process}\end{matrix}}{\text{moles of reactant in the fresh feed}}$$

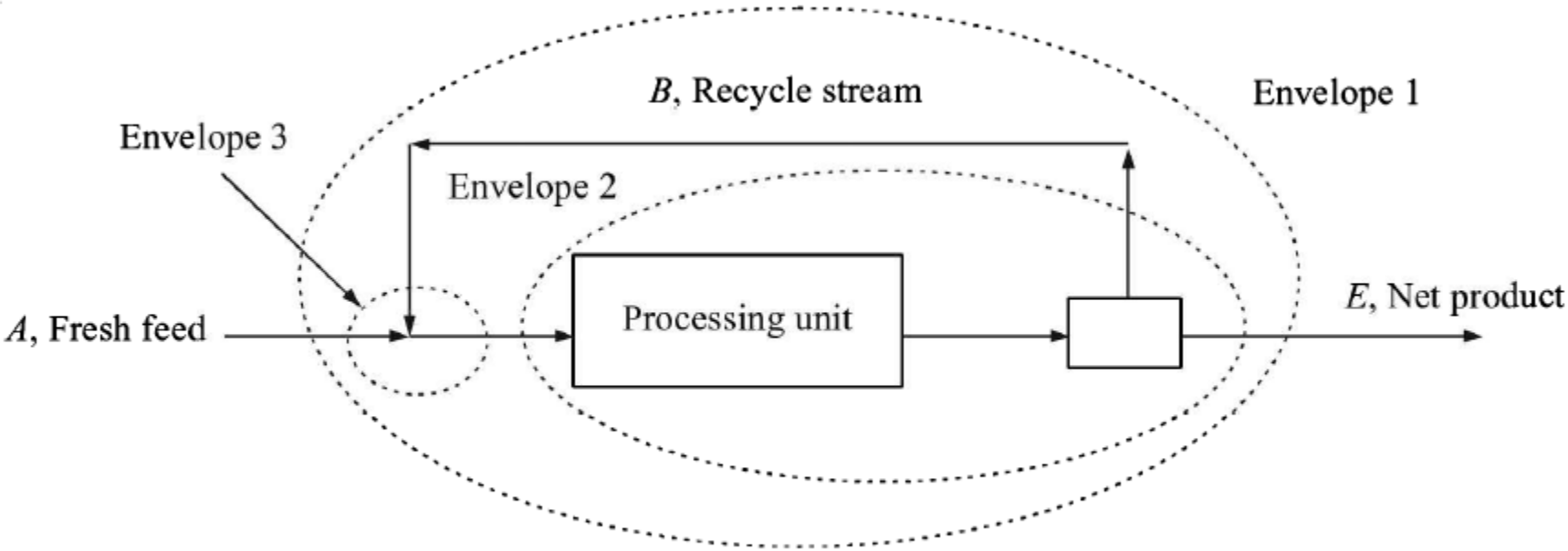
$$= \frac{\text{moles of reactant in stream } A - \text{moles of reactant in stream } E}{\text{moles of reactant in stream } A}$$

The single pass conversion is the fraction of the reactants that actually enter the reactor in a single pass that is converted.

$$\text{Single pass conversion} = \frac{\begin{matrix}\text{moles of reactant fed} \\ \text{into the reactor}\end{matrix} - \begin{matrix}\text{moles of reactant} \\ \text{leaving the reactor}\end{matrix}}{\text{moles of reactant fed into the reactor}}$$

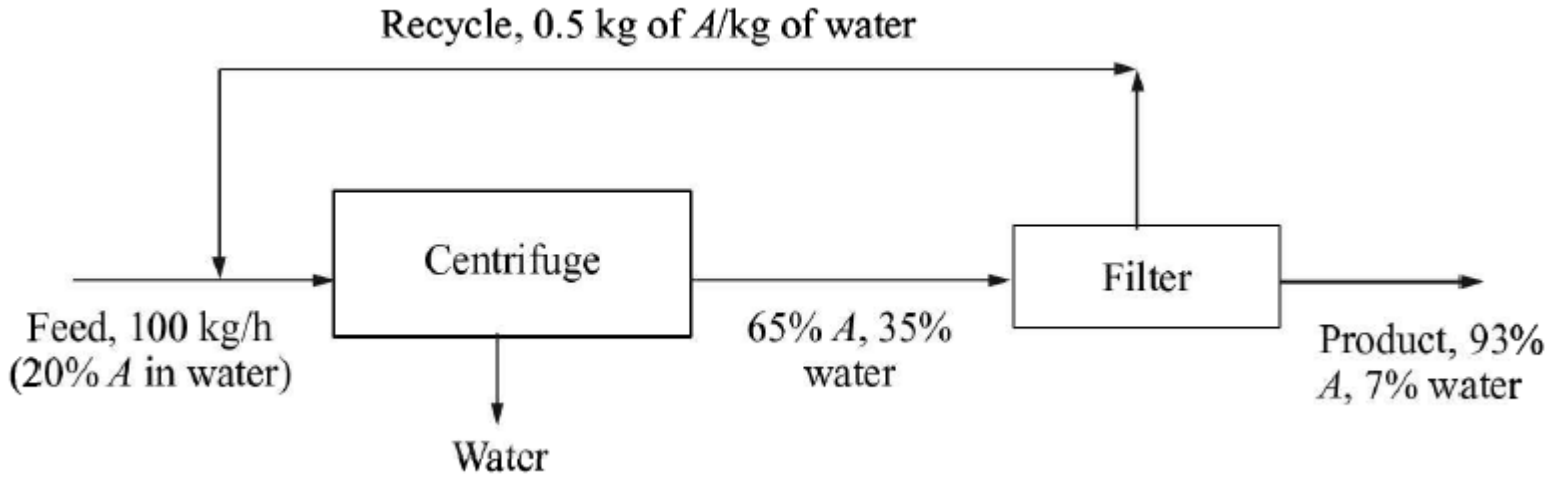
$$= \frac{\text{moles of reactant in stream } C - \text{moles of reactant in stream } D}{\text{moles of reactant in stream } C}$$

Material Balance in recycle operation



Material balance in recycle operation.

Final purification stage in the preparation of a pharmaceutical product *A* from natural sources requires centrifuging and continuous filtration as depicted in Figure Determine the flow rate of the recycle stream in kg/h.



Solution *Basis:* 100 kg/h fresh feed

Let *R* kg/h be the recycle stream, *P* kg/h be the product stream and *W* kg/h be the amount of water separated and removed in the centrifuge.

Considering the envelope which contains the centrifuge and the filter, the total material and component-A balance yield

$$100 = P + W \tag{A}$$

$$20 = 0.93\ P \tag{B}$$

Solving these equations, we get $P = 21.51$ kg/h and $W = 78.49$ kg/h.

Now, consider material balance about the filter. Let x be the flow rate of the streams entering the filter. Then, total material balance gives

$$x = P + R \tag{C}$$

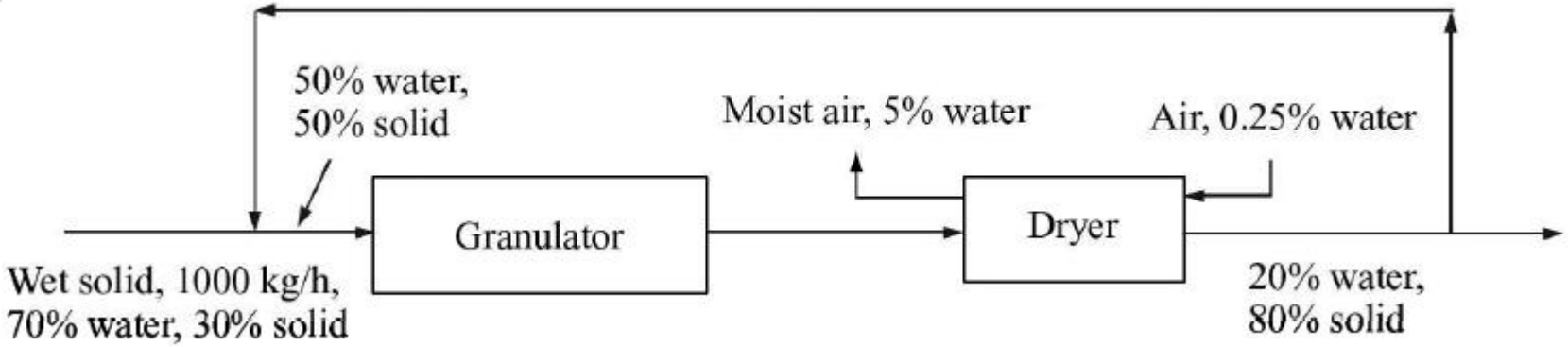
Component-A balance

$$0.65x = \frac{0.5}{1.5} R + 0.93P \tag{D}$$

Substitute $P = 21.51$ in Eqs. (C) and (D) and solve the equations simultaneously. The result is $R = 19$.

The amount of recycle stream = 19 kg/h.

A wet solid containing 70% water is mixed with recycled dry solid to reduce the water content to 50% before being admitted into the granulator. The solid leaving the granulator is fed to a drier where it is brought into contact with dry air initially containing 0.25% water by weight. In the drier, the air picks up moisture and leaves with a moisture content of 5%. The solids leaving the drier contain 20% water. A portion of this solid is recycled.



For 1000 kg/h of wet solid sent to the granulator as fresh feed, determine:

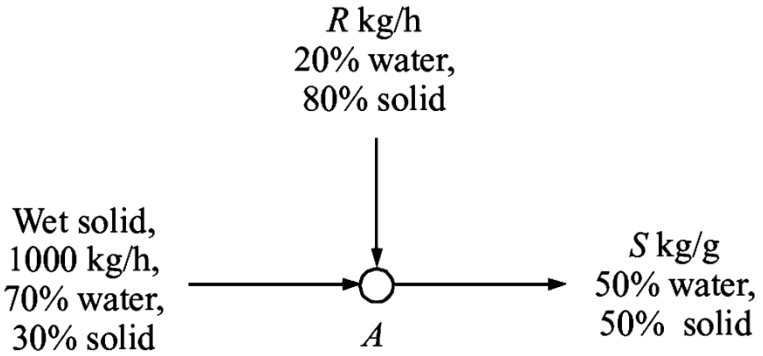
(a) The amount of solid recycled

(b) The circulation rate of air in the drier on a dry basis.

Solution *Basis:* 1000 kg/h of fresh solid feed containing 70% water. Let R be the amount of recycle stream, S be the amount of combined stream entering the granulator, P be the amount of dry solid product withdrawn from the dryer, G_1 be the amount of air admitted to the dryer and G_2 be quantity of air leaving the dryer. Consider the balance for moisture-free solid over the outer envelope which covers the granulator as well as the dryer.

$$1000 \times 0.3 = \mathrm{P} \times 0.8$$

which gives $P = 375$. Now, take material balance at point A where the recycle stream joins the fresh feed.



Total material balance,

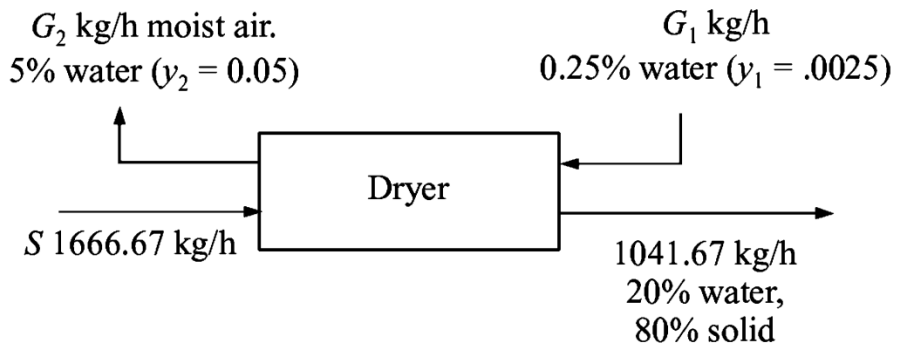
$$1000 + R = S$$

Water balance,

$$1000 \times 0.70 + R \times 0.20 = S \times 0.5$$

Solution of the above equations give $R = 666.67$ and $S = 1666.67$.

Refer to Figure 11.9 which shows the quantity and composition of material entering and leaving the dryer. The solids entering the dryer, $S = 1666.67$ kg, solids leaving $= P + R = 1041.67$ kg.



Since all the dry air entering the dryer is present in the air stream leaving the dryer, the material balance for the dry air is simply

$$G_1(1 - y_1) = G_2(1 - y_2)$$

Here, $y_1 = 0.0025$ and $y_2 = 0.05$ so that Eq. (9.58) becomes

$$G_2 = G_1 \frac{0.9975}{0.95} = 1.05 G_1 \qquad \text{(A)}$$

Finally, we can take a water balance around the drier, which is

$$S \times 0.5 + G_1 y_1 = G_2 y_2 + (P + R) \times 0.20 \qquad \text{(B)}$$

Using Eq. (A) and substituting known values into Eq. (B), we get

$$1666.67 \times 0.5 + 0.0025 G_1 = 1.05 G_1 \times 0.05 + 1041 \times 0.20$$

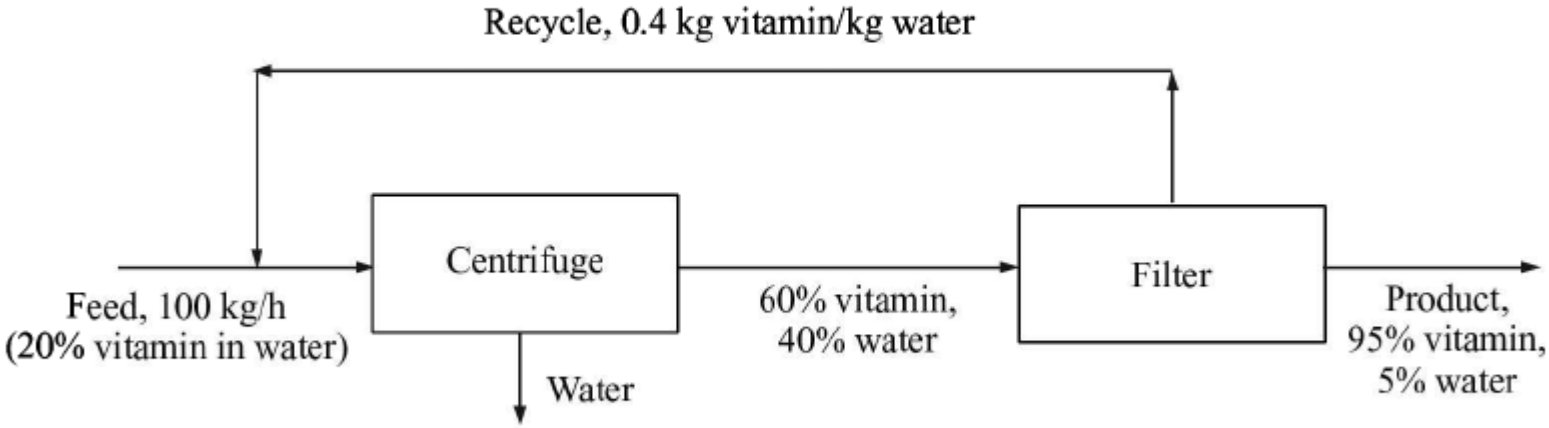
Therefore, $G_1 = 12502.7$ kg.

(a) The amount of solid recycled = $R = 666.67$ kg/h

(b) Circulation rate of dry air = $G_1(1 - y_1) = 12502.7 \times 0.9975 = 12500$ kg/h

Class work - 1

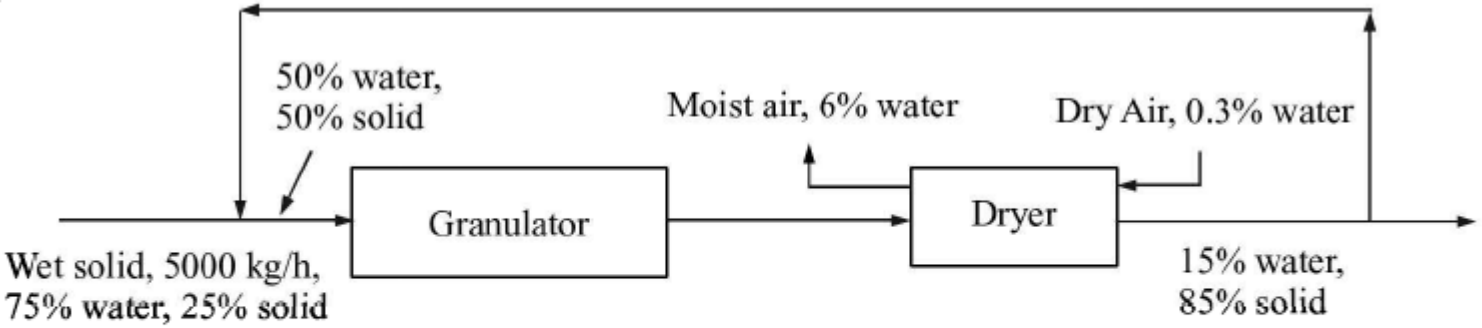
Final purification stage in the preparation of vitamins from natural sources requires centrifuging and continuous filtration, as depicted in Figure



Determine the flow rate of the recycle stream in kg/h.

Class work - 2

Wet solid containing 75% water is mixed with recycled dry solid to reduce the water content to 50% before being admitted into the granulator. The solid leaving the granulator is fed to a drier where it is brought into contact with dry air initially containing 0.3% water by weight. In the drier, the air picks up moisture and leaves with a moisture content of 6%. The solids leaving the drier contains 15% water. A portion of this solid is recycled.



For 5000 kg/h of wet solid sent to the granulator as fresh feed, determine:

(a) The amount of solid recycled
(b) The circulation rate of air in the drier on a dry basis.

Answer for Class work - 1

$100 = P + W$ — (1)

$100 \times \frac{20}{100} = P \times \frac{95}{100} + W \times 0$ (There is no vitamin in water)

$20 = 0.95P$

$P = 21.053$ kg/hr

$\therefore 100 = 21.053 + W$

$W = 78.947$ kg/hr

Filter N.B

$x = P + R$ — (2)

vitamin N.B

$0.6x = 0.95P + \frac{0.4R}{1.4}$

$0.6x = 0.95P + 0.285R$ — (3)

$0.6x - 0.285R = 20$ — (4)

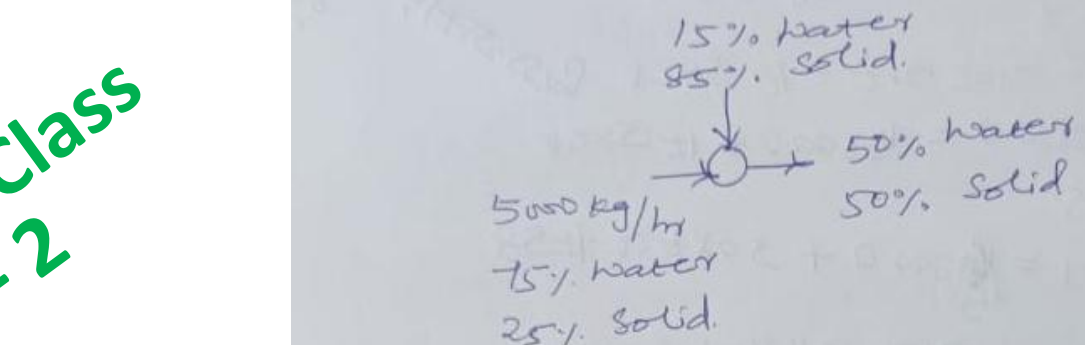
$x - R = 21.053$ — (5)

$x = 44.44$ kg/hr

$R = 23.391$ kg/hr

Answer for Class work - 2

a) Amount of solid recycled.



$$5000 \times \frac{25}{100} = P \times \frac{85}{100}$$

$$1250 = 0.85P$$

$$P = 1470.588 \ \frac{kg}{hr}$$

Overall MB

$$5000 + R = S \quad -(1)$$

$$5000 \times \frac{25}{100} + R\frac{85}{100} = S\frac{50}{100}$$

$$1250 + 0.85R = 0.5S \quad -(2)$$

$$R - S = -5000 \quad -(1)$$

$$0.85R - 0.5S = -1250 \quad -(2)$$

(1) × 0.5 $\quad 0.5R - 0.5S = -2500$

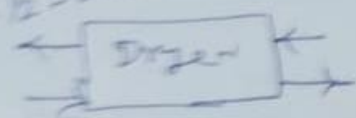
$0.85R - 0.5S = -1250$

$0.35R = -1250$

$R = 3571.42$

$S = 8571.429$

G_2 kg/hr
6% (y_2 = 0.06)



G_1 kg/hr
0.3% (y_1 = 0.003)

S
8571.429 kg/hr

5042.008 kg
15% water/hr
85% solid

$$G_1(1-y_1) = G_2(1-y_2)$$

$$G_1(1-0.003) = G_2(1-0.06)$$

$$\frac{G_1}{G_2} = \frac{0.94}{0.997} = 0.9428$$

$$G_1 = 0.943 G_2$$

$$G_2 = 1.06 G_1$$

$$S \times 0.5 + G_1 y_1 = G_2 y_2 + (P+R) \times 0.15$$

$$8571.429 \times 0.5 + 0.003 G_1 = 1.06 G_1 (0.06) + 5042.008 \times 0.15$$

$$4285.71 + 0.003 G_1 = 0.0636 G_1 + 756.3012$$

$$4285.71 - 756.3012 = 0.0636 G_1 - 0.003 G_1$$

$$\frac{3529.41}{0.0606} = G_1$$

$$G_1 = 58241.089 \text{ kg}$$

$\therefore G_2 = 61735.55$ kg

Process Calculations for Biotechnologist - 21BT32T

Unit - 4

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UNIT 4 ENERGY BALANCE

Fundamentals of energy balance calculations – Concepts of heat capacity, latent heat, sensible heat, vapour pressure and internal energy – energy balance with and without chemical reactions, Application of energy balance in Bioprocesses.

Total period required = 15 (9 + 6)

COURSE OUTCOMES

Make use of the energy balance concepts in various industrial processes

Heating values of fuels.

The heating value of a fuel is equal to its standard heat of combustion but of opposite sign.

Total heating value.

A fuel is heat evolved in its complete combustion under constant pressure at a temperature of 25°C.

Net heating value.

It is defined as final state of the water in the system after combustion is taken as vapour at 25°C.

Coke and Carbon.

The heat of formation of carbon is the form of the diamond in accurately known and is equal to 0.45 32 Kal per g-atom.

coal analysis?

It is addition to organic matter it contain minerals constituent of the plants from which it was formed and inclusion.

Ultimate analysis?

It is determination is made of each of the proximate analysis defined groups of moisture, volatile matter, fixed carbon and art.

Combustible.

The sum of the fixed carbon and volatile matter of a coal.

Rank of Coal.

The fuel ratio of a coal is defined as the Ratio of the percentage of fixed carbon to that of volatile matter.

Define Heating value of coal.

The total heating value of a coal may be determined by direct calorimetric measurement.

Molecular at of petroleum fraction.

The average molecular at of petroleum fractions may be average boiling point and gravity.

Hydrogen content.

The relationship between hydrogen content and characteristic factor fro material of constant boiling points.

Incomplete combustion of fuels.

The standard heating values at fuel correspond to conditions at complete combustion of all carbon to carbon dioxide gas.

enthalpy of water vapour.

The enthalpy of superheated water vapour referred to the liquid at 25°C

enthalpy of vapourisation.

The enthalpy of water is equal to the heat of sum vapourisation at 25°C.

Thermal efficiency?

The thermal efficiency after process may be defined as the percentage of the heat input that is effectively utilized.

hot thermal efficiency?

If the gas is cooled before use it is sensible heat is available.

cold thermal efficiency.

It is heating value can be classed as heat effectively utilized in the producer.

weight of dry gaseous products.

A direct Measurements of the dry gaseous products from a combustion process.

weight of Dry-Air supplied.

Direct Measurement of the ut (as) volume of air used in combustion to the gaseous products.

ut of moisture in air.

The ut of moisture per mole of dry air depends on the temperature pressure, relative humidity of the air.

The flue gas from an industrial furnace has the following composition by volume:

CO_2-11.73%, CO – 0.2%, N_2 -0.09% O_2 – 6.81% and N_2 -81.17%

Calculate the percentage excess air employed in the combustion if the loss of carbon in clinker and ash is 1% of the fuel used and the fuel has the following composition by weight:

C-74%, H_2 -5%, O_2-5% N_2-1% S-1%, H_2O-9% and ash -5%,

Basis : 100 kg of the fuel charged to the industrial furnace.
Reactions:

$$C + O_2 \rightarrow CO_2 \quad \ldots (1)$$

$$H_2 + \frac{1}{2} O_2 \rightarrow H_2O \quad \ldots . (2)$$

$$S + O_2 \rightarrow SO_2 \quad \ldots . (3)$$

Oxygen balance:
Oxygen required for complete combustion

$$= \frac{74}{12} + \frac{5}{2} \times 0.5 + \frac{1}{32} kg.mol \text{ (Ref.1,2 and 3)}$$

= 7.447 kg.mol.

Oxygen already present in fuel

$$= \frac{5}{32} = 0.157 kg.mol$$

Net oxygen demand from air

7.447-0.157=7.29 kg.mol

Carbon balance:
Carbon lost in clinker and ash = 1 kg
Carbon burnt =74-1.0=73 kg=6.08 kg atom
Let x kg mols of flue gas are formed.
Therefore,
(.1173+.002)x=6.8

x= 50.96 kg.mol.

From flue gas analysis,
N_2 in flue gas = 50.96 $\times$ 0.8117=41.36 kg.mol.
N_2 from fuel = 1 kg =0.036 kg mol.
N_2 from air = 41.360-0.036=41.324 kg.mol.
Oxygen supplied from air

$$= 41.324 \times \frac{21}{79} = 10.98 \text{ kg mol}$$

Excess oxygen = 10.98 -7.29 =3.69 kg.mol

Percentage excess air used

≡ Percentage excess oxygen used

$$= \frac{Excess}{Theoretical} \times 100 \times \frac{3.69}{7.29} \times 100 = 50.62.$$

Ex. No: 2

Octane is burnt with 10% excess air. Calculate the following:

(a) Air/fuel ratio by weight.

(b) Air/fuel ratio by volume.

(c)Weight of dry exhaust gas formed per unit weight of fuel.

(d) Mol of oxygen in the exhaust gas per unit weight o fuel.

(e) Mol of water vapour in exhaust gas per unit weight of fuel

(f) Volume of exhaust gas at 1atmosphere and 260° per unit weight f fuel.

The specific gravity of octane may be taken as 0.7

Basis : 1 kg mol of octane burnt.

Reaction: C_8H_{18} 12.5 $O_2 \rightarrow$ 8 CO_2 +9 H_2O

(a) Theoretical oxygen demand =12.5 kg. mol

Oxygen supplied by 10% excess air

=12.5 × 1.1 = 13.75 kg mols =440 kg.

Nitrogen supplied by air

$$= 13.75 \times \frac{79}{21} = 51.73 kg \text{ mol}$$

=1448.4 kg

Amount of air supplied

=13.75+51.73=65.48 kg mol

= 1888.4 kg

Molecular weight of fuel =114

$$\frac{Wt.of \text{ air}}{Wt.of \text{ fuel}} = \frac{1888.4}{114} = 16.56.$$

(b) Sp. Gravity of octane = 0.7

Density of octane = 0.7 gm/cc =700 kg/m^3

Volume of fuel = $\frac{114}{700}$ =0.163 m^3

Assuming ideal gas behaviour, volume of air at N.T.P

=65.48 × 22.4 =1466.75 m^3

$$\frac{\text{Volume of air}}{\text{Volume of fuel}} = \frac{1466.75}{0.163} = 8998.5$$

© Excess O_2 =supplied O_2- used O_2
=13.75-12.50=1.25 kg mol.

Dry flue gas analysis

Constituents	Amount kg mol	Amount kg
CO_2	8.00	352.0
O_2	1.25	40.0
N_2	51.73	1448.4
Total	60.98	1840.4

$$\frac{\text{Wt. of dry exhaust gas}}{\text{Wt. of fuel}} = \frac{1840.4}{114} = 16.4$$

$$(d)\ \frac{\text{Mol. of } O_2 \text{ in the exhaust gas}}{\text{Wt. of fuel}} = \frac{1.25}{144} = 0.011$$

$$(e)\ \frac{\text{Mol. of water vapour in the exhaust gast}}{\text{Wt. of fuel}} = \frac{9.0}{114} = 0.079$$

(f) Mols of exhaust gas (wet)

$$= 60.98 + 9.0 = 69.98$$

Applying ideal gas law, volume at 260°C and 1 atmosphere

$$\frac{nRT}{P} = \frac{69.98 \times 0.08206 \times (260 + 273)}{1.0}$$

$$= 3060.8 \text{ m}^3$$

$$\frac{\text{Volume of exhaust gas (wet)}}{\text{Wt. of fuel}} = \frac{3060.8}{114} = 26.85.$$

Ex. No: 3

A producer gas with the composition by volume, 27.3% CO, 5.4% CO_2, 0.6%-O_2, 66.7%-N_2 is burnt with 20% excess air. If the combustion is 98% complete, calculate the composition by volume of the fuel gases.

Basis: 100 kg mole of producer gas burnt.
Oxygen balance:

O_2 required for CO combustion
$= 27.3 \times 0.5 = 13.65$ kg mole

O_2 present in fuel =m 0.6 kg mole

Net O_2 required = 13.65 -0.6 =13.05 kg mol

O_2 supplied by 20% excess air

$= 13.05 \times 1.20 = 15.66$ kg mol

O_2 required for 98% combustion of CO

$=273 \times 0.5 \times 0.98 = 13.38$ kg mol

Total available oxygen = 15.66+0.6

= 16.26 kg mol.

O_2 in excess =16.26 – 13.38 =2.88kg mol.

Nitrogen balance:

N_2 from producer gas = 66.7 kg mol.

N_2 from air $= 15.66 \times \frac{79}{21}$ =58.91 kg.mol

N_2 from both the sources, which is in the flue gas

= 66.7+58.91=125.61 kg.mol.

Carbondioxide balance:

CO_2 from producer gas = 5.4 kg mol.
CO_2 from the combustion of CO

$= 27.3 \times 0.98 = 26.75$ kg mol

Total CO_2 in flue gas $= 5.4 + 26.75 = 32.15$ kg mol.

Carbon monoxide balance:
CO burnt = 26.75 kg mol.
CO left $= 27.3 - 26.75 = 0.55$ kg mol

Constituents	Amount	
	Kg.mol	Mol%
CO_2	32.15	19.44
CO	0.55	0.34
N_2	125.1	77.93
O_2	2.88	1.79
Total	161.19	100.00

Ex. No: 4

A furnace is fired with a natural gas that consists entirely of hydrocarbons (negligible inert gases and sulphur compounds). The Orsat analysis of the flue gas gives 9.5% CO_2, 2.0% O_2 and 1.8% CO.

What is the molar ratio of net hydrogen to carbon in the fuel?

What per cent of excess air is being used?

Basis: 100 kg mol of dry flue gas.
From Orsat analysis,

Mole of N_2 = 100-(9.5+1.8+2.0)=86.7.
Oxygen balance:

O_2 supplied by air = $86.7 \times \frac{21}{79}$ =23.05 kg mol

O_2 reported in fuel gas (dry)

$$=9.5+\frac{1}{2}\times 1.8 +2.0=12.4 \text{ kg mol}$$

O_2 unaccounted = 23.05 -12.4 =10.65 kg mol

= O_2 reacted with H_2.

(a)Mols of hydrogen reacted

$$= 10.65 \times 2 = 21.3$$

Amount of carbon = 9.5+1.8 =1.3 kg atom.

$$\frac{\text{Mols of } H_2}{\text{Atoms of } C} = \frac{21.3}{11.3} = 1.885.$$

(b) Mols of O_2 required for complete combustion

=Mol required for H_2+Mol required for C

=10.65+11.3=21.95

Amount of excess O_2 = 23.05 -21.95=1.1mol

% excess air ≡ % excess O_2 = $\frac{1.1}{21.95}\times 100 = 5.0$

Ex. No: 5

The exhaust gas from a hydrocarbon fuel oil fired furnace, shows 10.2% CO_2, 7.9% O_2 and 81.9% N_2 by Orsat analysis.

Calculate

(i) % excess air used, and

(ii) kg of dry air supplied per kg of oil burnt in the engine.

N_2 in flue gas = 81.9 kg mol

O_2 supplied from air $= 81.9 \times \frac{21}{79} = 21.77$ kg mol

O_2 reported in flue gas = 10.2 (as CO_2) +7.9 (as O_2)
=18.1 kg mol

O_2 unaccounted = 21.77-18.1
=3.67 kg ml =O_2 used for H_2.

O_2used actually = 10.2+3.67=13.87 kg mol.

O_2 excess in flue = 7.9 kg mol

(a) % excess air $\equiv$ % excess O_2

$$= \frac{7.9}{13.87} \times 100 = 56.96.$$

(b) O_2 used for H_2 = 3.67 kg mol.

Amount of H_2 in fuel $= 3.67 \times 2 = 7.34$ kg mol

$= 14.68$ kg

Carbon in fuel = 10.2 kg atom

$= 122.4$ kg.

Total weight of the hydrocarbon fuel oil.

= Wt. of H + Wt. of C

$= 14.68 + 122.4 = 137.08$ kg

Weight of air = Mol of $O_2 \times 32$ + Mol of $N_2 \times 28$

$= 21.77 \times 32 + 81.9 \times 28 = 2989.8$ kg

$$\frac{\text{Kg dry air}}{\text{Kg oil burnt}} = \frac{2989.8}{137.08} = 21.81.$$

Ex. No: 6

Determine the fuel gas analysis and air fuel ratio by weight when a medium fuel oil with 84.9% carbon,11.4% hydrogen, 3.2% sulphur, 0.4%Oxygen and 0.1% ash by weight is burnt with 20% excess air. Assume complete combustion.

Basis : 100 kg of medium fuel oil burnt
Oxygen balance:
Amounts of O_2 used are calculated with the help of Eqns.(1), (2) (3) of Ex.1

O_2 required for C=$84.9 \times \frac{32}{12} = 226.4kg$

O_2 required for S=$3.2 \times \frac{32}{32} = 3.2kg$

O_2 required for H =$11.4 \times 8 = 91.2\ kg$

Total	320.8 kg

O_2 present in fuel =0.4 kg
O_2 required from air =320.8-0.4=320.4 kg.

O_2 supplied $= 320.4 \times 1.2 = 384.48$ kg
O_2 excess $= 384.48 - 320.4 = 64.08$ kg

Air supplied $= 384.48 \times \frac{100}{23} = 1671.65$ kg

$$\frac{\text{Wt. of air}}{\text{Wt. of fuel}} = \frac{1671.65}{100} = 16.716.$$

Flue gas Analysis (Wet):

Amount of N_2 = $1671.65 \times \frac{77}{100} = 1287.17 Kg$

CO_2 produced $= \frac{84.9}{12} \times 44 = 311.3 = 7.075$ kg.mol.

SO_2 Produced $= 3.2 \times \frac{64}{32} = 6.4$ kg $= 0.10$ kg mol.

H_2O produced $= \frac{11.4}{2} \times 18 = 102.6$ kg $= 5.70$kgmol

O_2 excess = 64.08 = 2.003kg mol.

Flue gas analysis is given below:

Constituents	Amount kg mol	Amount kg
CO_2	7.075	11.63
SO_2	0.100	0.16
O_2	2.003	3.29
N_{2+}	45.970	75.55
H_2O	5.700	9.37
Total	60.848	100.00

Ex. No: 7

A boiler is fired using 200 kg hr, of a pure saturated hydrocarbon gas (C_nH_m) at atmospheric pressure and 20°C. The dry analysis of the fuel gas which leaves the boiler at atmospheric pressure and 300° C is CO_2-12%, O_2 -3% and N_2-85%. Estimate the formula of the fuel and total volumetric flow rate of the gas.

Basis: 100 kg mol of dry flue gas
Oxygen balance:

N_2 in flue gas = 85 kg mol.

O_2 supplied by air $= 85 \times \frac{21}{79} = 22.59\ kg$ mol

O_2 reported in flue gas = O_2 as CO_2 + O_2 as O_2
= 12+3.0 =15.0 kg mol.

O_2 unaccounted = O_2 reacted with H_2
= 22.59-15.00 =7.59 kg mol.

H_2 reacted = 7.59 ×2 = 15.18 kg mol = 30.36 kg

Formulated of the fuel (hydrocarbon)gas:

Amount of carbon = 12 kg atom

Amount of hydrogen = 30.36 kg atom

$$\frac{\text{Atom of H}}{\text{Atom of C}} = \frac{30.36}{12} = 2.53$$

As the ratio is more than 2., hence it is a paraffin of formula C_nH_{2n+2}.

$$\frac{n}{2n+2} = \frac{1}{2.53}$$

Solving, n= 3.77 =4.0
Hence the fuel is n-butane (C_4H_{10})
Volumetric flow rate:

$$\text{Amount of fuel} = \frac{200}{58} = 3.45 \frac{kg.mol}{hr.}$$

Assuming ideal gas law, volume at 20°C and 1 atmosphere is,

$$V = \frac{nRT}{P} = \frac{3.45 \times 0.8206 \times (273+20)}{1.0}$$

$$= 82.95 \text{ m}^3 / hr.$$

Ex. No: 8

A furnace is fired by a gas having the composition H_2-52%, CH_4-30%, CO-8%, C_nH_m-3.%, CO_2-2%, O_2-0.4% and rest N_2. Using a certain quantity of air excess over stoichiometric complete combustion of the gas is achieved, giving a dry water gas of 5 m^3 per m^3 of fuel burned. Estimate:

(a) composition by volume of dry waste gas formed

(b) Per cent excess air used

(c) Weight of water formed per m^3 of gas burned, neglecting considered as C_3H_6.

Basis: 100 kg mols of fuel burnt

Reactions : $CO+\frac{1}{2}O_2 \rightarrow CO_2$

$CH_4+2O_2 \rightarrow CO_2+2H_2O$

$C_3H_8\ (C_nH_m)+4.5O_2 \rightarrow 3CO_2+3H_2O$

$H_3+\frac{1}{2}O_2 \rightarrow H_2O$

Oxygen balance.

O_2 required for CO $= 8 \times \frac{1}{2} = 4$ kg mol

O_2 required for CH_4 $= 30 \times 2 = 60$ kg mol

O_2 Required for C_3H_8 $= 3.6 \times 4.5 = 16.2$ kg mol

O_2 required for H_2 $= 52 \times \frac{1}{2} = 26.0$ kg mol

Total O_2 required $= 106.2$ kg mol

O_2 in fuel $= 0.4$ kg mol

Net O_2 from air $= 106.2 - 0.4 = 105.8$ kg mol

Dry flue gas formed (with theoretical amount of air):

CO_2 formed = 8.0 (from CO) +30.0 (from CH_4)+10.8 (from C_3H_8) +2.0 (from fuel
= 50.8 kg mol.

N_2 from air $= 105.8 \times \frac{79}{21} = 398.0$ kg mol

Total N_2 = 398.0 +4.0 = 402.0 kg mol

Amount of dry flue gas = 50.8 +402.0 = 452.8 kg mol

$$\frac{Waste\ gas}{Fuel\ gas} = \frac{5m^3}{m^3}$$

Let it be assumed that the fuel gas the flue gas be reported at the same conditions. Hence volume ratio is identical to mol ratio.

Mols of waste gas = 5 × Fuel gas = 500 kg mol
Dry fuel gas with theoretical amount of air

$= 452.8$ kg mols

Hence excess air $= 500-452.8 = 47.2$ kg mol

Theoretical air $= 105.8 \times \frac{100}{21} = 503.81$ kg mol

(b) % excess air used $= \frac{47.2}{503.81} \times 100 = 9.39$

(a) Composition of waste gas

Air used $= 503.81 + 47.2 = 551.01$ kg mol

N_2 from air $= 551.01 \times \frac{79}{100} = 435.29$ kg mol

Total N_2 $= 435.29 + 4.0 = 439.29$ kg mol

Excess O_2 $= 47.2 \times \frac{21}{100} = 9.91$ kg mol

Waste gas analysis:

Constituents	Amount (mol)	Mol % or vol. %
CO_2	50.80	10.16
O_2	9.91	1.98
N_2	439.29	87.86
Total	500.00	100.00

(c) amount of water formed

= 52.0(from H_2)+60 (from CH_4) +10.8 (from C_3H_6)

=122.8 kg mols = 2210.4 kg

Amount of gas burned = 100 kg mol = 2240 m^3 at NTP

$$\frac{Wt.\text{ of water formed}}{m^3\text{ of gas burned}} = \frac{2210.4}{2240} = 0.987.$$

Ex. No: 9

The dry fuel gas from an oil fired furnace has a composition of 11.2% CO_2, 5.8% O_2 and 83% N_2 when analyzed by an Orsat apparatus. Calculate:

(a) Present excess air, and

(b) Weight of combustion air used per kg. of oil fired.

Assume fuel to have 82% C, 12%H, 3% S and balance impurities. Molecular weight of dry gas is 30.

Basis: 100 kg of oil fired.

Oxygen balance:

O_2 rqd. For 82 kg carbon = 218.67 kg = 6.833 kg mol.

O_2 rqd. for 12 kg hydrogen = $12 \times 8 = 96$ kg = 3.0 kg mol.

O_2 rqd. for 3 kg sulphur = 3 kg = 0.094 kg mol.

Total O_2 rqd. = 9.927 kg mol.

Let x kg mols of dry fuel gases are formed.

Carbon balance:

Carbon in fuel $= \frac{82}{12} = 6.833$ kg atom

Carbon in fuel gases = 0.112 x (from CO_2) kg atom.

So, $0.112\,x = 6.833$

$\therefore$ $x = 61.0$ kg mol.

Amount of N_2 in fuel gas = $61.0 \times 0.83 = 50.63$ kg mol.

This N_2 has come from combustion air.

So, O_2 from air $= 50.63 \times \frac{21}{79} = 13.46$ kg mol

O_2 used = 9.927 kg mol.

O_2 excess = $13.46 - 9.927 = 3.533$ kg mol.

% excess air $\equiv$ % excess O_2

$$= \frac{3.533}{9.927} \times 100 = 35.60$$

(b) Amount of combustion air

$$= 50.63 \times \frac{100}{79} = 64.09 \text{ kg mol}$$

Molecular weight of air = 28.84

Weight of air $= 64.09 \times 28.84 = 1848.36$ kg

$$\frac{\textit{Wt.} \text{ of combustion air}}{\textit{Wt.} \text{ of fuel}} = \frac{1848.36}{100} = 18.48.$$

Ex. No: 10

16. a. (i) A combustion reactor is fed with 50 kmol/ h of butane and 2100 kmol/h of air. Calculate the % air used. (8)

Sol. : Basis : 50 kmol/h of butane.

Reaction : $C_4H_{10} + \frac{13}{2} O_2 \rightarrow 4\,CO_2 + 5\,H_2O$

Air flow rate = 2100 kmol/h

O_2 in air supplied = $2100 \times (0.21) = 441$ kmol/h

From reaction, we have,

1 kmol $C_4H_{10} \equiv 6.5$ kmol O_2

$\therefore$ Theoretical O_2 requirement for 50 kmol butane per hour

$$= \frac{6.5}{1} \times 50 = 325 \text{ kmol/h}$$

$\therefore$ Theoretical requirement of air $= 325 \times \frac{100}{21} = 1547.6$ kmol/h

Air supplied to reactor = 2100 kmol/h

$$\% \text{ excess air} = \frac{\text{Air supplied} - \text{Air theoretically required}}{\text{Air theoretically required}} \times 100$$

$$= \frac{2100 - 1547.6}{1547.6} \times 100 = 35.7\%$$

b. Ethylene oxide is produced by oxidation to of ethylene. 100 kmol of ethylene are fed to the reactor and the product is found to contain 80 kmoi ethylene oxide and 10 kmol carbon dioxide. Calculate: (a) the percent conversion of ethylene and (b) the percent yield of ethylene oxide. (13)

Ex. No: 11

Sol. : Basis : 100 kmol of ethylene fed to a reactor.

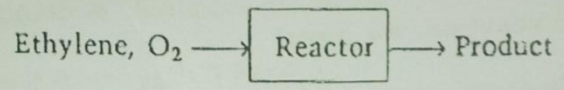

Fig. 4.4 : Production of ethylene oxide

Reaction 1 : $C_2H_4 + \frac{1}{2} O_2 \rightarrow C_2H_4O$

Reaction 2 : $C_2H_4 + 3\,O_2 \rightarrow 2\,CO_2 + 2\,H_2O$

Amount of C_2H_4O produced is 80 kmol and amount of CO_2 produced is 10 kmol.

From reaction - 1, 1 kmol C_2H_4 ≡ 1 kmol C_2H_4O

∴ C_2H_4 reacted to produce C_2H_4O i.e. by reaction - 1,

$$= \frac{1}{1} \times 80 = 80 \text{ kmol}$$

From reaction - 2, 1 kmol C_2H_4 ≡ 2 kmol CO_2

C_2H_4 reacted to produce 10 kmol CO_2 by reaction - 2,

$$= \frac{1}{2} \times 10 = 5 \text{ kmol}$$

C_2H_4 totally reacted = C_2H_4 reacted by reaction - 1
+ C_2H_4 reacted by reaction - 2
= 80 + 5 = 85 kmol

$$\% \text{ conversion of } C_2H_4 = \frac{85}{100} \times 100 = 85 \quad \text{... Ans. (a)}$$

$$\% \text{ yield of } C_2H_4O = \left(\frac{\text{kmol } C_2H_4 \text{ reacted to produce } C_2H_4O}{\text{kmol } C_2H_4 \text{ totally reacted}}\right) \times 100$$

$$= \frac{80}{85} \times 100 = 94.12 \quad \text{... Ans. (b)}$$

Ex. No: 12

(ii) In production of sulphur trioxide, 100 kmol of SO_2 and 200 kmol of O_2 are fed to a reactor. The product stream is found to contain 80 kmol SO_3. Find the percent conversion of SO_2. (7)

Sol. : Basis : 100 kmol of SO_2 entering the reactor.

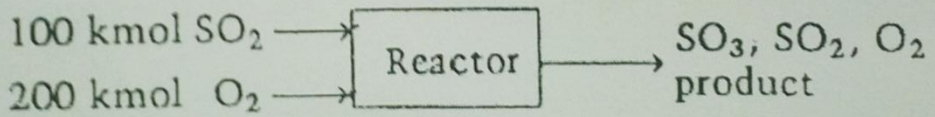

Fig. 4.1 : Production of sulphur trioxide

Reaction : $SO_2 + \frac{1}{2} O_2 \rightarrow SO_3$

$\therefore$ 1 kmol SO_2 $\equiv$ 1 kmol SO_3

i.e. 1 kmol SO_3 requires 1 kmol SO_2 to be reacted by reaction (1).

1 kmol SO_3 $\equiv$ 1 kmol SO_2

80 kmol SO_3 $\equiv$?

$\therefore$ SO_2 reacted $= 80 \times \frac{1}{1} = 80$ kmol

$$\% \text{ conversion of } SO_2 = \frac{\text{kmol } SO_2 \text{ reacted}}{\text{kmol } SO_2 \text{ charged}} \times 100$$

$$= \frac{80}{100} \times 100 = 80$$

Ex. No: 13

100 kg per hour of coke are fed to a furnace having a grate efficiency such that 95% carbon present in the coke is burnt. The coke composition is 90% carbon and 10% ash (by weight) 30% excess air is supplied for ensuring complete combustion. If 98% of carbon burnt is oxidized to dioxide and the rest to monoxide,

(a) Report the composition by volume of fuel gases.

(b) If the fuel gases are at a temperature of 300°C and a pressure of 750 mm Hg. Calculate their flow rate in cubic metres/minute.

O_2 rqd. For complete combustion.

$$= \frac{90}{12} \times 32 = 240 \text{ kg}$$

O_2 supplied	=	240×1.3=312kg
Carbon burnt	=	90×0.95=85.5kg
Carbon to CO_2	=	85.5×.98=83.79kg
Carbon to CO	=	85.5×.02=1.71kg

O_2 used for CO $= 1.71 \times \frac{16}{12} = 2.28 kg$

Total O_2 used	=	225.72kg
Excess O_2 in flue	=	312-225.72=86.28kg

(a) Flue Gas Analysis:

N_2 from air = $312 \times \frac{77}{23} = 1044.5kg$

CO_2 formed = 83.79+223.44=307.23 kg

CO formed = 1.71+2.28=3.99kg

Analysis is as follows:

Constituents	Amount, kg	Mol	Mol% (vol%)
CO_2	307.23	6.98	14.81
CO	3.99	0.14	0.30
O_2	86.28	2.70	5.73
N_2	1044.50	37.30	79.16
Total	1442.00	47.12	100.00

(b) P=750 mm Hg.
T=300°C=573°K

$$R = \frac{PV}{nT} = \frac{760 \times 22.4}{1 \times 273} = 62.36 \frac{(mmHg)(m^3)}{kg\ mol^{o}k}$$

$$\text{Volume of flue gas} = V = \frac{nRT}{P}$$

$$= \frac{47.12 \times 62.36 \times 573}{750}$$

$$= 2244.94 m^3/hr = 37.42 \frac{m^3}{min}$$

Ex. No: 14

A coal containing C-67.9%, H-4.4%,S-0.8%,N-16%,O-7.9% ash 4.5% and water 12.9% is burnt in a furnace. The product of combustion dry gas, analyses CO_{2-} -14.5%, O_2-4.7% and N_2 rest. Calculate:

a. **The theoretical volume of air used for the complete combustion of 100kg of coal;**
b. **The percent excess air used.**

O_2 required for carbon $= 67.9 \times \frac{1}{12} = 5.66 kg\ mol$

O_2 required for H_2 $= \frac{4.4}{2} \times \frac{1}{2} = 1.10$ kg mol

O_2 required for S $= 0.8 \times \frac{1}{32} = 0.025$ kg mol

Total O_2 required $= 6.785$ kg mol

O_2 present $= \frac{7.9}{32} = 0.247$ kg mol.

O_2 to be supplied $= 6.785 - 0.247 = 6.538$ kg mol.

(a) Air required $= 6.538 \times \frac{100}{21} = 31.13 kg$ mol

$= \frac{31.13 \times 0.082 \times 273}{1}$

$= 697.4 m^2$ at N.T.P.

(b) Let Y=mols of the flue gas obtained,

Carbon in flue =0.145Y.

Carbon in coal =5.66 kg atom.

So, 0.145 Y =5.66

Y=30.03 kg mol.

N_2 in flue gas =100-(14.5-4.7)=80.8%

Amount of N_2 =39.03 ×0.808=31.54 kg mol.

N_2 from air = Total N_2 – Fuel N_2

$$= 31.54 - \frac{16}{28} = 30.97 \text{ kg mol}$$

Air supplied $= 30.97 \times \frac{100}{79} = 39.2 \text{ kg mol}$

% excess air = % excess oxygen

$$= \frac{O_2 \text{ supplied - } O_2 \text{ requied}}{O_2 \text{ required}}$$

$$= \frac{(39.2 \times .21) - 6.538}{6.538} \times 100 = 25.9$$

Ex. No: 15

A coal tar fuel containing C – 90%, H – 5%, S – 0.5% , O – 2.5% and N – 2% and of gross calorific value of 38,000 kJ / kg and S.G. 1.1 is burnt with air as the atomizing agent at a rate of 50 kg per hour. Assuming that 20% excess air is used and the combustion is complete, calculate:

a. **The volume at N.T.P. of air supplied to the burner per hour.**
b. **The volume of the resulting product of combustion per hour at 700^0 C**
c. **The volumetric composition of the product of combustion.**

Basis : 1 hr operation

Coal tar fuel used = 50 kg / hr

Oxygen balance:

O_2 rqd. for C = $50 \times 0.9 \times \frac{32}{12} = 120 kg$

O_2 rqd. for H = $50 \times .05 \times \frac{16}{2} = 20 kg$

O_2 rqd. for S = $50 \times .005 \times \frac{32}{32} = 0.25 kg$

Total O_2 rqd = 140.25 kgs

O_2 in fuel = $50 \times \frac{2.5}{100} = 1.25 kg$

O_2 to be supplied by air = 140.25 – 1.25 = 139.0 kg

= 4.34 kg mol.

(a) Air required = $4.34 \times 1.2 \times \frac{100}{21}$ (Air supplied is 20% excess)

= 24.80 kg mol = 24.80 × 22.4

= 555.5 $\frac{m^3}{hr}$.(measured at NTP)

O_2 supplied	= 24.80 × 0.21 = 5.21 kg mol
O_2 excess	= 5.21 – 4.34 = 0.87 kg mol
(b) N_2 from air	= 24.80 × 0.79 = 19.59 kg mol.
N_2 from fuel	= 50 × 0.02 = 1.0 kg = 0.035 kg mol
Total N_2	= 19.625 kg mol
CO_2 produced	= 45 + 120 = 165 kg = 3.75 kg mol
H_2O produced	= 2.5 + 20 = 22.5 kg = 1.25 mol
SO_2 produced	= 0.25 + 0.25 = 0.5 kg = 0.008 kg mol

O_2 excess = 0.87 kg mol

Total flue gas = 25.503 kg mol

Volume at 700^0C = $25.503 \times 22.4 \times \frac{(700+273)}{273}$

= 2036 m^3 / hr

(c) Volumetric composition of the products of combustion

Constituents	Amount in mol	Volume (or mol)%
CO_2	3.750	14.70
O_2	0.870	3.41
N_2	19.625	76.96
H_2O	1.250	4.90
SO_2	0.008	0.03

Ex. No: 16

Blast furnace gas having an analysis by volume on basis of:

CO_2 – 13.0 %

CO – 25.0 %

H_2 – 3.5 %

N_2 – 58.5 %

In burned in a furnace.

Calculate:

Percentage of excess air when the dry product of combustion contains 3.5 % O_2

Percentage of excess air when the dry flue gases contain 19.5 % CO_2 5.8% o_2 AND 74.7 % N_2

Oxygen demand and flue gas formed are computed as under:

Constituents	Amount kg mol	Oxygen rqd., kg mol	Flue gas obtained
CO_2	13.0	0	13.0 (CO_2)
CO	25.0	12.50	25.0 (CO_2)
H_2	3.5	1.75	3.5 (H_2O)
N_2	58.5	0	58.5 (N_2)
Total	100.0	14.25	100.0

Let y_1 = kg mol of dry flue gas obtained
= kg mol of combustion air used

(a) N_2 from combustion air = 0.79 z

O_2 from combustion air = 0.21 z

O_2 excess in flue gas = 0.21 z – 14.25

Dry flue gas = (100 – water vapour) + 0.79 z + (0.21 z – 14.25)

= (100 – 3.5) + z – 14 .25 = 82.25 + z = y_1

$$\frac{\text{Oxygen}}{\text{Dry flue gas}} = \frac{0.21z - 14.25}{82.25 + z} = \frac{3.5}{100}$$

Solving, z = 97.88 kg mol

Oxygen from air = 97.88 × 0.21 = 20.55

Oxygen excess = 20.55 – 14.25 = 6.3 kg mol

% excess air = % excess oxygen = $\frac{6.3}{14.25} \times 100 = 44.2$

(b) Let y_2 = kg mol of dry flue gas in this case

By a carbon balance for the blast furnace and the flue (dry) gases,

$$0.195\, y_2 = 38$$

so, $y_2 = 194.87$ kg mol

Oxygen in flue gas is 5.8 %

Amount of oxygen = $194.87 \times 0.058 = 11.3$ kg mol

% excess air = % excess oxygen

$$= \frac{\text{Excess oxygen in flue}}{\text{Oxygen requrired}} \times 100$$

$$= \frac{11.3}{14.25} \times 100 = 79.3$$

Process Calculations for Biotechnologist - 21BT32T

Unit - 5

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UNIT 5 FUELS AND COMBUSTION

Types of fuels – solid, liquid & gaseous fuels – Ultimate and proximate analysis. Determination of composition by orsat analysis of products of combustion of solid, liquid and gas fuels – calculations of excess air from orsat techniques – problems on combustion process.

Total period required = 15 (9 + 6)

COURSE OUTCOMES

Develop different combustion equation and solve using basic principles of thermochemistry.

Internal energy.

The internal energy of a substances in defined as the total quantity of energy that in passes by virtue at the presence relative positions and mole marts at its component molecule atoms.

External energy.

The external energy of a body in dependent on its position and motion relative to the earth.

BTU.

It is defined as the British thermal unit no longer in bared on the energy required to heat a pound at water one degree Fahrenheit.

Flow Process.

It is one in which streams at materials continuously enter (or) leave the system.

Non flow process.

There in no continuous streams at material enter (or) leave the system deuce the gorge at operation.

Steady – flow process.

In the steady flow process in constancy at temperature and compositions at any given locations in the process.

Enthalpy.

In the energy equations for both flow and non flow process.

$4 = U + PV$

Heat balance.

Heat balance in a loose terms retry to a special form of energy balance which has come into general use in all thermal process.

Heat capacity.

It in an amount of heat required to increase the temperature of a body by one degree.

Specific heat.

It in ratio at the heat capacity at a body to the heat capacity at an equal nears of water.

Mean heat capacity.

The mean (or) average heat capacity other that tap range, where the mean molal heat capacity at const.

Kopp’s rule.

The heat capacity of a solid compound in approximately equal to the sum of the heat cap action of constituents.

Heat of fusion.

The fusion of a crystalline solid at its melting point to form a liquid at the same temperature.

Heat of vaporization.

The heat required to vaporize a substance consists of the energy absorbed in overcoming the intermolecular forces at attraction in the liquid.

Standard heat of reaction.

The heat of a chemical reaction in the heat absorbed in the course of the reaction.

Ex. No: 1

A stream of nitrogen flowing at a rate of 100 kmol/h is heated from 303 K (30°C) to 373 K (100°C). Calculate the heat that must be transferred.

Data: C_p^o for nitrogen = $29.5909 - 5.141 \times 10^{-3} T + 11.1829 \times 10^{-4} T^2 - 4.968 \times 10^{-9} T^3$.

Solution:

Basis : 100 kmol/h of nitrogen gas

$$Q = n\int_{T_1}^{T_2} C_p^0 dT$$

$$Q = n\int_{T_1}^{T_2} \left(29.5909 - 5.141\times10^{-3}T + 11.1829\times10^{-6}T^2 - 4.968\times10^{-9}T^3\right)dT$$

$$= n\left[29.5909\left(T_2 - T_1\right) - \frac{5.141\times10^{-3}}{2}\left(T_2^2 - T_1^3\right) + \frac{11.1829\times10^{-6}}{3}\left(T_2^3 - T_1^2\right) - \frac{4.968\times10^{-9}}{4}\left(T_2^4 - T_1^4\right)\right]$$

where n = 100 kmol/h

T_2 = 373 K

T_1 = 303 K

$$Q = 100\left[29.5909(373-303) \frac{5.141\times10^{-3}}{2}\left((373)^2 - (303)^2\right) + \frac{11.1829\times10^{-6}}{3}\left((373)^3 - (303)^3\right)\right.$$
$$\left. + \frac{4.968\times10^{-9}}{4}\left((373)^4 - (303)^4\right)\right]$$

= 202587.2 kJ/h
= 56.274 kJ/s = 56.274 kW
(As 1 J/s = 1 W)

Ex. No: 2

A stream of carbon dioxide flowing at a rate of 100 kmol/min is heated from 298 K (25°C) to 383 K (110°C). Calculate the heat that must be transferred using C_p°

Data:

$$C_p^\circ = a + bT + cT^2 + dT^3$$

Gas	a	b x 10^3	c x 10^6	d x 10^9
CO_2	21.3655	64.2841	-41.0506	9.7999

Solution:

Basis : 100 kmol/min of CO_2.

Q = Heat that must be transferred

$$= n\int_{T_1}^{T_2} C_p^o dT$$

$$Q = n\int_{T_1}^{T_2} \left(21.3655 + 64.2841\times10^{-3}T - 41.0506\times10^{-6}T^2 + 9.7999\times10^{-9}T^3\right)dT$$

$$= n\left[21.3655\left(T_2 - T_1\right) + \frac{64.2841\times10^{-3}}{2}\left(T_2^4 - T_1^4\right) - \frac{41.0506\times10^{-6}}{3}\right.$$

$$\left.\left(T_2^3 - T_1^3\right) + \frac{9.7999\times10^{-9}}{4}\left(T_2^4 - T_1^4\right)\right.$$

where n = 100 kmo1/min

T_2 = 383 K

T_1 = 298 K

$$Q = 100\left[21.3655\left(383 - 298\right) + \frac{64.2841\times10^{-3}}{2}\left[(383)^4 - (298)^4\right]\right.$$

$$\left. - \frac{41.0506\times10^{-6}}{3}\left((383)^3 - (298)^3\right) + \frac{9.7999\times10^{-9}}{4}\left((383)^4 - (298)^4\right)\right]$$

= 330335.5 kJ/min

= 5505.6 kJ/s = 5505.6 kW

Ex. No: 3

A stream following at a rate of 15000 mol/h containing 25 mole % N_2 and 75 mole % H_2 is to be heated from 298 K (25°C) to 473 K (200°C). Calculate the heat that must be transferred using C_p° data given below:

Data:

$$C_p^\circ = a + bT + cT^2 + dT^3$$

Gas	a	b x 10^3	c x 10^6	d x 10^9
N_2	29.5909	-5.41	13.1829	-4.968
H_2	28.6105	1.0194	-0.1476	0.769

Solution:

Basis: 15000 mol/h of $N_2 - H_2$ mixture.

Molal flow rate of gas mixture = 15 kmol/h

$$XN_2 = \frac{25}{100} = 0.25$$

$$XH_2 = \frac{75}{100} = 0.75$$

$$C_p^o \text{ mix} = \sum C_{p1}^o X_1$$

$$= XN_2 C_{p_{N_2}}^o - XH_2 C_{p_{H_2}}^o$$

$$= 0.25\left[29.5909 - 5.141\times10^{-3}T + 13.1829\times10^{-6}T^2 - 4.968\times10^{-9}T^3 + 0.75\right.$$

$$\left.\left[28.6105 + 1.0194\times10^{3}T - 0.1476\times10^{-6}T^2 + 0.769\times10^{-9}T^3\right]\right.$$

$$= 28.8556 - 0.5207\times10^{-3}T + 3.185\times10^{-6}T^2 - 0.6652\times10^{-9}T^3$$

Q = Heat transferred

$$= n\int_{T_1}^{T_2} C^{o}_{pmix}\,dT$$

$$= n\int_{T_1}^{T_2} \left(28.8556 - 0.5207\times10^{-3}T + 3.185\times10^{-6}T^2 - 0.6652\times10^{-9}T^3\right)dT$$

$$= \left[28.8556(T_2 - T_1) - \frac{0.5207\times10^{-3}}{2}(T_2^2 - T_1^2) - \frac{3.185\times10^{-6}}{3}(T_2^3 - T_1^3) - \frac{0.6652\times10^{-9}}{4}(T_2^4 - T_1^4)\right]$$

where n = 15 kmol/h

T_1 = 298 K

T_2 = 473 K

$$Q = 15\left[28.8556(473 - 298) - \frac{0.5207\times10^{-3}}{2}\left((473)^2 - (298)^2\right)\right.$$

$$\left. + \frac{3.185\times10^{-6}}{3}\left((473)^3 - (298)^3\right) - \frac{0.6652\times10^{-9}}{4}\left((473)^4 - (298)^4\right)\right]$$

$$= 15[5049.73 - 35.13 + 84.25 - 7.01]$$

$$= 15\times5091.84$$

$$= 76377.6\,kJ/h$$

$$= 21.216\,kJ/s$$

$$= 21.216\,kW\,(1J/s = 1W)$$

EXAMPLE 13.1 A gas mixture analyzing 20% (mol) CO, 30% (mol) H_2 and 50% (mol) N_2 is completely burned in air. The following heat of reaction data are available:

$$CO(g) + \frac{1}{2} O_2(g) \rightarrow CO_2(g) \qquad \Delta H_r^0 = -282.91 \text{ kJ/mol}$$

$$H_2(g) + \frac{1}{2} O_2(g) \rightarrow H_2O(g) \qquad \Delta H_r^0 = -241.83 \text{ kJ/mol}$$

(a) Determine the amount of heat liberated on the complete combustion of 100 mol of the gas mixture.

(b) If only 90% of the CO and 80% of the H_2 react, how much heat is liberated on the combustion of 100 mol of the mixture?

Solution *Basis:* 100 mol gas mixture burned

(a) The heats of reactions are negative, indicating that heat is liberated during the reaction. According to the stoichiometric equation, 1 mol CO on combustion liberates 282.91 kJ of heat. The number of moles of CO present is 20 mol. Therefore, the amount of heat liberated on combustion of CO = 20×282.91 kJ = 5658.2. Similarly, the amount of heat liberated on combustion of 30 mol hydrogen = 30×241.83 kJ = 7254.9 kJ. Therefore, the total amount of heat liberated is

$$5658.2 + 7254.9 = 12913.1 \text{ kJ}$$

(b) The number of moles of CO reacted is

$$0.9 \times 20 = 18 \text{ mol}$$

The number of moles of hydrogen reacted is

$$0.8 \times 30 = 24 \text{ mol}$$

The total amount of heat liberated is

$$18 \times 282.91 + 24 \times 241.83 = 10{,}896.3 \text{ kJ}$$

EXAMPLE 13.2 The heat of combustion of methane, carbon and hydrogen are –890.4 kJ/mol, – 393.51 kJ/mol and – 285.84 kJ/mol respectively. Calculate the heat of formation of methane.

Solution The desired reaction is

$$C(s) + 2H_2(g) \rightarrow CH_4(g) \qquad \Delta H_f^0 = ?$$

The heat of reaction (in this case, the heat of formation) can be calculated either by using the Hess's law or by using Eq. (13.2) directly.

Using Hess's law: Since the heat of combustion data are available, the following combustion reactions may be written:

$$CH_4(g) + 2O_2(g) \rightarrow CO_2(g) + 2H_2O(l) \qquad \Delta H^0 = -890.4 \text{ kJ} \qquad \text{(A)}$$

$$C(s) + O_2(g) \rightarrow CO_2(g) \qquad \Delta H^0 = -393.51 \text{ kJ} \qquad \text{(B)}$$

$$H_2(g) + \frac{1}{2}O_2(l) \rightarrow H_2O(l) \qquad \Delta H^0 = -285.84 \text{ kJ} \qquad \text{(C)}$$

Multiply Eq. (C) by 2 and add the result to Eq. (B) and subtract Eq. (A) from the sum. The result is the desired reaction. That is, Eq. (B) + 2 × Eq. (C) – Eq. (A) gives

$$C(s) + 2H_2(g) \rightarrow CH_4(g)$$

The same algebraic operations are to be carried out on the respective heat of reactions. By Hess's law, the heat of desired reaction is

$$\Delta H^0 \text{ (B)} + 2\Delta H^0 \text{ (C)} - \Delta H^0 \text{ (A)} = -393.51 + 2(-285.84) - (-890.4) = -74.79 \text{ kJ}$$

Using Eq. (13.2): Heat of reaction can be calculated from heat of combustion data using Eq. (13.2). The heat of reaction is the sum of the heat of combustion of all the reactants in the desired reaction minus the sum of the heat of combustion of all the products of the desired reaction. Here the reactants are one mole of carbon and two moles hydrogen, and the product is one mole of methane. Therefore, the heat of reaction is

$$1 \times (-393.51) + 2 \times (-285.84) - (-890.4) = -74.79 \text{ kJ}$$

Therefore, the heat of formation of methane is – 74.79 kJ.

EXAMPLE 13.5 Calculate the heat of reaction for the esterification of ethyl alcohol with acetic acid if the standard heats of combustion are: ethyl alcohol (l), −1366.91 kJ/mol; acetic acid (l), − 871.69 kJ/mol; ethyl acetate (l), −2274.48 kJ/mol.

Solution The esterification reaction may be represented as

$$C_2H_5OH\,(l) + CH_3COOH\,(l) \rightarrow C_2H_5COOCH_3\,(l) + H_2O\,(l) \quad \Delta H^0_{298} = ?$$

Using Eq. (13.2): To calculate heat of reaction from the heat of combustion data Eq. (13.2) may be directly used.

$$\Delta H^0 = \sum_{\text{Reactants}} \Delta H_c^0 - \sum_{\text{Products}} \Delta H_c^0$$

Here,

$$\sum_{\text{Reactants}} \Delta H_c^0 = -1366.91 - 871.69 = -2238.6 \text{ kJ}$$

$$\sum_{\text{Products}} \Delta H_c^0 = -2274.48 + 0 = -2274.48 \text{ kJ}$$

(Note that the heats of combustion of H_2O and CO_2 are zero.)

Substituting these into Eq. (13.2), we get

$$\Delta H^0 = -2238.6 - (-2274.48) = 35.88 \text{ kJ}$$

Using Hess's law: The given data on heat of combustion may be used to write the following chemical equations:

$$C_2H_5OH\,(l) + 3O_2(g) \rightarrow 2CO_2(g) + 3H_2O\,(l) \qquad \Delta H_c^0 = -1366.91 \text{ kJ} \qquad \text{(A)}$$

$$CH_3COOH\,(l) + 2O_2(g) \rightarrow 2CO_2(g) + 2H_2O\,(l) \qquad \Delta H_c^0 = -871.691 \text{ kJ} \qquad \text{(B)}$$

$$C_2H_5COOCH_3\,(l) + 5O_2(g) \rightarrow 4CO_2(g) + 4H_2O\,(l) \qquad \Delta H_c^0 = -2274.48 \text{ kJ} \qquad \text{(C)}$$

Add Eqs. (A) and (B) and subtract Eq. (C) from the result. We get

$$C_2H_5OH\,(l) + CH_3COOH\,(l) \rightarrow C_2H_5COOCH_3\,(l) + H_2O\,(l)$$

By Hess's law the same algebraic operations may be performed on the heat of combustion data as well, with the result that

$$\Delta H_{298}^0 = \Delta H_c^0(\text{A}) + \Delta H_c^0(\text{B}) + \Delta H_c^0(\text{C}) = -1366.91 - 871.69 - (-2274.48) = 35.88 \text{ kJ}$$

EXAMPLE 13.6 The vapour-phase hydration of ethylene to ethanol is represented by:

$$C_2H_4(g) + H_2O(g) \rightarrow C_2H_5OH(g)$$

Calculate the standard heat of reaction if the following data are available:

$$2CO_2(g) + 3H_2O(l) \rightarrow C_2H_5OH(l) + 3O_2(g) \quad \Delta H^0_{298} = 1366.91 \text{ kJ}$$

The standard heat of combustion of ethylene at 298 K is −1410.99 kJ/mol and heats of vaporization of water and ethanol are, respectively, 44.04 kJ/mol and 42.37 kJ/mol.

Solution The following equations may be formulated using the given data:

$$2CO_2(g) + 3H_2O(l) \rightarrow C_2H_5OH(l) + 3O_2(g) \qquad \Delta H^0_{298} = 1366.91 \text{ kJ} \qquad \text{(A)}$$

$$C_2H_4(g) + 3O_2(g) \rightarrow 2CO_2 + 2H_2O(l) \qquad \Delta H^0_{298} = -1410.99 \text{ kJ} \qquad \text{(B)}$$

$$H_2O(l) \rightarrow H_2O(g) \qquad \Delta H_{v,298} = 44.04 \text{ kJ} \qquad \text{(C)}$$

$$C_2H_5OH(l) \rightarrow C_2H_5OH(g) \qquad \Delta H_{v,298} = 42.37 \text{ kJ} \qquad \text{(D)}$$

Perform the following arithmetic operations on the above equations:

$$\text{Eq. (A)} + \text{Eq. (B)} + \text{Eq. (D)} - \text{Eq. (C)}$$

The result is

$$C_2H_4(g) + H_2O(g) \rightarrow C_2H_5OH(g)$$

$$\Delta H^0_{298} = \Delta H^0_{298}(A) + \Delta H^0_{298}(B) + \Delta H^0_{298}(D) - \Delta H^0_{298}(C) = -45.75 \text{ kJ}$$

EXAMPLE 13.7 Calculate the standard heat of formation of acetylene (C_2H_2) given that the standard heat of combustion of acetylene is -1299.61 kJ, the standard heat of combustion of carbon is -393.51 kJ and the standard heat of formation of liquid water is -285.84 kJ.

Solution We have the following data:

$$C_2H_2(g) + \frac{5}{2}O_2(g) \rightarrow 2CO_2(g) + H_2O(l) \qquad \Delta H^0_{298} = -1299.61 \text{ kJ} \qquad \text{(A)}$$

$$C(s) + O_2(g) \rightarrow CO_2(g) \qquad \Delta H^0_{298} = -393.51 \text{ kJ} \qquad \text{(B)}$$

$$H_2(g) + \frac{1}{2}O_2(g) \rightarrow H_2O(l) \qquad \Delta H^0_{298} = -285.84 \text{ kJ} \qquad \text{(C)}$$

We can apply Hess's law to calculate the heat of the desired formation reaction.

$$2C(s) + H_2(g) \rightarrow C_2H_2(g) \qquad \Delta H^0_{298} = ?$$

Multiply Eq. (B) by 2, add Eq. (C) to it and subtract Eq. (A) from the result. We get

$$2C(s) + H_2(g) \rightarrow C_2H_5(g)$$

$$\Delta H^0_{298} = 2 \times (-393.51) - 285.84 - (-1299.61) = 226.75 \text{ kJ}$$

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DEPARTMENT OF BIOTECHNOLOGY

BT3451- ANALYTICAL TECHNIQUES IN BIOTECHNOLOGY

LECTURE NOTES

REGULATION 2021

II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

ANALYTICAL TECHNIQUES IN BIOTECHNOLOGY

UNIT - I

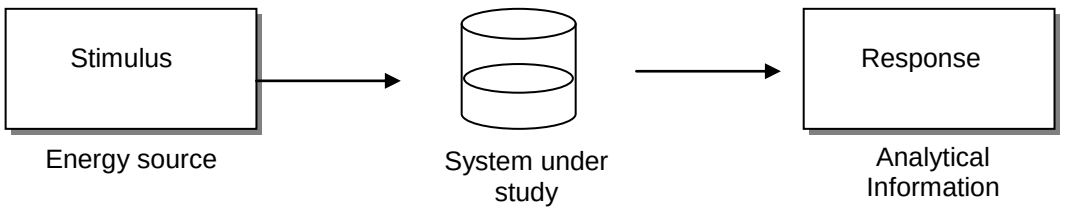
PART - A

1. How the classification of Analytical methods?

Analytical methods two types

(i) Classical Methods
(ii) Instrumental Methods

2. Draw the black Diagram of over all Instrumental measurement.



3. How many types of Electrical Domains?

1. Analog Domains
2. Time Domains
3. Digital Domains

4. Define Period and pulse width.

The time between Successive Lot HI transitions is called the period, and the time between a LO to HI and HI to LO transition is called the pulse width.

5. Define Serial Data:-

Data represented by binary coding on a single transmission line is called serial – coded binary data, (or) simply serial data.

A common example of serial data transmission is the computer modem, which is a device for transmitting data between computers by telephone over a single conductor.

6. What is a data domain?

The various modes of encoding information electrically are called data domains. A classification scheme has been developed based on this concept that greatly simplifies the analysis of instruments system and promotes under standing of the measurement process.

7. How to classify the Data domains?

1) Non electrical Domains
    a) Physical & chemical domains
2) Electrical Domains
    a) Analog domains
    b) Time domains
    c) Digital domains

8. What is a transducer in an analytical instrument?

1. Photocell
2. Photomultiplier tube
3. Electrodes
4. Glass-calomel electrodes
5. Photographic film.

9. What are analog domains?

The analog domain is encoded as the magnitude of one of the electrical quantities. Voltage current, charge (or) power

These quantities are continuous in both amplitude and time as shown by the typical analog signals. Magnitudes of analog qualities can be measured continuously (or) They can be sampled at specific points in time dictated by the needs of a particular experiment (or) instrumental method.

10. How to classify the calibration of instrumental method.

All types of analytical methods require calibration, a process that releases the measured analytical signal to the concentration of analyte. The three most common calibration methods include the preparation and use of a calibration curve, the standard addition method, and the internal standard method.

11. Describe the Calibration Curves.

The calibration curve technique, several standards containing exactly known concentrations of the analyte are introduced into the instrument, and the instrumental response is recorded.

Ordinarily, this response is corrected for the instrument output obtained with a blank.

Ideally the blank contains all of the components of the original sample except for the analyte. The resulting data are then plotted to give a graph of corrected instrument response versus analyte concentration.

12. Define Internal Standard Method.

An internal Standard is a substance that is added in a constant amount to all samples, blanks, and calibration standards in an analysis.

It may be a major Constituent of samples and standards that is present in a large enough amount that its concentration can be assumed to be the same in all cases.

Calibration then involves plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards.

13. Define Ohm's law.

Ohm's law describes the relationship among potential resistance, and current in a resistive series circuit. Ohm's law can be written in the form.

$$V = IR$$

Where V is the potential difference in volts between two points in a circuit, R is the resistance between the two points in ohms, and I is the resulting current in amperes.

14. What is digital multimeter (DMM)?

The instrument that contains circuits for measuring voltages, currents and resistances is usually called a digital multimeter (DMM).

15. What is Digital voltmeter (DVM)?

A digital voltmeter usually consists of a single integrated circuit, a power supply that is often a battery, and a liquid – crystal digital display. The heart of the integrated circuit is an analog – to – digital converter, which converts the input analog – signal to a number that is

proportional to the magnitude of the input voltage.

16. What is Alternative current circuits?

The electrical output from transducers of analytical signals often fluctuate periodically. These fluctuations can be represented by a plot of the instantaneous current or potential as a function of time. The period t_p for the signal is the time required for the completion of one cycle.

The reciprocal of the period is the frequency of the signal. That is, $F=1/t_p$

The unit of frequency is the hertz, Hz, which is defined as one cycle per second.

17. What is Transformers?

Alternating current is readily increased (or) decreased in voltage by means of a power transformer such as that shown schematically. The varying magnetic field formed around the primary coil in this device from the 110-V alternating current induces alternating currents in the Secondary coils; the potential V_x across each is given by

$$V_x=115 \times N_2/N_1$$

Where N_2 and N_1 are the number of turns in the secondary and primary coils, respectively

18. What is Oscilloscopes?

The oscilloscope is a most useful and versatile laboratory instrument that utilizes a cathode – ray tube as a read out device. Both analog and digital oscilloscopes are manufactured. Digital oscilloscope are used when sophisticated signal processing is required. Analog oscilloscopes are generally simpler than their digital counter parts, are usually portable, are easier to use, and are less expensive, costing as little as $ 500. We shall confine our discussion to simple analog instruments.

19. What are the Sources of Noise in Instrumental Analysis?

Chemical analyses are affected by two types of noise,

(i) Chemical noise
(ii) Instrumental noise

20. Define Signals and Noise.

The Analytical measurement is mode up of two components. One component, the signal,

carries information.

PART - B

1. Describe the classification of analytical methods.

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

CLASSIFICATION OF ANALYTICAL METHODS

Analytical methods are often classified as being either classical or instrumental. This classification is largely historical with classical methods, sometimes called wet chemical methods, preceding instrumental methods by a century or more.

Classical Methods

In the early years of chemistry, most analyses were carried out by separating the components of interest (the analytes) in a sample by precipitation, extraction, or distillation. For qualitative analyses, the separated components were then treated with reagents that yielded products that could be recognized by their colors, boiling or melting points, solubilities in a series of solvents, odors, optical activities, or refractive indexes. For quantitative analyses, the amount of analyte was determined by gravimetric or by titrimetric measurements. In gravimetric measurements, the mass of the analyte or some compound produced from the analyte was determined. In titrimetric procedures, the volume or mass of a standard reagent required to react completely with the analyte was measured.

These classical methods for separating and determining analytes still find use in many laboratories. The extent of their general applications is, however, decreasing with the passage of time and with the advent of instrumental methods to supplant them.

Instrumental Methods

Early in the twentieth century, chemists began to exploit phenomena other than those used for classical methods for solving analytical problems. Thus, measurements of physical properties of analytes – such as conductivity, electrode potential, light absorption or emission, mass to – charge ratio and fluorescence – began to be used for quantitative analysis of a variety of inorganic, organic, and biochemical analytes. Furthermore, highly efficient chromatographic and electrophoresis techniques began to replace distillation, extraction, and precipitation for the

separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are knows collectively as instrumental methods of analysis.

Many of the phenomena that instrumental methods are based on have been known for a century or more. Their application by most chemists, however, was delayed by lack of reliable and simple instrumentation. In fact, the growth of modern instrumental methods of analysis has paralleled the development of electronics and computer industries.

TYPES OF INSTRUMENTAL METHODS

For this discussion, it is useful to consider chemical and physical characteristics that are useful for qualitative or quantitative analysis. Table 1-1 lists most of the characteristic properties that are currently used for instrumental analysis. Most of the characteristics listed in the table require a source of energy to stimulate a measurable response from the analyte. For example, in atomic emission an increase in the temperature of the analyte is required to first produce gaseous analyte atoms and then to excite the atoms to higher energy states. The excited state atoms then emit characteristic electromagnetic radiation, which is the quantity measured by the instrument. Sources of excitation energy may take the form of a rapid thermal change as in the previous example, electromagnetic radiation from a selected region of the spectrum, application of one of the electrical quantities – voltage, current, or charge – or perhaps subtler forms intrinsic to the analyte itself.

Note that the first six entries in Table 1-1 involve interactions of the analyte with electromagnetic radiation. In the first property, radiant energy is produced by the analyte; the next five properties involve changes in electromagnetic radiation brought about by its interaction with the sample. Four electrical properties then follow. Finally, four miscellaneous properties are grouped together; mass – to – charge ratio, reaction rate, thermal characteristics, and radioactivity.

Table - Chemical and Physical Properties Employed in Instrumental Methods.

Characteristic Properties	**Instrumental Methods**
Emission of radiation	Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)
Absorption of radiation	Spectrophotometry and photometry (X-ray,UV, visible, IR);photoacoustic spectroscopy; nuclear magnetic resonance and electron spin resonance spectroscopy
Scattering of radiation	Turbidimetry; nephelometry; Raman spectroscopy

Refraction of radiation	Refractometry; interferometry
Diffraction of radiation	X-Ray and electron diffraction methods
Rotation of radiation	Polarimetry; optical rotary dispersion; circular dichroism
Electrical potential	Potentiometry; chronopotentiometry
Electrical charge	Coulometry
Electrical current	Amperometry; polarography
Electrical resistance	Conductometry
Mass	Gravimetry(quartz crystal microbalance)
Mass – to- charge ratio	Mass spectrometry
Rate of reaction	Kinetic methods
Thermal characteristics	Thermal gravimetry and titrimetry; differential scanning colorimetry; differential thermal analyses; thermal conductometric methods.
Radioactivity	Activatio and isotope dilution methods.

The second column in Table 1-1 lists the names of instrumental methods that are based upon the various physical easy to select an optimal method from among available instrumental techniques and their classical counterparts. Some instrumental techniques are more sensitive than classical techniques, but others are not. With certain combination of elements or compounds, an instrumental method may be more selective; with others, a gravimetric or volumetric approach may suffer less interference. Generalizations on the basis of accuracy convenience or expenditure of time are equally difficult to draw. Nor is it necessarily true that instrumental procedures employ more sophisticated or more costly apparatus; indeed, the modern electronic analytical balance used for gravimetric determinations is a more complex and refined instrument than some of those used in the other methods listed in Table 1-1.

As noted earlier, in addition to the numerous methods listed in the second column of Table 1-1, there is a group of instrumental procedure that are used for separation and resolution of closely related compounds. Most of these procedures are based upon chromatography or electrophoresis. One of the characteristics listed in Table 1-1 is ordinarily used to complete the analysis following chromatographic separations. Thus, for example, thermal conductivity, ultraviolet and infrared absorption, refractive index, and electrical conductance have been used for this purpose.

This text deals with the principles, the applications, and the performance characteristics of the instrumental methods listed in Table 1-1 and of chromatographic and electrophoretic separation procedures as well. No space is devoted to the classical methods, the assumption being that the reader will have encountered these techniques in earlier studies.

2. Describe the sources of Radiation and its types.

INSTRUMENTS FOR ANALYSIS

An instrument for chemical analysis converts information stored in the physical or chemical characteristics of the analyte to information that may be manipulated and interpreted by a human. Thus, an analytical instrument can be viewed as a communication device between the system under study and the investigator. To retrieve the desired information from the analyte, it is necessary to provide a stimulus, which is usually in the form of electromagnetic, electrical, mechanical, mechanical, or nuclear energy as illustrated in Figure 1-1. The stimulus elicits a response form the system under study whose nature and magnitude are governed by the fundamental laws of chemistry and physics. The resulting information is contained in the phenomena that results from the interaction of the stimulus with the analyte. A familiar example is the passage of a narrow band of wavelengths of visible light through a sample to measure the extent of its absorption by the analyte. The intensity of the light is determined before and after its interaction with the sample, and the ratio of these intensities provides a measure of the analyte concentration.

Generally, instruments for chemical analysis comprise just a few basic components, some of which are listed in Table 1-2. To understand the relationships among these instrument components and the flow of information from the characteristics of the analyte through the components to the numerical or graphical output produced by the instrument, it is instructive to explore the concepts of data domains.

Data Domains

The measurement process is aided by a wide variety of devices that convert information from one form to another. In order to investigate how instruments function, it is important to understand the way in which information is encoded, or transformed from one system of information to another, as a characteristic of electrical signals- that is, as voltage, current, charge, or encoding information electrically are called data domains. A classification scheme has been developed based on this concept that greatly simplifies the analysis of instrumental systems and promotes understanding of the measurement process. – C.G. Enke, Anal. Chem., 1971, 43, 69A.

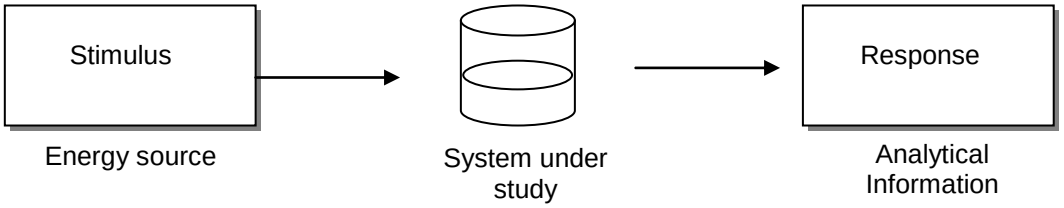


Figure Block diagram showing the overall process of an instrumental measurement

TABLE - Some Examples of Instrument Components

Instrument	Energy source (stimulus)	Analytical information	Input Transducer	Data Domains of Transduced Information	Information processor	Read out
Photometer	Tungsten lamp, glass filter	Attenuated light beam	Photocell	Electrical current	Meter scale	Current meter
Atomic emission Spectrometer	Flame	UV or visible radiation	Photo multiplier tube	Electrical potential	Amplifier, demodulator, monochromator chopper	Chart recorder
Coulometer	DC source	Cell current	Electrodes	Electrical current	Amplifier	Chart recorder
pH meter	Sample /glass electrode	Hydrogen ion activity	Glass – calomel electrodes	Electrical potential	Amplifier, digitizer	Digital unit
X-Ray powder diffractometer	X-Ray tube, sample	Diffracted radiation	Photographic film	Latent image	Chemical developer	Black images on film
Color comparator	Sunlight color Eye	Color	Eye	Optic nerve signal	Brain	Visual color response

As shows in the data domains map of Figure 1-2, data domains may be broadly classified into non electrical domains and electrical domains.

Non electrical Domains

The measurement process begins and ends in non electrical domains. The physical and chemical characteristics that are of interest in a particular experiment reside in theses data domains. Among these characteristics are lengths, density, chemical composition. Intensity of light, pressure and others listed in the first column of Table.

It is possible to make a measurement entirely in non electrical domains. For instance, the determination of the mass of an object using a mechanical equal-arm balance involves a comparison of the mass of the object, which is placed on one balance pan, with standard masses placed on a second pan.

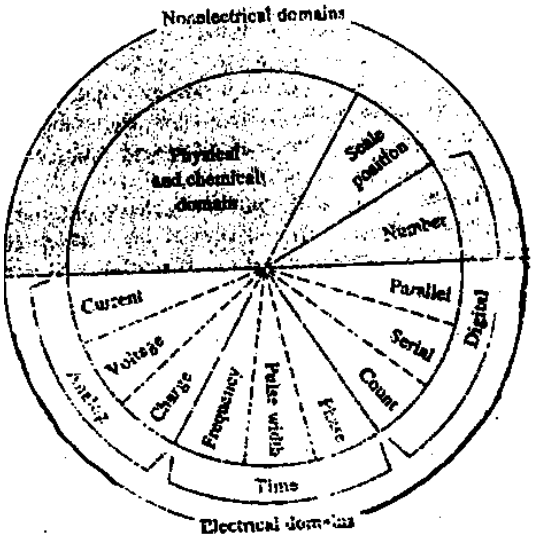


Figure Data domains map. The upper (shaded) half of the map comprises non electrical domains. The bottom half is made up of electrical domains. Note that the digital domain spans both electrical and non electrical domains.

The information representing the mass of the object in standard units is encoded directly by the experimenter, who provides information processing by summing the masses to arrive at a number. In certain other mechanical balances, the gravitational force on amass is amplified mechanical by making one of the balance arms longer than the other, thus increasing the resolution of the measurement.

The determination of the linear dimensions of an object with a ruler and the measurement of the volume of a sample of liquid with a graduated cylinder are other examples of measurements carried out exclusively in non – electrical domains. Such measurements are often associated with classical analytical methods. The advent of inexpensive electronic signal processors, sensitive transformer, and readout devices has led to the development of a host of electronic instruments, which acquire information from non electrical domains, process it in electrical domains, and finally present it in non electrical domains once again. Electronic devices process information and transform it from one domain to another in ways analogous to the multiplication of mass in mechanical balances with unequal arms. As a consequence of the availability of these electronic devices and their rapid and sophisticated information processing, instruments that rely exclusively on non electrical information transfer are rapidly becoming relics of the past. Nontheless, the information that we seek begins in the properties of the analyte and ends in a number, both of which are non electrical domains. The ultimate objective in all measurements is that the final numerical result must be in some manner proportional to the relevant chemical or physical characteristic of the analyte.

Electrical Domains

The modes of encoding information as electrical quantities can be subdivided into analog domains, time domains, and digital domains, as illustrated in the bottom half of the circular map in figure Note that the digital domain spans three electrical domains and one non electrical domain because numbers presented on any type of display convey digital information and can also be encoded electrically.

Any measurement process can be represented as a series of inter domain conversions. For example, Figure illustrates the measurement of the intensity of molecular florescence of a sample of tonic water containing a trace of quinine and, in a general way, some of the data domain conversions that are necessary to arrive at a number expressing the intensity.

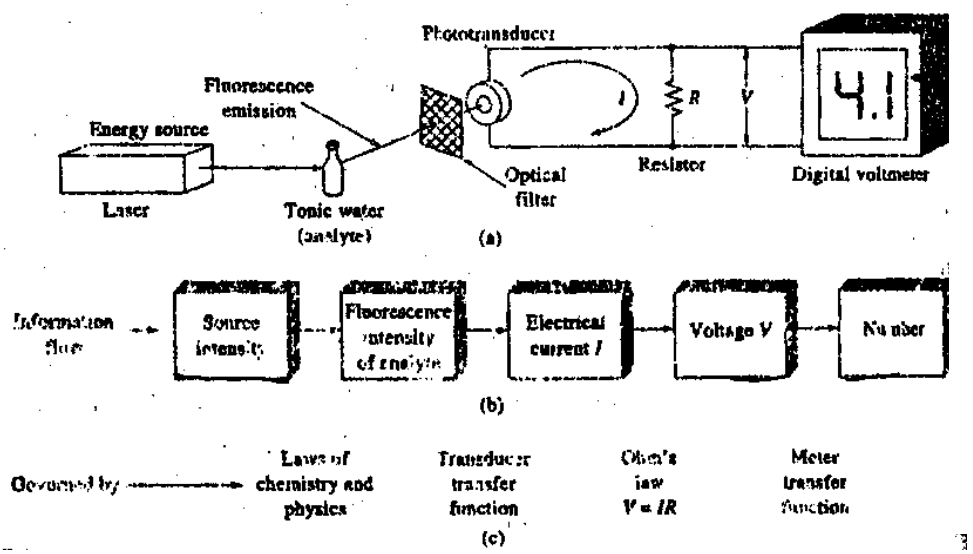


Figure A block diagram of a florometer showing (a) a general diagram of the instrument, (b) a diagrammatic representation of the flow of information through various data domains in the instrument, and (c) the rules governing the data domain transformations during the measurement process.

The intensity of the fluorescence is significant in this context because it is proportional to the concentration of the quinine in the tonic water, which is ultimately the information that we desire. The information begins in the solution of tonic water as the concentration of quinine. This information is teased from the sample by applying to it a stimulus in the form of electromagnetic energy form the laser shown in fig. The radiation interact with the quinine molecules in the tonic water to produce fluorescence emission in a region of the spectrum characteristic and of magnitude proportional to its concentration, Radiation, and thus information, that is unrelated to the concentration of quinine is removed form the beam of light by an optical filter, as shown in

Figure 1-3a. The intensity of the fluorescence emission, which is a non electrical domain, is encoded into an electrical domain by a special type of device called an input transducer. The particular type of device called an input transducer. The particular type of transducer used in this experiment is a photo transducer used in this experiment is a phototransducer, of which there are numerous types, some of which are discussed in chapter 7. In this example, the input transducer converts the fluorescence from the tonic water to an electrical current, I, proportional to the intensity of the radiation. The mathematical relationship between the electrical output and the input radiant power impinging on its surface is called the transfer function of the transducer.

The current from the photo transducer is then passed through a resistor R, which according to Ohm's law produces a voltage V that is proportional to 1, which is in turn proportional to the intensity of the fluorescence. Finally, V is measured by the digital voltmeter to provide a read out proportional to the concentration of the quinine in the sample.

Voltmeters, alphanumeric displays, electric motors, computer screens, and many other devices that serve to convert data form electrical to non electrical domains are called output transducers. The digital voltmeter of the fluorometer of Figure 1-3a is rather complex output transducer that converts the voltage V to a number on a liquid crystal display so that it may be read and interpreted by the user of the instrument. We shall consider the detailed nature of the digital voltmeter and various other electrical circuits and signals in chapter 2 through 4.

In both amplitude and time as shown by the typical analog signals of figure. Magnitudes of analog quantities can be measured continuously or they can be sampled at specific points in time dictated by the needs of a particular experiment or instrumental method as discussed in chapter 4. Although the data of figure are recorded as a function of time, any variable such as wavelength, magnetic field strength, or temperature may be the independent variable under appropriate circumstances. The correlation of two analog signals that result form corresponding measured physical or chemical properties is important in a wide variety of instrumental techniques, such as nuclear magnitude resonant spectroscopy, infrared spectroscopy, and different thermal analysis.

Analog signals are especially susceptible to electrical noise that results from interactions with in measurement circuits or from other electrical devices in vincinity of the measurement system. Such undesirable noise bears no relationship to the information of interest, and methods have been developed to minimize the effects of this unwanted information.

Time Domains

Information is stored in time domains as the time relationship of signal fluctuations. Rather than in the amplitudes of the signals. Figure illustrates three different time – domain signals recorded as an analog quantity versus time. The horizontal dashed lines represent an arbitrary

analog signal threshold that is used to decide whether a signal is HI(above the threshold) or LO (below the threshold). The time relationships between transitions of the signal form HI to LO or form LO to HI contain the information of interest. For instruments that produce periodic signals, the number of cycles of the signal per unit time is the frequency, and the time required for each cycle is tits period. Two examples of instrumental systems that produce information encoded in the frequency domain are Raman spectroscopy and instrumental neutron activation analysis. In these methods, the frequency of arrival of photons at a detector is directly related to the intensity of the emission from the analyte, which is proportional to its concentration.

The time between successive LO to HI transitions is called the period, and the time between a LO to HI and a HI to LO transition is called the pulse width.

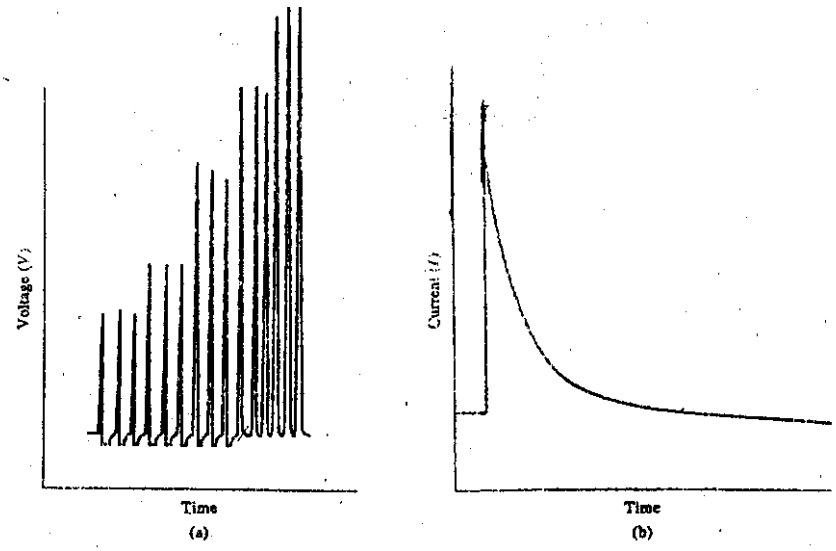


Figure Analog signals. (a) Instrument response from the photometric detection system of a flow injection analysis experiment. A stream of reaction mixture containing plugs of red $Fe(SCN)^{2+}$ flows past a monochromatic light source and a photo transducer, which produces a changing voltage as sample concentration changes. (b) The current response of a photomultiplier tube when the light from pulsed source falls on the photocathode of the device.

Devices such as voltage – to- frequency converter and frequency – to – voltage converters may be used to convert time – domain signals to analog – domain signals and vice versa. These and other such data domain converters will be discussed in Chapter 3 and 4 as a part of our treatment of electronic devices and will be referred to in other contexts throughout this book.

Digital Domains

Data are encoded in the digital domain in a two –level scheme. The information can be represented by the state of alight bulb, a light – emitting diode, a toggle switch, or a logic level

signal, to cite but a few examples. The characteristic that these devices share is that each of the must be in one of only two states. For examples, lights and switches may be only HI or LO. The definition of what constitutes ON and OFF for switches and lights is understood, but in the case of electrical signals, as in the case of time domain signals, an arbitrary signal level must be defined that distinguishes between HI and LO. Such a definition may depend on the conditions of an experiment. Or it may depend upon the characteristics of the electronic devices in use. For example, the signal represented in figure 1-5c is a train of pulses form a nuclear detector. The measurement task if to count the pulses during a fixed period of time to obtain a measure of the intensity of radiation. The dashed line represents a signal level that not only is low enough to ensure that no pulses are lost but also is sufficiently high to reflect random fluctuations in the signal that are unrelated to the nuclear phenomena of interest. If the signal courses the threshold fourteen times, as the case of the signal in figure then we may be confident that fourteen nuclear events occurred. After the events have been counted, the data are then encoded in the digital domain the form of the number 14.

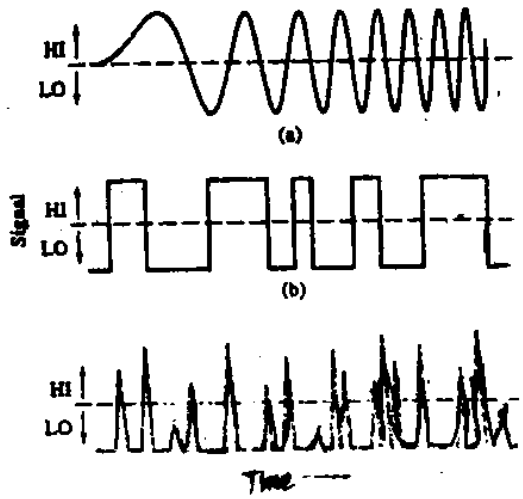


Figure Time – domain signals. The horizontal dashed lines represent signal thresholds, when each signal is above the thresholds. When each signal is above the threshold, the signal is HI, and when it is below the threshold, the signal is LO.

We shall explore the means for making HI-LO electronic decisions and encoding the information in the digital domain.

As suggested by the data domains map of figure, the digital domain spans both electrical and non-electrical domains in the example just cited, the nuclear events are accumulated by using an electronic counter and are displayed on a digital readout. When the experimenter reads and interprets the display, the number that represents the measured quality is once again in a non electrical domain. Each piece of HI-LO data that represents a nuclear event is a bit of information,

which is the fundamental unit of information in the digital domain. Bits of information that are transmitted along a single electronic channel or wire may be counted by an observer or by an electronic device that is monitoring the channel; such accumulated data is termed count digital data, which appears in the data – domains map of figure for example, the signal in figure corresponds to the number n=8 because there are eight complete cycles in the signal. The signal in the figure (b) corresponds to n = 5, and the signal in figure (c) corresponds to n=14. Although effective, this means of transmitting information is not very efficient.

A far more efficiently way to encode information is to use binary numbers to represent numeric and alphabetic data. To see how this type of encoding may be accomplished. Let us consider the signals in figure the count digital data of the signal in figure represent the number n=5 as before. We monitor the signal and count the number of complete oscillations. The process requires a period of time that is proportional to the number of cycles of the signal, or in this case, five times the length of a single time interval, as indicated in figure Not that the time intervals are numbered consecutively beginning with zero. In a binary encoding scheme, such as the one shown for the signal in figure, we assign a numerical value of each successive interval of time. For example, the zeroth time interval represents 2^0=1, the first time interval represents 2^1 =2, the second time interval represents 2^2 =4, and so forth, as shown in figure during each time interval, we need only decide whether the signal is HI or LO. If the signal is HI during any given time interval, then the value corresponding to that interval is added to the total. All intervals that are LO contribute zero to the total.

In figure the signal is HI only in interval 0 and interval 2, so the total value represented is $1 \times 2^0+0\times2^1+1\times2^2=5$. Thus, in the space of only three time intervals, the number n= 5 has been determined. In the count digital example of the signal in figure five time intervals were required to determined the same number. In this limited example, the binary – coded serial data is nearly twice as efficient as the count serial data. A more dramatic example may be seen in the counting of n=10 oscillation s similar to those of the signal in figure In the same ten time intervals, ten HI-LO bits of information in the serial binary coding scheme enable the representation of the binary number from 0 to 2^{10}=1024, or 0000000000 to 1111111111. The improvement in efficiency is 1024/10, or about 100-fold. In other words, the count serial scheme requires 1024 time intervals to represent the number 1024, while the binary coding scheme requires only ten time intervals. As a result of the efficiency of binary coding schemes, most digital information is encoded, transferred, processed, and decoded in some form of binary.

Data represented by binary coding on a single transmission line is called serial –coded binary data, or simply serial data. A common example of serial data transmission is the computer modem, which is a device for transmitting data between computers by telephone over a single conductor (and ground).

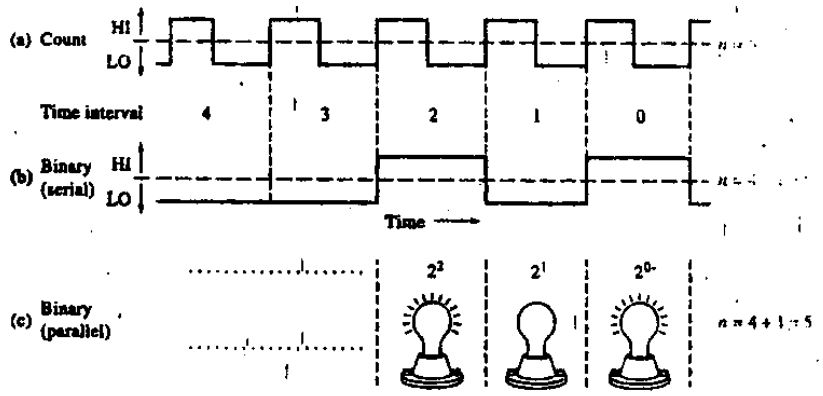


Figure Diagram illustrating three types of digital data: (a) count serial data, (b) binary coded serial data, and (c) parallel binary data. In all three cases, the data represent the number n = 5.

A still more efficient method for encoding data in the digital domain is seen in the signal of figure. Here we use three light bulbs to represent the three binary digits: 2^0=1; 2^1=2; 2^2=4. However, we could use switches, wires light – emitting diodes, or any of a host of electronic devices to encode the information. In this scheme, ON=1 and OFF=0, so that our number is encoded as shown in figure with the first and third lights ON and the middle light OFF, which represents 4+0+1=5. This scheme is highly efficient because all of the desired information is presented to us simultaneously, just as all of the digits on the face of the digital voltmeter in figure appear simultaneously. Data presented in this way are referred to as a parallel digital data. Data are transmitted within analytical instruments and computer by parallel data transmission. Since data usually travel relatively short distances within these devices, it is economical and efficient to use parallel information transfer. This economy of short distance is in contrast to the situation in which data must be transported over long distances from instrument to instrument or from computer to computer. In such instances, communication is carried out serially by using modems or other more sophisticated or faster serial data transmission schemes.

3. What is the uses of detectors, Transducers and 9 to 11, sensors, Readout Devices and Microprocessors and computers in instruments?

Detectors, Transducers, and Sensors

The terms detector, transducer, and sensor are often used synonymously, but in fact the terms have some what different meanings. The most general of the three terms, detector, refers to a mechanical, electrical, or chemical device that identifies, records, or indicates a change in one of the variables in its environment, such as pressure, temperature, electrical charge, electromagnetic radiation, nuclear radiation, particulates, or molecules. This term has become a catchall to the extent that entire instruments are often referred to as detectors. In the context of instrumental

analysis, we shall use the term detector in the general sense in which we have just defined it, and we shall use detection system to refer to entire assemblies that indicate or record physical or chemical quantities. An example is the UV (ultraviolet) detector often used to indicate and record the presence of eluted analytes in liquid chromatography.

The term transducer refers specifically to those devices that convert information in non electrical domains to information in electrical domains and the converse. Examples include photodiodes, photomultipliers, and other electronic photo detectors that produce current or voltage proportional to the radiant power of electromagnetic radiation that falls on their surfaces. Other examples include thermisters, strain gauges, and Hall effect magnetic field strength transducers. As suggested previously the mathematical relationship between the electrical output and the input radiant power, temperature, force, or magnetic field strength is called the transfer function of the transducer.

The term sensor also has become rather broad, but in this text we shall reserve the term for the class of analytical devices that are capable of monitoring specific chemical species continuously and reversibly. There are numerous examples of sensors throughout this text including the glass electrode and other ion-selective electrodes, which are treated in Chapter, the Clark oxygen electrode, which is described in Chapter, and optrodes, or fiber- optic sensors, which appear in chapter. Sensors consist of a transducer coupled with a chemically selective recognition phase. So, for example, optrodes consist of a photo transducer coupled with a fiber optic that is coated on the end opposite the transducer with a substance that responds specifically to a particular physical or chemical characteristic of an analyte.

A sensor that is especially interesting and instructive is the quartz crystal microbalance, or QCM. This device is based on the piezoelectric characteristics of quartz. When quartz is mechanically deformed, an electrical potential develops across its surface. Furthermore, when a voltage is impressed across the faces of a quartz crystal. The crystal deforms. A crystal connected in an appropriate electrical circuit oscillates at a frequency that is characteristic of the mass and shape of the crystal and that is amazingly constant –provided that the mass of the crystal is constant. This property of some crystalline materials is called the piezoelectric effect, and forms the basis for the quartz-crystal microbalance. Moreover, the characteristic constant frequency of the quartz crystal is the basis for modern high – precision clocks, time bases counters, timers, and frequency meters, which in turn have led to many highly accurate and precise analytical instrumental systems.

If a quartz crystal is coated with a polymer that selectively adsorbs certain molecules, the mass of the crystal increases if the molecules, the mass of the crystal increases if the molecules at preset, thus decreasing the resonant frequency of the quartz crystal. When the molecules are desorbed form the surface, the crystal returns to its original frequency. The relationship between

the change in frequency of the crystal Δ F and the change in mass of the crystal ΔM is given by

$$\Delta F = \frac{CF^2 \Delta M}{A}$$

where M is the mass of the crystal. A is its surface area, F is the frequency of oscillation of the crystal. And C is a proportionality constant. The relationship above indicates that it is possible to measure very small changes in the mass of the crystal if the frequency of the crystal can be measure precisely. As it turns out, it is possible to measure frequency changes of one part in 10^7 quite easily with inexpensive instrumentation. The limit of detection for a piezoelectric sensor of this type is estimated to be about 1 pg, or 10^{-12}g. these sensors have been used to detect a variety of gas – phase analytes including formaldehyde, hydrogen chloride, hydrogen sulfide, and benzene. They have also been proposed as sensors for chemical warfare agents such, as mustard gas and phosgene.

The piezoelectirc mass sensor presents an excellent example of a transducer converting a property of the analyte, mass in this case, to a change in an electrical quantity, the resonant frequency of the quartz crystal. This example also illustrates the distinction between a transducer and a sensor. In the quartz-crystal microbalance, the transducer is the quartz crystal, and the selective second phase is the polymeric coating. The combination of the transducer and the selective phase constitute the sensor.

Readout Devices

A read out device is a transducer that converts information from an electrical domain to that is understandable by a human observer. Usually, the transduced signal takes the form of the alphanumeric or graphic output of a cathode-ray tube, a series of numbers on a digital display, the position of a pointer on a meter scale, or occasionally, the blackening of a photo graphic plate, or a tracing on a recorder paper. In some instances, the readout device may be arranged to give the analyte concentration directly.

Microprocessors and Computers in Instruments

Most modern analytical instruments contain or are attached to one or more sophisticated electronic devices and data domain converts, such as operational amplifiers, integrated circuits, analog- to –digital and digital –to-analog converters, counters, microprocessor, and computers. In order to appreciate the power and limitations of such instruments, it is necessary that the scientist develop at least a qualitative understanding of how these devices function and what they can do.

4. Describe the calibration of Instrumental methods.

Calibration of instrumental methods

With two exceptions, all types of analytical methods require calibration, a process that relates the measured analytical signal to the concentration of analyte – The two exceptions are gravimetric and coulometric methods. In both of these cases, the relationship between the quantity measured and the concentration of analyte can be computed from accurately known physical constants. The three most common calibration methods include the preparation and use of a calibration curve, the standard addition method, and the internal standard method.

Calibration Curves

To use the calibration curve technique, several standards containing exactly known concentrations of the analyte are introduced into the instrument, and the instrumental response is recorded. Ordinarily, this response is corrected for the instrument output obtained with a blank. Ideally, the blank contains all of the components of the original sample except for the analyte. The resulting data are then plotted to give a graph of corrected instrument response versus analyte concentration.

Figure shows a typical calibration curve (also called a working curve or an analytical curve). Plots, such as this, that are linear over a significant concentration range (the dynamic rage) are often obtained and are desirable because they are less subject to error than are non linear plots are observed, which require a larger number of calibration data to establish accurately the relationship between the instrument response and concentration. Usually, an equation is developed for the calibration. Usually, an equation is developed for the calibration, curve by a least – squares technique (Appendix a1C) so that sample concentrations can be computed directly.

The success of the calibrations curve method is critically dependent upon how accurately the analyte concentrations of the standards are known and how closely the matrix – The term matrix refers to the collection of all of the various constituent making up an analytical sample. In addition to the analyte, the sample matrix includes all of the other constituents of the sample, which are sometimes referred to as the concomitants of the standards resemble that of the samples to be analyzed. Unfortunately, matching the matrix of complex samples is often difficult or impossible, and matrix effects lead to interference errors. To minimize matrix effects, it is often necessary to separate the analyte from the interferent before measuring the instrument response.

Standard Addition Methods

Standard addition methods are particularly useful for analyzing complex samples in which

the likelihood of matrix effects is substantial. A standard addition method can take several forms. See M. Bader, J. chem. Educ., 1980, 57,703. One of the most common forms involves adding one or more increments of a standard solution to sample aliquots of the same size.

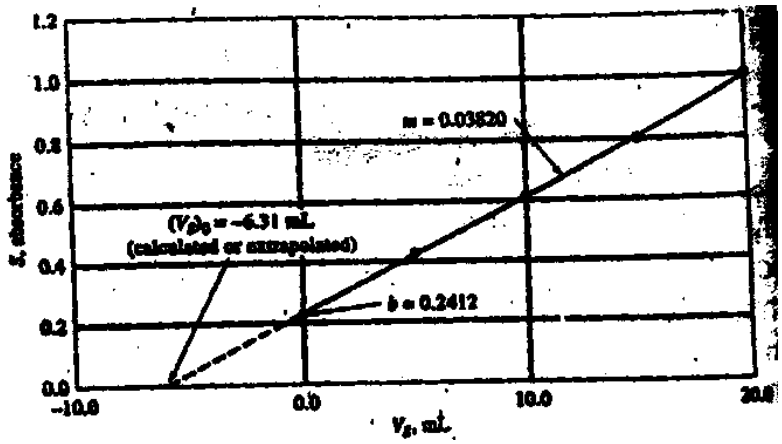


Figure Linear calibration plot for the method of standard additions. The concentration of the unknown solutions may be calculated from the slope m and the intercept b, or it may be determined by extrapolation as explained in the text.

This process is often called spiking the sample. Each solution is then diluted to a fixed volume before measurement. It should be noted that when the amount of sample is limited, standard additions can be carried out by successive introductions of increments of the standard to a single measured volume of the unknown. Measurements are made on the original sample and on the sample plus the standard after each additions. In most versions of the standard addition method, the sample matrix is nearly identical after each addition, the only difference being the concentration of the analyte or, in cases involving the addition of an excess of an analytical reagent, the concentration of the reagent. All other constituents of the reaction mixture should be identical because the standards are prepared in aliquots of the sample.

Assume that several identical aliquots V_x of the unknown solution with a concentration C_x are transferred to volumetric flasks having a volume V_t. To each of these flasks is added a variable volume V_s mL of a standard solution of the analyte having a known concentration c_s. suitable reagents are then added, and each solution is diluted to volume. Instrumental measurements are then made on each of these solutions to yield an instrument response S. if the instrument response is proportional to concentration, as it must be if the standard addition method is to be applicable, we may write

$$S = \frac{kV_sC_s}{V_t} + \frac{kV_xC_x}{V_t}$$

where k is a proportionality constant. A plot of S as a function of V_s is a straight line of the form

$$S=mV_s+b$$

Where the slope m and the intercept b are given by

$$m = \frac{kC_s}{V_t}$$

and

$$b=\frac{kV_xC_x}{V_t}$$

just such a plot is depicted in Figure

A least – square analysis (Section a 1C, Appendix 1) can be used to determine m and b; c_x can then be obtained from the ratio of these two quantities and the known values of C_α V_α and $V_{s'}$ Thus,

$$\frac{b}{m} = \frac{kV_xC_x}{kc_sV_t} = \frac{V_xC_x}{c_s}$$

or

$$C_x = \frac{bc_s}{mV_x}$$

A value for the standard deviation in c_x can then be obtained by assuming that the uncertainties in $C_{s'}$ $V_{s'}$ and V_t are negligible with respect to those in m and b. Then, the relative variance of the result $(s_c/c_s)^2$ is assumed to be the sum of the relative variances of m and b. that is,

$$\left(\frac{s_c}{c_x}\right)^2 = \left(\frac{s_m}{m}\right)^2 + \left(\frac{s_b}{b}\right)^2$$

where s_m is the standard deviation of the slope and where s_b is the standard deviation of the intercept. Taking the square root of this equation gives

$$s_c = c_x\sqrt{\left(\frac{s_m}{m}\right)^2 + \left(\frac{s_b}{b}\right)^2}$$

Alternatively, a manual plot of the data may be constructed, and the linear portion of the plot may be extrapolated to the left of the origin, as shown by the dashed line of figure. The difference between the volume of the standard added at the origin (zero) and the value of the volume at the intersection of the straight line with the x-axis, or the x-intercept$(V_x)_0$. is the volume of standard regent equivalent to the amount of analyte in the sample. In addition, the x-intercept corresponds to zero instrument response, so that we may write

$$S = \frac{kV_sC_s}{V_t} = \frac{kV_xC_x}{V_t} = 0$$

By solving Equation for C_α we obtain

$$c_x = -\frac{(V_s)_{0C_s}}{V_x}$$

In the interest of saving time or sample it is possible to perform a standard addition analysis by using only two increments of sample. Here, a single addition of V_s mL of standard would be added to one of the two samples, and we can write

$$S_1 = \frac{kV_xC_x}{V_t}$$
$$S_2 = \frac{kV_xC_x}{V_t} + \frac{kV_sC_s}{V_t}$$

where S_1 and S_2 are the analytical signals resulting from the diluted sample and the diluted sample plus standard, respectively. Dividing the second equation by the first gives upon rearrangement

$$S_1 = \frac{kV_xC_x}{V_t}$$
$$c_x = \frac{S_1C_sV_s}{(S_1 - S_2)V_x}$$

The Internal Standard Method

An Internal standard is a substance that is added in a constant amount to all samples, blanks, and calibration standards in an analysis. Alternatively It may be a major constituent of samples and standards that is present in a large enough amount that its concentration can be assumed to be the same in all cases. Calibration then in valves plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration can be assumed to be the same in all cases. Calibration then in valves plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards. This ratio for the samples is then used to obtain their analyte concentrations from a calibration curve.

An internal standard, if properly chosen and used, can compensate for several types of both random and systematic errors. Thus, if the analyte and internal standard signals respond proportionally to random instrumental and method fluctuations, the ratio of these signals is in depending of these fluctuations. If the two signals are influenced in the same way by matrix effects, compensation of these effects also occurs. In those instances where the internal standard is a major constituent of samples and standard, compensation for errors that arise in sample

preparation , solution, and cleanup may also occur.

A major difficulty in applying the internal standard method is that of finding a suitable substances to serve as the internal standard and of introducing that substance into both samples and standards in a reproducible way. The internal standard should provide a signal that is similar to the analyte signal in most ways but sufficiently different so that the two signals are readily distinguishable by the instrument. The internal standard must be known to be absent from the sample matrix so that the only source of the standard is the added amount. For example, lithium is a good internal standard for the determination of sodium or potassium in blood serum because the chemical behavior of lithium is similar to both analytes, but it does not occur naturally in blood.

As an example, the internal standard method is often used in the determination of trace elements in metals by emission spectroscopy. Thus, in determining part per million of antimony and tin in lead to be used for the manufacture of storage batteries, the relative intensity of a strong line for each of the minor constituents might be compared with the intensity of a weak line for lead. Ordinarily, these ratios would be less affected by variables that arise in causing the samples to emit radiation. In the development of any new internal standard method, we must verify that changes in concentration of analyte do not affect the signal intensity that results from the internal standard. In order for such a procedure to be successful, a good deal of time and effort would need to be expended in preparing a set of pure lead samples that contains exactly known concentrations of antimony and tin.

5. Describe the Direct current circuits and measurements using instruments.

DIRECT CURRENT CIRCUITS AND MEASUREMENTS

In this section, we consider some basic direct current circuits and how they are used in making current, voltage, and resistance measurements. A general definition of a circuit is a closed path that may be followed by an electric current. We begin our discussion of circuits with a survey of four important laws of electricity.

Laws of Electricity

Ohm's Law

Ohm's law describes the relationship among potential, resistance, and current in a resistive series circuit. In a series circuit, all circuit elements are connected in sequence along a unique path, head to tail, as are the battery and three resistors shown in Figure ohm's law can be written in the form.

$$V=IR$$

Where V is the potential difference in volts between two points in a circuit, R is the resistance between the two points in ohms, and I is the resulting current in amperes – Throughout most of the text, the symbol V will be used to denote electrical potential difference in circuits. In Chapters 22 through 24, however, the electrochemical convention will be followed in which electromotive force is designated as E.

Kirchhoff's Laws

Kirchhoff's current law states that the algebraic sum of currents around any point in a circuit is zero. Kisrchhoff's voltage law states that the algebraic sum of the voltages around a closed electrical loop is zero.

The applications of Kirchhoff's and Ohm's laws to basic dc circuits are considered in Section.

Power Law

The power P in watts dissipated in a resistive element is given by the product of the current in amperes and the potential difference across the resistance in volts:

$$P = IV$$

Substituting Ohm's law, gives

$$P = I^2R=V^2/R$$

Basic Direct Current Circuits

In this section we describe two types of basic dc circuits that find widespread use in electrical devices, namely series resistive circuits and parallel resistive circuits, and analyze their properties with the aid of the laws described in the previous section.

Series Circuits

Figure shows a basic series circuit, which consists of a battery, a switch, and three resistors in series.

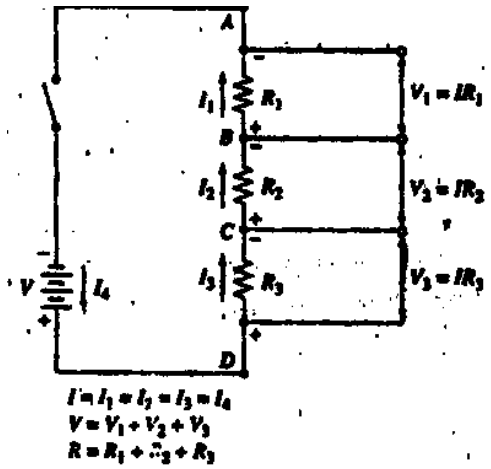


Figure Resistors in series; a voltage divider. The current in each resistor is the same in a series circuit.

Applying Kirchhoff's current law to point D in this circuit gives

$$I_A = I_3 = 0$$

Or

$$I_A = I_3$$

Note that the current out of D must be opposite in sign to the input current. Similarly, application of the law to point C gives

$$I_3 = I_2$$

Thus, the current is the some at all points in a series circuit: that is,

$$I = I_1 = I_2 = I_3 = I_4$$

Application of Kirchhoff's voltage law to the circuit in Figure yields

$$V - V_3 - V_2 - V_1 = 0$$

Or

$$V + V_1 + V_2 + V_3$$

Note that point D is positive with respect of point C, which in turn is positive with respect to point B; finally, B is positive with respect to A. The three voltages thus oppose the voltage of the battery and must be given signs that are opposite to V.

Substitution of Ohm's law into Equation gives

$$V=I(R_1+R_2+R_3)=IR_s$$

Note that the total resistance R_s of a series circuit is equal to the sum of the resistances of the individual components. That is,

$$R_s=R_1+R_2+R_3$$

Applying ohm's law to the part of the circuit from point B to A gives

$$V_1=I_1R_1=IR_1$$

Dividing by Equation yield

$$\frac{V_1}{V}=\frac{IR_1}{1(R_1+R_2+R_3)}$$

or

$$V_1=\frac{VR_1}{R_1+R_2+R_3}=V\left(\frac{R_1}{R_s}\right)$$

In a similar way, we may also write

$$V_2=VR_2/R_s$$

And

$$V_3=VR_3/R_s$$

Voltage Dividers

Series resistance are widely used in electrical circuits to provide potentials that are variable functions of an input voltage. Devices of this type are called voltage dividers. As shown in fig. one type of voltage divider provides voltages in discrete increments; the second type called a potentiometer – The word potentiometer is also used in a different context as the name for a complete instrument that utilizes a linear voltage divider for the accurate measurement of potentials provides a potential that is continuously variable.

In most potentiometer, such as the one shown in fig. the resistance is linear – that is, the resistance between one end, A. and any point. C, is directly proportional to the length, AC, of that portion of the resistor. Then R_{AC}=kAC where AC is expressed in convenient units of length and k is a proportionality constant. Similarly, R_{AB} = kAB. Combining these relationships with Equation yields.

$$V_{AC} = V_{AB}\frac{R_{AC}}{R_{AB}} = V_{AB}\frac{SC}{AB}$$

In commercial potentiometers, R_{AB} is generally a wire – wound resistor formed in a heretical coil. A movable contact, called a wiper; can be moved from one end of the helix to the other, allowing V_{AC} to be varied continuously from zero to V_{AB}.

Parallel Circuits

Figure depicts a parallel dc circuit. Applying Kirchoff's current law to point A in this figure, we obtain

$I_1+I_2+I_3-I_t=0$

Or

$$I_t = I_1+I_2+I_3$$

Applying Kirchhoff's voltage law to this circuit gives three independent equations. Thus, we may write, for the loop that contains the battery and R_1.

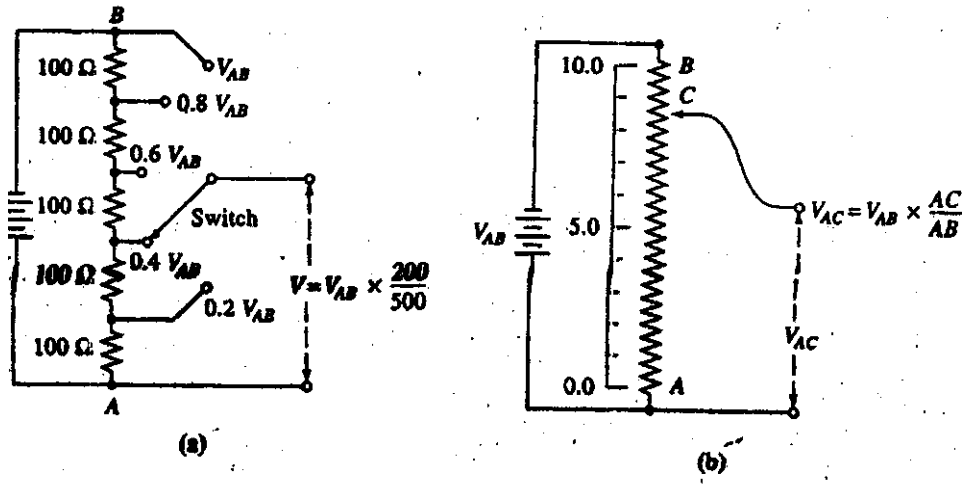


Figure: Voltage dividers: (a) Selector type and (b) continuously variable type (potentiometer).

$V - I_tR_1=0$

$V = I_tR_1$

For the loop containing V and R_2.

$V = I_2R_2$

For the loop containing V and R_3

$$V = I_3R_3$$

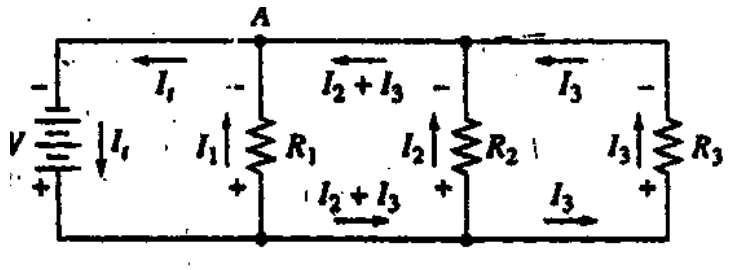


Figure: Resistors in parallel. The voltage across each resistor is equal to V, the battery voltage.

We could write additional equations additional equations for the loop containing R_1 and R_2 as well as the loop containing R_2 and R_3. However, these equations are not independent of the foregoing three. Substitution of the three independent equations into Equation yields.

$$I_t = \frac{V}{R_p} = \frac{V}{R_1} + \frac{V}{R_2} + \frac{V}{R_3}$$

By dividing the equation by V, we obtain

$$\frac{1}{R_p} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3}$$

Since the conductance G of a resistor R is given by G-I/R, we may then write

$$G_p = G_1 + G_2 + G_3$$

Equation shows that in a parallel circuit, in contrast to a series circuit, it is the conductance's G that are additive rather than the resistances.

Current Dividers from Parallel Circuits

Just as series resistances form a voltage divider, parallel resistances create a current divider. The fraction of the total current I_t that is present in R_1 in figure is

$$\frac{I_1}{I_t} = \frac{VIR_1}{VIR_p} = \frac{I/R_1}{I/R_p} = \frac{G_1}{G_P}$$

or

$$I_1 = I_t \frac{R_P}{R_1} = I_t \frac{G_1}{G_P}$$

As interesting special case occurs when two resistances R_1 and R_2, form a parallel circuit. The fraction of the current in R_1 is given by

$$\frac{I_1}{I_t} = \frac{G_1}{G_P} = \frac{I/R_1}{I/R_p} = \frac{I/R_1}{I/R_1 + I/R_2} =$$
$$\frac{R_2/R_1R_2}{R_2/R_1R_2 + R_2/R_1R_2} = \frac{R_2}{R_1 + R_2}$$

In a similar way, we can show that

$$\frac{I_2}{I_t} = \frac{R_1}{R_1 + R_2}$$

In other words, for two parallel resistors, the fraction of the current in one resistor is just the ratio of the resistance of the second resistor to the sum of the resistances of the two resistors. The equation above is often called the current – splitting equation.

6. Describe alternating current circuits.

The electrical output from transducers of analytical signals often fluctuate periodically. These fluctuations can be represented (as in figure) by a plot of the instantaneous current or potential as a functions of time. The period t_p for the signal is the time required for the completion of one cycle.

The reciprocal of the period is the frequency f of the signal. That is,

$$F=1/t_p$$

The unit of frequency is the hertz, Hz, which is defined as one cycle per second.

Sinusoidal Currents

The sinusoidal wave (figure) is the most frequently encountered type of periodic electrical signal. A common example is the alternating current produced by rotation of a coil in a magnetic field (as in an electrical generator). Thus, if the instantaneous current or voltage produced by a generator is plotted as a function of time, a sine wave results.

TABLE - Effect of Resistance e of the standard Resistor, R_{std}, on the Accuracy of Current Measurement*

Circuit Resistance R_D Ω	Standard Resistance R_{std} Ω	R_{std}/R_L	Relative Error %
1.0	1.0	1.0	-50
10	1.0	0.10	-9.1
100	1.0	0.010	-0.99
1000	1.0	0.0010	-0.10

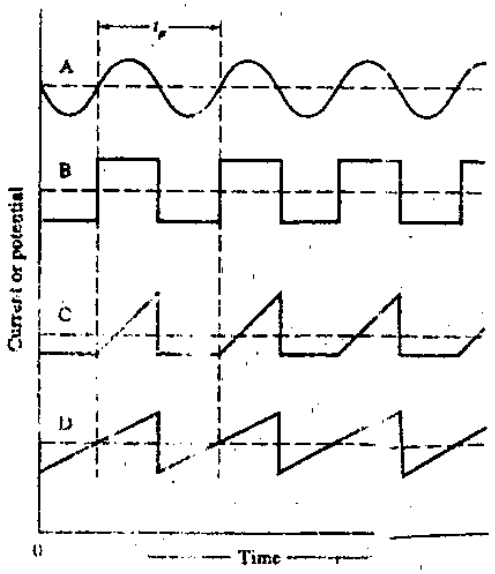


Figure: Examples of periodic signals (A) sinusoidal, (B) square wave, (C) ramp, and (D) sawtooth.

A pure sine wave is conveniently represented as a vector of length I_p (or V_p), which is rotating counter clockwise at a constant angular velocity ω. The relationship between the vector representation and the sine wave plot is shown in figure. The vector rotates at a rate of 2π radians in the period t_p; thus, the angular frequency is given by

$$\omega = \frac{2\pi}{t_p} = 2\pi f$$

If the vector quantity is current or voltage, the instantaneous current I or instantaneous voltage v at time t is given by – It is useful to symbolize the instantaneous value of time t is given by (see figure) – It is useful to symbolize the instantaneous value of time – varying current, voltage, or charge with the lower case letters I, v, and q. on the other hand. Capital letters are used for steady current, voltage, or charge or for a specially defined variable quantity such as a peak voltage current, that is, V_p and I_p.

$$i = I_p \sin\omega t = I_p \sin 2\pi ft$$

or, alternatively

$$v = v_p \sin\omega t = V_p \sin 2\pi ft$$

Where I_p and V_p, the maximum, or peak, current and voltage, are called the amplitude, A of the sine wave.

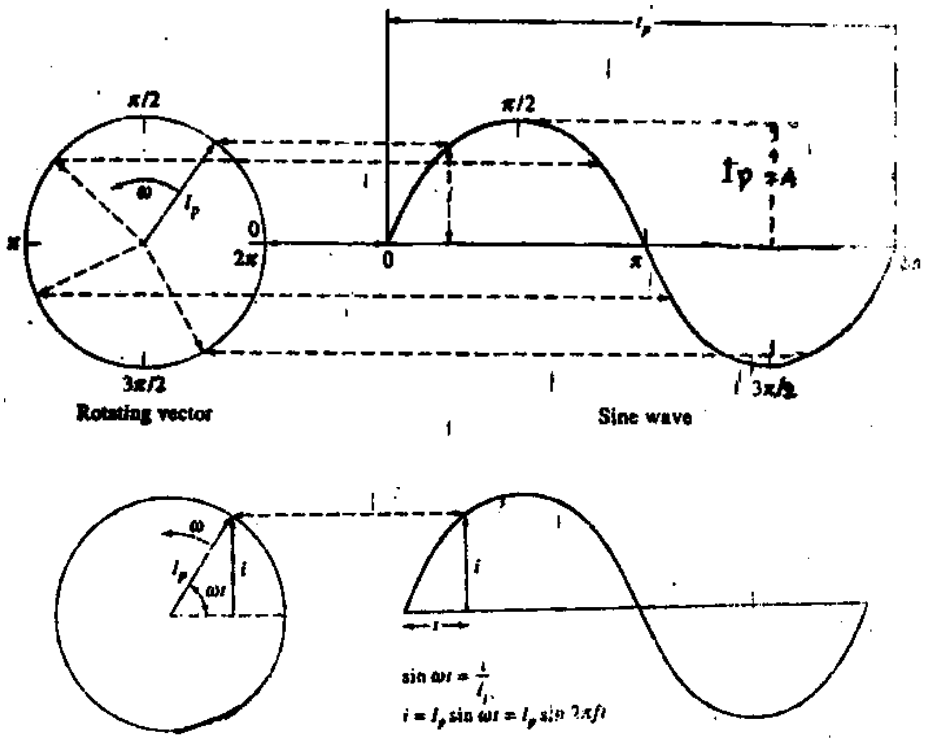


Figure Relationship between a sine wave of period t_p and amplitude I_p and the corresponding vector of length I_p rotating at an angular velocity of $\omega = 2\pi f$ radians/ second or a frequency of fHZ.

Figure shows two sine waves that have different amplitudes. The two waves are also out of phase by 90 degrees, or $\pi/2$ radians. The phase difference is called the phase angle, which arises when one vector leads or lags a second by this amount. A more generalized equation for a sine wave, then, is

$$I = I_p \sin(\omega t + \phi) = I_p \sin(2\pi ft + \phi)$$

Where ϕ is the phase angle from some reference sine wave. An analogous equation can be written in terms of voltage.

$$V = v_p \sin(2\pi ft + \phi)$$

The current or voltage associated with a sinusoidal current can be expressed in several ways. The simplest is the peak amplitude, I_P (or V_P), which is the maximum instantaneous current or voltage during a cycle; the peak – to - peak value, which is $2I_P$, is also used occasionally. The root mean squarer or rms current in an ac circuit will produce the same heating in a resistor as a direct current of the same magnitude. Thus, the rms current is important in power calculations (Equations and). The rms current is given by

$$I_{rms} = \sqrt{\frac{I_P^2}{2}} = 0.707 I_P \text{ and}$$

$$V_{rms} = \sqrt{\frac{V_P^2}{2}} = 0.707 V_P$$

Reactance in Electrical Circuits

When the current in an electrical circuit is increased or decreased, energy is required to change the electric and magnetic filed associated with the flow of charge. For example, if the circuit contains a coils of copper wire, or an inductor, the coil resists the change in the current as energy is stored in the magnetic field of the inductor As the current is reversed, the energy is retuned to the ac source; and as the second half of the cycle is completed, energy is once again stored in a magnetic field of the opposite sense. In a similar way, a capacitor in an ac circuit resists changes in voltage. The resistance of inductors to changes in current and the resistance. As we shall see, reactance in an ac circuit introduce phase shifts in the ac signal.

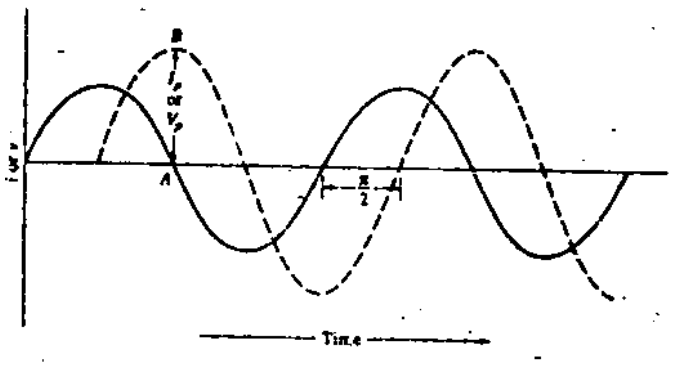


Figure: Sine waves with different amplitudes ($I_P$or V_P) and with a phase difference of 90 deg or π/2 radians.

The two types of reactance that characterize capacitors and inductors are capacitive reactance and inductive reactance, respectively.

Both capacitive reactance and inductive reactance are frequency – dependent quantities. At low frequency when the rate of change in current is low, the effects of reactance in most of the

components of a circuit are sufficiently small to be neglected with repaid changes, on the other hand, circuit elements such as switches, junctions, and resistors may exhibit reactance. When the effects of this type of reactance are undesirable, every effort is made to diminish its magnitude an thus its effects.

Capacitance and inductance are often deliberately introduced into a circuit with capacitors and inductors.

These devices play important roles in such useful functions as converting alternating current to direct current or the converse, discriminating among signals of different frequencies, separating ac and dc signals, differentiating signals, or integrating signals.

In the actions that follow, we shall consider only the properties of capacitors since most modern electronic circuits are based on these devices rather than on inductors.

Capacitors and Capacitance: Series RC circuits

A typical capacitor consist of a pair of conductors separated by a thin layer of a dielectric substance – that is, by an electrical insulator that contains essentially no mobile, current carrying, charged species.

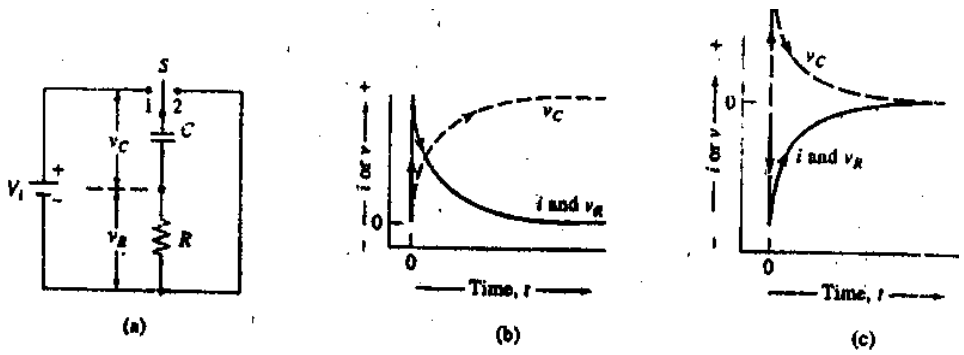


Figure (a) A series RC circuits. Time response of circuit when switch S is (b) in position 1 and (c) in position 2

The simplest capacitors consist of two sheets of metal foil separated by a thin film of a dielectric such as air, oil, plastic, mica, paper, ceramic, or metal oxide. Except for air and mica capacitors, the two layers of foil and the insulator are usually folded or rolled into a compact package a sealed to prevent atmospheric deterioration.

In order to describe the properties of capacitors, consider the series RC circuit shown in figure which contains a battery V_b a resistor R. and a capacitors C, in series. The capacitor is symbolized by a pair of parallel lines or identical length.

When the switch S is moved form position 2 to position 1, electrons flow from the negative terminal of the battery through the resistor R into the lower conductor, or plate, of the capacitor. Simultaneously, electrons are repelled form the upper plate and flow toward the positive terminal of the battery. This movement constitutes a momentary current, which quickly decays to zero as the potential of the battery V_P. When the current ceases, the capacitor is said to be charged.

If the switch then is moved form position 1 to position 2, electrons will flow from the negatively charged lower plate of the capacitor through the resistor R to the positive upper plate. Again, this movement constitutes a current that decays to zero as the oriental difference between the two plates disappears, here, the capacitor is said to discharged.

A useful property of capacitors is its ability to store an electrical charge for a period of time and then to given up the stored charge when needed. Thus, if S in figure is first held at 1 unit C is charged and is then moved to a position between 1 and 2, the capacitor will maintain its charge for an extended period. When S is moved to position2, discharge occurs in the same way as it would if the change from 1 to 2 had been rapid.

The quantity of electricity Q required to charge a capacitor fully depends upon the area do the plates, their shape, the spacing between them, and the dielectric constant of the material that separates them in addition, Q is directly proportional to the applied voltage. That is,

$$Q=CV$$

When V is the applied potential in volts and Q is the quantity of charge in coulombs, the proportionality constant C is the capacitance of a capacitor in farads F. A one-farad capacitor, then, stores on coulomb of charge per applied volt. Most of the capacitors used in electronic circuitry have capacitances in the microfarad (10^{-6}F) to picrofarad (10^{-12}F) ranges.

Capacitance is important in ac circuits, particularly because a voltage that varies with time gives rise to a time varying charge – that is, a current. This behavior is seen by differentiating Equation to give

$$\frac{dq}{dt} = C\frac{d_{vC}}{dt}$$

By definition, the current, I, is the time rate of change of change; that is , dq/dt=I, Thus,

$$i = C\frac{d_{vC}}{dt}$$

It is important to note that the current in a capacitor is zero when the voltage is time independent that is, when the voltage across the capacitor is constant. Furthermore, not that a very large current is required in order to make a rapid change in the voltage across a capacitor.

7. Describe the following compounds.

a) **Field Effect Transistors (FET)**
b) **Transformers**
c) **Cathode Ray Tubes**

Field Effect Transistors

Several types of field – effect transistors have been developed and are widely used in integrated circuits. One of these, the insulated – gate field – effect transistor, was the outgrowth of the need to increases the input resistance of amplifiers. Typical insulate – gate field – effect transistors have input impedances that range from 10^{o} to 10^{14} Ω . This type of transistor is most commonly referred to as a MOSFET, which is the acronym for metal oxide semiconductor field – effect transistor.

Figure shows the structural features of an n-channel MOSFET. Here, two isolated a n regions are formed in a p- type substrate. Covering both regions is a thin layer of highly insulating silicon dioxide, which may be further covered with a protective layer of silicon nitride. Opening s are etched through these layers so that electrical contact can be made to the two n regions. Two additional contacts are formed. One to the substrate and the other to the surface of the insulating layer. The contact with the insulating layer is termed the gate because the potential of this contact determines the magnitude of the positive current between the drain and the source. Note that the insulating layer of silicon dioxide between the gate lead and the substrate accounts for the high impedance of a MOSFET.

In the absence of a gate potential, essentially no current develops between drain and source because one of the two pn junctions is always reverse-biased regardless of the sign of the potential V_{DS}. MOSFET devices are designed to operate in either an enhancement or a depletion mode. The former type is shown in fig. where current enhancement is brought about by application of a positive potential to the gate. As shown, this positive potential induces a negative substrate channel immediately below the layer of silicon dioxide that covers the gate electrode.

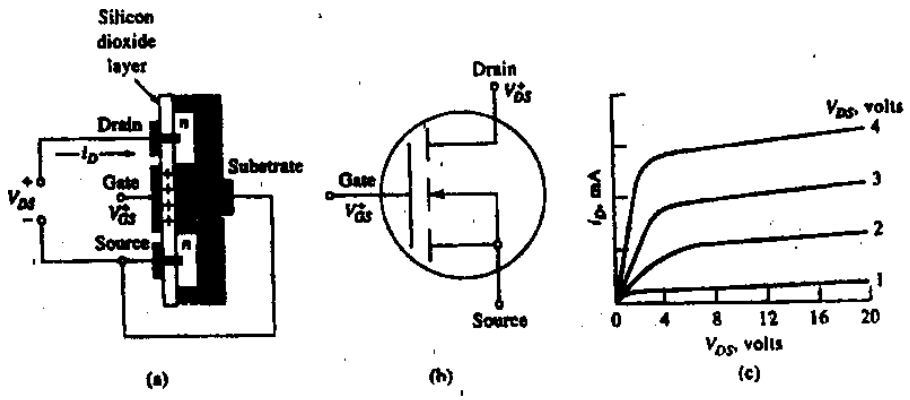


Figure An n-channel enhancement mode MOSFET. (a) Structure, (b) symbol,(c) performance

Characteristics.

The number of negative charges here, and thus the current, increases as the gate voltage V_{GS} increases. The magnitude of this effect is shown in figure. Also available are p – channel enhancement mode MOSFET devices in which the p and n regions are reversed from that shown in fig.

Depletion mode MOSFET devices are designed to conduct in the absence of a gate voltage and to become nonconducting as potential is applied to the gate An n- channel MOSFET of this type is similar in construction to the transistor shown figure. Except that the two n regions are now connected by a narrow channel of n-type semiconductor. Application of a negative voltage at V_{DS} repels electrons out of the channel and thus decreases the conduction through the channel. It is important to note that virtually zero current it required at the gate of a MOSFET device to initiate conduction between the source and rain. This tiny power requirements of BJT transistors. The characteristic low power consumption of field – effect devices make them ideal for portable applications requiring battery power.

Transformers

Alternating current is readily increased or decreased in voltage by means of power transformer such as that shown schematically in figure. The varying magnetic field formed around the primary coil in this device from the 110-V alternating current induces alternating currents in the secondary coils; the potential V_x across each is given by

$$V_x = 115 \times N_2/N_1$$

Where N_2 and N_1 are the number of turns in the secondary and primary coils, respectively. Power supplies with multiple taps, as in Figure are, available commercially; many voltage combinations may be obtained. Thus, a single transformer can serve as a power source for several components of an instrument.

Cathode – Ray Tubes

Figure is a block diagram that shows the main components of a cathode – ray tube. Here, the display is formed by the interaction of electron in a focused beam with a phosphorescent coating on the interior of the large curved surface of the evacuated tube. The electron beam is formed at a heated cathode. Which is maintained at ground potential. A multiple anode-focusing array produces a narrow beam of electrons that have been accelerated through a potential of several thousand volts. In the absence of input signals, the beam appears as a small bright dot in the center of the screen.

8. Describe the Sources of Noise in instrumental Analysis.

SOURCES OF NOISE IN INSTRUMENTAL ANALYSES

Chemical analyses are affected by two types of noise; chemical noise and instrumental noise.

Chemical Noise

Chemical noise arises from a host of uncontrollable variables that affect the chemistry of the system being analyzed. Examples include undetected variations in temperature of pressure that affect the position of chemical equilibria, fluctuations in relative humidity that cause changes in the moisture content of samples, vibrations that lead to stratification of powdered solids, changes in light intensity that affect photosensitive materials, and laboratory fumes that interact with samples or reagents. Details on the effects of chemical noise appear in later chapters that deal with specific instrumental methods. In this chapter we focus exclusively on instrumental noise.

Instrumental Noise

Noise is associated with each component of an instrument – that is, with the source, the input transducer, all signal- processing elements, and the output transducer. Furthermore, the noise from each of these elements may be of several types and may arise from several sources. Thus, the noise that is finally observed is complex composite that usually cannot be fully characterized. Certain kinds of instrumental noise are recognize: (1) thermal, or Johnson, noise; (2) shot noise; (3) flicker, or 1/f, noise; and (4) environmental noise; A consideration of the properties of the four kinds of noise is useful.

Thermal Noise, or Johnson Noise

Thermal noise is caused by the thermal agitation of electrons or other charge carriers in resistors, capacitors, radiation transducers; electrochemical cells, and other resistive elements in an instrument. This agitation of charged particles is random and periodically creates charge in homogeneities, which in turn create voltage fluctuations that then appears in the readout as noise. It is important to note that thermal noise is present even in the absence of current in a resistive element and disappears only at absolute zero.

The magnitude of thermal noise in a resistive circuit element is readily derived from thermodynamic considerations For example, see T Coor, j. chem. Educ., 1968,45,A534. and is given by

$$\overline{V}_{rms} = \sqrt{4KTR\Delta f}$$

where $\bar{V}_{rms}$ is the root – mean – square noise voltage lying in a frequency band width of ΔfHz, k is the Boltzmann constant (1.38×10^{-23}J/K), T is the temperature in Kelvin, and R is the resistance in ohms of the resistive element.

In section 3B-2 we discussed the relationship between the rise time t_r and the band width Δf of an operational amplifier. These variables are also used to characterize the capability of complete instruments to transduce and transmit information. Recall that

$$\Delta f = \frac{1}{3t_r}$$

The rise time of an instrument is tits response time in seconds to an abrupt change in input and normally is taken as the time required for the output to increase from 10% to 90% of its final value. Thus, if the rise time is 0.01 s, the band width is 33Hz.

Equation suggests that thermal noise can be decreased by narrowing the bandwidth. However, as the bandwidth narrows, the instrument becomes slower to respond to a signal change, and more time is required to make a reliable measurement.

As shown by Equation thermal noise can also be reduced by lowering the electrical resistance of instrument circuits and by lowering the temperature of instrument components. The thermal noise in transducers is often reduce by cooling. For example, lowering the temperature of a UV- visible photodiode array from a room temperature of 298 K to the temperature of liquid nitrogen of 77K will halve the thermal noise.

It is important ot note that thermal noise, while dependent upon the frequency band width, is independent of frequency, it self; thus, it is some times termed white noise by analogy to white light, which contains all visible frequencies. It is also noteworthy that thermal noise in resistive circuit elements is independent of the physical size of the resistor.

Shot Noise

Shot noise is encountered wherever electrons or other charged particles cross a junction. In typical electronic circuits, these junctions are found at pn interfaces; in photocells and vacuum tubes the junction consists of the evacuated space between the anode and cathode. The currents are comprised of a series of quantized events; that is, the transfer of individual electrons across the junction. These events occur randomly, however, and the rate at which they occur is thus subject ot statistical fluctuations which are described by the equation

$$i_{rms} = \sqrt{2Ie\Delta f}$$

where i_{rms} is the root – mean – square current fluctuation associated with the average direct current. i.e. is the charge on the electron of 1.60×10^{-19}C, and Δf is again the band width of frequencies being considered. Like thermal noise, shot noise is white noise.

Equation suggest that shot noise in a current measurement can be minimized only by reducing band width.

Flicker Noise

Flicker noise is characterized as having a magnitude that is inversely proportional to the frequency of the signal being observed; it is sometimes termed 1/f (one-over-f) noise as a consequence. The causes of flicker noise are not well understood; it is ubiquitous, however, and is recognizable by its frequency dependence. Flicker noise becomes significant at frequencies lower than about 100 Hz. The long – term drift observed in dc amplifiers, meters, and galvanometers is a manifestation of flicker noise. Flicker noise can be reduced significantly by using wire- wound or metallic film resistors rather than the more common carbon - composition type.

Environmental Noise

Environmental noise is a composite of different forms of noise that arise form the surroundings. Figure suggests typical sources of environmental noise in a university laboratory.

Much environmental noise occurs because each conductor in an instrument is potentially an antenna capable of picking up electromagnetic radiation and converting it to an electrical signal. There are numerous sources of electromagnetic radiation in the environment, including ac power lines, radio and TV stations, gasoline engine ignition system, arcing switches, brushes in electrical motors, lightning, and ionospheric disturbances. Note that some of these sources, such as power lines and radio stations, cause noise with relatively narrow frequency band widths.

It is also noteworthy that the noise spectrum shown in figure contains a large, continuous noise region at low frequencies. This noise has the properties of flicker noise; its sources are unknown. Superimposed upon the flicker noise are noise peaks associated with yearly and daily temperature fluctuations and other periodic phenomena associated with the use of a laboratory building.

Finally, two quiet- frequency regions in which environmental noise is low are indicated in figure the region extending from about 3Hz at almost 60Hz and the region from about 1 kHz to about 500kHz, or the frequency at which AM radio signals are prevalent. Often, signals are converted to frequencies in these regions in order to reduce noise during signal processing.

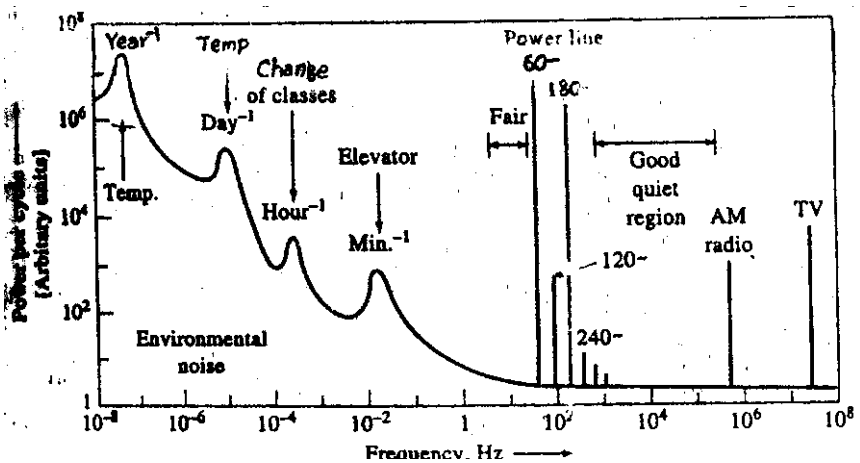


Figure some sources of environmental noise in a university laboratory. Note the frequency dependence and rgions where various types of interference occur. (form T.coor, J. Chem. Educ., 1968, 45, A540, With permission)

9. Describe the signal to Noise Enhancement.

SIGNAL – TO – NOISE ENHANCEMENT

Many laboratory measurements require only minimal effort to maintain the signal – to – noise ratio at an acceptable level. Examples include the weight determinations, made in the course of a chemical synthesis or the color comparison made in determining the chlorine content of the water in a swimming pool. For both examples, the signal is large relative to the noise and the requirements for precision and accuracy are minimal. When the need for sensitivity and accuracy increases, however, the signal – to – noise ratio often becomes the limiting factor in the precision of a measurement.

Both hardware and software methods are available for improving the signal – to – noise ratio of an instrumental method. Hardware noise reduction is accomplished by incorporating in to the instrument design components such as filters, choppers, shields, modulators, and synchronous detectors. These devices remove or attenuates the noise without affecting the analytical signal significantly. Software methods are based upon various computer algorithms that permit extraction of signals from noisy data. As a minimum, software methods requires sufficient hardware to conditions the output signal from the instrument and convert it from analog to digital from. Typically, data are collected by using a computer equipped with a data acquisition module as described in chapter. Signals may then be extracted from noise by using the data acquisition computer or another that is connected to it via a network.

Some Hardware Devices for Noise Reduction

This section contains a brief discussion of several hardware devices and techniques used for enhancing the signal – to – noise ratio.

Grounding and Shielding

Noise that arises from environmentally generated electromagnetic radiation can often be substantially reduced by shielding, grounding, and minimizing the lengths of conductors within the instrumental system. Shielding consists of surrounding a circuit, or the most critical of the wires in a circuit, with a conducting material that is attached to earth ground. Electromagnetic radiation is then absorbed by the shield rather than by the enclosed conductors. Noise pickup and possibly amplification of the noise by the instrument circuit may thus be minimized. We should note that the techniques of minimizing noise through grounding and shielding are often more art than science particularly in instruments that involve both analog and digital circuits. The optimum configuration is often found only after much trail and error. The guidelines given in the references serve as a useful starting point. For an excellent discussion of grinding and shielding, see H.V. Malmstadt, C.G. Enke, and S.R Crouch, Microcomputers and Electronic instrumentation; making the Right Connections, pp. 401-409. Washington, DC: American Chemical Society, 1994. A venerable but valuable reference is R. Morrison. Grounding and Shielding Techniques in Instrumentation. New York: Wiley-Inter science, 1967.

Shielding becomes particularly important when the output of a high – resistance transducer, such as the glass electrode, is being amplified. Here, even minuscule randomly induced currents produce relatively large voltage fluctuations in the measured signal.

Difference and Instrumentation Amplifiers

Any noise generated in the transducer circuit is particularly critical because it usually appears in an amplified from in the instrument readout. To attenuate this type of noise, most instruments employ a difference amplifier, such as that shown in figure fort the first stage of amplification. Common – mode noise in the transducers circuit generally appears in phase at both the inverting and non inverting input s of the amplifier and is largely subtracted out by the circuit so that the noise t its out put is diminished substantially. For cases in which a difference amplifier is insufficient to remove the noise, an instrumentation amplifier such as the one shown in fig. is used.

Instrumentation amplifiers are composed of three op amps configured as shown in figure. Op amp A and op amp B make up the input stage of the instrumentation amplifier in which the two op amps are cross coupled through three resistors R_1, R_1/a, and R_1. The second stage of the

module is the difference amplifier of op amp C. We will not derive the transfer function of the instrumentation amplifier; however, suffice to say that the overall gain of the circuit is given by

$V_0=K(2a+1)(v_2-v_1)$

Equation highlights two advantages of the instrumentation amplifier; (1) the over all gain of the amplifier may be controlled by varying a single resistor R_1/a, and (2) the second difference stage rejects common-mode signals. In addition, op amps A and B are voltage followers with very high input resistance, so the instrumentation amplifier presents a negligible load to its transducer circuit. The combination of the two stages can provide rejection of common- mode noise by a factor of 10^6 or more while amplifying the signal by as much as 1000.

These devices are sued often with low- level signals in noisy environments such as in the measurement of signals in biological organisms in which the organism acts an as antenna. Electrocardiographic instrumentation exploits the advantages of instrumentation amplifiers.

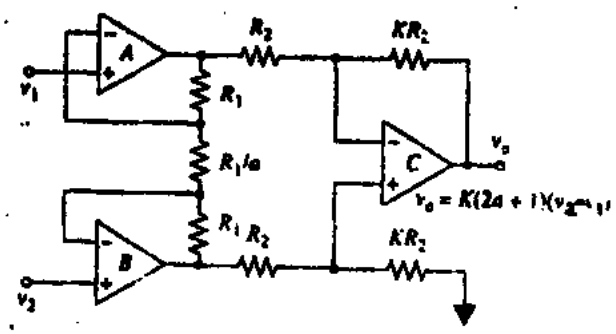


Figure An instrumentation amplifier for reducing the effects of noise common to both inputs. The gain of the circuit is controlled by resistors R_1/a and KR_2.

Another typical application is in computer data acquisition module such as the a programmable gain amplifier is under the control of a computer, which changes resistor R_1/a of figure by using solid – state switches under digital control.

Analog Filtering

One of the most common methods of improving the signal – to – noise ratio in analytical instruments is by use of low – pass analog filters such as that shown in figure. The reason for this widespread application is that many instruments signals are of low frequency with bandwidths extending over a range of only a few hertz. Thus, a low – pass filter characterized by the Bode diagram shown in figure will effectively remove many of the high – frequency components of the signal, including thermal or shot noise. Figure illustrates the use of a low – pass RC filter for reducing noise from a slowly varying dc signal.

High- pass analog filters such as that shown in fig. also find considerable application in analytical instruments where the analyte signal is at relatively high frequency. The high – pass filter reduces the effects of drift and other low – frequency flicker noise.

Narrow – band electronic filters are also available to attenuate noise outside an expected band of signal frequencies.

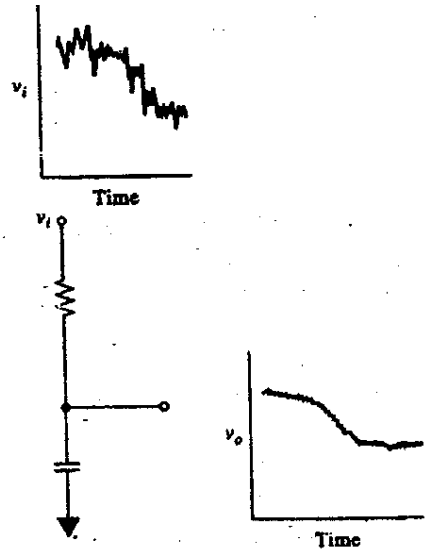


Figure use of a low – pass filter with a large time constant to remove noise from a slowly changing dc voltage.

We have pointed out that the magnitude of fundamental noise is directly proportional to the square root of the frequency band width of a signal. Thus, significant noise reduction can be achieved by restricting the input signal to a narrow band of frequencies and using an amplifier that is tuned to this band. It is important to note that the band pass of the filter must be sufficiently wide to carry all of the signal frequencies.

Modulation

Direct amplification of a low- frequency or dc signal is particularly troublesome when an instrument exhibits amplifier drift and flicker noise. Often, this 1/f noise is several times large than the types of noise that predominate at higher frequencies as is illustrated in the noise power spectrum of figure. For this reason, low frequency or dc signals form transducers are often converted to a higher frequency. Where 1/f noise is less troublesome. This process is called modulation. After amplification, the modulated signal can be freed from amplifier 1/f noise by filtering with a high – pass filter, demodulation and filtering with a low – pass filter then produce an amplified dc signal suitable for output.

UNIT – II

PART - A

1. What are the six phenomena of optical spectroscopic methods.

Optical Spectroscopic methods are based upon six phenomena's

(1) absorption
(2) fluorescence
(3) phosphorescence
(4) scattering
(5) emission (and)
(6) chemiluminescence's

2. How many components are present in spectroscopic instruments?

Typical spectroscopic instruments contain five components, including;
(1) a stable source of radiant energy.
(2) a transparent container for holding the sample
(3) a device that isolates a restricted region of the spectrum for measurement
(4) a radiation detector, which converts radiant energy to a usable signal.
(5) a signal processor and readout which displays the transduced signal on a meter scale, an oscilloscope face, a digital meter, (or) a recorder chart.

3. What are the sources used in the spectroscopic Instrument?

Note that these sources are of two types;

(i) **Continuum sources:-** which emit radiation that changes in intensity only slowly as a function of wavelength, and

(ii) **Line sources:-** which emit a limited number of lines (or) bands of radiation each of which sapans a limited range of wave lengths.

4. What is Mechanism of Laser Action?

Laser action can be understood by considering the four processes depicted.
(a) Pumping
(b) Spontaneous emission (fluorescence)

(c) Stimulated emission and
(d) Absorption

5. Define stimulated emission.

Stimulated emission, which is the basis of laser behavior, is depicted, here, the excited laser species are struck by photons that have precisely the same energies (Ey-Ex) as the photons produced by spontaneous emission. Collisions of this type cause the excited species to relax immediately to the lower energy state and to simultaneously emit a photon of exactly the same energy as the photon that stimulated the process.

6. Define Gas Lasers.

A variety of gas lasers are available commercially. These devices are of four types; (i) neutral atom lasers such as He/Ne; (2) ion lasers in which the active species is Ar^+ (or) kr^+; (3) molecular lasers in which the lasing medium is CO_2 (or) N_2 and (4) eximer lasers.

7. Define Dye lasers.

Dye lasers have become important radiation sources in analytical chemistry because they are continuously tunable over a range of 20 to 50 nm. The band width of a tunable laser is typically a few hundredths of a nanometer or less. The active materials in dye lasers are solutions of organic compounds capable of fluorescing in the ultraviolet, visible, or infrared region.

8. What are Filters?

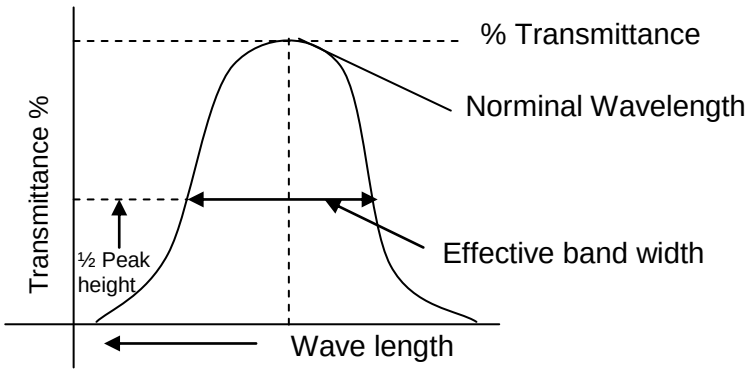
→ Two types of filers are employed for wave length selection;
→ Interference filters and absorption filters.

Absorption filters are restricted to the visible region of the spectrum; interference filter on the other hand are available for the ultraviolet, visible and well into the infrared region.

9. What is a Wave lengths selector?

For most spectroscopic analyses, radiation that consists of a limited, narrow, continuous group of wavelengths called a band is required.

A narrow bandwidth enhances the sensitivity of absorbance measurements, may provide selectivity to both emission and absorption methods, and is frequently a requirement from the stand point of obtaining a linear relationship between the optical signal and concentration.



10. Define Interference Filters.

As the name implies, interference filters rely on optical interference to provide narrow bands of radiation dielectric. An interference filter consits of a transparent dielectric that occupies the space between two semitransparent matallic filter. This array is sandwiched between two plates of glass or other transparent materials.

11. Define Absorption filters.

Absorption filters, which are generally less expensive than interference filters, have been widely used for band selection in the visible region.

Absorption filters have effective band widths that range from perhaps 30 to 250nm. Filters that provide the narrowest band widths also absorb a significant fraction of the desired radiation and may have a transmittance of 10% (or) less at their bank peaks.

12. Define Cut off filters.

Cut of filters have transmittances of nearly 100% over a portion of the visible spectrum, but then rapidly decrease to zero transmittance over the remainder. A narrow spectral band can be isolated by coupling cut off filter with a second filter.

13. What are the components of Monochromators?

(i) An entrance slit that provides a rectangular optical image,
(ii) A collimating lens (or) mirror that produces a parallel beam of radiation,
(iii) A prism (or) a grating that disperses the radiation into its component wavelengths.
(iv) A focusing element that remforms the image of the entrance slit and focuses it on a planar surface called a focal plane, and
(v) An exit slit in the focal plane that isolates the desire3d spectral band.

14. Define Monochromators.

It is necessary (or) desirable to be able to vary the wavelength of radiation continuously over a considerable range. This process is called scanning a spectrum. Monochromators aredesigned for spectral infrared radiation are all similar in mechanical construction in the sense that they employ slits, lenses, mirrors, windows and gratings or prisms.

15. Give the types of Monochromators.

Two types of dispersing elements are found in monochromators; reflection gratings and prisms.

For purposes of illustration, a beam made up of just two wavelengths, λ_1 and λ_2. This radiation enters the monochromators via a narrow rectangular opening (or) slit, is collimated and then strikes the surface of the dispersing element at an angle.

16. Define Grating Monochromators.

Dispersion of Ultraviolet, visible, and infrared radiation can be brought about by directing a polychromatic beam through a transmission grating or on to the surface of a reflection grating; the latter is by far the more common type. Replica gratings, which are used in most monchromators are manufactured from a master grating.

17. Define Monochromator slits.

The slits of a monochromator play an important role in determining the monochromators performance characteristics and quality. Slit jaw are formed by carefully machining two pieces of metal to give sharp edges.

Care is taken to assure that the edges of the slit are exactly parallel to one another and that they lie on the same plane. In some monochromators, the openings of the two slits are fixed; more commonly, the spacing can be adjusted with a micrometer mechanism.

18. Give any two types of photon Transducers.

(i) photo voltaic cells, in which the radiant energy generates a current at the interface of a semiconduct layer and metal.
(ii) Phototubes, in which radiation causes emission of electrons from a photosensitive solid surface.
(iii) Photomultiplier tubes, which contain a photoemissive surface as well as several additional surface that emit a cascade of electrons when struck by elelctron.

19. Define photomultiplier Tubes.

For the measurement of low radiant power, the photomultiplier tube (DMT) offers advantages over the ordinary phototube. The photo cathode surface is similar in composition to the surfaces of the phototubes described and it emits electrons when exposed to radiation. The tube also contains additional electrodes called dynodes.

20. Define photon Counting.

The output from a photomultiplier tube consists of a pulse of electrons for each photon that reaches the detector surface. Ordinarily this analog singal is filtered to remove undesirable fluctuations due to the random appearance of photons at the photocathode and measured as a dc voltage (or) current.

Radiant power is proportional to the number of pulses per unit time rather than to an average current (or) potential a measurement of this type is termed phototn counting.

21. Define fiber-optic Sensors.

Fiber-optic sensors, which are also called optrodes, consist of a reagent phase immobilized on the end of a fiber optic. Interaction of the analyte with the reagent crates a change in absorbance, reflectance, fluorescene (or) luminescence, which is then transmitted to a detector via the optical fiber. Fiber – optic sensors are genrally simple, inexpensive devices that are easily minimized.

22. Define resolution.

The resolution of a Fourier transform spectrometer can be described in terms of the difference in wave number between two lines that can be just separated by the instrument. That is

$$\Delta\bar{v} = \bar{v}_1 - \bar{v}_2$$

Where $\bar{v}_1$ and $\bar{v}_2$ are wave numbers for a pair of barely resoluable lines.

PART - B

1. Describe the general designs of optical instruments.

GENERAL DESIGNS OF OPTICAL INSTRUMENTS

Optical spectroscopic methods are based upon six phenomena; (1) absorption, (2) fluorescence, (3) phosphorescence, (4) scattering, (5) emission, and (6) chemiluminescence's. While the instruments for measuring each differ somewhat in configuration, most of their basic components are remarkably similar. Furthermore, the required properties of these components are the same regardless of whether they are applied to the ultraviolet, visible, or infrared portion of the spectrum.

Typical spectroscopic instruments contain five components, including: (1) a stable source of radiant energy; (2) a transparent container for holding the sample; (3) a device that isolates a restricted region of the spectrum for measurement; (4) a radiation detector, which converts radiant energy to usable signal (usually electrical); and (5) a signal processor and readout, which displays the transduced signal on a meter scale, an oscilloscope face, a digital meter, or a recorder chart. Figure illustrates the three ways in which these components are configured in order to carry out the six types of spectroscopic measurements mentioned earlier. As can be seen in the figure components (3), (4), and (5) are arranged in the same way for each type of measurement.

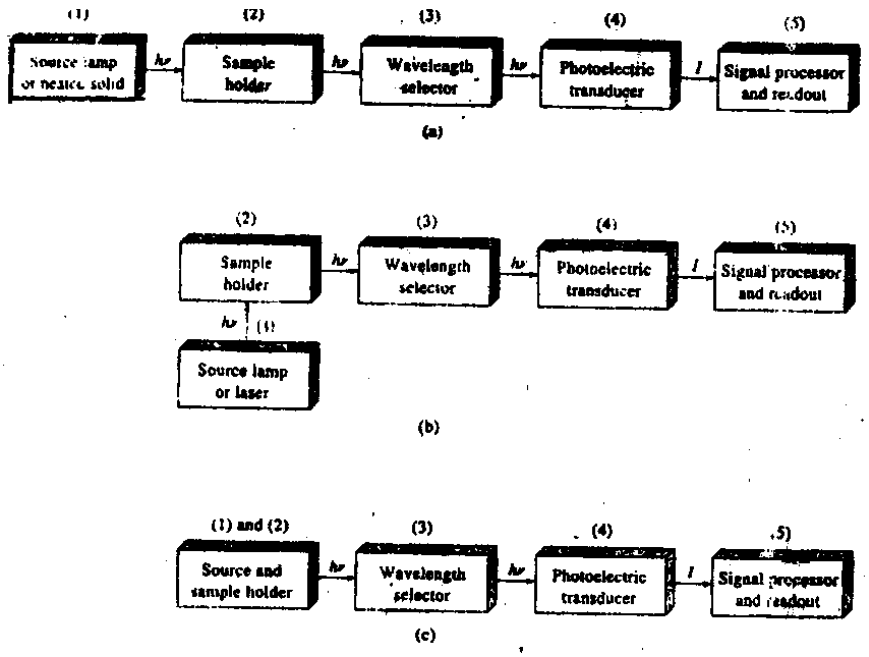


Figure: components of various types of instruments for optical spectroscopy (a) Absorption (b) fluorescence, phosphorescence, and scattering; (c) emission and chemiluminescence.

The first two instrumental configurations, which are used for the measurement of absorption, fluorescence, phosphorescence, and scattering, require an external source of radiant energy. For absorption, the e beam from the source passes through the sample directly into the wavelength selector, although in some instruments, the position of the sample and selector is reversed. In the latter three, the source induces the sample, held in a container, to emit characteristic fluorescence, phosphorescence, or scattered radiation, which is usually measured at an angle of 90 deg with respect to the source.

Emission spectroscopy and chemiluminescence spectroscopy differ from the other types in the respect that no external radiation source is required; the sample itself is the emitter figure. In emission spectroscopy, the same container is an arc, a spark, or a flame that both holds the sample and causes it to emit characteristic radiation. In chemiluminescence spectroscopy, the radiation source is a solution of the analyte plus reagents held in a glass sample h older. Emission is brought about by energy released in a chemical reaction in which the analyte takes part directly or in directly.

Figure summarizes the optical characteristics of all the components shown in figure with the exception of the signal processor and readout. Note that instrument components differ in detail, depending upon the wavelength region with in which they are to be used. Their design also depends on whether the instrument is to be used primarily for qualitative or quantitative analysis and on whether it is to be applied to atomic or molecular spectroscopy. Nevertheless, the general function and performance requirements of each type of component are similar, regardless of wavelength region and application.

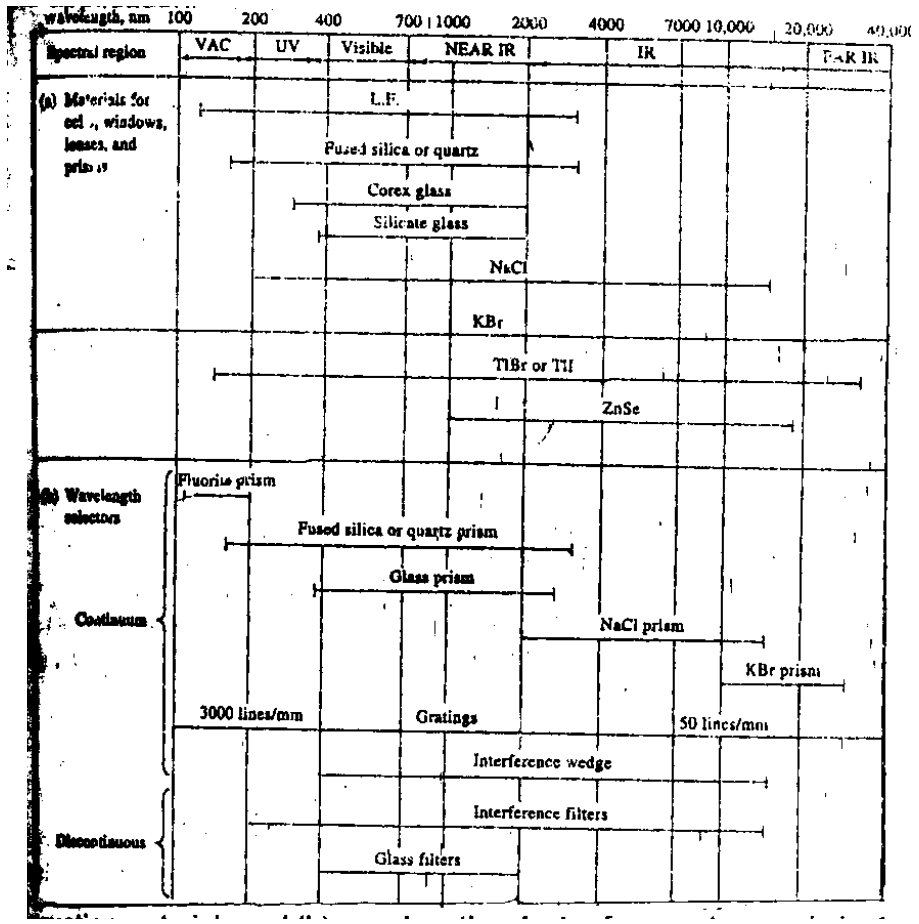


Figure (a) Construction materials and (b) wavelength selector for spectroscopic instruments.

2. Describe the source of Radiation & its types.

SOURCES OF RADIATION

In order to be suitable for spectroscopic studies, a source must generate a beam of radiation with sufficient power for easy detection and measurement. In addition, its out-put power should be stable for reasonable periods. Typically, the radiant power of a source varies exponentially with the voltage of its power supply. Thus, a regulated power source is often needed to provide the required stability. Alternatively, the problem of source stability is circumvented by double-beam designs in which the ratio of the signal from the sample to that of the source in the absence of sample serves as the analytical variable. In such designs, the intensities of the two beams are measured simultaneously or nearly simultaneously so that the effect of fluctuations in the source output is largelly canceled.

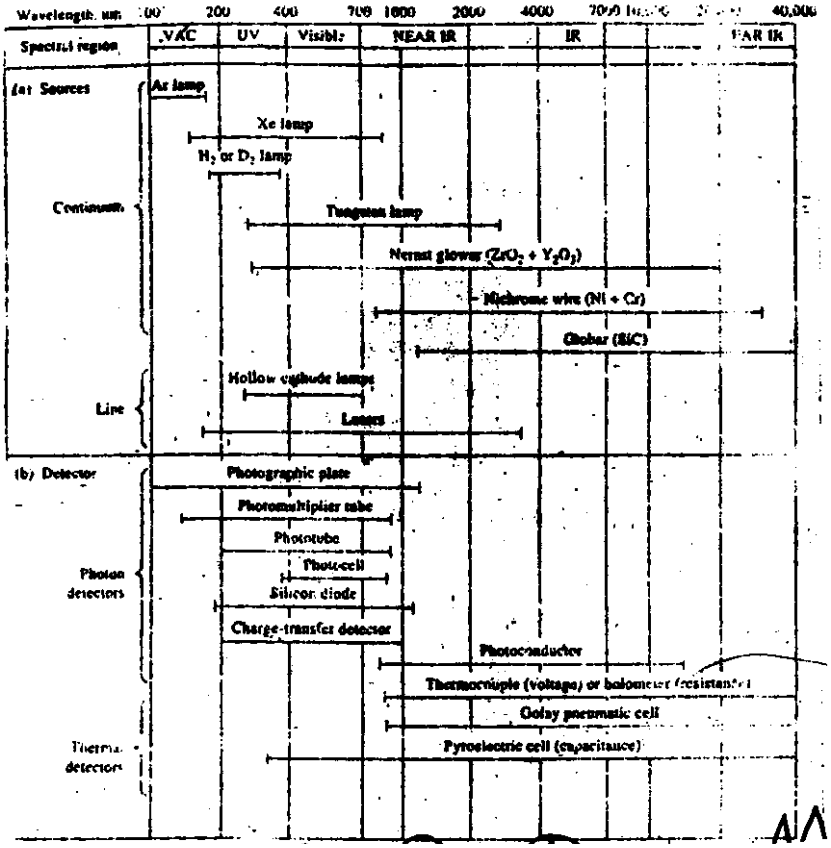


Figure (a) Sources and (b) Detectors for spectroscopic instruments

Figure lists the most widely used spectroscopic sources. Note that these sources are of two types: continuum sources, which emit radiation that changes in intensity only slowly as a function of wavelength, and line sources, which emit a limited number of lines or bands of radiation each of which spans a limited range of wavelengths.

Continuum Sources

Continuum source find widespread use in absorption and fluorescence spectroscopy. For the ultraviolet region, the most common source is the deuterium lamp. High-pressure, gas-filled are lamps that contain argon, xenon, or mercury serve when a particularly intense source is required. For the visible region of the spectrum, the tungsten filament lamp is used almost universally. The common infrared sources are inert solids heat to 1500 to 2000 K a temperature at which the maximum radiant output occurs at 1.5 to 1.9 μ m. Detail on the construction and behavior of these various continuum sources will be found in the chapter dealing with specific types of spectroscopic methods.

Line Sources

Sources that emit a few discrete lines find wide use in atomic absorption spectroscopy, atomic and molecular fluorescence spectroscopy, and Raman spectroscopy (refractometry and polarimetry also employ line sources). The familiar mercury and sodium vapor lamps provide a relatively few sharp lines in the ultraviolet and visible regions and are used in several spectroscopic instruments. Follow cathode lamps and electrode less discharge lamp are the fluorescence methods. Discussion of such sources is deterred to section.

Laser Sources

Lasers are highly useful sources in analytical instrumentation because of their high intensities, their narrow bandwidths, and the coherent nature of their outputs. The first laser was described in 1960. Since that time, chemists have found numerous useful applications for these sources in high-resolution spectroscopy, in kinetic studies of processes with lifetimes in the range 10^{-9} to 10^{-12} s, in the detection and determination of extremely small concentrations of species in the atmosphere, and in the induction of isotopic ally selective reactions. In addition, laser sources have become important in several routine analytical methods, including Raman spectroscopy, molecular absorption spectroscopy, emission spectroscopy, and as part of instruments for Fourier transform infrared spectroscopy.

The term laser is an acronym for light amplification by stimulated emission of radiation. As a consequence of their light-amplifying characteristics, lasers produce spatially narrow (a few hundredths of a micrometer), extremely intense beams of radiation. The process of stimulated emission, which will be described shortly, produces a beam of highly monochromatic (bandwidths of 0.01 nm or less) and remarkably coherent (section 6B-6) radiation. Because of these unique properties, lasers have become important sources for use in the ultraviolet, visible, and infrared regions of the spectrum. A limitation of early lasers was that the radiation from a given source was restricted to a relatively few discrete wavelengths or lines. Now, however, dye lasers are available that provide narrow bands of radiation at any chosen wavelength within a somewhat limited range of the source.

Components of lasers

Figure is a schematic representation that shows the components of a typical laser source. The heart of the device is the lasing medium. It may be a solid crystal such as ruby, a semiconductor such as gallium arsenide, a solution of an organic dye, or a gas such as argon or krypton. The lasing material is often activated, or pumped, by radiation from an external source so that a few photons of proper energy will trigger the formation of a cascade of photons of the same energy. Pumping can also b e accomplished by an electrical current or by an electrical discharge. Thus, gas lasers usually do not have the external radiation source shown in figure instead, the power supply is connected to a pair of electrodes contained in a cell filled with the gas.

A laser normally functions as an oscillator, or a resonator, in the sense that the radiation produced by the lasing action is caused to pass back and forth through the medium numerous times by means of a pair of mirrors as shown in figure. Additional photons are generated with each passage, thus leading to enormous amplification. The repeated passage also produces a beam that is highly parallel because nonparallel radiation escapes from the sides of the medium after being reflected a few times. One of the easiest ways to obtain a usable laser beam is to coat one of the mirrors with a sufficiently thin layer of reflecting material so that a fraction of the beam is transmitted rather than reflected.

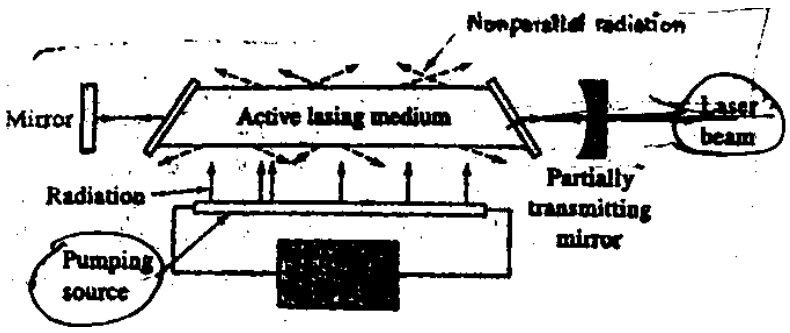


Figure: Schematic representation of a typical laser source

Mechanism of Laser Action

Laser action can be understood by considering the four processes depicted in figure namely (a) pumping (b) spontaneous emission (fluorescence), (c) stimulated emission, and (d) absorption. In this figure, we show the behavior of two of the many molecules that make up the lasing medium. Two of the several electronic energy levels of each are shown as having energies E_Y and E_X. Note that the higher electronic state for each molecule has several slightly different vibrational energy levels depicted as E_Y, E_Y', E_Y'', and so forth. We have not shown additional levels for the lower electronic state, although such usually exist.

3. Describe the following wavelength selectors.

(a) Filters

(b) Monochromators

c) ette Grating

Filters

Two types of filters are employed for wavelength selection; interference filters and absorption filters (the former are sometimes called Fabry-Perot filters). Absorption filters are restricted to the visible region of the spectrum; interference filters, on the other hand, are available for the ultraviolet, visible and well into the infrared region.

Interference Filters

As the name implies, interference filters rely on optical interference to provide narrow bands of radiation. An interference filter consists of a transparent dielectric[12] A dielectric material is an insulator that contains essentially no current-carrying charged particles. Die lectors are generally transparent in most spectral regions. (frequently calcium fluoride or magnesium fluoride) that occupies the space between two semitransparent metallic fierce. This array is sandwiched between two plates of glass or other transparent materials. The thickness of the dielectric layer is carefully controlled and determines the wavelength of the transmitted radiation. When a perpendicular beam of collimated radiation strikes this array, a fraction passes through the first metallic layer while the remainder is reflected. The portion that is passed undergoes a similar partition when it strikes the second metallic film. If the reflected portion from this second interaction is of the proper wavelength, it is partially reflected from the inner side of the first layer in phase with incoming light of the same wavelength. The result is that this particular wavelength is reinforced, while most other wavelengths, being out of phase, suffer destructive interference.

The relationship between the thickness of the dielectric layer t and the transmitted wavelength λ can be found with the aid of figure. For purposes of clarity, the incident beam is shown as arriving at an angle θ from the perpendicular. At point 1, the radiation is partially reflected and partially transmitted to point 1′ where partial reflection and transmission again takes place.

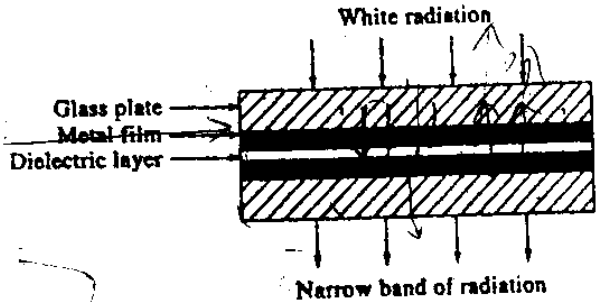


Figure: (a)

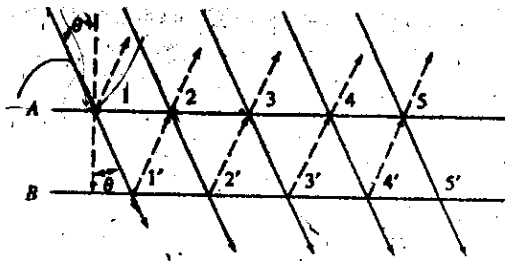


Figure: (b)

Figure (a) schematic cross section of an interference filter. Note that the drawing is not to scale and that the three central bands are much narrower than shown (b) schematic to show the conditions for constructive interference.

The same process occurs at 2, 2′, and so forth. For reinforcement to occur at point 2, the distance traveled by the beam reflected at 1′ must be some multiples of its wavelength in the medium λ′. Since the path length between surfaces can be expressed as t/cos θ, the condition for reinforcement is that

$$n\lambda' = 2t/\cos\theta$$

where **n** is a small whole number.

In ordinary use, θ approaches zero and cos θ approaches unity so that the foregoing equation simplifies to

$$n\lambda' \approx 2t$$

Where λ′ is the wavelength of radiation in the dielectric and t is the thickness of the dielectric. The corresponding wavelength in air is given by

$$\lambda = \lambda'\eta$$

Where η is the refractive index of the dielectric medium. Thus the wavelengths of radiation transmitted by the filter are

$$\lambda = \frac{2t\eta}{n}$$

The integer **n** is the order of interference. The glass layers of the filter are often selected to absorb all but one of the reinforced bands; transmission is thus restricted to a single order.

Figure illustrates the performance characteristics of typical interference filters. Ordinarily, filters are characterized, as shown, by the wavelength of their transmittance peaks, the percentage of incident radiation transmitted at the peak and their effective bandwidths.

Interference filters are available with transmitter peaks throughout the ultraviolet and visible regions and up to about 14 μm in the infrared. Typically, effective bandwidths are about 1.5% of the wavelength at peak transmittance, although this figure is reduced to 0.15% in some narrow-band filters; these have maximum transmittances of 1.

Interference wedges

An interference wedge consists of a pair of mirrored, partially transparent plates separated by a wedge-shaped layer of a dielectric material. The length of the plates ranges from about 50 to 200 mm. The radiation transmitted varies continuously in wavelength from one end to the other as the thickness of the wedge varies. By choosing the proper linear position along the wedge, a bandwidth of about 20 nm can be isolated.

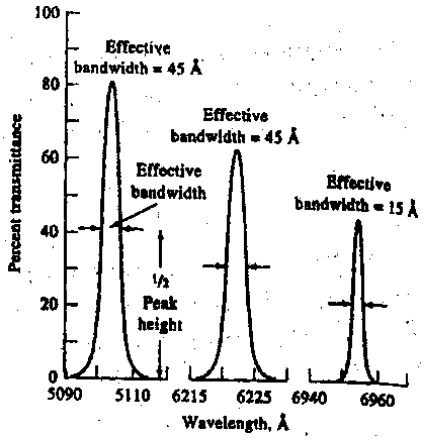


Figure: Transmission characteristics of typical interference filters

Interference wedges are available for the visible region (400 to 700 nm), the near-infrared region (1000 to 2000 nm), and for several parts of the infrared region (2.5 to 14.5μm). They can serve in place of prisms or gratings in monochromators.

Absorption Filters

Absorption filter, which are generally less expensive than interference filters, have generally less expensive than interference filters, have been widely used for band selection in the visible region. These filters function by absorbing certain portions of the spectrum. The most common type consists of colored glass or of a dye suspended in gelatin and sandwiched between glass or of a dye suspended in gelatin and sandwiched between glass plates. The former have the advantage of greater thermal stability.

Absorption filters have effective bandwidths that range from perhaps 30 to 250 nm. Filters that provide the narrowest bandwidths also absorb a significant fraction of the desired radiation and may have a transmittance of 10% of less at their band peaks. Glass filters with transmittance maxima through out the entire visible region are available commercially.

Cut-off filters have transmittances of nearly 100% over a portion of the visible spectrum, but then rapidly decrease to zero transmittance over the remainder. A narrow spectral band can be isolated by coupling a cut-off filter with a second filter.

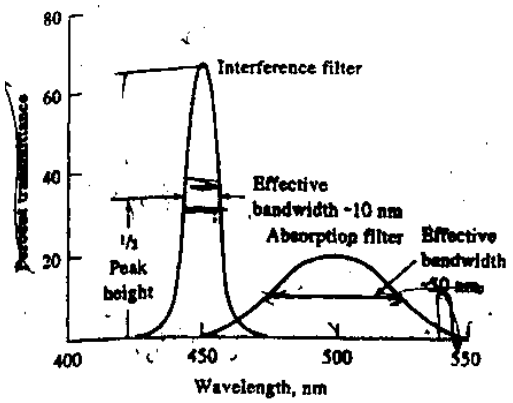


Figure: Effective bandwidths for two types of filters

It is apparent from figure that the performance characteristics of absorption filters are significantly inferior to those of interference-type filters. Not only are the bandwidths of absorption filters greater, but for narrow bandwidths the fraction of light transmitted is also less. Nevertheless, absorption filters are totally adequate for many applications.

Monochromators

For many spectroscopic methods, it is necessary or desirable to be also to vary the wavelength of radiation continuously over a considerable range. This process is called scanning a spectrum. Monochromators are designed for spectral scanning. Monochromators for ultraviolet, visible, and infrared radiation are all similar in mechanical construction in the sense that they employ slits, lenses, mirrors, windows, and gratings or prisms. To be sure, the materials from which these components are fabricated depend upon the wavelength region of intended use.

Components of Monochromators

Figure illustrates the optical elements found in all monochromators, which include the following (1) an entrance slit that provides a rectangular optical image, (2) a collimating lens or mirror that produces a parallel beam of radiation, (3) a prism or a grating that disperses the radiation into its component wavelengths, (4) a focusing element that reforms the image of the entrance slit and focuses it on a planar surface called a focal plane, and (5) an exit slit in the focal plane that isolates the desired spectral band. In addition, most monochromators have entrance and exit windows, which are designed to protect the components from dust and corrosive laboratory fumes.

As shown in figure two types of dispersing elements are found in monochromators: reflection grating and prisms. For purposes of illustration, a beam made up of just two wavelengths, λ_1 and $\lambda_2 (\lambda_1 > \lambda_2)$, is shown. This radiation enters the monochromators via narrow rectangular opening or slit, is collimated, and then strikes the surface of the dispersing element at

an angle. For the grating monochromator, angular dispersion of the wavelengths results from diffraction, which occurs at the reflective surface; for the prism, refraction at the two faces results in angular dispersal of the radiation, as shown. In both designs, the dispersal of the radiation, as shown. In both designs, the dispersed radiation is focused on the focal plane AB where it appears as two rectangular images of the entrance slit (one for λ_1 and one for λ_2). By rotating the dispersing element, one band or the other can be focused on the exit slit.

Historically, most monochromators were prism instruments. Currently, however, nearly all commercial monochromators are based upon reflection gratings because they are cheaper to fabricate, provide better wavelength separation for the same size dispersing element, and disperse radiation linearly along the focal plane. As shown in figure linear dispersion means that the position of a band along the focal plane for a grating caries linearly with its wavelength. For prism instruments, incontrast, shorter wavelengths are,

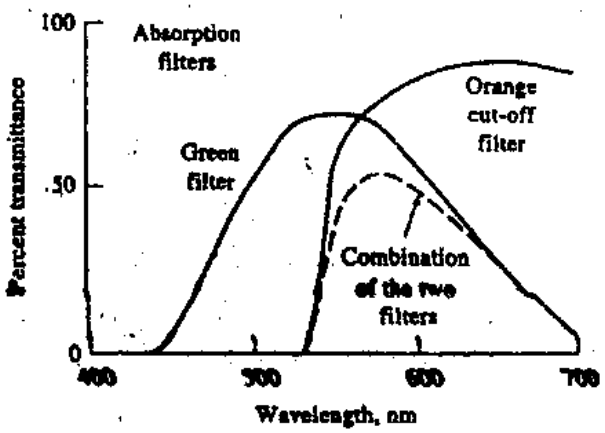
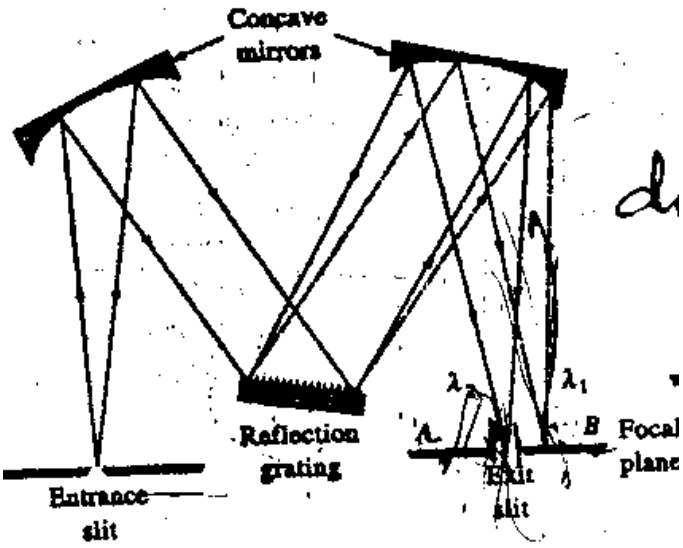
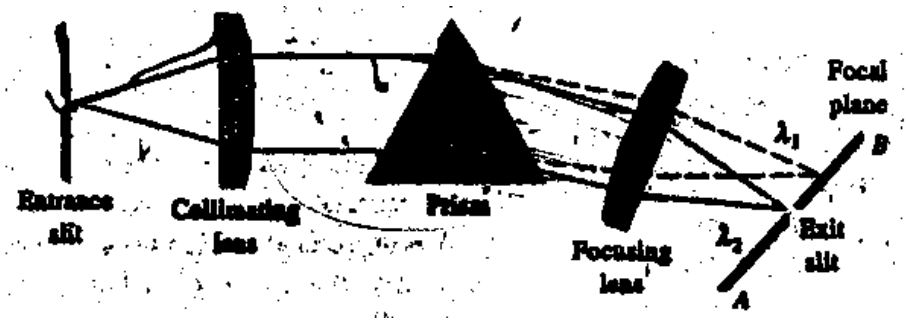


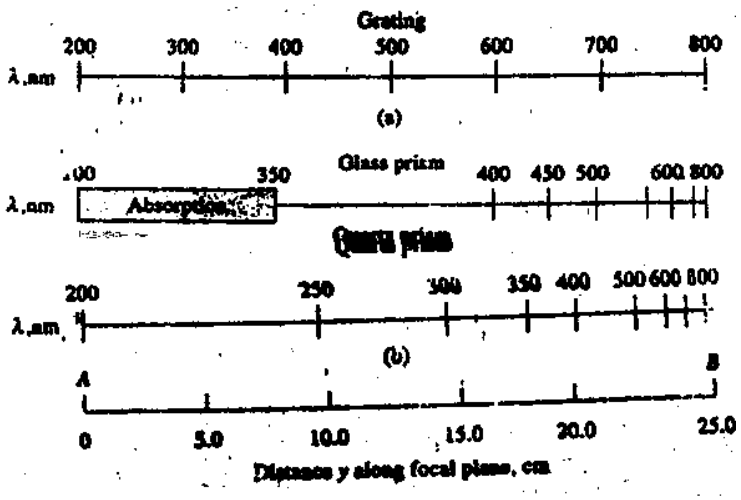
Figure: Comparison of various types of filters for visible radiation



(a)



(b)Figure: two types of monochromators (a) Czerney-Turner grating monochromator and (b) Bunsen prism monochromator (In both instances, $\lambda_1 > \lambda_2$)



(c)

Figure: Dispersion for three types of monochromators. The points A and B on the scale in (c) correspond to the points shown in figure

dispersed to a greater degree than are longer ones, with complicates instrument design. The nonlinear dispersion of two types of prism monochromators is illustrated by figure. Because of their more general use, we will largely focus over discussion on grating monochromators.

Prism Monochromators

Prisms can be used to disperse ultraviolet, visible, and infrared radiation. The material used fro their construction differs, however, depending upon the wavelength region.

Figure shows the two most common types of prism designs. The first is a 60 –deg prism, which is ordinarily fabricated from a single block of material. When crystalline (but not fused) quartz is the construction material, however, the prism is usually formed by cementing two 30-deg prisms together, as shown in figure; one is fabricated from right-handed quartz and the second from left-handed quartz. In this way, the optically active quartz causes no net polarization of the emitted radiation; this type of prism is called a cornu prism. Figure shows Bunsen monochromator, which employs a 60-deg prism, likewise often made of quartz.

As shown in figure the littrow prism, which permits more compact monochromator designs, is a 30-deg prism with a mirrored back. Refraction in this type of prism takes place twice at the same interface so that the performance characteristics are similar to those of a 60-deg prism in a Bunsen mount.

Grating Monochromators

Dispersion of ultraviolet, visible, and infrared radiation can be brought about by directing a polychromatic beam through a transmission grating or onto the surface

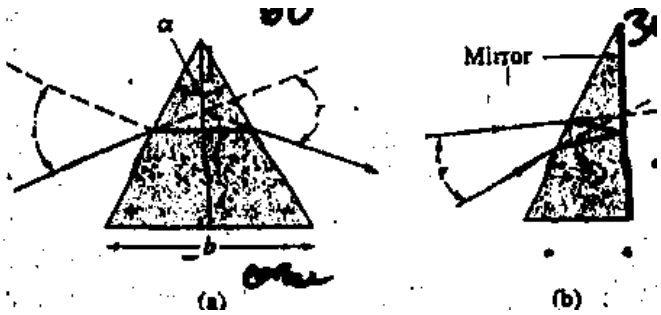


Figure: Dispersion by a prism: (a) quartz cornu types and (b) Littrow type

Of a reflection grating; the latter is by far the more common type. Replica gratings, which are used in most monochromators, are manufactured from a master grating. For an interesting in formative discussion of the manufacture, testing, and performance characteristics of gratings, see Diffraction Grating Handbook, Rochester, NY; Bausch and Lomb, Inc. (now Milton Roy Company), 1970. For a historical perspective on the importance of gratings in the advancement of science, see A.G. Ingalls, Sci. Amer 186(6), 45. The latter consists of a hard, optically flat, polished surface upon which have been ruled with a suitably shaped diamond tool a large number of parallel and closely spaced grooves. A magnified cross-sectional view of a few typical grooves is shown in figure. A grating for the ultraviolet and visible region will typically contain from 300 to 2000 grooves/mm, with 1200 to 1400 being most common. For the infrared region, 10 to 200 grooves/mm are encountered; for spectrophotometers designed for the most widely used infrared range of 5 to 15μm, a grating with about 100 grooves/mm is suitable. The construction of good

master grating is tedious, time consuming, and expensive because the grooves must be identical in size, exactly parallel, and equally spaced over the length of the grating (3 to 10 cm).

Replica gratings are formed from a master grating by a liquid resin casting process that preserves virtually perfectly the optical accuracy of the original master grating on a clear resin surface. This surface is ordinarily made reflective by a coating of aluminum, or sometimes gold or platinum.

The Echellette Grating: Figure is a schematic representation of an echellette-type grating, which is grooved or blazed such that it has relatively broad faces from which reflection occurs and narrow unused faces. This geometry provides highly efficient diffraction of radiation. Each of the broad faces can be considered to be a point source of radiation; thus interference among the reflected beams 1, 2, and 3 can occur. In order for the interference to be constructive, it is necessary that the path lengths differ by an integral multiple **n** of the wavelength λ of the incident beam.

In figure parallel beams of monochromatic radiation 1 and 2 are shown striking the grating at an incident angle i to the grating normal. Maximum constructive interference is shown as occurring at the reflected angle r. it is evident that beam 2 travels a greater distance than beam 1 and the difference in the paths is

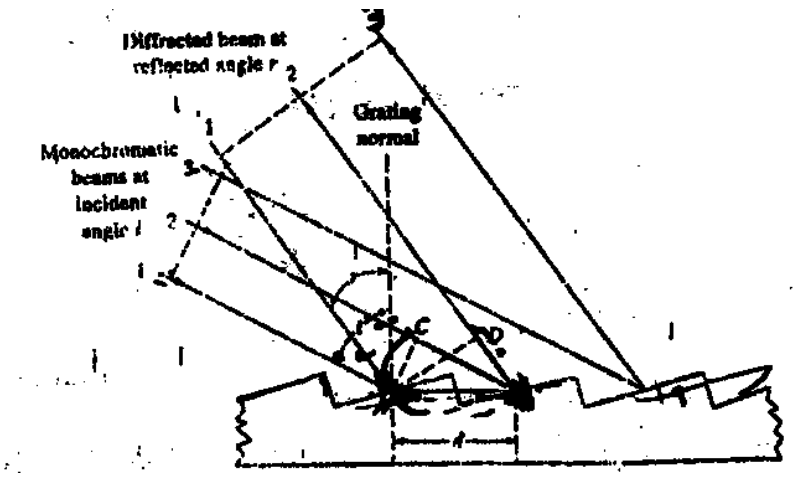


Figure: Mechanisms of diffraction from an enchellette type grating

Equal to $\left(\overline{CB}+\overline{BD}\right)$ (shown as a broader line in figure). For constructive interference to occur, this difference must equal nλ. That is,

$$\mathbf{n}\lambda=\left(\overline{CB}+\overline{BD}\right)$$

where **n** a small whole number, is called the diffraction order. Note, however, that angle CAB is equal to angle i and that angle DAB is identical to angle r. therefore, from simple trigonometry,

we may write,

$$\overline{CB} = d \sin i$$

where **n** d is the spacing between the reflecting surfaces. It is also seen that

$$\overline{BD} = d \sin r$$

Substitution of the last two expressions into the first gives the condition for constructive interference. Thus,

$$n\lambda = d (\sin i + \sin r)$$

Equation suggests that several values of λ exist for a given diffraction angle r. Thus, if a first-order line (n=1) of 900 nm is found at r, second-order (450-nm) and third-order (300-nm) lines also appear at this angle. Ordinarily, the first-order line is the most in tense; indeed, it is possible to design gratings that concentrate as much as 90% of the incident intensity in this order. The higher-order lines can generally be removed by filter. For example, glass, which absorbs radiation below 350 nm, eliminates the higher-order spectra associated with first-order radiation in most of the visible region. The example that follows illustrates these points.

Example:

An echellette grating that contains 1450 blazes/mm was irradiated with a polychromatic beam at an incident angle 48 deg to the grating normal. Calculate the wavelengths of radiation that would appear at an angle of reflection of+20,+10, and 0 dog.

To obtain d in Equation we write

$$d = \frac{1\text{mm}}{1450 \text{ blazes}} \times 10^6 \frac{\text{nm}}{\text{mm}} = 689.7 \frac{\text{nm}}{\text{blaze}}$$

When r in figure equals +20deg,

$$\lambda = \frac{689.7}{n} \text{nm} (\sin 48 + \sin 20) = \frac{748.4}{n} \text{nm}$$

and the wavelengths for the first-, second-, and third- order reflections are 748,374, and 249nm, respectively.
Further calculations of a similar kind yield the following data;

Wavelength (nm) for

r,deg	n=1	n=2	n=3
20	748	374	249
10	632	316	211
0	513	256	171

Concave Gratings. Gratings can be formed on a concave surface in much the same way as on a plane surface. A concave grating permits the design of a monochromator without auxiliary collimating and focusing mirrors or lenses because the concave surface both disperses the radiation and focuses it on the exit slit. Such an arrangement is advantageous in terms of cost; in addition, the reduction in number of optical surfaces in creases the energy throughput of a monochromator that contains a concave grating.

4. Describe the photon Transducers and its types.

Photon Transducers

Several types of photon transducers are available, including: (1) photovoltaic cells, in which the radiant energy generates a current at the interface of a semiconductor layer and a metal; (2) phototubes, in which radiation causes emission of electrons from a photosensitive solid surface; (3) photomultiplier tubes, which contain a photoemissive surface as well as several additional surfaces the emit a cascade of electrons when struck by electrons from the photosensitive area; (4) photoconductivity transducers in which absorption of radiation by a semiconductor produces electrons and holes, thus, leading to enhanced conductivity; (5) silicon photodiodes, in which photons increase the conductance across a reverse-biased pn junction; and (6) charge-transfer transducers, in which the charges developed in a silicon crystal as a result of absorption of photons are collected and measured.

For a comparison of the performance characteristics of the three most sensitive and widely used photon transducers, namely photomultipliers, silicon diodes, and charge-transfer devices, see W.E.L. Grossman,J.Chem. Educ., 1989, 66, 697.

Photovoltaic or Barrier-Layer Cells

The photovoltaic cell is a simple device that is used for detecting and measuring radiation in the visible region. The typical cell has a maximum sensitivity at about 550nm; the response falls off to perhaps 10% of the maximum at 350 and 750nm. Its range approximates that of the human eye.

The photovoltaic cell consists of a flat copper or iron electrode upon which is deposited a layer of semi conducting material, such as selenium. The outer surface of the semiconductor is coated with a thin transparent metallic film of gold or silver, which serves as the second or collector electrode; the entire array is protected by a transparent envelope. When radiation of sufficient energy reaches the semiconductor, covalent bonds are broke, with the result that conduction electrons and holes are formed.

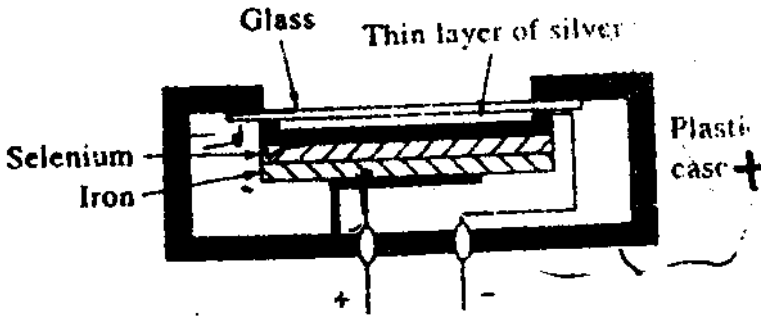


Figure: Schematic of a typical barrier-layer cell

The electrons then migrate toward the metallic film and the holes toward the base upon which the semiconductor is deposited. The liberated electrons are free to migrate through the external circuit to interact with these holes. The result is an electrical current of a magnitude that is proportional to the number of photons that strike the semiconductor surface. Ordinarily, the currents produced by a photovoltaic cell are large enough to be measured with a micro ammeter; if the resistance of the external circuit is kept small (<400Ω), the photocurrent is directly proportional to the power of the radiation that strikes the cell. Currents on the order of 10 to 100 μA are typical.

The barrier-layer cell constitutes a rugged, low cost means for measuring radiant power. No external source of electrical energy is required. On the other hand, the low internal resistance of the cell makes the amplification of its output less convenient. Consequently, although the barrier-layer cell provides a readily measured response at high levels of illumination, it suffers from lack of sensitivity at low levels. Another disadvantage of the barrier-type cell is that it exhibits fatigue in which its current output decreases gradually during continued illumination; proper circuit design and choice of experimental conditions minimize this effect. Barrier-type cell find use in simple, portable instruments where ruggedness and low cost are important. For routine analyses, these instruments often provide perfectly reliable analytical data.

Vacuum Phototubes

For a discussion of vacuum phototubes and photomultiplier tubes, see F.E.Lytle, Anal.chem, 1974, 46,545A.

A second type of photoelectric device is the vacuum phototube, which consists of semi cylindrical cathode and a wire anode sealed inside an evacuated transparent envelope.

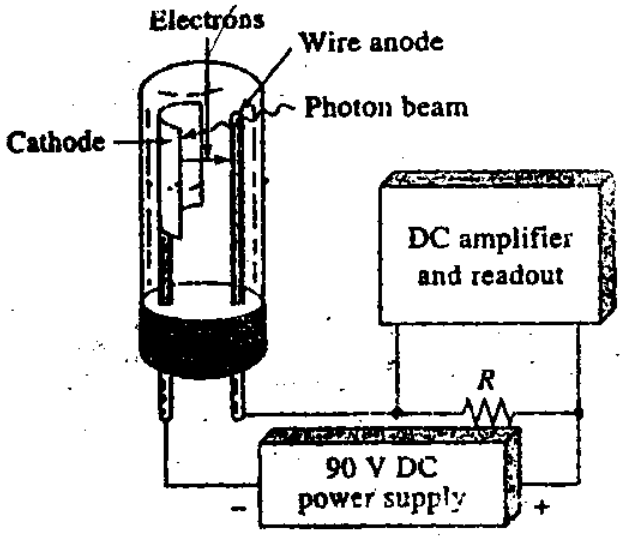


Figure: A phototube and accessory circuit. The photocurrent induced by the radiation causes a potential drop across R, with is then amplified to drive a meter or recorder.

The concave surface of the electrode supports a layer of photo emissive material that tends to emit electrons when it is irradiated. When a potential is applied across the electrodes, the emitted electrons flow to the wire anode generating a photocurrent that is generally about one tenth as great as that associated with a photovoltaic cell for a given radiant intensity. In contrast, however, amplification is easily accomplished since the phototube has a high electrical resistance.

The number of electrons ejected from a photo emissive surface is directly proportional to the radiant power of the beam that strikes that surface. As the potential applied across the two electrodes of the tube is increased, the fraction of the emitted electrons that reaches the anode rapidly increases; when the saturation potential is achieved, essentially all of the electrons are collected at the anode. The current then becomes independent of potential and directly proportional to the radiant power. Phototubes are usually operated at a potential of about90 V, which is well within the saturation region.

A variety of photomissive surfaces are used in commercial phototubes. Typical examples are shown in figure. From the user's standpoint, photoemissive surfaces fall into four categories; highly sensitive, red sensitive, ultraviolet sensitive, and flat response. The most sensitive cathodes are bialkali types such as number 117 in figure; they are made up of potassium, cesium, and antimony. Red-sensitive materials are multialkali types (for example, Na/K/Cs/Sb), or Ag/O/Cs formulations. The behavior of the latter is shown as S-11 in the figure. Compositions of Ga/In/As extend the red region up to about 1.1 μm. Most formulations are ultraviolet sensitive provided the tube is equipped with transparent windows. Flat responses are obtained with Ga/As compositions such as that labeled 128 in figure 7-28.

Phototubes frequently produce a small dark current that results from thermally induced electron emission and natural radioactivity from ^{40}K in the glass housing of the tube.

Photomultiplier Tubes

For the measurement of low radiant power, the photomultiplier tube (PMT) offers advantages over ordinary phototube. For a detailed discussion of the theory and applications of photo multipliers, see R.W.Engstrom, photomultiplier Handbook, Lancaster. PA: RCA Corporation, 1980. Figure is a schematic of such a device. The photo cathode surface is similar in composition to the surfaces of the phototubes described in figure and it emits electrons when exposed to radiation. The tube also contains additional electrodes called dynodes. Dynode 1 is maintained at a potential 90 V more positive than the cathode, and electrons are accelerated toward it as a consequence. Upon striking the dynode, each photoelectron causes emission of several additional electrons; these, in turn, are accelerated toward dynode 2, which is 90 V more positive than dynode 1. Again, several electrons are emitted for each electron that strikes the surface.

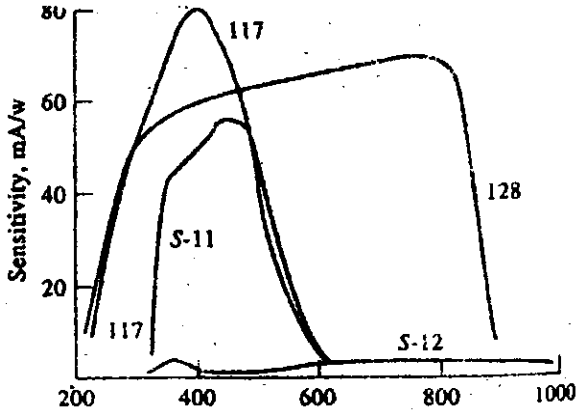
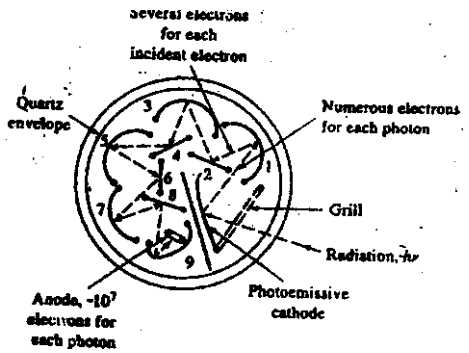
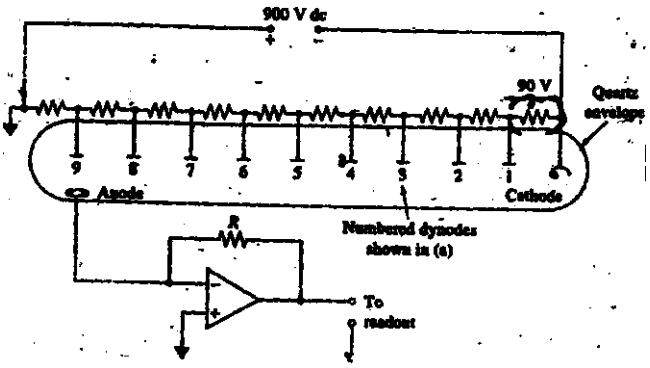


Figure: Spectral response of some typical photoemissive surfaces. (From F.E Lytle, Anal.chem., 1974,46, 546A.



(a)



(b)

Figure: photomultiplier tube: (a) cross-section of the tube and (b) electrical circuit.

By the time this process has been repeated nine times, 10^6 to 10^7 electrons have been formed for each incident photon; this cascade is finally collected at the anode and the resulting current is then electronically amplified and measured.

As shown by figure photomultipliers are highly sensitive to ultraviolet and visible radiation; in addition, they have extremely fast time responses. Often, the sensitivity of an instrument with a photomultiplier is limited by its dark-current emission. Because thermal emission is the major source of dark-current electrons, the performance of a photomultiplier can be enhanced by cooling. In face, thermal dark currents can be virtually eliminated by cooling the detector to -30^0 C. Transducer housings, which can be cooled by circulation of an appropriate coolant, are available commercially.

Photomultiplier tubes are limited to measuring low-power radiation because intense light causes irreversible damage to the photoelectric surface. For this reason, the device is always housed in a light-tight compartment, and care is taken to eliminate the possibility of its being exposed even momentarily to daylight or other strong light. With appropriate external circuitry, photomultiplier tubes can be used to detect the arrival of a single photon at the photocathode.

Silicon Diode Transducers

A silicon diode transducer consists of a reverse-biased pn junction formed on a silicon chip. As shown in figure, the reverse bias creates a depletion layer that reduces the conductance of the junction to nearly zero. If radiation is allowed to impinge on the chip, however, holes and electrons are formed in the depletion layer and swept through the device to produce a current that is proportional to radiant power.

Silicon diodes are more sensitive than vacuum phototubes but less sensitive than photomultiplier tubes. Photodiodes have spectral ranges from about 190 to 1100 nm.

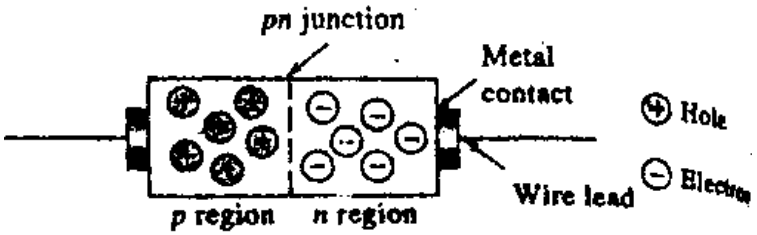
5. Describe the Multichannel photon Transducers & photoconductivity Transducers.

Multichannel Photon Transducers

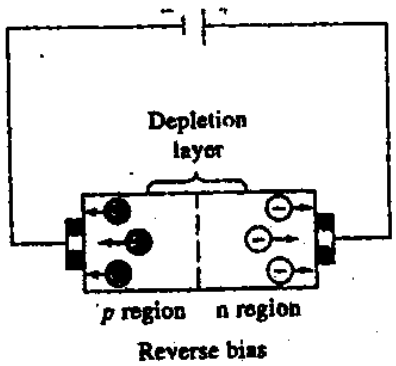
The first multichannel detector used in spectroscopy was photographic plate or a film strip that was placed along the length of the focal plane of a spectrometer so that all the lines in a spectrum could be recorded simultaneously. Photographic detection is relatively sensitive with some emulsions that respond to as few as 10 to 100 photons. The primary limitation of this type of detector is, however, the time required for developing the image of the spectrum and converting the blackening of the emulsion to radiant intensities.

Modern multichannel transducers consist of an array of small photoelectric-sensitive elements arranged either linearly or in a two-dimensional pattern on a single semiconductor chip. The chip, which is usually silicon and typically has dimensions of a few millimeters on a side, also contains electronic circuitry that makes it possible to determine the electrical output signal from each of the photosensitive elements either sequentially or simultaneously. For spectroscopic studies, a multichannel transducer is generally placed in the focal plane of a spectrometer so that various elements of the dispersed spectrum can be transduced and measured simultaneously.

At the present time three types of multichannel dev ices are used in commercial spectroscopic instruments;



(a)



(b)

Figure: (a) Schematic of a silicon diode. (b) Formation of depletion layer, which prevents flow of electricity under reverse bias.

Photodiode arrays (PDAs), charge-injection devices (CIDs), and charge-coupled devices (CCDs). Photodiode arrays are one-dimensional transducers in which the photosensitive elements are ranged in a line on the transducer face. In contrast, the individual photosensitive elements of charge-injection and charge-coupled devices are usually formed as two-dimensional arrays. Charge-injection and charge-coupled transducers both function by collecting photogene rated charges in various areas of the transducer surface and then measuring the quantity of charge accumulated in a brief period. In both devices, the measurement is accomplished by transferring the charge from a collection area to detection area. For this reason, the two types of transducers are sometimes called charge-transfer devices (CTDs). These devices have widespread use as image transducers for television applications and in astronomy.

Photodiode Arrays

In a photodiode array, the individual photosensitive elements are small silicon photodiodes each of which consists of reverse-biased pn junction. The individual photodiodes are part of a large-scale integrated circuit formed on a single silicon chip.

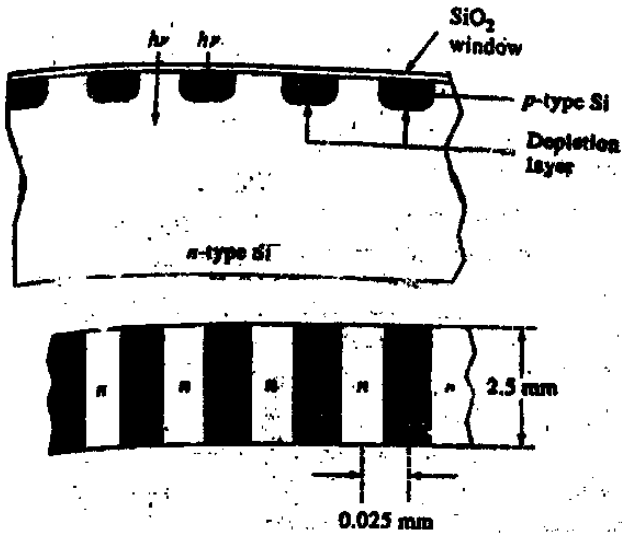


Figure: A reverse-biased linear diode-array detector: (a) cross section (b) top view

Figure shows the geometry of the surface region of a few of the transducer elements. Each element consists of a diffused p-type bar in an n-type silicon substrate to give a surface region that consists of a series of side-by-side elements that have typical dimensions of 2.5 by 0.025 mm. Light that is incident upon these elements creates charges in both the p and n regions. The positive charges are collected and stored in the p-type bars for subsequent integration (the charges formed in the n-regions divide themselves proportionally between the two adjacent p-regions. The number of transducer elements in a chip ranges from 64 to 4096 with 1024 being perhaps the most widely used.

The integrated circuit that makes up a diode array also contains a storage capacitor and switch for each diode as well as a circuit for sequentially scanning the individual diode-capacitor circuits. Figure is a simplified diagram that shows the arrangement of these components. Note that in parallel with each photodiode is a companion 10-pF storage capacitor. Each diode-capacitor pair is sequentially connected to a common out-put line by the N-bit shift register and the transistor switches. The shift register sequentially closes each of these switches momentarily causing the capacitor to be charged to-5 V, which then creates a reverse bias across the pn junction of the detector. Radiation that impinges upon the depletion layer in either the P or the n region forms charges (electrons and holes) that create a current that partially discharges the capacitor in the circuit. The capacitor charge that is lost in this way is replaced during the next cycle. The resultant charging current is integrated by the preamplifier circuit, which produces a voltage that is proportional to the radiant intensity. After amplification, the analog signal from the preamplifier passes into an analog-to-digital converter and to a microprocessor that controls the readout.

In using a diode-array transducer, the slit width of the spectrometer is usually adjusted so that the image of the entrance slit just fills the surface area of one of the diodes that make up the array. Thus the information obtained is equivalent to that recorded during scanning with a traditional spectrophotometer. With the array, however, information about the entire spectrum is accumulated essentially simultaneously and in discrete elements rather than in a continuous way.

Some of the photoconductor transducers mentioned in the previous section can also be fabricated into linear arrays for use in the infrared region.

Charge-Transfer Devices

Photodiode arrays cannot match the performance of photomultiplier tubes with respect to sensitivity, dynamic range, and signal-to-noise ratio. Thus, their use has been limited to experiments in which the multichannel advantage outweighs their shortcomings. In contrast, performance characteristics of charge-transfer devices appear to approach those of photomultiplier tubes in addition to having the multichannel advantage. As a consequence this type of transducer is now appearing in ever-increasing numbers in modern spectroscopic instruments. For details on charge-transfer devices, see J.V.Sweedler, Crit,Rev.Anal.Chem., 1993, 24, 59; J.V.Sweedler, R.B.Bilhorn, P.M.Epperson, G.R.Sims, and M.B.Denton, Anal.Chem., 1988,60,282A,327A; Charge-Transfer Devices in spectroscopy, J.V.Sweedler, K.L.Ratzlaff, and M.B.Dention, Eds, New York Wiley, 1994. A further advantage of charge-transfer devices is that they are two dimensional in the sense that individual transducer elements are arranged in rows and columns. For example, one detector that we describe in the next section consists of 244 rows of transducer elements, each row composed of 388 detector elements, resulting in a two-dimensional array of 19,672 individual transducers, or pixels, on a silicon chip with dimensions of

6.5 mm by 8.7 mm. With this device, it is possible to record an entire two-dimensional spectrum from an echelle spectrometer simultaneously.

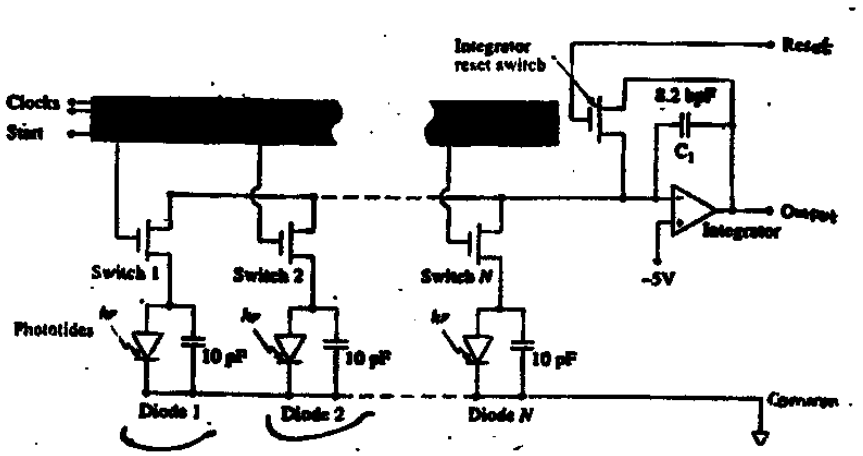


Figure: Block diagram of a photodiode-array detector chip

Charge-transfer devices operate much like a photographic film in that they integrate signal information as radiation strikes them. Figure is a cross-sectional depiction of one of the pixels that composes a charge transfer array. In this case, the pixel consists of two conductive electrodes that overlie an insulating layer of silica (note that a pixel in some charge-transfer devices is made up of more than two electrodes). This silica layer separates the electrodes from region of n-doped silicon. This assemblage constitutes a metal oxide semiconductor capacitor that stores the charges formed when radiation strikes the doped silicon. When, as shown, negative charge is applied to the electrodes, a charge inversion region is created under the electrodes, which is energetically favorable for the storage of holes. The mobile holes created by the absorption of photons then migrate and collect in this region. Typically, this region, which is called a potential well, is capable of holding as many as 10^5 to 10^6 charges before overflowing into an adjacent pixel. In the figure, one electrode is shown as more negative than the other making, the accumulation of charge under this electrode more favorable. The amount of charge generated during exposure to radiation is measured in either of two ways. In a charge-injection device, the voltage change that arises from movement of the charge from the region under one electrode to the region under the other is measured. In a charge-coupled device, the charge is moved to a charge sensing amplifier for measurement.

Charge-Injection Devices

Figure is a simplified diagram that shows the steps involved in the collection, storage, and measurement of the charge generated when one pixel of a semiconductor is exposed to photons. To monitor the intensity of the radiation that strikes the sensor element, the potentials applied to the capacitors are cycled as shown in steps (a) through (d) in the figure. In step (a) negative

potentials are applied to the two electrodes, which leads to formation of potential wells that collect and store holes formed in the n layer by absorption of photons. Because the electrode on the right is at a more negative potential, all the holes are retained under this electrode initially. The magnitude of the charge collected in some brief time interval is deter-mind in steps (b) and (c).

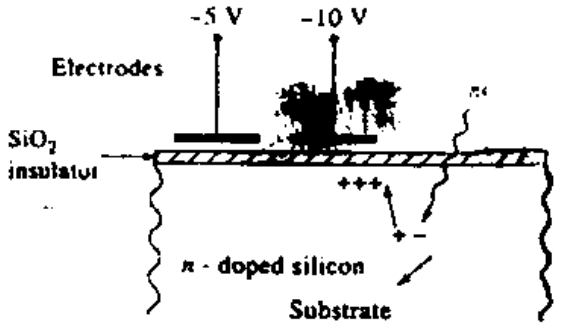


Figure: Cross section of a CTD detector in the charge integration mode. The positive hole produced by the photo hv is collected under the negative electrode
Figure: Duty cycle of a charge-injection device: (a) production and storage of charge,(b) first charge measurement, (c) second charge measurement after charge transfer, (d) reinjection of charge into the semiconductor

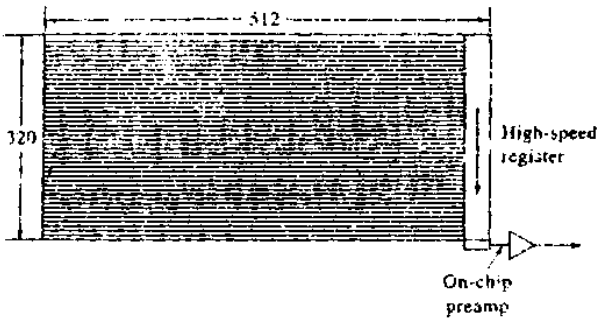
In (b), the potential of the capacitor on the left (V_1) is determined after removal of its applied potential. In step (c), the holes that have accumulated on the right electrode are transferred to the potential well under the left electrode by switching the potential applied to the former from negative to positive. The new potential of the electrode V_2 is then measured. The magnitude of the accumulated charge is determined from the difference in potential (V_1-V_2). In step (d), the detector is returned to its original state by applying positive potentials to both electrodes, which cause the holes to migrate toward the substrate. As an alternative to step (d), however, the detector can be returned to the condition shown in (a) without the loss of charge that has already accumulated. This process is called the nondestructive readout mode (NDRO). A major advantage of charge-injection devices over charge-coupled devices is that successive measurements can be made while integration is taking place.

As was true for the diode-array detector, the chip that contains the array of charge-injection transducer elements also contains appropriate integrated circuits for performing the cycling and measuring steps.

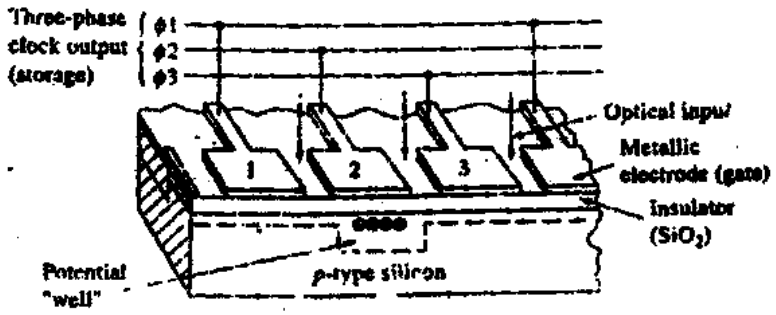
Charge-Coupled Device

Charge-coupled devices are marketed by several manufacturers and come in a variety of shapes and forms. Figure illustrates the arrangement of individual detectors in a typical array

that is made up of 512 × 320 pixels. Note that in this case the semiconductor is formed from p-type silicon, and the capacitor is biased positively so that electrons formed by the absorption of radiation collect in the well below the electrode, whereas holes move away from the n-type layer toward the substrate. Note also that each pixel is made up of three electrodes rather than two electrodes as in the charge-injection device. To measure the accumulated charge, a three-phase clock circuit is used to shift the charge in a stepwise manner to the right to the high speed shift register shown in figure. The charges are then transferred downward to a preamplifier and then to the readout.



(a)



(b)

Figure: A charge-coupled device array: (a) arrangement of 512 × 320 pixels and (b) Schematic showing four of the individual detectors

Thus, a row-by-row scan of the detector surface is accomplished. In contrast to the charge injection device, the readout in this case neutralizes the accumulated charge. The charge-coupled device offers the advantage of greater sensitivity to low light levels. A disadvantage in some cases, however, is the destructive nature of the readout process.

Photoconductivity Transducers

The most sensitive transducers for monitoring radiation in the near-infrared region (0.75 to 3 μm) are semiconductors whose resistances decrease when they absorb radiation within this range. The useful range of photoconductors can be extended into the far-infrared region by

cooling to suppress noise that arises from thermally induced transitions among closely lying energy levels. This application of photoconductors is important in infrared Fourier transform instrumentation. Crystalline semiconductors are formed from the sulfides, selenides, and stibnides of such metals as lead, cadmium, gallium, and indium. Absorption of radiation by these materials promotes some of their bound electrons into an energy state in which they are free to conduct electricity. The resulting change in conductivity can then be measured with a circuit such as that shown in figure.

Lead sulfide is the most widely used photoconductive material that offers the advantage that it can be used at room temperature. Lead sulfide transducers are sensitive in the region between 0.8 and 3μm (12.500 to 3300 cm^{-1}). A thin layer of this compound is deposited on glass or quartz plates to form the cell. The entire assembly is then sealed in an evacuated container to protect the semiconductor from reaction with the atmosphere. The sensitivity of cadmium sulfide, cadmium selenide, and lead sulfide transducers is shown by curves B, D, and G in figure.

6. Describe the types of optical instruments.

Types of optical instruments

In this section, we define the terminology we will use to describe various types of optical instruments. It is important to realize that the nomenclature proposed here is not agree upon and used by all scientists; it is simply a common nomenclature and the one that will be encountered throughout this book.

A spectroscope is an optical instrument used for the visual identification of atomic emission lines. It consists of a monochromator, such as one of those shown in figure, in which the exit slit is replaced by an eyepiece that can be moved along the focal plane. The wavelength of an emission line can then be determined from the angle between the incident and dispersed beam when the line is centered on the eyepiece.

We use the term colorimeter to designate to designate an instrument for absorption measurements in which the human eye serves as the detector using one or more color-comparison standards. A photometer consists of a source, a filter, and a photoelectric transducer as well as a signal processor and readout. It should be noted that some scientists and instrument manufacturers refer to photometers as colorimeters or photoelectric colorimeters. Filter photometers are commercially available for absorption measurements in the ultraviolet, visible, and infrared regions as well as emission and fluorescence in the first two wavelength regions. Photometers designed for fluorescence measurements are also called fluorometers.

A spectrograph is similar in construction to the two monochromators shown in figure except that the slit arrangement is replaced with a large aperture that holds a detector or transducer that is continuously exposed to the entire spectrum of dispersed radiation. Historically, the detector was a photographic film or plate. Currently, however, diode arrays or charge-transfer devices are often used as transducers in spectrographs.

A spectrometer is an instrument that provides in formation about the intensity of radiation as a function of wavelength or frequency. The dispersing modules in some spectrometers are multichannel so that two or more frequencies can be viewed simultaneously. Such instruments are sometimes called polychromators. A spectrophotometer is a spectrometer equipped with one or more exit slits and photoelectric transducers that permit the determination of the ratio of the power of two beams as a function of wavelength as in absorption spectroscopy. A spectrophotometer for fluorescence analysis is sometimes called a spectrofluorometer.

All of the instruments named in this section thus far employ filters or monochromators to isolate a portion of the spectrum for measurement. A multiplex instrument, in contrast, obtains spectral information with out first dispersing or filtering the radiation to provide wavelengths of interest. The term multiplex comes from communication theory, where it is used to describe systems in which many sets of information are transported simultaneously through a single chennel. Multiplex analytical instruments then are single-channel devoices in which all components of an analytical response are collected simultaneously. In order to determine the magnitude of each of these components, it is necessary to modulate the analyte signal in a way that permits subsequent decoding of the response into its components.

Most multiplex analytical instruments depend upon the Fourier transform (FT) for signal decoding and are consequently often called Fourier transform spectrometers. Such instruments are by no means confined to optical spectroscopy. Indeed, Fourier transform devices have been described for nuclear magnetic resonance spectrometry, mass, and microwave spectroscopy. Several of these instruments will be described in some detail in subsequent chapters. The section that follows describes the principles on which Fourier transform optical spectrometers are based.

7. Describe the principles of Fourier Transform optical measurements.

Principles of Fourier Transform optical measurements

Fourier transform spectroscopy was first developed by astronomers in the early 1950s in order to study the infrared spectra of distant stars; only by the Fourier technique could the very weak signals from these sources be isolated from environmental noise. The first chemical applications of Fourier transform spectroscopy, which were reported approximately a decade later, were to the energy-starved far-infrared region; by the late 1960s, instruments for chemical

studies in both the far-infrared (10 to 400 cm^{-1}) and the ordinary infrared regions were available commercially. Descriptions of Fourier transform instruments for the ultraviolet and visible spectral regions can also be found in the literature, but their adoption has been less widespread.

For more complete discussions of optical Fourier transform spectroscopy, consult the following references: A.G.Marshall and F.R.Verdun, Fourier Transforms in NMR, Optical and Mass Spectrometry. New York: Elsevier, 1990; A.G.Marshall, Fourier, Hadamard, and Hilbert Transforms in Chemistry. New York: Plenum Press, 1982; transform Techniques in chemistry, P.R.Griffiths, Ed. New York: Plenum Press, 1978. For brief reviews, see P.R.Griffiths, science. 1983,222, 297; W.D.Perkings, J.Chem. Educ., 1986, 63, A5, A296; L.Glasser, J.Chem. Educ., 1987, 64, A228, A260, A306.

Inherent Advantages of Transform Spectrometry

There are several major advantages to the use of Fourier transform instruments. The first is the throughput, or jaquinot, advantage, which is realized because Fourier transform instruments have few optical elements and no slits to attenuate radiation. As a consequence, the power of the radiation that reaches the detector is much greater than that in dispersive instruments, and much greater signal-to-noise ratios are observed.

A second advantage of Fourier transform instruments is their extremely high resolving power and wavelength reproducibility that make possible the analysis of complex spectra in which the sheer number of lines and spectral overlap make the determination of individual spectral features difficult.

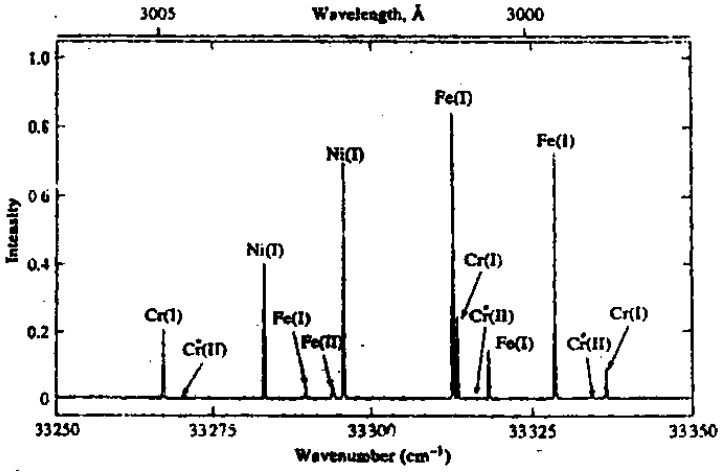


Figure: An iron emission spectrum illustrating the high resolving power of a Fourier transform emission spectrometer. (Reprinted with permission from A.P.Thorne, Anal. Chem, 199, 63,63A.

Which is part of an emission spectrum for steel, illustrates this advantage. The spectrum, which is figure extends from only 299.85 to 300.75 nm, contains 13 well separated lines of three elements. The wavelength resolution $(\Delta\lambda/\lambda)$ for the closest pair of lines is about 6 ppm.

A third advantage arises because all elements of the source reach the detector simultaneously. This characteristic makes it possible to obtain data for an entire spectrum in one second or less. Let us examine the consequences of this last advantage in further detail.

For purposes of this discussion, it is convenient to think of an experimentally derived spectrum as made up of m individual transmittance measurements at equally spaced frequency or wavelength intervals called resolution elements. The quality of the spectrum – that is, the amount of spectral detail – increases as the number of resolution elements becomes larger or as the frequency intervals between measurements become smaller. With a recording spectrophotometer, of course, individual point-by-point measurements are not made; nevertheless, the idea of a resolution element is useful, and the ideas generated from it apply to recording instruments as well. Thus, in order to increase spectral quality, m must be made larger; clearly, increasing the number of resolution elements must also increasing the number of resolution elements must also increase the time required for obtaining a spectrum with a scanning instrument.

Consider, for example, the derivation of an infrared spectrum from 500 to 5000cm^{-1}. If resolution elements of 3 cm^{-1} were chosen , m would be 1500; if 0.5s were required for recording the transmittance of each resolution element, 750 s or 12.5 min would be needed to obtain the spectrum. Reducing the width of the resolution element to 1.5 cm^{-1} would be expected to provide significantly greater spectral detail; it would also double the number or resolution elements as well as the time required for their measurement.

For most optical instruments, particularly those designed for the infrared region, decreasing the width of the resolution element has the unfortunate effect of decreasing the signal-to-noise ratio because narrower slits must be use, which lead to weaker source signals that reach the transducer. For infrared detectors, the reduction in signal strength is not, however, accompanied by a corresponding decrease in detector noise. Therefore, a degradation in signal-to-noise ratio results.

It was pointed out that marked improvements in signal-to-noise ratios accompany signal averaging. Here, it was shown that the signal-to-noise ratio S/N for the average of n measurements is given by

$$\frac{S}{N} = \sqrt{n}\,\frac{S_x}{\sqrt{\sum_{i=1}^{n}\left(S_x - S_i\right)^2}} = \frac{S_x}{N_x}\sqrt{n} \quad (7\text{-}20)$$

where S_x and N_x are the averaged signal and noise. The application of signal averaging to conventional spectroscopy is, unfortunately, costly in terms of time. Thus, in the example just considered, 750 s were required to obtain a spectrum of 1500 resolution elements. To improve the signal-to-noise ratio by a factor of 2 would require averaging 4 spectra, which would then require 4×750 s or 50 min.

Fourier transform spectroscopy differs from conventional spectroscopy in that all of the resolution elements for a spectrum are measured simultaneously, thus reducing enormously the time required to obtain a spectrum at any chosen signal-to-noise ratio. An entire spectrum of 1500 resolution elements can then be recorded in about the time required to observe just one element by conventional spectroscopy (0.5 s in our earlier example). This large decrease in observation time is often used to markedly enhance the signal –to – noise ratio of Fourier transform measurements. For example, in the 750 s required to derive the spectrum by scanning, 1500 Fourier transform spectra could be recorded and averaged. According to Equation 7-20, the improvement in signal-to-noise ratio would be $\sqrt{1500}$ or about 39. This inherent advantage of Fourier transform spectroscopy was first recognized by P. Fellgett in 1958 and is termed the Fellgett, or multiplex, advantage. It is worth noting here that for several reasons, the theoretical $\sqrt{n}$ improvement in S/N is seldom entirely realized. Nonetheless, major gains in signal-to-noise ratios are generally observed with the Fourier transform technique.

The multiplex advantage is important enough so that nearly all infrared spectrometers are of the Fourier transform type. Fourier transform instruments are much less common for the ultraviolet, visible, and near-infrared regions, however, because signal-to-noise limitations for spectral measurements with these types of radiation seldom lies in detector noise but instead in shot noise and flicker noise associated with the source. In contrast to detector noise, the magnitudes of both shot and flicker noise increase as the power of the signal increases. Furthermore, the total noise for all of the resolution elements in a Fourier transform measurement tends to be averaged and spread out uniformly over the entire transformed spectrum. Thus, the signal-to-noise ratio for strong peaks in the presence of weak peaks is improved by averaging but degraded for the weaker peaks. For flicker noise, such as is encountered in the background radiation from many spectral sources, degradation of S/N for all peaks is observed. This effect is sometimes termed the multiplex disadvantage and is largely responsible for the fact the Fourier transform has not been widely applied for ultraviolet/visible spectroscopy.

Time-Domain Spectroscopy

Conventional spectroscopy can be termed frequency domain spectroscopy in that radiant power data are recorded as a function of frequency or the inversely related wavelength. In contrast, time-domain spectroscopy, which can be achieved by the Fourier transform, is concerned with changes in radiant power with time figure illustrates the difference.

The plots in figures are conventional spectra of two monochromatic sources with frequencies v_1 and v_2 Hz. The curve in figure is the spectrum of a source that contains both frequencies. In each case, some measure of the radiant power, P(v) is plotted with respect to the frequency in hertz. They symbol in parentheses is added to emphasize the frequency dependence of the power; time-domain power will be indicated by P (t).

The curves in figure as show the time-domain spectra for each of the monochromatic sources. The two have been plotted together in order to make the small frequency difference between them more obvious. Here, the instantaneous power P(t) is plotted as a function of time. The curve in figure is the time-domain spectrum of the source that contains the two frequencies. As is shown by the horizontal arrow, the plot exhibits a periodicity or beat as the two waves go in and out of phase.

Examination of figure reveals that the time domain signal from a source that contains several wavelengths is considerably more complex than those shown in figure. Because a large number of wavelengths are involved, a full cycle is not completed in the time period shown. To be sure, a pattern of beats can be observed as certain wavelengths pass in and out of phase. In general, the signal power decreases with time as a consequence of the various closely spaced wavelengths becoming more and more out of phase.

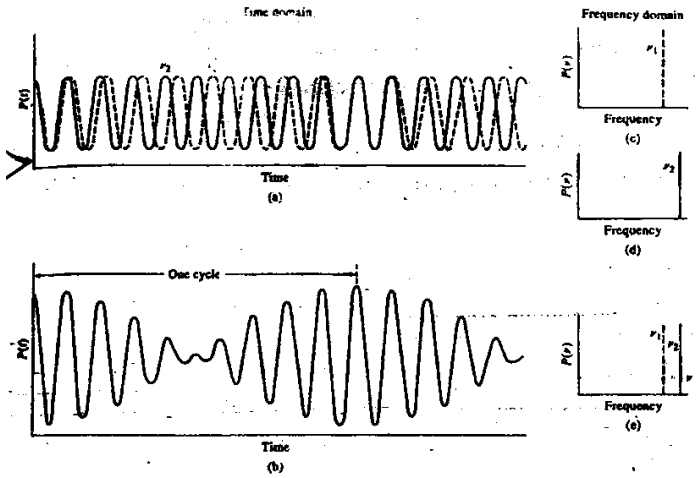


Figure: Illustrations of (1) time-domain plots (a) and (b) and (2) frequency-domain plots (c), (d), and (e).

It is important to appreciate that a time-domain signal contains the same information as a spectrum does in the frequency domain, and in fact, one can be converted to the other by numerical computations. Thus figure wad derived from figure by means of the equation

$$P(t) = k \cos(2\pi v_1 t) + k \cos(2\pi v_2 t)$$

where k is a constant and t is the time. The difference in frequency between the two lines was approximately 10% of v_2

The interference-conversion of time-and frequency-domain signals is exceedingly complex and mathematically tedious when more than a few lines are involved; the operation is only practical with a high-speed computer.

Obtaining Time-Domain Spectra with a Michelson Interferometer

Time-domain signals, such as those shown in figure cannot be acquired experimentally with radiation of the frequency range that is associated with optical spectroscopy (10^{12} to 10^{15} Hz) because there are no transducers that will respond to power variations at these enormous frequencies. Thus, a typical transducer yields a signal that will respond to power variations at these enormous frequencies. Thus, a typical transducer yields a signal that corresponds to the average power of high frequency signals and not to its periodic variation. To obtain time-domain signal requires, therefore, a methods of converting (or modulating) a high-frequency signal to one of measurable frequency without distorting the time relationships carried in the signal; that is, the frequencies in the modulated signal must be directly proportional to those in the original. Different signal – modulation procedures are employed for the various wavelength regions of the spectrum. The Michelson interferometer is used extensively to modulate radiation in the optical region.

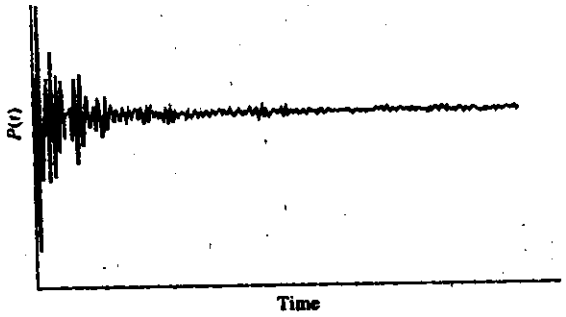


Figure: Time-domain signal of a source made up of several wavelengths.

The device used for modulating optical radiation is an interferometer similar in design to one first described by Michelson late in the nineteenth century. The Michelson interferometer is device that splits a beam of radiation into two beams of nearly equal power and then recombines them in such a way that intensity variations of the combined beam can be measured as a function of differences in the lengths of the paths of the two beams. Figure is a schematic of such an interferometer as it is used for optical Fourier transform spectroscopy.

UNIT – III

PART - A

1. Define principle of Spectrophotometry.

When beam of incident light of intensity, I_o passes through a solution, a part of it is reflected (I_r) a part absorbed (I_a) and rest transmitted (I_t) i.e.,

$$I_o = I_r + I_a + I_t$$

In colorimetric methods, I_r is eliminated because the measurement of I_o and I_t will be sufficient to determine I_a. For this purpose, the amount of light reflected (or I_r) is kept contant by using cell that have identical properties. I_o and I_t are then measured.

2. Define Lambert's law.

This law slates that the amount of light absorbed is directly proportional to the length (or) thickness of the solution under analysis. Thus,

$$A = \log_b \frac{I_o}{I_t} = a_s b$$

where A= absorbancy

as=absorbancy index characteristic for the solution

b= length or thickness of the medium.

3. Define Beer's Law.

This law states that the amount of light absorbed is directly proportional to the concentration of the solute in solution, thus,

$$\log_{10} \frac{I_o}{I_t} = a_s c$$

Where C= concentration of solute in solution.

4. How is molar absorption coefficient determined?

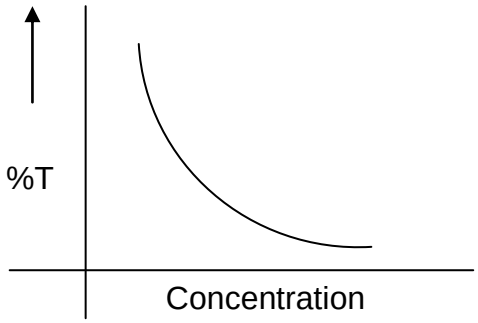
$$\text{Molar concentration} = \frac{\text{Grams per litre}}{\text{Molecular weight}}$$

$$\left.\begin{matrix}\text{Molar absorption} \\ \text{Co efficient } (a_m)\end{matrix}\right\} = \frac{\text{absorbance}}{\text{Molar concentation}} \times \text{moles per litre per concentration}$$

5. Define percentage transmission.

When light of an appropriate wavelength strikes a cuvette that contains a coloured sample, some of the light is absorbed by the solution. Ingeneral, objects that appear coloured absorb light at particular wavelength and reflect the other parts of the visible spectrum. After absorption the rest of the light is transmitted thought the sample to the detector. The proportion of the light that raches the detector is known as the percent transmission (%T) and represented by the equation.

$$\frac{I_t}{I_o} \times 100 = \%T$$



6. What are the Instrument components?

(i) Sources
(ii) Wavelength selectors
(iii) Sample containers
(iv) Radiation transducers and
(v) Signal processors and readout devices

7. Give the example of light sources.

1. Deuberium and hydrogen lamps
2. Tungsten Filament lamps
3. Xenon Arc Lamps

8. Give the types of Instruments.

We consider four general types of spectroscopic instruments;
(i) Single – beam
(ii) Double beam in space
(iii) Double beam in time
(iv) Multi channel

9. Define Dark current.

Dark current and amplifier noise are usually small compared with other sources of noise in photometric and spectrophotometric instruments and become important only under conditions of low photocurrents when the lamp intensity or the photodetector sensitivity is low. For example, such conditions are often encountered near the wavelength extremes for an instrument.

10. Define Probe-type photometers.

Probe type photometer an interesting. Commercially available, dipping – type photometer, which employes an optical fiber to transmit light from a source to a layer of solution lying between the glass seal at the end of the fiber and a mirror. The reflected radiation from the latter passes to a photodiode detector via a second glass fiber.

The photometer uses an amplifier with an electronic chopper that is synchronized with the light source, as a result, the photometer does not respond to extraneous radiaton.

11. Give the important characteristics of spectrophotometric and photometric method.

Important characteristics of spectrophotometric and photometric methods include;

(i) Wide applicability to both organic and inorganic systems.
(ii) Typical sensitivities of 10^{-4} to 10^{-5} M
(iii) Moderate to high selectivity
(iv) Good accuracy
(v) Case and convenience of data acquisition.

12. Define the terms 'micron', 'millimicron' and nanometer' .

One millionth of a meter is called micron (10^{-6}m). It is represented by the symbol μ.
One thousandth of a micron is known as millimicron (10^{-9}m) It is approximately equivalent to 10A^{o} units. It is represented as mμ. Nanometer is a preferred term for the designation of 10^{-9}m.

13. What is transmittance?

According to Beer- Lambert law, the amount light absorbed is proportional to the concentration of the absorbing substance and the thickness of the absorbing material

$$\log_{10} \frac{I_0}{I} = \varepsilon bc$$

Where I_o= is the intensity of the incident light

I=is the intensity of the transmitted light

b= is the absorbing thickness (path length)

c=is the concentration of the absorbing material.

ε=is the absorption coefficient

14. List out the differences between spectrophotometry and colorimetry.

Colorimetry	Spectrophotometry
1. Instruments are cheap	Instruments are costly
2. Requires very little maintenance	Requires closer monitoring and good maintenance
3. Tinted glass filter is used as monochromator	Prism or diffraction grating is used as monochromotor
4. Round glass cells are used	Quartz or fused silica rectangular cells are used
5. Photo voltaic cell is used as detector	Photomultiplier is used as detector.

15. Why are Spectrophotometers superior to Colorimeters?

Spectrophotometers are superior to colorimeters due to the following reasons:

(i) Spectrophotometers use a prison or a diffraction grating which allow them to have a bandpass of 0.5nm (or) less. Colorimeters use glass filters and they possess a hand pass of 10 to 20nm.

(ii) Spectrophotometers maintain greater accuracy than Colorimeters.

(iii) Small Sample Volume is required for spectrophotometers whereas comparatively large volume of samples is required for Colorimetric analysis.

16. Discuss fluorescence.

Fluorescence is an emission phenomenon. It occurs due to the energy transition from a higher to lower state with in the molecule. Fluorescence is measured by the detection of this emitted radiation. The reason for the transition from higher to lower states is an earlier excitation event. This earlier event is due to input of energy by absorption of electromagnetic radiation.

The wavelength of absorbed radiation must be at lower values (higher energy) than the emitted (fluoresced) wavelength. The difference between these two wave lengths is known as the stoke shift.

17. Define Phosphorescence.

While the molecule is in the excited state, it is possible for one electron to reverse its spin. The molecule is transferred to a lower – energy triplet state by a process called intersystem crossing. Through the processes of internal conversion and vibrational relaxation, the molecule rapidly attains the lowers vibrational level of the first excited triplet stage. From here, the molecule can return to the ground state by emission of photon. This emission is referred to as phosphorescence.

It is much longer – lived than fluorescence. Phosphorescence measurements are made by cooling samples to liquid nitrogen temperature (-196°C) to minimize collision with other molecules.

18. Write short notes on flours.

Flours are organic compounds mainly used in Spectroflourimetric analysis of non-fluorescent compounds. They are also called fluorescent probes. A flour should have the following properties.

a. It should tightly bind with the compound to be analyzed at a specific site
b. The binding should not destroy the compound to be analyzed.
c. The emitted florescence should be stable and sensitive to environmental changes.

19. Compare Spectroflourimetric technique with spectrophotometer technique.

	Spectroflourimetry	**Spectrophotonetry**
1.	Very low concentrations of samples can be accurately determined e.g → 100pg of serotonin is sufficient for determination	At very low concentration spectrophotometry is not accurate. It needs a minimum of 100 μg of serotonin for determination.
2.	Two monochromators are used. One selects the activating wavelength and the other selects the fluorescent wavelength	Only one monochromator is used.
3	Quenching due to impurities is a problem.	Quenching does not occur.

20. Give the application of Spectrofluorimetry.

1) Spectrofluorimetry is used for the quantitative analysis of riboflavin, thiamine, cortical, estrogen, serotonin, dopamine, organophosphorus pesticides, tobacco smoke, Carcinogens, LSD, barbiturates, porphyrins, cholesterol and even some metal ions.
2) Fluorescent probes are used to study the membrane structure. Studies with ANS (1-anilinonap – thalene – 8 sulphonate) probe have shown that structural changes occur in mitochondrial membrane during oxidation phosphorylation.

21. Define Near – Infrared Region.

The near – infrared (NIR) region, which meets the visible region at about 12,500Cm^{-1} (0.80μm) and extends to about 4000Cm^{-1} (2.50μm), there are many absorption bands that result from harmonic overtones of fundamental and combination bands often associated with hydrogen atoms. Among these are the first overtones of the O – N and N – H stretching vibrations near 7140cm^{-1} (1.40 μm) and 6667cm^{-1} (1.50μm), respectively, and combination bands that result from C-H stretching and deformation vibrations of alkyl groups at 4548 cm^{-1} (2.20μm) and 3850 cm^{-1} (2.60μm). The absorptivity of NIR bands is from to 1000 times less than that of mid – infrared bands. Thicker sample layers (0.5 – 10mm) compensate for these smaller molar absorptivities.

22. Give the types of Detectors using in Infrared Spectrometry.

The detectors used at longer wavelengths can be classified into two groups:

(i) Thermal detectors, in which the infrared radiation produces a heating effect that alters some physical property of the detector, and

(ii) Photon detectors, which use the quantum effects of the infrared radiation to change the electrical properties of a semiconductor.

23. Define Photon Detectors.

The more sensitive detectors rely on a quantum interaction between the incident photons and a semiconductor. The result produces electrons and holes. This is the internal photoelectric effect. A sufficiently energetic photo that strikes an electron in the detector can raise that electron from a non conducting state to a conducting state.

In an photoconductive detector, consisting of a homogeneous semiconductor chip, the presence of electrons in the conduction band lower the chip's resistance.

Photovoltaic detector generates a small voltage when exposed to radiation.

24. Define Raman Effect.

When Monochromatic radiation is scattered by molecules, a small fraction of the scattered radiation is observed to have a different frequency from that of the incident radiation; this is known as the Raman Effect.

The Raman Effect arises when a beam of intense monochromatic radiation passes through a sample that contains molecules that undergo a change is molecular polarizability as they vibrate.

25. Give any one comparison of Raman with Infrared Spectroscopy.

Raman Spectroscopy offers distinct advantages over the more direct infrared absorption measurement. Raman spectroscopy can be used to detect and analyze molecules with infrared inactive spectra, such as homonuclear diatomic molecules. For complicated molecules whose low symmetry does not forbid both Raman and infrared activity, certain vibrational modes are inherently stronger in the Raman Effect and weaker in, or apparently absent from, the infrared spectrum.

PART – B

1. Describe the basic principle of Beer's Law and Limitations to Beer's Law.

Molecular absorption spectroscopy is based on the measurement of the transmittance T or the absorbance A of solutions contained in transparent cells having a path length of b cm. Ordinarily, the concentration c of an absorbing analye is linearly related to absorbance as represented by the equation

$$A = -\log T = \log \frac{P_0}{P} = \in bc \quad .....(1)$$

All of the variables in this equation are defined in Table. This equation is a mathematical representation of Beer's law.

MEASUREMENT OF TRANSMITTANCE AND ABSORBANCE

Ordinarily, transmittance and absorbance, as defined Table, cannot be measured in the laboratory because the analyte solution must be held in some sort of a transparent container, or cell. As shown in figure, reflection occurs at the two air/wall interfaces well as at the two wall/solution interfaces. The resulting beam attenuation is substantial as we demonstrated where it was shown that about 8.5% of a beam of yellow light is lost by reflection passing through a glass cell containing water. In addition, attenuation of a beam may occur as a result of scattering by large molecules and sometimes from absorption by

Term and Symbol*	Definition	Alternative Name and Symbol
Radiant power P, P_0	Energy of radiation (in ergs) impinging on a 1-cm^2 area of a detector per second	Radiation intensity I, I_0

Absorbance A	$\log\frac{P_0}{P}$	Optical density D; extinction E
Transmittance T	$\frac{P}{P_0}$	Transmission T
Path length of radiation† b	-	*l, d*
Absorptivity † *a*	$\frac{A}{bc}$	Extinction coefficient k
Molar absorptivity ‡∈	$\frac{A}{bc}$	Molar extinction coefficient

**Terminology recommended by the American Chemical Society (Anal. 1990, 62, 91).*
†c may be expressed in g/L or in other specified concentration units; b may be expressed cm or in other units of length.
‡c is expressed in mol/L; b is expressed in cm.

the container walls. To compensate for these effects, the power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an identical cell containing only solvent. An experimental transmittance and absorbance that closely approximate the true transmittance and absorbance are then obtained with the equations

$$T = \frac{P_{solution}}{P_{solvent}} = \frac{P}{P_0} \quad .....(2)$$

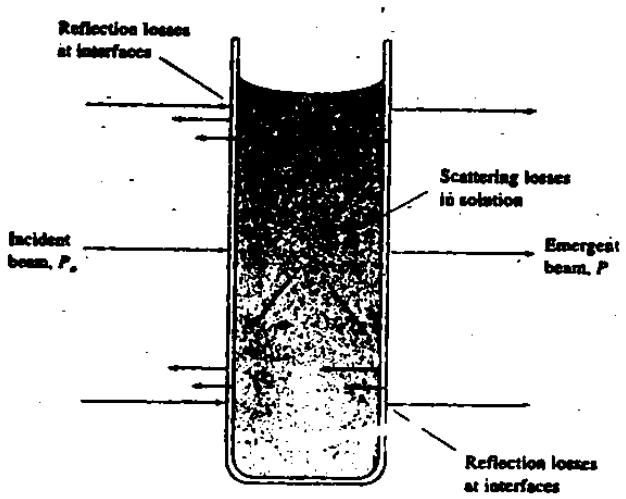


Fig. Reflection and Scattering losses

$$A = \log\frac{P_{solvent}}{P_{solution}} = \log\frac{P_0}{P} \qquad ...(3)$$

The terms P_0 and P, as used henceforth in this book, refer to the power of radiation after it has passed through cells containing the solvent and the analyte, respectively.

BEER'S LAW

Equation 1 represents Beer's Law. This relationship can be rationalized as follows – The discussion that follows is based on a paper by F, C. Strong, Anal. Chem., 1952, 24, 338.

Consider the block of absorbing matter (solid, liquid, or gas) shown in Figure. A beam of parallel monochromatic radiation with power P_0 strikes the block perpendicular to a surface; after passing through a length *b* of the material, which contains *n* absorbing atoms, ions, or molecules, its power is decreased to P as a result of absorption. Consider now a cross section of the block having an area S and an infinitesimal thickness *dx*. Within this section there are *dn* absorbing particles; associated with each particle, we can imagine a surface at which photon capture will occur. That is, if a photon reaches one of these areas by chance, absorption will follow immediately. The total projected area of these capture surfaces within the section is designated as dS; the ratio of the capture area to the total area, then, is dS/S. On a statistical average, this ratio represents the probability for the capture of photons within the section.

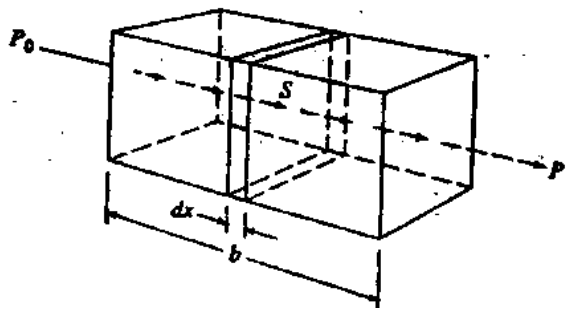


Fig. Attenuation of radiation with initial power P_0 by a solution containing c moles per liter of absorbing solute and with a path length of b cm. P < P_0

The power of the beam entering the section, P_x, is proportional to the number of photons per square centimeter and dP_x represents the quantity absorbed within the section; the fraction absorbed is then $-dP_x/P_x$, and this ratio also equals the average probability for capture. The term is given a minus sign to indicate that P undergoes a decrease. Thus,

$$-\frac{dP_x}{P_x} = \frac{dS}{S} \qquad \text{... (4)}$$

Recall, now, that dS is the sum of the capture areas for particles within the section; it must therefore be proportional to the number of particles, or

$$dS = adn \qquad \text{…. (5)}$$

where *dn* is the number of particles and *a* is a proportionality constant, which can be called the capture cross section. By combining Equations 4 and 5 and integrating over the interval between 0 and *n*, we obtain

$$-\int_{P_0}^{P} \frac{dP_x}{P_x} = \int_{o}^{n} \frac{adn}{S}$$

which, on evaluation of the integrals, gives

$$-ln\frac{P}{P_0} = \frac{an}{S}$$

On converting to base 10 logarithms and inverting the fraction to change the sign, we obtain

$$\log\frac{P_0}{P} = \frac{an}{2.303\ S} \qquad \text{....(6)}$$

Where *n* is the total number of particles within the block shown in figure. The cross-sectional area S can be expressed in terms of the volume of the block V in cm^3 and its length *b* in cm. Thus,

$$S = \frac{V}{b} cm^2$$

Substitution of this quantity into Equation (6) yields

$$\log\frac{P_0}{P} = \frac{anb}{2.303\ V} \qquad \text{..... (7)}$$

Note that since n/V has the units of concentration, that is, the number of particles per cubic centimeter, we can then readily convert n/V to moles per liter. Thus, the number of moles is given by

$$\text{number mol} = \frac{\text{n } \cancel{\text{particles}}}{6.02 \times 10^{23}\ \cancel{\text{particles}}/\text{mol}}$$

and *c* in mol/L is given by

$$c = \frac{n}{6.02\times10^{23}} mol \times \frac{1000\ \cancel{cm^3}\ /L}{V\ \cancel{cm^3}}$$

$$= \frac{1000\ n}{6.02\ \times10^{23}V} mol/L$$

Combining this relationship with Equation (7) yields

$$\log\frac{P_0}{P} = \frac{6.02\times10^{23}\ abc}{2.303\times1000}$$

Finally, the constants in this equation can be collected into a single term $\in$ to give

$$\log\frac{P_0}{P} = \in bc = A \qquad .....(8)$$

Which is a formulation of Beer's law

Application of Beer's Law to Mixtures

Beer's law also applies to a medium containing more than one kind of absorbing substance. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by

$$A_{total} = A_1 + A_2 + \ldots\ldots + A_n$$
$$= \in_1 bc_1 + \in_2 bc_2 + \ldots\ldots.. + \in_n bc_n \quad \ldots\ldots\ldots(9)$$

Where the subscripts refer to absorbing components 1, 2, ……n.

Limitations to Beer's Law

Few exceptions are found to the generalization that absorbance is linearly related to path length. On the other hand, deviations from the direct proportionality between the measured absorbance and concentration when b is constant are frequently encountered. Some of these deviations are fundamental and represent real limitations of the law. Others occur as a consequence of the manner in which the absorbance measurements are made or as a result of chemical changes associated with concentration changes; the latter two are sometimes known, respectively, as instrumental deviations and chemical deviations.

Real Limitations to Beer's Law

Beer's law is successful in describing the absorption behavior of media containing relatively low analyte concentrations; in this sense, it is a limiting law. At high concentrations (usually > 0.01M), the average distance between the molecules responsible for absorption is diminished to the point where each molecule affects the charge distribution of its neighbours. This interaction, in turn, can alter the ability of the molecules to absorb a given wavelength of radiation. Because the extent of interaction depends upon concentration, the occurrence of this phenomenon causes deviations from the linear relationship between absorbance and concentration. A similar effect is sometimes encountered in media containing low absorber concentrations but high concentrations of other species, particularly electrolytes. The close proximity of ions to the absorber alters the molar absorptivity of the latter by electrostatic interactions; the effect is lessened by dilution.

While the effect of molecular interactions is ordinarily not significant at concentrations below 0.01 MHz, some exceptions occur among certain large organic ions or molecules. For example, the molar absorptivity at 436 nm for the cation of methylene blue in aqueous solutions is reported to increase by 88% as the dye concentration is increased from 10^{-5} to 10^{-2} MHz; even below 10^{-6} MHz, strict adherence to Beer's law is not observed.

Deviations from Beer's law also arise because $\in$ is dependent upon the refractive index of the medium – G. Kortum and M. Seiler, Angew, Chem: 1939, 52, 687. Thus, if concentration changes cause significant alterations in the refractive index n of a solution, departures from Beer's law are observed. A correction for this effect can be made by substitution of the quantity $\in n(n^2 + 2)^2$ for $\in$ in Equation (8). In general, this correction is never very large and is rarely significant at concentrations less than 0.01M.

Apparent Chemical Deviation

Apparent deviations from Beer's Law arise when an analyte dissociates, associates, or reacts with a solvent to produce a product having a different absorption spectrum from the analyte. A common example of this behavior is found with aqueous solutions of acid/base indicators. For example, the color change associated with a typical indicator HIn arises from shifts in the equilibrium

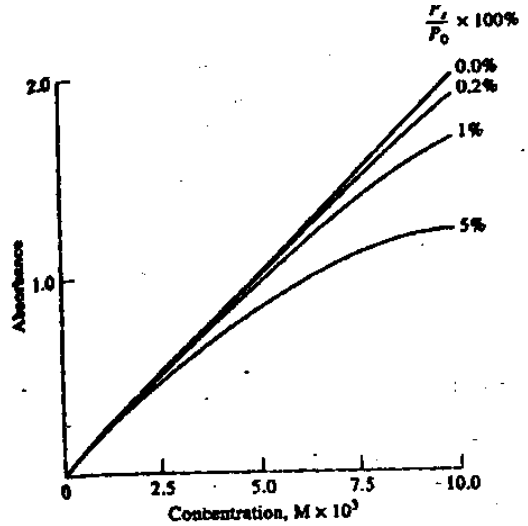
$$\underset{color\ 1}{HIn} \rightleftharpoons H^+ + \underset{color\ 2}{In^-}$$

Example demonstrates how the shift in this equilibrium with dilution results in deviation from Beer's Law.

2. Describe the Effects of Instrumental Noise on Spectrophotometric Analyses.

THE EFFECTS OF INSTRUMENTAL NOISE ON SPECTROPHOTOMETRIC ANALYSES

The accuracy and precision of Spectrophotometric analyses are often limited by the uncertainties or noise associated with the instrument. A general discussion of instrumental noise and signal-to-noise optimization is found; it may be helpful to review this material before undertaking a detailed study of this section.



Apparent deviation from Beer's law brought about by various Amounts of stray radiation

Instrumental Noise as a Function of Transmittance

As was pointed out earlier, a spectrophotometric measurement entails three steps: a 0% T adjustment, a 100% T adjustment, and a measure of % T with the sample in the radiation path. The noise associated with each of these steps combines to give a net uncertainity for the final value obtained for T. The relationship between the noise encountered in the measurement of T and the uncertainty in concentration can be derived by writing Beer's law in the form

$$c = -\frac{1}{\in b}\log T = -\frac{0.434}{\in b} ln\ T \qquad \text{..... (1)}$$

In order to relate the standard deviation in concentration σ_c to the standard deviation in transmittance σ_T we proceed, Appendix 1 by taking the partial derivative of this equation with respect to T, holding *b* and *c* constant. That is,

$$\frac{\partial c}{\partial T} = -\frac{0.434}{\in bT}$$

Application of Equation (Appendix 1) gives

$$\sigma_c^2 = \left(\frac{\partial c}{\partial T}\right)^2 \sigma_T^2 = \left(\frac{-0.434}{\in bT}\right)^2 \sigma_T^2 \quad .....(2)$$

Dividing Equation (2) by the square of Equation (1) gives

$$\left(\frac{\sigma_c}{c}\right)^2 = \left(\frac{\sigma_T}{T \ln T}\right)^2$$

$$\frac{\sigma_c}{c} = \frac{\sigma_T}{T \ln T} = \frac{0.434\ \sigma_T}{T \log T} .....(3)$$

For a limited number of measurements, we replace the population standard deviations σ_c and σ_T with the sample standard deviations s_c and straight and obtain

$$\frac{s_c}{c} = \frac{0.434\ s_T}{T \log T} \quad .....(4)$$

This equation relates the relative standard deviation of c (s_c/c) to the absolute standard deviation of the transmittance measurement (straight). Experimentally, s_T can be evaluated by making, say, 20 replicate transmittance measurements (N = 20) of the transmittance of a solution in exactly the same way and substituting the data into Equation.

From an examination of Equation (4) we see that the uncertainty in a photometric concentration measurement varies nonlinearly with the magnitude of the transmittance. The situation is somewhat more complicated than is suggested by Equation (4), however, because the uncertainty s_T is, under many circumstances, also dependent upon T.

Sources of Instrumental Noise

In a detailed theoretical and experimental study, Rothman, Crouch, and Ingle have described several sources of instrumental uncertainties and shown their net effect on the precision of absorbance or transmittance measurements. – L.D. Rothman, S.R. Crouch, and J.D. Ingle Jr., Anal. Chem., 1975, 47, 1226. These uncertainties fall into three categories depending upon how they are affected by the magnitude of the photocurrent and thus T. For Case I uncertainties, the precision is independent of T; that is, straight is equal to a constant k_1. For case II uncertainties, the precision is directly proportional to $\sqrt{T^2 + T}$. Finally, Case III uncertainties are directly proportional to T. Table summarizes information about the sources of these three types of uncertainty and the kinds of instruments where each is likely to be encountered.

Table: Types and Sources of Uncertainties in Transmittance Measurements

Category	Characterized by[a]	Typical Sources	Likely to be important in
Case I	$s_T = k_1$	Limited readout resolution	Inexpensive photometers and spectrophotometers having small transmittance meter scales
		Heat detector Johnson noise	IR and near-IR spectrophotometers and photometers
		Dark current and amplifier noise	Regions where source intensity and detector sensitivity are low
Case II	$s_T = k_2\sqrt{T^2 + T}$	Photon detector shot noise	High-quality UV/visible spectrophotometers
Case III	$s_T = k_3 T$	Cell positioning uncertainties	High-quality UV/visible and IR spectrophotometers
		Source flicker	Inexpensive photometers and spectrophotometers

ak_1, k_2 and k_3 are constants for a given system.

Case I: $s_T = k_1$

Case I uncertainties are often encountered with less expensive ultraviolet and visible spectrophotometers or photometers that are equipped with meters or digital readouts with limited resolution. For example, a typical instrument may b equipped with a meter having a 5 – to 7-in. scale which is readable to 0.2% to 0.5% of full scale. Here, the absolute uncertainty in T is the same from one end of the scale to the other. A similar limitation in readout resolution is found in some digital instruments.

Infrared and near-infrared spectrophotometers also exhibit Case I behavior. With these, the limiting random error usually arises from Johnson noise in the thermal detector. Recall that this type of noise is independent of the magnitude of the photocurrent; indeed, fluctuations are observed even in the absence of radiation and, therefore, net current.

Dark current and amplifier noise are usually small compared with other sources of noise in photometric and spectrophotometric instruments and become important only under conditions of low photocurrents where the lamp intensity or the photodetector sensitivity is low. For example, such conditions are often encountered near the wavelength extremes for an instrument.

The precision of concentration data obtained with an instrument that is limited by Case I noise can be obtained directly by substituting an experimentally determined value for $s_T = k_1$ into Equation (4). Here, the precision of a particular concentration determination depends upon the magnitude of T even though the instrumental precision is independent of T. The third column of Table shows data obtained with Equation 4 when an absolute standard deviation s_T of ± 0.003 or ± 0.3% T was assumed. A plot of the data is shown by curve A in figure.

An indeterminate uncertainty of 0.3% T is typical of many moderately priced spectrophotometers or photometers. Clearly, concentration errors of 1% to 2% relative are to be expected with such instruments. It is also evident that precision at this level can only be realized if the absorbance of the sample lies between about 0.1 and 1.

Table : Relative precision of Concentration Measurements as a Function of Transmittance and Absorbance for Three Categories of Instrument Noise

		Relative Standard Deviation in Concentration [a]		
Transmittance, T	**Absorbance, A**	**Case I Noise[b]**	**Case II Noise[c]**	**Case III Noise[d]**
0.95	0.022	± 6.2	± 8.4	± 25.3
0.90	0.046	± 3.2	± 4.1	± 12.3
0.80	0.097	± 1.7	± 2.0	± 5.8
0.60	0.222	± 0.98	± 0.96	± 2.5
0.40	0.398	± 0.82	± 0.61	± 1.4
0.20	0.699	± 0.93	± 0.46	± 0.81
0.10	1.00	± 1.3	± 0.43	± 0.56
0.032	1.50	± 2.7	± 0.50	± 0.38
0.010	2.00	± 6.5	± 0.65	± 0.28
0.0032	2.50	±16.3	± 0.92	± 0.23
0.0010	3.00	± 43.4	± 1.4	± 0.19

[a] $(s_c/c) \times 100\%$.
[b] From Equation (4) with $s_T = k_1 = \pm 0.0030$
[c] From Equation (5) with $k_2 = \pm 0.0030$
[d] From Equation (6) with $k_3 = \pm 0.013$

Case II: $s_T = k_2 \sqrt{T^2 + T}$

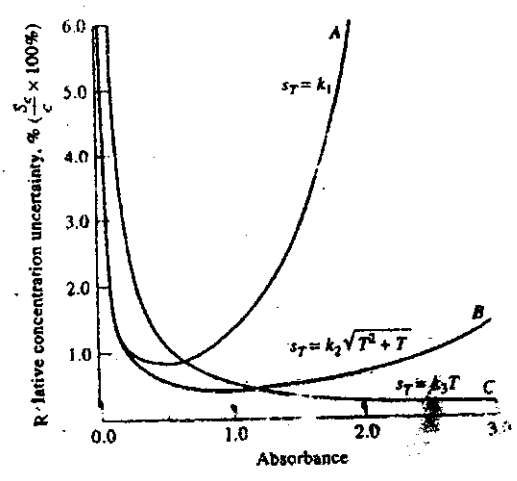


Fig. Relative concentration uncertainties arising from various categories of instrumental noise: A, Case I; B, Case II; C, Case III. The data are taken from Table.

This type of uncertainty often limits the precision of the highest quality instruments. It has its origin in shot noise, which must be expected whenever the passage of electricity involves transfer of charge across a junction, such as the movement of electrons from the cathode to the anode of a photomultiplier tube. Here, an electric current results from a series of discrete events (emission of electrons from a cathode), the number of which per unit time is distributed in a random way about a mean value. The magnitude of the current fluctuations is proportional to the square root of current. The effect of shot noise on s_c is derived by substitution of s_T into Equation. Rearrangement leads to

$$\frac{s_c}{c} = \frac{0.434\ k_2}{\log T}\sqrt{\frac{1}{T} + 1} \qquad .....(5)$$

The data in column 4 of Table were obtained with the aid of Equation (5). Figure, curve B is a plot of such data. Note the much larger range of absorbances that can be encompassed without serious loss of accuracy when shot noise, rather than Johnson noise, limits the precision. This increased range represents a major advantage of photon-type detectors over thermal types, which are represented by curve A in the figure. As with Johnson-noise-limited instruments, shot-limited instruments do not give very reliable concentration data at transmittances greater than 95% (or A < 0.02) .

Case III: $s_T = k_3 T$

One source of noise of this type is the slow drift in the radiant output of the source; this type of noise can be called source flicker noise. The effects of fluctuations in the intensity of a source can be minimized by the use of a constant voltage power supply or a split beam arrangement. With many instruments, source flicker noise does not limit performance.

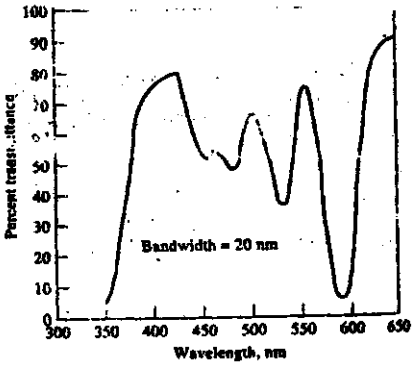


Fig. Effect of bandwidth on spectral detail. The sample was a didymium glass.

An important and widely encountered noise source, which proportional to transmittance, results from failure to position sample and reference cells reproducibly with respect to the beam during replicate transmittance measurements. All cells have minor imperfections. As a consequence, reflection and scattering losses vary as different sections of the cell window are exposed to the beam; small variations in transmittance result. Rothman, Crouch, and Ingle have shown that this uncertainty often is the most common limitation to the accuracy of high-quality ultraviolet/visible spectrophotometers. It is also a serious source of uncertainty in infrared instruments.

One method of reducing the effect of cell positioning with a double-beam instrument is to leave the cells in place during calibration and analysis; new standards and samples are introduced after washing and rinsing the cell in place with a syringe. Care must be taken to avoid touching or jarring the cells during this process.

The effect of uncertainties that are proportional to transmittance on analytical results can be obtained by substitution of $s_T = k_3T$ into Equation 4, which gives

$$\frac{s_c}{c} = -\frac{0.434\ k_3}{\log T} \quad .....(6)$$

Column 5 of Table contains data obtained from Equation (6) when k_3 is assumed to have a value of 0.013, which approximates the value observed in the Rothman, Crouch, and Ingle study. The data are plotted as curve C in Figure – To see how curve-fitting may be used to extract k_1, k_2 and k_3 from experimental absorption data, see F.J. Holler, Mathcad Applications for Analytical Chemistry, pp. 147-149. Philadelphia: Saunders College Publishing, 1994.

Effect of Slit Width on Absorbance Measurements

As shown in section, narrow slit widths are required to resolve complex spectra. – For a discussion of the effects of slit width on spectra, see Optimum Parameters for Spectrophotometry. Palo Alto, CA: Varian Instruments Division, 1977; F.C. Strong III, Anal. Chem., 1976, 48, 2155; D.D. Gilbert, J. Chem. Educ., 1991, 68, A278. For example, figure illustrates the loss of detail that accompanies the use of wider slits. In this example, the transmittance spectrum of a didymium glass was obtained at slit settings that provided effective bandwidths of 0.5, 9 and 20 nm. The progressive loss of spectral detail is clear. For qualitative studies, such losses often loom important.

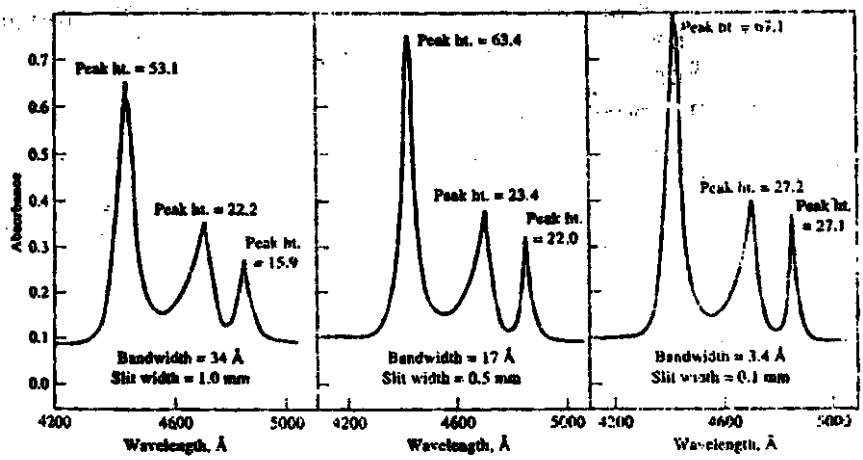


Fig. Effect of slit width and bandwidth on peak heights. Here, the sample was a solution of praseodymium chloride. (From Optimum Spectrophotometer Parameters, Application Report AR 14-2. Cary Instruments: Monrovia, CA. With permission)

Figure illustrates a second effect of slit width on spectra made up of narrow peaks. Here, the spectrum of a praseodymium chloride solution was obtained at slit settings of 1.0, 0.5, and 0.1 mm. Note that the peak absorbance values increase significantly (by as much as 70% in one instance) as the slit width decreases. At slit settings less than about 0.14 mm, absorbances were found to become independent of slit width. Careful inspection of figure reveals the same type of effect. In both sets of spectra, the areas under the individual peaks are the same, but wide slit widths result in broader lower peaks.

It is evident from both of these illustrations that quantitative measurement of narrow absorption bands demands the use of narrow slit widths or, alternatively, very reproducible slit-width settings.

Unfortunately, a decrease in slit width is accompanied by a second-order power reduction in the radiant energy; at very narrow settings spectral detail may be lost owing to an increase in the signal-to-noise ratio. The situation becomes particularly serious in spectral regions where the output of the source or the sensitivity of the detector is low. Under such circumstances, noise in either of these components or their associated electronic circuits may result in partial or total loss of spectral fine structure.

In general, it is good practice to narrow slits no more than is necessary for resolution of the spectrum at hand. With a variable slit spectrophotometer, proper slit adjustment can be determined by acquiring spectra at progressively narrower slits until peak heights become constant. Generally, constant peak heights are observed when the effective bandwidth of the instrument is 0.1 or less of the effective bandwidth of the absorption peak.

Effect of Scattered Radiation at Wavelength Extremes of an Instrument

We have already noted that scattered radiation may cause instrumental deviations from Beer's law. When measurements are attempted at the wavelength extremes of an instrument, the effects of stray radiation may be even more serious and on occasion may lead to the appearance of false absorption peaks. For example, consider a spectrophotometer for the visible region equipped with glass optics, a tungsten source, and a photovoltaic cell detector. At wavelengths below about 380 nm, the windows, cells, and prism begin to absorb radiation, thus reducing the energy reaching the transducer. The output of the source falls off rapidly in this region as well; so also does the sensitivity of the photo-electric device. Thus, the total signal for the 100% T adjustment may be as low as 1% to 2% of that in the region between 500 and 650 nm.

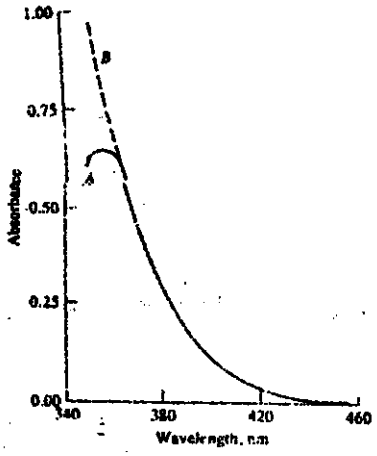


Fig. Spectra of cerium (IV) obtained with a spectrophotometer having glass optics (A) and quartz optics (B). The false peak in A arises from transmission of stray radiation of longer wavelengths.

The scattered radiation, however, is often made up of wavelengths to which the instrument is highly sensitive. Thus, its effects can be enormously magnified. Indeed, in some instances the output signal produced by the stray radiation may exceed that from the monochromator beam; under these circumstances, the measured absorbance is as much that for the stray radiation as for the radiation to which the instrument is set.

An example of a false peak appearing at the wavelength extremes of a visible-region spectrophotometer is shown in figure. The spectrum of a solution of cerium (IV) obtained with an ultraviolet/visible spectrophotometer, sensitive in the region of 200 to 750 nm, is shown by curve B. Curve A is a spectrum of the same solution obtained with a simple visible spectrophotometer. The apparent maximum shown in curve A arises from the instrument responding to stray wavelengths longer than 400 nm, which (as can be seen from the spectra) are not absorbed by the cerium (IV) ions.

This same effect is sometimes observed with ultraviolet/visible instruments when attempts are made to measure absorbances at wavelengths lower than about 200 nm.

3. Describe the Instruments Components and Types of Instruments.

Instrument Components

Instruments for measuring the absorption of ultraviolet, visible, and near-infrared radiation are made up of one or more (1) sources, (2) wavelength selectors, (3) sample containers, (4) radiation transducers, and (5) signal processors and readout devices. The design and performance of components 2, 4 and 5 have been described in considerable detail in Chapter and thus are not discussed further here. We will, however, consider briefly the characteristics of sources and sample containers for the region of 185 to 3000 nm.

Types of Instruments

In this section, we consider four general types of spectroscopic instruments: (1) single-beam, (2) double-beam in space, (3) double-beam in time, and (4) multichannel.

Single-Beam Instruments

Figure (a) is a schematic of a single-beam instrument for absorption measurements. It consists of one of the radiation sources described in an earlier section of this chapter, a filter or a monochromator for wavelength selection, matched cells that can be interposed alternately in the radiation beam, one of the transducers described in Section 7E, an amplifier, and a readout device. Normally, a single-beam instrument requires a stabilized voltage supply to avoid errors resulting

from changes in the beam intensity during the time required to make the 100% T adjustment and determine % T for the analyte.

Single-beam instruments vary widely in their complexity and performance characteristics. The simplest and least expensive consists of a battery-operated tungsten bulb as the source, a set of glass filters for wavelength selection, test tubes for sample holders, a photovoltaic cell as the transducer, and a small microammeter as the readout device. At the other extreme are sophisticated, computer-controlled instruments with a range of 200 to 1000 nm or more. These spectrophotometers have interchangeable tungsten/deuterium lamp sources, use rectangular silica cells, and are equipped with a high-resolution grating monochromator with variable slits. Photomultiplier tubes are used as transducers, and the output is often digitized and stored so that it can be printed or plotted in several forms.

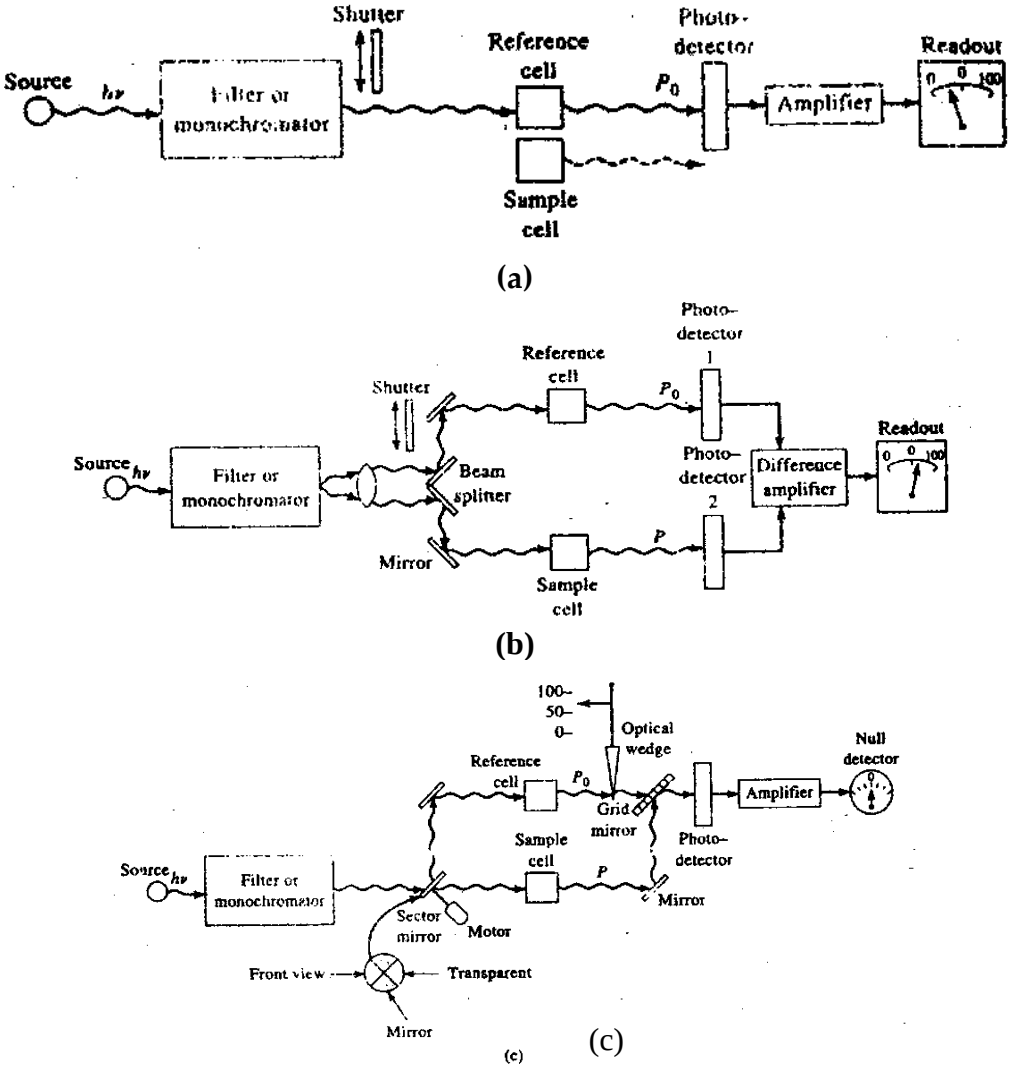


Fig. Instrument designs for photometers and spectrophotometers: (a) single-beam instrument, (b) double-beam instrument with beams separated in space, (c) double-beam instrument with beams separated in time.

Double-Beam Instruments

Many modern photometers and spectrophotometers are based upon a double-beam design. Figure (b) illustrates a double-beam-in-space instrument in which two beams are formed in space by a V-shaped mirror called a beam splitter. One beam passes through the reference solution to a phototransducer, and the second simultaneously traverses the sample to a second, matched transducer. The two outputs are amplified, and their ratio (or the log of their ratio) is determined electronically and displayed by the readout device. With manual instruments, the measurement is a two-step operation involving first the zero adjustment with a shutter in place between selector and beam splitter. In the second step, the shutter is opened and the transmittance or absorbance is read directly from the meter.

The second type of double-beam instrument is illustrated in figure (c). Here the beams are separated in time by a rotating sector mirror that directs the entire beam from the monochromator first through the reference cell and then through the sample cell. The pulses of radiation are recombined by another sector mirror, which transmits one pulse and reflects the other to the transducer. As shown by the insert labeled "front view" in figure (c), the motor-driven sector mirror is made up of pie-shaped segments, half of which are mirrored and half of which are transparent. The mirrored sections are held in place by blackened metal frames that periodically interrupt the beam and prevent its reaching the transducer. The detection circuit is programmed to use these periods to perform the dark-current adjustment.

The instrument shown in figure (c) is a null type, in which the beam passing through the solvent is attenuated until its intensity just matches that of the beam passing through the sample. Attenuation is accomplished in this design with an optical wedge, the transmission of which decreases linearly along its length. Thus, the null point is reached by moving the wedge in the beam until the two electrical pulses are identical as indicated by the null detector. The transmittance (or absorbance) is then read directly from the pointer attached to the wedge.

Double-beam instruments offer the advantage that they compensate for all but the most short-term fluctuations in the radiant output of the source as well as for drift in the transducer and amplifier. They also compensate for wide variations in source intensity with wavelength (see figure). Furthermore, the double-beam design lends itself well to the continuous recording of transmittance or absorbance spectra. Consequently, most modern ultraviolet and visible recording instruments are double-beam (usually in time). Scanning infrared spectrophotometers often are based on this design.

Multichannel Instruments

The most recent type of spectrophotometer, which appeared on the market in the early 1980s, is a single beam instrument based upon the diode array transducer described in Section 7E-

3. A simplified schematic of such a diode array spectrometer is shown in Figure. Radiation from a lamp is focused upon the sample or solvent container and then passes into a monochromator with a fixed grating. The dispersed radiation falls on a photodiode array transducer, which, as mentioned earlier, consists of a linear array of several hundred photodiodes that have been formed along the length of a silicon chip. Typically, the chips are 1 to 6 cm in length, and the widths of the individual diodes are 15 to 50 μm (see figure). This chip also contains a capacitor and an electronic switch for each diode. A computer-driven shift register sequentially closes each switch momentarily, which causes each capacitor to be charged to -5 V. Radiation impinging on any diode surface causes partial discharge of its capacitor. This lost charge is replaced during the next switching cycle. The resulting charging currents, which are proportional to the radiant power, are amplified, digitized, and stored in computer memory. The entire cycle is completed in a few milliseconds.

The monochromator slit width of a diode array instrument is usually made identical to the width of one of silicon diodes. Thus, the output of each diode corresponds to the radiation of a different wavelength, and a spectrum is obtained by scanning these outputs sequentially. Since the electronic scanning process is remarkably rapid, data for an entire spectrum are accumulated in 1 s or less.

A diode array instrument is a powerful tool for studies of transient intermediates in moderately fast reactions, for kinetic studies, and for the qualitative and quantitative determination of the components exiting from a liquid chromatographic column or a capillary electrophoresis column. The disadvantages of this type of instrument are its somewhat limited resolution (usually 1 to 2 nm) and its moderately high cost.

4. Describe the Structure of Single and Double beam Spectrophotometers.

Spectrophotometers

Numerous spectrophotometers are available from commercial sources. Some have been designed for the visible region only; others are applicable in the ultraviolet and visible regions. A few have measuring capabilities from the ultraviolet through the near infrared (185 to 3000 nm).

Instruments for the Visible Region

Several spectrophotometers designed to operate within the wavelength range of about 380 to 800 nm are available from commercial sources. These instruments are frequently simple, single-beam grating instruments that are relatively inexpensive (less than $1000 to perhaps $3000), rugged, and readily portable. At least one is battery operated and light and small enough to be handheld. The most common application of these instruments is for quantitative analysis, although several produce surprisingly good absorption spectra as well.

Figure shows a simple and inexpensive spectrophotometer, the Spectronic 20. The original version of this instrument first appeared in the market in the mid-1950s, and the modified version shown in the figure is still being manufactured and widely sold. Undoubtedly, more of these instruments are currently in use throughout the world than any other single spectrophotometer model. The instrument owes its popularity, particularly as a teaching tool, to its relatively low cost, its ruggedness, and its satisfactory performance characteristics.

The Spectronic 20 employs a tungsten filament light source, which is operated by a stabilized power supply that provides radiation of constant intensity. After diffraction by a simple reflection grating, the radiation passes through the sample or reference curettes to a phototube. The amplified electrical signal from the transducer then powers a meter with 5½ -in. scale calibrated in transmittance and absorbance.

The instrument is equipped with an occluder, which is a vane that automatically falls between the beam and the detector whenever the curette is removed from its holder; the 0% T adjustment can then be made. As shown in figure, the light control device in the Spectronic 20 consists of a V-shaped slot that can be moved in or out of the beam in order to set the meter to 100% T:

The wavelength range of the Spectronic 20 is from 340 to 625 nm; an accessory phototube extends this range to 950 nm. Other specifications for the instrument include a bandwidth of 20 nm and a wavelength accuracy of ± 2.5 nm. The Spectronic 20 has been upgraded recently to the Spectronic 20+; the newer instrument utilizes a solid-state transducer as well as other enhanced features.

Single-Beam Instruments for the Ultraviolet/Visible Region

Several instrument manufacturers offer non-recording single-beam instruments which can be used for both ultraviolet and visible measurements. The lower wavelength extremes for these instruments vary from 190 to 210 nm, and the upper from 800 to 1000 nm. All are equipped with interchangeable tungsten and hydrogen or deuterium lamps. Most employ photomultiplier tubes as transducers and gratings for dispersion. Some are equipped with digital readout devices; others employ large meters. The prices for these instruments range from $2000 to $8000.

As might be expected, performance specifications vary considerably among instruments and are related, at least to some degree, to instrument price. Typically, bandwidths vary from 2 to 8 nm; wavelength accuracies of ± 0.5 to ± 2 nm are reported.

The optical designs for the various grating instruments do not differ greatly from those shown in figures. One manufacturer, however, employs a concave rather than a plane grating; a

simpler and more compact design results. Instruments equipped with holographic gratings are also beginning to appear on the market.

Single-Beam Computerized Spectrophotometers

One manufacturer is now offering a line of computerized, recording, single-beam spectrophotometers, which operate in the range of 190 to 800 nm (or to 900 nm with an accessory). – W. Kaye, D. Barber, and R. Marasco, Anal. Chem., 1980, 52, 437A; V.A. Kohler and N. Brenner, Amer. Lab., 1981, 13 (9), 109. With these instruments, a wavelength scan is first performed with the reference solution in the beam path. The resulting transducer output is digitized in real time and stored in the memory of the computer. Samples are then scanned and absorbances calculated with the aid of the stored reference solution data. The complete spectrum is displayed on a cathode-ray tube within 2 s of data acquisition. Scan speeds as great as 1200 nm/min are feasible. The computer associated with the instrument provides several options with regard to data processing and presentation such as log absorbance, transmittance, derivatives, overlaid spectra, repetitive scans, concentration calculations, peak location and height determinations, and kinetic measurements.

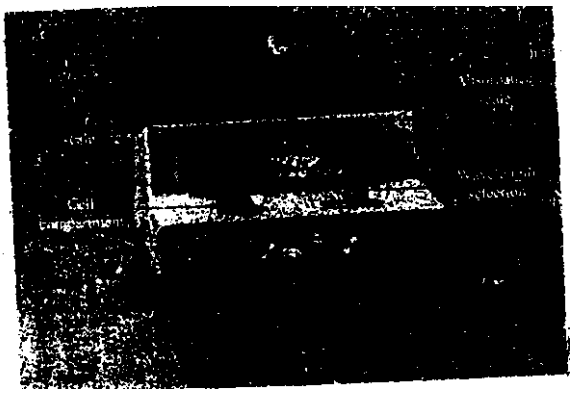


Figure (a) The Spectronic 20 spectrophotometer and (b) its optical diagram

As noted earlier, single-beam instruments have the inherent advantages of greater energy throughout, superior signal-to-noise ratios, and less cluttered sample compartments. On the other hand, the process of recording transducer outputs for reference and sample solutions successively for subsequent absorbance or transmittance calculations has heretfore not been very satisfactory because of the drift or flicker noise in sources and transducers. The manufacturer of these new single-beam instruments claims to have eliminated these instabilities by means of fundamentally new source design and a new electronic design that eliminates hysteresis or memory effects in the phototransducer.

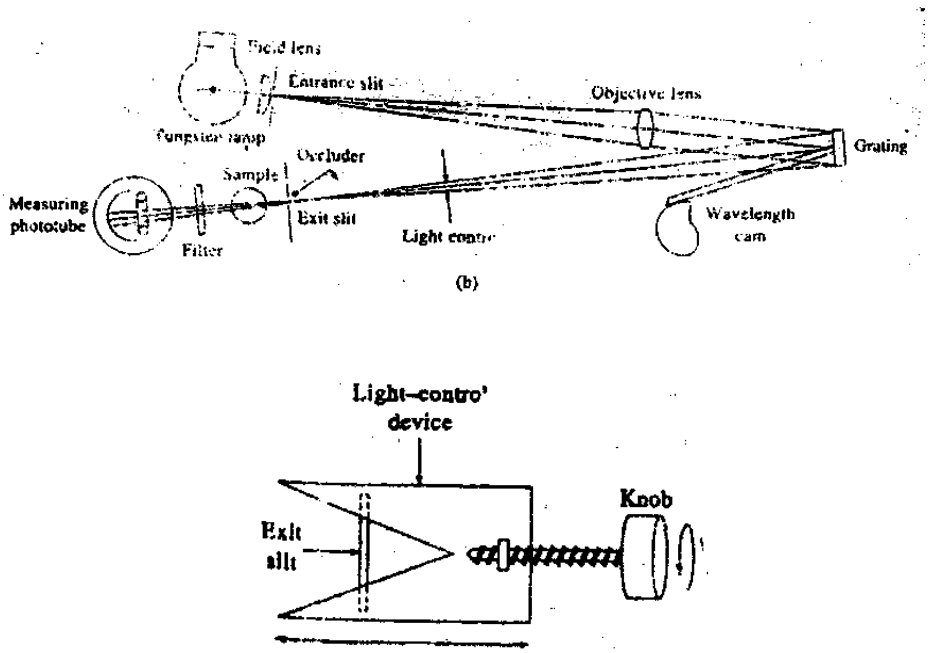


Figure. End view of the exit slit of the Spectronic 20 spectrophotometer pictured in figure (b)

The photometric accuracies of the new instruments are reported to be ± 0.005 A or ±0.3% T with a drift of less than 0.002 A/hr. Bandwidths of 0.5, 1, and 2 nm are available by manual interchange of fixed slits.

Double-Beam Instruments

Numerous double-beam spectrophotometers for the ultraviolet /visible region of the spectrum are now available. Generally, these instruments are more expensive than their single-beam counterparts, with the nonrecording variety ranging in cost from about $4000 to $15,000.

Figure shows construction details of a typical, relatively inexpensive, manual, double-beam ultraviolet/visible spectrophotometer. In this instrument, the radiation is dispersed by a concave grating, which also focuses the beam on a rotating sector mirror. The instrument design is similar to that shown in figure (c).

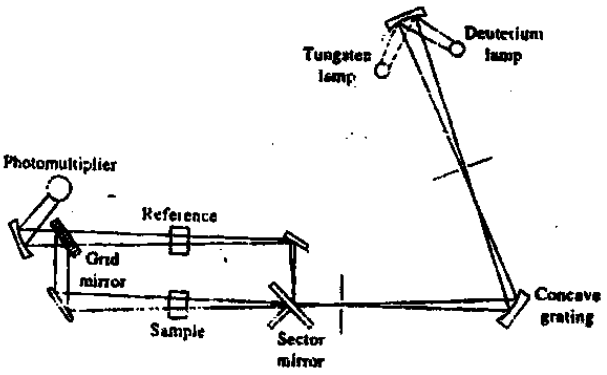


Figure. Schematic of a typical manual double-beam Spectrophotometer for the

ultraviolet/visible region.

The instrument has a wavelength range of 195 to 850 nm, a bandwidth of 4 nm, a photometric accuracy of 0.5% T, and a reproducibility of 0.2% A; stray radiation is less than 0.1% of P_0 at 240 and 340 nm. This instrument is typical of several similar ones offered by various instrument companies. Such instruments are well suited for quantitative work where acquisition of an entire spectrum is not often required.

Figure shows the optics of a more sophisticated, dual-channel (in time), recording spectrophotometer, which employs a 45 × 45-mm plane grating having 1440 lines/mm. Its range is from 190 to 750 nm with the option of extending this range to 900 nm. Bandwidths of 0.2, 0.5, 1.0, and 3.00 mm can be chosen by exchange of slits. The instrument has a photometric accuracy of ± 0.003 A; its stray radiation is less than 0.1% of P_0 at 220 and 340 nm. The performance of this instrument is significantly better than the double-beam instrument described previously; its price is correspondingly higher.

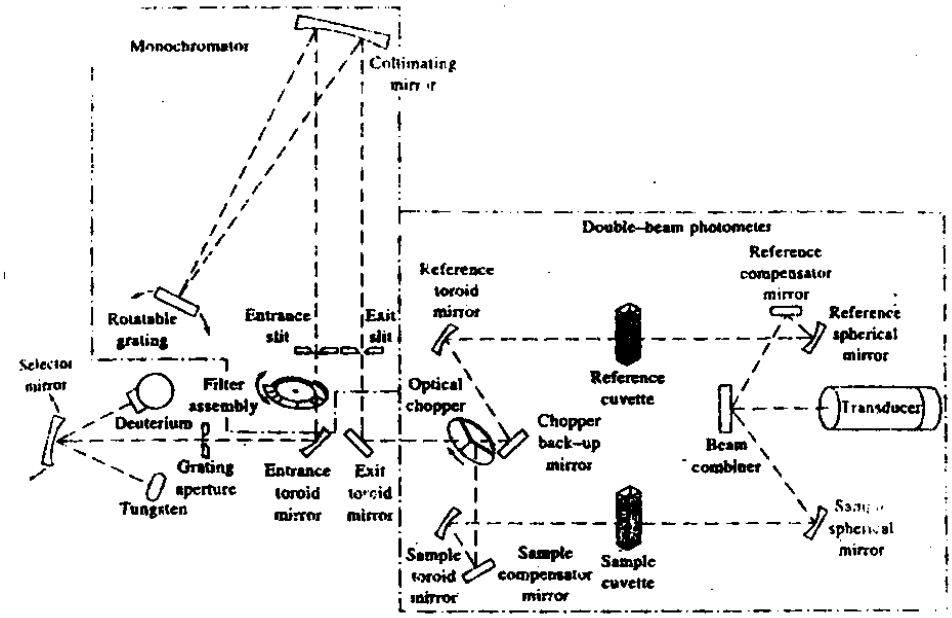


Figure. A double-beam recording spectrophotometer for the ultraviolet and visible regions; the Perkin-Elmer 57 Series

Double-Dispersing Instruments

In order to enhance spectral resolution and achieve a marked reduction in scattered radiation, a number of instruments have been designed with two gratings or prisms serially arranged with an intervening slit; in effect, then, these instruments consist of two monochromators in a series configuration.

The instrument shown in figure achieves the same performance characteristics with a single grating. Note that the radiation passing through entrance slit 1 is dispersed by the grating and travels through exit slit 1 to entrance slit 2. After a second dispersion by the grating, the beam emerges from exit slit 2 where it is split into a sample and a reference beam. The resolution of the instrument is reported to be 00.07 nm, and the stray light 0.0008% from 220 to 800 nm. The wavelength range is 185 to 3125 nm. For wavelengths greater than 800 nm, a lead sulfide photoconductive cell is used with the tungsten source.

Diode Array Instruments

In the mid-1970s, a number of scientific papers and review articles appeared in the literature describing applications of silicon diode arrays as transducers for spectrochemical measurements. Y.Talmi, Appl. Spectrosc., 1982, 36, 1; Y. Talmi, Anal. Chem., 1975, 47, 658A, 697A; Y. Talmi, Multichannel Image Detectors, Vol.1, 1979; Vol.2, 1983, Washington, DC: American Chemical Society; G. Horlick and E.G. Codding, Contemp. Top. Anal. Clin. Chem., 1977, 1, 195. With these devices located at the focal plane of a monochromator, a spectrum can be obtained by electronic rather than mechanical scanning; all of the data points needed to define a spectrum can thus be gathered essentially simultaneously. The concept of multichannel instruments is attractive because of the potential speed at which spectra can be acquired as well as their applicability to simultaneous multicomponent determinations. In about 1980, the first electronic multichannel spectrometer designed specifically for molecular absorption measurements in the ultraviolet/visible range became available from a commercial source. J.C. Milter, S.A. George, and B.G. Willis, Science, 1982, 218, 241. By now, several instrument companies offer such instruments.

Figure shows an optical diagram of a typical multichannel ultraviolet/visible spectrophotometer. Because of the few optical components, the radiation throughput is much higher than that of traditional spectrophotometers. As a result, a single deuterium lamp can serve as a source for not only the ultraviolet but also the visible region (upto 820 nm). After passing through the solvent or analyte solution, the radiation is focused on an entrance slit and then passes onto the surface of a holographic reflection grating. The transducer is a diode array made up of 328 elements, each having a dimension of 18 by 0.5 mm. the dispersion of the grating and the size of the diode elements are such that a resolution of 2 nm is realized throughout the entire spectral region. Because the system contains no moving parts, the wavelength reproducibility from scan to scan is exceedingly high, and signal averaging can be used to produce marked increases in signal-to-noise ratios.

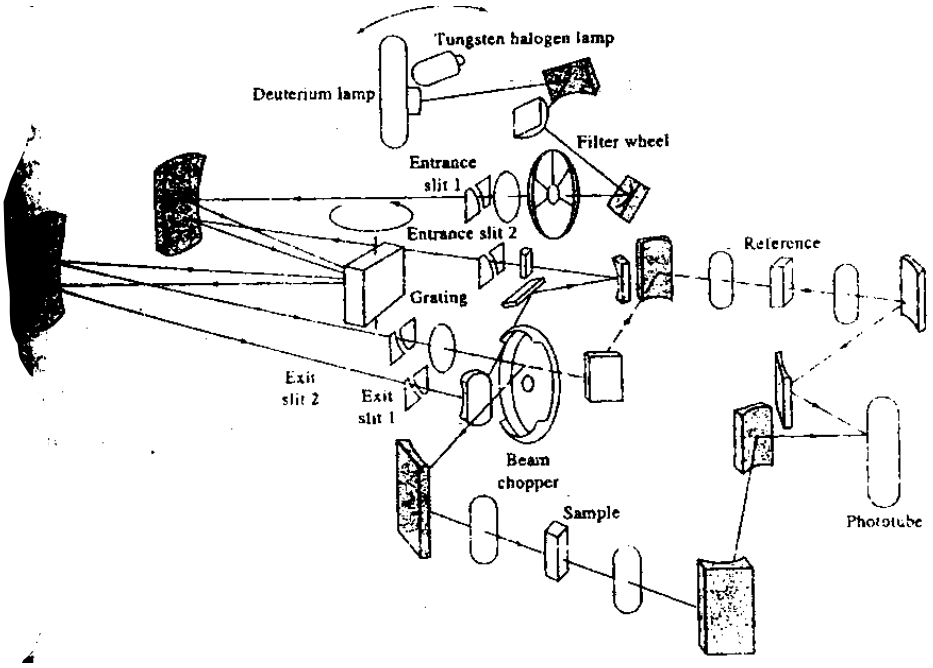


Figure A double-dispersing spectrophotometer.

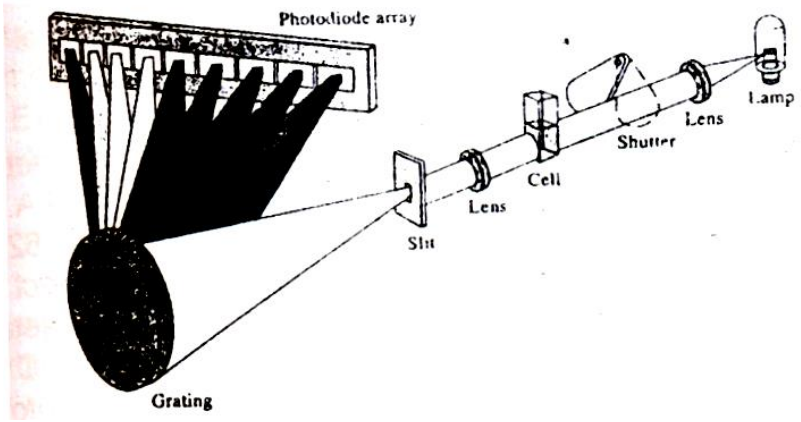


Figure A multichannel diode array spectrometer; the HP 8452A.

A single scan from 200 to 820 nm with this instrument requires 0.1s. In order to improve signal-to-noise ratios, however, spectra are generally scanned for a second or more with the data being accumulated in computer memory. With such short exposure times, photodecomposition of samples is minimized despite the location of the sample between the source and the monochromator. The stability of the source and the electronic system is such that the solvent signal needs to be observed and stored only every five to ten minutes.

The spectrophotometer shown in figure is designed to be interfaced with most personal computer systems. The instrument (without the computer) sells in the $7000 to $9000 price range.

5. Describe the application of Absorption measurement to Quantitative analysis.

QUANTITATIVE ANALYSIS BY ABSORPTION MEASUREMENTS

Absorption spectroscopy is one of the most useful and widely used tools available to the chemist for quantitative analysis. – For a wealth of detailed, practical information on spectrophotometric practices, see Techniques in Visible and Ultraviolet Spectrometry, Vol.I, Standards in Absorption Spectroscopy, C. Burgess and A. Knowles, Eds. London: Chapman and Hall, 1981; J.R. Edisbury, Practical Hints on Absorption Spectrometry. New York: Plenum Press, 1968. Important characteristics of spectrophotometric and photometric methods include: (1) wide applicability to both organic and inorganic systems, (2) typical sensitivities of 10^{-4} to 10^{-5} MHz (this range can often be extended to 10^{6} to 10^{-7} MHz by certain modifications) – See, for example: T.D. Harris, Anal. Chem., 1982, 54, 741A., (3) moderate to high selectivity, (4) good accuracy (typically, relative uncertainties of 1% to 3% are encountered, although with special precautions, errors can be reduced to a few tenths of a percent). (5) ease and convenience of data acquisition.

Scope

The applications of quantitative, ultraviolet/visible absorption methods not only are numerous, but also touch upon every field in which quantitative chemical information is required. For example, it has been estimated that in the field of health alone, 95% of all quantitative determinations are performed by ultraviolet/visible spectrophotometry and this number represents well over 3,000,000 daily tests carried out in the United States. – R.W. Birke and R. Mavrodineanu, Accuracy in analytical Spectrophotometry, NBS Special Publication 260-81, p. 1. Washington, DC: National Bureau of Standards, 1983.

The reader can develop a notion of the scope of spectrophotometry by consulting a series of review articles published periodically in Analytical Chemistry – see J.A. Howell and L.G. Hargis, anal. Chem., 19996, 68, 169R; 1994, 66, 445R; 1992, 64, 66R; 1990, 62, 155R; 1988, 60, 131R; 1986, 58, 108R; 1984, 56, 225R. and from monographs on the subject. For example, see E.B. Sandell and H. Onishi, Colorimetric Determination of Traces of Metals, 4th ed. New York: Interscience, 1978; Colorimetric Determination of Nonmetals, 2nd ed., D.F.Boltz and J.A.Howell, Eds. New York: Wiley, 1978; Z. Marczenko, Separation & Spectrophotometric Determination of Elements. New York: Halsted Press, 1986; M. Pisez and J. Bartos, Colorimetric and Fluorometric Analysis of Organic Compounds and Drugs. New York: Marcel Dekker, 1974; F.D. Snell, Photometric and Fluorometric Method of Analysis, Parts 1 and 2, "Metals"; Part 3, "Nonmetals." New York: Wiley, 1978-81.

TABLE : Solvents for the Ultraviolet and the Visible Regions

Solvent	Approximate[a] Transparency Minimum (nm)
Water	190
Ethanol	210
n-Hexane	195
Cyclohexane	210
Benzene	280
Diethyl ether	210
Acetone	330
1, 4-Dioxane	220

Applications to Absorbing Species

Spectrophotometric determination of any organic compound containing one or more of these groups is potentially feasible; many examples of this type of determination are found in the literature.

A number of inorganic species also absorb and are thus susceptible to direct determination; we have already mentioned the various transition metals. In addition, a number of other species also show characteristic absorption. Examples include nitrite, nitrate, and chromate ions; osmium and ruthenium tetroxides; molecular iodine; and ozone.

Applications to Nonabsorbing Species

Numerous reagents react selectively with nonabsorbing species to yield products that absorb strongly in the ultraviolet or visible regions. – For example, see the first reference in footnote 13. The successful application of such reagents to quantitative analysis usually requires that the color-forming reaction be forced to near completion. Itshould be noted that color-forming reagents are frequently employed as well for the determination of absorbing species, such as transition-metal ions; the molar absorptivity of the product will frequently be orders of magnitude greater than that of the uncombined species.

A host of complexing agents find application for the determination of inorganic species. Typical inorganic reagents include thiocyanate ion for iron, cobalt, and molybdenum; the anion of hydrogen peroxide for titanium, vanadium, and chromium; and iodide ion for bismuth, palladium, and tellurium. Of even more importance are organic chelating agents that form stable, colored complexes with cations. Examples include o-phenanthroline for the determination of iron, dimethyl glyoxime for nickel, diethyldithiocarbamate for copper, and diphenyldithiocarbazone for lead.

Procedural Details

The first steps in a photometric or spectrophotometric analysis involve the establishment of working conditions and the preparation of a calibration curve relating concentration to absorbance.

Selection of Wavelength

Spectrophotometric absorbance measurements are ordinarily made at a wavelength corresponding to an absorption peak because the change in absorbance per unit of concentration is greatest at this point; the maximum sensitivity is thus realized. In addition, the absorption curve is often flat in this region; under these circumstances, good adherence to Beer's law can be expected. Finally, the measurements are less sensitive to uncertainties arising from failure to reproduce precisely the wavelength setting of the instrument.

Variable that Influence Absorbance

Common variables that influence the absorption spectrum of a substance include the nature of the solvent, the pH of the solution, the temperature, electrolyte concentrations, and the presence of interfering substances. The effects of these variables must be known; conditions for the analysis must be chosen such that the absorbance will not be materially influenced by small, uncontrolled variations in their magnitudes.

Cleaning and Handling of Cells

Accurate spectrophotometric analysis requires the use of good-quality, matched cells. These should be regularly calibrated against one another to detect differences that can arise from scratches, etching, and wear. Equally important is the use of proper cell cleaning and drying techniques. Erickson and Surles - J.O. Erickson and T. Surles, Amer. Lab., 1976, 8 (6), 50 – recommend the following cleaning sequence for the outside windows of cells. Prior to measurement, the cell surfaces are cleaned with a lens paper soaked in spectrograde methanol. The paper is held with a hemostat; after wiping, the methanol is allowed to evaporate, leaving the cell surfaces free of contaminants. The authors showed that this method was far superior to the usual procedure of wiping the cell surfaces with a dry lens paper, which apparently leaves lint and films on the surface.

Determination of the Relationship Between Absorbance and Concentration

After deciding upon the conditions for the analysis, it is necessary to prepare a calibration curve from a series of standard solutions that bracket the concentration range expected for the

samples. Seldom, if ever, is it safe to assume adherence to Beer's Law and use only a single standard to determine the molar absorptivity. The results of an analysis should never be based on a literature value for the molar absorptivity.

Ideally, calibration standards should approximate the composition of the samples to be analyzed not only with respect to the analyte concentration but also with regard to the concentrations of the other species in the sample matrix in order to minimize the effects of various components of the sample on the measured absorbance. For example, the absorbance of many colored complexes of metal ions is decreased to a varying degree in the presence of sulfate and phosphate ions as a consequence of the tendency of these anions to form colorless complexes with metal ions. The color formation reaction is often less complete as a consequence, and lowered and absorbances are the result. The matrix effect of sulfate and phosphate can often be counteracted by introducing into the standards amounts of the two species that approximate the amounts found in the samples. Unfortunately, when complex materials such as soils, minerals, and plant ash are being analyzed, however, preparation of standards that match the samples is often impossible or extremely difficult. When this is the case, the standard addition method is often helpful in counteracting matrix effects.

The Standard Addition Method

The standard addition method can take several forms. See M. Bader, J. Chem. Educ., 1980, 57, 703. The one most often chosen for photometric or spectrophotometric analyses, and the one that was discussed in some detail in section 1E-2, involves adding one or more increments of a standard solution to sample aliquots of the same size. Each solution is then diluted to a fixed volume before measuring its absorbance. The application of this method to data from the photometric determination of iron (III) based upon the formation of the red thiocyanate complex.

In the interest of saving time or sample, it is possible to perform a standard addition analysis using only two increments of sample. Here, a single addition of V_s mL of standard would be added to one of the two samples. This approach is based upon the equation

$$c_x = \frac{A_1 c_s V_s}{(A_2 - A_1)V_x} \qquad .....(1)$$

EXAMPLE:-

A 2.00-mL urine specimen was treated with reagent to generate a color with phosphate, following which the sample was diluted to 100 mL. To a second 2.00-mL swample was added exactly 5.00 mL of a phosphate solution containing 0.0300 mg phosphate/mL, which was treated in the same way as the original sample. The absorbance of the first solution was 0.428; that of the second was 0.538. Calculate the concentration in milligrams of phosphate per millimeter of the specimen.

Here we substitute into Equation (1) and obtain

$$c_x = \frac{0.428 \times 0.0300\ (mg\ PO_4^{3-}/mL) \times 5.00mL}{(0.538 - 0.428) \times 2.00mL\ \text{sample}}$$

$$= 0.292\ \text{mg}\ PO_4^{3-}/mL\ \text{sample}$$

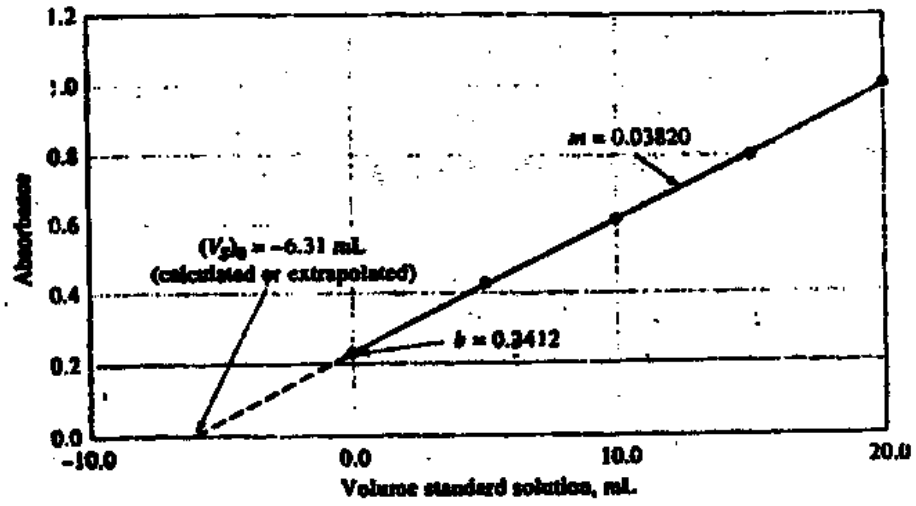
Analysis of Mixtures of Absorbing Substances

The total absorbance of a solution at a given wavelength is equal to the sum of the absorbances of the individual components present. This relationship makes possible the quantitative determination of the individual constituents of a mixture, even if their spectra overlap. Consider, for example, the spectra of MHz and N, shown in figure. Note that no wavelength exists at which the absorbance of this mixture is due simply to one of the components; thus, determination of either MHz or N is impossible by a single measurement. However, the absorbances of the mixture at the two wavelengths λ′ and λ″ may be expressed as follows:

$$A' = \in' Mbc_M + \in' Nbc_N \quad (\text{at } \lambda')$$

$$A'' = \in'' Mbc_M + \in'' Nbc_N \quad (\text{at } \lambda'')$$

The four molar absorptivities $\in'_M$, $\in'_N$, $\in''_M$, and $\in'_N$ can be evaluated from individual standard solutions of M and of N, or better, from the slopes of their Beer's law plots. The absorbances of the mixture, A′ and A″, are experimentally measurable, as is b, the cell thickness. Thus, from these two equations, the concentrations of the individual constituents, c_M and c_N, can be calculated. These relationships are valid only if Beer's law is followed and if the two components behave independently of one another. The greatest accuracy in an analysis of this sort is attained by choosing wavelength at which the differences in molar absorptivities are large.



Data for standard addition method for the determination of Fe^{3+} as the SCN^- complex

Mixtures containing more than two absorbing species can be analyzed, in principle at least, if a further absorbance measurement is made for each added component. The uncertainties in the resulting data become greater, however, as the number of measurements increases. Some of the newer computerized spectrophotometers are capable of reducing these uncertainties by overdetermining the system. That is, these instruments use many more data points than unknowns and effectively match the entire spectrum of the unknown as closely as possible by least-squares techniques utilizing the methods of matrix algebra. The spectra for standard solutions of each component are required for the analysis.

Derivative and Dual-Wavelength Spectrophotometry

Fc-additional information, see G. Talsky, Derivative Spectrophotometry, New York: VCH Publishers, 1994; T.C. O'Haver, Anal. Chem., 1979, 51, 91A; F. Sanchez Rajas, C. Bosch Ojeda, and J.M. Cano Pavon, Talanta, 1988, 35, 753; J.E. Cahill, Amer, Lab., 1979, 11 (a11) 79; J.E. Cahill and F.G. Padera, Amer. Lab., 1980, 12(4), 101; T.J. Porro, Anal. Chem., 1972, 44 (4), 93A.

In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance or transmittance with respect to wavelength as a function of wavelength. Often these plots reveal spectral detail that is lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of an interference can sometimes be made more easily or more accurately. Unfortunately, the advantages of derivative spectra are at least partially offset by a degradation in signal-to-noise ratio that accompanies obtaining derivatives. In many parts of the ultraviolet and visible regions, however, signal-to-noise ratio is not a serious limiting factor; it is here that derivative spectra are of most use. An additional disadvantage of derivative spectrophotometry is that the required equipment is generally more costly.

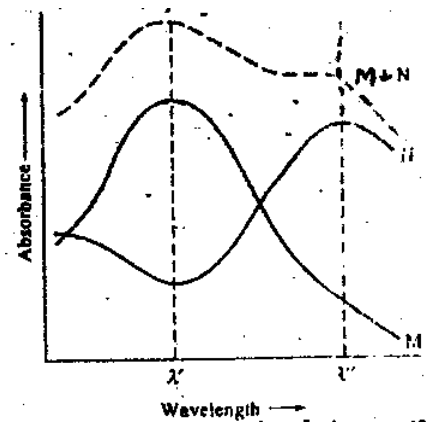


Figure Absorption spectrum of a two-component mixture. Optimum wavelengths λ′ and λ″ for the determination of the two components are indicated by the vertical dashed lines.

A variety of methods are used to obtain derivative spectra. For microprocessor-controlled digital spectrophotometers, the differentiation can be performed numerically using derivative least-squares polynomial smoothing. With analog instruments, derivatives of spectral data can be obtained by a suitable operational amplifier circuit. A third procedure is accomplished by wavelength modulation.

Wavelength Modulation Devices

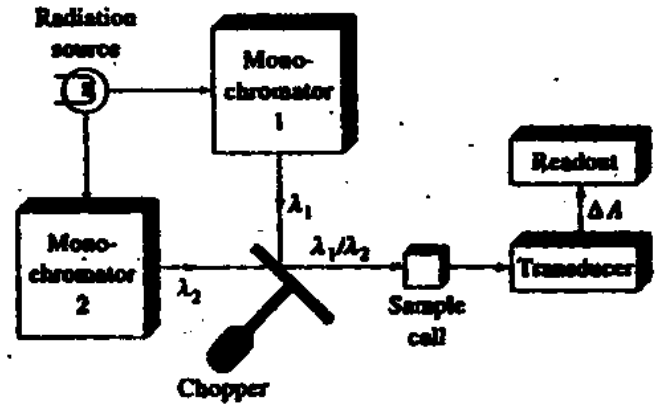


Figure Schematic of a dual-wavelength spectrophotometer.
The chopper causes λ_1 and λ_2 to pass through the sample alternately

Several procedures have been developed for wavelength modulation. In some of these procedures, a wavelength interval of a few nanometers is swept rapidly and repetitively while the spectrum is being scanned in the usual way. The amplitude of the resulting ac signal from the transducer is a good approximation of the derivative of the spectrum with respect to wavelength. The repetitive, rapid sweep has been obtained by any of a number of mechanical means, including oscillation or vibration of a mirror, slit, or dispersing element of a monochromator. A second scheme for wavelength modulation, and one that is offered by several instrument makers, involves the use of dual dispersing systems arranged in such a way that two beams of slightly different wavelengths (typically 1 or 2 nm difference) fall alternatively onto a sample cell and its transducer; no reference beam is used. The ordinate variable is the difference between the alternate signals, which provides a good approximation of the derivative of absorbance as a function of wavelength ($\Delta A/\Delta\lambda$). Figure is a schematic of a dual wavelength instrument. Note that a reference cell is not employed and the sample cell is alternately exposed to radiation from each of the monochromators. Generally, dual-wavelength instruments can also be operated in the single wavelength mode with the radiation passing alternately through a reference and standard cell.

6. Describe the structure of photo Acoustic Spectroscopy.

PHOTOACOUSTIC SPECTROSCOPY

Photoacoustic (PAS) or optoacoustic spectroscopy, which was developed in the early 1970s, provides a means for obtaining ultraviolet, visible, and infrared absorption spectra of solids, semisolids, or turbid liquids. Acquisition of spectra for these kinds of samples by ordinary methods is usually difficult at best and often impossible because of light scattering and reflection.

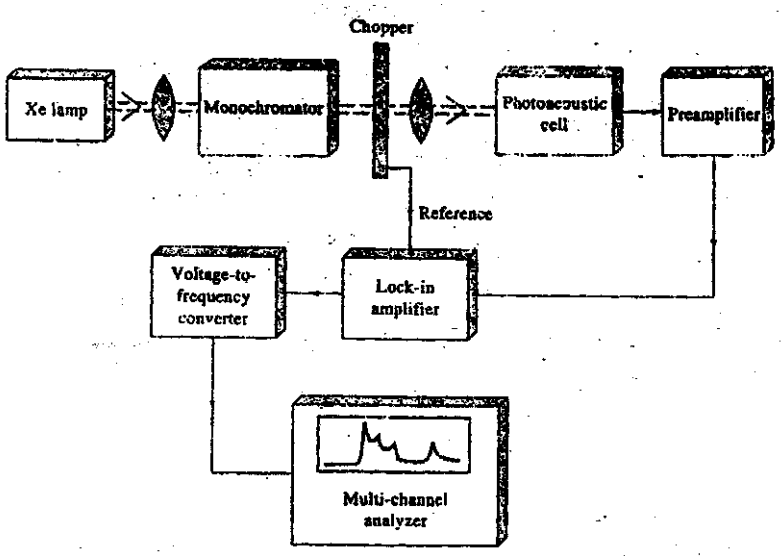


Figure Block diagram of a single-beam photoacoustic spectrometer with digital data processing.
The Photoacoustic Effect

Photoacoustic spectroscopy is based upon a light absorption effect that was first investigated in the 1880s by Alexander Graham Bell and others. This effect is observed when a gas in a closed cell is irradiated with a chopped beam of radiation of a wavelength that is absorbed by the gas. The absorbed radiation causes periodic heating of the gas, which in turn results in regular pressure fluctuations within the chamber. If the chopping rate lies in the acoustical frequency range, these pulses of pressure can be detected by a sensitive microphone. The photoacoustic effect has been used since the beginning of the 20th century for the analysis of absorbing gases and has recently taken on new importance for this purpose with the advent of tunable infrared lasers as sources. Of greater importance, however, has been the application of this phenomenon to the derivation of absorption spectra solids and turbid liquids. For a review on applications, see A. Rosencwaig, Anal. Chem., 1975, 47, 592A; J.W. Lin and L.P. Dubek, anal. Chem., 1979, 51, 1627; J.E. McClelland, Anal. Chem., 1983, 55, 89A; D. Betteridge and P.J.Meylor, CRC Crit. Rev. Anal. Chem., 1984, 14, 267; A. Rosencwaig, Photoacoustics and Photoacoustic Spectroscopy. New York: Wiley, 1980.

Photoacoustic Spectra

In photoacoustic studies of solids, the sample is placed in a closed cell containing air or some other nonabsorbing gas and a sensitive microphone. The solid is then irradiated with a chopped beam from a monochromator. The photoacoustic effect is observed provided the radiation is absorbed by the solid; the power of the resulting sound is directly related to the extent of absorption. Radiation reflected or scattered by the sample has no effect on the microphone and thus does not interfere. This latter property is perhaps the most important characteristic of the method.

The source of the photoacoustic effect in solids appears to be similar to that in gases. That is, nonradiative relaxation of the absorbing solid causes a periodic heat flow from the solid to the surrounding gas; the resulting pressure fluctuations in the gas are then detected by the microphone.

Instruments

Figure is a block diagram showing the components of a single-beam photoacoustic spectrometer. In this apparatus, the spectrum from the lamp is first recorded digitally, followed by the spectrum for the sample. The stored lamp data are then used to correct the output from the sample for variations in the lamp output as a function of wavelength. With this technique, it is necessary to assume an absence of drift in the source and detector systems. Double-beam instruments, which largely avoid the drift problem, have also been described. One such instrument is equipped with a pair of matched cells (and transducers), one of which contains the sample and the other a reference material such as finely divided carbon. A commercially available instrument is also based upon the split-beam principle. In this instrument, however, about 8% of the output from the grating monochromator is directed to a pyroelectric transducer, the rest passing into the sample cell. The output from the sample transducer is compared with that from the pyroelectric transducer to produce a spectrum that is corrected for variations in the lamp output as a functions of wavelength as well as time.

Applications

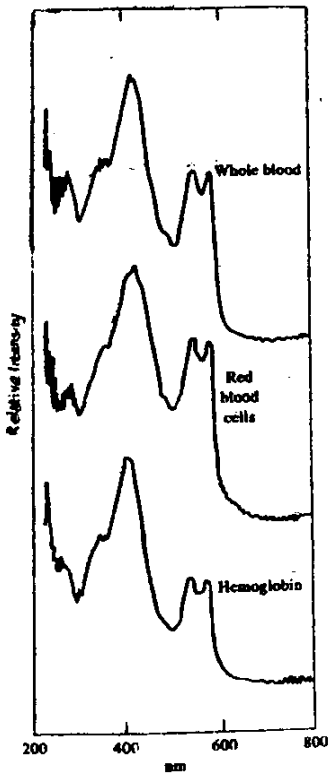
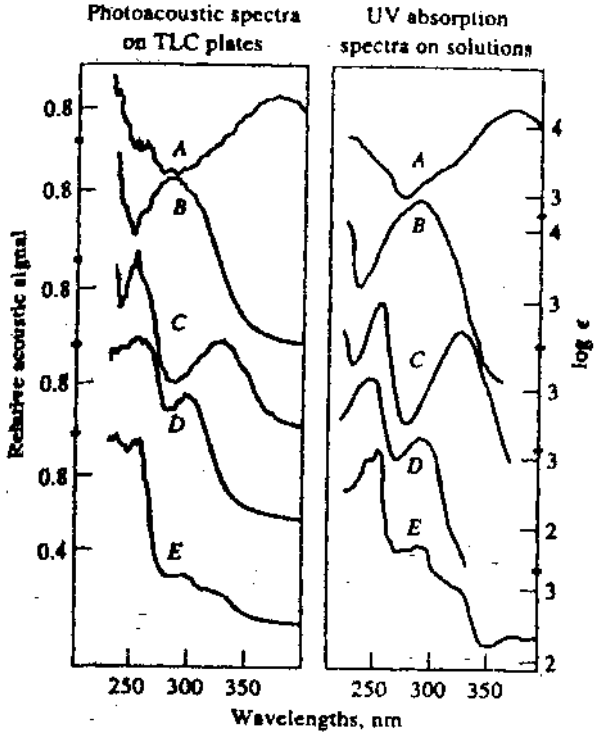


Figure Photoacoustic spectra of smears of blood and Blood components



Spectra of spots on a thin-layer chromatogram (left) and of solutions of the same compounds (right). The compounds are: (A) P-nitroaniline, (B) benzylidene acetone, (C) salicylaldehyde, (D) 1-tetraline and (E) fluorenone

Figure illustrates one application of ultraviolet/visible photoacoustic spectroscopy. Here, photoacoustic spectra of smears of whole blood, blood cells freed of plasma, and hemoglobin extracted from the cells are shown. Conventional spectroscopy, even with very dilute solutions of whole blood, does not yield satisfactory spectra because of the strong light-scattering properties of the blood cells, protein, and lipid molecules present, Photoacoustic spectroscopy permits spectroscopic studies of blood without the necessity of a preliminary separation of these large molecules.

7. Describe the Energy-Level Diagrams for photo luminescent molecules and Instruments for fluorescence and phosphorescence.

Fluorescence occurs in simple as well as in complex gaseous, liquid, and solid chemical systems. The simplest kind of fluorescence is that exhibited by dilute atomic vapors, which was described in Chapter. For example, the 3s electrons of vaporized sodium atoms can be excited to the 3p state by absorption of radiation of wavelengths 5896 and 5890 Å. After 10^{-5} to 10^{-8} s, the electrons return to the ground state, and in so doing emit radiation of the same two wavelengths in all directions. This type of fluorescence, in which the absorbed radiation is reemitted without a change in frequency, is known as resonance radiation or resonance fluorescence.

Many molecular species also exhibit resonance fluorescence. Much more often, however, molecular fluorescence (or phosphorescence) bands are found centered at wavelengths that are longer than the resonance line. This shift toward longer wavelengths, or lower energies, is termed the stokes shift.

Energy-Level Diagrams for Photoluminescent Molecules

Figure is a partial energy-level diagram for a typical photoluminescent molecule. The lowest heavy horizontal line represents the ground-state energy of the molecule, which is normally a singlet state and is labeled S_0. At room temperature, this state represents the energies of essentially all of the molecules in a solution.

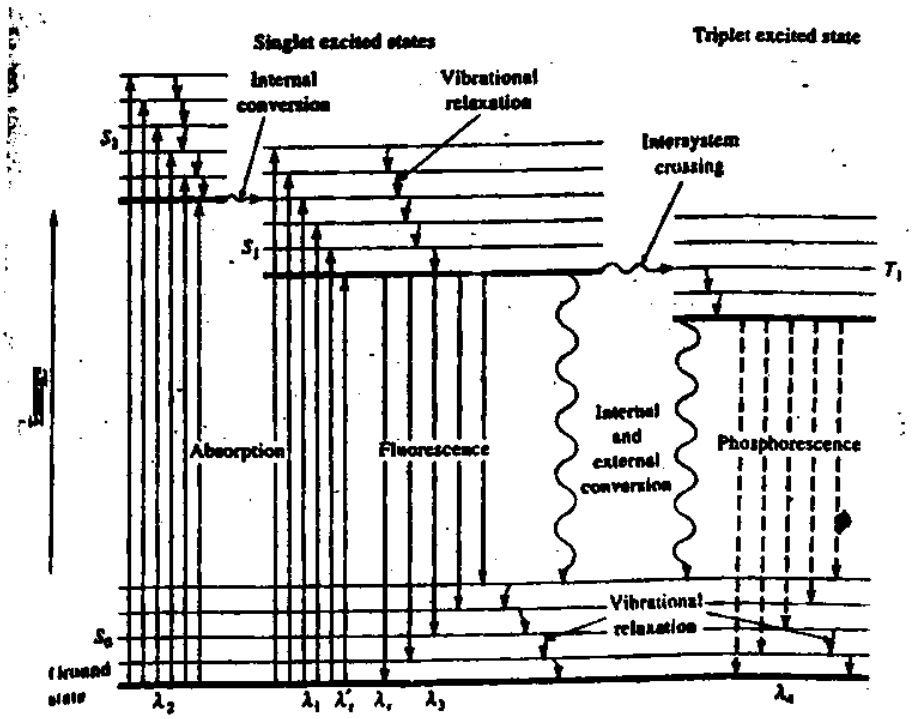


Figure Partial energy diagram for a photoluminescent system

The upper heavy lines are energy levels for the ground vibrational states of three excited electronic states. The two lines on the left represent the first (S_1) and second (S_2) electronic singlet states. The one on the right (T_1) represents the energy of the first electronic triplet state. As is normally the case, the energy of the first excited triplet state is lower than the energy of the corresponding singlet state.

Numerous vibrational energy levels are associated with each of the four electronic states, as suggested by the lighter horizontal lines.

As shown in figure, excitation of this molecule can be brought about by absorption of two bands of radiation, one centered about the wavelength λ_1 ($S_0 \rightarrow S_1$) and the second around the shorter wavelength λ_2 ($S_0 \rightarrow S_2$). Note that the excitation process results in conversion of the molecule to any of the several excited vibrational states. Note also that direct excitation to the triplet state is not shown because this transition does not occur to any significant extent since this process involves a change in multiplicity, an event which, as we have mentioned, has a low probability of occurrence (a low-probability transition of this type is called forbidden).

INSTRUMENTS FOR MEASURING FLUORESCENCE AND PHOSPHORESCENCE

The various components of instruments for measuring photoluminescence are similar to those found in ultraviolet/visible photometers or spectrophotometers. Figure shows a typical configuration for these components in fluorometers and spectrofluorometers. Nearly all

fluorescence instruments employ double-beam optics as shown in order to compensate for fluctuations in the power of the source. The sample beam first passes through an excitation filter or a monochromator, which transmits radiation that will excite fluorescence but excludes or limits radiation of the wavelength of the fluorescence emission. Fluorescence is propagated from the sample in all directions but is most conveniently observed at right angles to the excitation beam; at other angles, increased scattering from the solution and the cell walls may cause large errors in the measurement of intensity. The emitted radiation reaches a phototransducer after passing through a second filter or monochromator that isolates the fluorescence for measurement.

The reference beam passes through an attenuator that reduces its power to approximately that of the fluorescence radiation (the power reduction is usually by a factor of 100 or more). The signals from the reference and sample photomultiplier tubes are then fed into a difference amplifier whose output is displayed by a meter or recorder. Some fluorescence instruments are of the null type, this state being achieved by optical or electrical attenuators. Newer instruments use an analog divider circuit or digital data acquisition followed by data processing to complete the ratio of the fluorescence emission intensity to the excitation source intensity.

The sophistication, performance characteristics, and costs of fluorometers and spectrofluorometers differ as widely as do the corresponding instruments for absorption measurements. Fluorometers are analogous to absorption photometers in that filters are employed to restrict the wavelengths of the excitation and emission beams. Spectrofluorometers are of two types. The first utilizes a suitable filter to limit the excitation radiation and a grating or prism monochromator to produce the fluorescence spectrum. Several commercial spectrophotometers can be purchased with adapters that permit their use as spectrofluorometers.

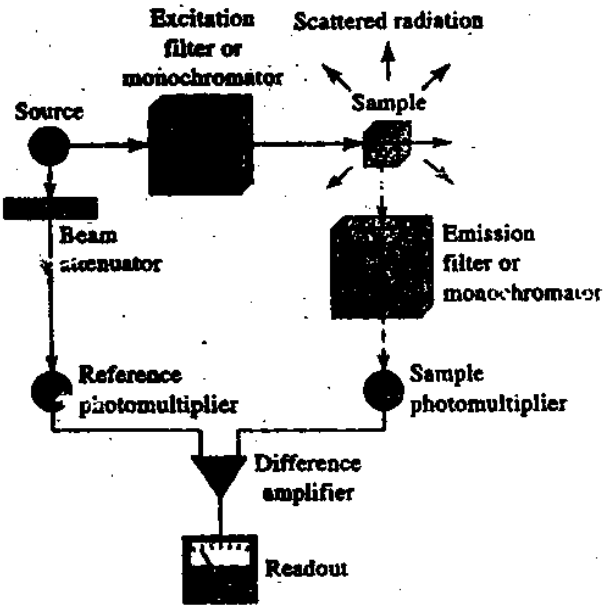


Figure Components of a fluorometer or a spectrofluorometer

The spectrofluorometers are specialized instruments equipped with two monochromators, one of which permits variation in the wavelength of excitation and the other of which allows production f a fluorescence emission spectrum. Figure (a) shows an excitation spectrum for anthracene in which the fluorescence emission was measured at a fixed wavelength, while the excitation wavelength was varied. With suitable corrections for variations in source output intensity and detector response as a function of wavelength, an absolute excitation spectrum is obtained that closely resembles an absorption spectrum.

Figure (b) is the fluorescence emission spectrum for anthracene; here, the excitation wavelength was held constant while scanning the emission wavelengths. These two spectra bear an approximate mirror image relationship to one another because the vibrational energy differences for the ground and excited electronic states are roughly the same.

The selectivity provided by spectrofluorometers is of prime importance to investigations concerned with the electronic and structural characteristics of molecules and is of value in both qualitative and quantitative analytical work. For concentration measurements, however, the information provided by simpler instruments is often entirely satisfactory. Indeed, relatively inexpensive fluorometers, which have been designed specifically to meet the measurement problems peculiar to fluorescence methods, are frequently as specific and selective as modified absorption spectrophotometers.

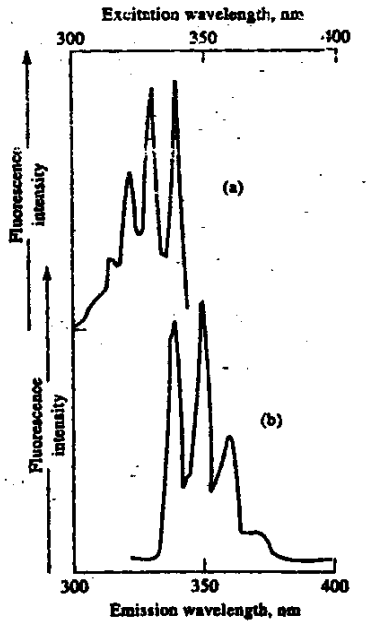


Figure Fluorescence spectra for 1 ppm anthracene in alcohol: (a) excitation spectrum; (b) emission spectrum

The discussion that follows is largely focused on the simpler instruments for fluorescence analysis.

8. Describe the Instrument Designs of Fluorometers and Spectrofluorometers.

Instrument Designs

Fluorometers

Filter photometers provide a relatively simple, low-cost way of performing quantitative fluorescence analyses. As noted earlier, either absorption or interference filters are used to limit the wavelengths of the excitation and emitted radiation. Generally, fluorometers are compact, rugged, and easy to use.

Figure is a schematic of a typical filter fluorometer that utilizes a mercury lamp for fluorescence excitation and a pair of photomultiplier tubes as transducers. The source beam is split bear the source into a reference beam and a sample beam. The reference beam is attenuated by the aperture disk so that its intensity is roughly the same as the fluorescence intensity. Both beams pass through the primary filter with the reference beam then being reflected to the reference photomultiplier tube. The sample beam is focused on the sample by a pair of lenses and causes emission of fluorescence. The emitted radiation passes through a second filter and then is focused on the second photomultiplier tube. The electrical outputs from the two transducers are fed into an analog divider to compute the ratio of the sample to reference intensities, which serves as the analytical variable.

The instrument just described is representative of the dozen or more fluorometers available commercially. Some of these are simpler single-beam instruments. The cost of such fluorometers ranges from a few hundred dollars to about $5000.

Spectrofluorometers

Several instrument manufacturers offer spectrofluorometers capable of providing both excitation and emission spectra. The optical design of one of these, which employs two grating monochromators, is shown in figure. Radiation from the first monochromator is split, part passing to a reference photomultiplier and part to the sample. The resulting fluorescence radiation, after dispersion by the second monochromator, is detected by a second photomultiplier.

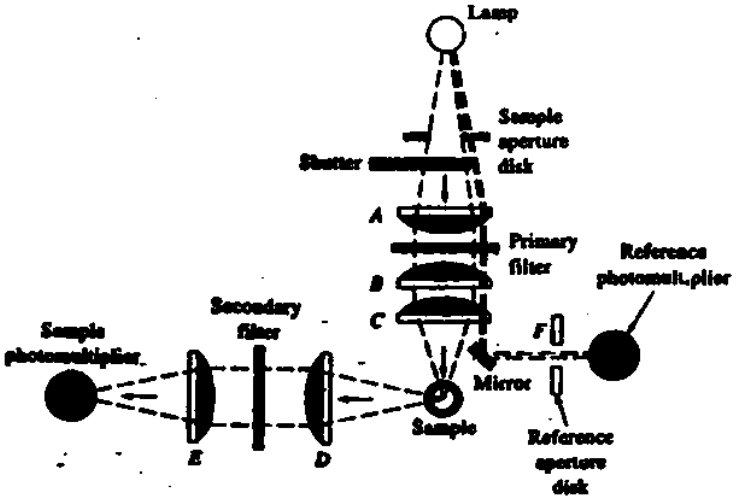


Figure A typical fluorometers

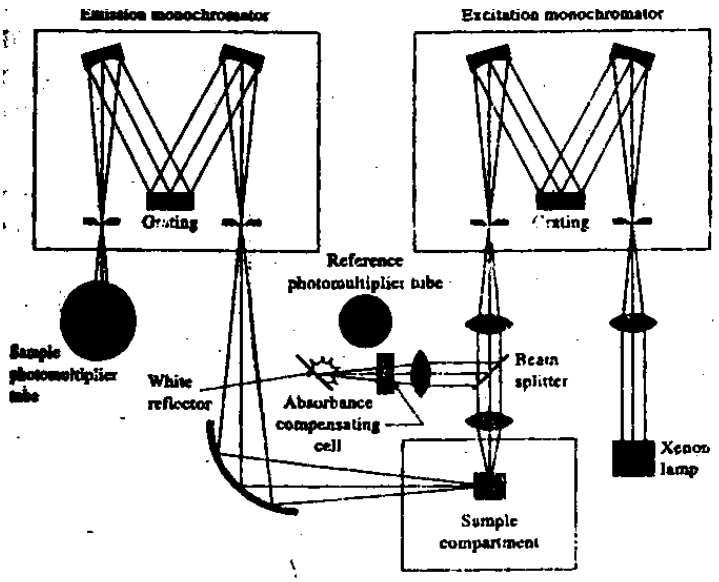


Figure A spectrofluorometer

An instrument such as that shown in figure provides perfectly satisfactory spectra for quantitative analysis. The emission spectra obtained will not, however, necessarily compare well with spectra from other instruments because the output depends not only upon the intensity of fluorescence but also upon the characteristics of the lamp, transducer, and monochromators. All of these instrument characteristics vary with wavelength and differ from instrument to instrument. A number of methods have been developed for obtaining a corrected spectrum, which is the true fluorescence spectrum freed from instrumental effects; many of the newer and more sophisticated commercial instruments provide a means for obtaining corrected spectra directly. – For a summary of correction methods, see N. Wotherspoon, G.K. Oster, and G. Oster, Physical Methods of Chemistry, A. Welssberger and B.R. Rossiter, Eds., Vol. I Part III B, pp.460-462 and pp. 473-478. New York: Wiley-Interscience, 1972.

Spectrofluorometers Based on Array Transducers

During the past two decades, a number of spectrofluorometers utilizing diode-array and charge-transfer devices have been described that permit fluorescence spectra to be obtained in fractions of a second. See, for example, Y. Talmi, D.C. Baker, J.R. Jadamec, and W.A. Saner, Anal. Chem., 1978, 50, 936A; D.W. Johnson, J.B. Callis, and G.D. Christian, Anal. Chem., 1977, 49, 747A; P.M. Epperson, R.D. Jalkian, and M.B. Denton, Anal. Chem., 1989, 61, 282. The principle of the most sophisticated of these is illustrated in figure (a). Here, the length of a sample cell is irradiated with an excitation beam that has been dispersed along the *xy* plane by a monochromator that has been rotated 90 deg with respect to its exit slit. The transducer is a two-dimensional charge-coupled device that sees the dispersed excitation radiation in the *xy* plane and the dispersed radiation from the emission monochromator in the *yz* plane. Figure (b) shows traditional excitation and emission spectra for a hypothetical molecular species. Figure (c) shows the total luminescence spectrum for this compound, which is an isometric projection, sometimes called a stack plot, of the complete excitation and emission spectra of the compound obtained with the arrangement shown in figure (a). Total spectra of this type can be obtained in a few seconds or less and are of particular use for analyzing mixtures of fluorescing species.

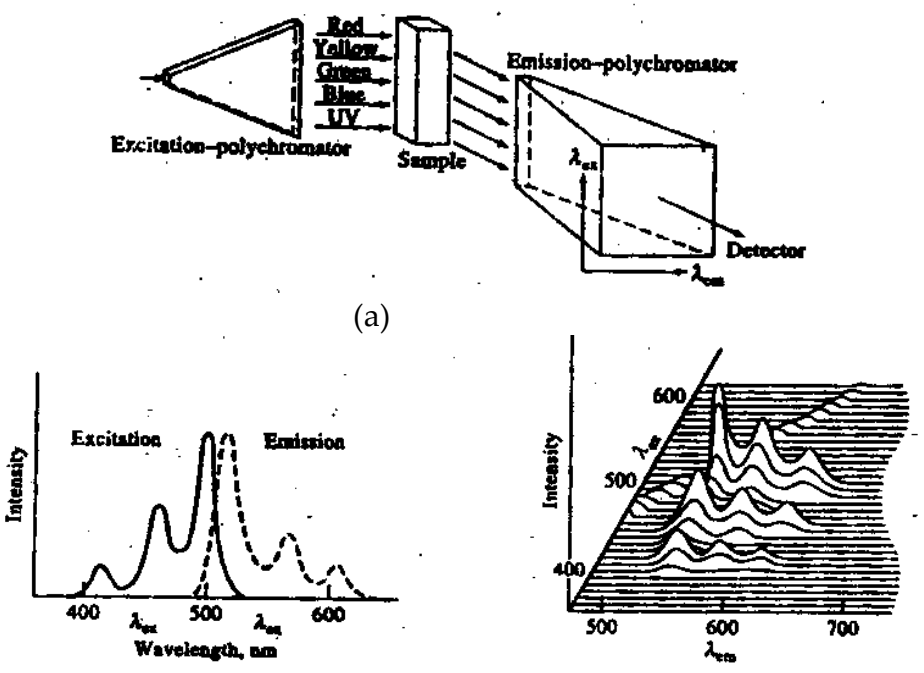


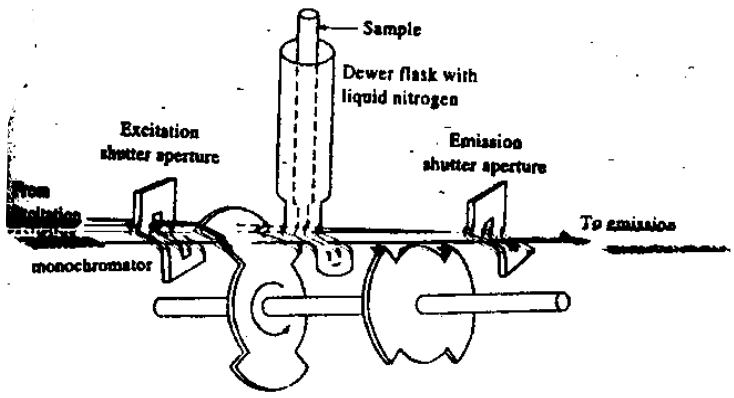
Figure (a) Schematic of an optical system for obtaining a a total luminescence spectrum with a two-dimensional charge-coupled device. (*with permission from G.W. Suter, A.J.Kallir, and U.I.Wild, Chimia, 1981, 37, 413)* (B) Excitation and emission spectra of a hypothetical compound (c) Total luminescence spectrum of compound in b.

Fiber Optic Fluorescence Sensors

Fiber optics has been used to demonstrate that several fluorescence analyses can be carried out at various locations well away from a source and a detector. Here, radiation from a laser source travels through an optical fiber and excites fluorescence in sample solutions. Fluorescence emission then travels back through the same fiber to a detector for measurement. The applicability of this type of device has been extended to nonfluorescing analytes by fixing a fluorescing indicator material to the end of the fiber. For a discussion of fiber optic fluorescence sensors, see O.S. Wolfbeis, in Molecular Luminescence Spectroscopy, S.G. Schulman, Ed., Part 2, Chapter 3. New York: Wiley, 1988. A discussion of the properties of fiber optics and applications to chemical instrumentation is found in Section 7G.

Phosphorimeters

Instruments that have been used for studying phosphorescence are similar in design to the fluorometers and spectrofluorometers just considered except that two additional components are required. See R.J. Hurtubise, Anal. Chem., 1983, 55, 669A; R.J. Hurtubise, Phosphorimetry: Theory, Instrumentation, and Applications, Chapter 3. New York: VCH Publishers, 1990. The first is a device that will alternately irradiate the sample and, after a suitable time delay, measure the intensity of phosphorescence. The time delay is required to differentiate between long-lived phosphorescence emission and short-lived fluorescence emission, both of which would originate from the same sample. Both mechanical and electronic devices are used, and many commercial fluorescence instruments have accessories for phosphorescence measurements. An example of one type of mechanical device is shown in figure.



Ordinarily, phosphorescence measurements are performed at liquid nitrogen temperature in order to prevent degradation of the output by collisional deactivation. Thus, as shown in figure, a Dewar flask with quartz windows is ordinarily a part of a phosphorimeter. At the temperature used, the analyte exists as a solute in a glass or solid solvent. A common solvent for this purpose is a mixture of diethylether, pentane, and ethanol.

9. Describe the Applications of Photoluminescence and Chemiluminescence.

APPLICATIONS AND PHOTOLUMINESCENCE METHODS

Fluorescence and phosphorescence methods are inherently applicable to lower concentration ranges than are absorbance-based spectrophotometric measurements and are among the most sensitive analytical techniques available to the scientist. The enhanced sensitivity arises from the fact that the concentration-related parameter for fluorometry and phosphorimetry F can be measured independently of the power of the source P_0. In contrast, an absorbance measurement requires evaluation of both P_0 and P, because absorbance, which is proportional to concentration, is dependent upon the ratio between these two quantities. The sensitivity of a fluorometric method can be improved by increasing P_0 or by further amplifying the fluorescence signal. In spectrophotometry, in contrast, an increase in P_0 results in a proportionate change in P and therefore fails to affect A. Thus, fluorometric methods generally have sensitivities that are one to three orders of magnitude better than the corresponding absorbance procedures. On the other hand, the precision and accuracy of photoluminescence methods is usually poorer than spectrophotometric procedures by a factor of perhaps two to five. Generally, phosphorescence methods are less precise than their fluorescence counterparts.

Fluorometric Determination of Inorganic Species

For a review of fluorometric determination of inorganic species, see A. Fernandez-Gutierrez and A.M. De La Pena, in Molecular Luminescence Spectroscopy, Part 1, Chapter 4. New York: Wiley, 1985.

Inorganic fluorometric methods are of two types. Direct methods involve the formation of a fluorescing chelate and the measurement of its emission. A second group is based upon the diminution of fluorescence resulting from the quenching action of the substance being determined. The latter technique has been most widely used for anion analysis.

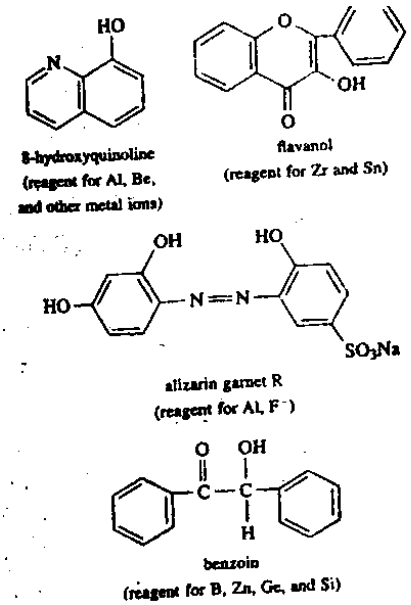
Cations that form Fluorescing Chelates

Two factors greatly limit the number of transition-metal ions that form fluorescing chelates. First, many of these ions are paramagnetic; this property increases the rate of intersystem crossing to the triplet state. Deactivation by fluorescence is thus unlikely although phosphorescence may be observed. A second factor is that transition-metal complexes are characterized by many closely spaced energy levels, which enhance the likelihood of deactivation by internal conversion. Nontransition-metal ions are less susceptible to the foregoing deactivation processes; it is for these elements that the principal inorganic applications of fluorometry are to be found. Note that nontransition-metal cations are generally colorless and tend to form chelates that are also without color. Thus, fluorometry often complements spectrophotometry.

Fluorometric Reagents

For a more detailed discussion of fluorometric reagents, see G.G. Guilbault, in Comprehensive Analytical Chemistry, G. Svehla, Ed., Vol. VIII, Chapter 2, pp. 167-178. New York: Elsevier, 1977; P.A. Straight. Johns, in Trace Analysis, J.D. Winefordner, Ed., pp. 263-271. New York: Wiley, 1976.

The most successful fluorometric reagents for cation analyses have aromatic structures with two or more donor functional groups that permit chelate formation with the metal ion. The structures of four common reagents follow:-



Selected fluorometric reagents and their applications are presented in Table.

TABLE : Selected Fluorometric Methods for Inorganic Species

Ion	Reagent	Wavelength, nm		Sensitivity μg/mL	Interference
		Absorption	Fluorescence		
Al^{3+}	Alizarin garnet R	470	500	0.007	$Be, Co, Cr, Cu, F^-, NO_3^-, Ni, PO_4^{3-}, Th, Zr$
F^-	Al complex of Alizarin garnet R (quenching)	470	500	0.001	$Be, Co, Cr, Cu, Fe, Ni, PO_4^{3-}, Th, Zr$
$B_4O_7^{2-}$	Benzoin	370	450	0.04	Be, Sb

Cd^{2+}	2-(o-Hydroxyphenyl)-benzoxazole	365	Blue	2	NH_3
Li^{+}	8-Hydroxyquinoline	370	580	0.2	Mg
Sn^{4+}	Flavanol	400	470	0.1	F^{-}, PO_4^{3-}, Zr
Zn^{2+}	Benzoin	-	Green	10	B, Be, Sb, Colored ions

Fluorometric Determination of Organic Species

The number of applications of fluorometric analysis to organic and biochemical species is impressive. For example, Weissler and White have listed methods for the determination of over 200 substances that include a wide variety of organic compounds, enzymes and coenzymes, medicinal agents, plant products, steroids, and vitamins. A. Weissler and C,E, White, Handbook of Analytical Chemistry, L. Meites, Ed., pp. 6-182 to 6-196. New York: McGraw-Hill, 1963. Without question, the most important applications of fluorometry are in the analyses of food products, pharmaceuticals, clinical samples, and natural products. See Molecular Luminescence Spectroscopy, S.G. Schulman, Ed., Part 1, Chapters 2, 3 and 5. New York: Wiley, 1985. For a review of bio-analytical applications of fluorescence spectroscopy, see F.V. Bright, Anal. Chem., 1988, 60, 1031A. The sensitivity and selectivity of the method make it a particularly valuable tool in these fields.

Phosphorimetric Methods

Phosphorescence and fluorescence methods tend to be complementary because strongly fluorescing compounds exhibit weak phosphorescence and vice versa. For a review of phosphorimetry, see R.J. Hurtubise, Anal. Chem.,1983, 55, 669A; R.J.Hurtubise, Phosphorimetry, Theory, Instrumentation, and Applications, New York: VCH Publishers, 1990. For example, among condensed ring aromatic hydrocarbons, those containing heavier atoms such as halogens or sulfur often phosphoresce strongly; on the other hand, the same compounds in the absence of the heavy atom tend to exhibit fluorescence rather than phosphorescence.

Phosphorimetry has been used for determination of a variety of organic and biochemical species including such substances as nucleic acids, amino acids, pyrine and pyrimidine, enzymes, petroleum hydrocarbons, and pesticides. The method has not, however, found as widespread use as fluorometry perhaps because of the need for low temperatures and the generally poorer precision of phosphorescence measurements. On the other hand, the potentially greater selectivity of phosphorescence procedures is attractive. The reason for this difference in behavior is that efficient phosphorescence requires rapid intersystem crossing to populate the excited triplet state, which in turn reduces the excited singlet concentration and thus the phosphorescence intensity.

During the past two decades, considerable effort has been expended in the development of phosphorimetric methods that can be carried out at room temperature. These efforts have taken two directions. The first is based upon the enhance4d phosphorescence that is observed for compounds adsorbed on solid surfaces, such as filter paper. In these applications, a solution of the analyte is dispersed on the solid, and the solvent is evaporated. The phosphorescence of the surface is then measured. The rigid matrix minimizes deactivation of the triplet state by collisional quenching has much more of an effect on phosphorescence than on fluorescence because of the much longer lifetime of the triplet state.

The second room temperature method involves solubilizing the analyte in detergent micelles in the presence of heavy metal ions. Apparently the micelles increase the proximity between the heavy metal ions and the phosphor, thus enhancing phosphorescence. See L.J. Cline Love, M. Skrilec, and J.G. Habarta, Anal. Chem., 1980, 52, 754; M. Skrilec and L.J. Cline Love, Anal. Chem., 11980, 52, 1559.

Application of Fluorometry and Phosphorimetry for Detection in Liquid Chromatography

Photoluminescence measurements provide an important method for detecting and determining components of a sample as they appear at the end of chromatographic or capillary electrophoresis columns.

Lifetime Measurements

The measurement of luminescence lifetimes was initially restricted to phosphorescent systems, where decay times were long enough to permit the easy measurement of emitted intensity as a function of time. Equipment is offered by several instrument manufacturers for studying rates of luminescence decay on the fluorescence time scale (10^{-5} to 10^{-8}s). This equipment employs mode-lock lasers that produce pulses of radiation having widths of 70 to 100 ps for excitation and fast-rise-time photomultiplier tubes for detection. Instruments of this kind provide information that is useful in basic studies of energy transfer and quenching. Furthermore, for analytical work, lifetime measurements enhance the selectivity of luminescence methods because they permit the analysis of mixtures containing two or more luminescent species with different decay rates.

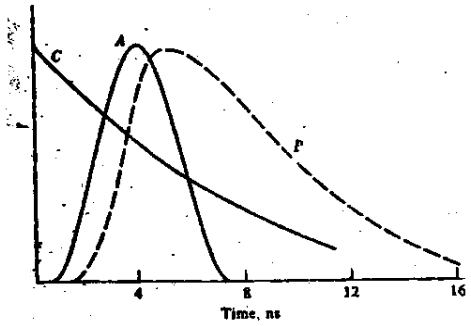


Figure shows curves from a typical fluorescence lifetime experience. Curve A gives the output of the source as a function of time, whereas curve B shows how the observed fluorescence signal decays. Curve B is a composite of the decay signal from the source and the decaying emission signal from the analyte. The true fluorescence decay signal C is then obtained by deconvolving the contribution of the source to the experimental signal. For details of the various methods for measuring fluorescence lifetimes, the reader is referred to the references in the footnote below. For references dealing with lifetime measurements, see L.B. Mc-Gown and F.V. Bright, CRC Crit. Rev. Anal. Chem., 1987, 18, 245; L.B. McGown, Anal. Chem., 1989, 61, 839A; G.M. Hieftje and G.R.Haugen, Anal. Chem., 1984, 56, 1401A.

CHEMILUMINESCENCE

The application of chemiluminescence to analytical chemistry is a relatively recent development. The number of chemical reactions that produce chemiluminescence is small, thus limiting the procedure to a relatively small number of species. Nevertheless, some of the compounds that do react to give chemiluminescence are important components of the environment. For these, the high selectivity, the simplicity, and the extreme sensitivity of the method account for its recent growth in usage. For reviews of analytical applications of chemiluminescence, see M.L. Grayeski, Anal. Chem., 1987, 59, 1243A; Bioluminescence and Chemiluminescence: Instruments and Applications, K. Van Dyke, Ed. Boca Raton; CRC Press, 1985; A.K. Campbell, Chemiluminescence: Principles and Applications in Biology and Medicine. New York: VCH Publishers, 1988.

The Chemiluminescence Phenomenon

Chemiluminescence is produced when a chemical reaction yields an electronically excited species that emits light as it returns to its ground state or that transfers its energy to another species, which then produces emission. Chemiluminescence reactions are encountered in a number of biological systems, where the process is often termed bioluminescence. Examples of species that exhibit bioluminescence include the firefly, the seapansy and certain jellyfish, bacteria, protozoa, and crustacea. The chemistry of the various natural bioluminescence processes is incompletely understood.

Over a century ago, it was discovered that several relatively simple organic compounds also are capable of exhibiting chemiluminescence. The simplest type of reaction of such compounds to produce chemiluminescence can be formulated as

$$A + B \rightarrow C^* + D$$
$$C^* \rightarrow C + h\nu$$

where C* represents the excited state of the species C. Here, the luminescence spectrum is that of the reaction product C. Most chemiluminescence reactions are considerably more complicated than is suggested by the foregoing equations.

For chemiluminescence, the radiant intensity I_{CL} (photons emitted per second) depends upon the rate of the chemical reaction (dC/dt) and the chemiluminescence quantum yield ϕ_{CL} (photons emitted per molecule reacted). The latter term is equal to the product of the excitation quantum yield ϕ_{EX} (excited states per molecule reacted) and the emission quantum yield ϕ_{EM} (photons per excited state). These relationships are described by the equation

$$I_{CL} = \phi_{CL} \frac{dC}{dt} = \phi_{EX} \phi_{EM} \frac{dC}{dt}$$

Chemiluminescence systems that are useful in analysis generally have values of ϕ_{CL} of 0.01 to 0.2.

Measurement of Chemiluminescence

The instrumentation for chemiluminescence measurements is remarkably simple and may consist of only a suitable reaction vessel and a photomultiplier tube. Generally, no wavelength selection device is necessary since the only source of radiation is the chemical reaction between the analyte and reagent. Several instrument manufacturers offer chemiluminescence photometers.

The typical signal from a chemiluminescence experiment as a function of time rises rapidly to a maximum as mixing of reagent and analyte is complete; then a more or less exponential decay of signal follows. Usually for quantitative analysis, the signal is integrated for a fixed period of time and compared with standards treated in an identical way. Alternatively, peak heights are used. Often a linear relationship between signal and concentration is observed over a concentration range of several orders of magnitude.

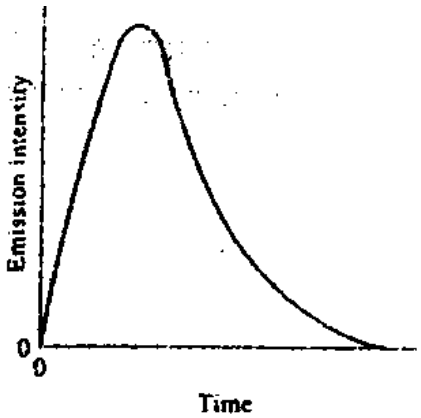


Figure Chemiluminescence emission intensity as a function of time after mixing reagents.

Analytical Applications of Chemiluminescence

For a discussion of various application of chemiluminescence in chemical analysis, see T.A. Nieman, in Handbook of instrumental Techniques for Analytical Chemistry, F. Settle, Ed., Chapter 27, p.541. Englewood Cliffs, NJ: Prentice-Hall, 1997.

Chemiluminescence methods are generally highly sensitive because low light levels are readily monitored in the absence of noise. Furthermore, radiation attenuation by a filter or a monochromator is avoided. In fact, detection limits are usually determined not by transducer sensitivity but rather by reagent purity. Typical detection limits lie in the parts per billion (or sometimes less) to parts per million range. Typical precisions are difficult to judge from the present literature.

Analysis of Gases

Chemiluminescence methods for determining components of gases originated with the need for highly sensitive means for determining atmospheric pollutants such as ozone, oxides of nitrogen, and sulfur compounds. One of the most widely used of these methods is for the determination of nitrogen monoxide; the reactions are

$$NO + O_3 \rightarrow NO_2^* + O_2$$
$$NO_2^* \rightarrow NO_2 + h\nu \quad (\lambda = 600 \text{ to } 2800 \text{ nm})$$

Ozone from an electrogenerator and the atmospheric sample are drawn continuously into a reaction vessel, where the luminescence radiation is monitored by a photomultiplier tube. A linear response is reported for nitrogen monoxide concentration of 1 ppb to 10,000 ppm. This procedure has become the predominant one for monitoring the concentration of this important atmospheric constituent from ground level to altitudes as high as 20 km.

The reaction of nitric oxide with ozone has also been applied to the determination of the higher oxides of nitrogen. For example, the nitrogen dioxide content of automobile exhaust gas has been determined by thermal decomposition of the gas at 700°C in a steel tube. The reaction is

$$NO_2 \rightarrow NO + O$$

At least two manufacturers now offer an instrument for determination of nitrogen in solid or liquid materials containing 0.1% to 30% nitrogen. The samples are pyrolyzed in an oxygen atmosphere under conditions whereby the nitrogen is converted quantitatively to nitrogen monoxide; the latter is then measured by the method just described.

Another important chemiluminescence method is used for monitoring atmospheric ozone. In this instance, the determination is based upon the luminescence produced when the analyte reacts with the dye rhodamine-B adsorbed on an activated silica gel surface. This procedure is sensitive to less than 1 ppb ozone. Ozone can also be determined in the gas phase based on the chemiluminescence produced when the analyte reacts with ethylene. Both reagents are reported to be specific for ozone.

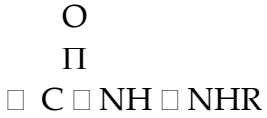
Still another important gas-phase chemiluminescence method is used for the determination of atmospheric sulfur compounds such as hydrogen sulfide, sulfur dioxide, and mercaptans. Here, the sample is combusted in a hydrogen flame to give a sulfur dimer, which then decomposes with the emission of light. For example with sulfur dioxide the reactions are

$$4H_2 + 2\,SO_2 \rightarrow S_2^* + 4H_2O$$
$$S_2^* \rightarrow S_2 + h\nu$$

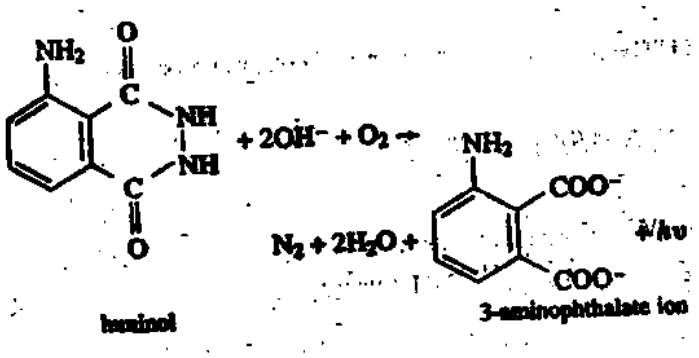
Here, the radiation occurs in the blue with peaks at 384 ands 394 nm. Its intensity is proportional to the concentration of the excited sulfur dimer. Similarly combustion of phosphorus compounds in a hydrogen flame gives emission due to HPO* at 526 nm. Linear working curves over four decades of concentration are reported. Both of these flame chemiluminscence techniques have been employed for detection of sulfur and phosphorus species in the effluent from gas chromatographic columns.

Analysis for Inorganic Species in the Liquid Phase

Many of the analyses carried out in the liquid phase make use of organic chemiluminescing substances containing the functional group



These reagents react with oxygen, hydrogen peroxide, and many other strong oxidizing agents to produce a chemiluminescing oxidation product. Luminol is the most common example of these compounds. Its reaction with strong oxidants, such as oxygen, hydrogen peroxide, hypochlorite ion, and permanganate ion, in the presence of strong base is given below. Often a catalyst is required for this reaction to proceed at a useful rate. The emission produced matches the fluorescence spectrum of the product, 3-aminophthalate anion; the chemiluminescence appears blue and is centered around 425 nm.



Within certain limits, the chemiluminescence intensity of luminal is directly proportional to the concentration of either the oxidant, the catalyst, or the luminal. Consequently, the reaction provides a sensitive method for determining any one of these species. For example, using hydrogen peroxide as the oxidant, the catalyst Co^{2+} can be estimated at concentrations down to 0.01 nmol/L. With a few cations, inhibition of luminescence occurs; in such cases the decrease in intensity permits determination of concentrations.

Analysis for Organic Species

To increase the selectivity of chemiluminescence reactions and to extend chemiluminescence to analytes not directly involved in chemiluminescence reactions, it is common practice to precede a chemiluminescence step by an enzyme reaction for which the desired analyte is the substrate and one of the products is detected by chemiluminescence. This is most commonly done in flow systems with columns of immobilized enzyme. Recently, however, attention has been directed toward biosensor designs using enzymes attached to optical fibers.

Oxidase enzymes that generate H_2O_2 are commonly used in the predetection step. Not only can H_2O_2 be determined with several different chemiluminescence systems, but the necessary oxidant (O_2) is already present in most samples, especially those in aqueous solution. Assuming quantitative conversion by the enzyme, substrates can be determined down to 10 to 100 nM, just as can H_2O_2. Substrates detected this way include glucose, cholesterol, choline, uric acid, amino acids, aldehydes, and lactate. For example:

$$\text{Uric acid} + O_2 \xrightarrow{\text{uricase}} \text{allantoin} + H_2O_2$$

The approach can be extended by using sequential enzyme steps to ultimately convert the analyte to an equivalent amount of chemiluminescent product. In this way, sugars other than glucose, glucosides, cholesterol esters, creatinine, and acetylcholine have been determined. C.A.K. Swindlehurst and T.A. Nieman, Anal. Chin. Acta, 1988, 205, 195. For example:

$$\text{sucrose} + H_2O \xrightarrow{\text{invertase}} \alpha - D - \text{glucose} + \text{fructose}$$

$$\alpha\text{-D-glucose} \rightleftharpoons \beta - D - \text{glucose}$$

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

Luminol plus a peroxidase catalyst appears to be the optimal system for H_2O_2 determination. Peak chemiluminescence intensity is reached in about 100 ms; the solvent is aqueous but is compatible with some organic components; the detection limit is about 0.1 pM, with linearity for three to four decades of concentration.

10. Describe the Infrared Sources and Infrared Instruments.

INFRARED SOURCES AND TRANSDUCERS

Instruments for measuring infrared absorption all require a source of continuous infrared radiation and a sensitive infrared transducer. The desirable characteristics of these instrument components were listed in Section 7B and 7E. In this section we describe sources and transducers that are found in current – day infrared instruments.

Sources

Infrared sources consist of an inert solid that is heated electrically to a temperature between 1500 and 2200K. Continuum radiation approximating that of a black body results 9 See figure) the maximum radiant intensity at these temperatures occurs between 5000 to 5900 cm^{-1} (2 to 1.7 μm). At longer wavelengths, the intensity falls off smoothly until it is about 1% of the maximum at 670 cm^{-1} (15μm). On the short wavelength side, the decrease is much more rapid, and a similar reduction in intensity is observed at about 10,000 cm^{-1} (1 μm).

The Nernst Glower

The Nernst glower is composed of rare earth oxides formed into a cylinder having a diameter of 1 to 2 mm and a length of perhaps 20mm. Platinum leads are sealed to the ends of the cylinder to permit electrical connection to what amounts to a resistive heating element. As current is passed though the device, temperatures between 1200 K and 2200K result. The Nernst glower has a large negative temperature coefficient of electrical resistance, and it must be heated externally to a dull red heat before the current is large enough to maintain the desired temperature. Because the resistance decreases with increasing temperature, the source circuit must be designed to limit the current; otherwise the glower rapidly becomes so hot that it is destroyed.

Figure shows the spectral output of a Nernst glower operated at approximately 2200K. Note that the curve resembles that of a blackbody; the small peaks and depressions are a function of the chemical composition of the device.

The Globar Source

A globar is a silicon carbide rod, usually about 50mm in length and 5mm in diameter. It also is electrically heated (1300 to 1500K) and has the advantage of a positive co-efficient of resistance. On the other hand, water cooling of the electrical contacts is required to prevent arcing. Spectral energies of the Globar and the Nerst glower are comparable except in the region below 5μm, where the Globar provides a significantly greater output.

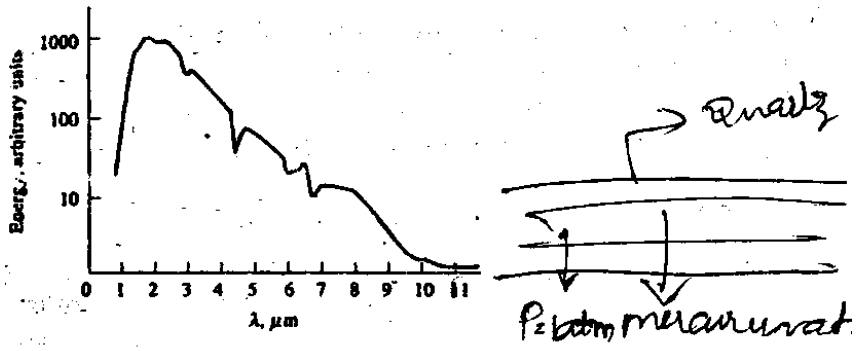


Figure Spectral distribution of energy from a Nernst glower operated approximately 2200K.

Incandescent Wire Source

A source of some what lower intensity but longer life than the Globar or Nernst glower is a tightly wound spiral of nichrome wire heated to about 1100K by an electrical current. A rhodium wire heater scaled in a ceramic cylinder has similar properties as a source.

The Mercury Arc

For the far- infrared region of the spectrum ($\lambda > 50$ μm), none of the thermal sources just described provides sufficient radiant power for convenient detection. Here, a high- pressure mercury arc is used. This device consists of a quartz-jacketed tube containing mercury vapor at a pressure grater than one atmosphere. Passage of electricity through the vapor forms an internal plasma source that provides continuum radiation in the far-infrared region.

The Tungsten Filament Lamp

An ordinary tungsten filament lam is a convenient source for the near –infrared region of 4000 to 12,800 cm^{-1} (2.5 to 0.78μm).

The Carbon Dioxide Laser Source

A tunable carbon dioxide laser is used as an infrared souce for monitoring the concentrations of certain atmospheric pollutants and for determining absorbing species in aqueous solutions. See H.R. Jones and P.A Wilks jt. Amer.Lab., 1982, 14 (3), 87;p LB Kreuzer, Anal. Chem 1974, 46,239A. A carbon dioxide laser produces a band of tradition in the 900 to 1100 cm^{-1} (11 to 9μm) range, which consists of about 100 closely spaced discrete lines. As described on page 151, any one of these lines can be chosen by tuning the laser. At though the range of wave lengths available is limited, the 900 to 1100 cm^{-1} region is one particularly rich in absorption bands arising from the interactive stretching modes of CO_2. Thus, this source is useful for quantitative determination of a number of important species such as ammonia, butadiene, benzene e, ethanol, nitrogen dioxide, and trichloroethylene. An important property of the laser source is the radiant power available in each line, which is several orders of magnitude greater than that of block body sources.

Three types of instruments for infrared absorption measurements are available from commercial sources. (1) dispersive grating spectrophotometers that are used primarily for qualitative work; (2) multiplex instruments, employing the Fourier transform (Section 71), that are suited to both qualitative and quantitative infrared measurements; and (3) nondispersive photometers that have been developed for quantitative determination of a variety of organic species in the atmosphere by absorption, emission, and reflectance spectroscopy.

Until the 1980s, the most widely used instruments for infrared measurements were dispersive spectrophotometers. Now, however, this type of instrument has been largely displaced by Fourier transform spectrometers because of their speed, reliability, and convenience. Only when cost is a major consideration are dispersive instruments still employed.

Fourier Transform Spectrometers

The theoretical basis and the inherent advantages of multiplex instruments were discussed in some details in Section, and the reader may find it worth while to review that section before proceeding further here. Two types of multiplex instruments have been described for the infrared region. In one, coding is accomplished by splitting the source into two beams whose path lengths can be varied periodically to give interference patterns. The Fourier transform is then used for data processing. For detailed discussions of Fourier transform infrared spectroscope see B.C. Smith, Fourier Transform Infrared Sepctroscopy. Boca Ratone CRC Prress, 1996; P.R. Griffiths and J.A. Dehaseth, Fourier Transformed Infrared spectroscopy, J. Ferraro and L. Basile, Eds. New Yourk; Acadamic Press, 1979. For review articles, see W.D. Perkins, j. Chem Editor 1986, 63, As, A269; L. Glaser, J. Chem educ., 1987, 64, A228, A2606.

The second is the Hadamard transform spectrometer, which is a dispersive instrument that employs a moving mask at the focal plane of a monochromator for encoding the spectral data. Hadmard transform infrared instruments have not been widely adopted and will, therefore, not be discussed further in this text. For a description of the Hadamard transform and Hadamard transform spectrometers, see M.O. Harwit and N.J.A Sloane, Hadamard Transform optics. New York; Acadamic press, 1979; Transform Techniques in Chemistry, P.R. Grifiths, Ed. New York ; Plenum Press, 1978. For a recent review of analytical applications of the Hadamard transform, see P.J. Treado and M.D. Morris, Anal, chem.., 1989, 61, 723A.

When Fourier transform infrared (FTIR) spectrometers first appeared in the marketplace, they were bulky, expensive (>$100,000), and required frequent mechanical adjustments. For these reasons, their use was limited to special applications where their unique characteristics (speed, high resolutions, sensitivity, and unparalleled wavelength precision and accuracy) were essential. Fourier transform instruments have now been reduced to benchtop size and have become reliable and easy to maintain. Furthermore, the price of simpler models has been reduced to the point where they are competitive with all but the simplest dispersive instruments ($15,000 to $ 20,000). For these reasons, Fourier transform instruments have largely displaced dispersive instruments in the laboratory. Sec S. A. Borman, Anal. Chem.., 1983, 55,1054A.

Components of Fourier Transform Instruments

The majority of commercially available Fourier transform infrared instruments are based upon the Michelson interferometer, although other types of optical systems are also encountered. We shall consider the Michelson design only, which is illustrated in figure, page 186. The Michelson interferometer was designed and built in 1891 by A.A. Michelson, He was awarded the 1907 Nobel Prize in physics for the invention of interferometry.

Drive Mechanism

A requirement for satisfactory interferograms (and thus satisfactory spectra) is that the speed of the moving mirror be constant and its position exactly known at any instant. The planarity of the mirror must also remain constant during its entire sweep of 10cm or more.

In the far-infrared region, where wavelengths range from 50 to 1000 μm(200 to 10cm^{-1}), displacement of the mirror by a fraction of a wave length, and accurate measurement of its position, can be accomplished by means of a motor-driven micrometer screw. A more precise and sophisticated mechanism is required for the mid-and near- infrared regions, however, Here, the mirror mount is generally floated on an air bearing held with in close – fitting stainless steel sleeves (See figure). The mount is driven by a linear drive motor and an electromagnetic coil similar to the voice coil in a loudspeaker; an increasing current in the coil drives the mirror at

constant velocity. After reaching its terminus, the mirror is returned rapidly to the starting point for the next sweep by a rapid reversal of the current. The length of travel varies form 1 to about 20cm; the scan rates range form 0.01 to 10cm/s.

Two additional features of the mirror system are necessary for successful operation. The first is a means of sampling the interferogram at precisely spaced retardation intervals. The second is a method for determining exactly the zero retardation point in order to permit signal averaging. If this point is not known precisely, the signals from repetitive sweeps would not be in phase; averaging would then tend to degrade rather than improve the signal.

The problem of precise signal sampling and signal averaging can be accomplished by using three interferometers rather than one, with a single mirror mount holding the three movable mirrors. Figure is a schematic showing such an arrangement. The components and radiation paths for each of the three interferometer systems are indicated by the subscripts, 1,2, and 3, respectively. System 1 is the infrared system that ultimately provides an interferogram similar to that shown as curve A in figure. System 2 is a so-called laser – fringe reference system, which provides sampling – interval information. It consists of a helium/neon laser S_2, an interferometric system including mirrors MM_2 and M_2, a beam splitter B_2, and a transducer T_2. The output from this system is a cosine wave, as shown in C of Figure. This signal is converted electronically to the square- wave form shown in D; sampling begins or terminates at each successive zero crossing. The laser –fringe reference system gives a highly reproducible and regularly spaced sampling interval. In most instruments, the laser signal is also used to control the speed of the mirror – drive system at a constant level.

The third interferometer system, sometimes called the white – light system, employs a tungsten source S_3 and transducer T_3 sensitive to visible radiation. Its mirror system is fixed to give a zero retardation that is displaced to the left from that for the analytical signal (see interferogram B, figure). Because the source is polychromatic, its power at zero retardation is much larger than any signal before and after that point.

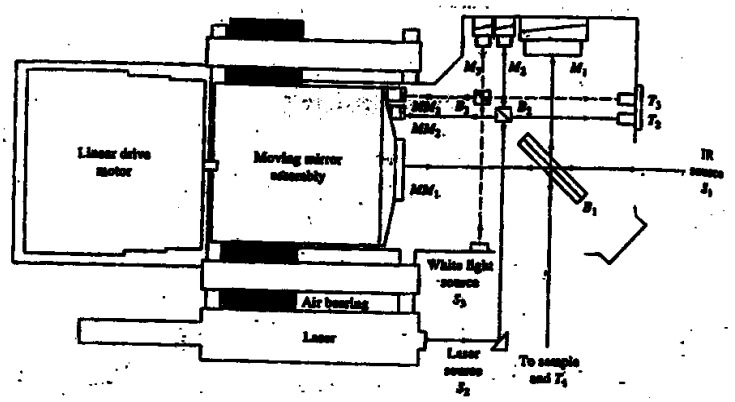


Figure Interferometers in an infrared Fourier transform spectrometer. Subscript 1 defined the radiation path in the infrared interferometer; subscripts 2 and 3 refer to the laser and white light interferometers, respectively.

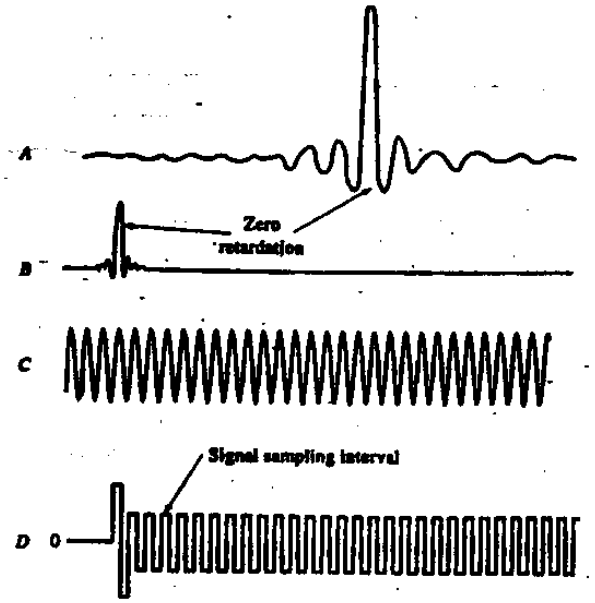


Figure Time domain signals for the three interferometers contained in a Fourier transform infrared instrument. Curve A: Infrared signal: curve B: white light signal: Curve C: laser – fringe reference signal; curve D square-wave electrical signal formed form the laser signal.

Thus, this maximum is used to trigger the start of data sampling for each sweep at a highly reproducible point.

The triple interferometer system just described leads to remarkable precision in determining spectral frequencies, which significantly exceeds that realizable with conventional grating instruments. This high reproducibility is particularly important when many scans are to be averaged. Contemporary instruments, such as the one shown in figure are able to achieve this same frequency precious with only a single interferometer. In this increment, the laser beam is ether parallel to or collinear with the infrared beam so that both beams traverse a single interferometer. Furthermore, no white- light source is employed, and the infrared interferogram is used to establish zero retardation. The maximum in the infrared interferogram is an excellent reference because this is the only point at which all wave lengths interfere constructively.

The system shown in figure is capable of providing spectra with a resolution of between 0.1 and 1 cm^{-1}. To obtain resolutions of 0.01 cm^{-1} requires a more sophisticated system for maintaining the alignment of the moving mirror. One mirror alignment system uses three laser – fringe reference systems that are directed at different points on the moving mirror instead of one. Because three points are adequate to define a plane, the use of three lasers significantly increases the accuracy with which the position and orientation of the mirror can be known at any instant.

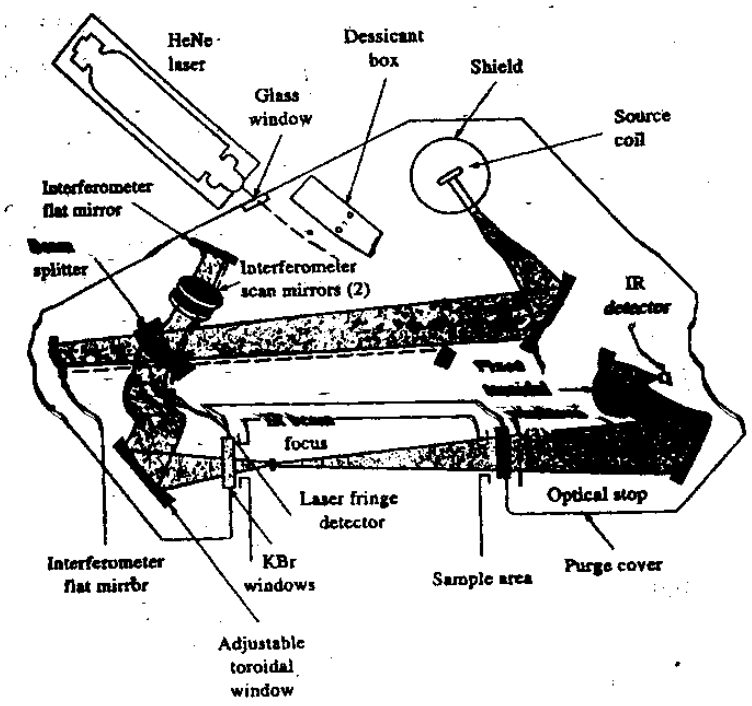


Figure A single – beam FTIR spectrometer.

Beam Splitters

Beam splitters are constructed of transparent materials with refractive indices such that approximately 50% of the radiation is reflected and 50% is transmitted. A widely used material for the far-infrared region is a thin film of Mylar sandwiched between two plates of a low-refractive-index solid. Thin films of germanium or silicon deposited on cesium iodide or bromide, sodium chloride, or potassium bromide are satisfactory for the mid – infrared region. A film of iron (III) oxide is deposited on calcium fluoride for work in the near – infrared.

Sources and Transducers

The sources for Fourier transform infrared instruments are similar to those discussed earlier in this chapter. Generally, thermal transducers are not readily adapted to Fourier transform instruments because of their slow response times. Triglycine sulfate pyroelectric transducers are widely used for the mid infrared region. Where better sensitivity or faster response times are required liquid –nitrogen cooled mercury/cadmium telluride or indium antimonide photoconductive transducers are employed.

Instrument Designs

Fourier transform infrared spectrometers are usually singe – beam instruments. Figure shows the optics of a less expensive spectrometer, which sells in the $16,000 to $20,000 range. A

typical procedure for determining transmittance or absorbance with this type of instruments is to first obtain a reference interferogram by scanning a reference (usually air) 20 or 30 times, coadding the data, and storing the results in the memory of the instrument computer (usually after transforming it to the spectrum). A sample is then inserted in the radiation path and the process repeated. The ratio of sample and reference spectra data is then computed to give the transmittance at various frequencies. Ordinarily, modern infrared sources and detectors are sufficiently stable so that reference spectra need to be obtained only occasionally.

Performance Characteristics of Commercial Instruments

A number of instrument manufactures offer several models of Fourier transform infrared instruments. The least expensive of these has a range of 7800 to 350 cm^{-1} (1.3 to 29μm) with a resolution of $4cm^{-1}$. This performance can be obtained with scan time as brief as one second. More expensive instruments with interchangeable beam splitters, sources, and transducers offer expanded frequency ranges and higher resolution for example, one instrument is reported to produce spectra form the far – infrared ($10cm^{-1}$ or 1000μm) through the visible region to 25,000 cm^{-1} or 400nm. Resolutions for commercial instruments vary form 8 to less than $0.01cm^{-1}$. Several minutes are required to obtain a complete spectrum at the highest resolution. D. Noble, Anal, Chem., 1995,67,381A.

Advantages of Fourier Transform

Spectrometers

For a comparison of performance characteristics of typical dispersive and interferometric instruments, see D.H. Chenery and N. Sheppard Appl. Spetrosc., 1978, 32,79; W.D Perkins, j. Chem Edu., 1987, 64, A269.

Over most of the mid – infrared spectral range Fourier transform instruments appear to have signal – to noise ratios that are better than those of a good – quality dispersive instrument by more than an order of magnitude. The enhanced signal-to- noise ration can, of course, be traded for rapid scanning, with good spectra being attainable in a few seconds in most cases. Interferometric instruments are also characterized by high resolutions (<0.1 cm^{-1}) and highly accurate and reproducible frequency determinations. The latter property is particularly helpful when spectra are to be subtracted for back ground correction.

A theoretical advantage of Fourier transform instruments is that their optics provide a much larger energy throughput (one or two orders of magnitude) than do dispersive instruments, which are limited in through put by the necessity of narrow slit width. The potential gain here, however, may be partially offset by the lower sensitivity of the fast-response detector required for

the interferometric measurements. Finally, it should be noted that the interferometer is free from the problem of stray radiation because each infrared frequency is, in effect chopped at a different frequency.

The areas of chemistry where the extra performance of interferometric instruments have been particularly useful include: (1) very high- resolution work that is encountered with gaseous mixtures having complex spectra resulting from the superposition of vibrational and rotational bands; (2) the study of samples with high absorbances; (3) the study of substances with weak absorption bands (for example, the study of compounds that are chemisorbed on catalyst surfaces);(4) investigations requiring fast scanning such as kinetic studies or detection of chromatographic effluents; (5) Collecting infrared data from very small samples, (6) Obtaining reflection spectra; (7) infrared emission studies.

11. Describe the theory of Raman Spectroscopy.

Theory of Raman Spectroscopy

Raman spectra are acquired by irradiating a sample with a powerful laser source of visible or near-infrared monochromatic radiation. During irradiation, the spectrum of the scattered radiation is measured at some angle (often 90 deg) with a suitable spectrometer. At the very most, the intensities of Raman lines are 0.001% of the intensity of the source; as a consequence, their detection and measurement are somewhat more difficult than are infrared spectra. An exception to this statement may be found in resonance Raman spectroscopy, which is considerably more sensitive than normal Raman spectroscopy. Resonance Raman spectroscopy is described in section 18D-1.

Excitation of Raman Spectra

Figure depicts a portion of a Raman spectrum that was obtained by irradiating a sample of carbon tetrachloride with an intense beam of an argon ion laser having a wavelength of 488.0nm (20492 cm^{-1}). The emitted radiation is of three types: stokes scattering, anti-Stokes scattering, and Rayleigh scattering. The last, whose wavelength is exactly that of the excitation source, is significantly more intense than either of the other two types.

As is usually the case for Raman spectra, the abscissa of figure is the wave number shift $\Delta\bar{\nu}$, which is defined as the difference in wave numbers (cm^{-1}) between the observed radiation and that of the source. Note that three Raman peaks are found on both side of the Rayleigh peak and that the pattern of shifts on each side is identical. That is, Stokes lines are found at wave numbers that are 218, 314, and 459 cm^{-1} smaller than the Rayleigh peak while anti-Stokes peaks occur at 218, 314, and 459 cm^{-1} greater than the wave number of the source. It should also be noted that additional

lines can be found at ±762 and ± 790 cm^{-1} as well. Quite generally, anti-Stokes lines are appreciably less intense than the corresponding Stokes lines. For this reason, only the Stokes part of a spectrum is generally used. Furthermore, the abscissa of the plot is often labeled simple frequency in cm^{-1} rather than wave number shift $\Delta\bar{\nu}$. it is noteworthy that fluorescence may interfere seriously with the observation of Stokes shifts but not with anti-Stokes. With fluorescing samples, anti-Stokes signals may, therefore, be more useful despite their lower intensities.

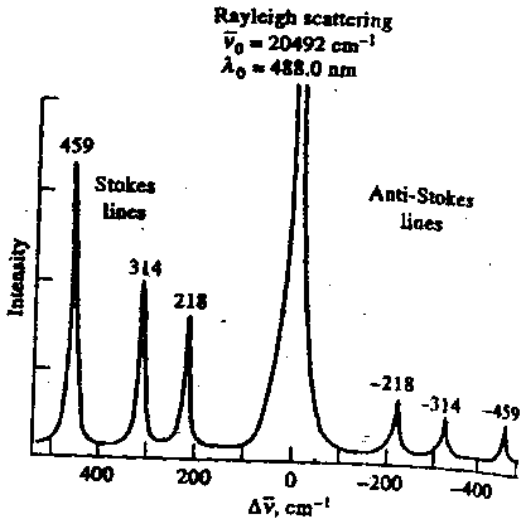


Figure: Raman spectrum for CCl_4 excited by laser radiation of λ_0=488 nm and $\bar{\nu}_0$=20,492 cm^{-1}. the number above the peaks is the Raman shift, $\Delta\bar{\nu} = \left(\bar{\nu}_s - \bar{\mu}_0\right) cm^{-1}$.

It is important to appreciate that the magnitude of Raman shifts are independent of the wavelength of excitation. Thus, shift patterns identical to those shown in figure would be observed for carbon tetrachloride regardless of whether excitations was carried out with a krypton ion laser (488.0), a helium/neon laser (632.8nm), or a Nd:YAG laser (1064nm).

Superficially, the appearance of Raman spectral lines at lower energies (longer wavelengths) is analogous to the Stokes shifts found in a fluorescence experiment; for this reason, Raman shifts to longer wavelengths are called Stokes shifts. We shall see, however, that Raman and fluorescence spectra arise from fundamentally different processes; thus, the application of the same terminology to both fluorescent and Raman spectra is perhaps unfortunate.

Mechanism of Raman and Rayleigh scattering

In Raman spectroscopy, spectral excitation is normally carried out by radiation having a wavelength that is well away from any absorption peaks of the analyte. The

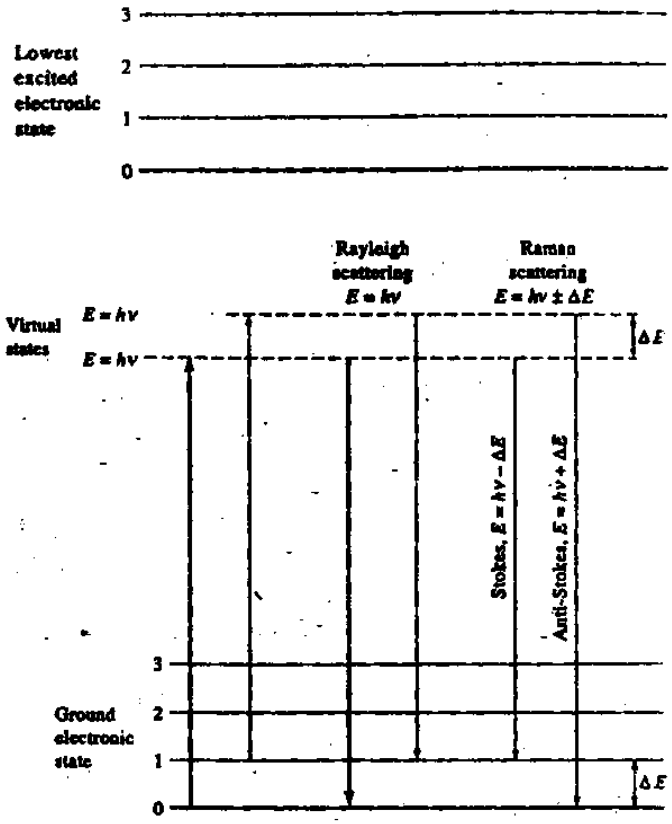


Figure: Origin of Rayleigh and Raman scattering

energy-level diagram in figure provides a qualitative picture of the sources of Raman and Rayleigh scattering. The heavy arrow on the far left depicts the energy change in the molecule when it interacts with a photon from the source. The increase in energy is equal to the energy of the photon *hv*. It is important to appreciate that the process shown is not quantized; thus, depending upon the frequency of the radiation from the source, the energy of the molecule can assume any of an infinite number of values, or virtual states, between the ground state and the first electronic excited state shown in the upper part of the diagram. The second and narrower arrow shows the type of change that would occur if the molecule encountered by the photon happened to be in the first vibrational level of the electronic ground state. At room temperature, the fraction of the molecules in this state is small. Thus, as indicated by the width of the arrows, the probability of this process occurring is much smaller.

The middle set of arrows depicts the changes that produce Rayleigh scattering. Again the more probable change is shown by the wider arrow. Note that no energy is lost in Rayleigh scattering. As a consequence, the collisions between the photon and the molecule are said to be elastic.

Finally, the energy changes that produce stokes and anti-stokes emission are depicted on the right. The two differ from the Rayleigh radiation by frequencies corresponding to $\pm\Delta E$, the

energy of the first vibrational level of the ground state. Note that if the bond were infrared active, the energy of its absorption would also be ΔE. Thus, the Raman frequency shift and the infrared absorption peak frequency are identical.

Note also that the relative populations of the two upper energy states are such that Stokes emission is much favored over anti-Stokes emission is much favored over anti-Stokes. In addition, Rayleigh scattering has a considerably higher probability of occurring than Raman because the most probable event is the energy transfer to molecules in the ground state and reemission by the return of these molecules to the ground state. Finally, it should be noted that the ratio of anti-stokes to stokes intensities will increase with temperature because a larger fraction of the molecules will be in the first vibrationally excited state under these circumstances.

Wave Model of Raman and Rayleigh scattering

Let us assume that a beam of radiation having a frequency v_{ex} is incident upon a solution of an analyte. The electric field E of this radiation can be described by the equation.

$$E=E_0 \cos (2\pi v_{ex} t)$$

where E_0 is the amplitude of the wave. When the electric field of the radiation interacts with an electron cloud of an analyte bond, it induces a dipole moment m in the bond that is given by

$$m=\propto E=\propto E_0 \cos (2\pi v_{ex} t)$$

where $\propto$ is a proportionality constant called the polarizability of the bond. This constant is a measure of the deformability of the bond in an electric field.

In order to be Raman active, the polarizability $\propto$ of a bond must vary as a function of the distance between nuclei according to the equation

$$\alpha = \alpha_0 + \left(r - r_{eq}\right)\left(\frac{\partial \alpha}{\partial r}\right)$$

where $\propto_0$ is the polarizability of the bond at the equilibrium internuclear distance r_{eq} and r is the internuclear separation at any instant. The change in internuclear separation varies with the frequency of the vibration v_v as given by

$$r - r_{eq} = r_m \cos (2\pi v_v t)$$

where r_m is the maximum internuclear separation relative to the equilibrium position. Substituting Equation gives

$$\alpha = \alpha_0 + \left(\frac{\partial \alpha}{\partial r}\right) r_m \cos\left(2\pi v_v t\right)$$

we can then obtain an expression for the induced dipole moment m by substituting Equation into Equation thus,

$$m = \alpha_0 E_0 \cos(2\pi v_{ex} t) + E_0 r_m \left(\frac{\partial \alpha}{\partial r}\right) \cos(2\pi v_v t) \cos(2\pi v_{ex} t)$$

Recall from trigonometry that

Cos x cos y = [cos (x+y) +cos (x-y)]/2

Applying this identity to Equation gives

$$m = \alpha_0 E_0 \cos(2\pi v_{ex} t) + \frac{E_0}{2} r_m \left(\frac{\partial \alpha}{\partial r}\right) \cos\left[2\pi (v_{ex} - v_v) t\right] + \frac{E_0}{2} r_m \left(\frac{\partial \alpha}{\partial r}\right) \cos\left[2\pi (v_{ex} + v_v) t\right]$$

The first term in this equation represents Rayleigh scattering, which occurs at the excitation frequency v_{ex}. The second and third terms in equation correspond respectively the Stokes and anti-Stokes frequencies of v_{ex}-v_v and v_{ex}+v_v. here, the excitation frequency has been modulated by the vibrational frequency of the bond. It is important to note that Raman scattering requires that the polarizability of a bond varies as a function of distance – that is, $\partial\alpha / \partial r$ in equation must be greater than zero for Raman lines to appear.

We have noted, for a given bond, the energy shifts observed in a Raman experiment should be identical to the energies of its infrared absorption bands, provided that the vibrational modes involved are active toward both infrared absorption and Raman scattering. Figure illustrates the similarity of the two types of spectra; it is seen that several peaks with identical $\bar{v}$ and $\Delta\bar{v}$ values exist for the two compounds. It is also note worthy, however, that the relative size of the corresponding peaks is frequently quite different; moreover, certain peaks that occur in one spectrum are absent in the other.

The differences between a Raman spectrum and an infrared spectrum are not surprising when it is considered that the basic mechanisms, although dependent upon the same vibrational modes, arise from processes that are mechanistically different. Infrared absorption requires that a vibrational mode of the molecule have a change in dipole moment or charge distribution associated with it. Only then can radiation of the same frequency interact with the molecule and promote it to an

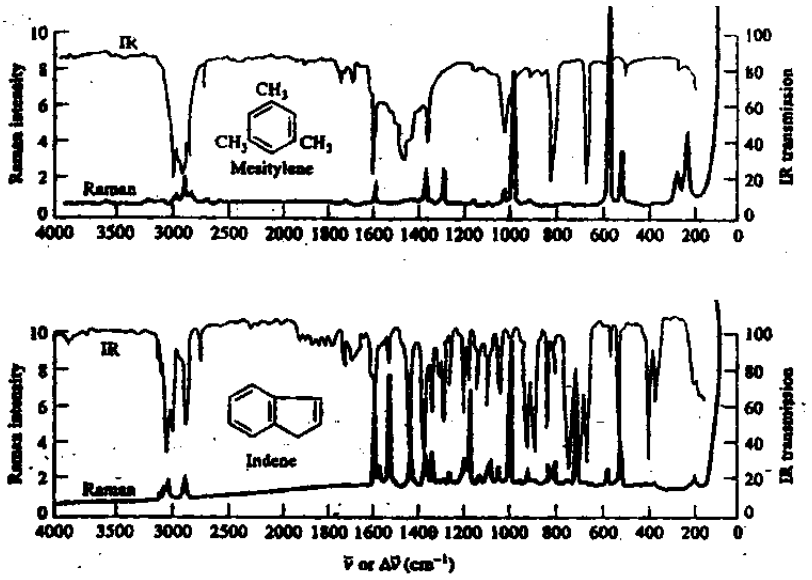


Figure: Comparison of Raman and infrared spectra.

excited vibrational state. In contrast, scattering involves a momentary distortion of the electrons distributed around a bond in a molecule, followed by reemission of the radiation as the bond returns to its normal state. In its distorted form, the molecule is temporarily polarized; that is, it develops momentarily an induced dipole that disappears upon relaxation and reemission. Because of this fundamental difference in mechanism, the Raman activity of a given vibrational mode may differ markedly from its infrared activity. For example, a homonuclear molecule such as nitrogen, chlorine, or hydrogen has no dipole moment either in its equilibrium position or when a stretching vibration causes a change in the distance between the two nuclei. Thus, absorption of radiation of the vibration frequency cannot occur. On the other hand, the polarizability of the bond between the two atoms of such a molecule varies periodically in phase with the stretching vibrations, reaching a maximum at the greatest separation and minimum at the closest approach. A Raman shift corresponding in frequency to that of the vibrational mode results.

It is of interest to compare the infrared and the Raman activities of coupled vibrational modes such as those described earlier for the carbon dioxide molecule. In the symmetric mode, no change in the dipole moment occurs as the two oxygen atoms move away from or toward the central carbon atom; thus, this mode is infrared inactive. The polarizability, however, fluctuates in phase with the vibration since distortion of bonds becomes easier as they lengthen and more difficult as they shorten. Raman activity is associated with this mode.

In contrast, the dipole moment of carbon dioxide fluctuates in phase with the anti-symmetric vibrational mode. Thus, an infrared absorption peak arises from this mode. On the other hand, as the polarizability of one of the bonds increases as it lengthens, the polarizability of

the other decreases resulting in no net change in the polarizability. Thus, the asymmetric stretching vibration is Raman inactive.

Often, as in the foregoing examples, parts of Raman and infrared spectra are complementary, each being associated with a different set of vibrational modes within a molecule. Other vibrational modes may be both Raman and infrared active. For example, all of the vibrational modes of sulfur dioxide yield both Raman and infrared peaks. The size of the peaks differ, however, because the probability for the transitions are different for the two mechanisms.

12. Describe the Instrumentation of Raman spectroscopy.

INSTRUMENTATION

Instrumentation for modern Raman spectroscopy consists of three components: a laser source, a sample –illumination system, and a suitable spectrometer. The performance requirements for these components are more stringent than for the molecular spectrometers we have already described, however, because of the inherent weakness of the Raman scattering signal compared with the signal produced by the Rayleigh scattering.

Sources

The sources used in modern Raman spectrometry are nearly always lasers because their high intensity is necessary to produce Raman scattering of sufficient intensity to be measured with a reasonable signal – to – noise ratio. Five of the most common lasers used for Raman spectroscopy are listed in Table. Because the intensity of Raman scattering varies as the fourth of power of the frequency, argon and krypton ion sources that emit in the blue and green region of the spectrum have an advantage over the other sources shown in the table for example, the argon line at 488 nm provides Raman lines that are nearly three times as intense as those exited by the helium/neon source, given the same input power.

The last two sources in the table, which emit near infrared radiation, are finding more and more use as excitation sources.

Type Sources	Wave lengh,nm
Argon ion	488.0 or 514.5
Krypton ion	530.9 or 647.1
Helium /Neon	632.8
Diode Laser	782 or 830
Nd/YAG	1064

Near-infrared sources have two major advantages over shorter wave length lasers. The first is that they can be operated at much higher power (up to 50 W) without causing photodecomposition of the sample. The second is that they are not energetic enough to populate a significant number of fluorescence – producing excited electronic energy states in most molecules. Consequently, fluorescence is generally much less intense or nonexistent with these lasers. The Nd/YAG line at 1064nm is particularly effective in eliminating fluorescence. The two lines of the diode array laser at 782 and 830nm also markedly reduce fluorescence in most cases.

Figure illustrates an example where the Nd/YAG source completely eliminates background fluorescence. The upper curve was obtained with conventional Raman equipment using the 514.5nm line form an argon-ion laser for excitation. The sample was anthracene, and most of the recoded signal arises from the fluorescence of that compound. The lower curve is for the same sample recorded with a Fourier transform spectrometer equipped with a Nd.YAG laser that emitted at 1064nm. Note the total absence of florescence back ground signal.

A variety of other laser sources are available; undoubtedly new and improved sources will appear in the future. There is a need for several sources because one must be chosen that is not absorbed by the sample (except with resonance Raman measurements) or the solvent.

Sample Illumination System

Sample handling for Raman spectroscopic measurements is simpler than for infrared spectroscopy because glass can be used for windows, lenses, and other optical components instead of the more fragile and atmospherically less stable crystalline halides. In addition, the laser source is easily focused on a small sample area and the emitted radiation efficiently focused on a slit.

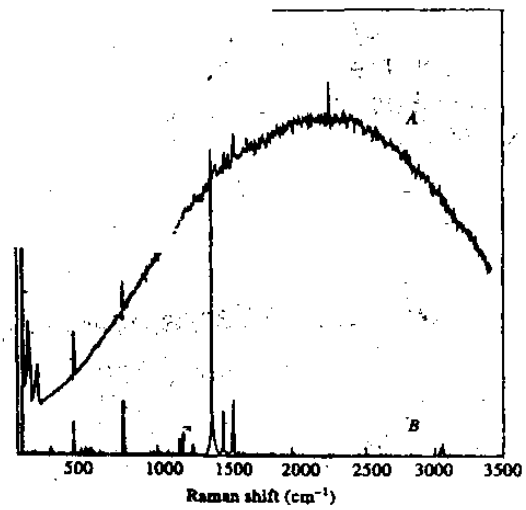


Figure Spectra of anthracene. A: conventional instruments, 5145 A excitation. B: FT instrument, 1.064 μm excitation.

Consequently, very small samples can be investigated. In fact, a common sample holder for nonabsorbing liquid samples is an ordinary glass melting – point capillary.

Liquid Samples

Figure shows two of many systems for handling liquid. Each contains a narrow band-pass interference filter that transmits a single line form the laser source and rejects (>99.9%) of the other lines. The size of the tube in figure b has been enlarged to show details of the reflection of the Raman radiation off the walls; in fact, the holder is a 1-mm o.d. glass capillary that is about 5 cm long.

A major advantage of sample handling in Raman spectroscopy compared with infrared arises because water is a weak Raman scatterer but a strong absorber of infrared radiation. Thus, aqueous solutions can be studied by Raman spectroscopy but not by infrared. This advantage is particularly important for biological and inorganic systems and in studies dealing with water pollution problems.

Solid Samples

Raman spectra of solid samples are often acquired by filling a small cavity with the sample after it has been ground to a fine powder. Polymers can usually be examined directly with no sample pretreatment. For unusually weak scatters, such as dilute gas samples, the call is some times placed between the mirrors of the laser source; enhanced excitation power results. Another means for producing Raman spectra form gas samples is the multipass gas cell. A cylindrical glass tube fitted with mirrors at both ends is filled with the sample gas. The excitation laser beam is passed through a small window in one of the mirrors and subsequently passes through the gaseous sample numerous times. The resulting Raman scattering perpendicular to the sample tube and to the excitation laser beam is then focused on the entrance slit of the spectrometer by a large lens.

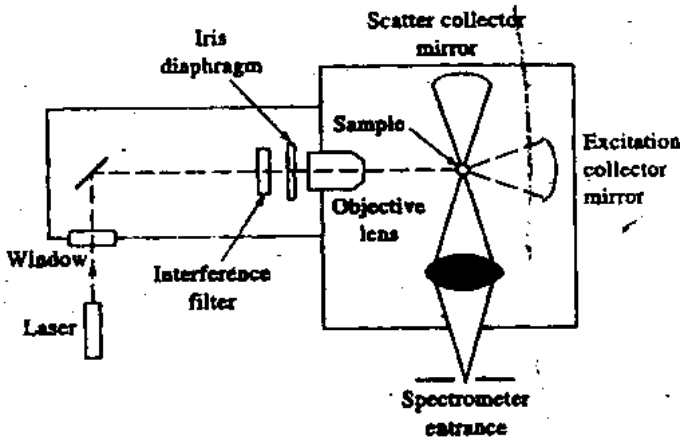
Fiber – Optic Sampling

One of the significant advantages of Raman spectrometry is that it is based upon visible or near – infrared radiation that can be transmitted for a considerable distance (as much as 100m or more) through optical fibers. Fig. is a schematic of typical instrumentation for sampling with a fiber-optic probe. Here, a microscope objective lens is used to focus the laser excitation beam onto one end of a bundle of input fibers, which carries the radiation to a fiber-optic probe that is immersed in the sample. As shown in figure the probe consists of the input fibers surrounded by several collection fiber that transport the scattered radiation to the monochromator. At the entrance to the spectrometer the collection fibers are arranged linearly as in figure so that they illuminate the entire slit.

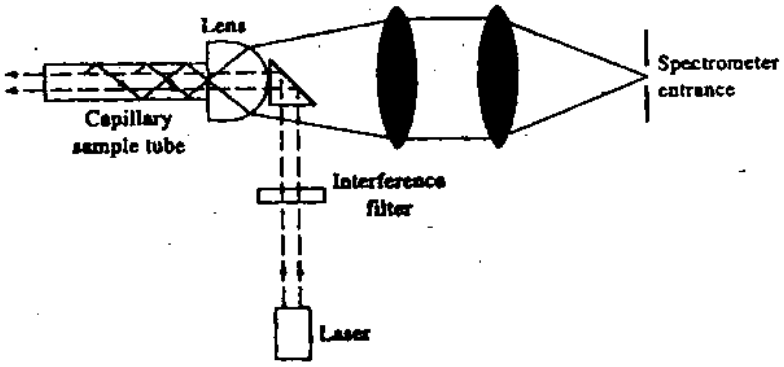
Figure illustrates how fiber-optic probes can be used to obtain spectra directly under relatively severe sampling conditions. Here, the probe encased in a protective glass sheath was inserted directly into sample of molten polyethylene.

Raman Spectrometers

Until the early 1980s, Raman spectrometers were similar in design and used the same type of components at the classical ultraviolet/visible dispersing instrument described in Section 13D-3. Most employed double grating systems to minimize the spurious radiation reaching the transducer. Photomultipliers served as transducers. Now, however, most Raman spectrometers being marketed are either Fourier transform instruments equipped with cooled germanium transducers or multi channel instruments based upon charge – coupled devices.



(a)



(b)

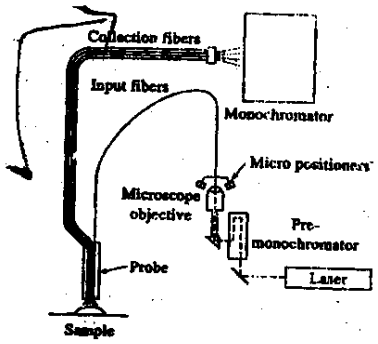
Figure Two sample excitation systems

These transducers, in contrast to photomultiplier tubes, are sensitive to radiation at 782nm produced by diode lasers, which provide Raman excitation of many compounds without significant fluorescence. Charge coupled devices are unfortunately not sensitive to the 1064nm radiation form a ND/ YAG laser.

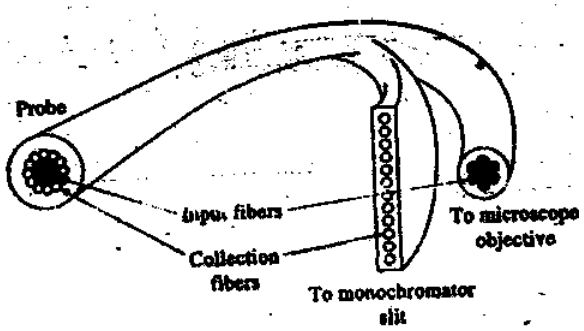
Currently, there is a controversy in the literature as to which is the better type of instrument for Raman measurements on samples that fluoresce: Fourier transform instruments or dispersing instruments based upon charge – coupled detectors at this point, it is not clear which of these types of instrument will be the most widely adopted in the future.

Figure is a schematic of an instrument for Fourier-transform Raman measurements. The interferometer is the same type as is used in infrared instruments. The transducer is a liquid nitrogen – cooled germanium photoconductor. Because the intensity of the Rayleigh line is several orders of magnitude greater than that of the Raman line, holographic interference filters called notch filters or a monochromator are usually used in the instrument to limit the radiation reaching the transducer to wave lengths longer than that of the source. With this arrangement, only the Stokes potion to the spectrum is used. Some Fourier transform instruments utilize filters designed to remove only the wavelength of the laser source. Regardless of the filter system used, the sharpness of the filter's transmittance profile limits the lowest frequencies that can be observed.

Figure is a schematic of a typical Raman dispersing spectrometer with a charge – coupled detector. The source is a diode laser and filter system that yields radiation of 783 nm. This beam is focused on the end of the excitation fiber by means of a lens and transmitted to a fiber probe that consists of the excitation fiber surrounded by 19 collection fibers. The later than carry the Raman emission to the slit of the monochromator,



(a)



(b)

Figure 18.7 (a) schematic of a system for obtaining Raman spectra with a fiber – optic probe: (b) end view of the prob: (c) end view of the collection fibers at the entrance slit of the monochrmator. The blackended circle represents the input fiber, and the hatched circles the collection fibers.

where it passes through a filter than rejects the Raleigh scattered radiation. A diffraction grating containing 600 grooves / mm disperses the radiation and reflects it onto a charge – coupled device that is made up of 258 by 1152 pixels. Each of the 258 vertical pixels is summed before being read out.

Figure shows some typical applications of dispersing spectrometer with a fiber – optic probe. To obtain the upper two spectra, the problem was immersed directly in the neat samples. The lower two spectra sere obtained by placing the probe approximately 3mm above the solid samples. Note that the Rhoda mine 6G is strongly fluorescent, making it extremely difficult to obtain a Raman spectrum with visible radiation. By using 783mm radiation from a diode laser, this problem is avoided.

13. What is Application of Raman Spectroscopy?

APPLICATIONS OF RAMAN SPECTROSCOPY

Raman spectroscopy has been applied to the qualitative and quantitative analysis of inorganic, organic, and biological systems. – See Analytical Raman Spectroscopy, J.G. Grasselli and B.J. Bulkin, Eds. New York: Wiley, 1991.

Raman Spectra of Inorganic Species

See K. Nakamoto, infrared and Raman spectra of Inorganic and coordination compounds, 5th ed. New York: Wiley,1996.

The Raman technique is often superior to infrared for spectroscopy investigating inorganic systems because aqueous solutions can be employed. In addition, the vibrational energies of metal –legend bonds are generally in the range of 100 to 700cm^{-1}, a region of the infrared that is experimentally difficulty to study. These vibrations are frequently Raman active, however, and peaks with $\Delta\bar{\nu}$ values in this ranges are readily observed. Raman studies are potentially useful sources of information concerning the composition, structure, and stability of coordination compounds. For example, numerous halogen and halogenoid complexes produce Raman spectra and thus are amenable to investigation by this means. Metal – oxygen bonds are also Raman active Spectra for such species as VO_4^{3-}, $AI(OH)^{-}{}_4$, $SI(OH)^{2-}{}_6$ and $Sn(OH)_6^{2-}$ have been obtained; Raman studies have been useful in determining the probable structures of such species. For example, in perchloric acid solutins, vanadium (IV) appears to be present as VO^{+2}(aq) rather than as $V(OH)_2^{2+}$ (aq). Studies of boric acid solutions shows that the anion formed by acid dissociates is the tetrahedral $B(OH)_4^-$ rather than $H_2BO_3^-$.

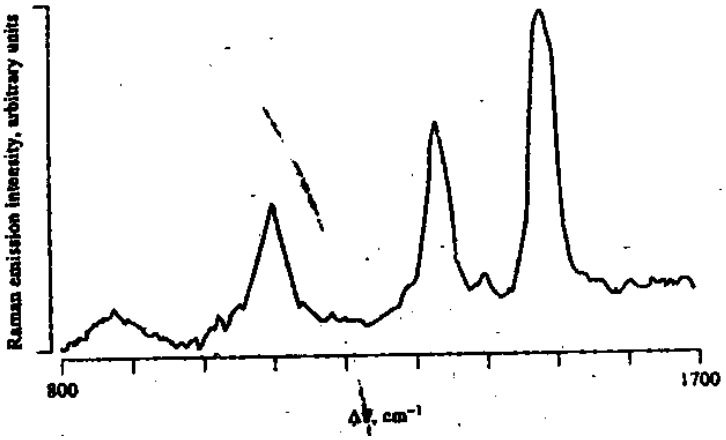


Figure Spectrum of molten polyethylene obtained using fiber-optic probe inserted into the polymer melt.

Dissociation constants for strong acids such as H_2SO_4, HNO_3, H_2SeO_4, and H_5IO_6 have been calculated from Raman spectroscopy for theoretical verification and structural studies of inorganic systems.

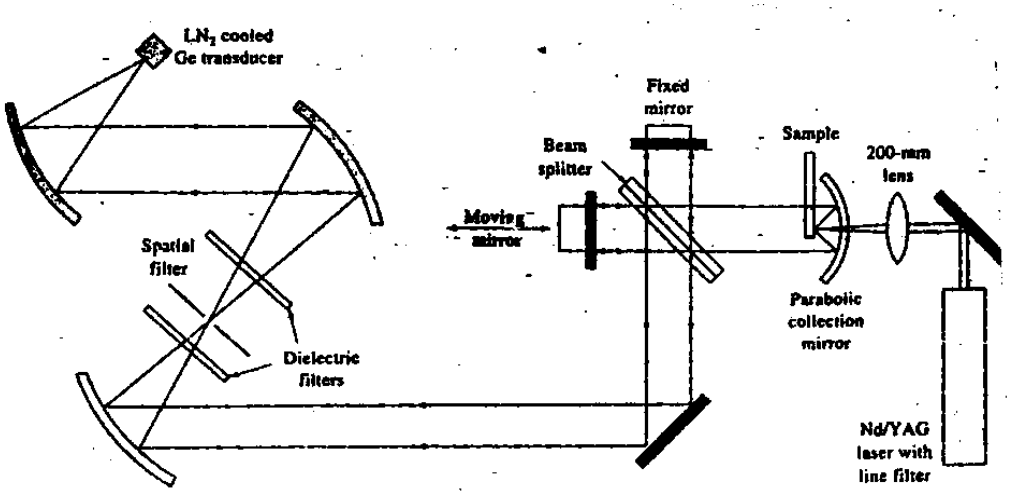


Figure Optical diagram of an FT-Raman spectrometer (LN_2=liquid nitrogen).

Raman Spectra of Organic Species

Raman spectra are similar to infrared spectra in that they have regions that are useful for functional group detection and fingerprint regions that permit the identification of specific compounds. Daimay element al. Have published a comprehensive treatment of Raman functional groups frequencies. L. Daimay, N.B Colthup, W.G Fately, and J.G. Grasselli, The Hand book of infrared and Raman Characteristic frequencies of Organic Compounds New York Wiley-Interscience.1971.

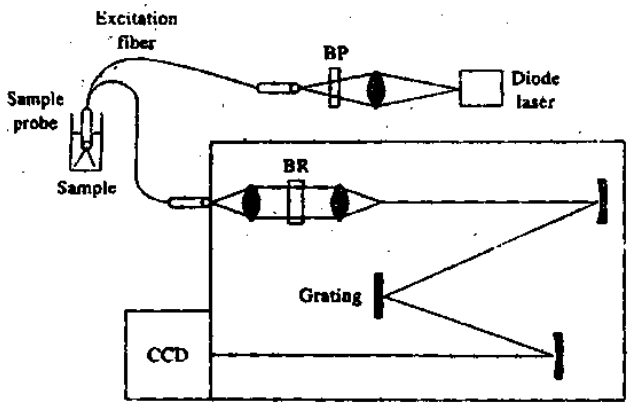


Figure Multi channel dispersive Raman spectrometer with a charge – coupled device (CCD). BP is an interference band – pass filter; BR is a Rayleigh band – rejection filter

Raman spectra yield more information about certain types of organic compounds than do their infrared counterparts. For example, the double – bond stretching vibration for – olefins results in weak and sometimes undetected infrared absorption. On the other hand, the Raman band (which like the infrared band, occurs at about 1600cm^{-1})is intense, and its position is sensitive to the nature of substituents as well as to their geometry. Thus, Raman studies are likely to yield useful information about the olefinic functional group that may not be revealed by infrared spectra.

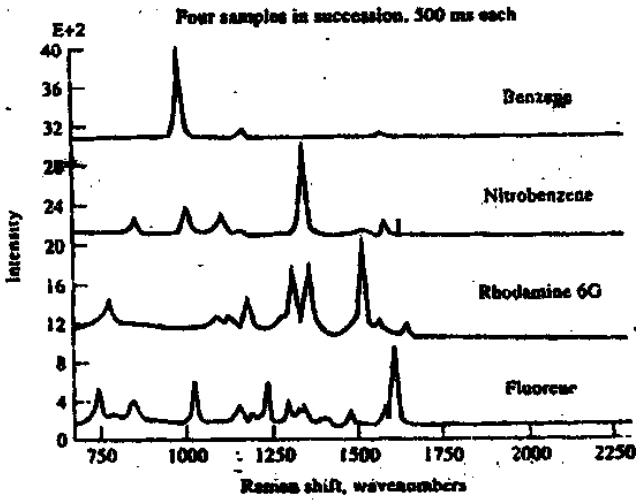


Figure Four Raman spectra obtained with the apparatus shown in figure

This statement applies to cycloparaffin derivatives as well; these compounds have a characteristic Raman peak in the region of 700 to 1200cm^{-1}. This peak has been attributed to a breathing vibration in which the nuclei move in and out symmetrically with respect to the center of the ring. The position of the peak decreases continuously from 1190cm^{-1} for cyclopropane to 700 cm^{-1} for cycloctane; Raman spectroscopy thus appears to be an excellent diagnostic tool for the estimation of ring size in paraffin's. The infrared peak associated with this vibration is weak or nonexistent.

Biological Applications of Raman Spectroscopy

Raman spectroscopy has been applied widely for the study of biological systems. For reviews of biological applications, see P.R. Carey, Biochemical Applications of Raman and Resonance Raman spectroscopy, New York; Academic Press, 1982; A.T.Tu, Raman spectroscopy in Biology New York; Wiley, 1982; I.W. Levin and E.N. Lewis, Anal. Chem, 1990, 62,1101A; P.V. Huong, in Analytical Raman Spectroscopy, J.G. Graselli and B.J. Bulkin, Eds., Chapter 11. New York; Wiley, 1991. The advantages of this technique include the small sample requirement, the minimal sensitivity toward interference by water, the spectral detail, and the conformational and environmental sensitivity.

Quantitative Applications

Raman spectra tend to be less cluttered with peaks than infrared spectra. As a consequence, peak overlap in mixtures is less likely, and quantitative measurements are simpler. In additions, Raman sampling devices are not subject to attack by moisture, and small amounts of water is a sample do not interface. Despite these advantages, Raman spectroscopy has not yet been exploited widely for quantitative analysis. This lack of use has been due largely to the rather high cost of Raman spectrometers relative to that of absorption instrumentation. This barrier is likely to become less important as suitable low – cost laser diodes proliferate.

Because laser beams can be precisely focused, it becomes possible to perform quantitative analyses on very small samples. For this work, instruments called laser microprobes are employed. Laser microprobes have been used to determine analytes in single bacterial cells, components in individual particles of smoke and fly ash, and species in microscopic inclusions in minerals.

OTHER TYPES OF RAMAN SPECTROSCOPY

With the development of tunable lasers, several new Raman spectroscopic methods were developed in the early 1970s. A brief discussion of the application some of these techniques follows.

Resonance Raman Spectroscopy

For brief reviews of this topic, see M.D. Morris and D.J. Wallan Anal. Chem., 1979, 51, 182A; D.P. Strommen and K. Nakamoto,/ Chem, Edu., 1977, 54, 474; S.A. Asher Anal Chem., 1993,65,59A 201A.

Resonance Raman scattering refers to a phenomenon in which Raman line intensities are greatly enhanced by excitation with wavelengths that closely approach that of an electronic

absorption peak of an analyte. Under this circumstance, the magnitudes of Raman peaks associated with the most symmetric vibrations are enhanced by a factor of 10^2 to 10^6. As a consequence, resonance Raman spectra have been obtained at analyte concentrations as low as 10^{-8} M. This level of sensitivity is in contrast to normal Raman studies, which are ordinarily limited to concentrations greater than 0.1M. Because resonance enhancement is restricted to the Raman bands associated with the chromophore, resonance Raman spectra usually consist of only a few lines. Furthermore, resonance Raman spectra may be obtained quite selectively because excitation can be targeted for specific absorption bands.

Figure (a) illustrates the energy change responsible for resonance Raman scattering. This figure differ from the energy diagram for normal Raman scattering in that the electron is promoted into an excited electronic state followed by an immediate relaxation to a vibrational level of the electronic ground state.

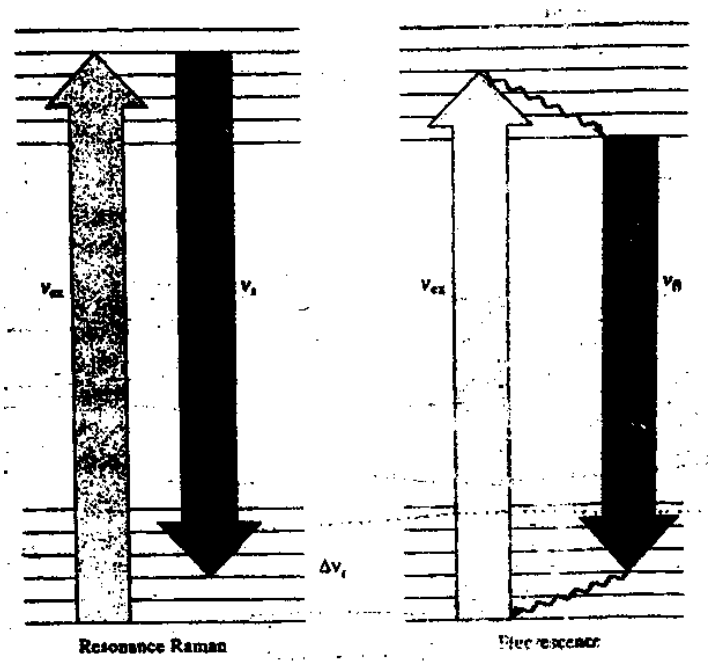


Figure: Energy diagram for (a) resonance Raman scattering and (b) fluorescence emission. Radiationless relaxation shown as wavy arrows.

As shown in the figure, resonance Raman scattering differs from fluorescence in that relaxation to the ground state is not preceded by prior relaxation to the lowest vibrational level of the excited electronic state. The time scales for the two phenomena are also quite different with Raman relaxation occurring in less than 10^{-14}s compared with the 10^{-6} to 10^{-8}s for fluorescence emission.

Line intensities in a resonance Raman experiment increase rapidly as the excitation wavelength approaches the wavelength of the electronic absorption peak. Thus, to achieve the greatest signal enhancement for a broad range of absorption maxima, a tunable laser is required.

With intense laser radiation, sample decomposition can become a major problem because electronic absorption peaks often occur in the ultraviolet region. Specifically, intense absorption cases local heating in the sample, which leads to decomposition of the analyte. To circumvent this problem, it is common practice to circulate the sample past the focused beam of the laser. Circulation is normally accomplished in one of two ways: by pumping a solution or liquid through a capillary mounted in the sample position, or by rotating a cylindrical cell containing the sample through the laser beam. Thus, only a small fraction of the sample is irradiated at any instant, and heating and sample decomposition are minimized.

Perhaps the most important application of resonance Raman spectroscopy has been to the study of biological molecules under physiologically significant conditions; that is, in the presence of water and at low to moderate concentration levels. As an example, the technique has been used to determine the oxidation state and spin of iron atoms in hemoglobin the and cytochrome-c. In these molecules, the resonance Raman bands are due solely to vibrational modes of the tetrapyrrole chromophore. None of the other bands associated with the protein is enhanced, and at the concentrations normally used these bands do not interfere as a consequence.

A major limitation to resonance Raman spectroscopy is interference by fluorescence either by the analyte itself or by other species presents in the sample.

UNIT – IV

PART - A

1. Define thermal methods.

Thermal analysis is; "A group of techniques in which a physical property of a substance and (or) its reaction products is measured as a function of temperature while the substance is subjected to a controlled temperature program.

Well over a dozen thermal methods can be recognized, which differ in the properties measured and the temperature programs.

2. Define thermo gram (or) thermal decomposition curve.

A thermo gravimetric analysis the mass of a sample in a controlled atmosphere is recorded continuously as a function of temperature (or) time as the temperature of the sample is increased. A plot of mass (or) mass percent as a function of time is called a thermo gram, or a thermal decomposition curve.

3. Give the common part of thermo gravimetric Instrumentation.

Modern commercial instruments for thermo gravimetry consists of; (1) a sensitive analytical balance (2) a furnace (3) a purge gas system for providing as inert (or) atmosphere (4) microcomputer / micro processor for instrument control and data acquisition and display.

4. Give the application of TG.

The information provided by Thermo gravimetric methods is more limited than that obtained with the other two thermal methods described; a temperature variation must bring about a change in mass of the analytic. Thus, thermo gravimetric methods are largely limited to decomposition and oxidation reactions and to such physical processes as vaporization, sublimation, and desorption.

Perhaps the most important applications of thermo gravimetric methods are found in the study of polymers.

Thermo grams provide information about decomposition mechanism for various polymeric preparations.

5. Define principle differential thermal analysis (DTA).

Differential thermal analysis is a technique in which the difference in temperature between a substance and a reference material is measured as a function of temperature while the substance and reference material are subjected to a controlled temperature program. Usually, the temperature program involves heating the sample and reference material in such a way that the temperature of the sample T_S increases linearly with time.

The difference in temperature ΔT between the sample temperature and the reference temperature T_r ($\Delta T = T_r - T_s$) is then monitored and plotted against sample temperature to give a differential thermo gram.

6. Give the application of Differential Thermal Analysis (DTA).

Differential thermal analysis finds wide spread use in determining the thermal behavior and composition of naturally occurring and manufactured products. The number of applications is impressive and can be appreciated by examination of a two – volume monograph and recent previous in Analytical chemistry.

PART – B

1. Describe the Instrumentation of Thermogravimetric methods (TG).

In a thermogravimetric analysis the mass of a sample in a controlled atmosphere is recorded continuously as a function of temperature or time as the temperature of the sample is increased (usually linearly with time). A plot of mass or mass percent as a function of time is called a thermogram, or a thermal decomposition curve, - For a brief review of thermogravimetry, sec C.M. Earnest, Anal. Chem., 1984, 56, 1471 A.

The Balance

A number of different thermobalance designs are available commercially that are capable of providing quantitative information about samples ranging in mass from 1 mg to 100 g. The most common type of balance, however, has a range of 5 to 20 mg. Although the sample holder must be housed in the furnace, the rest of the balance must be thermally isolated from the furnace. Fig. is a schematic of one thermobalance design. A change in sample mass causes a deflection of the beam, which interposes a light shutter between a lamp and one of two photodiodes. The resulting imbalance in the photodiode current is amplified and fed into coil E, which is situated between the poles of a permanent magnet F. The magnetic field beam to its original position. The amplified photodiode current is monitored and transformed into system. In most cases mass versus temperature data can either be plotted in real time or stored for further manipulation or display at a later time.

The Furnace

The temperature range for most furnaces for thermogravimetry is from ambient to 1500^0C. Often the heating and cooling rate of the furnace can be selected from somewhat greater than zero to as high as 200^0 C/min. Insulation and cooling of the exterior of the furnace is required to avoid heat transfer to the balance. Nitrogen or argon are usually used to purge the furnace and prevent oxidation of the sample. For some analysis, it is

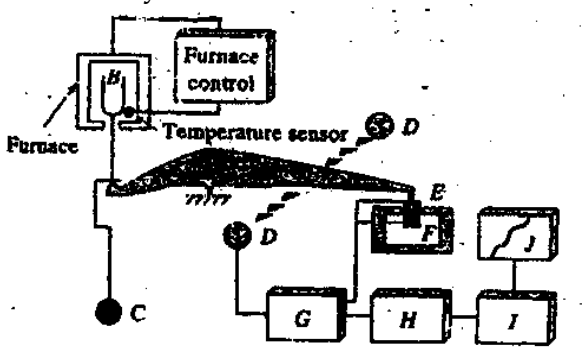


Figure: Components of a thermal balance: A, beam; B, sample cup and holder; C, counterweight; D, lamp and photodiodes; E, coil; F, magnet; G, control amplifier; H, tare calculator; I, amplifier; J, recorder.

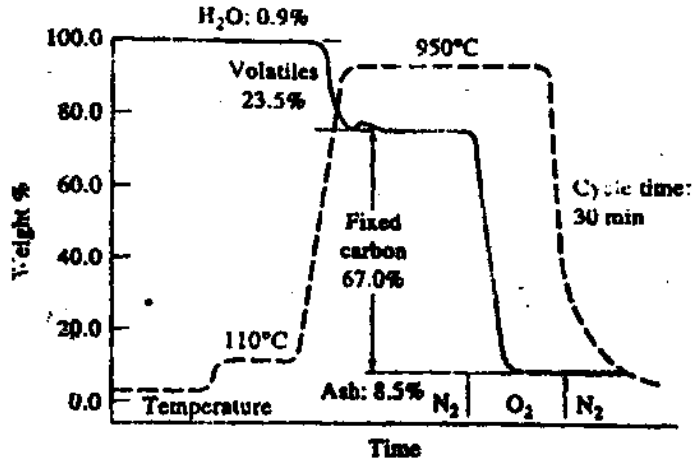


Figure: A controlled atmospheric thermogram for a bituminous coal sample. A nitrogen atmosphere was employed for approximately 18 min followed by an oxygen atmosphere for 4 to 5 min. The analysis was then completed in nitrogen.

desirable to switch purge gases as the analysis proceeds. Fig. provides an example in which the purge gas was automatically switched from nitrogen to oxygen and then back to nitrogen. The sample in this case was a bituminous coal. Nitrogen was employed during the first 18 min while the moisture content and the percent volatiles were recorded. The gas was then switched to oxygen for 4 to 5 min, which caused oxidation of carbon to carbon dioxide. Finally, the analysis was finished with a nitrogen purge to give a measure of the ash content.

Instrument Control / Data Handling

The temperature recorded in a thermogram is ideally the actual temperature of the sample. The temperature can, in principle, be obtained by immersing a small thermocouple directly in the sample. Such a procedure is seldom followed, however, because of possible catalytic decomposition of sample, potential contamination of samples, and weighing errors resulting from the thermocouple leads. As a consequence of these problems, recorded temperatures are generally measured with a small thermocouple located as close as possible to the sample container. The recorded temperatures are generally measured with a small thermocouple located as close as possible to the sample container. The recorded temperatures then generally lag or lead the actual sample temperature.

Modern thermobalance usually use a computerized temperature control routine that automatically compares the voltage output of the thermocouple with a voltage versus temperature table that is stored in read only memory (ROM). The microcomputer uses the difference between the temperature of the thermocouple and the temperature specified in ROM to adjust the voltage to the heater. Using this method it is possible to achieve excellent

agreement between the specified temperature program and the temperature of the sample. Typical run-to-run reproducibility for a particular program falls within ± 2^0C throughout an instrument's entire operating range.

Applications

The information provided by themogravimetric methods is more limited than that obtained with the other two thermal methods described in this chapter because here a temperature variation must bring about a change in mass of the analysis. Thus, thermogravimetric methods are largely limited to decomposition and oxidation reactions and to such physical processes as vaporization, sublimation, and desorption.

Perhaps the most important applications of thermogravimetric methods are found in the study of polymers. Thermograms provide information about decomposition mechanisms for various polymetric preparations. In addition, the decomposition patterns are characteristic for each kind of polymer and in some cases can be used for identification purposes. Fig. shows decomposition patterns for five polymers obtained by themogravimetry.

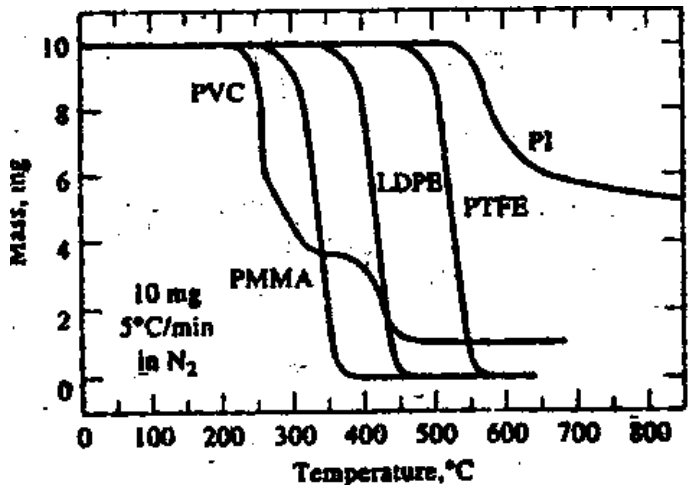


Figure: Thermograms for some common polymetric materials. PVC = polyvinyl chloride; PMMA = polymethyl methacrylate; LDPE = low density polyethylene; PTFE = ploytetrafluoroethlene; PI = aromatic polypyromel litimide.

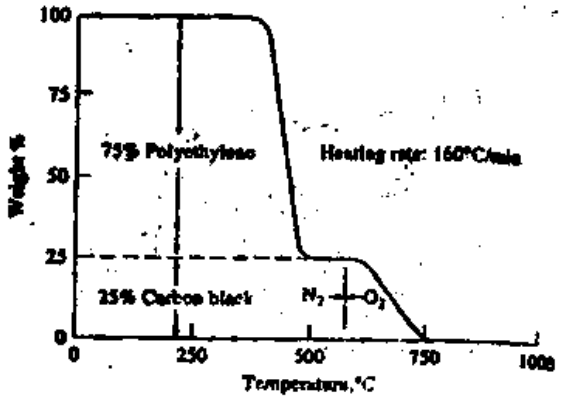


Figure: Thermogravimetric determination of carbon black in polyethylene

Figure illustrates how a thermogram is used for quantitative analysis of a polymeric material. The sample is polyethylene that has been formulated with fine carbon-black particles to inhibit degradation from exposure to sunlight. This analysis would be difficult by most other analytical methods.

Figure is a recorded thermogram obtained by increasing the temperature of pure CaC_2O_4. H_2O at a rate of 5^0C/min. The clearly defined horizontal regions correspond to temperature ranges in which the indicated calcium compounds are stable. This figure illustrates one of the important applications of thermogravimetry, namely, that of defining thermal conditions necessary to produce a pure form for the gravimetric determination of a species.

Figure (a) illustrates an application of thermogravimetry to the quantitative analysis of a mixture of calcium, strontium, and barium ions. The three are first precipitated as the monohydrated oxalates. The mass in the temperature range between 320^0 and 400^0C is that of the three anhydrous compounds, CaC_2O_4, SrC_2O_4, and BaC_2O_4, while the mass between about 580^0 and 620^0C corresponds to the weight of the three carbonates. The weight change in the next two steps results from the loss of carbon dioxide, as first CaO and then SrO are formed. Clearly, sufficient data are available in the thermogram to calculate the weight of each of the three elements present in the sample.

Fig. (b) is the derivative of the thermogram shown in fig. 6a. The data acquisition system of

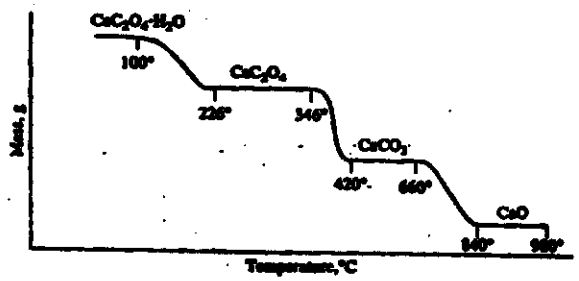


Figure: A thermogram for decomposition of CaC_2O_4 . H_2O in an inert atmosphere.

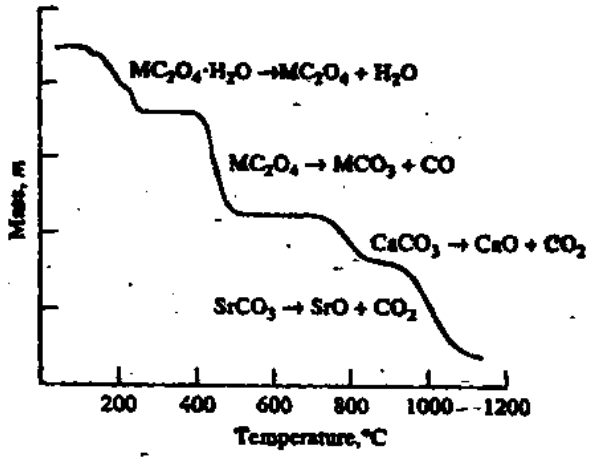


Figure: (a) Thermogram

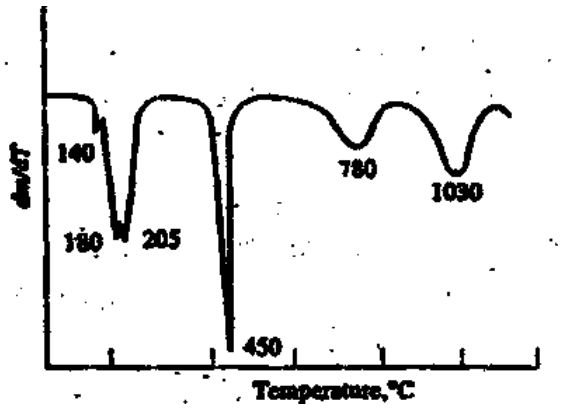


Figure : (b) Differential thermogram

Figure: Decomposition of CaC_4O_4. H_2O, SrC_2O_4. H_2O, and BaC_2O_4. H_2O. (From L. Erdey, G. Liptay, G. Svehla, and F.Paulik, Talanta, 1962,9,490. With permission).

most modern instruments are capable of providing such a curve as well as the thermogram itself. The derivative curve may reveal information that is not detectable in the ordinary thermogram. For example, the three peaks at 140^0, 180^0, and 205^0C suggest that the three hydrates lose moisture at different temperatures. However, all appear to lose carbon monoxide simultaneously and thus yield a single sharp peak at 450^0C.

2. Describe the Instrumentation and Application of Differential Thermal Analysis (DTA).

Differential thermal analysis is a technique in which the difference in temperature between a substance and a reference material is measured as a function of temperature while the substance and reference material are subjected to a controlled temperature program. Usually, the

temperature program involves heating the sample and reference material in such a way that the temperature of the sample T_s increases linearly with time. The difference in temperature Δ T between the sample temperature of the temperature $T_r(\Delta T = T_r - T_s)$ is then monitored and plotted against sample temperature to give a differential thermogram such as that shown in Figure. The significance of the various parts of this curve are described in Section.

Instrumentation

Figure is a schematic of the furnace compartment of a differential thermal analyzer. A few milligrams of the sample (S) and an inert reference substance (R) are

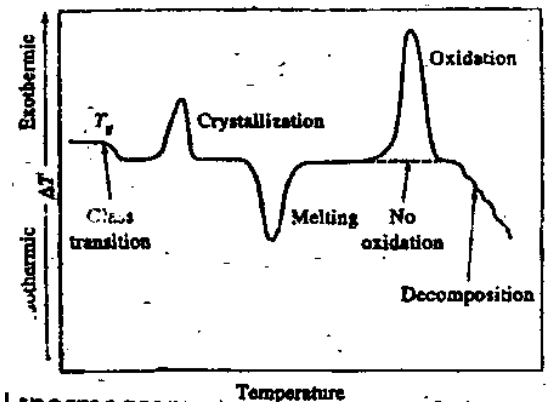


Figure: Schematic differential thermogram showing types of changes encountered with polymeric materials.

contained in small aluminum dishes that are located above sample and reference thermocouples in an electrically heated furnace.[4] For a description of several DTA instruments from commercial sources, see L. Voress,Anal.Chem., 1994,66,1035A. The reference material is an inert substance such as alumina, silicon carbide, or glass beads.

The output potential E_s from the sample thermocouple passes into a microcomputer where it is made to control the current input to the furnace in such a way that the sample temperature increases linearly and at a predetermined rate. The sample thermocouple signal is also converted to temperature T_s and is then recorded as the abscissa of the differential thermogram. The output across the sample and reference thermocouples ΔE is amplified and converted to a temperature difference ΔT, which serves as the ordinate of the thermogram.

Generally, the sample and reference chamber in differential thermal apparatus are designed to permit the circulation of an inert gas, such as nitrogen, or a reactive gas, such as oxygen or air. Some systems also have the capability of operating at high and low pressures.

General Principles

Figure is an idealized differential thermogram obtained by heating a ploymer over a sufficient temperature range to cause its ultimate decomposition. The initial decrease in

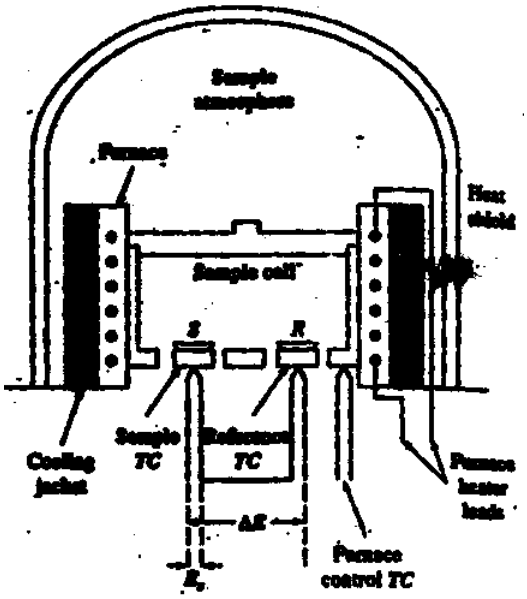


Figure: Schematic of a typical instrument for differential thermal analysis (Tc = thermocouple).

ΔT is due to the glass transition, a phenomenon observed initially when many polymers are heated. The glass transition temperature T_g is the characteristic temperature at which glassy amorphous ploymers become flexible or rubber-like because of the onset of the concerted motion of large segments of the ploymer molecules. Upon being heated to a certain temperature T_g the polymer changes from a glass to a rubber. Such a transition involves no absorption or evolution of heat so that no change in enthalpy results__ that is, $\Delta H = 0$. The heat capacity of the rubber is, however, different from that of the glass, which results in the lowering of the baseline, as shown in the figure. No peak results during this transition, however, because of the zero enthalpy change.

Two maxima and a minimum are observed in the thermogram in Figure. all of which are called peaks. The two maxima are the result of exothermic processes in which heat is evolved from the sample, thus causing its temperature to rise; the minimum labeled "melting " is the consequence of an endothermic process in which heat is absorbed by the analyte. Upon being heated to a characteristic temperature, many amorphous polymers begin to crystallize as microcrystals, giving off heat in the process. Crystal formation is responsible for the first exothermic peak shown in Figure. The area under such a peak becomes larger the slower the heating rate because more and more crystals have time to form and grow under this circumstance.

The second peak in the figure is endothermic and involves melting of the microcrystals

formed in the initial exothermic process. The third peak is exothermic and is encountered only if the heating is performed in the presence of air or oxygen. This peak is the result of the exothermic oxidation of the polymer. The final negative change in ΔT results from the endothermic decomposition of the polymer to produce a variety of products.

As suggested in Figure, differential thermal analysis peaks result from both physical changes and chemical reactions induced by temperature changes in the sample. Physical processes that are endothermic include fusion, vaporization, sublimation, absorption, and desorption. Adsorption and crystallization are generally exothermic. Chemical reactions may also be exothermic or endothermic. Endothermic reactions include dehydration, reduction in a gaseous atmoshphere, and decomposition. Exothermic include oxidation in air or oxygen, polymerization, and catalytic reactions.

Peak areas in differential thermograms depend upon the mass of the sample, m, the enthalpy, ΔH, of the chemical or physical process, and certain geometric and heat conductivity factors. These variables are related by the equation

$$A = -kGm\Delta H = -k'm\Delta H$$

where A is the peak area, G is a calibration factor that depends upon the sample geometry and k is a constant related to the thermal conductivity of the sample. The convention of assigning a negative sign to an exothermic enthalpy change accounts for

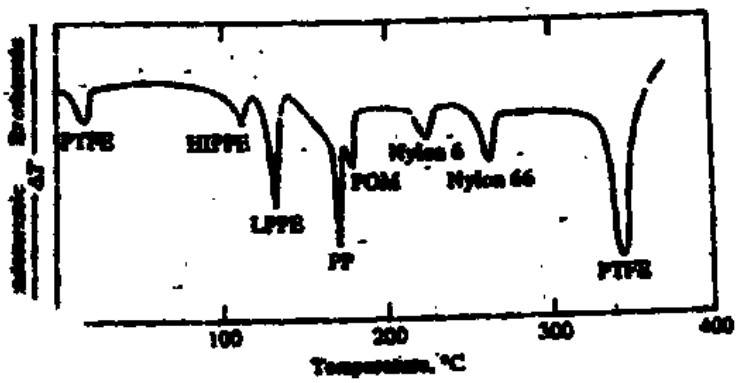


Figure: Differential thermogram for a mixture of seven polymers. PTFE = Polytetrafluoroethylene; HIPPE = high-pressure (low-density) polyethylene; LPPE = low-pressure (high-density) polyethylen; PP=polypropylene; POM = polyoxymethylene.

the negative sign in the equation. For a given species, K' remains constant provided that a number of variables such as heating rate, particle size, and placement of the sample relative to the sample thermocouple are carefully controlled. Under these circumstances Equation31-1 can be used to determine: 91) the mass of a particular analyte if k' and ΔH can be determined by

calibration; (2) enthalpy change if k′ and m are known.

Applications

Differential thermal analysis finds widespread use in determining the thermal behavior and composition of naturally occurring and manufactured products. The number of applications is impressive and can be appreciated by examinations is of a two-volume monograph and recent reviews in Analytical Chemistry. - Differential Thermal Analysis, R.C.Mackenzie, Ed.New York: Academic Press, 1970; D. Dollimore, Anal.Chem.,1996,68,63R;1994,66,17R;1992,64,147R; 1990,62,44R; 1998,60,274R. A few illustrative applications follow.

Differential thermal analysis is a powerful and widely used tool for studying and characterizing polymers. Figure illustrates the type of physical and chemical changes in polymeric materials that can be studied by differential thermal methods. Note that thermal transitions for a ploymer often take place over an extended temperature range because even a pure polymer is a mixture of homologs, and not a single chemical species.

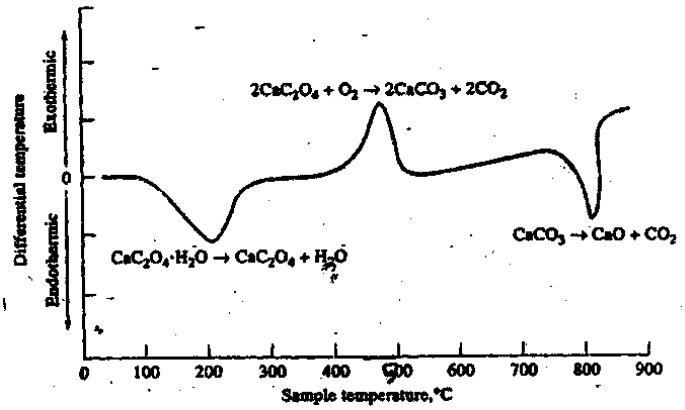


Fig Differential thermogram of $CaC_2O_4 . H_2O$ in the prescence of O_2 ; the rate of temperature increase was 8˚C/min.

Figure is a differential thermogram of a physical mixture of seven commercial polymers. Each peak corresponds to the melting point of one of the components. Polytetrafluoroethylene (PTFE) has an additional low-temperature peak that arises from a crystalline transition. Clearly, differential thermal analysis has the potential use of identifying polymers.

Differential thermal measurements have been used for studies of the thermal behaviour of pure inorganic compounds as well as such inorganic substances as silicates, ferrites, clays, oxides, ceramics, catalysts, and glasses. Information is provided about such processes as fusion desolvation, dehydration, oxidation, reduction, adsorption, and solid-state reactions.

Figure demonstrates the use of differential thermal analysis for studying the thermal

behaviour of a simple inorganic species. The differential thermogram was obtained by heating calcium oxalate monohydrate in a flowing stream of air. The two minima indicate that the sample became cooler than the reference material as a consequence of the two endothermic reactions that are shown by the equations below the minima. The single maximum indicates that the oxidation of calcium oxalate to give calcium carbonate and carbon and carbon dioxide is exothermic. When an inert gas, such as nitrogen, is substituted for air as the purge gas, three minima are encountered because decomposition of calcium oxalate is now endothermic, with the products being calcium carbonate and carbon monoxide.

An important use of differential thermal analysis is for the generation of phase diagrams and the study of phase transitions. An example is shown in Figure, which is a differential thermogram of sulfur, in which the peak at 113°C corresponds to the solid-phase change from the rhombic to the monoclinic form, whereas the peak at 124°C corresponds to the melting point of the element. Liquid sulfur is know to exist in at least three forms, and the peak at 179°C apparently involves these transitions, while the peak at 446°C corresponds to the boiling of sulfur.

The differential thermal method provides a simple and accurate way of determining the melting, boiling,

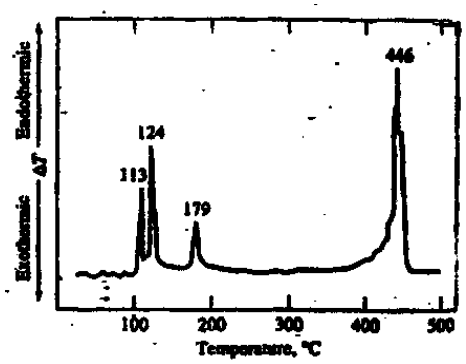


Figure: Differential thermogram for sulfur. (Reprinted with permission from J.Chiu,Anal.Chem., 1963,35,933. Copyright 1963 American Chemical Society).

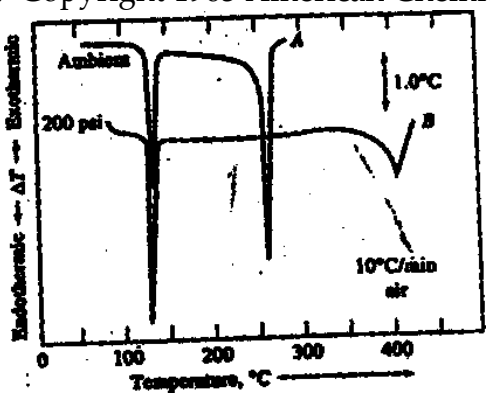


Figure: Differential thermogram for benzoic acid. Curve A: at atmospheric pressure; curve B: at 200 Ibs/in^2.

and decomposition points of organic compounds. Generally, the data appear to be more

consistent and reproducible than those obtained with a hot stage or a capillary tube. Figure. shows thermograms for benzoic acid at atmospheric pressure (A) and at 200 psi (B). The first peak corresponds to the melting point and the second to boiling point of the acid.

3. Describe the Differential Scanning Calorimetry (DSC).

Differential scanning calorimetry is a thermal technique in which differences in heat flow into a substance and a reference are measured as a function of sample temperature while the two are subjected to a controlled temperature program. The basic difference between differential scanning calorimetry and differential thermal analysis is that the former is a calorimetric method in which differences in energy are measured. In contrast, in differential thermal analysis, differences in temperature are recorded. The temperature programs for the two methods are similar. Differential scanning calorimetry has by now become the most widely used of all thermal methods.

Instrumentation

Two types of methods are used to obtain differential scanning calorimetry data. In power compensated DSC the sample and reference material are heated by separate heaters in such a way that their temperature are kept equal while these temperatures are increased (or decreased) linearly. In heat flux DSC, the difference in the sample temperature is increased—or decreased—formation, the instrumentation for the two is fundamentally different.[6] For a description of several commercially available Instruments of both types, see D. Noble, Anal.Chem., 1995,67,323A.

Power Compensated DSC

Figure is a schematic showing the design of a power compensated calorimeter for performing DSC measurements. The instrument has two independent furnaces, one for heating the sample and the other for heating the reference. In a commercial model based upon this design, the furnaces are small, weighing about a gram each, a feature that leads to rapid rates of heating, cooling, and equilibration. The furnaces are imbedded in a large temperature-controlled heat sink. Above the furnaces are the sample and reference holders, which have platinum resistance thermometers imbedded in them to monitor the temperatures of the two materials continuously.

Two control circuits are employed in obtaining differential thermograms with the instrument shown in Figure one for average-temperature control and one for differential-temperature control. in the average-temperature control circuit, a programmer provides an electrical signal that is proportional to the desired average temperature of the sample and reference holders as a function of time.

UNIT – V

SEPARATION METHOD

PART – A

1. Define Chromatography.

Chromatography is a powerful separation method that finds application to all branches of science. Chromatography was invented and named by the Russian botanist Mikhail Tswett at the beginning of the twentieth century. He employed the technique to separate various plant pigments such as chlorophylls and xanthophylls by passing solution of these compounds through a glass column packed with finely divided calcium carbonate. The separated species appeared as coloured bands on the column, which accounts for the name he close for the methods (Greek : Chroma meaning "color" and graphein meaning to "write").

2. Explain Absorption chromatography.

In absorption chromatography, the stationary phase is finely divided absorbent such as alumina or silica gel and the mobile phase can be a gas or more commonly a liquid. Eg. Column, thin layer (TLC), Gas solid (GSC).

3. Explain partition chromatography.

Partition chromatography involves partition between two liquids rather than absorption by a solid from a liquid. Here the stationary phase is a liquid which is held on an inert porous supporting liquid.

E.g. Column, paper (PPC), thin layer (TLC), gas liquid (GLC).

4. Define Chromatogram.

If a detector that responds to solute concentration is placed at the end of the column and its signal is plotted as function of time (or of volume of the added mobile phase). A series of peaks is obtained such a plot, called a chromatogram is useful for both qualitative and quantitative analysis. The position of peaks on the time axis may serve to identify the components of the sample; the areas under the peaks provide a quantitative measure of the amount of each component.

5. What is partition co-efficient?

The distribution equilibria involved in chromatography are described by relatively straight forward equation that involve the transfer of an analyte between the mobile and stationary phase. Thus, for the solute species A, we may write

A_{mobile} $A_{stationary}$.

The equilibrium constant K for this reaction is called distribution constant, the partition ratio, or the partition co-efficient and is defined as

$$K = \frac{C_s}{C_M}$$

where

C_s – is the molar concentration of the solute in the stationary phase.

C_M – is the molar concentration in the mobile phase.

K – is constant over a wide range of solute concentration; that is C_s is directly proportional to C_M.

6. Define Retention time and dead time.

The time it takes after sample injection for the analyte peak to reach the detector is called the Retention time and is given the symbol t_R. The species that is not retained by the column. Often the sample or the mobile phase will contain an unretained species. When they do not, such a species may be added to aid in peak identification. The time t_M for the unretained species to reach the detector is sometimes called the dead time. The rate of migration of the unretained species is the same as the average rate of motion of the mobile phase molecules.

7. Define Retention factor.

The Retention factor, or capacity factor, is an important parameter that is widely used to describe the migration rates of solutes on columns. For a solute A. The retention factor KA' is defined as

$$K_A' = \frac{K_A V_s}{V_M}$$

where

K_A is distribution constant for the species A.

8. What is kinetic theory of chromatography?

The rate or kinetic theory of chromatography successfully explains in quantitative terms the shapes of chromatographic peaks and the effects of several variable on the breadth of these peaks. A detailed discussion of this theory, which is based upon a random – walk mechanism, is beyond the scope of this text. We can, however, give a qualitative picture of chromatographic zone and why the zones broaden as they move down a column. This discussion then leads to a consideration of variables that improve column efficiency by reducing broadening.

9. What are the methods for describing column efficiency?

Two related terms are widely used as quantitative measures of chromatographic column efficiency.

- Plate height H
- Plate count plates N.

The two are related by the equation.

$$N = L/H$$

Where

L is the length of the column packing.

The efficiency of chromatographic column increases as the plate count becomes greater and as the plate height becomes smaller.

10. Explain optimization of column performance.

A chromatographic separation is optimized by varying experimental condition until the components of a mixture are separated cleanly with a minimum expenditure of time. Optimization experiments are aimed at either

- Reducing zone broadening
- Altering relative migration rates of the components.

The zone broadening is increased by those kinetic variable that increase the plate height of a column. Migration rates, on the other hand, are varied by changing those variables that affect retention and selectivity factors of the solution.

11. Give two types of gas chromatography.

Two types of gas chromatography are encountered: 1. gas – solid chromatography (GSC) 2. gas – liquid chromatography (GLC).

Gas liquid chromatography finds widespread use in all fields of science, where it name is usually shortened to gas chromatography (GC) despite the fact that this usage neglects gas – solid chromatography as a legitimate kind of chromatography.

12. Define Retention Volumes.

To take into account the effects of pressure and temperature in gas chromatography, it is sometimes useful to use retention volumes rather than the retention times that were employed. The relationship between the two is

$V_R = t_R F$

$V_M = t_M F$

where

F – is the average volumetric flow rate within the column.

V & t – are retention volumes and times, respectively, and

R & M – subscripts, refer to species that are retained and not retained on the column.

13. What is relationship between V_g and K?

It is of interest to relate V_g to the distribution constant K. To do so, we substitute the expression relating t_R and t_M to K′ which gives

$$V_g = \frac{jFt_M K'}{W} \times \frac{273}{T_c}$$

Combining this expression with equation $V_R^o = jt_R F$ and $V_M^o = jt_M F$, yields.

$$V_g = \frac{V_M^o K'}{W} \times \frac{273}{T_c}$$

Substituting equation $\bar{v} = u \times \frac{1}{1 + KV_s / V_M}$ for K′ gives (here V_M^o and V_M are identical).

$$V_g = \frac{KV_s}{W} \times \frac{273}{T_c}.$$

The density of ρ_s of the liquid on the stationary phase is given by

$$\rho_s - \frac{W}{V_s}.$$

Thus

$$V_g = \frac{K}{\rho_s} \times \frac{273}{T_c}$$

Note

V_g – at a given temperature depends only upon the distribution constant of the solute and the density of the liquid making up the stationary phase.

14. Give the character of Ideal Detector.

The ideal detector for gas chromatography has the following characteristics.

1. Adequate sensitivity. Just what constitutes adequate sensitivity cannot be described in quantitative terms.

Example : The sensitivities of the detectors we are about to describe differ by a factor of 10^7. Yell all are widely used and clearly adequate for certain tasks: the least sensitive are not, however, satisfactory for certain applications. In general, the sensitivities of present day detectors lie in the range of 10^{-8} to 10^{-15} g solute/s.

2. Good stability and reproducibility.
3. A linear response to solute that extends over several orders of magnitude.
4. A temperature range from room temperature to at least 400°C.
5. A short response time that is independent of flow rate.
6. High reliability and ease of use. The detector should, to the extent possible, be foolproof in the hands of inexperienced operators.
7. Similarity in response toward all solutes or alternatively a highly predictable and selective response toward one or more classes of solutes.
8. Non destructive of sample.

15. Define electron capture detector.

The electron – capture has become one of the most widely used detectors for environmental samples because this detector. Selectivity detects halogen containing compounds, such as pesticides and polychlorinated biphenyls. This type of detector operators in much the same way as a proportional counter for measurement of x – radiation.

The electron – capture detector is selective in it response, being highly sensitive to

molecules containing electronegative functional groups such as halogens, peroxides, quinines and nitro groups.

16. Define flame photometry detector.

The flame photometric detector (FDD) has been widely applied to the analysis of air and water pollutants, pesticides and coal hydrogenation products. It is a selective detector that is primarily responsive to compounds containing sulfur and phosphorous. In this detector, the eluent is passed into a low temperature hydrogenation / air flame.

17. What are the characters of stationary phase in GLC?

Desirable properties for the immobilized liquid phase in a gas – liquid chromatography column include

1. low volatility
2. Thermal stability
3. chemical inertness
4. solvent characteristics such that K′ and α values for the solutes to be resolved fall within a suitable range.

18. What is the scope of H-PLC?

High – performance liquid chromatography is the most widely used of all of the analytical separation techniques, with annual scales of HPLC equipment approaching the billion dollar mark. The reasons for the popularity of the methods is it sensitivity, it ready adaptability to accurate quantitative determinations, its suitability for separating non volatile species or thermally fragile ones, and above all its widespread applicability to substance that are of prime interest to industry to many fields of science and to the public.

Examples of such materials include amino acids, proteins, nucleic acid, hydrocarbon, carbohydrates, drugs, terpenoids, pesticides, antibiotics steroids, metal – organic species and a variety of inorganic substance.

19. Give the types of column packing in HPLC.

Two basic types of packings have been used in liquid chromatography, pellicular and porous particle. The former consists of spherical, non porous, glass or polymer – beads with typical diometer of 30 to 40 μm. A thin, porous layer of silica, alumina a polystyrene-di-vinyl benzene synthesis resin or an ion-exchange resin is deposited on the surface of these beads.

20. Give the list of detectors using in HPLC.

Liquid chromatographic detectors are of two basic types. Bulk property detectors responds to a mobile phase bulk property such as refractive index dielectric constant, or density which is modulated by the presence of solutes. In contrast, solute property detectors respond to some property of solutes, such as or absorbance, fluorescence or diffusion current, that is not possessed by the mobile phase.

21. Give the application of partition chromatography.

Typical application of partition chromatography.

Field	Typical Mixtures
Pharmaceuticals	Antibiotics, Sedative, Steroids, Analgesics
Biochemical	Amino acids, proteins, carbohydrates, lipids
Food products	Artificial sweetness, Antioxidants, Aflatoxins, Additives
Industrial chemicals	Condensed aromatics, surfactants, propellants, Dyes.
Pollutiants	Pesticides, Herbicides, Phenols, PCBS.
Forensic chemistry	Drugs, poisons, blood alcohols, narcotics
Clinical medicine	Bile acids, Drugs metabolites, Urine extracts, Estrogens.

22. Give the application of Absorption chromatography.

Liquid solid chromatography is best suited to samples that are soluble in non polar solvents and correspondingly have limited solubility in aqueous solvents such as those used in the reversed – phase partition procedure. As with partition chromatography, compounds with differing kinds or numbers of functional groups are usually separable. A particular strength of absorption chromatography, which is not shared by other methods is it ability to differentiate among the components of isomeric mixture.

23. Define size exclusion chromatography.

Size – exclusion chromatography, which has also been called gel permeation, or gel filtration chromatography is a powerful technique that is particularly applicable to high – molecular – weight species. Packing for size – exclusion chromatography consists of small silica or polymer particles containing a network of uniform pores into which solute and solvent molecules can diffuse.

24. Give the application of size exclusion chromatography.

Size – exclusion methods are subdivided into gel filtration and gel permeation chromatography. The former use aqueous solvent and hydrophilic packings the latter are based upon non polar organic solvents and hydrophobic package. The methods are complementary in the sense that the one is applied to water – soluble samples and the other to substances soluble in less polar organic solvent.

25. Define Capillary electrode process.

Electrophoretic separation are currently performed in two quite different formats. One is called slab electrophoresis and the other capillary electrophoresis. The first is the classical method that has been used for many years to separate complex, high molecular – weight species of biological and biochemical interest slab separation are carried out on a thin flat layer or slab of a porous semisolid gel containing an aqueous buffer solution within it pores.

PART – B

1. Describe the Instrumentation of Thermogravimetric methods (TG).

INSTRUMENTS FOR GAS-LIQUID CHROMATORGRAPHY

Today, well over 30 instrument manufacturers offer some 130 different models of gas-chromatographic equipment at costs that vary form perhaps $ 1500 to $ 40,000 . In the last two decades, many changes and improvements in gas chromatographic instruments have appeared in the marketplace. In the 1970s, electronic integrators and computer-based data-processing equipment became common. The 1980s saw computers being used for automatic control of most instrument parameters, such as column temperature, flow rates, and sample injection; development of very high-performance instruments at moderate costs; and perhaps most important, the development of open tubular columns that are capable of separating a multitude of analytes in relatively short times.

The basic components of an instrument for gas chromatography are illustrated in Fig. A description of each component follows. Note that in the illustration, the gas flow is split before it enters the column. This type of arrangement is employed when a type of detector is used that measures a change in property of the gas stream in the presence of analyte molecules. With other types of detectors, flow splitters are normally not used.

Carrier Gas Supply

Carrier gases, which must be chemically inert, include helium, nitrogen, and hydrogen. As will be shown later, the choice of gases is often dictated by the type of detector used.

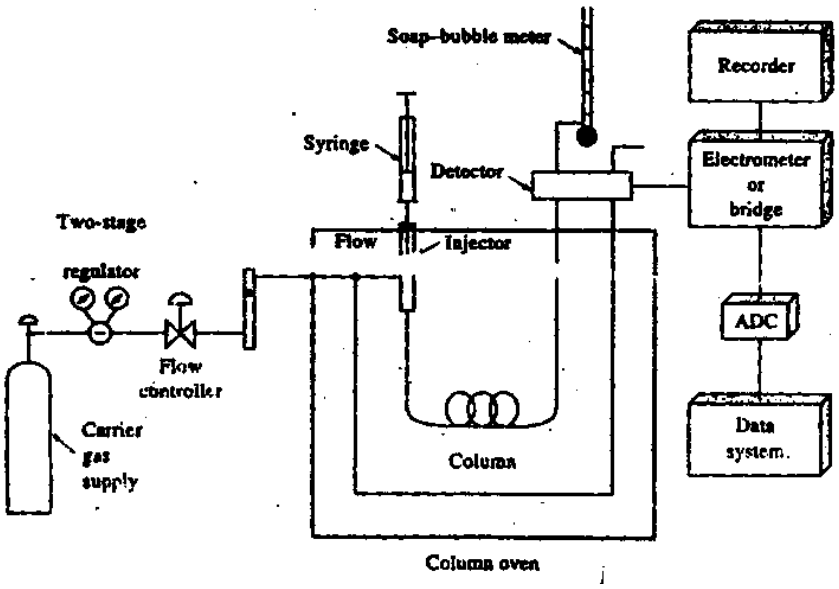


Figure: Schematic of a gas chromatograph.

Associated with the gas supply are pressure regulators, gauges, and flow meters. In addition, the carrier gas system often contains a molecular sieve to remove water or other impurities.

Flow rates are normally controlled by a two-stage pressure regulator at the gas cylinder and some sort of pressure regulator or flow regulator mounted in the chromatograph. Inlet pressures usually range form 10 to 50 psi (above room pressure), which lead to flow rates of 25 to 150 mL/min with packed columns and 1 to 25 mL/min for open-tubular capillary columns. Generally, it is assumed that flow rates will be constant if the inlet pressure remains constant. Flow rates can be established by a rotameter at the column head; this device, however, is not as accurate as a simple soap-bubble meter, which, as shown in Fig. is located at the end of the column. A soap film is formed in the path of the gas when a rubber bulb containing an aqueous solution of soap or detergent is squeezed; the time required for this film to move between two graduations on the buret is measured and converted to volumetric flow rate (see figure). Many modern commercial gas chromatographs are equipped with electronic flow meters that are computer controlled to maintain the flow rate at any desired level.

Sample Injection System

Column efficiency requires that the sample be of suitable size and be introduced as a "plug" of vapor; slow injection of oversized samples causes band spreading and poor resolution. The most common method of sample injection involves the use of micro syringe to inject a liquid

or gaseous sample through a self-sealing, silicone-rubber diaphragm or septum into a flash vaporizer port located at the head of the column (the sample port is ordinarily about 50^0C above the boiling point of the least volatile component of the sample).

Figure: A soap-bubble flow meter

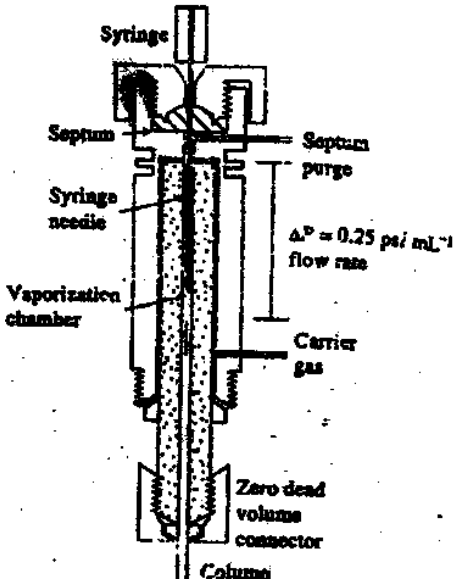


Figure Cross-sectional view of a microflash vaporizer direct injector.

Fig. is a schematic of a typical injection port. For ordinary analytical columns, samples sizes vary from a few tenths of a micro liter to 20 μL. Capillary columns require much smaller samples (≈ 10^3 μL); here, a sample splitter system is employed to deliver only a small fraction of the injected sample to the column head, with the remainder going to waste.

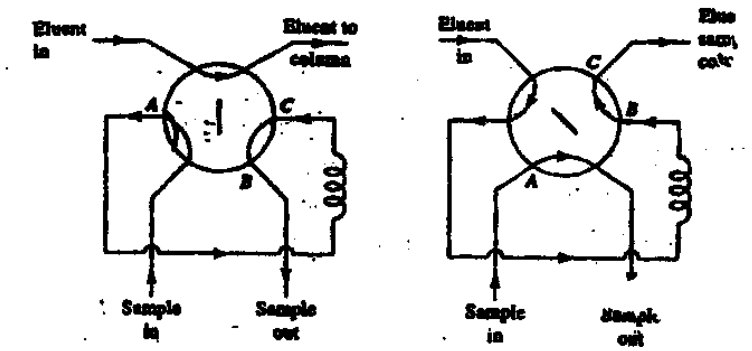


Figure A rotary sample valve; valve position (a) for filling sample loop ACB and (b) for introduction of sample into column

For quantitative work, more reproducible sample sizes for both liquids and gases are obtained by means of a rotary sample valve such as that shown in Figure. With such a device, errors due to sample size can be reduced to 0.5% to 2% relative. The sampling loop in Figure. is filled by injection of an excess of sample. Rotation of the valve by 45 deg then introduces the reproducible volume ACB into the mobile phase. Solid samples are introduced as solutions or, alternatively, are sealed into thin-walled vials than can be inserted at the head of column and punctured or crushed from the outside.

Column Configurations and Column Ovens

Two general types of columns are encountered in gas chromatography, packed and open tubular, or capillary. To data, the vast majority of gas chromatography has been carried out on packed columns. Currently, however, this situation is changing rapidly, and it seems probable that in the near future, packed columns will be replaced by the more efficient and faster open tubular columns except for certain special applications.

Chromatographic columns vary in length from less than 2 m to 50 or more. They are constructed of stainless steel, glass, fused silica, or Teflon. In order to fit into an oven for thermo stating, they are usually formed as coils having diameters of 10 to 30 cm. A detailed discussion of columns, column packings, and stationary phases is found in Section 27c.

Column temperature is an important variable that must be controlled to few tenths of a degree for precise work. Thus, the column is ordinarily housed in a thermostated oven. The optimum column temperature depends upon the boiling point of the sample and the degree of separation required. Roughly, a temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time (2 to 30 min). For samples with a broad boiling range, it is often desirable to employ temperature programming, whereby the column temperature is increased either continuously or in steps as the separation proceeds. Figure shows the improvement in a chromatogram brought about by temperature programming.

In general, optimum resolution is associated with minimal temperature; the cost of lowered temperature, however, is an increase in elution time and therefore the time required to complete an analysis. figure 27-5a and 27-5b illustrate this principle.

Detection Systems

Dozens of detectors have been investigated and used during the development of gas chromatography. In the sections that follow immediately, we describe the most widely used of these. In section, we consider instruments in which gas chromatographs are coupled to mass spectrometers and infrared spectrophototmeters. Here, the spectral device serves not only to detect the appearance of analytes at the end of the column but also to identify them.

Characteristics of the Ideal Detector

The ideal detector for gas chromatography has the following characteristics:

1. Adequate sensitivity. Just what constitutes adequate sensitivity cannot be described in quantitative terms. For example, the sensitivities of the detectors we are about to describe differ by a factor of 10^7. Yet all are widely used and clearly adequate for certain tasks; the least sensitive are not, however, satisfactory for certain applications. In general, the sensitivities of present-day detectors lie in the range of 10^{-8} to 10^{-15}g solute/s.

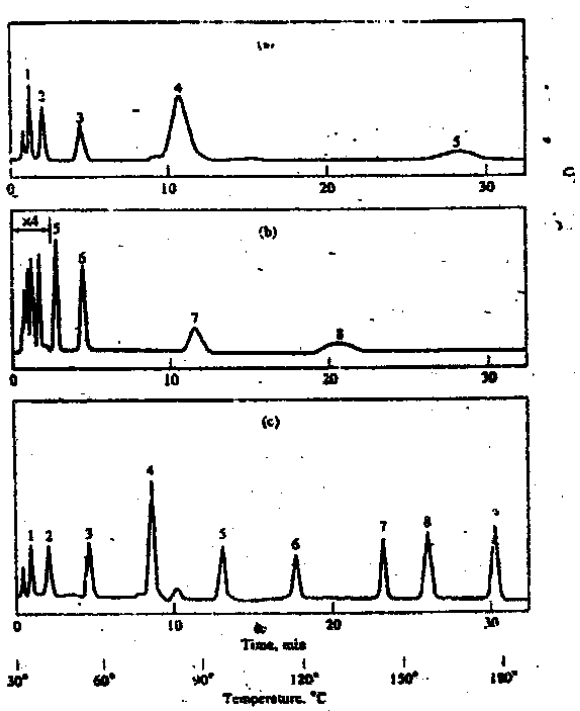


Figure Effect of temperature on gas chromatograms: (a) Isothermal at 45°C; (b) isothermal at 145°C; (c) programmed at 30° to 180°C.

2. Good stability and reproducibility.
3. A linear response to solutes that extends over several orders of magnitude.
4. A temperature range room temperature to at least 400^0C.
5. A short response time that is independent of flow rate.
6. High reliability and ease of use. The detector should, to the extent possible, be foolproof in the hands of inexperienced operators.
7. Similarity in response toward all solutes or alternatively a highly predictable and selective response to ward one or more classes of solutes.
8. Nondestructive of sample.

Needless to say, no detector exhibits all of these characteristics, and it seems unlikely that such a detector will ever be designed.

2. Describe the following.

Flame Ionization Detectors (FID)

The flame ionization detector is the most widely used and generally applicable detector for gas chromatography. With a burner such as that shown in Figure, the effluent from the column is mixed with hydrogen and air and then ignited electrically. Most organic compounds. When pyrolyzed at the temperature of a hydrogen/air flame, produce ions and electrons that can conduct electricity through the flame. A potential of a few hundred volts is applied across the burner tip and a collector electrode located above the flame. The resulting current ($\approx 10^{12}$ A) is then directed into a high-impedance operational amplifier measurement.

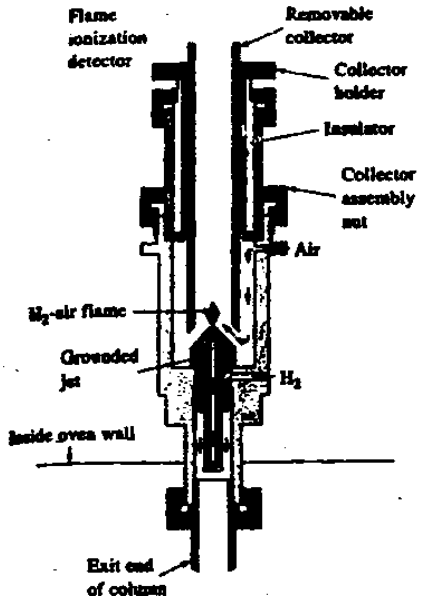


Figure A typical flame ionization detector. (Courtesy of Hewlett-packard Company)

The ionization of carbon compounds in a flame is a poorly understood process, although it is observed the number of ions produced is roughly proportional to the number of reduced carbon atoms in the flame. Because the flame ionization detector responds to the number of carbon atoms entering the detector per unit of time, it is a mass-sensitive, rather than a concentration-sensitive, device.

Functional groups, such as carbony1, alcohol, halogen, and amine, yield fewer ions or none at all in a flame. In addition, the detector is insensitive toward noncombustible gases such as H_2O, CO_2, SO_2, and NO_2. These properties make the flame ionization detector for the analysis of most organic samples, including those that are contaminated with water and the oxides of nitrogen and sulfur. The insensitivity of the flame ionization detector to water makes it particularly useful for the detection of pollutants in natural water samples.

The flame ionization detector exhibits a high sensitivity (10^{-13}g/s), large linear response range (-10^7), and low noise. It is generally rugged and easy to use. A disadvantage of the flame ionization detector is that it is destructive of sample.

Thermal Conductivity Detectors (TCD)

A very early detector for gas chromatography, and one that still finds wide application, is based upon changes in the thermal conductivity of the gas stream brought about by the presence of analyte molecules. This device is sometimes called a katharometer. The sensing element of a katharometer is an electrically heated element whose temperature at constant electrical power depends upon the thermal conductivity of the surrounding gas. The heated element may be a fine platinum, gold, or tungsten wire or, alternatively, a semiconducting thermistor. The resistance of the wire or thermistor gives a measure of the thermal conductivity of the gas; in contrast to the wire detector, the thermistor has a negative temperature coefficient. Figure is a cross-sectional view of one of the temperature-sensitive elements in a thermal conductivity detection system.

Figure (b) shows the arrangement of detector elements in a typical detector unit. Two pair of elements are employed, one pair being located in the flow of the effluent from the column and the other in the gas stream ahead of the sample injection chamber. (These elements are labeled “sample” and “reference” in Figure (b). Alternatively, the gas stream may be split as shown in Figure. In either case, the effect of thermal conductivity of the carrier gas is canceled, and the effects of variation in flow rate, pressure, and electrical power are minimized. The resistances of the twin-detector pairs are usually compared by incorporating them into two arms of a simple Wheatstone bridge circuit such as that shown in Figure (b).

A modulated single-filament thermal conductivity detector was introduced in 1979; this device offers higher sensitivity, freedom from baseline drift, and reduced equilibration time. Here,

the analytical and reference gases are passed alternately over a tiny filament held in a ceramic detector cell, which has a volume of only 5 μL. The gas-switching device operates at a frequency of 10 Hz. The output from the filament is thus a 10 –Hz electrical signal whose amplitude is proportional to the difference in thermal conductivity of the analytical and reference gases. Because the amplifier circuit responds only to a 10-Hz signal, thermal noise in the system is largely eliminated.

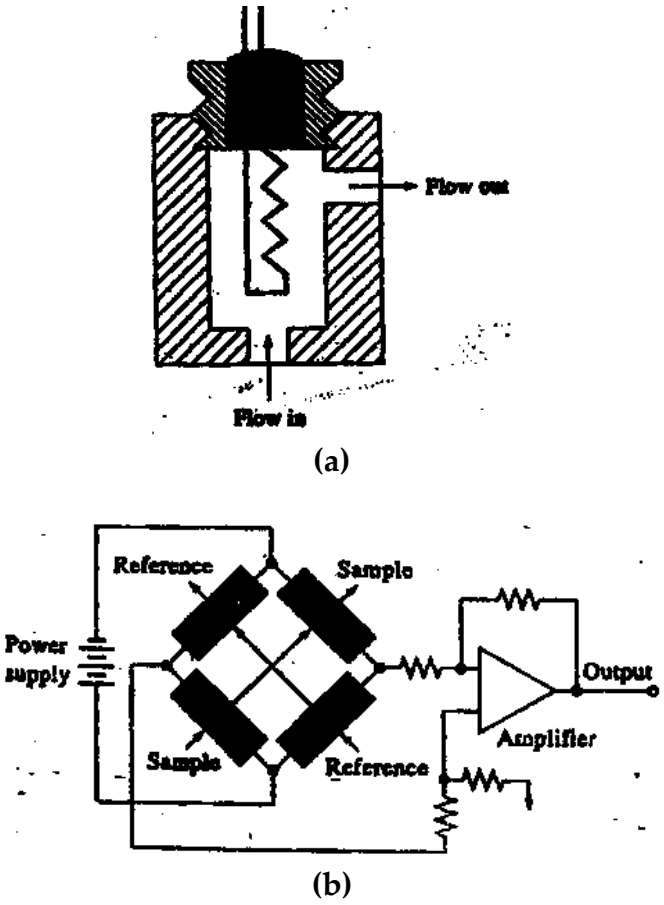


Figure: Schematic of (a) a thermal conductivity detector cell, and (b) an arrangement of two sample detector cells and two reference detector cells.

The thermal conductivities of helium and hydrogen are roughly six to ten times greater than those of most organic compounds. Thus, in the presence of even small amounts of organic materials, a relatively large decrease in the thermal conductivity of the column effluent takes place; consequently, the detector undergoes a carrier gases more closely resemble those of organic constituents; therefore a thermal conductivity detector dictates the use of hydrogen or helium in order to achieve good sensitivity.

The advantage of the thermal conductivity detector is its simplicity, its large linear dynamic range (10^5), its general response to both organic and inorganic species, and its nondestructive character, which permits collection of solutes after detection. A limitation of the katharometer is

its relatively low sensitivity (10^{8}g solute/mL carrier gas). Other detector exceed this sensitivity by factors as large as 10^{4} to 10^{7}. It should be noted that the low sensitivity of thermal detectors often preclude their use with capillary columns because of the very small samples that can be accommodated by such columns.

Atomatic Emission Detectors (AED)

The atomic emission detector is available commercially[5]. See B.D. Quimby and J.J. Sullivan, Anal.Chem., 1990,62, 1027,1034. In this device (see Figure 27-9), the eluent is introduced into a microwave-energized helium plasma that is coupled to a diode array optical emission spectrometer. The plasma is sufficiently energetic to atomize all of the elements in a sample and to excite their characteristic atomic emission spectra. These spectra are then observed with a spectrometer that employs a movable, flat diode array capable of detecting emitted radiation from about 170 to 780 nm. As shown on the right of the figure, the possitionable doide array is capable of monitoring simultaneously two to four elements at any given setting. At the present time, the software supplied with the detector allows measurement of the concentration of 15 elements. Presumably, future software will permit detection of other elements.

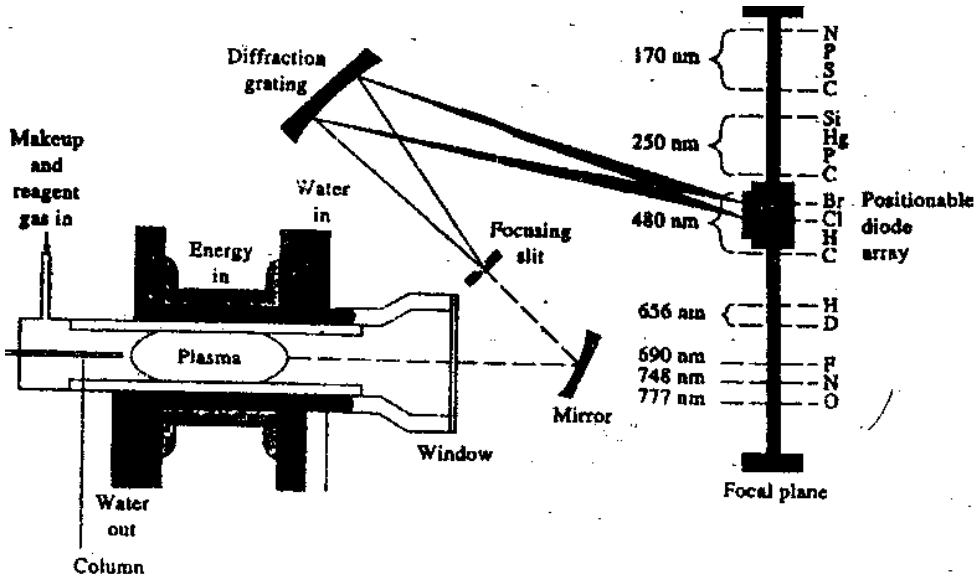


Figure: An atomic emission detector.

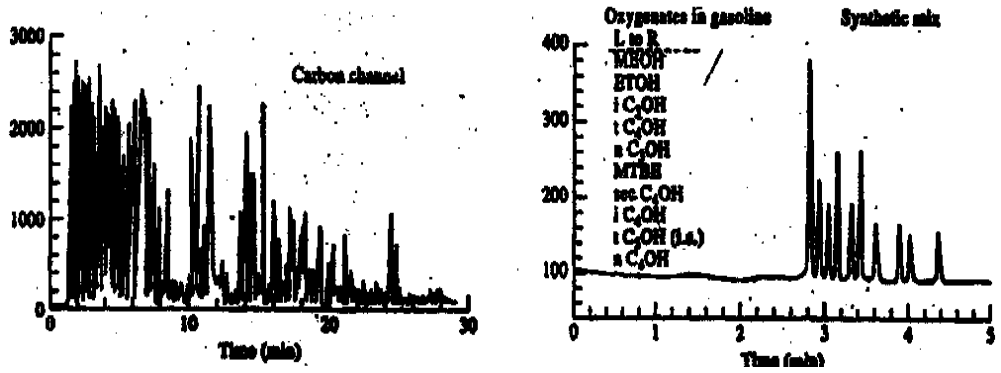


Figure Chromatograms for a gasoline sample containing a small amount of MTBE and several aliphatic alcohols: (a) monitoring the line for carbon; (b) monitoring the line for oxygen.

Figure illustrates the power of this type of detector. The sample in this case consisted of a gasoline containing a small concentration of methyl tertiary butyl ether (MTBE), an antiknock agent, as well as several aliphatic alcohols in low concentrations. The upper spectrum, obtained by monitoring the carbon emission line at 198 nm, consists of a muriad of peaks that would be impossible to sort out an identify. In contrast, when the oxygen line at 777 nm is used to obtain the chromatogram (Figure-b), peaks for the various alcohols and for MTBE are clearly evident and readily identifiable.

3. What is application of Gas liquid Chromatography (GLC)?

APPLICATIONS OF GAS-LIQUID CHROMATOGRAPHY (GLC)

In evaluating the importance of GLC, it is necessary to distinguish between the two roles the method plays. The first is as a tool for performing separations; in this capacity, it is unsurpassed when applied to complex organic, metal-organic, and biochemical systems that are made up volatile species or species that can be derivitized to yield volatile substances. The second, and distinctly different, function is that of providing the means for completion of an analysis. Here, retention times or volumes are employed for qualitative identification, while peak heights or peak areas provide quantitative information. For qualitative purposes, GLC is much more limited than most of the spectroscopic methods considered in earlier chapters. As a consequence, an important trend in the field has been in the direction of combining the remarkable fractionation qualities of GLC with the superior identification properties of such instruments as mass, infrared, and NMR spectrometers.

Qualitative Analysis

Gas chromatograms are widely used as criteria of purity for organic compounds. Contaminants, if present, are revealed by the appearance of additional peaks; the areas under these peaks provide rough estimates of the extent of contamination. The technique is also useful for evaluating effectiveness of purification procedures.

In theory, retention times should be useful for the identification of components in mixtures. In fact, however, the applicability of such data is limited by the number of variables that must be controlled in order to obtain reproducible results. Nevertheless, gas chromatography provides an excellent means of confirming the presence or absence of a suspected compound in a mixture, provided an authentic sample of the substance is available. No new peaks in the chromatogram of the mixture should appear upon addition of the now compound, and enhancement of an existing peak should be observed. The evidence is particularly convincing if the effect can be duplicated on different columns and at different temperatures.

Selectivity Factors

We have seen (Section 26B-3) that the selectivity factor α for compounds A and B is given by the relationship $\alpha = \frac{K_B}{K_A} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} = \frac{(t_R)_B}{(t_R)_A}$ If a standard substance is chosen as compound B, then α can provide an index for identification of compound A, which is largely independent of column variables other than temperature; that is, numerical tabulations of selectivity factors for pure compounds relative to a common standard can be prepared and then used for the characterization of solutes. Unfortunately, finding a universal standard that yields selectivity factors of reasonable magnitude for all types of analysis is not possible. Thus, the amount of selectivity factor data available in the literature is presently limited.

The Retention Index

The retention index I was first proposed by Kovats in 1985 as a parameter for identifying solutes from chromatograms[9]. E. Kovats, Helv. Chim.Acta, 1958,41,1915. The retention index for any given solute can be derived form a chromatogram of a mixture of that solute with at least two normal alkanes having retention times that bracket that of the solute. That is, normal alkanes are the standards upon which the retention index scale is based. By definition, the retention index for a normal alkane is equal to 100 times the number of carbons in the compound regardless of the column packing, the temperature, or other chromatographic than normal alkanes vary, often by several hundred retention index unit, with column variables.

It has long been know that within a homologous series, a plot of the logarithm of adjusted retention time t_R ($t_R - t_M$) versus the number of carbon atoms is excluded. Such a plot for C_4 to C_9 normal alkane standards is shown in figure 27-12. Also indicated on the ordinate are log retention times for three compounds on the same column and at the same temperature. Their retention indexes are then obtained by multiplying the corresponding abscissa values by 100. Thus, the retention index for toluene is 749, while for benzene it is 644.

Normally a graphical procedure is not required in determining retention indexes. Instead adjusted retention data are derived by interpolation from a chromatogram of a mixture of the solute of interest and two or more alkane standards.

It is important to reiterate that the retention index for a normal alkane is independent of temperature and column packing. Thus, I for heptane, by definition, is always 700. In constrast, retention indexes of all other solutes may, and often do, vary widely from one column to another. For example, the retention index for acenapthene on a cross-linked polydimethyl siloxane

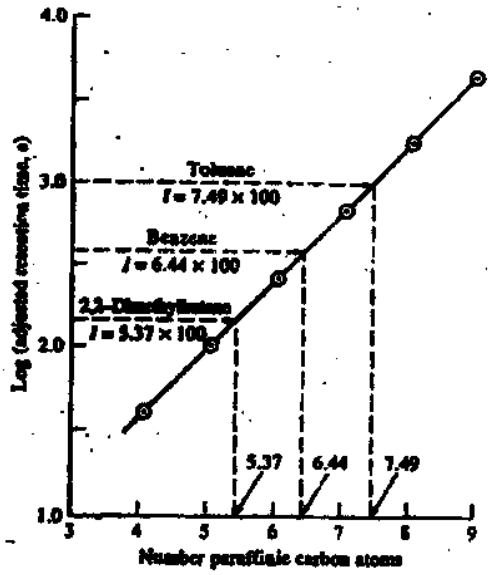


Figure: Graphical illustration of the method for determining retention indexes for three compounds. Stationary phase: squalane.Temperature:60°C.

stationary phase at 140°C is 1460. With 5% phenyl-ploydimethyl siloxane as the stationary phase, it is 1500 at the same temperature, while with polyethylene glycol as the stationary phase, the retention index is 2084.

The retention index system has the advantage of being upon readily available reference materials that cover a wide boiling range. In addition, the temperature dependence of retention indexes is relatively small. In 1984, Sadtler Research Laboratories introduced a library of retention indexes measured on four types of fused-silica open tubular columns. The computerized format of the database allows retention index searching and possible identity recall with a desktop computer[10]. –See J.F. Sprouse and A. Varano, Amer, Lab., 1984 (9), 54.

Quantitative Analysis

The detector signal from a gas-liquid chromatographic column has had wide use for quantitative and semi – quantitative analysis. An accuracy of 1% relative is attainable under carefully controlled conditions. As with most analytical reliability is directly related to the control

of variables; the nature of the sample also plays a part in determining the potential accuracy. The general discussion of quantitative chromatographic analysis given in Section applies to gas chromatography as well as to other types; therefore, no further consideration of this topic is given here.

Interfacing Gas Chromatography with Spectroscopic Methods.

Gas chromatography is often coupled with the selective techniques of spectroscopy and electrochemistry, thus giving so-called hyphenated methods that provide the chemist with powerful tools for identifying the components of complex mixtures.,[11] For a review on hyphenated methods, see T. Hirschfeld, Anal.Chem., 1980,52,297A; C.L. Wilkins, Science, 1983, 222 291; Anal.Chem., 1987,59,571A; P,R. Griffiths et al., Anal.Chem., 1986,58,1349A.

In early hyphenated methods, the eluates from the chromatographic column were collected as separate fractions in a cold trap after being detected by a nondestructive and nonselective detector. The composition of each fraction was then investigated by nuclear magnetic resonance, infrared, or mass spectroscopy, or by electroanalytical measurements. A serious limitation to this approach was the very small (usually micromolar) quantities of solute contained in a fraction; nonetheless, the general procedure proved useful for the qualitative analysis of many multicomponent mixtures.

A second general method, which now finds wide spread use, involves the application of a selective detector to monitor the column effluent continuously. Generally, these procedures require computer control of instruments and computer memory for storage of spectral data for subsequent display as spectra and chromatograms.

Gas Chromatography/Mass Spectrometry (GC/Ms).

Several instrument manufacturers offer gas-chromatographic equipment that can be directly interfaced with rapid-scan mass spectrometers of various types. - For additional Information, see G.M. Message, Practical Aspects of Gas Chromatography/Mass Spectrometry. New York: Wiley, 1984; J.Masucci and G.W. Caldwell, in Modern Practice of Gas Chromatography, 3rd ed., R.L. Grob,Ed., Chapter 6 New York: Wiley-Interscience, 1995; C.L. Wilkins, Anal.Chem., 1987,59,571A; J.Fjeld-sted and J.Truche, Amer, Lab., 1989 (10), 33. The flow rate from capillary columns is generally low enough that the column output can be fed directly into the ionization chamber of the mass spectrometer. A schematic of a typical system is shown in Figure. For packed columns and megabore capillary columns, however, a jet separator such as that shown in figure. must be employed to remove most of the carrier gas from the analyte. In this device, the exit gases flow creases the momentum of the heavier analyte molecules so that 50% or more of them travel in a more or less straight path to the skimmer. The light helium atoms, in contrast, are deflected by the vacuum and are thus pumped away.

Most quadrupole and magnetic sector mass spectrometers are offered with accessories that permit interfacing with gas-chromatographic equipment. In addition, the Fourier transform mass spectrometer described in Section has been coupled to gas-liquid columns. - E.B. Ledford et at., Anal.Chem., 1980,52,2550. Its speed and high sensitivity are particularly advantageous for this application.

Beginning in the late 1970s, several mass spectrometers designed specifically as gas chromatographic detectors appeared on the market. Generally, these are compact quadrupole instruments, which are less expensive ($ 25,000 to $ 50,000) and easier to use and maintain than the multipurpose mass spectrometers described in Chapter 20· - For descriptions of several benchtop GC/MS instruments, see.Wach, Anal.Chem., 1994,66,927A.

The simplest mass detector for use in gas chromatography is the ion trap detector (ITD). - G.C. Stafford Jr.P.E. Kelley, and D.c. Bradford, Amer.Lab., 1983 15 (6), 51; S.A. Borman,Anal.Chem., 1983.,55,726A. (see Section 20C-3 and Figure 20-15). In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio-frequency field (see Figure 27-15). The trapped ions are then ejected from the storage area to an electron multiplier detector. The ejection is controlled so that scanning on the basis of mass-to-charge ratio is possible. The ion trap detector is remarkably compact and less expensive than quadrupole instruments.

Mass spectrometric detectors ordinarily have several display modes, which fall into two categories: real time and computer reconstructed. Within each of these categories are a choice of total ion current chromatograms (a plot of the sum of all ion currents as function of time), selected ion current chromatogram (a plot of ion currents for one or a few ions as a function of time), and mass spectra of various peaks. Real-time mass spectra appear on an oscilloscope screen equipped with mass markers; the mass chromatogram may appear on the oscilloscope screen or as a real-time plot. After a separation is complete, computer-reconstructed chromagrams can be displayed on the screen or can be printed out. Reconstructed mass spectra for each peak can also be displayed or printed. Some instruments are further equipped with spectral libraries for compound identification.

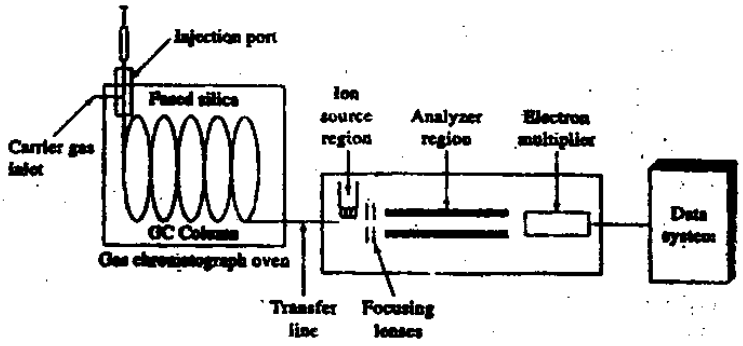


Figure Schematic a typical capillary gas chromatography/mass spectrometer

Gas chromatography/mass spectrometry instruments have been used for identification of hundreds of components that are present in natural and biological systems. For example, these procedure have permitted characterization of the odor and flavor components of foods, identification of water pollutants, medical diagnosis based on breath components, and studies of drug metabolites.

An example of one application of GC/MS is shown in figure. The upper figure is a computer-reconstructed mass chromatogram of a sample trapped from an environmental chamber during the combustion of cloth treated with a fire-retarding chemical. the ordinate here is the total ion current while the abscissa

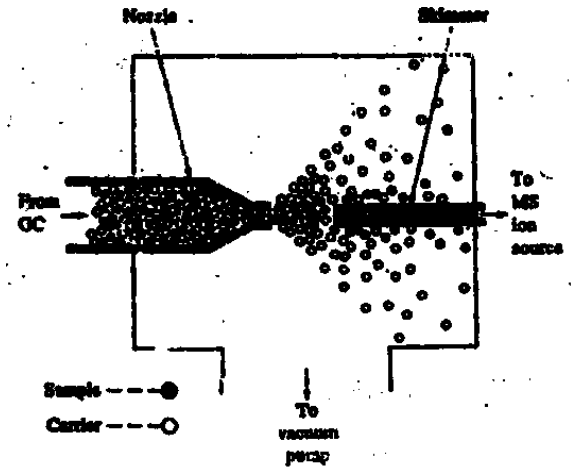


Figure: Schematic of a jet separator.

is retention time. The lower figure is the computer reconstructed mass spectrum of pead 12 the chromatogram. Here, relative ion currents are plotted as a function of mass number.

Gas Chromatography/Fourier Transform Infrared Spectroscopy (GC/FTIR)

Coupling capillary column gas chromatographs with Fourier transform infrared spectrometers provides a potent means for separating and identifying the components of difficult mixtures. Several instruments of this type are now offered commercially. – See C. Fujimoto and K. Jinno, Anal. Chem., 1992, 64, 476A; P.R. Griffiths, S.L. Pentoney, A. Giorgetti, and K.H. Shafter, Anal. Chem., 1986, 58, 1349A; c.l. Wilkins, Science, 1983, 222, 291; P.R. Griffiths, J.A. de Haseth, and L.V. Azarraga, Anal. Chem., 1983, 55, 1361A; S.A. Borman, Anal. Chem., 1982, 54, 901A.

4. Describe the Instruments for liquid chromatography.

Sample Injection Systems

Often, the limiting factor in the precision of liquid chromatographic measurement lies in the reproducibility with which samples can be introduced into the column packing. The problem is exacerbated by band broadening, which accompanies overloading columns. Thus, the volumes used must be minuscule—a few tenths of a microliter to perhaps 500 μL. Furthermore, it is convenient to be able to introduce the sample without depressurizing the system.

The earliest and simplest means of sample introduction was syringe injection through a self-sealing elastomeric septum. for this purpose, microsyringes designed to withstand pressures up to 1500 psi are used. In stop—flow injections, the flow of solvent is stopped momentarily, a fitting at the column head is removed, and the sample is injected directly onto the head of the column packing. After replacing the fitting, the system is again pressurized. The advantage of this technique is its simplicity. Unfortunately, the reproducibility of syringe injection is rarely better than 2% to 3% and is often considerably worse.

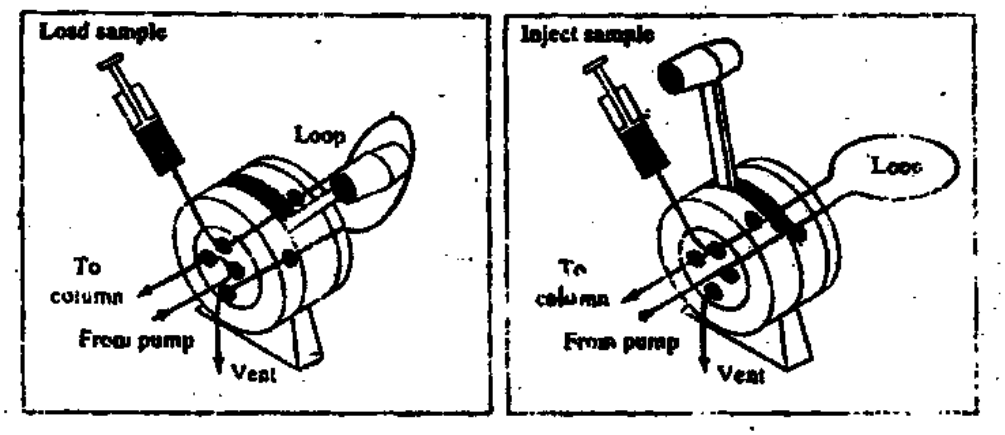


Figure: A sampling loop for liquid chromatography.

The most widely used method of sample introduction in liquid chromatography is based upon sampling loops, such as that shown in Figures and Theses devices are often an integral part of liquid-chromatographic equipments and have interchangeable loops providing a choice of sample sizes from 5 to 500 μL. Sampling loops of this type permit the introduction of samples at pressure up to 7000 psi with precisions of a few tenths percent relative. Micro sample injection valves, with sampling loops having volumes of 0.5 to 5 μL, are also available.

Liquid-Chromatographic Columns

Liquid-chromatographic columns are ordinarily constructed from smooth-bore stainless steel tubing, although heavy-walled glass tubing is occasionally encountered. The latter is restricted to pressure that are lower than about 600 psi. Hundreds of packed columns differing in size and packing are available from several manufacturers. Their costs range from $200 to $500 generally.- For descriptions of recent commercially available HPLC columns, see R.E. Majors, LC-GC, 1995,13(8), 202;1994, 12(12),890.

Analytical Columns

The majority of liquid-chromatographic columns range in length from 10 to 30 cm. Normally, the columns are straight, with added length, where needed, being gained

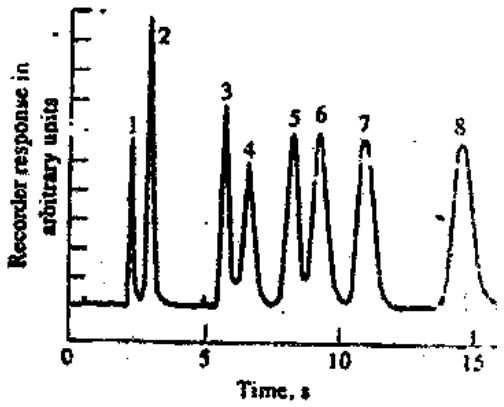


Figure: High-speed isocratic separation. Column dimensions: 4cm length; 0.4 cm i.d. Packing: 3-μm sperisorb, Mobile phase: 4.1% ethy1 acetate in n-hexane. Compounds: (1) p-xylene, (2) anisole, (3) benzyl acetate, (40 diocty1 phthalate, (5) dipenty1 phthalte, (6) dibuty1 phthalate, (7) dipropy1 phthalate, (8) diethy1 phthalate. (From R.P.W. Scott, Small Bore Liquid Chromatography Columns: Their Properties and Uses, p. 156. New York: Wiley, 1984. Reprinted with permission of John Wiley & Sons, Inc.)

by coupling two or more columns together. Occasionally coiled columns are encountered, although someloss in efficiency results from this configuration. The inside diameter of liquid columns is often 4 to 10mm: the most common particle size of packings is 5 or 10μm. Perhaps the most common column currently in use is one that is 25 cm in length, 4.6mm in inside diameter, and packed with 5 μm particles. Columns of this type contain 40,000 to 60,000 plates/meter.

Recently, manufacturers have producing high-speed, high-performance columns, which have smaller dimensions that those and just described.[5] See Microcolumn High-Performance Liquid Chromatography, P. Kucera, Ed. New York: Elsevier, 1984; Small Bore Liquid

Chromatography Columns: Their Properties and Uses, R.P.W. Scott, Ed. New York: Wiley, 1984; M. Novotny, Anal.Chem., 1988,60,500A. Such columns may have inside diameters that range from 1 to 4.6mm and be packed with 3 or 5 μm particles. Often, their lengths are as short as 3 to 7.5 cm. Such columns contain as many as 100,000 plates/meter and have the advantage of speed and minimum solvent required for liquid chromatography are expensive to purchase and to dispose of. Figure illustrates the speed with which a separation can be performed on this type of column. Here, eight components of diverse type are separated in about 15 s. The column was 4 cm in length and had an inside diameter of 4 mm; it was packed with 3- μm particles.

Guard Columns

Usually, a short guard column is introduced before the analytical column to increase the life of the analytical column by removing not only particulate matter and contaminants from the solvents but also sample components that bind irreversibly to the stationary phase. In addition, in liquid-liquid chromatography, the guard column serves to saturate the mobile phase with the stationary phase so that losses of this solvent from the analytical column are minimized. The composition of the guard-column packing should be closely similar to that of the analytical column; the particle size is usually larger, however, to minimize pressure drop. When the guard column has become contaminated, it is repacked or discarded and replaced with a new one of the same type. Thus, the guard column is sacrificed to protect the more expensive analytical column.

Column Thermostats

For many applications, close control of column temperature is not necessary, and columns are operated at ambient temperature. Often, however, better chromatograms are obtained by maintaining column matograms are obtained by maintaining column temperatures constant to a few tenths degree centigrade. Most modern commercial instruments are temperatures to few tenths of a degree from near ambient to 100^0 to 150^0C. Columns may also be fitted with water jackets fed from a constant temperature bath to give precise temperature control.

Types of Column Packings

Two basic types of packings have been used in liquid chromatography, pellicular and porous particle. The former consist of spherical, nonporous, glass or polymer beads with typical diameters of 30 to 40 μm. S thin, porous layer of silica, alumina, a polystyrene-divinyl benzene synthetic resin, or an ion-exchange resin is deposited on the surface of these beads. For some applications, an additional coating is applied, which consists of a liquid stationary phase that is held in place by adsorption. Alternatively, the beads may be treated chemically to given an organic surface layer. Currently, pellicular packings are used largely for guard columns and not for analytical columns.

The typical porous particle packing for liquid chromatography consists of porous microparticles having diameters ranging from 3 to 10 μm; for a given size particle, every effort is made to minimize the range of particle sizes. The particles are composed of silica, alumina, the synthetic resin polystyrene-divinyl benzene, or an ion-exchange resin. Silica is by far the most common packing in liquid chromatography. Silica particles are prepared by agglomerating submicron silica particles under conditions that lead to larger particles having highly uniform diameters. The resulting particles are often coated with thin organic films, which are chemically or physically bonded to the surface.

Detectors

For more detailed discussion of liquid chromatographic detectors, see R.P.W.Scott, Liquid Chromatographic Detectors, 2nd ed. Amsterdam: Elsevier, 1986; E.S. Yeung and R.E. Synovec,Anal.Chem., 1986,58,1237A;C.A.Dorschel et al., Anal.Chem.,1989,61,951A.

Unlike gas chromatography, liquid chromatography has no detectors that are as universally applicable and as reliable as the flame ionization and thermal conductivity detectors described in Section. A major challenge in the development of liquid chromatography has been in detector improvement.

Characteristics of the Ideal Detector

The ideal detector for liquid chromatography should have all of the properties listed on page 706 for gas chromatography with the exception that the liquid chromatography detector need not be responsive over as great a temperature range. In addition, an HPLC detector should have minimal internal volume in order to reduce zone broadening.

Types of Detectors

Liquid chromatographic detectors are of two basic types. Bulk property detectors respond to a mobile phase bulk property, such as refractive index, dielectric constant, or density, which is modulated by the presence of solutes. In constrast, solute property detectors respond to some property of solutes, such as UV absorbance, or diffusion current, that is not possessed by mobile phase.

Table lists the most common detectors for HPLC and some of their more important properties. A 1982 survey of 365 published papers in which liquid chromatography played an important role revealed that 71% were based upon detection by UV absorption. 15% by fluorescene, 5.4% by refractive index, 4.3% by electrochemical measurements, and another 4.3% by

other measurements. - See Anal.Chem. 1982,54,327A. Of the UV absorption detectors, 39% were based upon one of the emission lines of mercury, 13% upon filtered radiation from a deuterium source, and 48% upon radiation emitted from a grating monochromator.

Absorbance Detectors

Figure is a schematic of a typical, Z-shaped, flow through cell for absorbance measurements on eluents from a chromatographic column. In order to minimize extra-column band broadening, the volume of such a cell is kept as small as possible. Thus, typically, volumes are limited to 1 to 10 μL and cell lengths to 2 to 10 mm. Most cells of this kind are restricted to pressures no greater than about 600 psi. Consequently, a pressure reduction device is often required.

Many absorbance detectors are double-beam devices in which one beam passes through the eluent cell and the other through a filter to reduce its intensity.

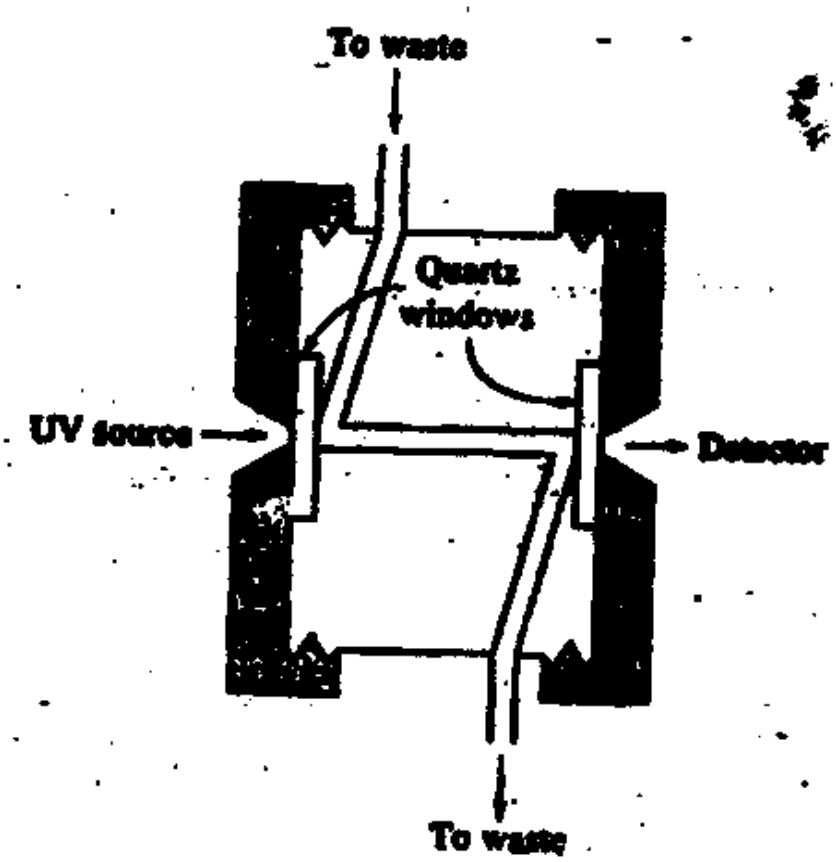


Figure: Ultraviolet detector cell for HPLC.

TABLE Performances of LC Detectors

LC Detector	Commercially Available	Mass LOD (commercial detectors)[a]	Mass LOD (state of the art)[b]
Absorbance	Yes[c]	100 pg-1 ng	1 pg
Fluorescence	Yes[c]	1-10 pg	10 fg
Electrochemical	Yes[c]	10pg-1 ng	100 fg
Refractive index	Yes	100 ng-1 μg	10 ng
Conductivity	Yes	500 pg-1 ng	500 pg
Mass spectrometry	Yes[d]	100 pg-1 ng	1 pg
FT-IR	Yes[d]	1 μg	100 ng
Light scattering[e]	Yes	10 μg	500 ng
Optical activity	No	-	1 ng
Element selective	No	-	10 ng
Photoionization	No	-	1 pg-1 ng

[a]Mass LOD is calculated for injected mass that yields a signal equal to five times the σ noise, using a mol wt of 200 g/mol, 10 μL injected for conventional or 1 μL injected for microbore LC.
[b]Same definition as a, above, but the injected volume is generally smaller.
[c]Commercially available for microbore LC also.
[d]Commercially available, yet costly.
[e]Including low-angle light scattering and nephelometry.
(From E.S. Yeung and R.E. Synovec,Anal.Chem., 1986,58,1238. With permission).

Matched photoelectric detectors are then used to compare the intensities of the two beams. Alternatively, a chopped beam system similar to that shown in Figure.13b is used in conjunction with a single phototube. In either case, the chromatogram consists of a plot of the log of the ratio of the two transduced signals as a function of time. Single-beam instruments of the solvent system are stored in a computer memory and ultimately recalled for the calculation of absorbance.

Ultraviolet Absorbance Detectors with Filters.

The simplest UV absorption detectors are filter photometers with a mercury lamp as the source. Most commonly the intense line at 254 nm is isolated by filters; with some instruments, lines at 250, 313,334, and 365 can also be employed by substitution of filters. Obviously this type of detector is restricted to solutes that absorb at one of these wavelengths. As shown in Section 14B, several organic functional groups and a number of inorganic species exhibit broad absorption bands that encompass one or more of these wavelengths.

Deuterium or tungsten filament sources with interference filters also provide a simple means of detecting absorbing species as they are eluted from a column. some modern instruments are equipped with filter wheels containing several filters that can be rapidly switched to detect various species as they are eluted. Such devices are particularly useful for repetitive, quantitative analyses where the qualitative composition of the sample is known so that a sequence of appropriate filters can be chosen. Often, the filter changes are computer controlled.

Ultraviolet Absorbance Detectors with Monochromators.

Most HPLC manufacturers offer detectors that consist of a scanning spectrophotometer with grating optics. Some are limited to ultraviolet radiation; others encompass both ultraviolet and visible radiation. Several operational modes can be chosen. For example, the entire chromatogram can be obtained at a single wavelength; alternatively, when eluent peaks are sufficiently separated in time, different wavelengths can be chosen for each peak. Here again, computer control is often used to select the best wavelength for each eluent. Where entire spectra are desired for identification purpose, the flow of eluent can be stopped for a sufficient period to permit scanning the wavelength region of interest.

The most powerful ultraviolet spectrophotometric detectors are diode-array instruments as described in Section and Figure 13-22[8]. __ See J.C.Miller, S.O.George, and B.G. Willis, Science, 1982,218,241; S.A. Bormkan, Anal.Chem., 1983,55,836A; Diode Array Detection in HPLC, I.Huber and S.A. George, Eds. New York: Marcel Dekker, 1993. Several manufacturers offer such instruments, which permit collection of data for an entire spectrum in approximately one second. Thus, spectral data for each chromatographic peak can column. One form of presentation of the spectral data, which is helpful in identification of spectra were obtained at successive five-second intervals. The appearance and disappearance of each of three steriods in the eluent is clearly evident.

Infrared Absorbance Detectors

Two types of infrared detectors are offered commercially. The first is similar in design to the instruments shown in Figure 16-14, with wavelength scanning being provided by three

semicircular filter wedges. The range of this instrument is from 2.5 to 14.5 μm or 4000 to 690 cm^{-1}.

The second, and much more sophisticated, type of infrared detector is based upon Fourier transform instruments similar to those discussed in Section 16C-1. Several of the manufactures of Fourier transform instruments offer accessories that permit their use as HPLC detectors.

Infrared detector cells are similar in construction to those used with ultraviolet radiation except that windows are constructed of sodium chloride or calcium fluoride. Cell lengths range from 0.2 to 1.0 mm and volumes from 1.5 to 10μL.

The simpler infrared instruments can be operated at one or more single wavelength settings; alternatively, the spectra for peaks can be scanned by stopping the flow at the time of elution. The Fourier transform instruments are used in an analogous way to the diode-array instruments for ultraviolet absorbance measurement described in the previous section.

A major limitation to the use of infrared detectors lies in the low transparency of many useful solvents. For example, the broad infrared absorption bands for water and the alcohols largely preculde the use of this detector for many applications.

Fluorescence Detectors

Fluorescence detectors for HPLC are similar in design to the fluorometers and spectrofluorometers described in Section. In most, fluorescence is observed by a photoelectric detector located at 90 deg to the excitation

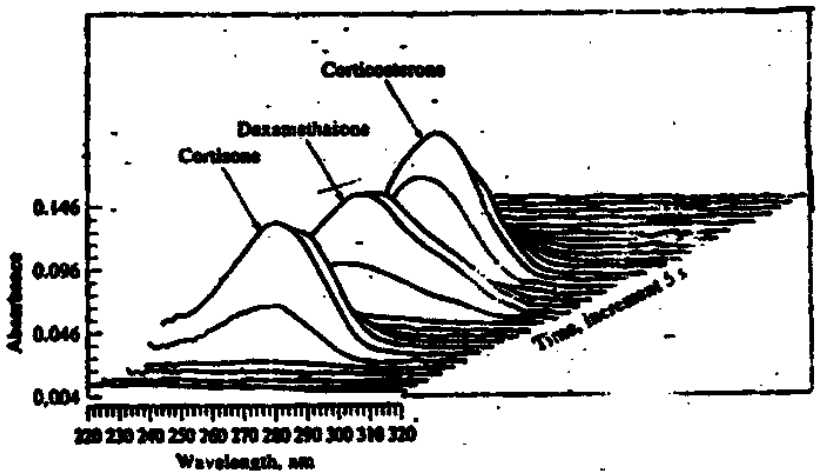


Figure: Absorption spectra of the eluent from a mixture of three sterolds taken at 5-second intervals.

beam. The simplest detectors employ a mercury excitation source and one or more filters to isolate a band of emitted radiation. More sophisticated instruments are based upon a Xenon

source and employ a grating monochromator to isolate the fluorescent radiation. Future development in fluorescence detectors will probably be based upon tunable laser sources, which should lead to enhanced sensitivity and selectivity.[9] See R.B. Green, Anal.Chem., 1983,55,20A; E.S. Yeung and M.J. Sepanlak, Anal.Chem., 1980,52,1465A.

An inherent advantage of fluorescence methods is their high sensitivity, which is typically greater by more than an order of magnitude that most absorbance procedures. This advantage has been exploited in liquid chromatography for the separation and determination of the components of samples that fluorescent compounds are frequently encountered in the analysis of such materials as pharmaceuticals, natural products, clinical samples, and petroleum products. Often, the number of fluorescing species can be enlarged by preliminary treatment of samples with reagents that form fluorescent derivatives. For example, dansylchloride (5-dimethylaminonapthalene-1-sulphonyl chloride), which reacts with primary and secondary amines, amino acids, and phenols to given fluorescent compounds, has been widely used for the detection of amino acids in protein hydrolyzates.

Refractive-Index Detectors

Figure is a schematic of a differential refractive index detector in which the solvent passes through one half of the cell on its way to the column; the eluate then flows through the other chamber. The two compartments are separated by a glass plate mounted at an angle such that bending of the incident beam occurs if the two solutions differ in refractive index. The resulting displacement of the beam with respect to the photosensitive surface of a detector causes variation in the output signal, which, when amplified and recorded, provides the chromatogram.

Refractive-index detectors have the significant advantage of responding to nearly all solutes. That is, they are general detectors analogous to flame or thermal conductivity detectors in gas chromatography. In addition, they are reliable and unaffected by flow rate. They are, however, highly temperature to a few thousandths of a degree centigrade. Furthermore, they are not as sensitive as most other types of detectors.

5. Describe the following

1. Partition Chromatography

2. Adsorption Chromatography.

1. Partition chromatography

Partition chromatography has become the most widely used of all of the four types of liquid chromatographic procedures. In the past, more of the applications have been to nonionic, polar compounds of low to moderate molecular weight (usually < 3000). Recently, however, methods have been developed (derivatization and ion pairing) that have extended partition

separations to ionic compounds.

Partition chromatography can be subdivided into liquid-liquid and bonded-phase chromatography. The difference in these techniques lies in the method by which the stationary phase is held on the support particles of the packing. With liquid-phase, the stationary phase is bonded chemically to the support surfaces. Early partition chromatography was exclusively of the liquid-liquid type; by now, however, the bonded-phase method has become predominate because of certain disadvantages of liquid-liquid systems. One of these disadvantages is the loss of stationary phase by dissolution in the mobile phase, which requires periodic recoating of the support particles. Furthermore, stationary-phase solubility problems prohibit the use of liquid-phase packings for gradient elution. Our discussions will focus exclusively on bonded-phase partition chromatography. - For a report on retention mechanism of bonded-phase chromatography, see.J.G. Dorsey and W.T. Cooper, anal, Chem., 1994,66,857A.

2. Adsorption chromatography.

Adsorption, or liquid-solid, chromatography is the classic form of liquid chromatography first introduced by Tswett at the beginning of the twentieth century. In more recent times, it has been adapted to and has become an important member of HPLC methods - See D.L. Saunders,/Chromatogr.Sci., 1977,15,372.

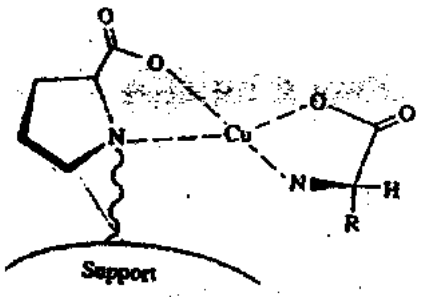


Figure: Schematic of the ternary complex formed between an L-proline bonded-phase, an analyte amino acid, and a copper (II) ion

The only stationary phases that are used for liquid-solid HPLC are silica and alumina, with the former being preferred for most, but not all, applications because of its higher sample capacity and its wider range of useful forms. With a few exceptions, the adsorption characteristics of the two substances parallel one another. With both, the order of retention times is: olefins < aromatic hydrocarbons < esters ≈ aldehydes≈ ketones < alcohols≈ amines < sulfones < sulfoxides < amides < carboxylic acids. - Silica and alumina surfaces are highly polar, and elutions are usually performed with some of the less polar mobile phases. Thus, some chromatographers treat adsorption chromatography as a type of normal-phase partition chromatography. (For example, see J.G. Dorsey et al., anal.Chem., 1990,62, 326R).

6. Describe the Ion Exchange Chromatography.

Ion-exchange chromatography (IC), which is often shortened to ion chromatography refers to modern and efficient methods of separating and determining ions based upon ion-exchange resins. Ion chromatography was first developed in the mid-1970s when it was shown that anion or cation mixtures can be readily resolved cation-exchange resins. At that time, detection was generally performed with conductivity measurements. Currently, other detectors are also available for ion chromatography. - For a brief review of modern ion chromatography, see J.F. Fritz, Anal. Chem., 1897,59,335A; P.K. Dasgupta, Anal. Chem., 1992, 64,775A. For monographs on the subject, see H.Small, Ion Chromatography. New York: Plenum Press, 1989; E.T. Gjerde and J.s. Fritz, Ion Chromatography, 2nd ed. Mamaroneck, NY: Huethig, 1987; R.E. Smith, Ion Chromatography Applications, Boca Raton, FL: CRC Press, 1987; J.Weiss, Ion Chromatography, 2nd P.E. Jackson, Ion Chromatography: Principles and Applications. New York: Elsevier, 1990. For a description of several commercially available IC systems, see D.Noble, anal. Chem., 1995,67,205A.

Ion chromatography was on outgrowth of ion-exchange chromatography, which during the Manhattan project was developed for the separation of closely related rare earth cations with cation-exchange resins. This monumental work, which laid the theoretical groundwork for ion-exchange separations, was extended after World War II to many other types of materials; ultimately it led to automated methods for the separation and detection of amino acids and other ionic species in complex mixtures. The development of modern HPLC began in the late 1960s, but its application to ion-exchange separation of ionic species was delayed by the lack of a sensitive general method of detecting such eluted ionic species as alkali and earth cations and halide, acetate, and nitrate anions. This situation was remedied in 1975 by the development by workers at Dow Chemical Company of an eluent suppressor technique, which made possible the conductometric detection of eluted ions. - H.Small, T.S. Stevens, and W.C.Bauman, Anal.Chem., 1975,47,1801. This technique is described in Section.

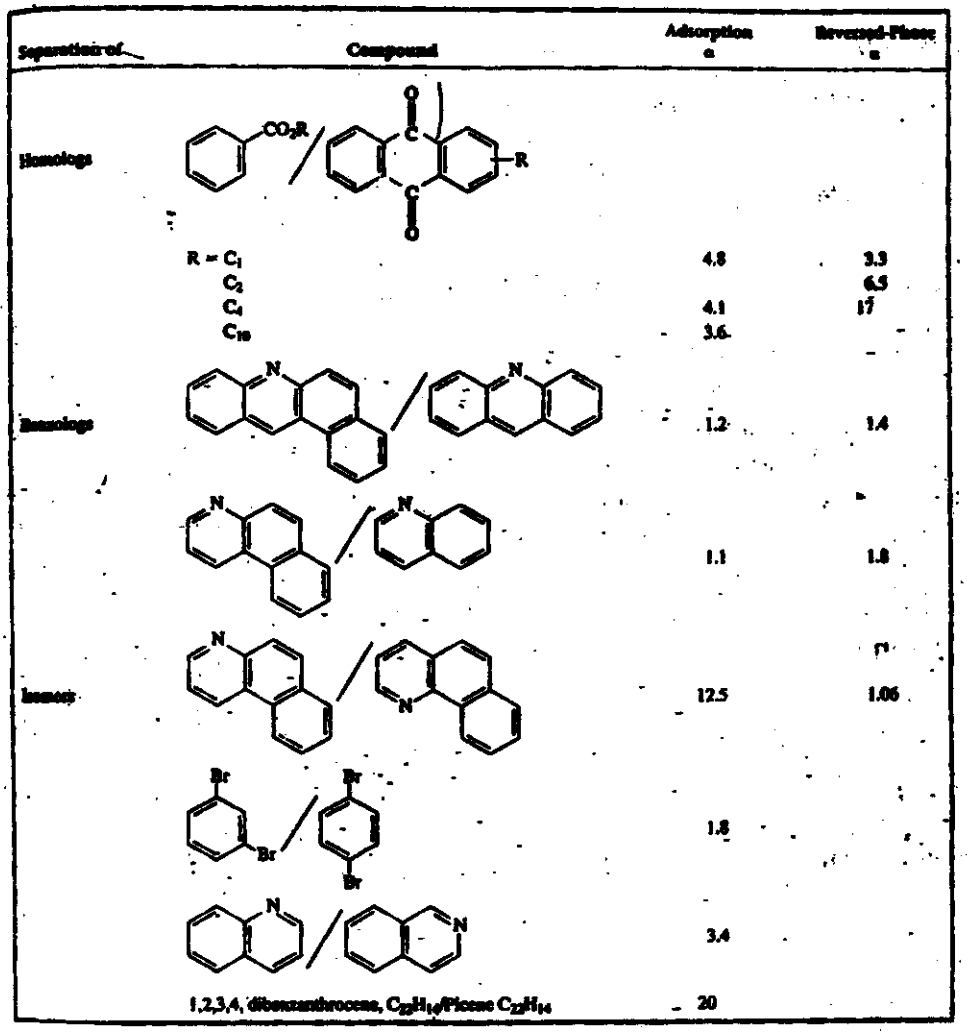


TABLE – Comparison of Selectivities of Adsorption and Reversed-Phase Chromatography*

*** Data from: L.R. Snydeer and J.J. Kirkland, Introduction to Modern Liquid Chromatography.**

Ion-Exchange Equilibria

Ion-exchange processes are based upon exchange equiplibria between ions in solution and ions of like sign on the surface of an essentially insoluble, high-molecular-weight solid. Natural ion-exchangers, such as clays and zeolites, have been recognized and used for several decades. Synthetic ion-exchange resins were first produced in the mid-19f30s for water softening, water deionization, and solution purification. The most common active sites for cation-exchange resins are the sulfonic acid group $-SO_3^- H^+$, a strong acid, and the sulfonic acid group $-COO^- H^+$, a weak acid. Anionic exchangers contain tertiary amine groups $-N(CH_3)_3^+ OH^-$ or primary amine groups

$-NH_3^+OH^-$; the former is a strong base and the latter a weak one.

When a sulfonic acid ion-exchanger is brought in contact with an aqueous solvent containing a action M^{x+}, an exchange equilibria is set up that can be described by

$$xRSO_3^-H^+ + M^{x+} \rightleftharpoons \left(RSO_3^-\right)_x M^{x+} + xH^+$$

Solid Solution Solid Solution

where $RS^-{}_3H^+$ represents one of many sulfonic acid groups attached to a large polymer molecule. Similarly a strong base exchange interacts with the anion A^{x-} as shown by the reaction

$$xRN(CH_3)_3OH^- + A^{x-} \rightleftharpoons \left[RH(CH_3)_3^+\right]_x A^{x-} + xOH^-$$

Solid Solution solid solution

As an example of the application of the mass-action law to ion-exchange equilibria, we will consider the reaction between a singly charged ion B^+ with a sulfonic acid resin held in a chromatographic column. From a neutral solution, initial retention of B^+ ions at the head of the column occurs because of the reaction.

$$RSO_3^-H^+(s) + B^+(aq) \rightleftharpoons RSO_3^-(s) + H^+(aq)$$

Here, the (s) and (aq) emphasize that the system contains a solid and an aqueous phase. Elution with a dilute solution of hydrochloric acid shifts the equilibrium in Equation to the left, causing part of the B^+ ions in the stationary phase to be transferred to the mobile phase. These ions then move down the column in a series of transfers between the stationary and mobile phases.

The equilibrium constant K_{ex} for the exchange reaction shown in Equation takes the form

$$\frac{\left[RSO_3^-B^+\right]_s\left[H^+\right]_{aq}}{\left[RSO_3^-H^+\right]_s\left[B^+\right]_{aq}} = K_{ex}$$

Here, $\left[RSO_3^-B^+\right]_s$ and $\left[RSO_3^-H^+\right]_s$ are concentrations (strictly activities) of B^+ and H^+ in the solid phase. Rearranging yields.

$$\frac{\left[RSO_3^-B^+\right]_s}{\left[B^+\right]_{aq}} = K_{ex}\frac{\left[RSO_3^-H^+\right]_s}{\left[H^+\right]_{aq}}$$

During the elution, the aqueous concentration of hydrogen ions in much larger than the concentration of the singly charged B^+ ions in the mobile phase. Further more, the exchanger has

an enormous number of exchange sites relative to the number of exchange sites relative to the number of B^+ ions being retained. Thus, the overall concentrations $[H^+]_{aq}$ and $\left[RSO_3^-B^+\right]_s$ are not affected significantly by shifts in the equilibrium. Therefore, when $\left[RSO_3^-H^+\right]_s$ □ and $[H^+]_{aq}$ □ $\left[B^+\right]_{aq}$ the right-hand side of Equation 28-7 is substantially constant, and we can write

$$\frac{\left[RSO_3^-B^+\right]_s}{\left[B^+\right]_{aq}} = K = \frac{cS}{cM}$$

where K is a constant that corresponds to the distribution constant as defined by Equation. All of the equations in Table can then be applied to ion-exchange chromatography in the same way as to the other types, which have already been considered.

Note that K_{ex} in Equation represents the affinity of the resin for the ion B^+ relative to another ion (here,H^+). Where K_{ex} is large, a strong tendency exists for the solid phase to retain B^+; where K_{ex} is small, the reverse obtains. By selecting a common reference ion such as H^+, distribution ratios for different ions on a given type of resin can be experimentally compared. Such experiments reveal that polyvalent ions are much more strongly held than singly charged species. Within a given charge group, however, differences appear that are related to the size of the hydrated ion as well as to other properties. Thus, for a typical sulfonated cation exchange resin, values for K_{ex} decrease in the order

$$H^+ > Li^+.\ \text{For divalent cations, the order is} Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} >$$
$$Ni^{2+} > Cd^{2+} > Cu^{2+} > Co^{2+} Zn^{2+} > Mg^{2+} > UO_2^{2+}.$$

For anions, K_{ex} for a strong base resin decreases in the order
$SO_4^{2-} > C_2O_4^{2-} > I^- > NO_3^- > Br^- > Cl^- > HCO_-^2 > HCO_2^- > CH_3CO_2^- > OH^- > F^-$. This sequence is somewhat dependent upon type of resin and reaction conditions and should thus be considered only approximate.

Ion-Exchange Packings

Historically, ion-exchange chromatography was performed on small, porous beads formed during emulsion copolymerization of styrene and divinylbenzene. The presence of divinylbenzene (usually ≈ 8%) results in cross-linking, which imparts mechanical stability to the beads. In order to make the polymer active toward ions, acidic or basic functional groups are then bonded chemically to the structure. The most common groups are sulfonic acid and quaternary amines.

Figure shows the structure of a strong acid resin. Note the cross-linking that holds the

linear polystyrene molecules together. The other types of resins have similar except for the active functional group.

Porous polymeric particles are not entirely satisfactory for chromatographic packings because of the slow rate of diffusion of analyte molecules through the micropores of the ploymer matrix and because of the compressibility of the matrix. To overcome this problem, two newer types of packings have been developed and are in more general use than the porous polymer type. One is a pellicular bead packing in which the surface of a relatively large, (30 to 40 μm) nonporous, spherical, glass, or polymer bead is coated with a synthetic ion-exchange resin. A second type of packing is prepared by coating porous microparticles of silica, such as those used in adsorption chromatography, with a thin film of the exchanger. With either type, faster diffusion in the polymer film leads to enhanced efficiency. On the other hand, the sample capacity of these particles is less, particularly for the pellicular type.

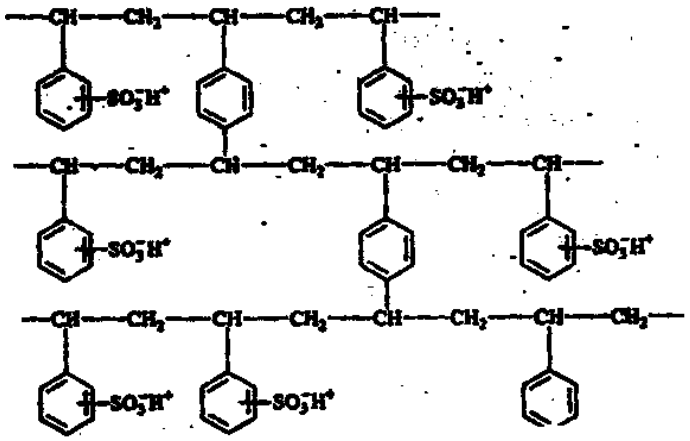


Figure Structure of a cross-linked polystyrene ion-exchange resin, Similar resins are used in which the $-SO_3^-H^+$ group is replaced by $-COO^{-H^+}$, $-NH_3^+OH^-$, and $-N(CH_3)_3^+OH^-$ groups

Inorganic Applications of Ion-Exchange Chromatography

The mobile phase in ion-exchange chromatography must have the same general properties that are required for other types of chromatography. That is, it must dissolve the sample, have a solvent strength that leads to reasonable retention times (correct k' values), and interact with solutes in such a way as to led to selectivity (suitable α values). The mobile phases in ion-exchange chromatography are aqueous solutions that may contain moderate amounts of methanol or other water-miscible organic solvents; these mobile phases also contain ionic species, often in the form of a buffer. Solvent strength and selectivity are determined by the kind and concentration of these added ingredients. In general, the ions of the mobile phase compete with analyte ions for the active sites on the ion-exchange packing.

Ion-Exchange Chromatography with Eluent Suppressor Columns

As noted earlier, the widespread application of ion chromatography for the determination of inorganic species was inhibited by the lack of a good general detector, which would permit quantitative determination of ions on the basis of chromatographic peak areas. Conductivity detectors are an obvious choice for this task. They can be highly sensitive, they are universal for charged species, and as a general rule, they respond in a predictable way to concentration changes. Further more, such detectors are simple, inexpensive to construct and maintain, easy to miniaturize, and ordinarily construct and maintain, easy to miniaturize, and ordinarily given prolonged, trouble-free service, The only limitation to conductivity detectors proved to be a serious one, which delayed their general use. This limitation arises from the high electrolyte concentration required to elute most analyte ions in a reasonable time. As a consequence, the conductivity from the mobile-phase components tends to swamp that from analyte ions, thus greatly reducing the detector sensitivity.

In 1975, the problem of high eluent conductance was solved by the introduction of a so-called eluent suppressor column immediately following the analytical ion-exchange column. The suppressor column is packed with a second ion-exchange resin that effectively converts the ions of the solvent to a molecular species of limited ionization without affecting the analyte ions. For example, when cations are being separated and determined, hydrochloric acid is often chosen as the eluting reagent, and the suppressor column is an anion-exchange resin the hydroxide form. The product of the reaction in the suppressor is water. That is,

$$H^+(aq) + Cl^-(aq) + Resin^+OH^-(s) \rightarrow Resin^+Cl^-(s) + H_2O$$

The analyte cations are, of course, not retained by this second column.

For anion separations the suppressor packing is the acid form of a cation-exchange resin. Here, sodium bicarbonate or carbonate may serve as the eluting agent. The reaction in the suppressor is then

$$Na^+(aq) + HCO_3^-(aq) + Resin^-H^+(s) \rightarrow Resin^-Na^+(s) + H_2CO_3(aq)$$

Here, the largely undissociated carbonic acid does not contribute significantly to the conductivity.

An inconvenience associated with the original suppressor columns was the need to regenerate them periodically (typically, every 8 to 10 hr)in order to convert their packings back to the original acid or base form. Recently, however, fiber membrane suppressors have become available that operate continuously, as shown in Figure. Here, the eluent and the suppressor

solution flow in opposite directions on either side of the permeable ion-exchange membranes labeled M. For the analysis of anions, the membranes are cation-exchange resins; for examples, sodium ions are to be removed from the eluent, acid for regeneration flow continuously in the suppressor stream. Sodium ions from the eluent exchange with hydrogen ions of the membrane and then migrate across the membrane where they exchange with hydrogen ions of the regeneration reagent. The device has a remarkably high exchange rate; for example, it is capable of removing essentially all of the sodium ions from a 0.1 M.

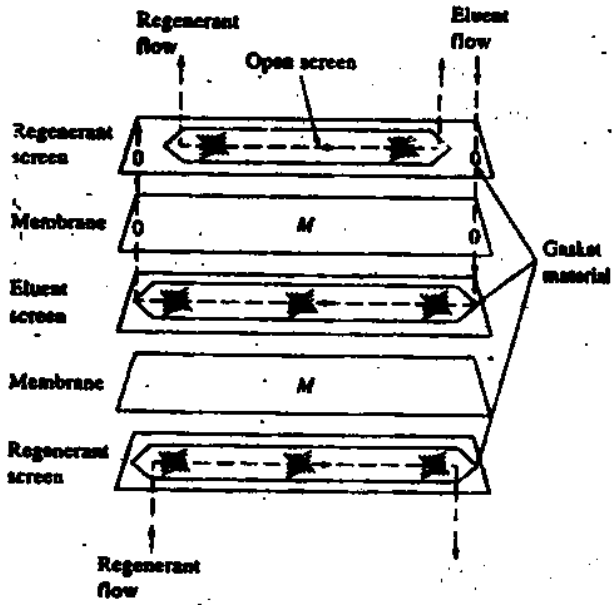


Figure: A micromembrane suppressor. Eluent flows through a narrow channel that contains a plastic screen that reduces that void volume and appears to increase mass transfer rates. The eluent is separated from the suppressor solution by 50-μm exchange resins. Regenerant flow is in the direction opposite to eluent flow.

solution of sodium hydroxide when the eluent flow rate is 2 mL min.

In recently designed commercial instruments, regeneration of suppressor solutions is performed automatically with electrogenerated hydrogen or hydroxyl ions so that interruptions in the use of the instruments for regeneration are not required. - See A. Henshall et al., Amer.Lab.,1992 (11),20R;R.Saari-Nord-haus and J.M.Anderson,Amer.Lab.,1996 (2), 33N.

Figure shows two applications of ion chromatography based upon suppressor column and conduct metric detection.

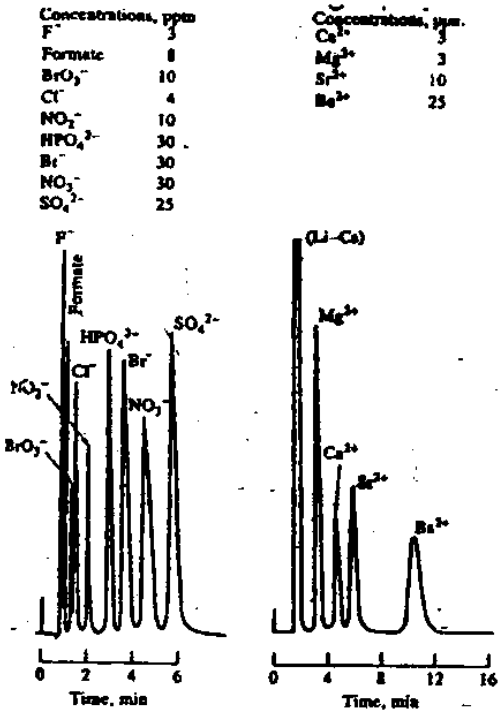


Figure: Typical applications of ion chromatography, (a) Separation of anions on an anion-exchange column. Eluent: 0.0028 M $NaHCO_3$/0.0023 M $NaCO_3$. Sample size: 50μL. (b) Separation of alkaline earth ions on a cation-exchange column. Eluent:0.025 M phenylenediamine dihydrochloride/0.0025 M HCl. Sample size: 100μL.

In each case, the ions were present in the parts per million range, and the sample sizes were 50μL in one case and 100 μL in the other. The method is particularly important for anion analysis because no other rapid and convenient method for handling mixtures of this type now exists.

Single-Column Ion Chromatography

See D.T.Gjerde and J.S.Fritz, Ion Chromatography, 2nd ed. Mamaroneck, NY: Huethig Velag, 1987.

Equipment has also become available commercially for ion chromatography in which no suppressor column is used. This approach depends upon the small differences in conductivity between the eluted sample ions and the prevailing eluent ions. To amplify these differences, low-capacity exchangers are used, which makes possible elution with species having low equivalent conductances. - See J.S.Fritz,D.J.Gjerde,and R.M. Becker, Anal.Chem., 1980,52,1519. Single-column chromatography tends to be somewhat less sensitive and to have a more limited range than ion chromatography with a suppressor column.

An indirect photometric method that permits the separation and detection of nonabsorbing

anions and cation without suppressor column has recently been described. - H.Small and T.E. Miller, Anal.Chem., 1982,54,462. Here also, no suppressor column is used, but instead, anions or cations that do absorb are used to displace the analyte ions from the column. When the analyte ions are displace from the exchanger, their place is taken by an equal number of eluent ions (provided, of course, that the charge on the analyte and eluent ions is the same). Thus, the absorbance of the eluate decreases as analyte ions exit from the column. Figure show a chromatogram obtained by this procedure. Here, the eluent was a dilute solution of disodium phthalate, phthalate ion being the ultraviolet-absorbing displacing ion. Indirect detection has more restricted working range than does direct detection.

Organic and Biochemical Applications of Ion-Exchange Chromatography

Ion-exchange chromatography has been applied to a variety of organic and biochemical systems, including drugs and their metabolites, serums, food preservatives, vitamin mixtures, sugars, and pharmaceutical preparations. An example of one of these applications is shown in Figure in which 1 x 10^{-8} mol each of 17 amino acids was separated on a cation-exchange column.

Figure: Indirect photometric detection of several anions by elution. Eluent: 10^{-3} M disodium phthalate, 10^{-3}M boric acid, pH 10. Flow rate: 5mL/min. Sample volume: 0.02 mL. UV detector, Sample ions(a) 18-µg carbonate; (b) 1.4-µg chloride, (c) 3.8-µg phosphate; 9d) 5-µg azide; (e) 10-µg nitrate.

Ion-Exclusion Chromatography

Ion-exclusion chromatography is not a form of ion chromatography because neutral species rather than ions are being separated. Nevertheless, it is convenient to discuss it here because ion-exclusion chromatography, like ion chromatography, employs ion-exchange columns to achieve

separations.

The theory and applications of ion-exclusion chromatography are conveniently illustrated by the separation of simple carboxylic acids shown by the chromatogram in Figure. Here, the stationary phase was a cation-exchange resin in its acidic form, and elution was accomplished with a dilute solution of hydrochloric acid. The analytical column was followed by a suppressor column that was packed with a cation-exchange resin in the silver form. The hydrogen ions of the eluent were exchanged for silver ions, which then precipitated the chloride ions, thus removing the ions contributed by the eluent. The undissociated form of the analyte acids were distributed between the mobile phase in the column and the immobilized liquid held in the pores of the packing. The distribution constants for the various acids are primarily related to the inverse of their dissociation constants, although other factors also play a part in the extent to which various species are distributed between the two phases.

Ion-exclusion chromatography finds numerous applications for identification and determination of acidic species in such materials as milk, coffee, wine, and many other products of commerce. Salts of weak acids can also be analyzed because they are converted to the corresponding acid by the hydrogen ions in the exchanger. Weak bases and their salts can also be determined by ion-exclusion chromatography. In these applications an anion-exchange column in the hydroxide form is employed.

7. Describe the Thin Layer chromatography.

Planar chromatographic methods include thin-layer chromatography (TLC) and paper chormatography (PC), and electrochromatography. Each makes use of a flat, relatively thin layer of material that is either self supporting or is coated on a glass, plastic, or metal surface. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or an electrical potential. Planar chromatography is sometimes called two-dimensional chromatography is though this description is not strictly correct inasmuch as the stationary phase does have a finite thickness.

Currently, most planar chromatography is based upon the thin-layer technique, which is faster, has better resolution, and is more sensitive than its paper counterpart. This section is devoted to thin-layer methods only.

The Scope of Thin-Layer Chromatography

In terms of theory, the types of stationary and mobile phases, and applications, thin-layer and column liquid chromatography are remarkably similar. In fact, an important use of thin-layer chromatography, and the reason this topic is included here, is to serve as a guide to the

development of optimal conditions for performing separations by column liquid chromatography. The advantages of following this procedure are the speed and low cost of the exploratory thin-layer experiments. Some chromatographers, in fact, take the position that thin-layer experiments should always precede column experiments.

In addition to its use in developing column chromatographic methods, thin-layer chromatography has become the workhorse of the drug industry for the all important determination of product purity. It has also found widespread use in clinical laboratories and is the backbone of many biochemical and biological studies. Finally, it finds widespread use in the industrial laboratories. - Monographs devoted to the principles and applications of thin-layer chromatography include: R.Hamilton and S.Hamilton, Thin-layer Chromatography: New York: Wiley, 1987; B.Fried and J.Sherma, Thin-Layer Chromatography, 3rd ed. New York: Marcel Dekker, 1994; J.C. Touchstone,Practice of Thin-Layer Chromatography, 2nd ed. New York: Wiley, 1983. For briefer reviews, see D.C. Fenimore and C.M. Davis, Anal.Chem., 1981,53,253A; S.J. Costanzo, J.Chem., Educ., 1984,61,1015; C.F. Poole and S.K. Poole, Anal.Chem., 1989,61, 1257A. As a consequence of these many areas of application, it has been estimated that at least as many analyses are performed by thin-layer chromatography as by high-performance column liquid chromatography. - T.H. Mauch II, Science, 1982, 216,161; J.C. Touchstone, LC-GC,1993,11(6), 404.

Thin-Layer Plates

Thin-layer plates are available from several commercial sources at costs that range from $ 1 to $ 10 per plate. The common plate sizes in centimeters are 5 ×20, 10 × 20, and 20 × 20. Commercial plates come in two categories, conventional and high-performance. The former have thicker-layers (200 to 250 μm) of particles having nominal particle sizes of 20 μ m or greater. High-performance plates usually have film thickness of 100 μm and particle diameters of 5μm or less. High-performance plates, as their name implies, provide sharper separations in shorter times. Thus, a conventional plate typically will exhibit 2000 theoretical plates in 12 cm with a development time of 25 min. the corresponding figures for a high-performance plate are 4000 theoretical plates in 3 cm requiring 10 min for development. High-performance plates suffer from the disadvantage of having a significantly smaller sample capacity.

Sample Application

Sample application is perhaps the most critical aspect of thin-layer chromatography, particularly for quantitative measurements. Usually the sample, as a 0.01% to 0.1% solution, is applied as a spot 1 to 2 cm from the edge of the plate. For best separation efficiency, the spot should have a minimal diameter—about 5f mm for qualitative work and smaller for quantitative analysis. For dilute solutions, three or four repetitive applications are used, with drying in between.

Manual application of samples is performed by touching a capillary tube containing the sample to the plate or by use of a hypodermic syringe. A number of mechanical dispensers, which

increase the precision and accuracy of sample application, are now offered commercially.

Plate Development

Plate development is the process in which a sample is carried through the stationary phase by a mobile phase; it is analogous to elution in liquid chromatography. The most common way of developing a plate is to place a drop of the sample near one edge of the plate and mark its position with a pencil. After the sample solvent has evaporated, the plate is placed in a closed container saturated with vapors of the developing solvent. One end of the plate is immersed in the developing solvent, with care being taken to avoid direct contact between the sample and the developer (Figure). The developing solvent travels up the plate, being drawn by capillary action between the fine particles. As the developing solvent travels past the point of sample application, it dissolves the sample

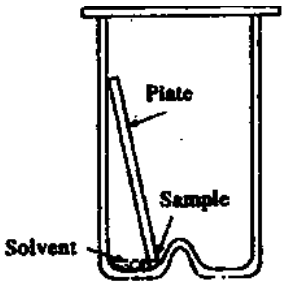


Figure: A typical development chamber.

and carries it up the plate, with the sample distributing itself between the moving solvent and the stationary phase. After the developer has traversed one half or two thirds of the length of the plate, the plate is removed from the container and dried. The positions of the components are then determined in any of several ways.

Locating Analytes on the Plate

Several methods are employed to locate sample components after separation. Two common methods, which can be applied to most organic mixtures, involve spraying with a solution of iodine or sulfuric acid, both of which react with organic compounds to yield dark products. Several specific reagents (such as ninhydrin) are also useful locating separated species.

Another method of detection is based upon incorporating a fluorescent material into the stationary phase. After development, the plate is examined under ultraviolet light. The sample components quench the fluorescence of the material so that all of the plate fluoresces except where the nonfluorescing sample components are located.

Figure is an idealized drawing showing the appearance of a plate after development. Sample 1 contained two components, whereas sample 2 contained one. Frequently, the spots on a

real plate exhibit tailing, giving spots that are not symmetrical are those in the figure.

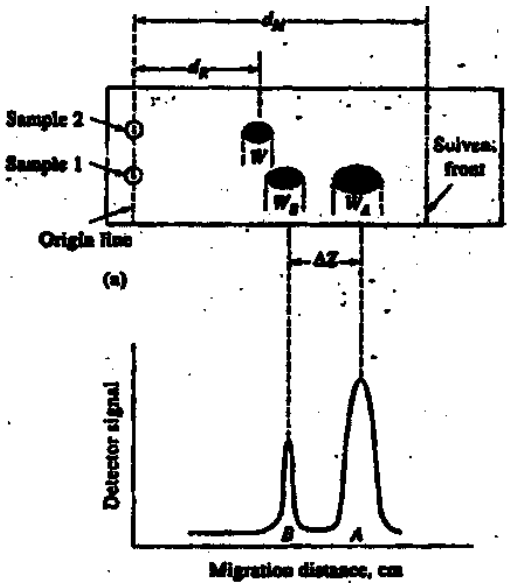


Figure: Thin-Layer chromatograms.

Performance Characteristics of Thin-Layer Plates

Most of the terms and relationships developed for column chromatography in Section 26B can, with slight modification, be applied to thin-layer chromatography as well. One new term, the retardation factor or R_F factor, is required.

The Retardation Factor

The thin-layer chromatogram for a single solute is shown as chromatogram 2 in. The retardation factor for this solute is given by $R_F = \frac{d_R}{d_M}$ where d_R and d_M are linear distances measured from the origin line. Values for R_F can vary from 1 for solutes that are not retarded to a value that approaches 0. It should be noted that if the spots are not symmetric, as they are in Figure, the measurement of d_F is based on the position of maximum intensity.

The Retention Factor

All of the equations in Table are readily adapted to thin-layer chromatography. In order to apply this equation, it is only necessary to relate d_R and d_M as defined in Figure to t_R and t_M. Which are defined in figure. To arrive at this relationship, consider the single solute that appears in chromatogram 2 in Figure. Here, t_M and t_R correspond to times required for the mobile phase and the solute to travel a fixed distance—in this case, d_R. For the mobile phase, this time is equal

to the distance divided by its linear velocity u, or

$t_M = d_R/u$

The solute does not reach this same point, however, until the mobile phase has traveled the distance d_M. Therefore,

$t_R = d_M/u$

Substitution of Equations and into Equation yields $k' = \frac{d_M - d_R}{d_R}$

The retention factor k′ can also expressed in terms of the retardation factor by rewriting Equation 28-15 in the form $k' = \frac{1 - d_R/d_M}{d_R/d_M} = \frac{1 - R_F}{R_F}$ Retention factors derived in this way can be used for method development in column chromatography as described in Section 28D-2. Obtaining retention factors by thin-layer chromatography is, however, usually simpler and more rapid than obtaining the data from experiments on a column.

Plate Heights

Approximate plate heights can also be derived for a given type of packing by thin-layer chromatographic measurement. Thus, for sample 2 in Figure, the plate count is given by the equation

$$N = 16\left(\frac{d_R}{W}\right)^2$$

where d_R and W are defined in the figure. the plate height is then given by

$H = d_R/N$

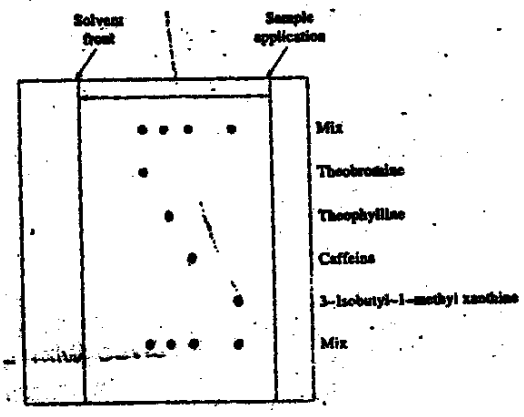


Figure: Separation of xanthine derivatives on a C-18 reversed-phase plate. Mobile phase: methanol/0.1 M $k_2 HPO_4$ (55.45 v/v). Detection: iodine vapor. Development time: 1 hr,R_F values: theobromine 0.68, theophylline 0.56, caffeine 0.44, 3-isobuty1-methy1 xanthine 0.21.

Application of Thin-Layer Chromatography

Qualitative Thin-Layer Chromatography

The data from a single chromatogram usually do not provide sufficient information to permit identification of the various species present in a mixture because of the variability of R_F values with sample size, the thin-layer plate, and the conditions extant during development. In addition, the possibility always exists that two quite different solutes may exhibit identical or nearly identical R_F values under a given set of conditions.

Variable That Influence R_F.

At best, R_F values can be reproduced to but two significant figures; among several plates, one significant figure may be a more valid statement of precision. The most important factors that determine the magnitude of R_F include thickness of the stationary phases, temperature, degree of saturation of the developing chamber with mobile phase vapor, and vapor, and sample size. Complete control of these variables is generally not practical. Partial amelioration of their effects can often be realized, however, by substituting a relative retention factor R_X for R_F, where

$$R_X = \frac{\text{travel distance of analyte}}{\text{travel distanceof as standard substance}}.$$

Use of Authentic Substances

A method that often provides tentative identification of the components of a sample is to apply to the plate the unknown and solutions of purified samples of species likely to be present in that unknown. A match in R_F values between a spot for the unknown and that for a standard provides strong evidence as to the identify of one of the components of the sample (see Figure0. Confirmation is always necessary, however. A convenient confirmatory test is to repeat the experiment with different stationary and mobile phases as well as with different visualization reagents.

Elution Methods

The identity of separated analyte species can also be confirmed or determined by a scraping and dissolution technique. Here, the area containing the analyte is scraped from the plate with a razor or a spatula and the contents collected on a piece of glazed paper. After transfer to a test tube or other container, the analyte is dissolved with a suitable solvent and separated from the stationary phase by centrifugation or filtration. Identification is then carried out by such techniques as mass spectrometry, nuclear magnetic resonance, or infrared spectroscopy.

Two-Dimensional Planner Chromatography

Figure illustrates the separation of amino acids in a mixture by development in two dimensions. The sample was placed in one corner of a square plate and development was performed in the ascending direction with solvent A. This solvent was then removed by evaporation, and the plate was rotated 90 deg, following which an ascending development with solvent b was performed. After solvent removal, the positions of the amino acids were determined by spraying with ninhydrin, a reagent that forms a pink to purple product with amino acids. the spots were identified by comparison of their positions with those of standards.

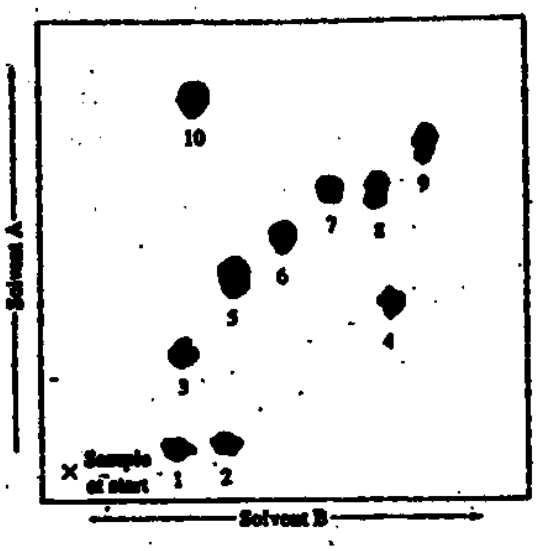


Figure: Two-dimensional thin-layer chromatogram (silica gel) of some amino acids. Solvent A: toluene/2-chloroethanol/pyridine. Solvent B: chloroform/benzy1 alcohol/acetic acid. Amino acids: (1) aspartic acid, (2) glutamic acid, (3) serine, (4) β-alanine, (5) glycine, (6) alanine, (7) methionine, (8) valine, (9) isoleucine, and (10) cysteine.

Quantitative Analysis

A semi-quantitative estimate of the amount of a component present can be obtained by comparing the area of a spot with that of a standard. Better data can be obtained by scraping the spot from the plate, extracting the analyte from the stationary-phase solid, and measuring the analyte by a suitable physical or chemical method. In a third method, a scanning densitometer can be employed to measure the radiation emitted from the spot by fluorescence or reflection.

8. Describe the Capillary Electrophoresis.

CAPILLARY ELECTROPHORESIS

As useful as conventional slab electrophoresis has been, and continues to be, this type of electrophoretic separations is typically slow, labor intensive, difficult to automate, and does not yield very precise quantitative information. During the mid- and late 1980s, there was explosive growth in research and application of electrophoresis performed in capillary tubes as well as the appearance of several commercially available instruments. Capillary electrophoresis yields high-speed, high-resolution separations on exceptionally small sample volumes (0.1 to nL in contrast to slab electrophoresis, which requires samples in the μL range). Additionally, the separated species are eluted from one end of the capillary, so quantitative detectors, like those of HPLC, can be used instead of cumbersome staining techniques of slab electrophoresis.[2]

For review articles on capillary electrophoresis, see J.W.Jorgenson, Anal.Chem., 1986, 58, 743A; A.G.Ewing, R.A.Wallingford, and T.M.Olefirowicz., Anal. Chim.,1989, 61, 292A; H.H.Lauer and J.B.Ooms. Anal. Chim. Acta, 1991,250,45. For recent books, see D.R.Baker, Capillary Electrophoresis. New York: Wiley, 1995; R.Weinberger, Practical Capilary Electrophoresis. New York: Academic Press, 1993; S.Y.Li, Capillary Electrophoresis. New York: Elsevier, 1992; Capillary Electrophoresis, P.Camilleri, Ed.Boca Raton: CRC Press, 1993; F.Foret, L.Krivankova, and P.Bocek, Capillary Zone Electrophoresis. New York: VCH, 1993.

Instrumentation for Capillary Electrophoresis

As shown in figure the instrumentation for capillary electrophoresis is simple. - For a review of current commercially available capillary electrophoresis instruments, see C.Henry, Anal. Chem., 1996, 64, 747A. A buffer-filled fused-silica capillary, that is typically 10 to 100μm in internal diameter and 40 to 100 cm long, extends between two buffer reservoirs that also hold platinum electrodes. Sample introduction is performed at one end, and detection at the other.

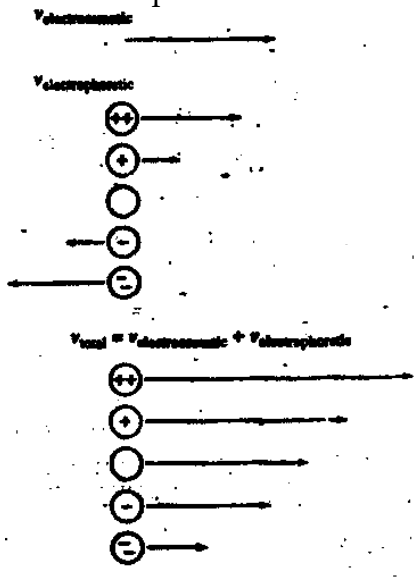


Figure: Velocities in the presence of electro osmotic flow. The length of the arrow next to an ion indicates the magnitude of its velocity; the direction of the arrow indicates the direction of motion. The negative electrode would be to the right, and the positive electrode to the left of this section of solution.

The polarity of the high-voltage power supply can be as indicated, or can be reversed to allow rapid separation of anions.

Although the instrumentation is conceptually simple, there are significant experimental difficulties in sample introduction and detection due to the very small volumes involved. Since the volume of a normal capillary is 4 tov5μL, injection and detection volumes must be on the order of a few nanoliters or less.

Sample Introduction

The most common sample introduction methods are electrokinetic injection and pressure injection. With electrokinetic injection, one end of the capillary and its electrode are removed from their buffer compartment and placed in a small cup containing the sample. A potential is then applied for a measured time, causing the sample to enter the capillary by a combination of ionic migration and electroosmotic flow. The capillary end and electrode are then placed back into the regular buffer solution for the duration of the separation. This injection technique discriminates by injecting larger amount of the more mobile ions relative to the slower moving ions.

With pressure injection, the sample introduction end of the capillary is also placed momentarily into a small cup containing the sample, and a pressure difference is then used to drive the sample, and pressure difference is then used to drive the sample solution into the capillary. The pressure difference can come from applying a vacuum at the detector end, by pressurizing the sample, or by elevating the sample end. Pressure injection does not discriminate due to ion mobility, but cannot be used in gel-filled capillaries.

For both electrokinetic injection and pressure injection, the volume injected is controlled by the duration of the injection. Injections of 5 to 50 nL are common, and volumes below 100 pL have been reported. For a buffer with density and viscosity near the values for water, a height differential of 5 cm for 10 s injects about nL with a 75-μm inside diameter capillary.

J.D Olecho, J.M.Y.Tso, J.Thayer, and A. Wainright, Amer. Lab., 1990, 22(18), 30

Microinjection tips constructed from capillaries pulled down to very small diameters allow sampling from picoliter environments such as single cells or sub-structures within single cells. This technique has been employed to study amino acids and neurotransmitter from single cells.

Detection

Because the separated analytes move past a common point in most types of capillary electrophoresis, detectors are similar in design and function to those described for HPLC. One difference in behavior of detectors is encountered, however, because in capillary electrophoresis each ion migrates at a rate determined by its electrophoretic mobility. Thus, analyte bands pass through the detector at different rates, which results in peak areas that are somewhat dependent upon retention times. In contrast, in HPLC all species pass through the detector at the velocity of the mobile phase, and peak areas are independent of retention times. Ordinarily this time dependence is of no concern.

Table lists the detection methods that have been reported through 1988 for capillary electrophoresis. The second column of the table lists representative detection limits for these detectors.

Absorbance Methods

Both fluorescence and absorbance detectors are widely used in capillary electrophoresis, although the latter are more common be cause they are more generally applicable. In order to keep the detection volume on the nL scale or smaller, detection is performed on-column. In this case a small section of the protective polyimide coating is removed from the exterior of the capillary by burning, dissolution, or scraping. That section of the capillary then serves as the detector cell. Unfortunately, the path length for such measurements is no more than 50 to 100 μm, which restricts detection limits in concentration terms; because such small volumes are involved, however, mass detection limits are equal to or better than those for HPLC.

In order to improve the sensitivity of absorbance measurements, several techniques have been suggested for increasing the path length of the measurements. Three of these are shown in figure. One of these, which is commercially available, involves bending the end of the capillary into a "z" shape as shown in figure giving a path length of 3 mm. Unfortunately, the improvement in sensitivity with this technique is much smaller than would be expected, probably because of inadequate focusing of the light.

Figure (b) shows a second stratagem for increasing path lengths. Here a bubble is formed near the end of the capillary. In the commercial version of this technique, the bubble for a 50-μm capillary has an inside diameter of 150 μm, thus giving a threefold increase in path length.

Table Detection Modes Developed for Capillary Electrophoresis[a]

Detection Principle	Representative Detection Limit[b] (moles

	detected)
Spectrometry	
Absorption[c]	10^{-15}-10^{-13}
Fluorescence	
Precolumn derivatization	10^{-17}-10^{-20}
On-column derivatization	8×10^{-16}
Postcolumn derivatization	2×10^{-17}
Indirect fluorescence	5×10^{-17}
	4×10^{-17}
Thermal lens[c]	2×10^{-15}
Raman[c]	1×10^{-17}
Mass spectrometry	
Electrochemical	1×10^{-16}
Conductivity[c]	Not reported
Potentiometers	7×10^{-19}
Amperometry	1×10^{-19}
Radiometry[c]	

[a]From A.G.Ewing, R.A.Wallingford, and T.M.Olefirowicz, Anal.Chem., 1989,61,298A. With permission.
[b]Detection limits quoted have been determined with variety of injection volumes that range from 18 pI to 10Nl.
[c]Mass detection limit converted from concentration detection limit using a 1-nL injection volume.

A third method for increasing the path length of radiation by reflection is shown in figure. In this technique, a reflective coating of silver is deposited on the end of the capillary. In this case the radiation beam undergoes numerous reflections until it exits the capillary.

Indirect Detection

Indirect absorbance detection has been used for detection of species that are difficult to detect because of low molar absorptivities without derivatization. An ionic chromophore is placed in the electrophoresis buffer. The detector then receives a constant signal due to the presence of this substance. The analyte displaces some of these ions, just as in ion-exchange chromatography, so that the detector signal decreases during the passage of an analyte band through the detector. The analyte is then determined from the decrease in absorbance. The electropherogram in figure was generated by using indirect absorbance detection with 4-mM chromate ion as the chromophore; this ion absorbs strongly at 254 nm in the buffer.

Fluorescence Detection

Just as in HPLC, fluorescence detection yields increased sensitivity and selectivity for fluorescent analytes of fluorescent derivatives. Laser-based instrumentation is preferred in order to focus the excitation radiation on the small capillary and to achieve the low detection limits available from intense sources. Laser fluorescence, detection has allowed detection of only 10 zeptomoles or 6000 molecules. S.Wu and N.Dovichi, J.Chromatogr., 1989,480,141.

Electrochemical Detection

Two types of electrochemical detection have been used with capillary electrophoresis: conductivity and amperometry. One of the problems with electrochemical detection has been that of isolating the detector electrodes from the high potential required for the separation. One method for isolation involves inserting of a porous glass or graphite joint between the end of the capillary and second capillary containing the detector electrodes.

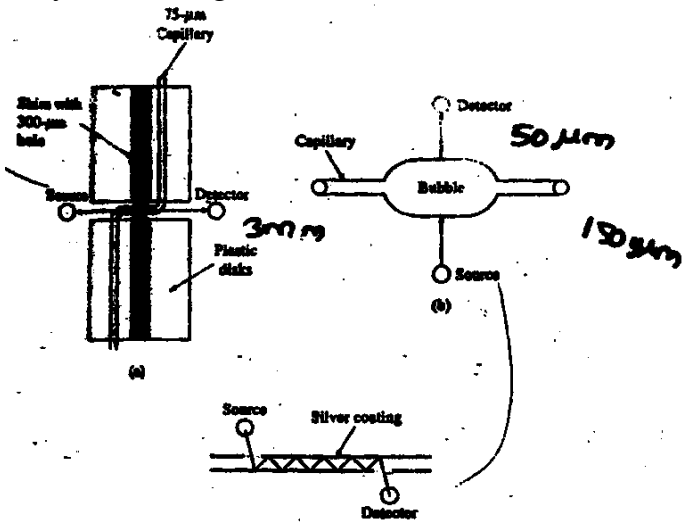


Figure: Three types of cells for improving the sensitivity of detection by absorbance measurements: (a) the 3-mm z cell, (b) the 150-μm bubble cell, (c) the multireflection cell.

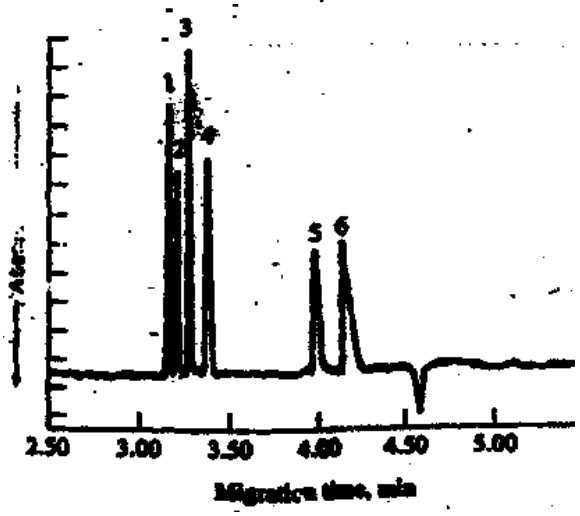


Figure: Electropherogram of a six-anion mixture by indirect detection with 4-nM chromate ion

at 254 nm.

Peak: (1) bromide (4ppm), (2) chloride (2ppm), (3) sulfate (4ppm), (4) nitrate (4ppm), (5) fluoride (1ppm), (6) phosphate (6ppm).

Mass Spectrometric Detection

The very small volumetric flow rates of under 1 μL/min from electrophoresis capillaries makes it feasible to couple the effluent from the capillary of an electrophoretic device directly to the ionization source of a mass spectrometer. Currently the most common sample introduction/ionization interface used for this purpose is electrospray although fast atom bombardment has also been applied. Capillary electrophoresis with mass-spectrometric detection is only a decade and a half old but has become of major interest to biologists and biochemists for the determination of large molecules that occur in nature, such as proteins, DNA fragments, and peptides.

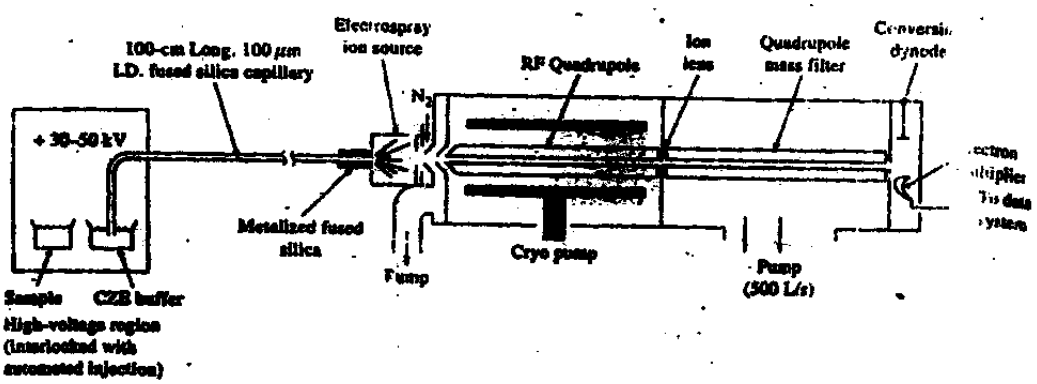


Figure: An instrument for capillary electrophoresis/mass spectrometry. The voltage between the buffer solution on the left and the metalized silica capillary is 30 to 50kV. The flow of nitrogen is 3 to 5kV. The flow of nitrogen at ≈ 70^0 C for desolvation is 3 to 6 L/min.

Figure is a schematic of atypical electrospray interface coupled to a quadrupole mass spectrometer. Note that the second buffer compartment has been dispense with, and the 30 – to – 50 –kV potential for the electrophoretic separation is applied between the remaining buffer compartments and the opposite end of the capillary, which is metalized. A 5-kV potential is also applied between the capillary tip and ground to charge the electrospray.

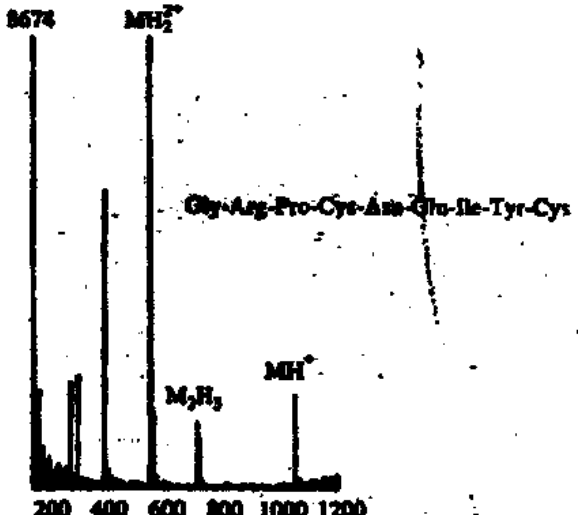


Figure: ESI mass spectrum for vasotocin

Figure shows the electrospray mass spectrum obtained for vasotocin, a polypeptide having a molecular weight of 1050. Note the presence of doubly and triply charged species. With higher-molecular-weight species, ions bearing a dozen or more charges are often encountered. This fact makes it possible to detect high-molecular-weight analytes with a quadrupole instrument with a relatively modest mass range.

Typical detection limits for capillary electrophoresis/mass spectrometry (CE/MS) are on the order of a few tens of femtomoles for molecules having a molecular weight of 100,000 or more.

9. What is Application of Capillary electrophoresis?

APPLICATIONS OF CAPILLARY ELECTROPHORESIS

For an excellent review of applications of capillary electrophoresis and electrochromatography to the biological sciences, see B.L.Karger, A.S.Cohen, and A.Gutman, J.Chromatogr., 1989,492,585.

Capillary electrophoretic separations are performed in several ways called modes. It is noteworthy that these modes were first employed in slab electrophoresis and were subsequently adapted for capillary electrophoretic separations. These modes include capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP). The sections that follow illustrate typical applications of each of these techniques.

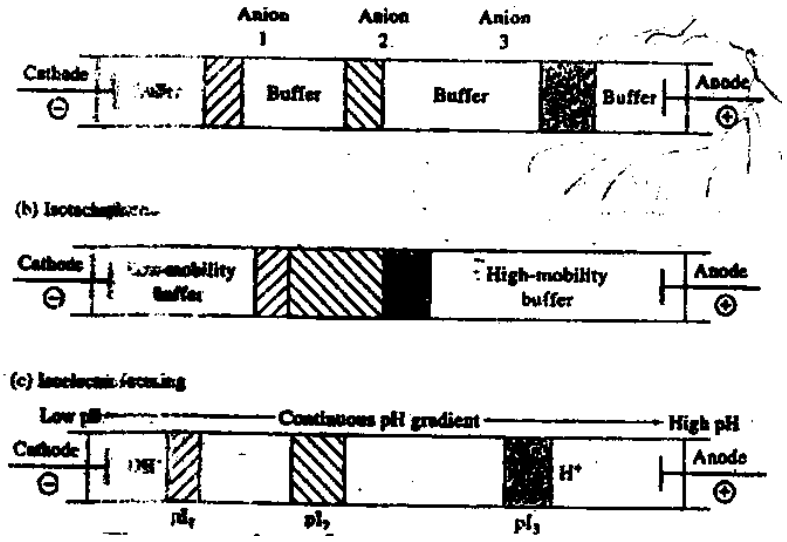


Figure: Three modes of separation by electrophoresis

Capillary Zone Electrophoresis

In capillary zone electrophoresis (CZE), the buffer composition is constant throughout the region of the separation. The applied potential causes the different ionic components of the mixture to each migrate according to its own mobility and to separate into zones that may be completely resolved or may be partially overlapped. Completely resolved zones have regions of buffer between them. The situation is analogous to elution column chromatography, where regions of mobile phase are located between zones containing separated analytes.

Separation of Small Ions

For most electrophoretic separations of small ions, it has been found best from the standpoint of decreased time to have the analyte ions move in the same direction as the electroosmotic flow. Thus, for cation separatio0ns the walls of the capillary are untreated, and the electroosmotic flow and the cation movement is toward the cathode. For the separation of anions, on the other hand, the electroosmotic flow is usually reversed by treating the walls of the capillary with an alkyl ammonium salt, such as cetyl trimethylammonium bromide. The positively charged ammonium ions become attached to the negatively charged silica surface and, in turn, create a negatively charged double layer of solution, which is attracted toward the anode, thus reversing the electroosmotic flow.

In the past, the most common methods for analysis of small anions has been ion-exchange chromatography. For cations, the preferred techniques have been atomic absorption spectroscopy and inductively coupled plasma emission spectroscopy. Recently, however, capillary electrophoretic methods have begun to compete with these traditional methods for small-ion analysis. Several major reasons for adoption of electrophoretic methods have been recognized:

lower equipment costs, smaller sample size requirements, much greater speed, and better resolution.

The initial cost of equipment and the expense of maintenance for electrophoresis is generally significantly lower than those for ion chromatographic and atomic spectroscopic instruments. Thus, commercial

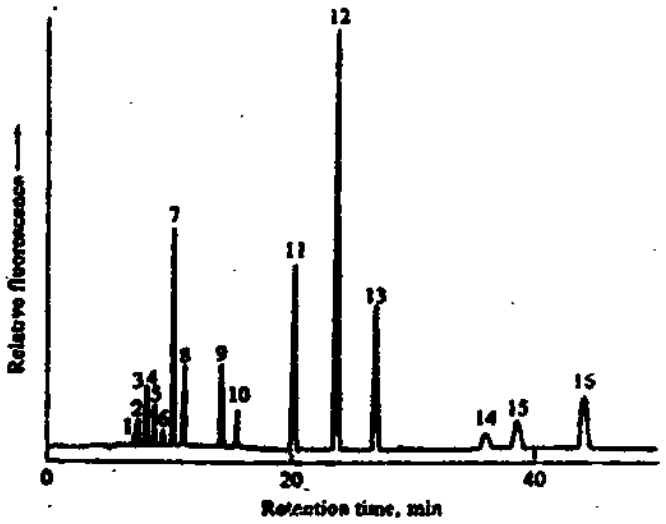


Figure: Electro chromatogram showing the electro chromatographic separation of the 16 PAHs. The peaks are identified as follows: (-10^{-6} to 10^{-8} M of each compound): (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5)phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz[a]anthracene, (10)chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13)benzo[a]pyrene, (14) dibenz[a,h]anthracene, (15) benzo[ghi]perylene, and (16) indeo [1,2,3-cd]pyrene,

matic phenols and nitro compounds with equipment such as that shown in figure. This technique involved introduction of surfactant, such as sodium dodecyl sulfate (SDS), at a concentration level at which micelles form. Micelles form in aqueous solutions when the concentration of an ionic species having a long chain hydrocarbon tail is increased above a certain level called the critical micelle concentration (CMC). At this point the ions begin to form spherical aggregates made up to 40 to 100 ions whose hydrocarbon tails are in the interior of the aggregate and whose charged ends are exposed to water on the outside. Micelles constitute a stable second phase that is capable of absorbing nonpolar compounds into the hydrocarbon interior of the particles, thus solubilizing the nonpolar species. Solubilization is commonly encountered when a greasy material or surface is washed with a detergent solution.

Capillary electrophoresis carried out in the presence of micelles is termed micelles electrokinetic capillary chromatography and given the acronym MECC or MEKC. In this technique, surfactants are added to the operating buffer in amounts that exceed the critical micelle

concentration. For most applications to date, the surfactant has been sodium dodecyl sulfate. The surface of an anionic micelle of this type has a large negative charge, which gives it a large electrophoretic mobility toward the positive electrode. Most buffers, however, exhibit such a high electroosmotic flow rate toward the negative electrode that the anionic micelles are carried toward that electrode also, but at a much reduced rate. Thus, during an experiment, the buffer mixture consists of a faster-moving aqueous phase and a slower-moving micellar phase. When a sample is introduced into this system, the components distribute themselves between the aqueous phase and the hydrocarbon phase in the interior of the micelles. The positions of the resulting equilibria depend upon the polarity of the solutes. With polar solutes the aqueous solution is favored; with nonpolar compounds, the hydrocarbon environment is preferred.

The system just described is quite similar to what exists in a liquid partition chromatographic column except that the "stationary phase" is moving along the length of the column but at a much slower rate than the length of the column but at a much slower rate than the mobile phase. The mechanism of separations is identical in the two cases and depends upon differences in

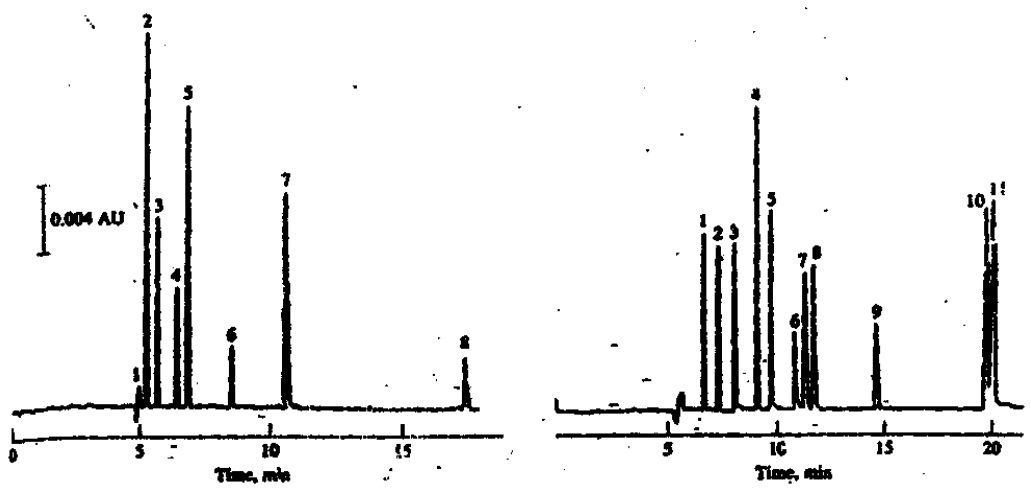


Figure: Typical separation by MECC. (a) some test compounds: 1=methanol, 2=resorcinol, 3=phenol,4=p-nitroaniline, 5=nitrobenzene, 6=toluene,7=2-naphthol,8=sudan III; capillary, 0.05-mm I.D., 500 mm to the detector; applied voltage. Ca.15 kV; current detection absorbance at 210nm. (b) Analysis of a cold medicine; 1=acetaminophen,2=caffeine, 3=sulpyrine, 4=naproxen,5=guaiphenesin,10=noscapine, 11=chloropheni-ramine and tipepidine; applied voltage. 20kV; capillary, as in (a); detection absorption at 220nm.

distribution coefficients for analytes between the mobile aqueous phase and the hydrocarbon pseudo stationary phase. The process is thus true chromatographic one; hence, the name micellar electrokinetic capillary chromatography. Figure illustrates two typical separations by MECC.

Capillary chromatography in the presence of micelles appears to have a promising future. One advantage that this hybrid technique has over HPLC is much higher column efficiencies (100,000 plates or more). In addition, changing the second phase in MECC is simple, involving only the changing of the micellar composition of the buffer. In contrast, in HPLC, the second phase can only be altered by changing the type of column packing.

B.E. / B.TECH DEGREE EXAMINATION. MAY / JUNE 2006.

FOURTH SEMESTER

BIO - TECHNOLOGY

BT 1254 – INSTRUMENTAL METHODS OF ANALYSIS

PART - A

1. Name atleast two methods of calibration of an instrument.
2. Suggest two methods by which you can separate salt from DNA?
3. What is the van Deemter's equation?
4. What are amphiprotic compounds? Give examples.
5. How does the purity of DNA measured?
6. What is fluorescence resonance energy transfer?
7. What is Augers spectroscopy?
8. The infrared spectrum of CO shows a vibrational absorption peak at 2170cm^{-1}
9. Why do infra red spectra seldom show regions at which the transmittance is 100%?
10. Distinguish fluorescence and phosphorescence.

PART - B

11. (a)(i) What are the causes of absorption bands in materials?

    (ii) Why do we prefer to express the Beer – Lambert law using absorbance as a measure of the absorption rather than % T?

    (iii) What is the significance of the molar absorbtivity, e?

    Or

12. (a) (i) How are thermal methods used to study protein folding?

    (ii) What is flame absorption spectroscopy?

    Or

    (b) (i) In thermal analysis methods why is the thermocouple for measuring sample temperature seldom directly immersed into the sample?

(ii) How would you study protein ligand binding using Isothermal Titration Microcalorimetery(ITC).

13. (a) (i) Why is degassing of solvents important prior to HPLC purification?

(ii) How would you separate the following:

(1) Chlorophyll from leaves

(2) Formaldehyde from cigarette.

Or

(b) (i) Write short notes on Raman Spectroscopy.

(ii) Explain Jablonski's diagram.

14. (a) (i) Application of FRET.

(ii) Why is Fourier transformation important in spectroscopy

(b) (i) Explain size exclusion chromatography.

(ii) Ion exchange chromatography.

15. (a) (i) Explain the instrumentation of HPLC

(ii) What are the various detection systems?

Or

(b) (i) Why is HETP an important parameter in chromatography?

(ii) Explain the differences between electrophoresis chromatography.

B.E. / B.TECH. DEGREE EXAMINATION, MAY / JUNE 2007.

FOURTH SEMESTER

BIO - TECHNOLOGY

BT 1254 – INSTRUMENTAL METHODS OF ANALYSIS

(REGULATION 2004)

PART - A

1. What is a figure of merit?
2. What are the two types of memories?
3. Define signal to noise ratio.
4. Name the type of noise that can be reduced by, decreasing the temperature of a measurement?
5. Define black body radiation.
6. Define the term effective band width of a filter.
7. State Beer's law.
8. Name any two compounds which are Raman active.
9. What is electro osmotic flow?
10. Define ground state of a molecule.

PART - B

11. (a) What are the factors that are of vital importance in selecting an analytical method?

Or

(b) Write a brief note on Feed back circuits, and the voltage follower circuit.

12. (a) (i) Explain thermo gravimetric analysis?

(ii) Why are the applications of TGA more limited than those for DSC and DTA?

Or

(b) (i) Explain differential scanning calorimetry?

(ii) Write a short note on heat flux DSC?

13. (a) Write short notes on supercritical chromatography and capillary electrophoresis.

Or

(b) (i) What are differences between GC and HPLC?

(ii) What are the various types of detectors used in gas chromatography?

(iii) What are the various pumps system used in HPLC?

(iv) For a reverse phase separation predict the order of elution of ethy acetate, diethyl either, nitromethane.

14. (a) (i) Explain Jablonski's diagram.

(ii) Write short notes on infrared spectroscopy?

Or

(b) (i) Write a brief note on the importance of Raman spectroscopy in biological studies?

(ii) With a diagram explain double beam spectrophotometer?

15. (a) (i) What are the various methods of optical spectroscopy, based upon working phenomena?

(ii) What are the major components of a spectroscopic instrument?

(iii) What are the various types of radiation sources? Explain two of them.

Or

(b) (i) Contrast between spontaneous and stimulated emission.

(ii) Describe the advantage of four level laser system over a three level type.

(iii) Why is glass better than fused silica as a prism construction material for a monochromator to be used in the region of 400 to 800nm?

B.E. / B.TECH. DEGREE EXAMINATION, APRIL/MAY 2008.

FOURTH SEMESTER

BIOTECHNOLOGY

BT 1254 –INSTRUMENTAL METHODS OF ANALYSIS

(REGULATION 2004)

PART - A

1. How is signal – to – noise ration relevant in measurement?
2. What is a transistor and what role does it play in an electronic circuit?
3. What is the source of UV in a spectrophotometer?
4. What is transducer?
5. Write the Lambert Beer's law and define the various parameters
6. Indicate the region of IR in the electromagnetic spectrum
7. Name any two chromophores that absorb in the Visible region
8. What is plate number and how is it related to resolution?
9. What is the principle of gas chromatography?
10. Write a short note on flow through detectors.

PART - B

11. (a) Write an essay on the importance of calibration and what methods are available for calibrating any three common analytical instruments used in Biotechnology?

Or

(b) Write an essay on the principle of working and uses of various optical instruments used in Biotechnology.

12. (a) Describe the theory and instrumentation associated with Raman Spectroscopy.

Or

(b) Describe how IR is useful in analytical spectrometry as well as in other

instrumentations relevant to biological research.

13. (a) Give a detailed account of functioning of differential scanning calorimetery and its utility.

Or

(b) Give a comprehensive account of any four spectroscopic measurements employed in the analysis of biological materials.

14. (a) Describe how Fourier transform measurements are utilized in various optical methods or spectrometric methods.

Or

(b) Describe the various parameters that govern separation in a chromatography set – up.

15. (a) Describe the salient features of HPLC that contribute to high resolution separation.

Or

(b) Write an essay on the principle, types and uses of ion – exchange chromatography.

B.E. / B.TECH. DEGREE EXAMINATION, MAY/ JUNE 2009.

FOURTH SEMESTER

BIOTECHNOLOGY

BT 1254 –INSTRUMENTAL METHODS OF ANALYSIS

(REGULATION 2004)

PART-A

1. What are semiconductors? Give an example.
2. What are hyphenated methods/ Give two example.
3. Give the advantages of grating monochromators over prism monochromators.
4. What are the general requirements of a radiation source?
5. Distinguish between adsorption and emission spectrum.
6. What are the advantages of using lasers in Raman spectroscopy?
7. mention any four factors which affect the DTA curves.
8. What is meant by ion exchange capacity of a resin? How is it determined?
9. List the variables that lead to zone broadening.
10. What is meant by retention time and retention Factor?

PART – B

11.(a) 1.Give an account of software signal enhancement techniques.
2. What is environmental noise? Mention the sources and measures to minimize it.
3.Explain the internal standard methods of calibration.

(or)

(b)1. Discuss in detail the classification of instrumental techniques.
2. Explain in detail the principle of any two readout devices.

12. (a) 1.With neat diagrams describe the working of bolometer any Golay cell detectors.
2. Give the principle, merits and demerits of any four infrared radiation sources.

(or)

(b) 1. Describe any four ultraviolet radiation sources.

2. Explain the working of photovoltaic cell and vaccum phototubes. Mention their advantages and disadvantages.

13. (a) 1. With a neat diagram describe the various components of a Raman Spectrophotometer.

2. Illustrate the function of various components of a double beam ultraviolet Spectrophotometer.

(or)

(b) 1. With neat diagrams of the optical systems compare the working principles of single beam, double

beam and Fourier transform infrared Spectrophotometer.

2. State and explain Beer – Lamberts law. Explain the factors which lead to the deviation from Beer-

Lamberts law.

14 (a) 1. Describe thr instrumentation for differential thermal analysis.

2. Briefly explain any four detection methods used in capillary electrophoresis.

Or

(b)1. Describe the instrumentation for differential scanning colorimetry.

2. Write notes on van Deemter equation and electron capture detector.

15.(a) 1.Discuss in detail the various components of a HPLC instrument.

2.Write a note on column packing used in ion exchange and size exclusion chromatography.

Or

(b)1. With a neat block diagram explain thr supercritical fluid chromatography instrumentation.

2. Discuss the functions of different components of GC instrumentation.

Reg. No. : | | | | | | | | | | | | |

Question Paper Code: E3040

B.E./B.Tech. DEGREE EXAMINATION, APRIL/MAY 2010

Fourth Semester

Biotechnology

BT2254 — INSTRUMENTAL METHODS OF ANALYSIS

(Regulation 2008)

Time: Three hours Maximum: 100 Marks

Answer ALL Questions

PART A — (10 × 2 = 20 Marks)

1. Explain, what is meant by significant figures?

2. What type of noise can be used for reduction of frequency?

3. Explain the term chromophore and give two examples.

4. What is the relation between wavelength and energy of electromagnetic radiations?

5. What is Rayleigh scattering?

6. Define transmittance.

7. Name the factors, which affect the thermogravimetric curve.

8. Explain how DTA differs from TGA?

9. Write short notes on electrophoresis.

10. Explain, what is meant by Rf value?

PART B — (5 × 16 = 80 Marks)

11. (a) Enumerate the software techniques used for signal to noise enhancement.

Or

(b) Describe what are the types of errors encountered in analytical measurement?

12. (a) List out the advantages of fast Fourier transformation over ensemble averaging as a signal enhancement technique.

Or

(b) Give brief answers:

(i) Wave length sectors

(ii) Radiation transducers.

13. (a) Explain the various components of UV spectrophotometer.

Or

(b) Discuss the various applications of IR spectroscopy in analytical field.

14. (a) Draw the block diagram of the instrument DTA. Discuss DTA thermogram of calcium acetate monohydrate.

Or

(b) Discuss the applications and merits of thermogravimetric analysis.

15. (a) Describe the general principle underlying the electrophoresis method for separation of mixture.

Or

(b) Discuss the principle and applications of ion exchange chromatography.

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Reg. No. : | | | | | | | | | | | |

Question Paper Code : 11185

B.E./B.Tech. DEGREE EXAMINATION, APRIL/MAY 2011

Fourth Semester

Biotechnology

BT 2254 — INSTRUMENTAL METHODS OF ANALYSIS

(Regulation 2008)

Time : Three hours Maximum : 100 marks

Answer ALL questions

PART A — (10 × 2 = 20 marks)

1. Mention the sources of environmental noise.
2. Distinguish between digital and analogue oscilloscopes.
3. What are the advantages of laser sources?
4. List the desirable properties of a transducer.
5. Why is iodine introduced into a tungsten lamp?
6. Distinguish between photometers and colorimeters.
7. What are monochromators? Mention their types.
8. What is meant by retention factor?
9. Give any four applications of thermogravimetry.
10. List the variables that lead to zone broadening.

PART B — (5 × 16 = 80 marks)

11. (a) (i) What are alphanumeric displays? Explain the properties and applications of LEDs and LCDs. (8)

(ii) What is the principle and functions of transformers, rectifiers and voltage regulators? (8)

Or

(b) (i) What is meant by signal to noise enhancement? Explain any three methods used for the same. (8)

(ii) Discuss the classification of instrumental methods. (8)

v

12. (a) (i) What are optical filters? Describe the principle of interference filters. (8)

(ii) How does a photomultiplier tube function? Give its merits and demerits. (8)

Or

(b) (i) Describe the principle and advantages of photovoltaic cells. (8)

(ii) What are the advantages of Fourier transform spectroscopy? How does it differ from conventional spectroscopy? (8)

13. (a) (i) State and explain Beer–Lambert's Law. Mention its limitations. (8)

(ii) Explain the differences among single beam, double beam and multi channel instruments for absorption measurements. List the advantages of one over the other. (8)

Or

(b) (i) With a neat diagram explain a double beam spectrometer for the UV and visible region. (8)

(ii) Give a detailed account of the various UV and IR sources. (8)

14. (a) (i) Describe the principle and applications of differential thermal analysis. (8)

(ii) Explain the principle of non-dispersive IR spectrometry. (8)

Or

(b) (i) What is the principle of differential scanning calorimetry? Discuss its instrumentation. (8)

(ii) Describe the principle and applications of ion-exchange chromatography. (8)

15. (a) (i) How does a flame ionization detector work? Explain with a neat figure. (8)

(ii) Discuss the theory and applications of size-exclusion chromatography. (8)

Or

(b) (i) Describe the principle and applications of supercritical chromatography. (8)

(ii) Write a note on capillary electrophoresis. (8)

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Reg. No. : | | | | | | | | | | | |

Question Paper Code : 53060

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2010

Fourth Semester

Biotechnology

BT 2254 — INSTRUMENTAL METHODS OF ANALYSIS

(Regulation 2008)

Time : Three hours Maximum : 100 Marks

Answer ALL questions

PART A — (10 × 2 = 20 Marks)

1. What is the significance of signal-to-noise ratio?

2. What is a thermostat and how is it useful in Biotech instruments?

3. State the working principle of an optical filter.

4. Write a note on photodiode.

5. What is the significance of Beer Lamberts law?

6. Write a short note on IR.

7. What is Raman Effect?

8. Define theoretical plate.

9. Apply Ohm's law with respect to gel electrophoresis.

10. Give any two essential properties of chromatographic matrix.

Reg. No. : | | | | | | | | | | | |

Question Paper Code : 53060

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2010

Fourth Semester

Biotechnology

BT 2254 — INSTRUMENTAL METHODS OF ANALYSIS

(Regulation 2008)

Time : Three hours Maximum : 100 Marks

Answer ALL questions

PART A — (10 × 2 = 20 Marks)

1. What is the significance of signal-to-noise ratio?

2. What is a thermostat and how is it useful in Biotech instruments?

3. State the working principle of an optical filter.

4. Write a note on photodiode.

5. What is the significance of Beer Lamberts law?

6. Write a short note on IR.

7. What is Raman Effect?

8. Define theoretical plate.

9. Apply Ohm's law with respect to gel electrophoresis.

10. Give any two essential properties of chromatographic matrix.

PART B — (5 × 16 = 80 Marks)

11. (a) Describe the working principle of any two instruments highlighting how signal-to-noise ratio is enhanced.

Or

(b) Explain the electromagnetic spectrum and indicate various Biotechnological instruments that are operated.

12. (a) Describe the various optical phenomena used in Biotech instrumentation.

Or

(b) Describe the operating principle and design of a fluorescence microscope.

13. (a) Schematically draw the various parts of a UV-Vis spectrophotometer and explain the functioning of each major component.

Or

(b) Explain the working and uses of differential scanning calorimeter.

14. (a) Describe the typical chromatographic arrangement with explanation on the functional significance of the components.

Or

(b) What are the differences between column chromatography and HPLC? Explain.

15. (a) Write explanatory notes on Supercritical chromatography and capillary chromatography.

Or

(b) Explain the roles of mobile and stationary phases in the interaction chromatography.

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********** END **********