DEPARTMENT OF BIOTECHNOLOGY

BT 3362-
CELL & MICROBIOLOGY LABORATORY MANUAL

REGULATION 2021

II YEAR & III SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

DEPARTMENT OF BIOTECHNOLOGY
BT3362 CELL & MICROBIOLOGY LABORATORY MANNUAL

OBJECTIVES

To demonstrate various techniques to learn the morphology, identification and propagation of cells and microbes.
To learn the staining techniques and culturing of microorganism.

LIST OF EXPERIMENTS

1. Introduction, Laboratory Safety, Use of Equipment; Sterilization Techniques
2. Microscopy – Working and care of Microscope, phase contrast and fluorescent microscopy 3. Culture Media-Types and Use; Preparation of Nutrient broth and agar
4. Culture Techniques, Isolation and Preservation of Cultures- Broth: flask, test tubes; Solid:Pour plates, streak plates, slants, stabs
5. Identification of given plant, animal, bacterial cells and yeast/mould
6. Staining Techniques Simple, Differential- Gram's Staining, spore /capsule staining, Giemsa, and Leishman Staining
7. Quantification of Microbes: Sampling and Serial Dilution; Bacterial count in Soil – TVC
8. Effect of Disinfectants- Phenol Coefficient, Antibiotic Sensitivity Assay
9. Osmosis and Tonicity and Tryphan Blue Assay
10. Growth Curve in Bacteria and Yeast
11. Staining for different stages of mitosis in AlliumCepa (Onion)
12. Effect of pH, Temperature, UV radiation on Growth Bacteria

TOTAL : 60 PERIODS

OUTCOMES:

This practical course will facilitate the students

- Understand the advanced technical information pertaining to laboratory bio-safety and preventive measures from pathogenic microorganism.
- Know the various aseptic techniques and sterilization methods.
- Develop the minimum skills to work on several important techniques for the study of microorganisms in the laboratory.
- Learn the various techniques of culturing of microorganisms and media preparation.
- Study the growth of microorganisms by varying the growth conditions.
- Identify the various stages of mitosis

Equipment Needed for 30 Students

Autoclave	1
Hot Air Oven	1
Incubators	2
Light Microscopes	4
Incubator Shaker	1
Colorimeter	2
Lamina Flow Chamber	2

Glassware: Petridish, Test tubes,Microscopic slides, Inoculation , loop, Gas burner

REFERENCES

1. Cappuccino, J.G. and N. Sherman "Microbiology: A Laboratory Manual", 4th Edition, Addison-Wesley, 1999. 72.

Exp. No. 1

INTRODUCTION, LABORATORY SAFETY, USE OF EQUIPMENT; STERILIZATION TECHNIQUES

Microbiology is one of the biological sciences that deal with the study of microorganisms that cannot be seen with naked eye. Safety in microbiology laboratory is important in the prevention of infection that might be caused by the microorganisms being studied. In addition to microorganisms there are some chemicals used in the laboratory that are potentially harmful. Many procedures involve glassware, open flames, sharp objects that can cause damage if used improperly.

The laboratory procedures must be read prior to attending that laboratory session. By being knowledgeable on the procedure, we will assure the safety of ourselves. The following precautions should be taken to avoid the problem that could potentially occur.

1. Laboratory coats must be worn and buttoned while in the laboratory.
2. Only closed toe shoes are to be worn in the laboratory.
3. Long hair must be tied back to minimize fire hazard or contamination of the experiment.
4. Hands must be cleaned and nails should be cut properly. Keep hands and other objects away from face, nose, eyes, ears, and mouth.
5. No food or drinks are permitted in the laboratory at any time.
6. All unnecessary books, purses, briefcases, etc. must be kept off the work table.
7. Glassware should be washed with soap and water and then rinsed with distilled water.
8. When handling chemicals, the hazard code on the bottle should be noted, the appropriate precautions indicated are to be followed.
9. Persons should be careful around Bunsen flames, cannot always been seen flaming of inoculation loops, wires or needles that should be done before, immediately after use to transfer biological material. Persons should not walk about the laboratory with transfer loops, wires, needles or pipettes containing inflectional materials. Bunsen burner is a fire hazard. Flammable liquids should not be kept near the flame.

10. Pipetting anything by mouth (including water) should not be done; instead pipetting devise should be used.
11. Caps on reagent solution bottle and bacterial culture should be used and replaced properly.
12. Everything should be labeled clearly with applicable information such as media, date of preparation, organism name, batch number, etc.
13. All culture should be handled as being potentially pathogenic and the following precaution should be observed at all times.
    a) Cultures must always be carried in a test tube rack when moving around the laboratory.
    b) Cultures must be kept in a test tube rack on the bench tops when not in use.
    c) Broth cultures must never be pipetted by mouth. Always should use a suction aide when filling a pipette with biological/chemical reagents.
    d) Spilt cultures should be covered with paper towel and hence saturated with disinfectant solution. Following 15 mins of reaction time, the towels should be removed and disposed off in the manner indicated by the instructor.
14. Bio-hazardous fluids should not be pour down the sink, contaminated too, swabs, pipette or other instruments should not be placed on the laboratory bench. These items should be sterilized in a Bunsen burner (inoculation loop) or placed in disinfectant solution (swabs, pipettes) to kill the microorganisms.
15. Spills, cuts and other accident should be reported to the instructor in case further treatment is necessary.
16. All chemicals, reagents, cultures and glasswares should be returned to their appropriate plates.
17. Hands must be thoroughly washed with soaps after working with microorganisms. Disinfectant soaps are useful but any soap will remove only surface bacteria.

STERILIZATION TECHNIQUE

Aim

To study the sterilization technique using autoclave.

Principle

Sterilisation is the freezing of an article from all living organisms including bacteria and their spores. Sterilisation of culture media, containers and instrument is essential in cell biology work for isolation of cells. In surgery and medicine, the sterilization of instruments, drugs and other supplier is important for the prevention of infection.

Water is heated in a closed container and saturated steam was produced under pressure with the temperature was over 100`C, most type of microorganism including bacteria (but not all viruses) were killed when apparatus was heated for 20 minutes at 120`C in the steam under pressure.

Materials Required

1. Autoclave
2. Sterilization material
3. pure water
4. power supply etc

Procedure

1. The bottom of the autoclave is filled with water (upto the bucket support). It was made thus the water doesn't touch the bucket. The water in excess is drained off.
2. The bucket contains culture materials to be sterilized in the autoclave along with the paper turn black when the correct temperature was reached.
3. The lid was closed and it was made sure thus the rubber washer was its 'groove'. The rid clamps were crewed down evenly and firmly but not too tightly.
4. The air outlet was opened. The heating of the autoclave began. The air outlet was extended for 2-4 minutes until the jet of steam was uniform. This showed that all the air has been driven out of the autoclave.
5. The air outlet was closed. The lid clamps were tightened and heat was reduced slightly.
6. The temperature gauge was watched. When the desired temperature was reached (i.e.) 120'C. The heat was regulated till the needle on the lid remained on the temperature selected. Then timing was started at this point.

Notes

The important things to be noted for sterilized technique in the autoclave.

1. The content must not be too tightly packed. There must be free circulation of steam. Too tight packing will prevent steam to reach every part of the material
2. Ensure complete removal of air before closing the steam escaping vent.

3. Use adequate sterility control such as Browne's sterilizing control tubes containing a red solution which turns green when heated to 110'C for 15 minutes alternatively paper impregnated with this solution can be used.
4. Cotton plug tubes or containers must be covered with brown paper.
5. After the run material is sterilized, it should be tested for sterility.

Result

Thus sterilized technique was studied and number of microbial growth was seen in a sterilized sample.

Exp. No. 2

MICROSCOPY – WORKING AND CARE OF MICROSCOPE, PHASE CONTRAST AND FLUORESCENT MICROSCOPY

Introduction

Microscopy is the use of a microscope (an instrument) that magnifies the size of the image of an object too small to be visible with the naked eye. The microscope has been a valuable tool in the development of scientific theory. Microscopes, of which there are many types, are the basic tools employed by cell biologist for the observation of cell and microorganisms. With the aid of microscope, various types of cells and microorganisms can be observed. The size of a microorganisms or a microbial structure determines the degree of magnification needed to se it. At magnifications of 1000x, bacteria and larger microorganism (fungi, algae and protozoa) can be viewed in light microscope. A series of lenses are made use in order to magnify minute objects. Microscope is of two categories, light or optical and electron depending upon the principle on which magnification is based. Light microscope, in which the magnification is obtained by a system of optical lenses using light waves includes.

1. Bright- Field
2. Dark Field
3. Fluorescence
4. Phase-contrast

Visualization of smaller microorganisms, like viruses, as well as the internal structure of bacterial cells, requires the use of higher magnification (10,000X to 100,000X) and better resolution, such high magnifications can be achieved with electron microscopes that use electron microscopes that use electrons instead of visible light.

Bright Field Microscopy

Aim

To study the principle of bright field microscope

Principle

In Bright field microcopy, the microscopic field (the area observed) is brightly lighted and the microorganism appears dark because they absorb some of the light. Ordinarily, microorganisms do not absorb much light, but staining them with a dye greatly increases their light absorbing ability resulting in greater contrast and color differentiation.

Working:

This microscope has three units **Condenser lens system:** Gathers the light rays and makes them pass thought the object **Objective lens system:** it is near the object and resolves the image

1. **Eye piece system:** it is near the receiver's eye and magnifies the image formed by the objective system

Resolving power: It is the ability to distinguish two objects rather point adjacent to each other as distinct and separate. It is given by the formula

$$r = \frac{0.61\lambda}{NA}$$

Where λ is the wavelength of light

NA is the numerical aperture, which is determined by the concentration of line entering the objective lens and the refractive index of the medium between lens and the specimen.

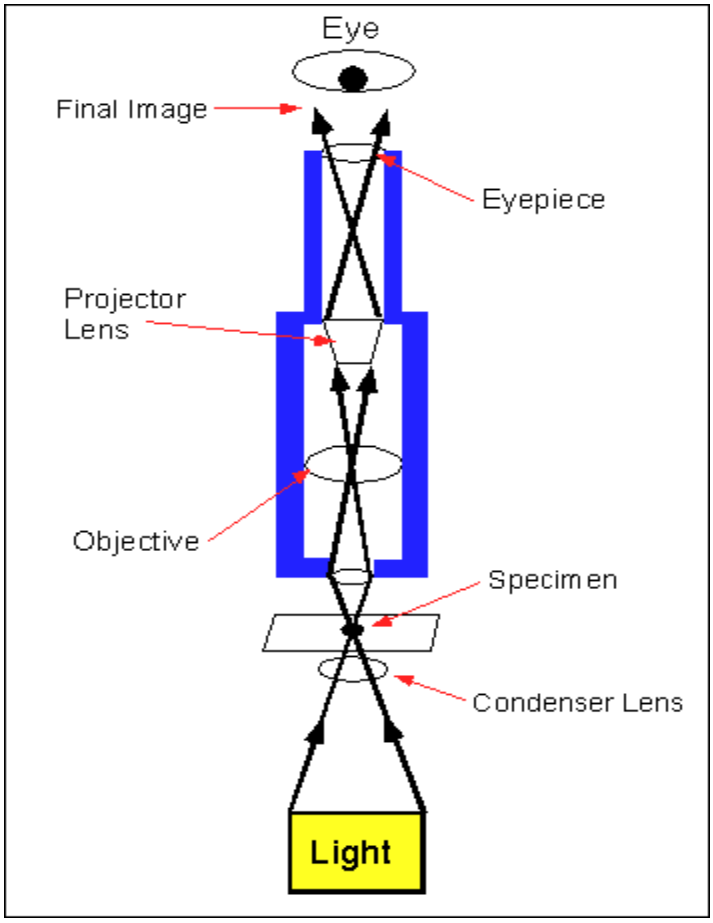


Figure 1. Working mechanism of light microscopy (Light path)

Applications

1. The examination of fixed and stained tissue by bright field microscopes is the standard approach for the analysis of tissue specimens in histology laboratories.
2. Nucleic acid probes and antibodies can be labeled with a variety of tags, that allows their visualization in the compound microscope making it possible to determine the location of specific molecules within individual cells.
3. It is used for gross morphological examination of different cells, tissues, bactieria, yest etc.

Advantages

The power of the microscope can be enhanced by the use of video cameras and computers for image analysis and processing

Disadvantages

1. Small and thin line structures such as bacterial flagella can not be sufficiently resolved
2. Staining procedure kill cells and are not suitable for experiments in which the observation of living cells is desired

Fluorescence Microscope

Aim

To study the principle of Fluorescence microscope

Principle:

Fluorescence microscope forms an image by using the fluorescent light emitted by the sample of fluorescent dye is used to label the molecule of interest. The dye is a molecule that absorbs light at one wavelength and emits light at the second wavelength. The fluorescence is detected by illuminating the specimen with a wavelength of light that excites the fluorescent dye and then using appropriate filters to detect the specific wavelength of light that the dye emits.

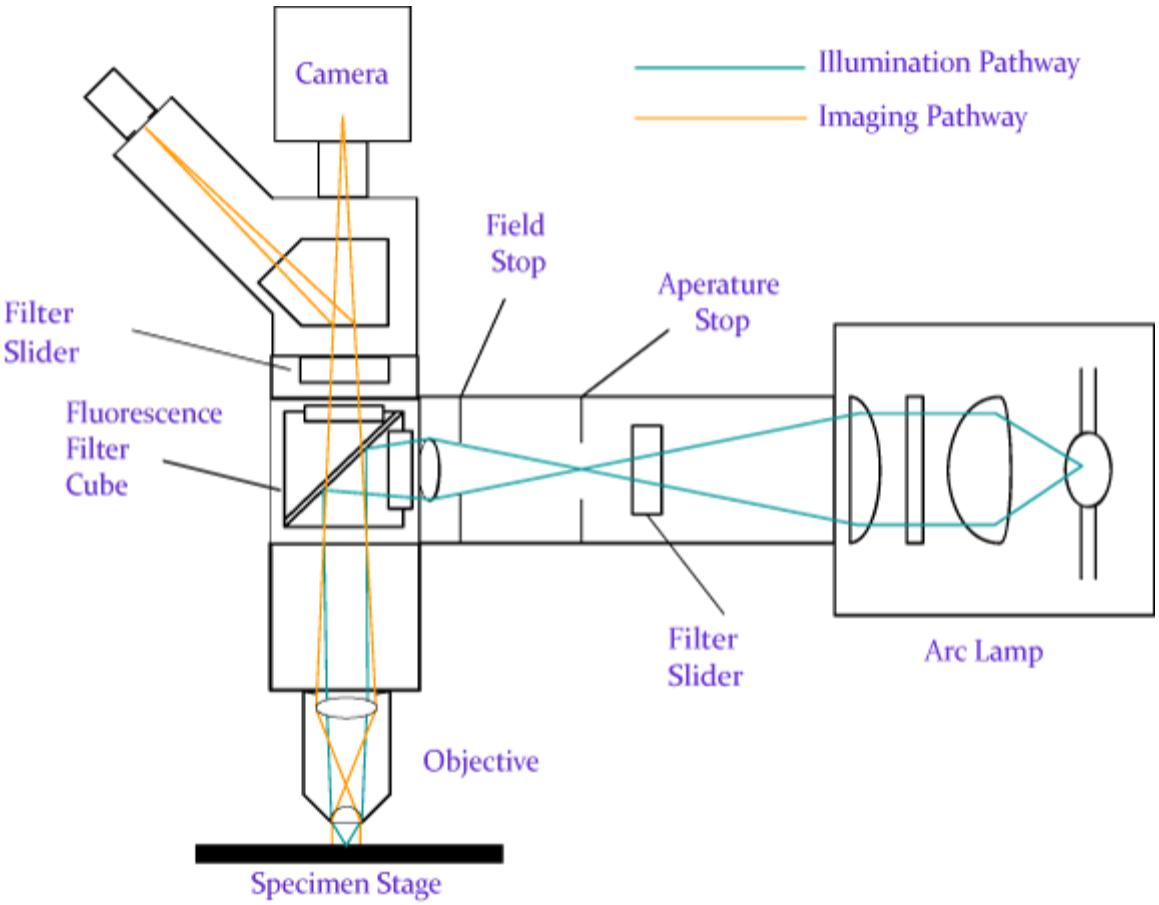


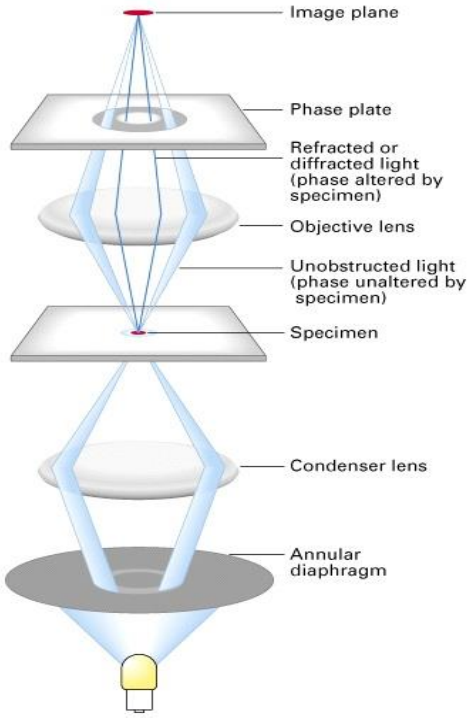
Figure 2. Working mechanism of Fluorescence microscopy (Light path)

Working

A high intensity mercury lamp is used as a light source and emits white light. The excitor filter transmits only blue light to specimen and blocks out all other colors. The blue light reflected downward to the specimen by a dischroic mirror (which reflects light of certain colors but transmits light of other colors) the specimen is stain with a fluorescent dye certain portions of the specimen retains the dye others do not. The stained portions retains the dye, others do not. The stained portions absorb blue light and emit green light, which passes upwards, penetrates the dischroic mirror and reaches the barrier filter. The filter allows the green light pass to the eye and blocks out any residual blue light from the specimen, which many not have been, completely deflected bye the dischroic mirror. Thus the eye perceives the stained portions of the specimen as glowing green against a jet-black background, whereas the unstained portions of the specimen are invisible

Applications

1. The fluorescent microscope is an essential tool in medical microbiology and microbial ecology
2. Bacterial pathogens can be identified by staining them with flurochormes or specifically labeling them with fluorescent antibodies using immunofluorescent procedures
3. One frequent application is to label antibodies directed against a specific protein with flucoroscent dyes so that the intracellular distribution of the protein can be determined



Advantages:

Fluorescence microscopy is used to study a variety of molecules within cells. It is a widely used and very sensitive method for intra cellular distribution of molecules

Disadvantages

1. The fixations added to preserve cell architecture destroy the protein antigenicity which hinders the attachment of the dye embedded antibody
2. Viewing cell sections which is thin is very difficult
3. The embedding material is often fluorescent. This obscured signals from the antibody carrying the fluorescent dye

Phase contrast microscope

Aim

To study the principle of bright field microscope

Principle:

This is based on the fact that light passing thought the on material and into another material of a slightly different refractive Indices or thickness will undergo a change in shape. Phase. The difference in phase or wave front irregularities is translated into variations of brightness of the structures and hence they are detectable by the eye.

Figure 3. Working mechanism of Fluorescence microscopy (Light path)

Working

This microscope uses a conventional light microscope fitted with a phase contract objective and phase contrast condenser. This special optical system makes it possible to distinguish unstained structures within a cell, which differ only in their refractive indices or thickness. The condenser of this microscope has annular stop (an opaque disc with a a thin transparent ring), which produces a hollow conc. of light. As this cone passes thought a cell, some light rays are bent due to variation in density and the refractive index with the specimen. These rays are retarded by about ¼ wavelengths. The deviated light is focused to form an image of the object. The undeviated light rays strike a phase ring in phase plate, a special optical disk located in the objective, while the deviated rats miss the ring and pass through most of the rest of the plate. The background formed by the undeviated light is bright, while the unstained object remains dark and well defined.

Applications

1. It is used to detect bacterial component such as endospores and inclusion bodies containing poly beta hydroxy butrate, poly metaphosphate or other substances
2. These microscopes are used in studying eukaryotic cells and living unstained cells.
3. They are used for examination of minute cellular structure and detail in living cells.

Advantages:

1. The living micro organisms in their material state can be studied, since the specimen does not have to be fixed or stained
2. Minute structures and detail scan be observed

Disadvantage

It produces a halo around dark circles in the specimen

Ex.No:3 CULTURE MEDIA-TYPES AND USE; PREPARATION OF NUTRIENT BROTH AND AGAR

AIM

To prepare the nutrient broth and nutrient agar and explain the types and use of culture media.

CULTURE MEDIA

Culture media is a gel or liquid that contains nutrients and is used to grow bacteria or microorganisms. They are also termed growth media. Different cell types are grown in various types of medium. Nutrient broths and agar plates are the most typical growth media for microorganisms. Some microorganisms or bacteria need special media for their growth. **Significance –** Culture media is used in order to identify the causative agent from infected material.

TYPES OF CULTURE MEDIA

The culture media are classified in many different ways:

1. Based on the physical state
    - Liquid media
    - Solid media
    - Semisolid media

   Based on the presence or absence of oxygen
    - Anaerobic media
    - Aerobic media

   Based on nutritional factors
    - Simple media
    - Synthetic media
    - Complex media
    - Special media

PREPARATION OF NUTRIENT AGAR

AIM

To prepare the nutrient broth medium for cultivatable of bacteria.

PRINCIPLE

Nutrient agar is made with various nutrients which allow the growth of a wide variety of microorganisms that do not usually require specific nutrients or supplements. The primary constituents of the media are peptone, beef extract, and agar. In addition to these nutrients, some vitamins and some trace ingredients necessary for the growth of bacteria are also added. The peptone is the source of nitrogen or protein that acts as a source of amino acids for the

bacteria. The beef extract is the primary source of carbon which is essential for the formation of carbohydrates in the bacteria. It also contains other components like some vitamins, different trace minerals, organic compounds, and salts, which further enhance the growth of different organisms. Besides, sodium chloride is added to the medium in order to maintain the osmotic equilibrium of the medium and prevent the change in pH of the medium during growth.

PROCEDURE

1. Dissolve the dehydrated medium in the appropriate volume of distilled water i.e., 23 gm dehydrated nutrient agar (*see the manufacturer's instruction*) in 1000 mL distilled water.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Sterilize the medium by autoclaving (121°C for 15 min)
4. Dispense the medium into tubes *(i.e. 3 ml to make nutrient agar slopes, 5 ml to make nutrient agar deeps)* or plates.
5. Leave the agar medium to solidify.
6. Date the medium and give it a batch number.
7. Store in a cool dark place.

OBSERVATION

Nutrient agar plate were observed after 24 hours of contamination.

RESULT

There was no microbial growth on agar plate were stores under 4 degree Celsius for further use.

PREPARATION OF NUTRIENT BROTH

AIM

To prepare nutrient broth medium for cultivation of bacteria.

MATERIALS REQUIRED

1. Conical flask
2. Cotton
3. Distilled water
4. Measuring cyclinder
5. Autoclave

INGREDIENTS

1. Peptone- 5gms
2. Sodium chloride- 5 gms
3. Yeast extract- 5gms
4. Beef extract- 5 gms

PRINCIPLE

Bacteria, in contrast to Fungi, are routinely cultured in a liquid medium i.e. Nutrient Broth Medium (NBM). The liquid basal medium, used in bacteriology laboratory constitutes the 3 essential components –Beef Extract – It is the beef derivative which is a rich source of Organic Carbon, Nitrogen, Vitamins and Inorganic Salts that supports the rapid growth of bacterium in the laboratory at an optimum temperature, pH, and Osmotic Pressure. Peptone – It is a semi-digested protein which is soluble in water and easily metabolized by the bacterial cell, provides the rich source of protein to the bacterial cell of the rapid growth. Sodium Chloride – It maintains the osmotic pressure in the broth medium so that the movement of molecules takes place in and out of the bacterial cell. It must be present in right proportion otherwise it will lead to the lysis of the bacterial cell.

PROCEDURE

Weigh the quantity of Peptone, Beef Extract, and Sodium Chloride using the weighing scale for 1000 ml of Nutrient Broth as follows:

⇒ Put the butter paper on the weighing scale and transfer the required quantity of peptone on to the paper using the spatula. Repeat the step to obtain the required quantity of beef extract and sodium chloride.

⇒ Take a clean and dry Conical Flask/ Erlenmeyer flask.

⇒ Pour 500 ml of distilled water to the flask and add the weighed quantity of Peptone, Beef Extract, and Sodium Chloride.

⇒ Mix the content and Heat it with continuous agitation to dissolve the constituents.

⇒ Now add more distilled water to the medium and make the volume 1000 ml.

⇒ Check the pH of the solution using pH strip, it should be 7.2 ± 0.2. If required, adjust the pH by adding either 1N HCl (acid) or 1N NaOH (base) as per the case.

⇒ Mix well the content and apply the Non-absorbent cotton plug to the flask.

⇒ Autoclave the content at 121 °C and 15 psi pressure for 15 minutes.

⇒ Allow the content to cool and then inoculate the specimen to be cultured.

OBSERVATION

Nutrient broth is observed after 2 hours for turbidity.

RESULT

The nutrient broth was cleaned and of any microbial growth media was stored under 4 degree Celsius for future use.

Exp. No. 5

Identification of given plant, animal, bacterial cells and yeast/mould

Aim

To observe the plant cell, animal cell and bacterial cell under microscope.

Materials Required

Slides and microscope

Procedure

Prepared slides were focused under high power and differences between these cells were observed.

Plant Cell

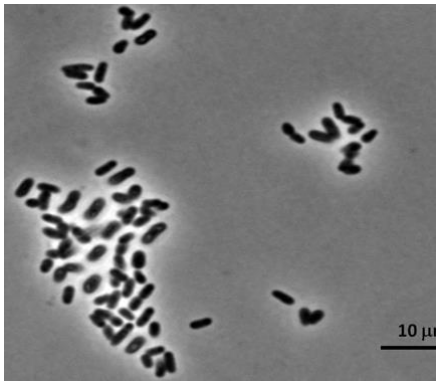
A rigid cell wall of hexagonal shape was observed. The cell contains a large nucleus, plenty of cytoplasm, vacuoles and other organelles under the high power.

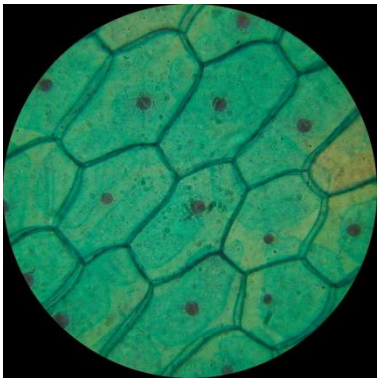
Animal Cell

An irregular shaped cell was observed within a thin plasma membrane seen under high power. The cell contained nucleus and cytoplasm.

Bacterial Cell

Bacilli were observed. These are rod shaped organisms. They were observed in groups of two.





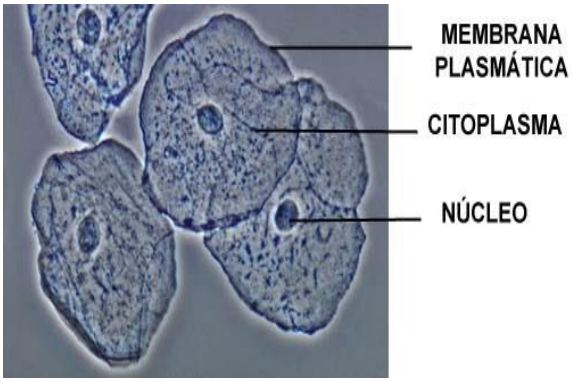


Figure 4. Bacterial cells **Figure 5. Plant cells** **Figure 6. Animal cells**

Result

The plant cell, animal cell and bacterial cell were observed.

Exp. No. 6

STAINING TECHNIQUES SIMPLE, DIFFERENTIAL- GRAM'S STAINING, SPORE /CAPSULE STAINING, GIEMSA, AND LEISHMAN STAINING

Aim

To differentiate the two principle groups of bacteria- gram positive and gram negative

Principle

In gram staining procedure, all bacteria are stained purple by crystal violet, the primary stain. Bacteria cells that have a thick peptidoglycan layer retain the crystal violet during subsequent decolorization and counter stain steps. These bacteria appear purple when viewed under the microscope and referred to as gram positive

Bacterial cell that have a thin peptidoglycan layer lose the crystal violet during decolorization step and take up the counter stain safranin. These bacteria appear red when viewed under the microscope and are referred to as gram negative.

Materials Required

Cultures (18 -24 hours broth or agar)
Baacillus cereus (Gram Positive rod)
Excherichia coli (Gram negative rod)

Equipment: Light microscope, Laminar flow

Procedure

1. Transfer a loopful of the bacterial suspension to the surface of a clean glass slide, and spread it over a small area. Allow the slide to air dry or fix the cells by passing the slide briefly through the Bunsen burner flame.

2. Flood the slide for one minute with a crystal violet solution. Wash off briefly with tap water for 2 sec.

3. Flood slide with Gram's iodine solution and allow to sit for one minute. Wash off with tap water for 2sec

4. Flood slide with 95% alcohol and pour off immediately. Then wash off with tap water for 2sec...

5. Flood the slide with safranin solution and allow it to stain for at least one minute. Wash off with tap water. Drain the slide and blot it dry with absorbent paper

6. Observe the slide under the microscope.

Observation- Gram Stain

Magnification	Slide A	Slide B	Observation	
10 X				
40 X				
100 X				

Observation	Slide A	Slide B
Cell Shape		
Cell Colour		
Gram Reaction		

Result:

The cell in the given Sample A was identifies as _____
The cell in the given Sample B was identifies as _____

Blood Smear Using Leishman stain

Aim:

To observe blood cells in blood smear.

Reagent Required

1. Leishman stain;

0.15% Leishman powder in 100% methanol. Use after 24 hours

2. Phosphate buffer:

Stock A: 0.2 M sodium di hydrogen orthophosphate (MW. 156). To prepare, dissolve 3.12 g in 100ml of distilled water.

Stock B: 0.2 M di-sodium hydrogen orthophosphate (MW. 142. To prepare, dissolve 2.83 g in 100ml distilled water. For pH 6.8 add 26.5 mo of A to 24.5 ml of B and Make upto 100ml with distilled water

PROCEDURE

Preparation of Blood smears:

To prepare blood smear clean slides may be used. The index finger is sterilized by cotton soaked in methylated spirit. Sterilize the lancet and puncture the skin, wipe away the first blood which comes out as soon as anther drop falls touch the slide to the blood. So that a small drop is deposited towards one end of the slide at an angle of about 30 to the slide; move it backwards to make contact with the drop. The blood should run quickly along the contact line. With a steady movement of the hand, spread the drop of blood along the slide. The spreader must not be lifted off the slide until the last trace of blood has been spread out; the film should be about 3 cm in length. It is important that the film of blood finishes at least 1 cm before the end of slide. Wave the slide though the air to day the film. The best result is obtained by freshly prepared film.

Staining procedure

Leishman's stain is poured on the blood film and allowed for three minutes: as the stain's formulation includes methanol, this will fix the cells. Dilute the stain on the slide with an equal amount of buffered water, pH 6.8, adding the water slowly with a plastic Pasteur pipette and mixing by sucking the stain up and down the pipette. Leave for approximately 12 minutes: the appearance of polychromatic components can be ignored. Was off, the excess stain with slowly running tap water and flood slide for one minute with buffered water, pH 6.8. Dry the slide and mount with a cover slip if required, using DPX mounting medium.

Inference

Blood is opaque, from a viscous fluid tissue made up of formed elements suspended in a complete fluid matrix known as plasma. The formed elements that are observed includes.

6. Red blood corpuscles or erythrocytes which are not nucleated appear red in color due to the presence of haemoglobin.
7. White blood corpuscles which was seen as two types
   a. Granular leucocytes or granulocytes
   b. Agranular leucocytes or aranulocytes

a. Granular Leucocytes

The WBC's has a granulated cytoplasm which appear as faint pink neutrophills, basophills and eosinophills.

i.**Neutrophills**

The cells have a faint pink cytoplasm in colored granular and multilobed nucleus.

ii.**Eosinophills**

Cytoplasm is stained faint pink and contained dark red orange granules and nucleus is bilobed.

iii.**Basophills**

Cytoplasm granules are large dark blue black which fill the cells and nucleus is S shape or irregular in shape.

b. Agranulocytes

These cells do not posses granular in their cytoplasm. These includes monocytes and lymphocytes.

i.**Monocytes**

Larges in size of all white cells. The cytoplasm appears greenish blue with kidney shaped.

ii.**Lymphocytes**

They posses dark violet color nucleus which almost fills the entire cell and has a ring of clear blue cytoplasm.

Result:

Blood smear was taken and neutrophills, eosinophills, basophills, monocytes and lymphocytes were observed.

Blood Smear Using Giemsa stain

Aim:

To observe blood cells in blood smear.

Reagent Required

1. **Giemsa Stain.** 0.15% of stain in 100% methanol. Use after 24 hours

2. Giemsa buffer:

Stock A: 0.2 M sodium di hydrogen orthophosphate (MW. 156). To prepare, dissolve 3.12 g in 100ml of distilled water.

Stock B: 0.2 M di-sodium hydrogen orthophosphate (MW. 142. To prepare, dissolve 2.83 g in 100ml distilled water. For pH 6.8 add 26.5 mo of A to 24.5 ml of B and Make upto 100ml with distilled water

PROCEDURE

Preparation of Blood smears:

To prepare blood smear clean slides may be used. The index finger is sterilized by cotton soaked in methylated spirit. Sterilize the lancet and puncture the skin, wipe away the first blood which comes out as soon as anther drop falls touch the slide to the blood. So that a small drop is deposited towards one end of the slide at an angle of about 30 to the slide; move it backwards to make contact with the drop. The blood should run quickly along the contact line. With a steady movement of the hand, spread the drop of blood along the slide. The spreader must not be lifted off the slide until the last trace of blood has been spread out; the film should be about 3 cm in length. It is important that the film of blood finishes at least 1 cm before the end of slide. Wave the slide though the air to day the film. The best result is obtained by freshly prepared film.

Staining procedure

Giemsa's stain is poured on the blood film and allowed for three minutes: as the stain's formulation includes methanol, this will fix the cells. Dilute the stain on the slide with an equal amount of buffered water, pH 6.8, adding the water slowly with a plastic Pasteur pipette and mixing by sucking the stain up and down the pipette. Leave for approximately 3-4 minutes: the appearance of polychromatic components can be ignored. Was off, the excess stain with slowly running tap water and flood slide for one minute with buffered water, pH 6.8. Dry the slide and mount with a cover slip if required, using DPX mounting medium.

Observation:

The shape and color of cell were observed

Result:

The various types of cells were observed under the microscope after staining with Giemsa's stain

Ex.No.: 7 QUANTIFICATION OF MICROBES: SAMPLING AND SERIAL DILUTION; BACTERIAL COUNT IN SOIL – TVC

AIM

To quantification of microbes in soil by serial dilution method.

PRINCIPLE

Dilution is the process of making a solution weaker or less concentrated. In microbiology, serial dilutions (log dilutions) are used to decrease a bacterial concentration to a required concentration for a specific test method, or to a concentration which is easier to count when plated to an agar plate. Serial dilution involves the process of taking a sample and diluting it through a series of standard volumes of sterile diluent, which can either be distilled water or 0.9 % saline. Then, a small measured volume of each dilution is used to make a series of pour or spread plates. Depending on the estimated concentration of cells/organisms in a sample, the extent of dilution is determined. For e.g., if a water sample is taken from an extremely polluted environment, the dilution factor is increased. In contrast, for a less contaminated sample, a low dilution factor might be sufficient. Serial two-fold and ten-fold dilutions are commonly used to titer antibodies or prepare diluted analytes in the laboratory. The dilution factor in a serial dilution can be determined either for an individual test tube or can be calculated as a total dilution factor in the entire series.

MATERIALS REQUIRED

1. Soil sample.
2. Test tubes
3. Petri plates
4. Distilled water
5. Nutrient agar

PROCEDURE

1. The sample/culture is taken in a test tube and six test tubes, each with 9 ml of sterile diluents, which can either be distilled water or 0.9% saline, are taken.
2. A sterile pipette is taken.
3. 1 ml of properly mixed sample/culture is drawn into the pipette.
4. The sample is then added to the first tube to make the total volume of 10 ml. This provides an initial dilution of 10^{-1}.
5. The dilution is thoroughly mixed by emptying and filling the pipette several times.
6. The pipette tip is discarded, and a new pipette tip is attached to the pipette.
7. Now, 1 ml of mixture is taken from the 10^{-1} dilution and is emptied into the second tube. The second tube now has a total dilution factor of 10^{-2}.
8. The same process is then repeated for the remaining tube, taking 1 ml from the previous tube and adding it to the next 9 ml diluents.
9. As six tubes are used, the final dilution for the bacteria/cells will be 10^{-6} (1 in 1,000,000).

RESULT

By serial dilution, and colony streaking skills. The hypothesis was confirmed, having shown that each dilution decreases number of cells per ml, and that the more set of streaks we perform, the more isolated colonies we get.

Ex. No. 8

EFFECT OF DISINFECTANTS- PHENOL COEFFICIENT, ANTIBIOTIC SENSITIVITY ASSAY

Aim

To determine the susceptibility of a pathogen to a range of antibiotics

Principle

Antibiotics are natural antimicrobial agents produced by microorganisms. One type of penicillin, for example, is produced by the mold *Penicillium notatum*. The Kirby-Bauer test, also called the disc diffusion test, is a valuable standard tool for measuring the effectiveness of antimicrobics against pathogenic microorganisms. In the test, antimicrobics-impregnated paper disks are placed on a plate that is inoculated to form a bacterial lawn. The plates are incubated to allow growth of the bacteria and time for the agent to diffuse into the agar. As the drug moves through the agar, it establishes a concentration gradient. If the organism is susceptible to it, a clear zone will appear around the disk where growth has been inhibited.

The size of this zone of inhibition depends upon the sensitivity of the bacteria to the specific antimicrobial agent and the point at which the chemical's minimum inhibitory concentration (MIC) is reached. Some drugs kill the organism and are said to be bactericidal. Other drugs are bacteriostatic; they stop growth but don't kill the microbe.

Materials and Methods

Broth culture, Muller- Hinter agar Plates, antibiotic disc, cotton swab and sterile forceps

Procedure

- The test organism was inoculated on to sterile peptone water and was incubated at 37°C for 2-4 hours.
- After proper incubation, the suspension was then swabbed on to sterile Muller – Hinter Agar using sterile cotton swab and kept in position for some time.
- The antibiotic discs of known potency were placed on to agar surface using sterile forceps and gently pressed it
- Incubated the plates at 37°C for 24 hours and observed it carefully.

Observation

Observed the zone of growth inhibition around the disc and measured it and compared the value with standard antibiogram. Based on the comparison the organism can be differentiated in to sensitive, intermediate sensitive and resistant.

Exp. No. 9

OSMOSIS AND TONICITY AND TRYPHAN BLUE ASSAY

Aim

To determine the effect of osmosis on RBC's

Materials required

Slide, 3 solutions A, B, C, sterilized needle

Solution A: 0.15M concentration of Nacl
Solution B: 0.075M concentration of Nacl
Solution C: 0.3M concentration of Nacl

Principle

This is based on principle of osmosis which is the movement of water from region of higher to lower concentration. All cells have specific tonicity and movement of particles in and out of it is allowed by semi permeable membrane. When cells are kept in solution of same tonicity, i.e isotonic, there is no movement of water molecules. When cells are in solution of lower tonicity, water molecules move into cell. The cell swells until it burst and dies. Such a solution is hypotonic. When cells are kept in higher concentration, water moves out. The cell shrinks and dies.

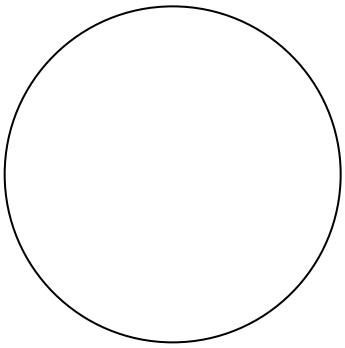
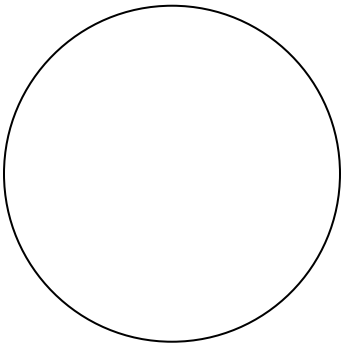
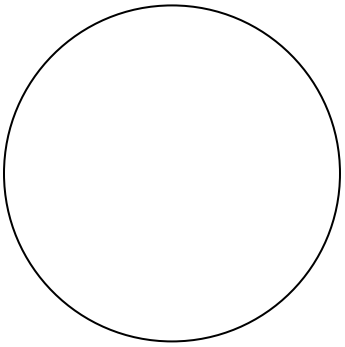
Procedure

A slide was taken and three drops of blood were added. To each a solution of different tonicity (A, B, C) was added and allowed to stand for 15 minutes. The slide was then observed under a microscope.

When a cell is placed in a hypotonic solution (i.e. one in which the concentration of the solute is lower than in the cytosols) animal cells swell owing to the osmotic flow of water inwards. Conversely, when placed in a hypertonic solution (i.e. one in which the concentration of the solute is higher than in the cytosol). Animal cells shrink as cytosolic water leaves the cell by osmotic flow. In an isotonic medium which has the solute concentration identical to that of the cell cytosol, the RBC size remains the same.

Observation

The flowing observation were seen

S.No.	Cells	Structure of RBC
1.	Isotonic cells	
2.	Hypotonic cells	
3.	Hypertonic cells	

Isotonic : RBC in the solution remains same

Hypertonic: RBC in the solution was swollen

Hypotonic: RBC in the solution was shrunk

Result: The effect of osmosis on RBC's was studied and observed under microscope

TRYPHAN BLUE ASSAY

Aim

To estimate the viability of cells by dye exclusion (tryphan blue).

Principle

Viable cells are impermeable towards tryphan blue and a number of other dyes.

Materials Required

Growth medium, tryphan blue, haemocytometer, microscope.

Procedure

Prepare the cell suspension at a high concentration. A clean haemocytometer slides with its cover slip, in place was focused the counting grid, under the low power. Mix 1 drop of cell suspension with 1 drop of tryphan blue and loaded in the counting chamber of haemocytometer. The slide was kept aside about 1 minute and the total number of cells and the number of stained cells was counted.

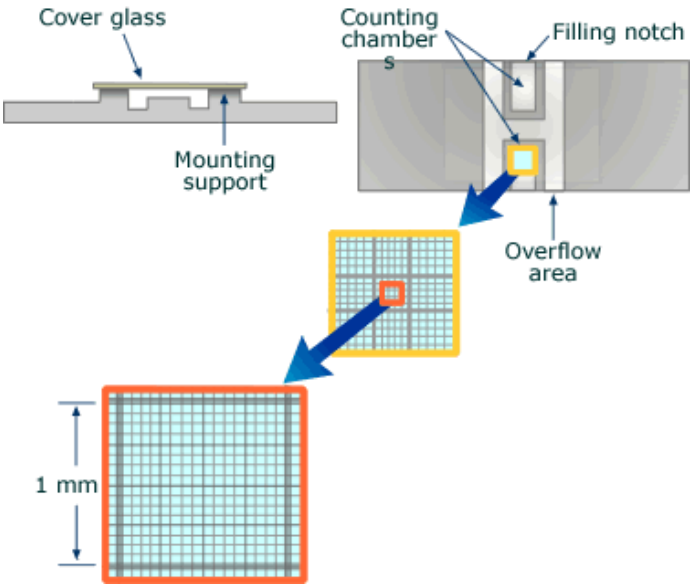
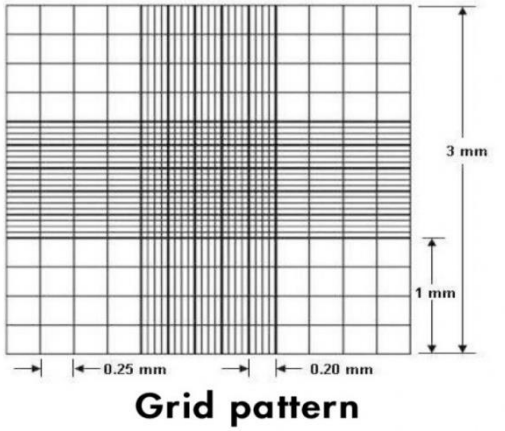


Figure 6. Haemocytometer and its grid lines



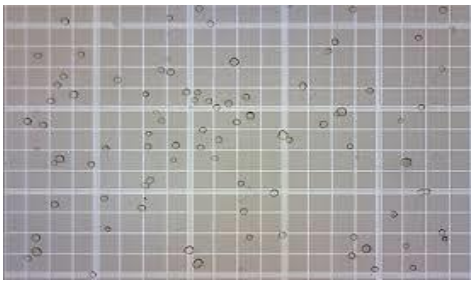


Figure 7. Grid pattern and cells located in the grid pattern for counting

Result

The cells were observed and counted.

Ex.No: 10 # GROWTH CURVE IN BACTERIA AND YEAST

AIM

To plot the growth curve for the given microorganism by bidometric method.

INTRODUCTION

Growth is defined as an increase in cellular considerations and may result in an increase in the microbes size population number the growth curve includes in phases.

LAG PHASE

There is no appreciable increase in number through these may be an increase in the size of cells. The initial period is to require for the adaption to the new environment during which the necessary enzymes and metabolizes intermediate one built up during which the multiplication. The phase varies with culture medium and environment factor such as temperature.

LOG PHASE

Following the log phase, the cell starts building and there number increase exponentially growth rack is proportional to bacterial to bacterial biomass.

STATIONARY PHASE

Cell division concentration to a rate due to depletion of nutrients and accumulation of toxic production. The numbers of cells that in just enough to replace the number of cells that die. The vessels count remains stationary as a equilibrium exists between the dying cells and the newly forms the cells.

DECLINE PHASE

This is the phase where the population decrease due to the death of cells. Besides nutritional exhaustion and toxic accumulation cell death may be causes by catalytic enzymes also. A variety of cells one available to monitor the bacterial growth curve either by number of cells or total mash by their colonies forming units in agar plates.

PRINCIPLES

In spectrophotometer a beam of light is transmitted through the bacterial suspension to a right sensitive detector as bacteria number increases less light will reach the detector because of the absorbance of the capital density. Other methods viable count: viable cell count one used to culture was incoculate into flash prepared 30 ml nutrient broth and the zeroth low was adjusted at 540 nm also absorbent 100 degree Celsius transmitters at zero optical density with steroids nutrient. The sample was test fixed at 80 degree Celsius for 10 minutes and it was transferred into the cuvette the optical density. The same procedure was repeated at regular intervals for 24 hours.

OBSERVATION

The turbidometric reading at each time intervals were observed.

RESULT: The growth curve was successfully plotted according for the reading obtained.

Exp. No. 11

STAINING FOR DIFFERENT STAGES OF MITOSIS IN ALLIUMCEPA (ONION)

Aim:

To prepare temporary slide of onion root tip squash and study the various stages of mitosis in cells

Materials Required

Slides cover slip, filter paper, onion root tip, aecta carmine stain, microscope.

Principle

Root tips are made of apical meristematic tissue. The cells are in a continuous of division. 1 N HCL is used to soften the tips to make sit easy to prepare a uniform squash. Acetocarmine stain is used to stain is used to stain chromosomes.

Procedure

1. The onion roots were washed till they became white
2. The tips were carefully cut and placed in a petridish containing preheated 1N HCL.
3. The tips were placed in 1N HCl at 65 C for about 15 min
4. The slide was flooded with acetocarmine for 15 min
5. The slide was placed and the tips were kept carefully
6. The cover slip was placed to the tip gently.
7. The root was squashed by the thumb and uniform squash was obtained
8. The slide was viewed under the microscope.

Observation

i. **Prophase:** Nucleus was seen without disappearance of nuclear membrane. This confirms prophase.

ii. **Metaphase:** The chromosomes were in the equatorial plane. This confirms metaphase.

iii. **Anaphase:** The splitting of chromosomes into 2 and the movement towards the poles confirms anaphase.

iv. **Telophase:** Formation of invagination and nuclear membrane confirms telophase.

Result

Thus the various stages of mitosis were identified and studied in the temporary slide of onion root tip squash.

Ex.No : 12 EFFECT OF pH ON BACTERIAL GROWTH

AIM:

To study the effect of pH on bacterial growth.

PRINCIPLE

These are optimal hydrogen ion concentration for mass organism althrough they grow at of their microorganism natural habitat. The microbes itself act as a result of its metabolism plays a role in setting the pH of its environment. Acid production bacterial molds and yeast increases the hydrogen ion concentration of their environment and tend to grow best at moderately low pH values, other bacteria especially protein into basic amines and ammonia raise the pH of their environment and thrive in alkaline condition.

MATERIALS REQUIRED

Petri Plates, test tubes, nutrient agar culture of E.Coli sodium hydroxide HCL and standard lab wares.

PROCEDURE

6. Subject the bacterial culture to grow at two different pH by heating few drops of HCL.
7. Turbidometric cell mass, after 30 minutes 50 ml of the bacteria was taken and its optical density of 540 nm was measured using a calorimeter as a spectrophotometer. Sterile medium was taken as blank.

OBSERVATION

Compared to control plate the E.Coli growth in media acid and alkaline pH was found to be low.

RESULT

The growth of E.Coli which was subject of disc and alkaline pH was found to be decreased by 13% to 25% respectively.

EFFECT OF TEMPERATURE ON BACTERIAL GROWTH

AIM

To study the effect of temperature on bacterial growth.

PRINCIPLE

Different type of bacteria have distinct requirement, the temperature at which they grow. A maximum temperature above which culture will not develop and a minimum temperature below in between range in which growth occur the most growth tubes place within a limited range called the optimum temperature.

The optimum temperature for growth of a partial microbial species is correlated with the temperature of the temperature on the microbial growth is actually is a reflection of the effect of

the temperature on the enzymatic reactor in the cell. As the temperature is lowered the enzyme and growth of the cell is slowed.

PROCEDURE

1. The bacterial culture was subjected to grow at two different temperature and at 50 degree Celsius.
2. Turbidometric method is laso used in quantitative the bacterial culture was taken and its cell mass after 30 minutes of the bacterial culture was taken and its optical density at 540nm was measured using a calorimeter as a spectrophotometer sterile medium was taken as a blank.

OBSERVATION

When control plate, the E.Coli growth in media which were kept at 20 degree Celsius.

RESULT

At 20 degree Celsius 50% change in the growth of E.Coli. At 50 degree Celsius the growth of E.Coli was found to be decreased to 40% respectively.

EFFECT OF UV RADIATON ON BACTERIAL GROWTH

AIM

To study the effect of UV radiation on bacterial growth.

PRINCIPLE

UV light is capable of producing a effect in cells when exposed to the low penetrating wavelength in the range of 210nm to 300 nm cellular components capable obsorbing UV Length one the nucleic acid with the DNA acting as the primary bite of damage.

When the pyrimidinese absorbed UV wavelength the effect of this form of radiation is thymine dimentions which is molecules in one nucleus acid stand in the DNA molecules dimmer formation disturb the configuration of the DNA molecules and the distortion interfere with DNA replication transcription during protein synthesis.

MATERIALS REQUIRED

Petriplates, test tubes, nutrient agar culture of E.Coli, refrigerator incubator and other standard lab wares.

PROCEDURE

1. Subject the bacterial culture to UV exposure by keeping the culture under upto 30 minutes.
2. After time interval of 30 minutes take 30 ml of the culture and measure the turbidity of the cell growth by using calorimeter at 620nm of the sterile medium is used as blank. Repeat the procedure for evening 30 minutes interval.

OBSERVATION:

The turbidometric readings of the culture whose UV wave measured and graph of optical densities in this was plotted.

RESULT

The growth of E.Coli was seen standard under UV whose compared to bacteria growing under normal condition.

DEPARTMENT OF BIOTECHNOLOGY

BT 3361-
BIOCHEMISTRY LAB MANUAL

REGULATION 2021

II YEAR & III SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

GENERAL GUIDELINES FOR WORKING IN BIOCHEMISTRY LAB

Laboratories can be dangerous places in which to work and all users need to be aware of the potential hazards and to know what to do in cases of emergency. When starting work in a new laboratory, it is important to become familiar with the layout of the room and the location of the safety equipments.

1. **Personal Protection:**
   - Goggles or safety spectacles - should always be worn when carrying out any procedures where there is risk.
   - Gloves must be worn when handling corrosive substances such as strong acids or alkalis.
   - Protecting clothing – laboratory coats are symbols of protection not status. Cotton laboratory coats always should use for protect from chemical splashes and infectious material.
   - Face masks – These are not always necessary but need to be worn when there is risk of dust from chemicals or an aerosol of micro-organisms.
2. **Chemcial hazards**
   - Hazard working symbols, which are black on an orange background pruent on reagent bottles to warn of specific dangers and must be needed.
   - Corrosive and intent substances – A corrosive substance is one that destroys living tissue and the inherent dangers of strong acid or alkalis coming in contact with the skin .

Toxic compounds:

- Compounds are graded as toxic or highly toxic depending on the dose required to kill 50 percent of a populatin of animals (LD 50) Ninhydrin, Ethidium bromide are Carcinogenic.

Flammability hazards: -

- Flammable substances are these with a low flash and all naked flames in the laboratory should be extinguished when handling them.
- Oxidizing substances may not be flammable themselves but may cause a fire when brought into contact with combustible materials should avoid.
- Explosive reagents such as Picric acid are explosive and must be handled with extreme caution.

3. **Physical hazards**
   - Fire, pressure, ionizing radiation, non-ionizing radiation should handled carefully.

4. **Biological hazards**
   - Microorganisms – should handle carefully.
   - Safety Cabinets. Microorganisms should be handled in safety cabinets or hood and not in open air.
   - Wire loops, used for the plating should be handled carefully.
   - Animals and body fluids should be handled carefully.

Reference: An Introduction to practical Biochemistry by David T Plummor (3^{rd} ed.)

1. STUDY OF UNITS, VOLUME, WEIGHT, SENSITIVITY, SPECIFICITY, PRECISION AND ACCURACY

AIM:

To study Units, volume and weight sensitivity, specificity, precision and accuracy**.**

MEASUREMENT AND CONCENTRATION UNITS:

1.UNITS OF MASS:

1 kg	=	1000 g	=	1000000 mg
1g	=	1000 mg	=	1000000 µg
1µg	=	1000 ng	=	1000000 pg
1pg	=	1000 pg		

Kilogram:	kg
Gram:	g
Milligram:	mg
Microgram:	µg
Nanogram :	ng
Picogram:	pg

Gram.(g)

A unit of mass in the metric system. The gram was originally defined to be the mass of one cubic centimetre of pure water, but is now defined to be 1/1000 of the mass of the standard kilogram.

Kilogram.(kg)

The SI base unit of mass is equal to the mass of the international prototype of the kilogram: a piece of platinum-iridium alloy kept at the International Bureau of Weights and Measures, Sévres, France. One kilogram equals exactly 1000 gram and is approximately the mass of a litre of water.

2.UNITS OF VOLUME

1 millimole	=	10^{-3} moles
1 micromole	=	10^{-6} moles
1 nanomole	=	10^{-9} moles
1 picomole	=	10^{-12} moles
1 litre	=	1000 ml
1 ml	=	1000 µl
1 µl	=	1000 nl

Litre (L or l)

The metric unit of volume. The litre was originally defined to be the volume occupied by a kilogram of water and equal to one cubic decimeter. In the SI, one kilogram water occupies about 1.000 028 cubic decimeter

Mole(mol)
The SI base unit of the amount of a substance. The mole is defined as the amount of substance of a system, which contains as many elementary entities as there are atoms in 0.012 kilogram of carbon 12.

3.UNITS OF HEAT:

1 kilogram calorie (kcal) = 4.184 kilojoule (kJ) or 10^3 cal
1 kilojoule (kJ) = 0.239 kilogram calorie (kcal) or 10^3 J

Joule (J): The SI derived unit of energy.One joule is defined as the amount of work or energy exerted when a force of one newton is applied over a displacement of one metre. One joule is the equivalent energy of one watt of power radiated or dissipated for one second.

Calorie (cal): The CGS unit of heat energy. This calorie (also called a gram calorie or small calorie) is the amount of heat required at a pressure of one atmosphere to raise the temperature of one gram of water by one degree Celsius.

4.UNITS OF PRESSURE:

1 Pa ≡ 1 N/m² = 10^{-5} bar = 9.8692×10^{-6} atm = 7.5006×10^{-3} Torr = 145.04×10^{-6} psi

1 bar ≡ 10^6 dyn/cm² =100 000 Pa = 0.98692 atm = 750.06 Torr = 14.504 psi

1 atm ≡ 1 atm = 101 325 Pa = 1.01325 bar = 760 Torr = 14.696 psi

1 torr ≡ 1 mmHg = 133.322 Pa = 1.3332×10^{-3} bar = 1.3158×10^{-3} atm = 19.337×10^{-3} psi

1 psi ≡ 1 lbf/in² = 6 894.76 Pa = 68.948×10^{-3} bar = 68.046×10^{-3} bar = 51.715 Torr

Bar(bar) :A unit of pressure, equal to 10^5 pascal. One bar is roughly the same as the average pressure of the Earth's atmosphere (atm), which is 1.013 25 bar. A barometer an instrument for measuring barometric pressure of the atmosphere, usually in units of millibar (mbar) or as the height in millimeters, of a column of mercury (mmHg).

Pascal(Pa) :The SI derived unit of pressure. The pascal is equal to one newton per square metre or one "kilogram per metre per second per second.

ATOMIC WEIGHT:
Atomic weight of an element is defined as average weight of atom of specified isotopes relative to weight of Carbon atom taken as 12.

MOLECULAR WEIGHT:

The molecular weight of a compound is the sum of the atomic weights of the atoms in the molecules that form these compounds.

ATOMIC NUMBER:

A unit of measurement, equal to the number of electrons surrounding a neutral (uncharged) atom, and also to the number of protons in the nucleus.

GRAM ATOM:

The atomic weight of an element expressed in grams.

GRAM EQUIVALENT:

The weight of substance in grams, which will combine with (or) replace 1 g of hydrogen (or) 8 g of oxygen is called gram equivalent.

GRAM MOLECULE:

The molecular weight of a substance expressed in grams.

MOLARITY

The molar concentration of a solution, usually expressed as the number of moles of solute per liter of solution.

MOLALITY:

The molal concentration of a solute, usually expressed as the number of moles of solute per 1,000 grams of solvent.

NORMALITY:

Normality is a measure of concentration. It is equal to the number of gram equivalents of a solute per liter of solution.

SENSITIVITY:

Sensitivity of a method is defined as its ability to detect small amounts of test substance. For example the smallest reading after zero that can consistently detected and measured. the slope of calibration curve is conventional way of expressing sensitivity and is particularly useful when comparing two methods.

SPECIFICITY:

Specificity is ability to detect only the test substance. Lack of specificity will result in positive results if methods is qualitative and positive bias in quantitative results. It is important to appreciate that specificity is often linked to sensitivity.

PRECISION:

Precision (or) reproducibility of a method is the extent to which a number of replicate measurements of sample agree with one another and is affected by random error of the method. It is measured as the imprecision, which is expressed numerically in terms of standard deviation statistically. It is represented as co efficient of variation, which is

V = (Standard deviation/mean) x 100

ACCURACY:

Accuracy is closeness of the mean of set of replicate analysis to true value of sample. It id often only possible to accesses the accuracy of one method relative to one another, which for one reason is assumed to give a true mean value. Accuracy is expressed in terms of error, which is between true result and measured value.

QUALITATIVE TEST FOR CARBOHYDRATES

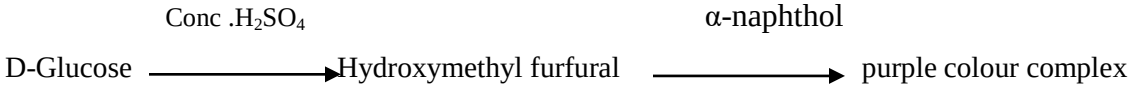
AIM:

To analyze the carbohydrates in sample qualitatively.

MOLISCH'S TEST

PRINCIPLE

This is a general test for all carbohydrates. Concentrated sulphuric acid hydrolyses glycosidic bonds to yield monosaccharides, which in presence of an acid gets dehydrated to form furfural and its derivatives. These products reacts with sulphonated α naphthol to give a purple colour complex. Polysaccharides and Glycoproteins also give positive reactions.



REAGENTS REQUIRED:

- Concentrated Sulphuric acid
- α-naphthol: 5% (w/v) in ethanol.

PROCEDURE:

To 2 ml of the test solution add 2 drops of Molisch's reagent. Then carefully pour Sulphuric acid along the sides of the test tube.

OBSERVATION:

A violet colored ring appears at the junction of the two liquids.

INFERENCE:

Presence of carbohydrates

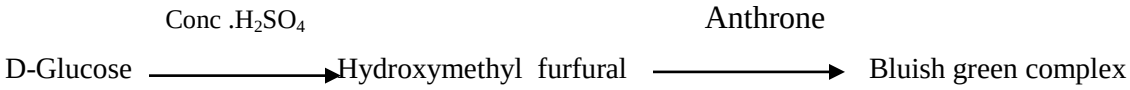
TABULATION: MOLISCH'S TEST

SI No	SAMPLE	OBSERVATION	INFERENCE
1	A		
2	B		
3	C		
4	D		
5	E		
6	F		

ANTHRONE TEST

PRINCIPLE

Anthrone reaction is another general test for carbohydrates. In this furfural produced reacts with anthrone to give bluish green coloured complex.



MATERIALS REQUIRED:

- Concentrated Sulphuric acid
- Anthrone reagent: 0.2% (w/v) in conc H_2SO_4.
- Boiling water bath

PROCEDURE:

- To 1 ml of the test solution add 1 ml of Anthrone reagent and mix thoroughly.
- Keep it in water bath for 10 mins.

OBSERVATION:

Formation a bluish green colour complex.

INFERENCE:

Presence of carbohydrates

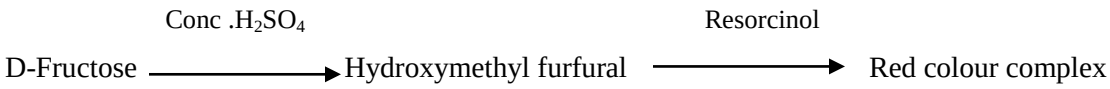
TABULATION :ANTHRONE TEST

SI No	SAMPLE	OBSERVATION	INFERENCE
1	A		
2	B		
3	C		
4	D		
5	E		
6	F		

SELIWANOFF'S TEST

PRINCIPLE

This is due to dehydration of ketoses to give furfural derivatives,which then condense with resorcinol to form red complex.prolonged heating will hydrolyse disaccharides and other monosacchasrides will also eventually give colour. This test is used to distinguish aldoses from ketoses.



MATERIALS REQUIRED:

- Seliwanoff's reagent: 0.05% (w/v) Resorcinol in 3N HCl.
- Boiling water bath

PROCEDURE:

- To 1 ml of the test solution add 1 ml of Seliwanoff's reagent.
- Keep it in water bath for 2-3 mins.

OBSERVATION:

Formation of cherry red color complex.

INFERENCE:

Presence of Ketoses.

TABULATION: SELIWANOFF'S TEST

SI No	SAMPLE	OBSERVATION	INFERENCE
1	A		
2	B		
3	C		
4	D		
5	E		
6	F		

FEHLING'S TEST

PRINCIPLE

Fehling's test is a specific and highly sensitive for the detection of reducing sugars.formation of yellow (or) red precipitate of cuprous oxide denotes presence of reducing sugar.Rochelle salt acts as chelating agent in this reaction.

D-Glucose + 2 CuO ⟶ D-Gluconic acid + Cu_2O (Red colour precipitate)

MATERIALS REQUIRED:

- Fehling's reagent A
- Fehling's reagent B
- Boiling water bath

PROCEDURE:

- To 1 ml of the test solution add 1 ml of Seliwanoff's reagent.
- Keep it in water bath for 10 mins.

OBSERVATION:

Reddish precipitate is obtained.

INFERENCE:

Presence of reducing sugar.

TABULATION: FEHLING'S TEST

SI No	SAMPLE	OBSERVATION	INFERENCE
1	A		
2	B		
3	C		
4	D		
5	E		
6	F		

BARFOED'S TEST

PRINCIPLE

It is test for detection of monosaccharides. The monosaccharides react with Barfoed's reagent to give red coloured product.

Monosaccharides + Barfoed's reagent ⟶ Red colour precipitate

MATERIALS REQUIRED:

- Barfoed's reagent
- Boiling water bath

PROCEDURE:

- To 1 ml of the test solution add 1 ml of Barfoed's reagent.
- Keep it in water bath for 5 mins.

OBSERVATION:

Formation of red colour.

INFERENCE:

Presence of Monosaccharides.

TABULATION : BARFOED'S TEST

SI No	SAMPLE	OBSERVATION	INFERENCE
1	A		
2	B		
3	C		
4	D		
5	E		
6	F		

IODINE TEST

PRINCIPLE

Iodine forms coloured adsorption complexes with polysaccharides. Starch gives blue colour with iodine while glycogen gives reddish brown colour complex. It is rapid test for amylose, amylopectin and glycogen.

MATERIALS REQUIRED:

- Iodine solution: Prepare 0.005 N iodine solution in 3% (w/v) potassium iodide solution.
- 1 % test solutions of glucose,sucrose,starch etc.

PROCEDURE:

- To 1 ml of the test solution add 1 ml of Barfoed's reagent.
- Keep it in water bath for 5 mins.

OBSERVATION:

i) Formation of Violet/blue/purple color
ii) Formation of Deep red color .
iii) Formation of Reddish precipitate .

INFERENCE:

Violet or blue colour	-	Presence of Starch
Deep red colour	-	Presence of Glycogen
Reddish precipitate	-	Presence of Dextrin

SI No	SAMPLE	OBSERVATION	INFERENCE
1	A		
2	B		
3	C		
4	D		
5	E		
6	F		

RESULT:

The given sample contains

A	
B	
C	
D	
E	
F	

3. PREPARATION OF BUFFER SOLUTION

AIM:
To prepare certain buffer solutions and to study theory involved.

PRINCIPLE:
A buffer is a mixture of weak acid that resists changes in pH of a solution. They are added whenever pH of solution needs to be maintained at a constant and predetermined level. Buffer solution of desired pH can be prepared by using Henderson – Hosselbalch equation.

PREPARATION OF BUFFER SOLUTIONS:

1.Acetate Buffer
Stock solutions:
A.0.2 M Acetic acid (11.5 ml/L)
B.0.2 M Sodium acetate (16.4g/L)
X ml of A +**Y** ml of B, make upto a total volume of 100 ml.

X	Y	pH	X	Y	pH

2.Phosphate Buffer

Stock solutions:
A.0.2 M Monobasic Sodium phosphate (27.8 g/L)
B.0.2 M Sodium phosphate (53.65 g of $Na_2HPO_4.7H_2O$ or
71.7g of $Na_2HPO_4.12H_2O$ in 1L)
X ml of A + **Y** ml of B, make upto a total volume of 100 ml.

X	Y	pH	X	Y	pH

3.Tris (Hydroxymethyl) Amino methane Buffer or Tris Buffer

Stock solutions:
A.0.2 M Tris (Hydroxymethyl) Amino methane (24.2 g/L)
B.0.2 M HCl

50 ml of A + **Y** ml of B, make upto a total volume of 200 ml.0.05M Tris Hcl buffer will be obtained.

X	Y	pH	X	Y	pH

RESULT:

4. ESTIMATION OF PROTEIN BY BIURET METHOD

AIM:

To estimate the amount of protein present in the given sample by Biuret method.

PRINCIPLE:

This is based on principle that –CO-NH- groups of proteins will form a purple coloured complex with copper ions in an alkaline medium. Since all the proteins contain peptide bonds, this method is fairly specific and there is little interference with other compounds.

MATERIALS REQUIRED:

- Biuret Reagent
- Distilled water
- Test solution
- Protein standard
- Spectrophotometer.

PREPARARTION OF BIURET REAGENT;

Biuret reagent is prepared by dissolving 3g of copper sulphate and 9 g of sodium potassium triturate in 500ml of 0.2 N sodium hydroxide. To this 5g of potassium iodide is added and volume is made up to 1000 ml with 0.2 N sodium hydroxide.

PREPARATION OF PROTEIN STANDARD:

1 mg of BSA is dissolved in 1 ml of distilled water. This is used as stock solution. This is serially diluted and prepare a standard curve by taking absorbance values at different concentration of given samples at 550 nm

PROCEDURE:

- Take 0.2 ml to 1 ml of standard solution in test tube and make up to 4 ml with distilled water.
- Add 6 ml of alkaline. $CuSO_4$ to all the test tubes.
- Incubate at 37°C for 15 minutes.
- Take OD at 550 nm and plot the standard graph
- For test solution same procedure is followed as done above.
- Amount of protein present in the sample can be calculated by using standard graph

TABULATION:

S.NO	Working std (µl)	Concentration (µg / ml)	Vol of distilled H_2O	Biuret reagent	INCUBATE AT 37°C FOR 15 MIN	OD at 550 nm
1						
2						
3.						
4.						
5.						
6.						
7.						
8.						

CALCULATION:

RESULT

The given test sample contains _____ in _____ of test solution**.**

5. ESTIMATION OF PROTEIN BY LOWRY'S METHOD

AIM:

To estimate the amount of protein present in given sample.

PRINCIPLE:

The phenolic group of tyrosine and trytophan residues (amino acid) in a protein will produce a blue purple color complex , with maximum absorption in the region of 650 nm wavelength, with Folin-Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. The intensity of the colour development is directly proportional to the concentration of the protein in the sample.Thus the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins. Most proteins estimation techniques use Bovin Serum Albumin (BSA) universally as a standard protein, because of its low cost, high purity and ready availability. The method is sensitive down to about 10 µg/ml and is probably the most widely used protein assay despite its being only a relative method , subject to interference from Tris buffer, EDTA, nonionic and cationic detergents, carbohydrate, lipids and some salts. The incubation time is very critical for a reproducible assay. The reaction is also dependent on pH and a working range of pH 9 to 10.5 is essential.

MATERIALS REQUIRED:

- Spectrophotometer.
- Test tubes,
- Mortar pestle,
- Pipette

REAGENTS REQUIRED:

1. 2 % sodium carbonate in 0.1 N NaOH
2. 0.5 % $CuSO_4$ in 1% sodium potassium tartarate.
3. Alkaline $CuSO_4$: Mix (1) d (2) in 50 : 1 ratio
4. Folin's reagent: Phenol and water 1:2 ratio
5. Working standard (BSA): 25 mg of BSA dissolved in 100 ml of double distilled water.
6. The unknown solution is diluted to 200 times (200 dilutions) with distilled water.

PROCEDURE:

- Take 0.2 ml to 1 ml of standard solution in test tube and make up to 4ml with distilled water .
- Add 5.5 ml of alk. CuSO4 to all the test tubes.
- Keep in water bath for 10 minutes.
- Add 0.5 ml of Folin's Phenol reagent in all the test tubes and incubate for 30 minutes at room temperature.
- Take OD at 650 nm and plot the standard graph
- For test solution take 2ml from homogenate and same procedure is followed as done above.
- Amount of protein present in the sample can be calculated by using standard graph

TABULATION:

S.NO	Working std (µl)	Concentration (µg)	Vol of distilled H_2O (ml)	$CuSO_4$ reagent (ml)	INCUBATE AT 37°C FOR 10 MIN	Folin's (ml)	INCUBATE AT 37°C FOR 30 MIN	OD at 650 nm
1								
2								
3.								
4.								
5.								
6.								
7.								
8.								

CALCULATION:

RESULT:

Amount of protein present in the given sample = _____

6. ESTIMATION OF PROTEIN BY BRADFORD METHOD

AIM:

To estimate the amount of protein present in given sample by Bradford assay

PRINCIPLE:

The assay is based on the specific binding of Coomassie Brilliant Blue G-250 dye to arginine, tryptophan, tyrosine, histidine and phenylalanine residues in proteins. The dye binds approximately 8 times as well to arginine residues as the other listed aromatic residues. Therefore, the overall reliability of the assay is highly dependent on the primary structure of the proteins being assayed, as well as on the protein chosen to prepare the standard curve.

MATERIALS REQUIRED:

- Spectrophotometer.
- Test tubes,
- Mortar pestle,
- Pipette

REAGENTS PREPARARTION:

1. **Sample Extract:**
   Take 100 mg of tissue sample and homogenize it with Tris –Hcl buffer and centrifuge it**.** Take the supernatant for analysis.

2. **Bradford reagent:**
   Dissolve 100 mg of Coomassie Brilliant Blue G-250 in 50 ml of ethanol, add 100 ml of 5% phosphoric acid and make the volume to 1 liter with distilled water.

3. **0.1 M Tris –HCl buffer.(7.2)**

4. **Standard protein solution:**
   Dissolve 5 mg of BSA in 50 ml of Distilled water. This solution contains 100 µg/ml of protein.

PROCEDURE:

- Take 0.2 ml to 1 ml of standard solution in test tube and make up to 2 ml with distilled water.
- Add 3 ml of Bradford reagent in all the test tubes .Mix it well
- Incubate for 5 minutes at room temperature.
- Add 2 ml of distilled water in all the test tubes.
- Take OD at 595 nm and plot the standard graph
- For test solution take 0.2 ml of supernatant and add 1.8 ml of distilled water. Mix it well and record the absorbance at 595 nm.
- Amount of protein present in the sample can be calculated by using standard graph

TABULATION:

S.NO	Working std (µl)	Concentration (µg)	Vol of distilled H_2O (ml)	Bradford reagent (ml)	OD at 595 nm
1					
2					
3.					
4.					
5.					
6.					
7.					
8.					

CALCULATION:

RESULT:

Amount of protein present in the given sample = _____**7.**

7. QUANTIFICATION OF FREE AMINO ACIDS BY NINHYDRIN METHOD.

AIM:

To quantify free amino acids present in the given sample**.**

MATERIALS REQUIRED:

- Spectrophotometer
- Test tubes
- Mortar and pestle
- Pipette.

REAGENT PREPARATION:

1. **Sample Extract:**
   Take 100 mg of tissue sample and homogenize it with 80 % ethanol and centrifuge it**.** Take the supernatant for analysis.

2. **Ninhydrin reagent:**
   Dissolve 200 mg of Ninhydrin in 100 ml of acetone.

3. **80% Ethanol**

4. **Standard protein solution:**
   Take 100 mg of Leucine and dissolve it in 100 ml of water.

PROCEDURE:

- Take 0.2 ml to 1 ml of standard solution in test tube and make up to 1 ml with distilled water.
- Add 1 ml of Ninhydrin reagent in all the test tubes and boil it in water bath for 8 min.
- Cool it immediately and add 1 ml of 50 % ethanol and 3 ml of distilled water.
- Take OD at 530 nm and plot the standard graph
- Amount of free amino acids present in the sample can be calculated by using standard graph

TABULATION:

SI. NO	Working std (µl)	Conc (µg)	Vol of distilled H_2O (ml)	Ninhydrin reagent (ml)	BOIL IT FOR 8 MIN AND COOL	50% Ethanol (ml)	Vol of distilled H_2O (ml))	OD at 530 nm
1								
2								
3.								
4.								
5.								
6.								
7.								
8.								

CALCULATION:

RESULT:

The amount of free amino acids present in the given sample =_____

8. QUANTIFICATION OF AMINO ACID BY THIN LAYER CHROMATOGRAPHY.

AIM:

To analyze the given sample for its components using TLC system.

PRINCIPLE:

The principle of thin-layer chromatography (TLC) is that a suitable adsorbent is spread in a thin layer on a glass plate, or other suitable support plate. The drop of solution to be analyzed is applied at a known starting point. The plate is placed in a sealed chromatographic chamber with a suitable solvent system. By capillary action the solvent creeps up through the stationary layer and separates the components of the sample into a number of spots. After separation, the plates are dried and the spots identified by the addition of reagents.

REAGENTS REQUIRED:

- Amino Acid Mixtures
- Glass slides
- Silica gel
- Alumina (0.3%)
- Ninhydrin solution
- Iodine
- Butanol
- Glacial acetic acid
- Distilled water.

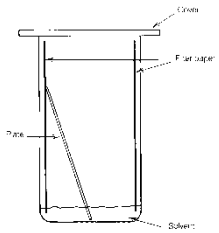
PROCEDURE:

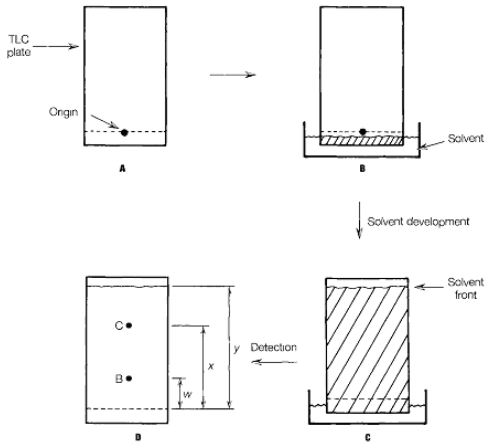
- Mix 3 g of absorbent with 6 ml of deionized water to form a paste.
- Take a glass slide and spread the absorbent smoothly over the surface to form a thin layer using a glass rod.
- Incubate in the oven for 30 min at 100 -120 ºC.
- Mark a line on the TLC plate and load the sample.
- Keep the TLC plate inclined in the solvent chamber and allow stand for 30 min.
- Identify the spot using locating reagent and expose the plates to vapours of Ammonium hydroxide.
- Calculate the R_f value and identify the amino acid by comparing with the standard values.

FORMULA:

$$R_f = \frac{\textbf{Distance traveled by the substance}}{\textbf{Distance traveled by the solvent}}$$

Solvent Chamber





Development of Solvent on TLC plate

Amino Acids	SOLVENT	
	Propanol/Water	Butanol/Water/Glacial Acetic acid

TABULATION:

CALCULATION:

RESULT:

The following amino acids are resolved using TLC and the R_f valve observed to be

9. ESTIMATION OF GLUCOSE BY GOD/POD METHOD

AIM:

To estimate the amount of Glucose present in the given sample by Glucose oxidase method.

PRINCIPLE:

Glucose oxidase catalyses the oxidation of α-D Glucose to D Glucono-1,5 lactone (Gluconic acid) with the formation of hydrogen peroxide.The oxygen liberated from hydrogen peroxide by peroxidase reacts with O- dianisidine and oxidizes it to a red chromophore product.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} H_2O_2 + \text{Gluconic Acid}$$

$$H_2O_2 + \text{O-dianisidine} \xrightarrow{\text{Peroxidase}} \text{Red coloured product}$$

MATERIALS REQUIRED:

Glucose Oxidase Peroxidase Reagent:
Dissolve 25 mg O-dianisidine completely in 1 ml of methanol. Add 49 ml of 0.1 M phosphate buffer (pH 6.5). Then add 5 mg of peroxidase and 5 mg of glucose oxidase to the above-prepared O-dianisidine solution.

Working Standard:
Dissolve 100 mg glucose in 100 ml water. Dilute 10 ml of this sock to 100 ml to obtain the working standard.

PROCEDURE:

- Take 0.2 ml to 1 ml of standard solution in test tube and make up to 1 ml with distilled water.
- Add 1 ml of Glucose oxidase reagent to all the test tubes.
- Incubate all the tubes at 35ºC for 40 minutes.
- Terminate the reaction by the addition of 2 ml of 6N HCl
- Take OD at 540 nm and plot the standard graph
- For test sample solution same procedure is followed as done above.
- Amount of glucose present in the sample can be calculated by using standard graph.

TABULATION:

S.NO	Vol of Working std (ml)	Vol of distilled H_2O (ml)	Vol of distilled GOD/POD reagent (ml)	INCUBATE THE TUBES AT 35°C FOR 40 MINS	OD at 540 nm
1					
2					
3.					
4.					
5.					
6.					
7.					
8.					

CALCULATION:

RESULT:

Amount of Glucose present in the given sample = _____

10. ESTIMATION OF GLUCOSE
BY DINITROSALICYLIC ACID METHOD

AIM:

To estimate the amount of Glucose present in the given sample by Dinitrosalicylic Acid Method

PRINCIPLE:

Reducing sugar treated with Dinitrosalicylic acid, then it is reacted with alkaline Rochelle salt produces dark red colour .The red colour developed is then compared with a set of standards in a colorimeter at 510 nm.

MATERIALS REQUIRED:

- **Dinitrosalicylic Acid (DNS) Reagent:**
  Dissolve 1 gm of DNS in 200 mg of Crystalline Phenol and 50 mg Na_2SO_4 in 100 ml of 1% Soduim Hydroxide.Store this solution in 4°C.
- **40% Rochelle Salt Solution (Potassium Sodium Tartarate)**
- **Standard Glucose solution.(Stock solution)**
  Dissolve 100 mg of Glucose in 100 ml of distilled water.
- **Working Standard: (100 µg/ml)**
  Dilute the 10 ml of stock solution to 100 ml using distilled water.
- **HCl, concentrate (37.3%, 11.9 N) solution**
- **KOH, 5N solution**

PROCEDURE:

- Add 1 drop, or 20 µl, of concentrate HCl solution to 1 ml of the sucrose solution. Allow the hydrolysis to proceed at 90ºC for 5 minutes.
- Add 3 drops, or 0.05 ml, of the 5 N KOH solutions to neutralize the acid, because the DNS method must be applied in an alkaline condition to develop the red brown color, which represents the presence of reducing sugars.
- Take 0.5 ml to 3 ml of standard solution in test tube and make up to 3ml with distilled water
- Add 3 ml of DNS reagent to all the test tubes and Keep in water bath for 10 minutes.
- Add 1 ml of Rochelle salt soution in all the test tubes and cool the content
- Take OD at 510 nm and plot the standard graph
- For test solution same procedure is followed as done above.
- Amount of glucose present in the sample can be calculated by using standard graph.

TABULATION:

S.NO	Vol of Working std (ml)	Vol of distilled H_2O (ml)	Vol of distilled DNS reagent (ml)	HEATING IN WATER BATH FOR 10 MIN	OD at 510 nm	Amount of Glucose (µg)
1						
2						
3.						
4.						
5.						
6.						
7.						
8.						

CALCULATION:

RESULT:

Amount of Glucose present in the given sample = _____

11. ESTIMATION OF RNA BY ORCINOL METHOD

AIM:

To estimate the amount of RNA present in the given unknown solution.

PRINCIPLE:

Acid hydrolysis of RNA releases ribose and this in the presence of strong acid dehydrates to yield furfural. Then orcinol reacts with furfural in the presence of ferric chloride to give a green color. Purine nucleotide is generally more reactive than pyrimidine.

Reagents required:

1. Stock standard solution:

100 mg of RNA was weighed and dissolved in 100 ml of distilled water.

2. Stock standard solution:

10 ml of stock was diluted to 100 ml using distilled water.

3. Orcinol reagent:

It was prepared by dissolving 300 mg of orcinol in 10 ml of ethanol and added 3.5 ml of the above solution to 0.1 % solution of ferric chloride in concentrated sulphuric acid just before use.

PROCEDURE:

- Pipette out 0.2ml to 1.0 ml of RNA solution into different test tubes.
- Take 0.2ml and 0.4ml of unknown solution in a separate test tube.
- Added 3.0 ml of orcinol reagent to each tube and mixed well.
- Kept the tubes in a boiling water bath for 15 minutes after which cooled to room temperature.
- The color developed is measured calorimetrically at 665 nm.

TABULATION:

S.No	Contents	B	S_1	S_2	S_3	S_4	S_5	U_1	U_2
1.	Volume of working standard in (m1)	-	0.2	0.4	0.6	0.8	1.0	-	-
2.	Concentration in μg	-	20	40	60	80	100	-	-
3.	Volume of unknown in (m1)	-	-	-	-	-	-	0.2	0.4
4.	Volume of distilled water in (ml)	3	2.8	2.6	2.4	2.2	2.0	2.8	2.6
5.	Volume of oricinol reagent in ml	3	3	3	3	3	3	3	3
Heated in a boiling water bath for 15 minutes									
6.	Optical density at 595 mm								

CALCULATION:

RESULT:

The given amount of RNA present in the 100 ml of the given unknown solution

=_____

12. Enzyme Kinetics - Michelis Menton parameters

AIM:

To study the Michelis-Menton kinetics of α-amylase enzyme and hence to determine Vmax and Km.

THEORY:

Kinetics of simple enzyme catalyzed reactions is referred to as Michelis Menton kinetics or saturation kinetics. These models are based on data from batch reaction weight constant volume in which the initial substrate (So) and enzyme (Eo) concentration are known. Saturation kinetics can be obtained from a simple reaction scheme that involves a reversible step for enzyme – substrate complex formation and a dissociation step of the ES complex.

Michelis Menton equation for steady state kinetics approximation is

$$V = \frac{Vmax\ S}{Km+S} \quad .....(1)$$

Where V_{max} – velocity of enzyme reaction of saturating substrate concentration

S - Substrate concentration

Km - Michelis Menton constant, measure of affinity of enzyme for substrate

Km = [S] at V = V_{max} /2 from the graph V versus [S].

Lineweaver – Burk plot:

If is the reciprocal of Michelis Menton approximation, A plot of 1/V versus 1/[S] gives slope of Km/V_{max}; and Y-intercept of 1/V_{max} and X intercept of -1/Km.

Eddie – Hofsfee plot:

A plot of V versus s V/[S] results in a line of slope –Km and y-intercept of V_{max} and X intercept of V_{max} /Km.

Hans – Woolf plot:

A plot of [S]/V versus [S] results in a line of slope 1/ V_{max} ; Y intercept of Km/ V_{max} ; X intercept of –Km.

In this experiment, the glucose formed can be estimated calorimetrically using DNS solution

MATERIALS REQUIRED

Starch Solution (1 %), DNS solution, Test tubes, pipette, spectrophotometer and α-amylase.

PROCEDURE

Prepare 10 ml of 1% starch solution (10 mg/ml). Take 11 test tubes and mark them 0, 1 to10, ”0” as blank.

Add aliquots of 0.1 ml to 1 ml of 1% starch solution to test tubes labeled 1 to 10. Don’t add starch solution to the tube’0’

Add varying volumes of distilled water and raise the final volume of the reaction mixture to 2 ml and add 2 ml of distilled water to blank (0).

Add 0.5 ml of α-amylase enzyme solution (10 mg/ml) in each tube including blank.

Mix well and keep the test tubes at 35 – 37 ºC for 10 minutes.

Add 0.5 ml 0.1 N HCl solution to stop the enzymatic digestion process.

Find the amount of glucose formed by α-amylase using DNS method.

Find reaction rate in µmol/ml.sec (V) which is equal to d[P]/dt.

Plot a graph between reaction rate (V) and substrate concentration [S], and (Lineweaver Burk plot) reciprocal of substrate concentration (1/[S]) and reciprocal of reaction rate (1/V).

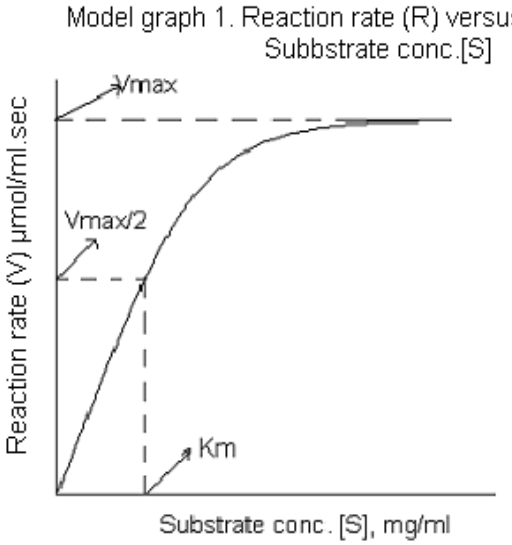
Find the values of Vmax and Km from graphs.

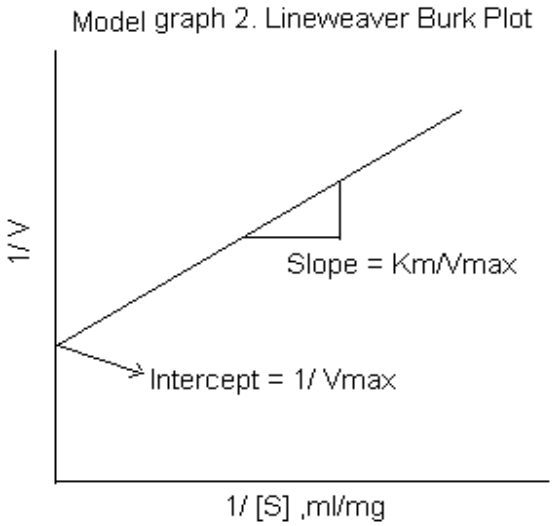
OBSERVATIONS

TABLE 1.

Sl. No.	Vol. of 1 % starch (ml)	Make up 3 ml of Water (ml)	Sub. Conc. (starch) [S] mg/ml	Volume of DNS solution	OD at 540 nm	Conc. of glucose [P] mg/ml	Conc. of glucose [P] µmol/ml	V µmol/ml.sec	1/V	1/[S]

MODEL GRAPH





MODEL CALCULATION

Glucose Conc. in mg/ml**, P** = (OD sample/OD Standard)* Concentration of standard.

P = P (mg/ml) *1000)/180, **µmol/ml**
Vmax = P (µmol/ml) / time in sec, **µmol/ml Sec**

RESULT:

The value of Vmax and Km are,

1. From optical density versus substrate concentration [S] plot

   Km =……mg/ml, and Vmax = …….. µmol/ml.sec

2. From Lineweaver Burk Plot,

Km=…….mg/ml, and Vmax = …….. µmol/ml.sec

DEPARTMENT OF BIOTECHNOLOGY

BT 3411-
CHEMIACAL ENGINEERING LABORATORY MANUAL

REGULATION 2021

II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

GENERAL PRECAUTIONS

1. Safety glasses are to be worn at all times in the laboratory except in the study area adjacent to the lab.

2. No horseplay. This includes running, throwing of any item (ice included), or the squirting of any liquid (water and liquid nitrogen included).

3. Know the location of fire-extinguishers in case of fire.

4. Know the location of sinks or eye washes in case a chemical needs to be washed off.

5. Label all containers that hold gas mixtures corrosive or dangerous materials with a list of the dangerous compounds, so appropriate action can be taken in case of accident.

6. After a project is completed, properly dispose of all used and unneeded chemicals.

7. No equipment should be left running unattended, Switch of the equipments once the experiment is completed.

8. All tools must be returned to their appropriate places before leaving the lab. All glassware should be cleaned before leaving. All materials (tape, chemicals, etc.) should be returned as soon as you are finished with them.

9. Report all accidents to your instructor.

10. If a serious injury occurs, report immediately to the health center after applying first aid.

11. Keeping your work area clean of superfluous material and organized will avoid most accidents (i.e. practice good housekeeping!!).

12. Make sure that all the valves in the experimental setup are closed before carrying out the experiment.

13. Switch off the device once the experiment is completed.

SAFETY IN HANDLING LIQUIDS

1. Always protect your skin from corrosive liquids by wearing Latex gloves which offer some protection

2. Always protect yourself by Lab Coat whenever the experiments are concerned with very hot or cold liquids

3. Protect your eyes with safety glasses or shields when working with liquids that are boiling or being agitated and could splash.

4. Always wear safety glasses when working with liquids under pressure.

5. Always wash your hands thoroughly after handling poisonous or corrosive liquids.

6. Do not drink any liquids from any laboratory glassware or equipment. Do not eat in an area where chemicals are in use.

7. Keep flammable liquids away from open flames and electrical motors.

8. Dispose of all used and un-needed liquids. However, don't pour anything down the sink except water.

9. Always clean-up spills immediately - this especially includes mercury which is poisonous and requires special clean-up procedures. Floor spills of any liquid can cause slips and falls. Mops and brooms are available. Don't dispose of mercury by pouring it down the drain.

10. Use extreme caution when neutralizing strong acids and bases or when dissolving chemicals that produce large amounts of heat when added to a solvent.

11. Never use your mouth as suction for pipeting liquids. Pipette bulbs are available.

12. Clean up broken glass and wrap it in old newspaper before putting it in the waste containers.

13. Keep walkways clear of hoses, extension cords and other tripping hazards.

FLOW THROUGH ORIFICEMETER

EXPT. NO: 1

AIM:

To determine the coefficient of discharge of orificemeter.

THEORY:

Orificemeter is the oldest flow measuring device for pipe flow. It is the simplest form. It consists of thin plate with circular tube clamped between the flanges of the joint in a pipeline such that its plane is perpendicular to the axis of the pipe.

APPARATUS REQUIRED:

Orificemeter, Stopwatch, Collecting tank, Meter scale.

FORMULA:

$$\text{Actual discharge, } Q_{act} = \frac{Ah}{t} \ (m^3/s)$$

Where,

Area of collecting tank, A = 1600×10^{-4} (m^2)

Rise in water level, h = 5×10^{-2} (m)

Time taken for 5 cm rise in water level (s)

$$H = \frac{\Delta h (\rho_m - \rho_f)}{\rho_f}$$

ρ_m = 13600 (kg/m^3)

ρ_f = 1000 (kg/m^3)

H_1 = (m)

$$\text{Theoretical discharge, } Q_{th} = \frac{a_1 a_2 \sqrt{2gH}}{\sqrt{a_1^2 - a_2^2}} \ (m^3/s)$$

Area of main pipe, a_1 = 4.9078 x 10^{-4} (m^2)

Area of throat, a_2 = 1.7668 x 10^{-4} (m^2)

Acceleration due to gravity = 9.81 (m/s^2)

Q_{th1} = (m^3/s)

$$\text{Coefficient of discharge, } C_d = \frac{Q_{act}}{Q_{th}}$$

Q_{act} - Actual discharge

Q_{th} - Theoretical discharge

PROCEDURE:

- The diameter of main pipe and throat are measured.
- The inlet valve is fully opened.
- Outlet valve is opened and the manometer head of both the limits are noted.
- Outlet value of collecting tank is closed and the time taken for 5cm is observed.
- Procedure is to be repeated by gradually increasing the flow rate.

GRAPH:

The coefficient of discharge of orificemeter is found by plotting Q_{act} vs $\sqrt{H}$

MODEL CALCULATION:

$$H = \frac{\Delta h (\rho_m - \rho_f)}{\rho_f}$$

$H_1 =$ (m)

$$\text{Actual discharge, } Q_{act} = \frac{Ah}{t} (m^3/s)$$

$Q_{act} =$ (m^3/s)

$$\text{Theoretical discharge, } Q_{th} = \frac{a_1 a_2 \sqrt{2gH}}{a_1^2 - a_2^2} \ (m^3/s)$$

$Q_{th} =$ (m^3/s)

$$\text{Coefficient of discharge, } C_d = \frac{Q_{act}}{Q_{th}}$$

$C_d =$

Mean C_d = Average of all C_d

RESULT:

Thus the coefficient of discharge of orificemeter was found to be:

Theoretical value:

Graphical value :

S.No	Manometer Reading $\times 10^{-2}$ (m)			$H = \frac{\Delta h (\rho_m - \rho_f)}{\rho_f}$ x 10^{-2} (m)	$\sqrt{H}$	Time taken for 5 cm rise in water level (s)	$Q_{act} = \frac{Ah}{t}$ x 10^{-4} (m^3/s)	$Q_{th} = \frac{a_1 a_2 \sqrt{2gH}}{a_1^2 - a_2^2}$ x 10^{-4} (m^3/s)	$C_d = \frac{Q_{act}}{Q_{th}}$
	h_1	h_2	Δh						

FLOW THROUGH VENTURIMETER

EXPT. NO: 2

AIM:

To determine the coefficient of discharge of venturimeter.

THEORY:

Venturimeter is a device for measuring the rate of pipeline if consist essentially of a converging entrance cone, a throat and a difference or diverging cone. After passing through the throat section the fluid is made to flow through the throat the diverging cone in order to restore the pressure back to the original value as nearby as possible. In order to reduce separation of flow and eddy formation the diverging cone angle are kept small generally between 5 to 6 degree.

APPARATUS REQUIRED:

Venturimeter, Stopwatch, Collecting tank, Meter scale.

FORMULAE:

$$\text{Actual discharge, } Q_{act} = \frac{Ah}{t} (m^3/s)$$

Where,

Area of collecting tank, A $= 1600 \times 10^{-4}$ (m^2)

Rise in water level, h $= 5 \times 10^{-2}$ (m)

Time taken for 5 cm rise in water level (s)

$$H = \frac{\Delta h (\rho_m - \rho_f)}{\rho_f}$$

ρ_m = 13600 (kg/m^3)

ρ_f = 1000 (kg/m^3)

H_1 = (m)

$$\text{Theoretical discharge, } Q_{th} = \frac{a_1 a_2 \sqrt{2gH}}{\sqrt{a_1^2 - a_2^2}} \ (m^3/s)$$

Area of main pipe, a_1 = 4.9078 x 10^{-4} (m^2)

Area of throat, a_2 = 1.7668 x 10^{-4} (m^2)

Acceleration due to gravity = 9.81 (m/s^2)

Q_{th1} = (m^3/s)

$$\text{Coefficient of discharge, } C_d = \frac{Q_{act}}{Q_{th}}$$

Q_{act} - Actual discharge

Q_{th} - Theoretical discharge

PROCEDURE:

- Diameter of inlet and throat was recorded.
- Keeping the outlet value closed. The inlet value is open fully.
- Outer value is collecting tank and time 5cm rise in water in tank is measured.
- The above procedure is repeated.

GRAPH:

The coefficient of discharge of venturimeter is found by plotting Q_{act} vs $\sqrt{H}$.

MODEL CALCULATION:

$$H = \frac{\Delta h(\rho_m - \rho_f)}{\rho_f}$$

H_1 = (m)

$$\text{Actual discharge, } Q_{act} = \frac{Ah}{t} (m^3/s)$$

Q_{act} = (m^3/s)

$$\text{Theoretical discharge, } Q_{th} = \frac{a_1 a_2 \sqrt{2gH}}{a_1^2 - a_2^2} \ (m^3/s)$$

Q_{th} = (m^3/s)

$$\text{Coefficient of discharge, } C_d = \frac{Q_{act}}{Q_{th}}$$

C_d=

Mean C_d = Average of all C_d

RESULT:

The coefficient of discharge of venturimeter was found to be:

Theoretical =

Graphical =

S.No	Manometer Reading $\times 10^{-2}$ (m)			$H = \frac{\Delta h (\rho_m - \rho_f)}{\rho_f}$ x 10^{-2} (m)	$\sqrt{H}$	Time taken for 5 cm rise in water level (s)	$Q_{act} = \frac{Ah}{t}$ x 10^{-4} (m^3/s)	$Q_{th} = \frac{a_1 a_2 \sqrt{2gH}}{a_1^2 - a_2^2}$ x 10^{-4} (m^3/s)	$C_d = \frac{Q_{act}}{Q_{th}}$
	h_1	h_2	Δh						

EXPERIMENTAL STUDY OF ROTAMETER

EXPT. NO: 3

AIM:

To determine the percentage error between the rotameter and actual discharge by performing an experiment on rotameter.

MATERIALS REQUIRED:

Rotameter fitted in pipeline and a collecting tank fitted with piezometer.

THEORY:

A Rotameter is a variable art flow meter. It consists of vertical tube having a tapered base in which a float can move up and down. As the upward rate of fluid in tube increases or decreases, the float results in change of height of float while pressure difference remains constant. The level of float then becomes a measurement of flow rate. The height of the total float is to read out from the graduation of the pipe.

WORKING PRINCIPLE:

When the rotameter is mounted vertically and fluid flow through the tapered tube .The float rise up to a level above the upward thrust of the fluid passing through the annular space between the float and the wall of the tube plus the buoyancy of the float in the fluid will be balanced by downward gravitational roll on the float .Thus the height to which the float will rice will give an indication of flow rate.

Rotameter is generally stainless steel bore silicate glass and perplex plastic tube are commonly used for comparatively low pressure and moderate temperature useful for hot water steam, organic gases or solvents ,fluorescent, alkaline etc. It can measure flow rises from a cubic millimeter to 10 cubic millimeter per minute.

FORMULA:

$$Q = \frac{Ah}{t} \ (m^3/s)$$

Where,

Area of collecting tank, A $= 900 \times 10^{-4} \ (m^2)$

Height rise in water level, h $= 5 \times 10^{-2} \ (m)$

Time taken for rise in water level (s)

PROCEDURE:

- Open the inlet valve of pipeline where the rotameter is fixed.
- Note down the discharge from rotameter.
- Compare the discharge valve with the wattmeter reading and actual discharge with collecting fitted with piezometer.
- Inlet valve opening is increased further and the discharge is compared.

TABLE 1

S.No	Rotameter (l/min)	Time taken for 5 cm rise in water level (s)	$Q=\frac{Ah}{t}$ (m^3/s)	Measured Discharge (l/min)

GRAPH:

Actual discharge vs Theoretical discharge was plotted.

MODEL CALCULATION:

Actual discharge, $Q_{act} = \frac{Ah}{t}$ (m^3/s)

Measured discharge, $Q_{M.D} = Q_{act} \times 1000 \times 60$ (l/min)

RESULT:

Thus the experimental study was carried out by rotameter and the discharge was measured.

PRESSURE DROP IN FLOW THROUGH PIPES

EXPT. NO: 4

AIM:

- To find the loss of head between two points in the flow of fluid through annular pipe using a given manometer.
- To calculate the mean velocity.
- Compute the coefficient of friction by applying correct formula.

APPARATUS REQUIRED:

Annular Pipe Apparatus Setup, Stop Watch, Meter Side.

FORMULAE:

$$h_f = \frac{\Delta h (\rho_m - \rho_f)}{\rho_f}$$

where

ρ_m — density of manometer fluid = 13600 kg/m^3

ρ_f — density of water = 1000 kg/m^3

$$Q = \frac{Ah}{t} \quad (m^3/s)$$

where

A — Area of collecting tank (1600 x 10^{-4} m^2)

h — Rise in water level (5 x 10^{-2} m)

t — Time taken for rise in water level (s)

$$v = \frac{Q}{A} \text{ (m/s)}$$

where

Q — Actual discharge (m^3/s)

A — Area of pipe = $\prod/4$ (D_i^2 - d_o^2) (m^2)

$$\text{Coefficient of Friction (f)} = \frac{2gD_h h_f}{4Lv^2}$$

where

D_h — hydraulic diameter = $\frac{\text{Area of pipe}}{\text{Wetted perimeter}} = \frac{\frac{\Pi}{4}(D_i^2 - d_0^2)}{\frac{\Pi}{4}(D_i + d_0)} = (D_i - d_0)$

h_f — Difference in pressure

L — length of pipe = 2m

v — Velocity of fluid

$$\text{Reynold's Number } N_{Re} = \frac{D_H v \rho}{\mu}$$

where

D_h — hydraulic diameter = D_i - d_o

ρ — density of fluid = 1000 kg/m^3

v — velocity of fluid

μ — viscosity of fluid = 0.84 x 10^{-3} kg/m^3

PROCEDURE:

- Open the inlet valve to allow the flow through one of the annular pipe.
- Open the inlet and outlet valve simultaneously into the limits of the manometer.
- Open the drain cork all the bottom of the manometer simultaneously and drain air.
- Note the manometer reading both sides.
- Collect water in collecting tank for entry rise of 5cm level of water and note the time (t) in seconds.
- Repeat the same procedure for different flow rate.

MODEL CALCULATION:

Pipe - I calculation

- Actual discharge, $Q_1 = \frac{Ah}{t}$ (m^3/s)

= (m^3/s)

- $v = \frac{Q}{A}$ (m/s)

$A = \frac{\Pi}{4}(D_i^2 - d_0^2)$ (m^2)

= (m/s)

- Coefficient of Friction (f) $= \frac{2gD_h h_f}{4Lv^2}$

$h_f = \frac{\Delta h(\rho_m - \rho_f)}{\rho_f}$

$D_h = (D_i - d_0)$

f =

- Reynold's Number $N_{Re} = \frac{D_H v \rho}{\mu}$

N_{Re} =

Pipe - II calculation

- Actual discharge, $Q_2 = \frac{Ah}{t}$ (m^3/s)

= (m^3/s)

- $v = \frac{Q}{A}$ (m/s)

$A = \frac{\Pi}{4}(D_i^2 - d_0^2)$ (m^2)

= (m/s)

- Coefficient of Friction (f) $= \frac{2gD_h h_f}{4Lv^2}$

$h_f = \frac{\Delta h(\rho_m - \rho_f)}{\rho_f}$

$D_h = (D_i - d_0)$

f =

- Reynold's Number $N_{Re} = \frac{D_H v \rho}{\mu}$

N_{Re} =

TABULATION:

Manometer reading		Δh =	Rise in	Time taken for rise	$Q = \frac{Ah}{t}$	$v = \frac{Q}{A}$	v^2	$f = \frac{2gD_h h_f}{4Lv^2}$	$N_{Re} = \frac{D_H v \rho}{\mu}$
$h_1 \times 10^{-2}$ (m)	$h_2 \times 10^{-2}$ (m)	$(h_1-h_2) \times 10^{-2}$ (m)	water level $\times 10^{-2}$ (m)	in water level (s)	$\times 10^{-4}$ (m^3/s)	(m/s)	(m/s)		

RESULT:

Loss of head between two points in the flow of fluids in annular pipe is calculated. The friction factor chart is drawn between friction factor and Reynold's number of pipe I and pipe II.

PRESSURE DROP IN FLOW THROUGH PACKED COLUMN

EXPT. NO: 5

AIM:

To determine the friction factor for the flow of liquid through a packed bed using Ergun`s equation.

APPARATUS REQUIRED:

Experimental setup, Spring balance, Bucket and Stopwatch.

THEORY:

Packed bed towers are used for continuous count of liquid and gas in both counter current and current flow.

These are vertical columns which have been filled with packing materials to increase the surface area for the contact between the two phases. There are two major modes of packing, random and regular.

Random packing consists in simple dumping the packing material into tower during installation and allowed to fall at random. Regular packing in general consists of metal crate like devices installed in the pipe, which breaks each stream into a large no. of small streams.

If the given experimental setup has saddler as packing material then one has to consider its equivalent diameter and sphericity. The equivalent diameter of a non-spherical particle is defined as the surface volume ratio for sphere of diameter (d_p) divided by surface – volume ratio for the particle whose nominal size as d_p.

As the liquid passes the bed it does through the empty spaces (voids) in bed, the voids from continuous channels throughout the bed. These channels need not be of same length and diameter, while the flow may be laminar through some channels are turbulent through others Vs the velocity in channel is difficult to estimate; therefore it is substituted by the velocity of liquid `v` as if it is passing through empty column into porosity of packed bed.

PROCEDURE:

- The U-Tube manometer is connected between the tapings on the packed bed.
- By opening the gate valve the water is allowed to flow through the packed bed at minimum flow rate.
- Care is to be taken to see that there are no air bubbles in the U-Tube manometer and if they are present they should be removed.
- After steady state is reached the manometer readings are noted and the flow rate of water is noted by collecting water for a specified known amount of time using the stop watch and weighing the same.
- Such readings are taken for different flow rates by manipulating the gate valve and are tabulated.

FORMULA:

Difference in manometer readings

$$R_m = (h_1 - h_2) \times 10^{-2} \text{ (m)}$$

Pressure drop

$$\Delta P = R_m \frac{(\rho_m - \rho_f)}{\rho_f}$$

Velocity

$$v_o = \frac{Q}{A}$$

Particle, Reynold`s number

$$N_{Re,p} = \frac{d_p v_o \phi_s \rho}{\mu}$$

f (experimental)

$$f_{exp} = \frac{\Delta P \varepsilon^2 \phi_s d_p}{\rho h (1-\varepsilon) v_o^2}$$

f (theoretical)

$$f_{th} = \frac{150(1-\varepsilon)}{N_{Re,p}} + 1.75$$

MODEL CALCULATION:

- $R_m = (h_1 - h_2) \times 10^{-2}$ (m)

  R_m =_____m

- $\Delta P = R_m \frac{(\rho_m - \rho_f)}{\rho_f}$

  ΔP_1 =_____m

- $Q_1 = \frac{A_o h}{t}$

  Q_1 =_____m^3/s

- $v_o = \frac{Q}{A}$

  v_o =_____m/s

- $N_{Re,p} = \frac{d_p v_o \phi_s \rho}{\mu}$

  $N_{Re,P}$ =_____

- $f_{exp} = \frac{\Delta P \varepsilon^2 \phi_s d_p}{\rho h (1-\varepsilon) v_o^2}$

  f_{exp} =_____

- $f_{th} = \frac{150(1-\varepsilon)}{N_{Re,p}} + 1.75$

  f_{th} =_____

SPECIFICATION

Diameter of column D =_____m
Length of column L =_____m
Diameter of particle D_p =_____m
Sphericity of particle $\emptyset s$ =_____
Viscosity of fluid μ = _____Kg/m.s
Density of fluid ρ =_____ Kg/m^3
Pore volume V_P =_____m^3

C.S of column A = $\frac{\Pi D^2}{4}$ =_____(m^2)

Fraction voltage in bed ε = $\frac{\text{pore volume}}{\text{total volume}}$ = _____

GRAPH:

Graphs were plotted against Reynolds no vs experimental and theoretical friction factors.

S.No	Manometric Readings		R_m x 10^{-2} (m)	Time Taken For 5cm Rise In Water Level (min)	$Q = \frac{A\ h}{t}$ x $10^{-5} m^3/s$	Pressure Drop (ΔP) N/m^2	Velocity V_o (m/s)	Particle Reynolds No. $N_{Re,P}$	Experimental Friction Factor F_{exp}	Theoretical Friction Factor F_{th}
	h_1 (cm)	h_2 (cm)								

RESULT:

Thus the graph of Reynold`s no. vs friction factor was plotted and friction factor was found to be _____

PRESSURE DROP IN FLOW THROUGH FLUIDIZED BED

EXPT. NO: 6

AIM:

To determine the relationship between the modified friction number to modified Reynolds number and to find the variation in porosity with the Reynolds number of the given fluidized bed apparatus.

THEROY:

The liquid or gas flowing at low velocities through porous bed as particles are poured in bed and not causing the particles to move. If the fluid velocity is increased steadily however, appoint is eventually reached at which the particle is no longer stationary but bed is suspended or fluidized, behaviour like a dense liquid at low, velocity. The pressure drop becomes constant on the bed starts to expand or the bed starts to be fluidized. The velocity is said to be minimum fluidization velocity:

FORMULA:

Pressure drop

$$\Delta \boldsymbol{P} = \frac{\Delta \boldsymbol{h}(\boldsymbol{\rho_m} - \boldsymbol{\rho_f})}{\boldsymbol{\rho_f}}$$

ρ_m : Density of mercury (13600 kg/m^3)

ρ_f: Density of water (1000 kg/m^3)

Actual discharge:

$$\mathbf{Q} = \frac{\boldsymbol{Ah}}{\boldsymbol{t}} \left(\frac{\boldsymbol{m^3}}{\boldsymbol{s}}\right)$$

Where,

A = area if collecting tank (m^2)

H = height of rise in water level (m)

T = time taken for rise in water level (s)

Superficial velocity:

$$\mathbf{V_o} = \frac{\boldsymbol{Q}}{\boldsymbol{AC}} \left(\frac{\boldsymbol{m}}{\boldsymbol{s}}\right)$$

Where

Q = Actual discharge ($\frac{m^3}{s}$)

Ac = Area of column = $\frac{\pi}{4} Dc^2 (m^2)$

Reynolds number:

$$\boldsymbol{N_{Re} = \frac{D_p V_\circ \rho_f}{\mu}}$$

Where

D = diameter of the particles(m)

ρ_f = density of fluid ($\frac{Kg}{m^3}$)

$V_\circ$= superfacial velocity ($\frac{m}{s}$)

μ= viscosity of the fluid ($\frac{Kg}{ms}$)

$$\boldsymbol{1\text{-}\varepsilon = \frac{L_H}{L_f}}$$

$$\boldsymbol{N_{Rem} = \frac{D_p V_\circ \rho_f}{\mu(1-\varepsilon)}}$$

$$\boldsymbol{F_m = \frac{\Delta P\ g\ D_p}{V_\circ^2 L_f} \times \frac{\varepsilon^3}{1-\varepsilon}}$$

PROCEDURE:

- A known weight and size of particle was fed into a transparent column.
- The water inlet was opened and the flow rate was measured by collecting a know volume of water in a definite time.
- The height of the bed and the pressure across the bed are measured in term of mercury column.
- The reading are noted by increasing static bed height.
- The boiling bed stage was continued for the transition range at the exits of fluidization.

- From the date, the fluid Reynolds no (N_{RE}). Modified Reynolds no ($N_{Re,m}$) , modified friction factor and co-relation parameters are calculated.

RESULT:

The fluidization experiment were conducted with a given column and the characteristics of flow were analysed by plotting graph between

- Porosity vs Reynolds number.
- Modified Reynolds number (N_{Rem}) vs modified friction factor.

PERFORMANCE STUDY OF CENTRIFUNGAL PUMP

EXPT. NO: 7

AIM:

To determine the efficiency of a centrifugal pump.

Apparatus required:

Centrifugal pump setup, meter scale, stopwatch.

Formula:

Efficiency (η):

$$\eta = \frac{output\ power}{input\ power} \times 100$$

Output power (P_o):

$$P_o = \frac{W \times Q_{act} \times H_T}{1000}\ (\text{Kw})$$

W : specified weight of water = 9810N

Q_{act} : actual discharge ($\frac{m^3}{s}$)

H_T : Total heat = Pressure heat (G) + Vaccum heat (V) + Datum heat (Z)

Q_{act}:

$$Q_{act} = \frac{Ah}{t}\ (\frac{m^3}{s})$$

A : Area of tank (m^2)

h : Height of water rise in collecting tank (m)

t : time taken for 10 cm rise in collecting tank (s)

Input Power (P_i):

$$P_i = \frac{n \times 3600 \times n_m}{NT}$$

N : Energy meter constant

n : Number of revolution of energy unit

n_m : predetermined efficiency

T : Time taken for 'n' revolution

THEORY :

Centrifugal pump works on the principle that a fluid mass is given a force, it is thrown outward radially. The main parts of the centrifugal pump include suction eye, vanes, Impeller, casing, suction pipe, discharge pipe. The suction pipe is connected with strains thus restricting any foreign particles entering into the pump. Generally, as the length of the suction pipe is less, the friction loss also will be less.

The other end of the suction pipe is connected to the suction eye of the pump. The suction eye is the first pipe is connected to the above level where the fluid has to be delivered.

Since the length of the discharge pipe is long the friction loss will also be higher at the discharge. The casing of the pump is designed of gradually increasing cross sectional area. It means that the velocity of the fluid decreased in order to attain pressure energy. So the casting does the work of reducing the velocity of the fluid.

An increase in the fluid pressure from the pump inlet to its outlet is created when the pump is in operation. This pressure difference drives the fluid through the system (or) plant. The centrifugal pump creates an increase in pressure by transferring mechanical energy from the motor to the fluid through the rotating impeller. The fluid flows from the inlet to the impeller centre and out along its blades. The centrifugal force hereby increase the fluid flows the inlet to the impeller centre and out along its blades. The fluids velocity and consequently the kinetic energy transfer to the pressure energy (Thermodynamic energy).

PROCEDURE:

1. Open the outlet valve until it is fully opened.
2. Primary of centrifugal pump.
3. Switch on the centrifugal pump.
4. By closing the outlet valve of the delivering pipe, the required pressure in the presence gauge is set.
5. The suction pressure in the vacuum gauge reading is noted.

6. The time taken for five revolution of disc in the energy meter is observed.
7. Energy meter constant from energy metre gauge is noted.
8. Repeat the step 4 to 6 for a required number number of time.
9. Area of collecting tank is measured.

RESULT:

The efficiency of centrifugal pump has been determined by conducting the performance pump.

LEAF FILTER

EXPT. No: 9

AIM:

To find the filter medium resistance and specific cake resistance for given $CaCO_3$ slurry.

MATERIALS REQUIRED:

Leaf filter apparatus setup, 3% by weight of $CaCO_3$, Stop watch.

PRINCIPLE:

One method of supporting the filter medium foils either vacuum or positive filtration is found in a leaf filter

The frame is made up of heavy screen suspended from an outlet pipe, and enclosed in heavy filter cloths. When a leaf is submerged in liquid magma and maximum vacuum supplied to the outlet pipe. The liquid is drawn through the cloth, while the solid adhere to the outside of the leaf. In addition to the diminished pressure maintained with filter leaf, a positive pressure may be exerted upon the liquid in which the mass is submerged on the leaf.

The loaded leaf with vacuum still may be transferred second vessel filled with water and enough water is drawn through the wash. The precipitate as the magma may be drawn through the wash. The precipitate or magma may be drawn from the first vessel and replaced with water without moving the leaf. The charge of solids adhering to forcing air or water through the leaf is in the reverse direction. It is then again ready to be immersed in the filtration tank.

PROCEDURE:

- Prepare 3% by weight of $CaCO_3$ in the given vessel
- Pour the slurry into the tank and continuously agitate slowly by an agitator at low speed
- Apply approximately 15 mm of Hg suction to the outlet pipe by adjusting the valve
- Start the stop watch when the filtrate is at 0 level in receiver
- After complete filtration, release the vacuum and switch off the suction pipe collect some of the filter cake in the watch glass
- Weight the wet filter cake dry it and weigh the dry cake
- Carry out the above, steps at an increased level of suction at 250mm of Hg.

TABLE 1

S.No	Height (m)	Volume x 10^{-4} (m^3)	$\overline{V}$ x 10^{-4} (m^3)	ΔV x 10^{-4} (m^3)	At 150mm of Hg			At 250mm of Hg		
					t (s)	Δt (s)	$\Delta t/\Delta v$ x 10^4 ($s/m^{3)}$	t (s)	Δt (s)	$\Delta t/\Delta v$ x 10^4 (s/m^3)

MODEL CALCULATION:

At 150mm of Hg:

- Volume:

  Radius of cylinder = ____(m)

  Volume of cylinder (V) = $\pi r^2 h$ (m^3)

  V = (m^3)

- $\frac{\Delta t}{\Delta v}$

Where $\Delta v = v_2 - v_1$ = _____

$\frac{\Delta t}{\Delta v}$ = (s/m^3)

- m_f = (Weight of $CaCo_3$ cake + watch glass) – (weight of empty watch glass)

Where:

Empty watch glass weight =

Wet weight =

Dry weight =

m_f =

- m_c = (weight of dry cake + watch glass) – (weight of empty watch glass)

m_c =

$$C_s = \frac{\text{Mass of } CaCO_3}{\frac{\text{Mass of } CaCO_3}{\text{Density of } CaCO_3} + \frac{\text{Mass of } H_2O}{\text{Density of } H_2O}}$$

C_s =

$$C = \frac{C_s}{\left[1 - \left(\frac{m_f}{m_c} - 1\right)\frac{C_s}{\rho}\right]}$$

C =

- Area**:**

  Diameter of filter tank (D) = _____

  Area of filter tank (A) = $\frac{\Pi}{4} D^2 (m^2)$

  Diameter of the leaf filter (d) = _____

  Area of filter ($A^{'}$) = $\frac{\Pi}{4} d^2 (m^2)$

- Specific cake resistance:

$$\alpha = \frac{K_p \Delta P A^2}{C\mu}$$

α =

- Filter medium resistance:

$$R_m = \frac{\beta \Delta P A'}{\mu}$$

R_m =

At 250mm of Hg:

- Volume:

  Radius of cylinder = ____(m)

  Volume of cylinder (V) = $\pi r^2 h$ (m^3)

  V = (m^3)

- $\frac{\Delta t}{\Delta v}$

Where $\Delta v = v_2 - v_1$ = _____

$\frac{\Delta t}{\Delta v}$ = (s/m^3)

- m_f = (Weight of $CaCo_3$ cake + watch glass) – (weight of empty watch glass)

Where:

Empty watch glass weight =

Wet weight =

Dry weight =

m_f =

- m_c = (weight of dry cake + watch glass) – (weight of empty watch glass)

m_c =

$$C_s = \frac{\text{Mass of } CaCO_3}{\frac{\text{Mass of } CaCO_3}{\text{Density of } CaCO_3} + \frac{\text{Mass of } H_2O}{\text{Density of } H_2O}}$$

C_s =

$$C = \frac{C_s}{\left[1 - \left(\frac{m_f}{m_c} - 1\right)\frac{C_s}{\rho}\right]}$$

C =

- Area**:**

Diameter of filter tank (D) = _____

Area of filter tank (A) = $\frac{\Pi}{4} D^2 (m^2)$

Diameter of the leaf filter (d) = _____

Area of filter (A') = $\frac{\Pi}{4} d^2 (m^2)$

- Specific cake resistance:

$$\alpha = \frac{K_p \Delta P A^2}{C\mu}$$

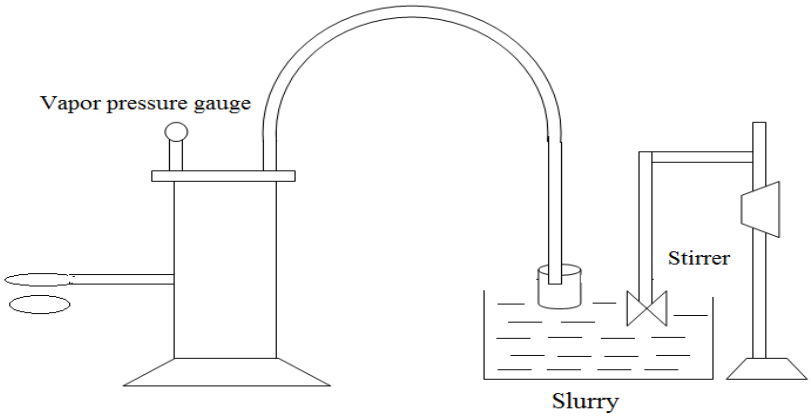
α =

- Filter medium resistance:

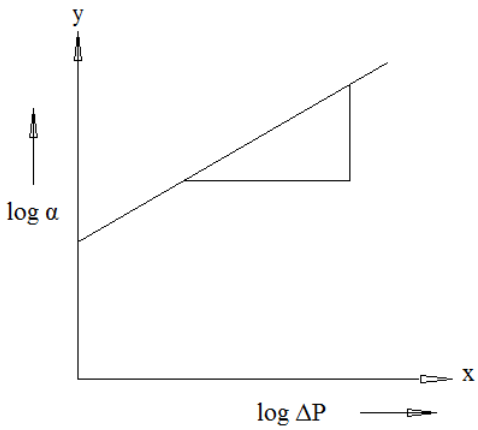
$$R_m = \frac{\beta \Delta P A'}{\mu}$$

R_m =

BLOCK DIAGRAM



MODEL GRAPH:



RESULT:

For 150mm of Hg of pressure

- Specific cake resistance, α =_____
- Filter medium resistance, R_m = _____

For 250mm of Hg of pressure

- Specific cake resistance, α =_____
- Filter medium resistance, R_m = _____

COUNTER CURRENT FLOW IN DOUBLE PIPE HEAT EXCHANGER

EXPT. NO: 10

AIM:

To study and compare the temperature distribution individual heat transfer coefficient and overall heat transfer coefficient for counter current flow pattern in double pipe heat exchanger.

MATERIALS REQUIRED:

Double pipe heat exchanger, Thermocouple, Measuring flask, Thermometer and Stop watch.

PROCEDURE:

- Thermocouple is kept in position of the flow of both hot and cold water
- Electric heater is switched on & flow rates are adjusted on hot water side and the cold water side
- Flow rate is kept constant till steady state is reached
- The temperature of hot and cold fluids in the inlet and outlet are measured

FORMULA:

Heat gained by cold water, $Q_c = \dot{m}_c C_{p_c} \Delta T_c$

$= \dot{m}_c C_{p_c} (T_{c_o} - T_{c_i})$

Heat lost by hot water, $Q_h = \dot{m}_h C_{p_h} \Delta T_h$

$= \dot{m}_h C_{p_h} (T_{h_i} - T_{h_o})$

Heat lost by hot water = heat gained by the cold water

$$Q_h = Q_c$$

$$\dot{m}_h C_{p_h} (T_{h_i} - T_{h_o}) = \dot{m}_c C_{p_c} (T_{c_o} - T_{c_i})$$

$$\dot{m}_h = \frac{\dot{m}_c C_{p_c} (T_{c_o} - T_{c_i})}{C_{p_h} (T_{h_i} - T_{h_o})}$$

Log mean temperature difference (LMTD)

$$LMTD = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)}$$

$$\Delta T_1 = T_{h_i} - T_{c_o}$$

$$\Delta T_2 = T_{h_o} - T_{c_i}$$

Overall heat transfer coefficient

$$U = \frac{Q}{A_o LMTD}$$

A_o = Area of outlet tube (m^2)

Effectiveness

$$\varepsilon = \frac{q}{q_{max}}$$

$$q_{max} = C_{min}(T_{h_i} - T_{c_i})$$

$$q = \dot{m}_c C_{p_c} \Delta T_c = \dot{m}_h C_{p_h} \Delta T_h$$

Heat capacity rate

$$C_{min} = \dot{m} C_p$$

If $(\dot{m}C_p)_h < (\dot{m}C_p)_c$

$$\varepsilon = \frac{\dot{m}_h C_{p_h} \Delta T_h}{C_{min}(T_{h_i} - T_{c_i})} \quad C_{min} = \dot{m}_h C_{p_h}$$

If $(\dot{m}C_p)_c < (\dot{m}C_p)_h$

$$\varepsilon = \frac{\dot{m}_c C_{p_c} \Delta T_c}{C_{min}(T_{h_i} - T_{c_i})} \quad C_{min} = \dot{m}_c C_{p_c}$$

Hot side calculation:

- Area = $\frac{\Pi d_i^{\,2}}{4}$
- Velocity = $\frac{\dot{m}_h}{\rho A_h}$
- Reynolds number = $\frac{d_i v \rho}{\mu}$
- To find h:

$$Nu = 0.023\, Re^{0.8}\, Pr^{0.4}$$

$$h = \frac{Nu \times k}{d_i}$$

Cold side calculation:

- Equivalent diameter $D_e = \frac{D_i^2 - d_o^2}{d_o}$
- Velocity = $\frac{\dot{m}_c}{\rho A_c}$
- Area = $\frac{\Pi (D_i^2 - d_o^2)}{4}$
- Reynolds number = $\frac{D_e v \rho}{\mu}$
- To find h

$$Nu = 0.023\ Re^{0.8}\ Pr^{0.3}$$

$$h = \frac{Nu \times k}{D_e}$$

OBSERVATION:

TABLE-I

Flow rate	Cold water temperature		Hot water temperature		Volumetric flow rate of hot water (l/s)	Volumetric flow rate of cold water (l/s)
	T_{C_i} (^oC)	T_{C_o} (^oC)	T_{h_i} (^oC)	T_{h_o} (^oC)		

PHYSICAL PROPERTIES OF COLD WATER (TABLE – II)

Cold mean temperature (˚C)	Density (ρ) (Kg/m^3)	Viscosity (μ) x 10^{-3} (kg/ms)	Specific heat capacity (C_p) x 10^3 (J/kg K)	Thermal conductivity (W/m K)	Prandtl no

PHYSICAL PROPERTIES OF HOT WATER (TABLE – III)

Hot mean temperature (˚C)	Density (ρ) (Kg/m^3)	Viscosity (μ) x 10^{-3} (kg/ms)	Specific heat capacity (C_p) x 10^3 (J/kg K)	Thermal conductivity (W/m K)	Prandtl no

Specifications

Inner diameter of inner tube; di = 10.5×10^{-3} m

Outer diameter of inner tube; d_0 = 12.5×10^{-3} m

Inner diameter of outer tube; D_i = 27.5×10^{-3} m

Outer diameter of outer tube; D_0 = 33.5×10^{-3} m

Length of tube, l = 1.65 m

$$LMTD = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)}$$

Calculation of LMTD:

Model calculation:

$$\Delta T_{LMTD} = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)}$$

ΔT_1 =

ΔT_2 =

ΔT_{LMTD} =

HOT SIDE CALCULATION:

Model calculation:

Area A = $\frac{\Pi d_i^{\,2}}{4}$

Velocity v = $\frac{\dot{m}_h}{\rho A_h}$

Reynolds number = $\frac{d_i v \rho}{\mu}$

N_{Re} =

Prandtl number $= \frac{C_p \mu}{k}$

N_{Pr} =

Nusselt number: Dittus Boelter equation,

$Nu = 0.023\ Re^{0.8}\ Pr^{0.4}$

Nu_1 = _____

To find h:

$Nu = \frac{h d_i}{k}$

$h = \frac{Nu \times k}{d_i}$

h_1 = _____

COLD SIDE CALCULATION:

Model calculation:

Area $= \frac{\Pi (D_i^{\,2} - d_o^{\,2})}{4}$

Velocity, v $= \frac{\dot{m}_c}{\rho A_c}$

Reynolds number $= \frac{D_e v \rho}{\mu}$ where $D_e = \frac{D_i^{\,2} - d_o^{\,2}}{d_o}$

Nusselt number: Dittus Boelter equation,

$Nu = 0.023\ Re^{0.8}\ Pr^{0.3}$

Nu_1 = _____

To find h:

$Nu = \frac{h D_e}{k}$

$h = \frac{Nu \times k}{D_e}$

h_1 = _____

Effectiveness

Hot side

$$q=\dot{m}_h C_{p_h}(T_{h_i}-T_{h_o})$$

$$C_{min}=\dot{m}_h C_{p_h}$$

Cold side

$$q=\dot{m}_c C_{p_c}(T_{c_o}-T_{c_i})$$

$$C_{min}=\dot{m}_c C_{p_c}$$

If $(C_{min})_c < (C_{min})_h$

$$\varepsilon=\frac{\dot{m}_c C_{p_c}\Delta T_c}{\dot{m}_c C_{p_c}(T_{h_i}-T_{c_i})}$$

If $(C_{min})_h < (C_{min})_c$

$$\varepsilon=\frac{\dot{m}_h C_{p_h}\Delta T_h}{\dot{m}_h C_{p_h}(T_{h_i}-T_{c_i})}$$

Overall heat transfer coefficient:

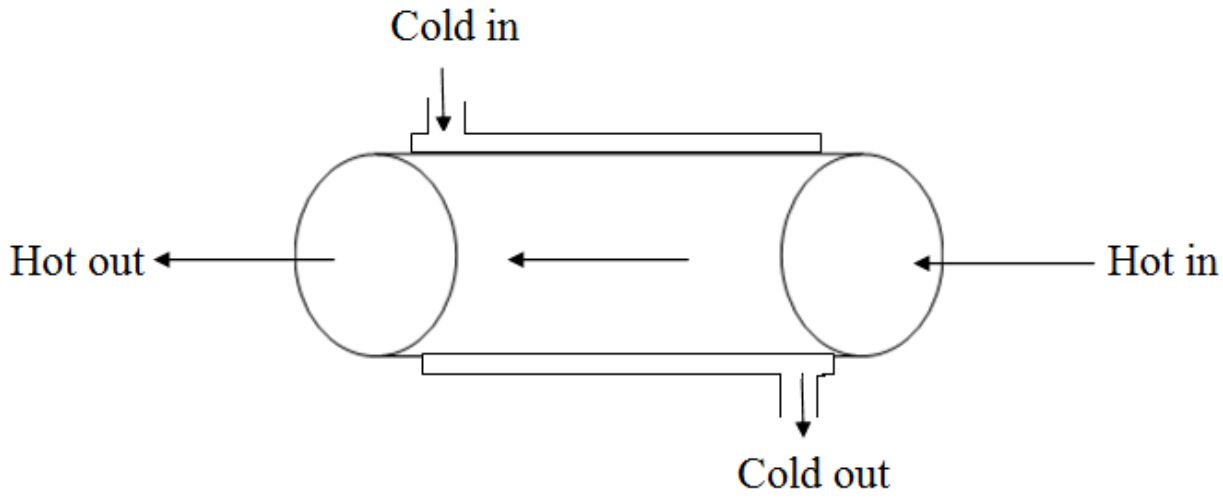
$$U=\frac{Q}{A_o LMTD}\ (W/m^2\ K)$$

A_o = Area of outlet tube

$A_0 = \pi d_i l = \pi \times 10.5 \times 10^{-3} \times 1.65 = 0.054\ m^2$

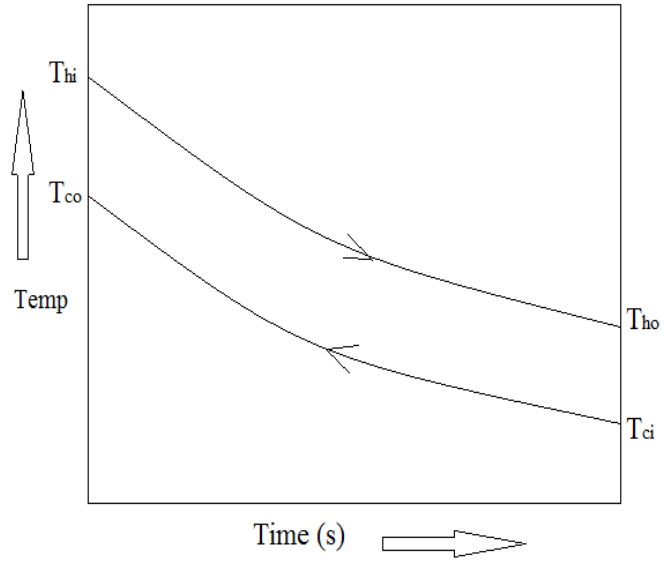
U_1 = (W/m^2 K)

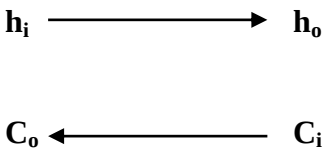
MODEL GRAPH



Temperature Profile

Counter Flow





RESULT:

The individual and overall heat transfer coefficient for counter flow pipe heat exchanger is studied

Mass flow rate of cold water	U ($W/m^2 K$)	h_h ($W/m^2 K$)	h_c ($W/m^2 K$)	Effectiveness for counter flow

PARALLEL FLOW IN DOUBLE PIPE HEAT EXCHANGER

EXPT. NO: 10

AIM:

To study and compare the temperature distillation of individual heat transfer coefficient and over all heat transfer coefficient for parallel flow pattern in double pipe heat exchanger

MATERIALS REQUIRED:

Double pipe heat exchanger, Thermocouple, Measuring flask, Thermometer and Stop watch.

PROCEDURE:

- Thermocouple is kept in position of the flow of both hot and cold water
- Electric heater is switched on, flow rates are adjusted on hot water side.
- Flow rate is kept constant till steady state is reached
- The temperature of hot and cold fluids are measured

FORMULA:

Heat gained by cold water, Q_c $= \dot{m}_c C_{p_c} \Delta T_c$

$= \dot{m}_c C_{p_c} (T_{c_o} - T_{c_i})$

Heat lost by hot water, Q_h $= \dot{m}_h C_{p_h} \Delta T_h$

$= \dot{m}_h C_{p_h} (T_{h_i} - T_{h_o})$

Heat lost by hot water = heat gained by the cold water

$$Q_h = Q_c$$

$$\dot{m}_h C_{p_h} (T_{h_i} - T_{h_o}) = \dot{m}_c C_{p_c} (T_{c_o} - T_{c_i})$$

$$\dot{m}_h = \frac{\dot{m}_c C_{p_c} (T_{c_o} - T_{c_i})}{C_{p_h} (T_{h_i} - T_{h_o})}$$

Log mean temperature difference (LMTD)

$$LMTD = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)}$$

$$\Delta T_1 = T_{h_o} - T_{c_o}$$

$$\Delta T_2 = T_{h_i} - T_{ci}$$

Overall heat transfer coefficient

$$U = \frac{Q}{A_o LMTD}$$

A_o = Area of outlet tube (m^2)

Effectiveness

$$\varepsilon = \frac{q}{q_{max}}$$

$$q_{max} = C_{min}(T_{h_i} - T_{ci})$$

$$q = \dot{m}_c C_{p_c} \Delta T_c = \dot{m}_h C_{p_h} \Delta T_h$$

Heat capacity rate

$$C_{min} = \dot{m} C_p$$

If $(\dot{m}C_p)_h < (\dot{m}C_p)_c$

$$\varepsilon = \frac{\dot{m}_h C_{ph} \Delta T_h}{C_{min}(T_{h_i} - T_{ci})} \quad C_{min} = \dot{m}_h C_{p_h}$$

If $(\dot{m}C_p)_c < (\dot{m}C_p)_h$

$$\varepsilon = \frac{\dot{m}_c C_{p_c} \Delta T_c}{C_{min}(T_{h_i} - T_{ci})} \quad C_{min} = \dot{m}_c C_{p_c}$$

OBSERVATION:

TABLE-I

Flow rate	Cold water temperature		Hot water temperature		Volumetric flow rate of hot water (l/s)	Volumetric flow rate of cold water (l/s)
	T_{C_i} (^oC)	T_{C_o} (^oC)	T_{h_i} (^oC)	T_{h_o} (^oC)		

PHYSICAL PROPERTIES OF COLD WATER (TABLE – II)

Cold mean temperature ($^\circ C$)	Density (ρ) (Kg/m^3)	Viscosity (μ) x 10^{-3} (kg/ms)	Specific heat capacity (C_p) x 10^3 (J/kg K)	Thermal conductivity (W/m K)	Prandtl no

PHYSICAL PROPERTIES OF HOT WATER (TABLE – III)

Hot mean	Density (ρ)	Viscosity (μ)	Specific heat	Thermal	Prandtl

temperature (˚C)	(Kg/m^3)	x 10^{-3} (kg/ms)	capacity (C_p) x 10^3 (J/kg K)	conductivity (W/m K)	no

Specifications

Inner diameter of inner tube, d_i = 10.5×10^{-3}m
Outer diameter of inner tube, d_0 = 12.5×10^{-3}m
Inner diameter of outer tube, D_i = 27.5×10^{-3}m
Outer diameter of outer tube, D_0 = 33.8×10^{-3}m
Length of tube, l = 1.65m

$$LMTD = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)}$$

Calculation of LMTD:

Model calculation:

$$\Delta T_{LMTD} = \frac{\Delta T_1 - \Delta T_2}{\ln\left(\frac{\Delta T_1}{\Delta T_2}\right)}$$

ΔT_1 =

ΔT_2 =

ΔT_{LMTD} =

HOT SIDE CALCULATION:
Model calculation:

Area A = $\frac{\Pi d_i^{\,2}}{4}$

Velocity v = $\frac{\dot{m}_h}{\rho A_h}$

Reynolds number = $\frac{d_i v \rho}{\mu}$

N_{Re} =

Prandtl number = $\frac{C_p \mu}{k}$

N_{Pr} =

Nusselt number: Dittus Boelter equation,

$Nu = 0.023\ Re^{0.8}\ Pr^{0.4}$

$Nu_1 =$ _____

To find h:

$$Nu = \frac{hd_i}{k}$$

$$h = \frac{Nu \times k}{d_i}$$

$h_1 =$ _____

COLD SIDE CALCULATION:

Model calculation:

$$\text{Area} = \frac{\Pi (D_i^{\,2} - d_o^{\,2})}{4}$$

$$\text{Velocity} = \frac{\dot{m}_c}{\rho A_c}$$

$$\text{Reynolds number} = \frac{D_e v \rho}{\mu} \text{ where } D_e = \frac{D_i^{\,2} - d_o^{\,2}}{d_o}$$

$N_{Re} =$

$$\text{Prandtl number} = \frac{C_p \mu}{k}$$

$N_{Pr} =$

Nusselt number: Dittus Boelter equation,

$Nu = 0.023\ Re^{0.8}\ Pr^{0.3}$

$Nu_1 =$ _____

To find h:

$$Nu = \frac{hD_e}{k}$$

$$h = \frac{Nu \times k}{D_e}$$

$h_1 =$ _____

Effectiveness

Hot side

$$q = \dot{m}_h C_{ph} (T_{h_i} - T_{h_o})$$

Cold side

$$q = \dot{m}_c C_{pc} (T_{c_o} - T_{c_i})$$

$$C_{min} = \dot{m}_h C_{p_h} \qquad C_{min} = \dot{m}_c C_{p_c}$$

If $(C_{min})_c < (C_{min})_h$

$$\varepsilon = \frac{\dot{m}_c C_{p_c} \Delta T_c}{\dot{m}_c C_{p_c} (T_{h_i} - T_{c_i})}$$

If $(C_{min})_h < (C_{min})_c$

$$\varepsilon = \frac{\dot{m}_h C_{p_h} \Delta T_h}{\dot{m}_h C_{p_h} (T_{h_i} - T_{c_i})}$$

Overall heat transfer coefficient:

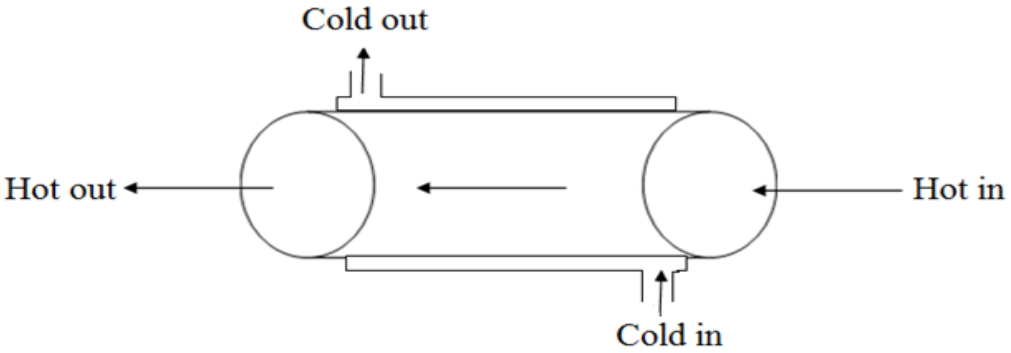
$$U = \frac{Q}{A_o LMTD}$$

A_o = Area of outlet tube

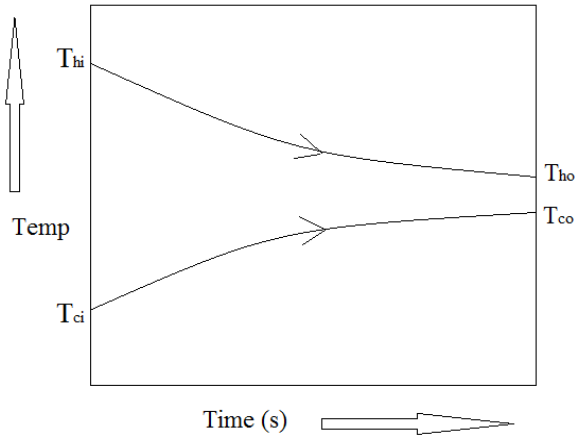
$A_0 = \pi d_i l = \pi \times 10.5 \times 10^{-3} \times 1.65 = 0.054\, m^2$

$U_1 =$ $(W/m^2 K)$

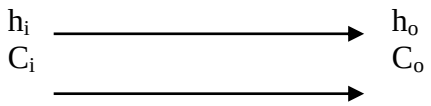
MODEL GRAPH



Temperature Profile



Parallel Flow



RESULT:

The individual and overall heat transfer coefficient for parallel flow double pipe heat exchanger is studied

Mass flow rate of hot water (kg/s)	U (W/m^2K)	h_h (W/m^2K)	h_c (W/m^2K)	Effectiveness for parallel flow

SIMPLE DISTILLATION

EXPT. NO: 11

AIM:

To verify Rayleigh`s equation through simple distillation process

THEORY:

The simplest form of batch still consists of a heated vessel (pot or boiler), a condenser and one or more receiving tanks. No trays or packing are provided. Feed is changed into the vessel and through brought to boiling vapours are condensed and collected in a receiver. No reflux is returned. The rate of vapourization is sometimes controlled to prevent "lomping". The charge and to avoid overloading the condenser, both other are minimal. This process is often referred to as Rayleigh distillation.

If we represent the moles of vapour by v, moles of liquid in the pot by L, the mole fraction of the mole volatile components in this liquid by x, and mole fraction of the same component in vapour by y, a material balance yields.

$$-y.dv=d(Lx)$$

Rearranging and integrating gives,

$$\ln \frac{Mi}{Mf} = \int_{xf}^{xi} \frac{dx}{y-x}$$

Where subscript 'i' represents the initial conditions and f represents the final condition of the liquid in the still pot. Integration limits have been reversed to obtain a positive integral. If equilibrium is assumed between liquid and vapour, the right hand side of equation may be evaluated by plotting $\frac{1}{y-x}$ vs x on measuring the area under the area between limits xi and xf. If the mixture is a binary system for which relative volatility 'α' is constant or if an average value that will serve for the range considered can be found, then the relationship that are relative volatility.

$$\alpha = \frac{\frac{y}{x}}{\frac{(1-y)}{1-x}}$$

$$\ln \frac{M_f}{M_i} = \frac{1}{\alpha - 1} \ln \left[\frac{x_f (1-x_i)}{x_i (1-x_f)} \right] + \ln \left[\frac{1-x_i}{1-x_f} \right]$$

PROCEDURE:

- 300ml of feed was prepared by mixing 200ml of isopropyl alcohol and 100ml of water.
- The density of feed solution was determined.
- The feed of the distillate flask was charged, the process was started.
- After a considerable amount of distillate was collected, the distillate and residue volumes were determined.
- The densities of distillates and residue were determined.
- The experiment was separated for a different composition of 100ml of isopropyl alcohol and 200ml water.
- The Rayleigh`s equation was verified.

FORMULAE:

Rayleigh`s equation

$$\ln \frac{F}{w} = \int_{xw}^{xF} \frac{dx}{y*-x}$$

Relative volatility

$$\alpha = \frac{\frac{y}{x}}{\frac{(1-y)}{1-x}}$$

$$\ln \frac{F}{W} = \frac{1}{\alpha - 1} \ln \left[\frac{x_F (1-x_W)}{x_W (1-x_F)} \right] + \ln \left[\frac{1-x_W}{1-x_F} \right]$$

where,

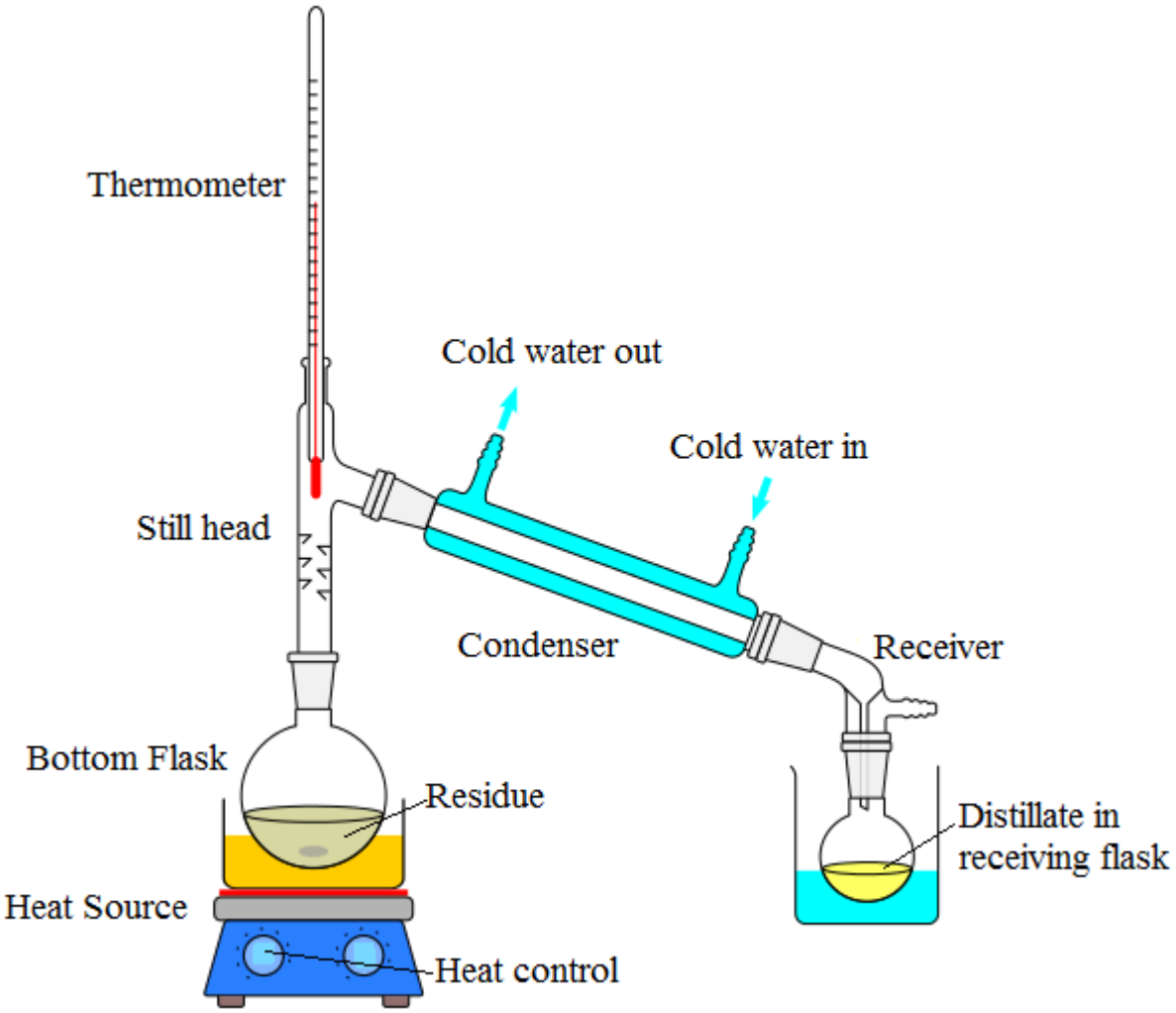
F → moles of feed

W → moles of residual liquid

xF → Composition of feed (mole fraction)

xW → Composition of residues (mole fraction)

DIAGRAM:



MODEL CALCULATION:

Estimation of Density: 1

Mixture number: 1

Density of feed:

Weight of empty bottle = _____gm

Weight of empty bottle + sample in it =_____gm

Volume of sample taken =_____ml

Weight of sample taken =_____gm

Density of sample =_____g/cc

Estimation of average molecular weight and moles:

Avg. mol. wt. of feed

Mol. Wt of compound A (MWA) =_____gm/gm mole

Mol. Wt of compound B (MWB) =_____gm/gm mole

Mole fraction of compound A (xA)=

Mole fraction of compound B (xB) =

Average molecular weight = MwA *xA + MWB *xB =_____gm/gm mole

Volume of feed (v) = _____ml

Density of feed (ρ)=_____gm/ml

Weight of feed (w) = v*ρ=_____gm

Mole of feed = Weight/ Mol.Wt = _____gm/ mole

Verification of Rayleigh`s equation:

$$\ln \frac{F}{w} = \int_{xw}^{xF} \frac{dx}{y*-x}$$

Moles of feed =_____gm mole

Moles of residue =_____gm mole

$$\ln \frac{F}{w} =$$

The R.H.S should be evaluated graphically. For evaluating the integral prepare a plot of $\frac{1}{y-x}$ against x. The x-y dates from perry engineering hand took for the concerned liquid system.

Area under the curve between the limits =

Estimation of relative volatility

$$\text{Relative volatility} = \alpha = \frac{\frac{y}{x}}{\frac{(1-y)}{1-x}}$$

Mole fraction of A in residue (x) =

Mole fraction of A in distillate (y) =

Relative volatility =

RESULT:

The Rayleigh`s equations is verified for two different feed compositions and relative volatility is determined.

Mixture l : α =

Mixture ll : α =

TABLE 1

Weight of	Mixture 1 : Compound A: 200ml Compound B: 100ml			Mixture 2 : Compound A : 100ml Compound B: 200ml		
	Feed	Distillate	Residue	Feed	Distillate	Residue
Empty Bottle (gm)						
Bottle + Sample (gm)						
Sample Taken (gm)						
Volume of Sample (ml)						
Density						

TABLE 2

Mixture	Volume of feed (ml)		Volume of distillate (ml)			Density (g/cc)			Mole fraction of A			Avg mol weight gm /gm mole			Moles of		
	A	B	Distillate	Residue	Feed	Distillate	Residue	Feed	Distillate	Residue	Feed	Distillate	Residue	Feed	Distillate	Residue	Fe
1																	
2																	

STEAM DISTILLATION

EXPT. NO: 11

AIM:

To verify the law of vapour pressure of immiscible liquid and to calculate the vapourization efficiency and the thermal efficiency of steam distillation and also to calculate the molecular weight of given liquid

THEORY:

The process of steam distillation is applicable to liquid which are not miscible with water phase rule states that the number of degree of freedom F, number of components c and the number of phase P are related by the equation

$F = c + P - 2$

In a system of 2 immiscible liquids the number of components is 2 and number of phase is 3 (2 liquid phase + 1 vapour phase) substituting these values in the phase rule we get that the number of degree of freedom is 1. Fixing either the pressure or the temperature completely defines such a system for ex, if the temperature is fixed, total vapour pressure is fixed (that is the total vapour pressure is fixed), then the temperature at which the distillation takes place is fixed.

From the above discussion it can be seen that per system compressing of 2 immiscible liquids A and B the sum of the vapour pressure of the vapour pressure of the two components equals to the external pressure P.

Thus if P_A is the vapour pressure of the first component at the boiling point, then the vapour pressure of the second component B, P_s is equal to $(P-P_A)$. If the vapour is in equilibrium with a some of mixture which is condensed.

$$\frac{W_A}{W_B} = \frac{M_A P_A}{M_B(P-P_A)}$$

Where,

W_A and W_B → mass of the components of A & B

M_A and M_B → molecular weight of A & B

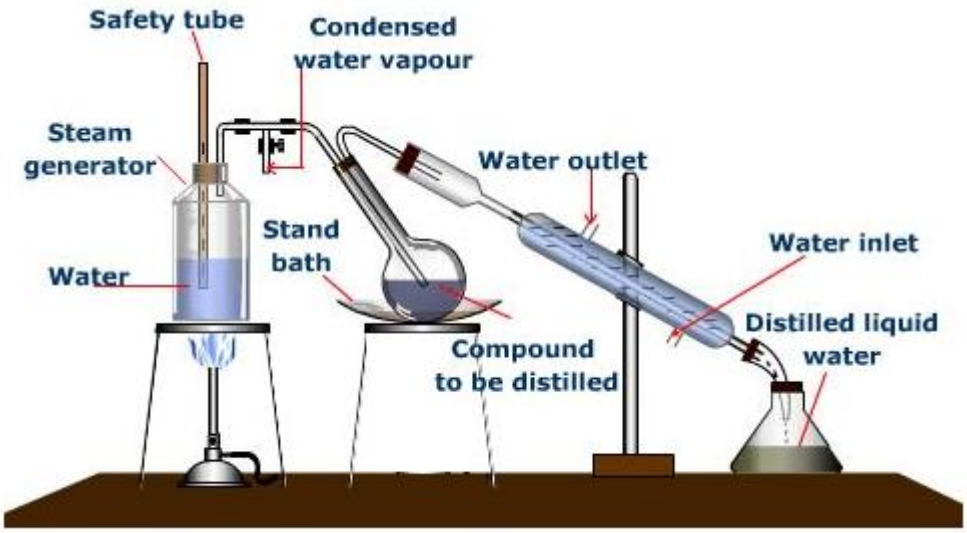
- ✓ In direct distillation inert gas is used instead of steam.
- ✓ The conditions to be fulfilled for steam distillation
- ✓ Component must be insoluble in water
- ✓ Component to be distilled are thermally unstable or if the component present and hence normal distillation cannot be carried out.

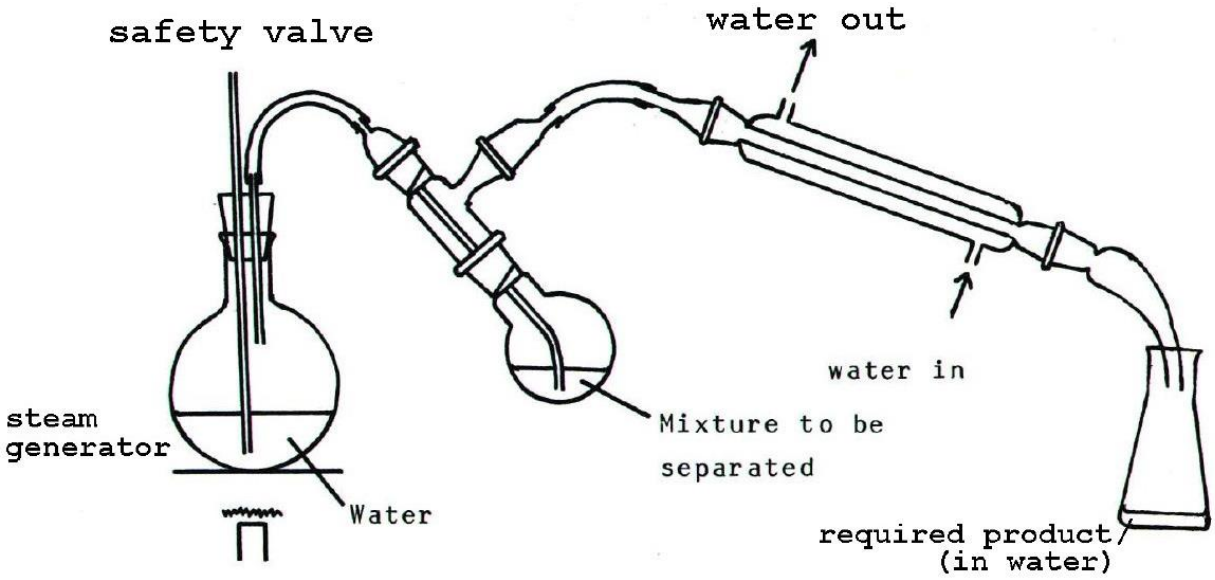
- ✓ When a feed containing a small quantity of volatile matter, as an impurity are to be purified

PROCEDURE:

- ➢ The apparatus was arranged
- ➢ About 200ml of the given liquid taken in the bottomed flask of steam distillation setup, arranged with steam boiler and condenser
- ➢ Steam at constant pressure was passed into sound bottomed flask
- ➢ The vapour after condensing in the condenser was called as distillate
- ➢ The distillate was continued till about 1/3rd of initial liquid taken distillates out
- ➢ The steam supply was then stopped
- ➢ The residue was cooled
- ➢ The volume and specific gravity of water and nitro benzene in the residue and distillate were found out for the determination of thermal and vapourization efficiencies.

DIAGRAM:





OBSERVATION:

Room temperature =

Distillation temperature =

Volume of sample in residue =

volume of sample in distillate =

Volume of water in residue =

Volume of water in distillate =

Weight of	Water	Water in distillate	Water in residue	NB in distillate	NB in residue
Empty bottle(gm.)					
Sample + bottle (gm)					
Sample taken (gm)					
Volume of sample taken (ml)					
Density					

DATA:

P_s : vapour pressure of sample at distillation temperature : 20mm of Hg

P_w : vapour pressure of water distillation temperature : 707.27mm of Hg

M_s : Molecular weight of sample : 123 kg/kmol

M_w : Molecular weight of water : 18kg/kmol

λ_w : Latent heat of water : 2.269 x 10^3 kJ/kmol

C_{ps} : Specific heat of $C_6H_5NO_2$: 1.386 kJ/kg.K

C_{pw}: Specific heat of water : 4217 kJ/kg.K

CALCULATIONS:

Vaporization efficiency, Ev $= \frac{\left(\frac{W_s}{W_w}\right)actual}{(W_s/W_w)ideal} \times 100$

$\left(\frac{W_s}{W_w}\right)actual = \frac{(volume \times density)\ of\ given\ sample\ in\ distillate}{(volume \times density)\ of\ water\ in\ distillate}$

$(W_s/W_w)ideal = \frac{P_s \times M_s}{P_w \times M_w}$

E_v =

Thermal efficiency, E_t

Reference temperature (T_{RF}) = 304k

Thermal efficiency = $\frac{Heat\ required}{Heat\ supplied} \times 100$

Heat supplied:

Heat input = Steam used x $\{\lambda_w + C_{pw}(373 - T_{RF})\}$

Steam used = Weight of water in distillate + Weight of water in residue

Heat input =

Heat required:

Heat required to distill the sample =

(Mass of sample in distillate) x [{λ sample at $(T_{RF}+T_p)/2$} +{C_p sample(T_D-T_{RF})}]

Estimation of λ sample at $(T_{RF}+T_b)/2$ (λ_{vf}) using <u>WATSON Equation</u>.

λ sample at $T_k = \lambda\left(\frac{1-T_{rt}}{1-T_{rB}}\right)^{0.38}$

Reduced temperature at T_k ($T_r k$) = T/T_c =

Reduced temperature at boiling point (T_{rB}) = T_B/T_C =

λ_{v1}at boiling point $\left(\frac{\lambda_{v1}}{T_B}\right) = 8.75 + 4.751 \log T_B$

Latent heat of sample at T K = _____

Mass of sample in distillate = volume of distillate collected x density of sample in distillate

Heat required to distill the sample = _____

E_t =____

E_f =____

Estimation of Molecular Weight:

Molecular weight =$\frac{P_S \times M_w}{P_S \times W_w}$ x W_s

Mass of sample (W_s) = Volume of sample in distillate x Density of sample

Mass of water (M_W) = Volume of water in distillate x Density of water

P_s = Partial pressure of sample = ____mm of Hg

P_w = partial pressure of water = _____mm of Hg

W_s = ____

M_W = ____

Molecular weight of sample, M_S = _____kg/kg mol.

RESULT:

Steam distillation of given liquid $C_6H_5NO_2$ was analyzed and the following were calculated

- Distillation temperature observed = K
- Vaporization efficiency = %
- Theoretical thermal efficiency = %
- Calculated molecular weight of the substrate = kg mol

DEPARTMENT OF BIOTECHNOLOGY

BT 3461-
ANALYTICAL INTSTRUMENTATION
LABORATORY MANUAL

REGULATION 2021

II YEAR & IV SEMESTER

MADHA ENGINEERING COLLEGE
MADHA NAGAR
CHENNAI- 600 069

MADHA ENGINEERING COLLEGE
DEPARTMENT OF BIOTECHNOLOGY
BT8512 ANALYTICAL METHODS OF INSTRUMENTATION LABORATORY MANUAL

Year and Semester : III / V **L T P C**
0 0 4 2

OBJECTIVES:

- To train the students
- To have a practical hands on experience on Absoprtion Spectroscopic methods
- To acquire experience in the purification by performing chromatography
- To validate and analysis using spectrometric and microscopic techniques

LIST OF EXPERIMENTS

1. Precision and validity in an experiment using absorption spectroscopy .
2. Validating Lambert-Beer's law using KMnO4
3. Finding the molar absorbtivity and stoichiometry of the Fe (1,10 phenanthroline)3 using absorption spectrometry.
4. Finding the pKa of 4-nitrophenol using absorption spectroscopy.
5. UV spectra of nucleic acids.
6. Chemical actinometry using potassium ferrioxolate.
7. Estimation of SO4-- by nephelometry.
8. Estimation of Al3+ by Flourimetry.
9. Limits of detection using aluminium alizarin complex.
10. Chromatography analysis using TLC.
11. Chromatography analysis using column chromatography.

TOTAL: 60 PERIODS

REFERENCES:

1. Skoog, D.A. etal. "Principles of Instrumental Analysis", Vth Edition, Thomson / Brooks – Cole,1998.
2. Braun, R.D. "Introduction to Instrumental Analysis", Pharma Book Syndicate, 1987.
3. Willard, H.H. etal. "Instrumental Methods of Analysis", VIth Edition, CBS, 1986.
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INDEX

S.No	Date of Experiment	Topic	Date of submission	Signature
1		PRECISION AND VALIDITY IN AN EXPERIMENT USING ABSORPTION SPECTROSCOPY		
2		VERIFICATION OF LAMBERT-BEER'S LAW USING KMnO4		
3		FINDING THE MOLAR ABSORBTIVITY AND STOCHIOMETRY OF FE3+(1, 10 PHENANTHROLINE) USING ABSORPTION SPECTROMETRY		
4		FINDING THE Pka OF 4-NIROPHENOL USING ABSORPTION SPECTROSCOPY		
5		UV SPECTRA OF NUCLEIC ACIDS		
6		ESTIMATION OF SULPHATE BY NEPHLOMETRY		
7		ESTIMATION OF AL+++ BY FLOURIMETRY		
8		CHROMATOGRAPHY ANALYSIS USING TLC		
9		CHROMATOGRAPHY ANALYSIS USING COLUMN CHROMATOGRAPHY		
10		UV – SPECTRA OF PROTEINS		

Exp:No:1 **Date:**

PRECISION AND VALIDITY IN AN EXPERIMENT USING ABSORPTION SPECTROSCOPY

Aim:

To validate Beer-Lambert's law and to find out the precision percentage for $KMnO_4$

Principle:

The Beer – Lambert's law is a linear relationship between absorbance and concentration of an absorbing species. **Beer's law** states that "the intensity of a beam of monochromatic light decreases exponentially with increase in concentration of the absorbing species arithmetically".

I =Intensity of incident light

C= concentration

k= Proportionality constant

$$\mathbf{-\ln I = kc + b} \quad \text{…….. Equation 1}$$

(On integration, b is constant of integration)

When concentration = 0, there is no absorbance. Hence $I = I_0$

Substituting in equation 1,

$-\ln I_0 = kX_0 + b \; -\ln I_0 = b$

Substituting the value of b, in equation 1, $-\ln I = kc - \ln I_0$

$\ln I_0 - \ln I = kc$

$$\ln \frac{\mathbf{I0}}{I} = kc$$

(since $\log A - \log B = \log \frac{\mathbf{A}}{B}$)

$$\frac{\mathbf{I0}}{I} = {}_e kc$$

$$\mathbf{I = I_0 e^{-kc}} \text{ (Beer's law)…….equation 2}$$

Lambert's law states that the rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light. This equation can be simplified, similar to equation 2 to get the following equation (by replacing 'c' with't')

$$\mathbf{I = I_0 e^{-kt}}$$ ……equation 3

Equation 2 and 3 can be combined to get

$I = I_0 e^{-kct}$ (converting natural logarithm to base 10& K =kx0.4343)

$$\frac{I}{I0} = 1_0^{-Kct}$$ (rearranging term)

$$\frac{I0}{I} = 1_0^{Kct}$$ (inverse on the both sides)

$$\log \frac{I0}{I} = Kct$$

(Taking log on both sides) ……equation 4

it can be learnt that Transmittance (T) = $\frac{I}{I0}$ and Absorbance (A) = $\frac{1}{T}$

Hence A = $\log \frac{1}{\frac{I}{I0}}$

$$A = \log \frac{I0}{I}$$ ……equation 5

Using equation 4 &5, since A = log and log= Kct we can infer that

(instead of K, we can use ε)

Mathematical equation for Beer – Lambert's Law is

$$\mathbf{A=\varepsilon ct}$$

i.e... A is proportional to c or A=εc the above equation is y=mx for m=ε

Where A = Absorbance or optical density or extinction co-efficient

ε = Molecular extinction coefficient

c = concentration of sample (mmol/lit)

t = path length (normality10 mm or 1 cm)

Chemicals Required:

1. Potassium permanganate
2. Distilled water

Apparatus required:

1. Spectrophotometer/ colorimeter

2. glass cuvettes
3. standard flasks

Procedure:

1. A series of test tubes were labeled 1-10 (1, 2, 3…..10) respectively.
2. The stock $KMnO_4$ solution (10 mM of $KMnO_4$ is prepared by adding 0.158 g of $KMnO_4$ and is made up to 1000 ml) diluted into 10%, 20%, 30%, 40%.....100% i.e. KMnO4 was pipetted out into (1, 2, 3, 4….10) ml in respective tubes which was made up to 10ml by distilled water.
3. Absorbance was read at 600 nm for all the diluted samples and for blank.
4. Graph was plotted for absorbance vs concentration and precision percentage was calculated.

TABLE I:

Reagent	**Blank**	**0.2mg/ml**	**0.4mg/ml**	**0.6mg/ml**	**0.7mg/ml**	**0.8mg/ml**

TABLE II:

CONCENTRATION(mg/ml)	**OD(440nm)I**	**OD(440nm)IIA**	**OD(440nm)IIIB**

TABLE III:

B-A	**Avg of B-A**	**% of error by instrument**	**II-I**	**Avg of I,II**	**% of error by Experiment**

CALCULATION:

GRAPH:

RESULT:

Exp:No:2 **Date:**

VERIFICATION OF LAMBERT-BEER'S LAW USING KMnO4

Aim:

To verify Beer-Lambert's law and to find out the molar extinction and its co-efficient or molar absorptivity.

Principle:

The Beer – Lambert's law is a linear relationship between absorbance and concentration of an absorbing species. **Beer's law** states that "the intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically".

I =Intensity of incident light

C= concentration

k= Proportionality constant

$$-\ln I = kc + b \quad \text{…….. Equation 1}$$

(On integration, b is constant of integration)

When concentration = 0, there is no absorbance. Hence $I = I_0$

Substituting in equation 1,

$-\ln I_0 = kX_0 + b$ $-\ln I_0 = b$

Substituting the value of b, in equation 1, $-\ln I = kc - \ln I_0$

$\ln I_0 - \ln I = kc$

$$\ln \frac{I0}{I} = kc$$

(since $\log A - \log B = \log \frac{A}{B}$)

$$\frac{I0}{I} = {}_e kc$$

$$I = I_0 e^{-kc} \text{ (Beer's law)…….equation 2}$$

Lambert's law states that the rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light. This equation can be simplified, similar to equation 2 to get the following equation (by replacing 'c' with't')

$$\mathbf{I = I_0 e^{-kt}}$$ ……equation 3

Equation 2 and 3 can be combined to get

$I = I_0 e^{-kct}$ (converting natural logarithm to base 10& K =kx0.4343)

$$\frac{I}{I0} = 1_0^{-Kct}$$ (rearranging term)

$$\frac{I0}{I} = 1_0^{Kct}$$ (inverse on the both sides)

$$\log \frac{I0}{I} = Kct$$

(taking log on both sides) ……equation 4

it can be learnt that Transmittance (T) = $\frac{I}{I0}$ and Absorbance (A) = $\frac{1}{T}$

Hence A = $\log \frac{1}{\frac{I}{I0}}$

$$A = \log \frac{I0}{I}$$ ……equation 5

Using equation 4 &5, since A = log and log= Kct we can infer that

(instead of K, we can use ε)

Mathematical equation for Beer – Lambert's Law is

$$\mathbf{A=\varepsilon ct}$$

i.e... A is proportional to c or A=εc the above equation is y=mx for m=ε

Where A = Absorbance or optical density or extinction co-efficient

ε = Molecular extinction coefficient

c = concentration of sample (mmol/lit)

t = path length (normality10 mm or 1 cm)

Chemicals Required:

1. Potassium dichromate ($K_2Cr_2O_7$)
2. Distilled water

Apparatus required:

1. Spectrophotometer/ colorimeter
2. Glass cuvettes
3. standard flasks, etc

Procedure:

1. A series of test tubes were labeled 1-10 (1, 2, 3…..10) respectively.
2. The stock $K_2Cr_2O_7$ solution (10 mM of $K_2Cr_2O_7$ is prepared by adding 0.158g of $K_2Cr_2O_7$ and is made up to 1000 ml) diluted into 10%, 20%, 30%, 40%.....100% i.e., $K_2Cr_2O_7$ was pipetted out into (1, 2, 3, 4….10) ml in respective tubes which was made up to 10 ml by distilled water.
3. Absorbance was read at 600 nm for all the diluted samples
4. Graph was plotted for absorbance vs concentration and molar extinction coefficient was calculated from slope (m) and by calculation $A=\varepsilon ct$

Reagent	B	S1	S2	S3	S4	S5
KMnO4						
Distilled Water						
KMnO4						
Distilled Water						

Observation:

Set I

Conc(mg/ml)	Absorption(440nm)

Set II

Conc(mg/ml)	Absorption(440nm)

CALCULATION:

GRAPH:

RESULT:

Exp:No:3 **Date:**

FINDING THE MOLAR ABSORBTIVITY AND STOCHIOMETRY OF FE^{3+}(1, 10 PHENANTHROLINE) USING ABSORPTION SPECTROMETRY

Aim:

To determine the molar absorbtivity and stoichiometry of Fe^{3+} - Phenonthroline complex by using absorption spectrum.

Principle:

The reaction of Ferrous Ammonium Sulphate and 1, 10 – Phenonthroline forms a complex which absorbs in UV and Visible region. The reaction stochiometry for the formation of a metal ion complex Fe^{3+} - Phenonthroline can be determined easily. Metal ions especially transition metal ions, possess the ability to form complexes with both organic and inorganic molecules called ligands. These complexes are produced when lone pair electrons from the ligand are donated into empty orbitals of the metal ion (resulting in a coordinate covalent bond). Here, iron(II) cations will be mixed with the ligand 1,10-phenanthroline to produce an iron(II)-phenanthroline complex:

$$x\ \mathrm{Fe2+} + y\ \mathrm{phen} \rightarrow \mathrm{Fe}x(\mathrm{phen})y^{2+}$$

where phen = 1,10-phenanthro

+9line

both Fe2+ and phenanthroline solutions will have the same molarity concentration. Mixtures prepared with the same total number of moles will therefore have the same total volume. As an additional consequence, in each prepared mixture the volume ratio of reactants used will be identical to the mole ratio of reactants used:

$$n\ \mathrm{Fe} = M\ \mathrm{Fe} \times V\ \mathrm{Fe} \text{ and } n\ \mathrm{phen} = M\ \mathrm{phen} \times V\ \mathrm{phen}$$

$$\text{if } M\ \mathrm{Fe} = M\ \mathrm{phen}$$

$$\text{then } n\ \mathrm{Fe}{:}n\mathrm{p}\ \mathrm{hen} \equiv V\ \mathrm{Fe}{:}V\ \mathrm{phen}$$

Thus it will be more convenient to analyze the amount of complex product formed as a function of reactant volumes, rather than as a function of reactant moles. Since the complex is a red-orange color (while both reactants are colorless) the technique of Absorption Spectroscopy will be used. When visible light is directed at a colored compound in solution, the compound will absorb some wavelength of the light while transmitting other wavelengths. The higher the concentration of the colored compound, the lighter it will absorb. The transmitted light gives rise to the solution color that we see. Since the iron(II)-phenanthroline complex is a red-orange color, it is expected to absorb blue-green wavelengths, between 460 and 550 nm. Beer's Law quantitatively describes the relationship

between the light absorbed (*A*) and the concentration of the colored species in solution (*c*) as

$$\mathbf{A=\varepsilon ct}$$

Where A = Absorbance or optical density or extinction co-efficient

ε = Molecular extinction coefficient

c = concentration of sample (mmol/lit)

t = path length (normality10 mm or 1 cm)

Chemicals required:

1. Ferrous Ammonium Sulphate – 0.02M (dissolved water (pH= 4.5) as a solvent and add 1ml Conc. Sulphuric acid)
2. 1, 10 – Phenonthroline – 0.02 M (dissolved in 0.2 w/v% in alcohol).
3. Distilled water

Apparatus Required:

1. Spectrophotometer/ Colorimeter
2. Glass Cuvettes
3. Standard flasks, etc

Procedure:

1. Prepare 30-ml of 2.50 x 10^{-4} M Fe^{+2} and 40-ml of 2.5 x 10^{-4} M phenanthroline in each of your small beakers.
2. A series of 11 test tubes were taken and labellaed as 1,2 3…11.
3. Mix each solution in various ratios as 4.5:5ml, 4:1ml, 3.5:1.5ml ……0.25:4.75ml.
4. Mix each solution well by covering the tube with Parafilm (or a small stopper) and inverting it many times. The red-orange color of the iron (II)-phenanthroline complex should fully develop about ten minutes after mixing.
5. The molar absorbtivity was calculated using the formula, A=εct.
6. The stochiometry of iron(II)-phenanthroline complex can be calculated from the graph. From the point of intersection the volume mixture of reactants used to obtain the maximum absorbance can be determined.(The maximum amount of complex formed). Finally, using this volume mixture, whole number volume ratio of Fe^{+2}:Phen (identical to the mole ratio), can be determined which is the stochiometric ratio for this reaction.

Tabulation

Wavelength	FAS	PHENONTHRALIN	COMPLEX

Calculation

Result:

Exp:No:4 **Date:**

FINDING THE Pka OF 4-NIROPHENOL USING ABSORPTION SPECTROSCOPY

Aim:

To determine the Pka of P-Nitrophenol solution.

Principle:

The undissociated form of P-Nitrophenol in acidic medium does not absorbs radiant energy in visible region, while quinanoid in alkaline medium absorbs strongly. The pH at which 50% ionization occurs gives half of pH gives half of the absorbance value obtained in alkaline solution assuming 100% ionization of P-Nitrophenol.

Chemicals required:

1. Phosphate buffer (0.2 M)
2. Carbonate-Bicarbonate buffer (0.1)
3. Citrate buffer (0.05)
4. P-Nitrophenol

Apparatus required:

1. Spectrophotometer/ colorimeter
2. Glass cuvettes
3. Standard flasks, etc

Preparation of buffer solution:-

Buffer 1: (Citrate Buffer)

A). Citric acid – 0.05 M 210.14 g/l (10.5 g/l 0.05 M) B). Sodium citrate- 0.05M 294.00g/l (14.7 0.05M)

A-145 ml + B-355= 500 ml (make up to 500 ml) + 0.139 gm (2x 10-3) P-Nitrophenol is mixed with this buffer solution.

Buffer 2: (Phosphate Buffer)

A). KOH- 0.2M 56.11 g/l (11.22 g/l 0.2 M)

B). KH_2PO_4- 0.2M 136.09 g/l (27.22 g/l 0.2 M)

A-65 ml + B- 250 ml + water -185 ml (make up to 500 ml) + 0.139 gm ($2x\ 10^{-3}$) P-Nitrophenol is mixed with this buffer solution.

Buffer 3: (Carbonate Buffer)

A). Sodium carbonate -0.1 M 10.599 g/l (10.6 g/l 0.1M)

B). Sodium bicarbonate -0.1M 84 g/l (8.4 g/l 0.1M)

A-5ml + B- 465 ml (make up to 500 ml) + 0.139 gm ($2x\ 10^{-3}$) P-Nitrophenol is mixed with this buffer solution.

Procedure:

1. Each buffer solution were prepared at different pH ranging from 4 to 10.

2. Buffer without 4 nitrophenol was set as Blank for all respective buffers.

3. Absorbance were recorded at 430nm.

4. A graph was plotted for absorbance against pH of the solutions.

5. The pH corresponding to the maximum absorbance value was divided by two to give the pka – value of P-Nitrophenol.

Tabulation

S.no	Name of Buffer	PH value	Absorbance at 480nm

Calculation:

Result:

Exp:No:5 **Date:**

UV SPECTRA OF NUCLEIC ACIDS

Aim:

To obtain absorption spectra of Nucleic acid in the Ultra-Violet range.

Principle:

Ultra violet spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 to 400 nm. Compounds which are colored absorb radiation from 400 to 800 nm. But compounds which are colorless absorb radiation in the UV region. In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, thereby the molecule undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and path length as given by Beer-Lambert's law.

The types of electrons present in any molecule may be conveniently classified as

'σ ' electrons: these are the ones present in saturated compounds. Such electrons do not absorb near UV, but absorb vacuum UV radiation (<200nm).

'π 'electrons : These electrons are present in unsaturated compounds (eg) double or triple bonds.

'n' electrons: these are non bonded electrons which are not involved in any bonding (eg) lone pair of electrons like in S, O, N and Halogens.

The nucleic acid absorbs strongly in UV region of spectrum due to the conjugated double bond system of the constituents purine and pyrimidine. They show characteristic maxima at 260 nm and minima at 230 nm.

Chemicals required:

DNA powder
Saline solution
Dis.H_2O

Apparatus required:

1. Spectrophotometer
2. Glass cuvettes
3. Standard flasks, etc

Procedure:

1. 10 mg of DNA is weighed accurately and dissolved and made up to 100 ml of saline solution. And the following concentrations were prepared, 2mg %, 4mg %, 6mg %, 8mg %, …..20mg %.

2. The absorbance was measured at 210nm for all the solutions taking distilled water as blank and adjusting the spectrophotometer to 100% T with blank.

3. Plot a graph of absorbance against DNA concentration (in mg %) gives a straight line passing through the origin.

Tabulation

Wavelength(nm)	Absorbance

Graph:

Result:

Exp:No 6 **Date:**

ESTIMATION OF SULPHATE BY NEPHLOMETRY

Aim:

To estimate the amount of sulphate ion present in water sample by using Nephlometry.

Principle:

Nephlometry is the measurement of scattered light as a function of concentration of suspended particles (less than, approximately 100 mg/liter, high concentrations).

Sulphate ions can be detected by the help of light scattering method which involves precipitation of the ions by Barium Chloride ($BaCl_2$) to form a stable colloidal solution. The light scattering ability of suspension is highly dependent upon the size of particles. The amount of scattered light absorbed by photocell is directly proportional to the extent of turbidity of a solution which is observed from a digital display which is denoted as nephlometric unit.

Equation can be used in Nephlometry relating light scattering of a particle at specific angle of observation and concentration of solution. The intensity of transmitted light is expressed using an equation similar to that of Beer – Lambert's law, i.e,

$$\mathbf{P = P_0 e^{-Tb}}$$

Where
P = Power of transmitted beam
P_0 = Power of incident beam
T= Turbidity or turbidity Co-efficient
b = Path length

$$\mathbf{Tb = \log \frac{P0}{P}}$$

'**T**' was found to be proportional to the concentration (c) of suspended particles.

Hence, as **T = kc,** $\mathbf{kcb = \log \frac{P0}{P}}$

Wavelength:

It is expressed by the following equation.

$$\mathbf{T = S/\lambda^t}$$

Where
T = Turbidity
S = Constant for a given sample
λ = Wavelength
t = depends on size of particles and is '4' when particle size is smaller than wavelength.

Chemicals and Equipments required:

- Solution A- Hydrazine Sulphate (5gm in 400ml of distilled water)
- Solution B- Hexamethylene tetramine (5gm in 400ml of distilled water)
- Solution A and B were mixed and made up to 1000ml by distilled water and stored.
- Working standard – 10ml of the stock was made up to 100ml by distilled water.

Test solution:

A. Sodium Sulphate (0.01M) (0.142 gm/100 ml)

B. Barium Chloride (0.01M) (0.244 gm/100 ml)

Mix 5ml of A and 5 ml of B to prepare test solution.

Apparatus:

1. Nephlo – Turbidimeter
2. Glass cuvettes

Procedure:

1. The solution A and B was mixed and made up to 1000ml by distilled water and allowed to settle for 48 hrs at room temperature to get 4000 NTU.
2. A series of 11 test tubes were serially diluted with working standard and labelled as 1, 2 3…10
3. Test solution was taken in 11^{th} test tube and readings were taken.
4. A graph was plotted to find the turbidence of test solution.

Tabulation

Flask number	**Conc of Sulphate(mg/ml)**	**Turbidity Reading using Nephlometry**

Graph:

Result:

Exp:No:7 **Date:**

ESTIMATION OF AL^{+++} BY FLOURIMETRY.

Aim:

To Estimate the Aluminium^{+++} ions by Fluorimetry method.

Principle:

Molecular fluorescence is the optical emission from molecules that have been excited to higher energy levels by absorption of electromagnetic radiation. The main advantage of fluo6rescence detection compared to absorption measurements is the greater sensitivity achievable because the fluorescence signal has in principle a zero background. Analytical applications include quantitative measurements of molecules in solution and fluoresecence in detected in liquid chromatography.

Reagents Required:

Ethanoic morin solution
Acetate buffer.
Sephadex.
Pipette.
Al(III) solution
AAS

General procedure:

1. A 500 ml water sample containing 0.4-1.6 tg dm 3 of Al(III) was transferred to a 1-dm3 polyethylene bottle and 2 cm3 of 6.2X103% of ethanolic morin solution.

2. 10 cm3 of 0.1 mol dm 3 acetate buffer solution (pH 5.30) and 60 mg of Sephadex SP C-25 ion exchanger were added.

3 The mixture was shaken mechanically for 15 min. The gel beads were then collected by filtration under suction and, with the aid of a pipette, were packed into a 1-mm cell together with a small volume of the filtrate.

4. A blank solution containing all reagents, except aluminum, was prepared and treated in the same way as the sample. The fluorescence intensity (20.0±0.5°C) for both the sample and the blank was measured at a Lem=496n m, for a Lx=426 nm. A calibration curve was constructed in the same way, using an Al (III) solution of known concentration.

Procedure for natural waters:

1.The above-mentioned reagents were added to a volume of natural water sample containing an adequate amount of Al(III), levelled off at 500-cm3 with doubly distilled water and placed into a 1-dm3 polyethylene bottle, in the same manner as described in the general procedure.

2. Both calibration curve and standard addition methods were used for calibration purposes. Reference method A determination of aluminum by AAS with a dinitrogen oxide-acetylene flame after extraction with 8-quinolinol (oxine)-4-methyl-2-pentanone (MIBK) was used as a reference method.

Treatment of the sample:

1. Natural water was filtered through 0.45-µm membrane filter paper (Millipore), treated with conc. HN03 (0.25 cm3/ 1000 cm3) and collected in a polyethylene container, which had previously been washed with nitric acid.

Result:

Exp: No:8 **Date:**

CHROMATOGRAPHY ANALYSIS USING TLC

Aim:

To separate the constituents of plant pigments (leaf chlorophyll) by TLC.

Principle:

In TLC the compound under examination moves along the surface of the adsorbent. The moving substance is attracted by the polar sites on the surface of the adsorbent by electrostatic forces and this binding is reversible. The solvent also interacts with the adsorbent and the compound interacts with the solvent. This three fold competitive interaction among solute, solvent and adsorbent establishes the relative rates at which the solvent front and solute ascend the layer of adsorbent on the glass plate. A more polar solute is attracted to the adsorbent more strongly than a less polar solute. This is reflected in the faster movements of the less polar compounds.

The experiment is based on the principle of adsorption. The chlorophyll extract of plant leaves get adsorbed by the solvent molecules. The extent of adsorption of plant pigment decides partition and separation. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried and separated components of the mixture are visualized. If the components are colored, visualization is straight forward. Usually the components are not colored so a UV lamp is used to visualize the plate.

Chemicals and equipments required:

1. Acetone
2. Petroleum ether
3. Mortar and Pestle
4. Developing Chamber
5. Glass plate
6. Distilled Water

Procedure:

1. Green leaves were crushed and/or squashed using a mortar and pestle until a green pulp was obtained.
2. Mixture of acetone, petroleum ether (7:3), was added to dissolve the chlorophyll. The green extract was centrifuged and chlorophyll extract was obtained as a supernatant.
3. The slurry (stationary phase + water) is prepared and poured on to a glass plate which is maintained on a leveled surface. The slurry is spread uniformly on the surface of the glass plate. After setting, the plates are dried in an oven.
4. Add a sufficient mixture of solvent system (acetone, petroleum ether (7:3)) and cover the bottom of the tank to a depth of 0.5cm. Allow it to stand for 15minutes.

5. Select a TLC plate, draw a very fine line with a pencil above 1cm from one edge of the plate and apply mixtures as spot on to the plate.
6. Place the plate into the tank and allow the solvent to develop up to 15 cm.
7. Remove the plate from the tank, and allow dry it.
8. Dry the chromatogram in an oven at 60°C for 10-15 minutes. Spots will be visualized.

Tabulation:

Band Observed	**Distance Travelled**	
	Solute	**Solvent**

Calculation:

Result:

Exp:No: 9 **Date:**

CHROMATOGRAPHY ANALYSIS USING COLUMN CHROMATOGRAPHY

Aim:

To separate the various pigments of a concentrated leaf extract using adsorption column chromatography.

Principle:

A solid stationary phase and a liquid mobile phase are used and the principle of separation is adsorption. When a mixture of components dissolved in the mobile phase is introduced in to the column, the individual components move with different rates depending upon their relative affinities. The compound with lesser affinity towards the stationary phase (adsorbent) moves faster and hence it is eluted out of the column first. The one with greater affinity towards the stationary phase (adsorbent) moves slower down the column and hence it is eluted later. Thus the compounds are separated. The type of interaction between the stationary phase (adsorbent) and the solute is reversible in nature. The rate of movement of a component (R) is given as follows:

Requirements:

Chromatography (20 cm X 1 cm)
Fresh green leaves
Alumina
Calcium carbonate
Sucrose
Sodium Sulphate
Petroleum ether
Methanol
Benzene
Mortar and pestle

Procedure:

Homogenize 5gm of green leaves in a mortar and pestle, and extracted by shaking with a mixture of petroleum ether, methanol and benzene (45: 15: 5). Remove the residue by filtration and wash the filtrate four times with water to remove methanol. Avoid vigorous shaking or an emulsion will form. Remove the last traces of water by adding anhydrous sodium sulphate, filter to remove the solid, and concentrate the extract to a few milliliters by careful evaporation in a fume chamber.

Preparation of column:

Prepare slurries of the column materials (alumina, calcium carbonate and sucrose) in petroleum ether and pack the column with alumina (5cm), calcium carbonate (7cm) and sucrose (7cm), inserting a filter paper disc between each adsorbent . Gentle suction may be applied to the bottom of the column to assist packing. Wash the column with several volumes of the eluting solvent, a mixture of benzene and petroleum ether (1:4).

Separation and elution of the pigments:

When the top of the column is almost dry, add the extract and elute the solvent. If the flow rate is too slow, apply gentle pressure to the top of the column.

Observation:

Time(mins)	Concentration

Graph:

Result:

Exp:No:10 **Date:**

UV – SPECTRA OF PROTEINS

Aim:

To obtain absorption spectra of protein in the Ultra-Violet range.

Principle:

Ultra violet spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 to 400 nm. Compounds which are colored absorb radiation from 400 to 800 nm. But compounds which are colorless absorb radiation in the UV region. In both UV as well as visible spectroscopy, only the valence electrons absorb the energy, thereby the molecule undergoes transition from ground state to excited state. This absorption is characteristic and depends on the nature of electrons present. The intensity of absorption depends on the concentration and path length as given by Beer-Lambert's law.

The types of electrons present in any molecule may be conveniently classified as

1. 'σ ' electrons: these are the ones present in saturated compounds. Such electrons do not absorb near UV, but absorb vacuum UV radiation (<200nm).

2. 'π 'electrons : These electrons are present in unsaturated compounds (eg) double or triple bonds.

3. 'n' electrons: these are non bonded electrons which are not involved in any bonding (eg) lone pair of electrons like in S, O, N and Halogens.

Below 230 nm, the extinction of a protein solution rises steeply reaching a maximum at 190 nm, this is mainly due to the peptide bond. In practice, it is more convenient to measure the extinction at 210 nm where the specific extinction coefficient is about 200 for most proteins. All proteins here since the peptide bond content is similar.

Tyrosine and tryptophan absorb at 275 nm and 280 nm and so proteins containing these amino acids will also absorb in this region. The specific extinction coefficient varies according to how much of these amino acids are present in the particular protein.

Chemicals required:

1. Standard Bovine serum albumin

2. 9% saline solution

3. Protein in serum diluted 1: 10,000 with 9% saline solution

4. Dis.H_2O

Apparatus required:

1. Spectrophotometer

2. Glass Cuvettes

3. Standard flasks, etc

Procedure:

1. Dissolve 10mg of Bovine Serum Albumin in 100 ml.distilled water.

2. Add a few drops of 0.1N NaOH to preserve the solution. Prepare the following concentrations of Bovine Serum Albumin: 2mg%, 4mg%, 6mg%, 8mg%, and 10mg% as given below:

3. Now measure the extinction of all the solutions at 210 nm taking distilled water as blank and adjusting the spectrophotometer to 100% T with blank.

4. Plot a graph of extinction against protein concentration (in mg %). You get a straight line graph passing through the origin.

5. Determine the concentration of protein in the unknown solution by extrapolation.

Tabulation

Wavelength	**Absorbance**

Result: