PRACTICAL 1

Aim- To study the general structure types and function of microscopes.

Introduction

1. Microscopy Used to study and observe objects and structures that are too small to be seen with the naked eye .
2. Microscopes are instruments which are lenses which use lenses and optical components to magnify and resolve tiny objects whose principle is totally based upon properties of light .
3. In early times people were using simple glass to magnify objects which later led to discovery of simple microscopes and later on compound microscopes in the 16th century.

Principle

Light microscope principle is based on general properties of light(visible light) and its interaction with the optical components including lenses to magnify or resolve microscopic objects .

Some major properties of light include :

1. Wave nature of light : light is an electromagnetic wave having specific wavelength and frequency (visible range : 380 nm -700nm)
2. Refraction: When a light ray passes through a different medium having different refractive index, it changes its path direction which is known as bending of light. It is a crucial aspect of lenses in light microscopy.
3. Focusing : Light microscope uses convex lens to focus all the incoming light in one point creating a magnified and sharp image .
4. Magnification:Total magnification of a microscope is the power of magnifying of both the lenses (objective and ocular)
5. Resolution. It refers to the ability to distinguish two objects close to each other. It is called resolving power which helps to create a clear and sharp image.

Types of microscope :

There are of two types of microscope:
- Light /optical microscope
- Electron microscope

Light microscopes are generally categorised into simple and compound microscopes .
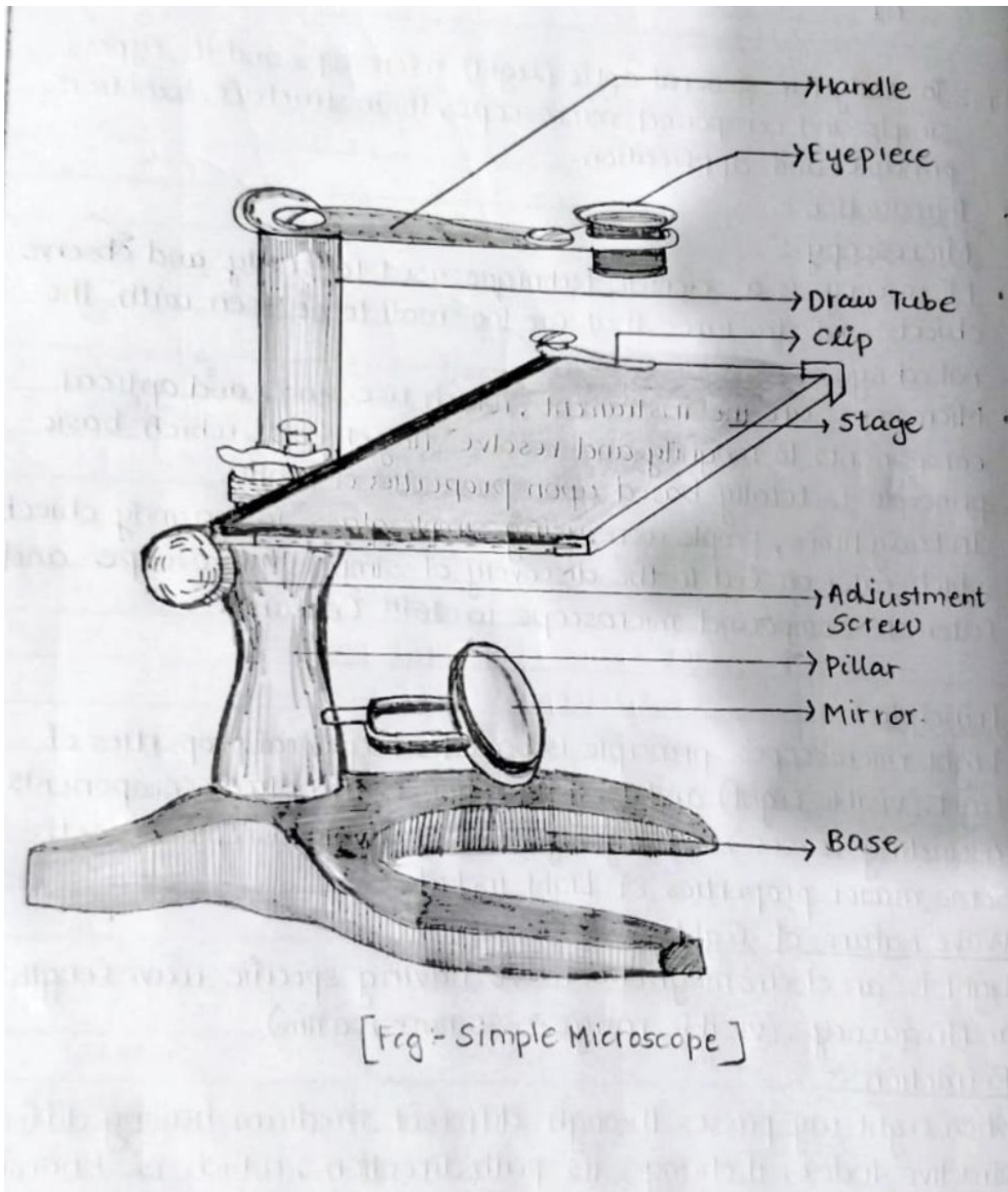
Simple microscope

- It consists of a single magnifying glass made of a single convex lens with a short focal length which magnify the object through angular magnification , thus producing an erect and virtual image of the object near the lens .

- If a tiny object is placed on the focal length of the microscope, it produces a magnified image.
- This has a general 10 X magnification power .

Structure

- Structure of a simple microscope is very simple .
- It has a body , stage made of thick glass for placing slides , a handle , an eyepiece placed with a handle and the mirror.
- The mirror is used for the reflection of the light source , illuminates the stage .
- There is also an adjustment knob by which the eyepiece may be moved upward and downward .
- The entire body stands on the base or foot .

Labels on figure:
→ Handle
→ Eyepiece
→ Draw Tube
→ Clip
→ Stage
→ Adjustment Screw
→ Pillar
→ Mirror.
→ Base

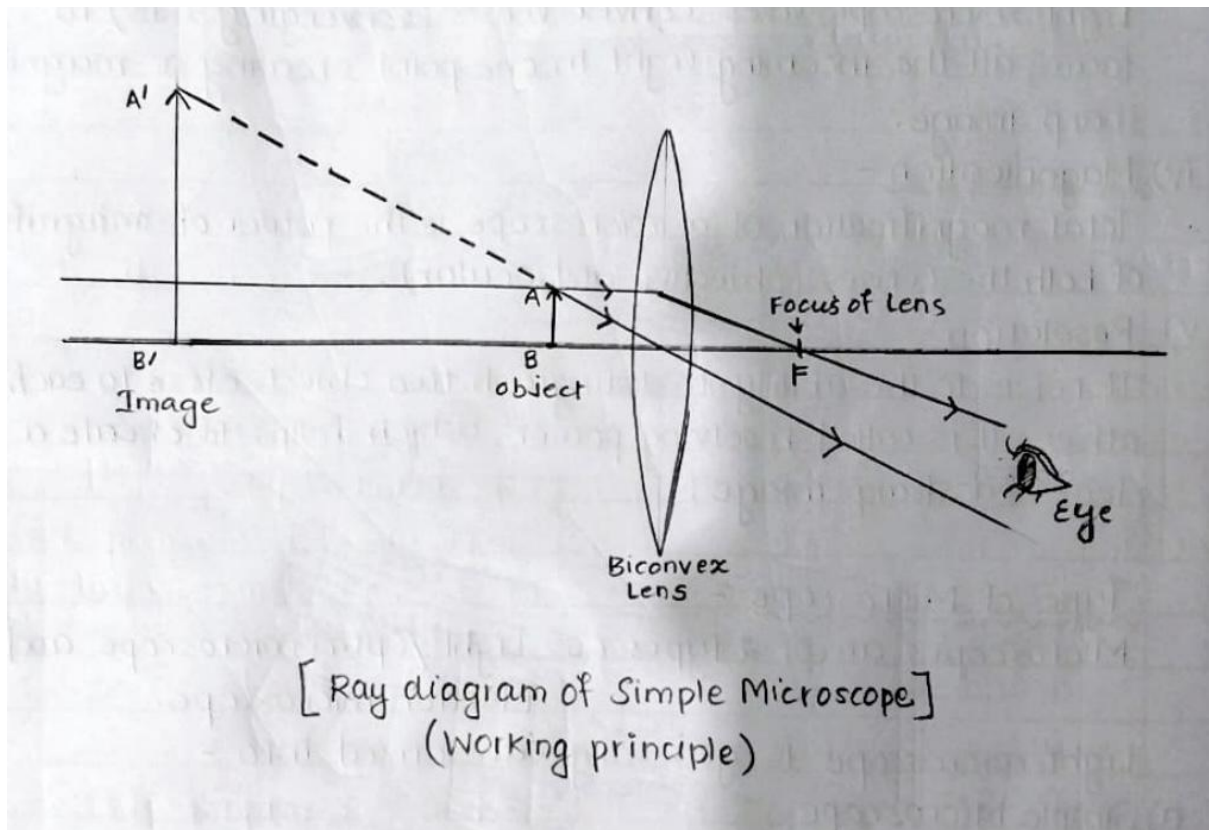[Fig - Simple Microscope]

Functioning

At first the slide is fixed on the stage with the help of clips .

The light should be focused on the slide by moving the mirror

Then keeping the eye at the eyepiece , the adjustment knob is moved and set to a position where the object is observed most clearly .

The specimen is carefully observed and dissected with a forcep or needle if necessary.

[Ray diagram of Simple Microscope]
(working principle)

Applications

Simple microscope are used in

- Botanical studies (to study, plant destruction and plant anatomy)
- Entomology and insect Observation (to study anatomy of insects arthropods- antennae , appendages, wing and other features.)

Compound Microscope

A compound microscope is a structurally quiet complex and functionally more advanced microscope than the simple microscope.

The different parts of a compound microscope is classified as mechanical parts and optical parts .
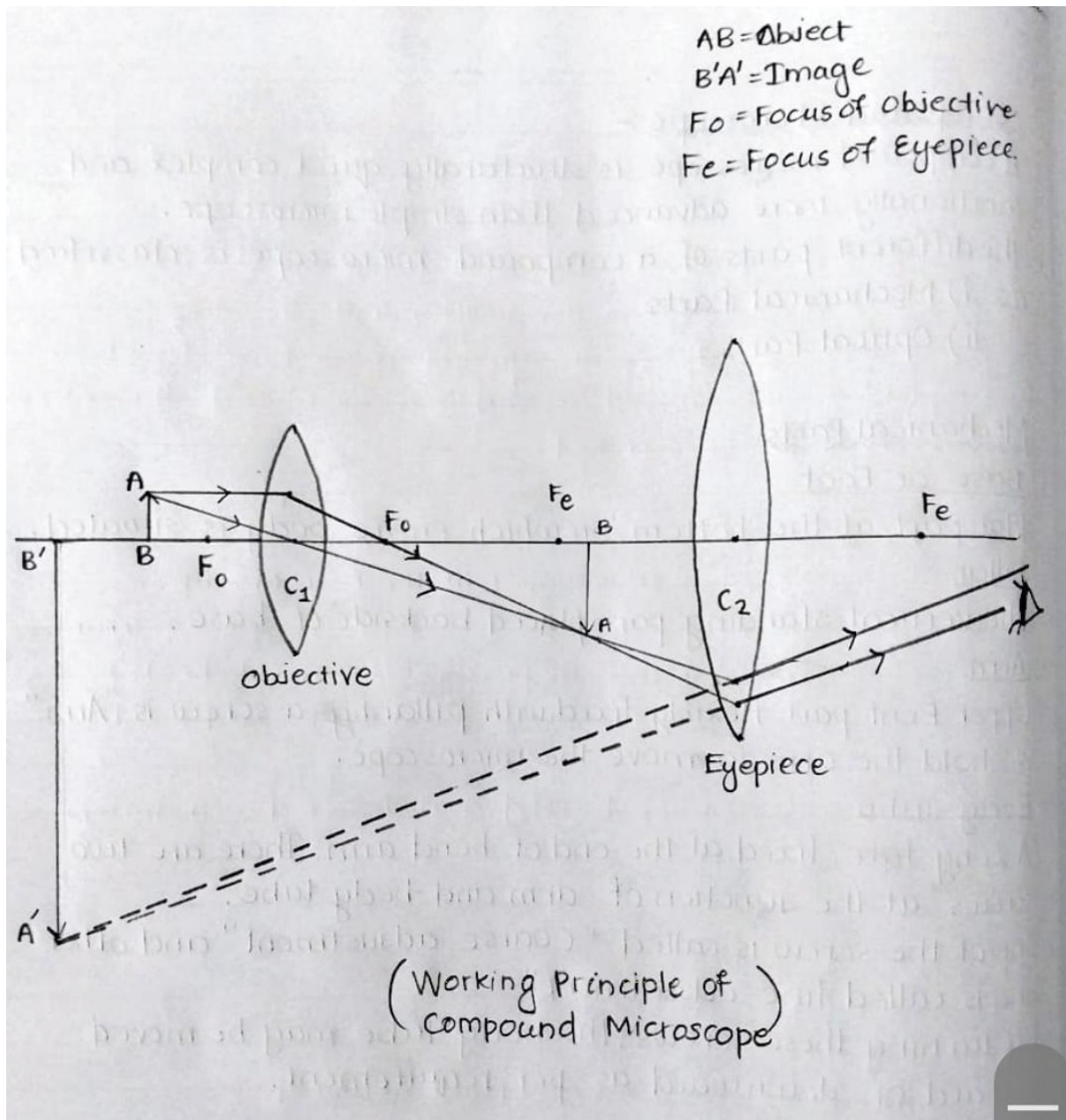
[Fig - Compound Microscope]
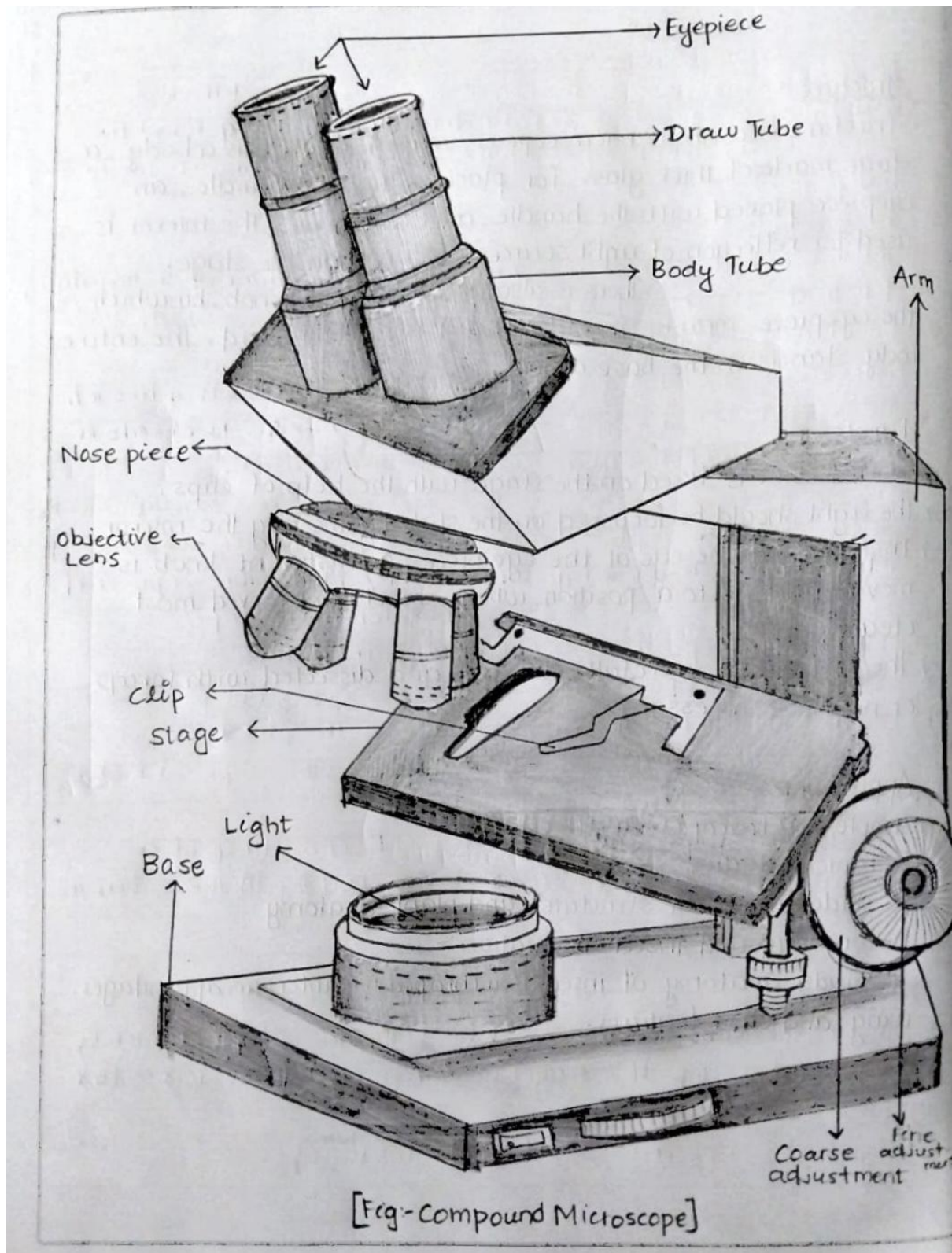
Mechanical parts

- **Base or foot** : flat part at the bottom on which the entire body is situated .
- **Pillar**. The vertical standing part , placed behind the base .
- **Arm** : upper bent part flexibly fixed with a pillar by a screw is "Arm" .We hold the arm to move the microscope .

- **Body tube** : A long tube , fixed at the end of the bent arm .These are two screws at the junction of the arm and the bodytube. One of the screws is called **coarse adjustment** and the other one is called **fine adjustment .** By turning these screws ,the body tube may be moved upward or downward as per requirement.
- **Draw tube** : This is inserted inside the body tube and maybe pulled upwardly when necessary .
- **Nose piece** : this is a circular body fixed at the lower end of the body tube. It is provided with three holes with spiral threads in its inner wall where objects of different magnification are attached.
- **Stage** : this is a rectangular plate not transparent attached with the arm at the base. There is a hole at the centre of the stage through which light is focused. Two clips are present to hold the slide properly.
- **Diaphragm** : by expansion and contraction of its part, as certains the amount of light to fall on the slide .

Optical parts
- **Eyepiece** : it is a small tube which can be inserted into the draw tube. There are two lenses fitted at its two ends. Keeping the eyes on this eyepiece , an object (specimen) placed on the stage is to be observed.
- **Objective :** there are also small tubes , like eyepieces and they are provided with lenses .Usually three objectives of different magnification are attached in three holes of the nosepiece .Magnification power is inscribed on the body of the eyepiece and objectives.
- **Condenser** : it is simply a combination of two lenses . It is attached beneath the hole of the stage . Light being converged through this condenser falls in the specimen placed on the slide.
- **Light source** : It is used as light source for illumination .

AB = Object
B'A' = Image
Fo = Focus of objective
Fe = Focus of Eyepiece.

(Working Principle of Compound Microscope)

Function

Light starts its journey at the base of the microscope from the source of illumination. Light travels upwards through the condenser and aperture , where it passes through the contents of the stage.

The image of the slide or specimen of the stage is picked up by the powerful magnification of the objective lens above it .(4x, 10x, 40x, and 100x)

Then light moves up the head of the microscope, reaches the eyepiece and is again magnified by the ocular lens (5x , 10x , 30x eyepiece is by far the most common)

Advantages

● It provides a detailed , sharp , 2d image of the subject.

- It comes fitted with their own light source .
- It is easy to use .

Disadvantage
- Magnification is limited i.e. 2000 X
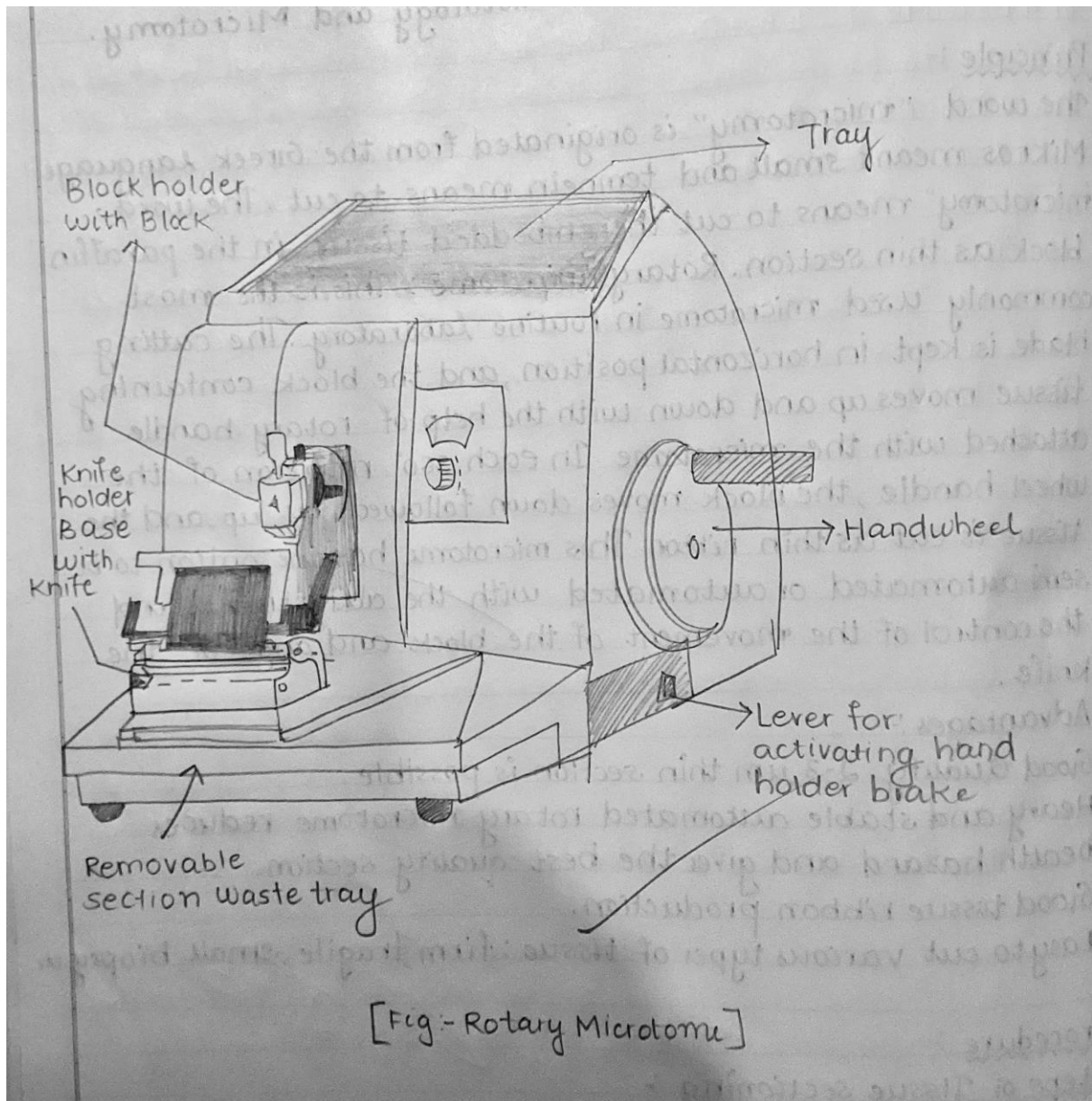
Practical 2

Aim : To study the structure and use of microtome (steps of histology)

Principle:

The word microtomy originated from the Greek language. Mikros means small and temnein means to cut . The word 'microtomy' means to cut the tissue in a thin section. It is the main instrument by which we cut the embedded tissue in the paraffin block as a thin section .

Rotatory microtome :

This is the most commonly used microtome in routine laboratories . The cutting blade is kept in a horizontal position and the block containing tissue moves up and down with the help of a rotary handle attached with the microtome . In each 360 rotation the block moves down followed by up and the tissue is cut as a thin ribbon .This microtome has the option to be semi automated or automated with the adjustment and the control of the movement of the block and the angle of the knife .

Block holder with Block

Tray

Knife holder

Base with Knife

Handwheel

Lever for activating hand holder brake

Removable section waste tray

[Fig - Rotary Microtome]

Advantages:
1. Good quality 2 to 3um thin section is possible
2. Heavy and stable automated rotary microtome reduces health hazard and give the best quality section
3. Good tissue - ribbon production
4. Easy to cut various type of(firm fragile small biopsy etc)

Procedure: (Steps of tissue sectioning)
1. **Trimming the tissue** : Trimming the tissue is needed to expose the tissue piece within the paraffin wax for cutting. This block is fixed in the chuck of the microtome and the paraffin is cut till the tissue is fully exposed.
2. **Cooling the block** : After the initial trimming the block are kept for cooling for 15-20 min . This will help to maintain the same consistency of paraffin and tissue .

3. **Cutting proper** : The block is fixed in the chunk of microtome . The cutting surface of the block should be parallel to the knife. The tissue in the blog is cut by gentle smooth slow stroke. The ribbon like tissue section are produced .The tip of the ribbon is held by forceps and the end part of the ribbon is removed from the knife as by brush.
4. **Floating the ribbon** : the ribbon of tissue is floated in the water bath and this makes the tissue flat and remove any wrinkling of the tissue the temperature of the water bath should be constantly maintained below the melting point of the paraffin wax.
5. **Picking up the tissue :** the slide is placed vertically within the water bath in front of the tissue and when the tissue is touched the slide is withdrawn vertically from the water to prevent any mix up the water bath should be clean immediately after cutting each block.
6. **Drying the section** : The slide containing the picked up section is kept in the slide rack. The slides are now kept in the hot oven to dry the temperature of the oven should be slightly more than the melting point of paraffin.
7. **Staining the section** : the slide with the tissue section are stained, and are required by the staining procedure. Example :- hematoxylin and eosin stain .

Practical 3

Aim: Preparation of buffer and determination of pH using PH metre.

A) Preparation of Buffer Solution

Introduction   A Buffer Solution is an acid or a base aqueous Solution consisting of a mixture of a weak acid and its conjugate base or vice versa. Its pH changes very little when a small amount of Strong acid or base is added to it. Buffer Solutions is used as a means of keeping pH at a nearly Constant value in a wide variety of chemical applications .

Principles of Buffering : Buffer Solutions resist pH changes because of a chemical equilibrium between the weak acid (HA) and its conjugate base(A)
and

$$HA \rightleftharpoons (H+) + (A-)$$
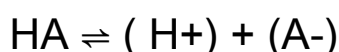
Types of Buffer
(1) Acidic buffer :
It has a pH lower than 7. The acidic buffer contains a weak acid and its conjugate base as salt . To change the pH of the solution we need to after the ratio between the acid and the salt . An example of a weak acid and its conjugate base/ salts is acetic acid and sodium acetate.

$$CH3COOH \longrightarrow (CH3COO-)aq. + (H+)aq.$$
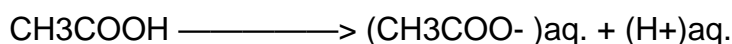
(2) Basic buffer :
Basic buffer has a pH of more than 7 . The basic buffer contains a weak base and conjugate acid as a salt . A weak base and it's conjugate acid is ammonia and ammonium chloride. The equilibrium is heading towards the left when we add ammonium chloride ammonium will sum up the solution. And at this instance Le chatelier principle will reach to the left side .

$$NH3 + H2O \longrightarrow (NH4+) + (OH-)$$
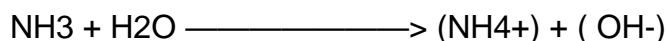
Preparation of Buffer :
By controlling the ratio of salt acid or salt base we can prepare a buffer solution if association constant (PKa) of acid and (PKb) of base are known .

Properties of Buffer solution:
Buffers are well known to avoid the change in pH but somehow if any adequate preparation of strong base or acid is added it can change. Buffer capacity is a rise in

the total concentration of the particles of the buffer solution. Therefore , it has a specific pH value .

FORMULA

Formula

$$Weight\ (in\ gm) = \frac{Molecular\ weight \times Required\ Molarity \times Required\ volume}{1000}$$

Q. Make a Buffer solution of 2 milimolar, pH of 7.4 and 500 ml Tris Buffer of molecular weight → 121.14.

Ans:- Required Molarity = 2 milimolar = 0.002 Molar

pH = 7.4

Volume = 500 ml .

Molecular weight = 121.14

$$Reqd.\ weight\ (in\ gm) = \frac{121.14 \times 0.002 \times 500}{1000} = 0.12114\ gm.$$

0.12114 gm. Tris Buffer is first dissolved in 200● ml. distilled water.

HCl is added to maintain the pH 7.4.

After maintaining it, total volume is made to be 500ml.

Another Formula, $\dfrac{C_1}{V_1} = \dfrac{C_2}{V_2}$.

B) Measurement of pH using pH metre :

Introduction

The biochemical experiments are done using buffered solution ,since many reactions are very sensitive to the pH and some reactions use or produce H+ ions .

Buffer is a solution whose pH does not change very much when small amounts of acid (H+) or base (OH-) are added. This does not mean that no change occurs, only that it is small compared to the amount of acid or base added the more the pH will change.

$$pH = pKa + \log \frac{[\text{base form}]}{[\text{acid form}]}$$

pH Meter and pH electrode :

- The most commonly used electrode is made from borosilicate glass, which is permeable to H+, but not to other cations and anions.
- Inside is a 0.1M HCl solution; outside there is a lower H+ concentration; thus the passage of H+ ion from inside to the outside. This leaves negative ions behind, which generates an electrode potential across the membrane.

$$V = E_{constant} + \frac{2.303\, RT}{F}\, pH$$

$\hookrightarrow (Eq. \to 3.2)$

V = Voltage
R = Gas constant.
T = Absolute Temperature
F = Faraday's constant.
E = Referrence Electrode for potential.

- A pH measurement is usually taken by immersing a glass or plastic combination electrode into a solution and reading the pH directly from a metre. At one time, pH measurement requires two electrodes.
- ➜ a pH-dependent glass electrode sensitive to H+ ions
- ➜ a pH-independent calomel reference electrode.
- The potential difference that develops between two electrodes is measured as a voltage.
- A pH metre is standardised with buffer solutions of known pH before a measurement of an unknown solution is laken.
- It should be noted from Equation -3.2 that the voltage depends on temperature.
- Hence the pH metre must have some means of temperature correction.
- Older Instruments usually have a Knob labelled `Temperature control', which is adjusted by the user to the temperature of the measured solution. Newer ph metres automatically displays a temperature corrected pH value.
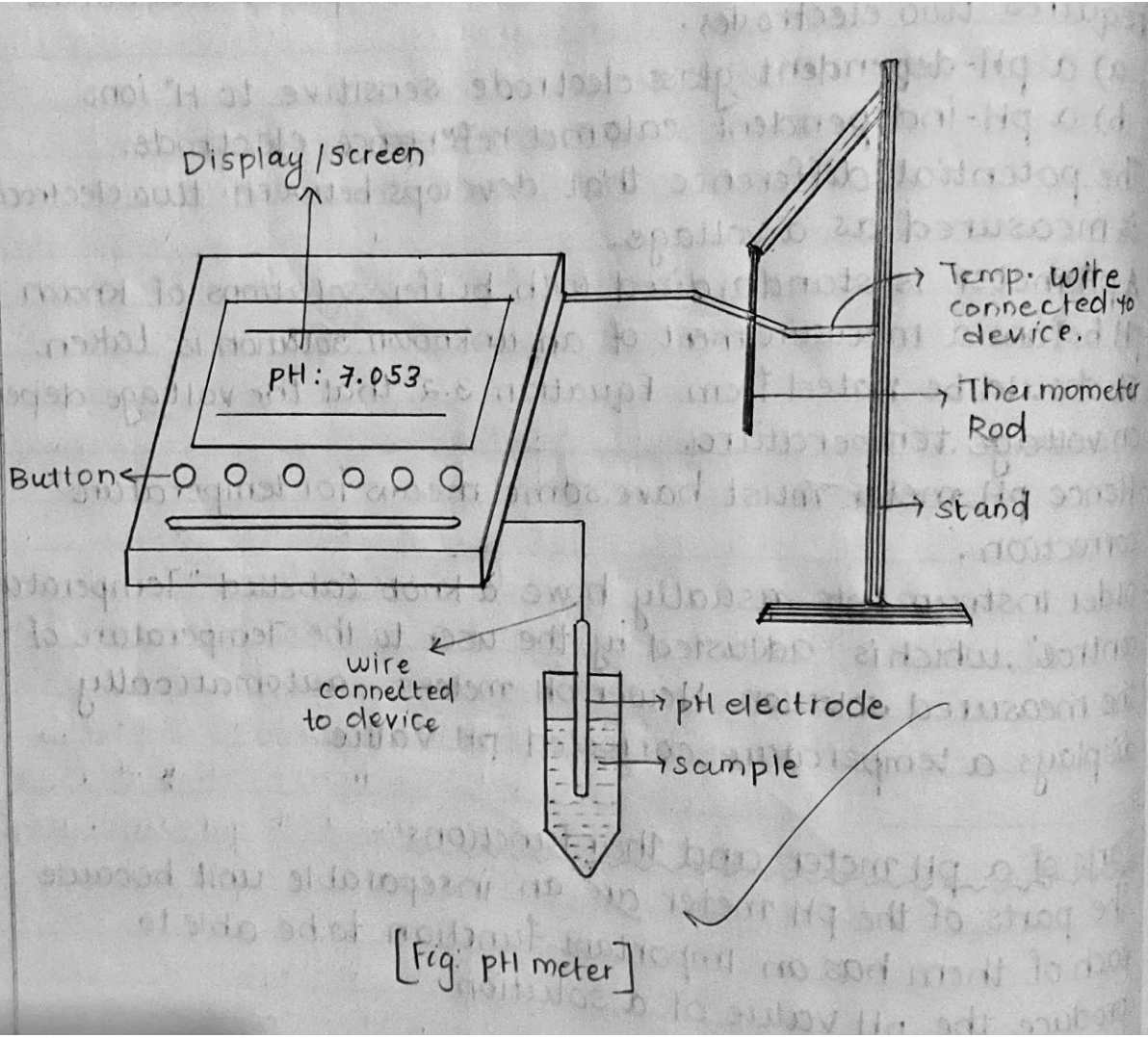
Parts of a pH meter and their functions:-

The parts of the pH metre are an inseparable unit because each of them has an important function to be able to produce the pH value of a solution.

1. **Glass Electrode** : Between the two electrodes immersed in the solution, the glass electrode component acts as one of the poles. This component also consists of a bulb or a round layer at the end. Its function is to become a place

for positive ion exchange which causes a potential difference between the two electrodes under test.

2. **Reference Electrode:**- The reference electrode is the opposite pole of the glass electrode. When these two electrodes are energised and immersed in a type of solution, an electrical circuit Is formed that determines the pH level of the solution.

3. **Thermometer**: Another component that must be owned by a pH meter is a temperature sensor or thermometer. This is in accordance with the principle of calculating the degree of acidity which can be affected by the temperature of the solution being tested. If the solution has a neutral pH of 7, the change in temperature is not considered to have an effect.

4. **Amplifier** :In parts of a pH metre, a voltage amplifier better known as an amplifier - is an important component that plays a role in determining the pH value. Just as a thermometer amplifier's calculations involve temperature, the amplifier will also make the pH reading more accurate.

5. **Microprocessor** : In a pH metre working system, the microprocessor plays a role in translating the voltage value of the amplifier, calculating the temperature measurement, and reading the pH value of a solution. In other words, this component will ensure that all processes carried out by other components are running properly so that it displays the pH value of the solution on the LCD screen contained in a pH metre unit.

Display /screen

pH: 7.053

Buttons

Temp. wire connected to device.

Thermometer Rod

Stand

wire connected to device

pH electrode

sample

[Fig: pH meter]

Practical 4

Aim- To study about UV-visible Spectrophotometry.

Theory

A UV-visible spectrophotometer is a scientific instrument used for analysing the absorption and transmission of light in the Ultra-violet (UV) and Visible (Vis) regions of "Electromagnetic spectrum ."

The Electromagnetic spectrum is composed of a continuous range of waves (light) having specific wavelengths and energy. These are X-rays (upto 7 nm.), Ultraviolet ray(180-340 nm.), visible (340-800 nm.), Infra-red (1000-100,000 nm) and finally radio waves (10-10,00,00,00,000nm).
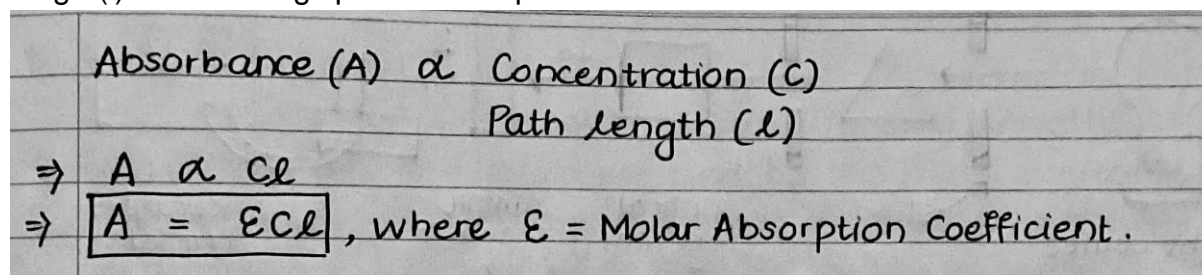
In this experiment, we will focus on UV and Visible regions and their absorption by molecules, which helps in measuring concentration of the solution.

Principle

UV-visible Spectrophotometry is based on the principle that uv and visible range light / wave have sufficient energy to elicit excitation of valence electrons of a molecule to a higher energy state. This causes absorption of energy as light. The amount of light absorbed is directly proportional to the concentration of the absorbing substance.

Beer-Lambert's Law:-

This Law forms the fundamental basis of UV-vis spectro-scopy. It states that Absorbance (A) of a solution is directly proportional to Concentration (C) and Path length(l) of absorbing species / sample.

$$\text{Absorbance (A)} \propto \frac{\text{Concentration (C)}}{\text{Path length } (l)}$$

$$\Rightarrow A \propto Cl$$

$$\Rightarrow \boxed{A = \varepsilon Cl}, \text{ where } \varepsilon = \text{Molar Absorption Coefficient.}$$

- Light has wave as well as particle nature. Light is quantified into its smallest unit called "Photon" , which are small packets containing energy .
- When a photon of specific energy interacts with a molecule, one of the two processes may occur: Either the photons may be scattered with unchanged frequency (called Rayleigh Scattering) or it may transfer its energy to the molecule, producing an excited state of the molecule (called Absorption)
- UV -vis spectroscopy primarily deals with electronic transitions.

- When a molecule absorbs UV or visible light, it promotes an electron from the ground state to an excited state. The energy difference between the ground and excited states corresponding to a specific wavelength is used.
- A Spectrophotometer is a device which uses light intensity to measure the absorbance of light by particles.

Spectrophotometer has some Key components and following parts :-

1. **Light source**: Light source emits a broad spectrum of light, typically including Ultraviolet (UV) and Visible (Vis) wavelengths.The choice of light source can vary. c.g. - tungsten-halogen lamps for visible light and deuterium lamps for UV rays .
2. **Monochromator** : It Is responsible for selecting a specific wavelength of light from the broad spectrum emitted by the light source. It does this by diffracting the light and allowing only the desired wavelength to pass through to the sample,
3. **Sample compartment** : It holds the sample that is being analysed.
4. **Cuvette or Sample Holder :** cuvette is made of glass or quartz and is designed to be transparent to the wavelengths being measured. The sample holder ensures uniform and consistent light transmission through the sample.
5. **Detector** : It detects the transmitted light after it passes through the sample.Such as → Photomultiplier tubes (PMTs) ,Charged-coupled Device (CCDs) .They convert the optical signal into electrical signal for analysis.
6. **Wavelength selector / Grating** : This component allows for the adjustment of the wavelength of light used for analysis.
7. **Beam Splitter :** It splits the incident light into 2 paths; one passes through the sample and other bypasses the sample.
8. **Control and Data Analysis Unit :** A control unit, operating spectrophotometer.
9. Display and output devices : A display showing real time information about measurement.
10. **Power Supply** : It provides electric power to the instrument.

[Fig :- Spectrophotometer (UV-Vis)]

[Fig :- Cuvette]
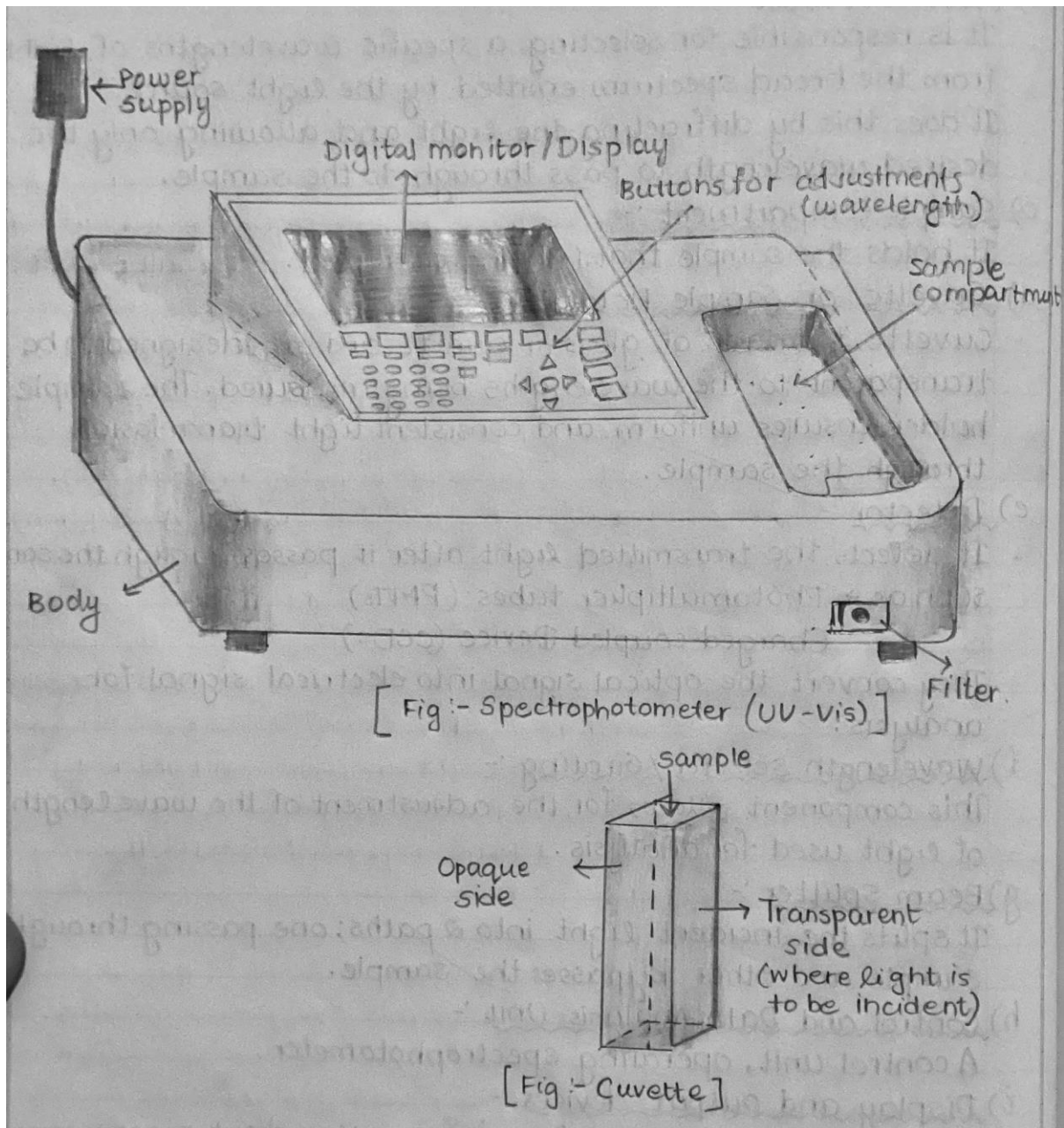
Application:-

1. Quantitative Analysis of biomolecules
2. Qualitative Analysis biomolecules
3. Pharmaceuticals for drug analysis
4. Environmental monitoring
5. Biochemical Assay
6. Food and Beverage Industry
7. Material science and Forensic Analysis.

Practical 5

Aim- Characterization of protein and nucleic acid by Gel electro-phoresis.

Theory

Get Electrophoresis

- Gel Electrophoresis is a widely used technique in molecular biology and biochemistry that helps in the separation of macromolecules such as Nucleic Acid (DNA, RNA) and proteins based on their size and charge.
- This technique was invented by A. Tiselius in the 1930s.
- Modern electrophoresis technique involves a "polymerized gel-like matrix, which acts as a support medium.
- Migration of molecule is influenced by
  I. Size, shape, charge and chemical composition of molecules.
  II. Rigid, maze-like matrix of gel.
  III. Applied electric field.

Introduction:

The movement (velocity V) of a charged molecule (q) in a medium subjected to electric field (E) is represented by -

$$V = \frac{Eq}{f}$$

, where f = frictional coefficient
E = Electric Field (Volt/cm.)
q = Net charge.
V = velocity.

The movement of charged particle in a sample subjected to electric field(E) is represented as :-

$$\mu = \frac{V}{E}$$

μ = mobility (It is the velocity per unit electric field)

This equation can be modified as $\mu = \frac{Eq}{Ef}$ $\Rightarrow$ $\boxed{\mu = \frac{q}{f}}$

Method of Gel Electrophoresis

The major difference among the various methods is the type of gel used.

The gel matrix is generally made from either agarose or polyacrylamide, the choice depends upon the size range of biomolecules.

Ex:- Cellulose and Cellulose acetate gels → low molecular wt. biomolecules

(Amino Acids, Carbohydrates)

Agarose Gel → Larger DNA Fragments.

Polyacrylamide Gel → higher resolution separation of smaller DNA

Composition of Gels :-
I) Agarose Gel:- Agarose is a natural polysaccharide derived From
Sea-weed. It is mixed with a buffer solution and heated to form a solid gel when
cooled.
II) Polyacrylamide Gel - Polyacrylamide gels are formed by polymerizing
acrylamide and a cross-linking agent , such as bisacrylamide , in the presence of a
catalyst like ammonium persulfate and a reducing agent like "TEMED" (N,N, N, N'-
Tetramethylethylenediamine)

Procedure -
a) Preparing the Gel
- Appropriate type of gel is chose on the basis of size range of bio-molecules
  that are to be separated.
- Weigh the required amount of agarose or polyacrylamide powder and different
  percentages of gels are prepared. Generally, agarose gels are prepared at
  0.5% to 2%, while polyacrylamide gels range from 6% to 15%
- Powdered acrylamide or agarose is mixed with a suitable buffer solution [TAE
  (Tris-Acetate - EDTA) or TBE (Tris-Borate-EDTA) for DNA electrophoresis.
- It is heated, stirred and dissolved completely. Polymerization is done by
  addition of catalyst Ammonium persulfate and a reducing agent like TEMED.
- Melted gel solution is poured into a "Gel-casting tray. Insert a comb at one
  end of the tray to create wells for sample loading .
- Alow the gel to solidify by cooling at room temp or in a ger-casting apparatus

b) Sample Preparation -
- DNA /RNA/Protein sample is prepared and mixed with "loading buffer"
  containing tracking dyes for visualization and density agents, to ensure that
  samples sink into the well.
- Then It is heated for a Few minutes at 65-70°C.

c) Sample Loading:-
- Carefully remove the comb trom the gel tray
- Load the samples into the wells using micropipette
- Include molecular weight markers (DNA ladders).

d) Electrepheresis -
- Submerge the geltray in electrophoresis chamber filled wih electrophoresis
  buffer.
- Connect the electrodes of the power supply to the gel tank.
- Red electrode (Anode) → should be placed al the end of sample loading
- Black electrode (Cathode) > should be placed at the opposite end.
- Set the desired voltage and run time on the power supply.
- For agarose gel, 100-150 V is typical.

e) Running the Electropherers: -
- Turn on the power pack to supply electric field across the gel.
- Molecules move through gel towards anode (positive electrode)
- The time period can vary from 20 mins to few hours.

f) Visualization -
- After the process, turn off the power supply and carefully remove the gel from the chamber.
- Stain the gel to visualize the separated molecules.
- For DNA, common stains like ethidium bromide or SYBR Green are used.
- Place the gel on a UV-transilminator and take a photograph.

Analysis-
1. Measure the distance between separated bands.
2. Size, thickness of bands are Studdied.
3. Desired strands are isolated.
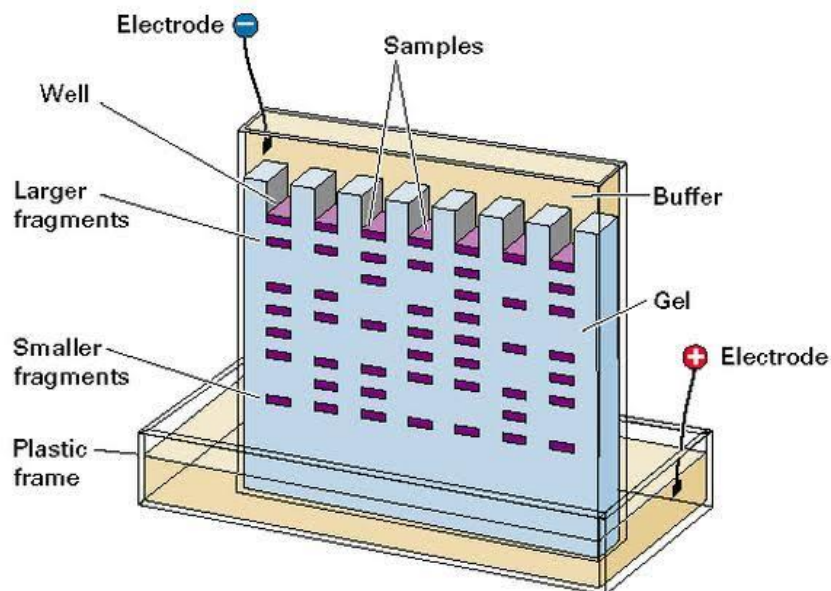
Practical 6

Aim - Purification and Analysis of Biomolecules (proteins) by paper chromatography.

Principle:-

Chromatography, the most important technique for isolating and purifying biomolecules , was developed by Mikhail Tswett in 1902. All types of chromatography are based on a very simple concept: the sample to be examined is allowed to interact with two physically distinct entities - " a mobile phase" and a stationary phase". The sample mast often contains a mixture of several components to be separated. The molecules targeted for analysis are called "analytes".

The mobile phase, which may be a "liquid / gas" , moves the sample components through a region containing the solid or liquid stationary phase, which is called the "sorbent".

Stationary phases differ according to chromatography types and it has the ability to bind some types of analytes.

The molecular components in the sample, distribute themselves between the mobile phase and the sorbent and thus have the opportunity to interact intimately with the stationary phase. The molecules (analytes) which show more affinity towards the sorbent, spend more time with the mobile phase and are retarded In their movement through the chromatographic system.

Molecules that show weak affinity for the sorbent spend more time with the mobile phase and are more easily removed or eluted from the system.

The general process of moving a sample mixture through a chromatographic system is called development.

The mobile phase, now called "effluent" contains the purified analytes, which are purified or isolated.

The purpose of chromatography is to obtain purified material for further characterization and studies.

Paper Chromatography

It is a kind of partition Chromatography , which is used
for separating proteins and amino acids based on their molecular characteristics.

Requirements:

a) Filter paper strips (rectangular)

b) sample solution: It contains the mixture of proteins and amino
acids (analytes), which is dissolved in a suitable solvent.

c) Solvent: It include includes water, methanol or a mixture of
these.

d) Chromatography chamber - A container / Beaker to hold the
Solvent.

e) Cover for chromatography tank.

7) staining reagents - This is used for visualisation of proteins or amino acids.

g) Pipettes or capillary tubes

h) Ruler for measuring

t) Laboratory gloves.

* *Solvent preparation: - 20 ml. Butanol + 5ml. Acetic Acid + 25 ml Water.*

* *Staining Reagent * Ninhydrin (0.37 gm + 100 ml Alcohol)*

Procedure -

1) Preparation of Chromatography Chamber :-

- Firstly , Filter papers are cut into rectangles, which fit into the chamber (Beaker) without touching the walls.
- Solvent is prepared and taken in the Beaker.
- Filter paper strip is placed in such a way that it barely touches the solvent.
- Close the tank with alid to prevent evaporation.

2)Spotting the sample solution :-

- Mark a line 1.5 cm above from the bottom of the rectangular paper strip and mark 3 points, each from the same distance.
- Use a capillary tube to spot a small amount of amino add solution onto the baseline of the chromatography paper strip.
- Repeat for each of the three amino acids.

3)Chromatography Development :-

- Place the spotted paper strip into the chromatography tank, ensuring that the baseline of the chromatography paper
- The solvent is allowed to move up the paper by capillary action until it reaches near the top of the strip.
- This may take about 1-2 hours maximum.

4)Drying the paper :-

- Air Dry the paper till it dries completely.

5) Staining :-

- Spray the dried paper with ninhydrin solution .
- The amino acids will react with ninhydrin, producing coloured spots(Blue, purple , yellow).

6) Visualization:-

- Observe the appearance of coloured spots on the paper.
- Record the Retardation Factor (Rf) values for each amino acid.
- The Rf value is calculated as the distance travelled by the amino acid divided by the distance travelled by the solvent.

[Rf = Distance travelled by amino acid/Distance travelled by solvent.]

- Measure the distance From baseline to each amino acid spot and the total distance travelled by the solvent.
- Calculate the Rp values and plot a standard graph.

Calculation -

Distance travelled by solvent = 5.9 cm.

Amino Acid samples (A, B,C) covered the following distance

(A) → 1.3 cm

(B) → 3.3 cm

(C) → 2.8 cm

Rf values will be (A) → $R_f = \dfrac{1.3}{5.9} = 0.22$

(B) → $R_f = \dfrac{3.3}{5.9} = 0.55$

(C) → $R_f = \dfrac{2.8}{5.9} = 0.47$

Result

The obtained Rf values were compared to the standard Rf values and amino acid sample A is found to be **Aspartic acid** , B is found to be **methionine** and C is found to be **tyrosine .**