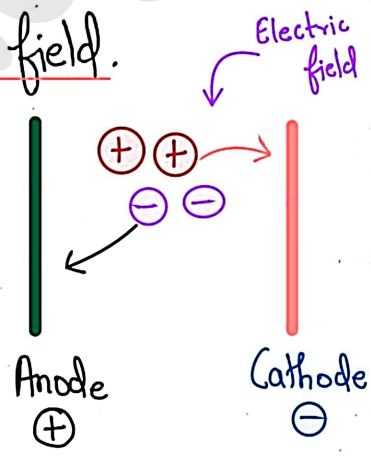
Electric field. ← Electrophoresis. → Migration.

- Electrophoresis Can be defined as Electrophoresis is a Method of Separation where Charge molecule Migrates in Differential speed when we applied Electical field.

Electro Phoresis
↓ ↓
Electricfield Migration.



Principle →

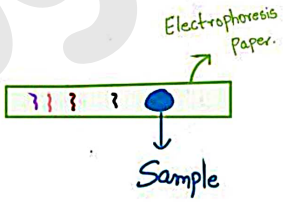
- The Charged molecule under the influence of electric field Migrate towards oppositely Charged electrodes.

- Those molecules who have ⊕ve Charges move towards Cathode & ⊖ve Molecule move towards Anode.

☆ But the movement is Influenced by Molecular weight & Charge on the molecule. 1. Charge ∝ Mobility 2.

2. Electrophoretic mobility ∝ $\frac{1}{\text{Mol. wt.}}$

- If Charge on Molecule is more then Migration is faster.
- If Charge on Molecule is less then Migration is Slower.
- More molecular weight Molecule Migrates Slowly
- Small molecular weight molecule Migrates faster.



☆ Iso, when a mixture is placed on the electrophoresis paper or Agarose gel different bands are seen along the paper after process.

$\mu = \frac{Q}{6\pi r \eta}$

- μ → Electrophoretic mobility.
- Q → Charge of the molecule.
- η → Viscosity
- r → radius of Molecule (cm).

Types of Electrophoresis.

1. Slab Electrophoresis
   - further divided into 3 types (Based on principle Used for separation)
     - ⓐ Zone electrophoresis.
       - ① Paper Electroph.
       - ② Gel Electrophoresis.
     - ⓑ Isoelectro-focusing
     - ⓒ Immuno electrophoresis
2. Capillary Electrophoresis

3.

* Factor affecting Electrophoretic Mobility.

The following factors which affect Migration of Ion are as follows →

1. Charge of Ions →

Electrophoretic Mobility ∝ Charge of the Molecule.

Example → X^+ & X^{+2}

X^{+2} moves faster as Compared to X^+.

2. Size of the Ion →

$$\text{Electrophoretic Mobility} \propto \frac{1}{\text{Size of Ions/Molecule}}$$

3.) Viscosity of Medium →

$$\text{Electrophoretic Mobility} \propto \frac{1}{\text{Viscosity of Medium}}$$

4. Voltage applied → Higher the Voltage applied faster the separation of Band.

But Voltage ↑ then Heat generated ↑, So we have to prevent evaporation of Buffer/Solvent by diff. Methods.

5.) pH of Buffer & Ionic Strenght →

4.

$$\text{Ionic Strenght of Buffer} \propto \frac{1}{\text{Migration of Compound}}$$

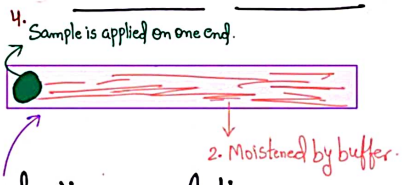
6) Temprature →

$$\text{Electrophoretic Mobility} \propto \text{Temprature}$$

7) Electrophoretic Mobility also depends on the Shape of Molecules.

ELECTROPHORESIS TECHNIQUES

1. Paper Electrophoresis →



- It is a technique which employ a 1. Whattman filter paper No.1 which is moistened by a buffer & 3. then Connected at two ends to two opposite charged Electrode.

* Then sample is applied on the one end & 5. let for separation of Component Under Electric Gradient.

6. After separation paper is dried & Stained.

5. • After separation, the paper is dried & stained to get Colour band

Components of Paper Electrophoresis →

• In Paper electrophoresis, 1. Paper is Used as the Supporting medium.

2. • Normally Whatman filter Paper (Grade 3MM or No. 1).

3. • The paper is Used to be washed with distilled water followed by 0.1M HCl or 0.01M EDTA to remove Impurities.

(ii) Electrodes & Voltage to be applied. →

• The 1. electrode in the form of a thin wire made up of Carbon or Platinum.

• A 2. DC voltage of about 8-15 V/cm lenght of paper is normally applied.

✱ There are two types of Paper Electrophoresis based on the Voltage applied → (i) Low Voltage electrophoresis ↓

(ii) High Voltage Electrophoresis →
The Voltage across the two electrode 50 – 215 V.

The Voltage across two electrode is about 100–300 V.

(iii) Buffers → 6.

- Buffers of different pH & Ionic strenght are Used in Separation Process.
- The pH of buffer to be Used depend upon the types of Compound to be separated.

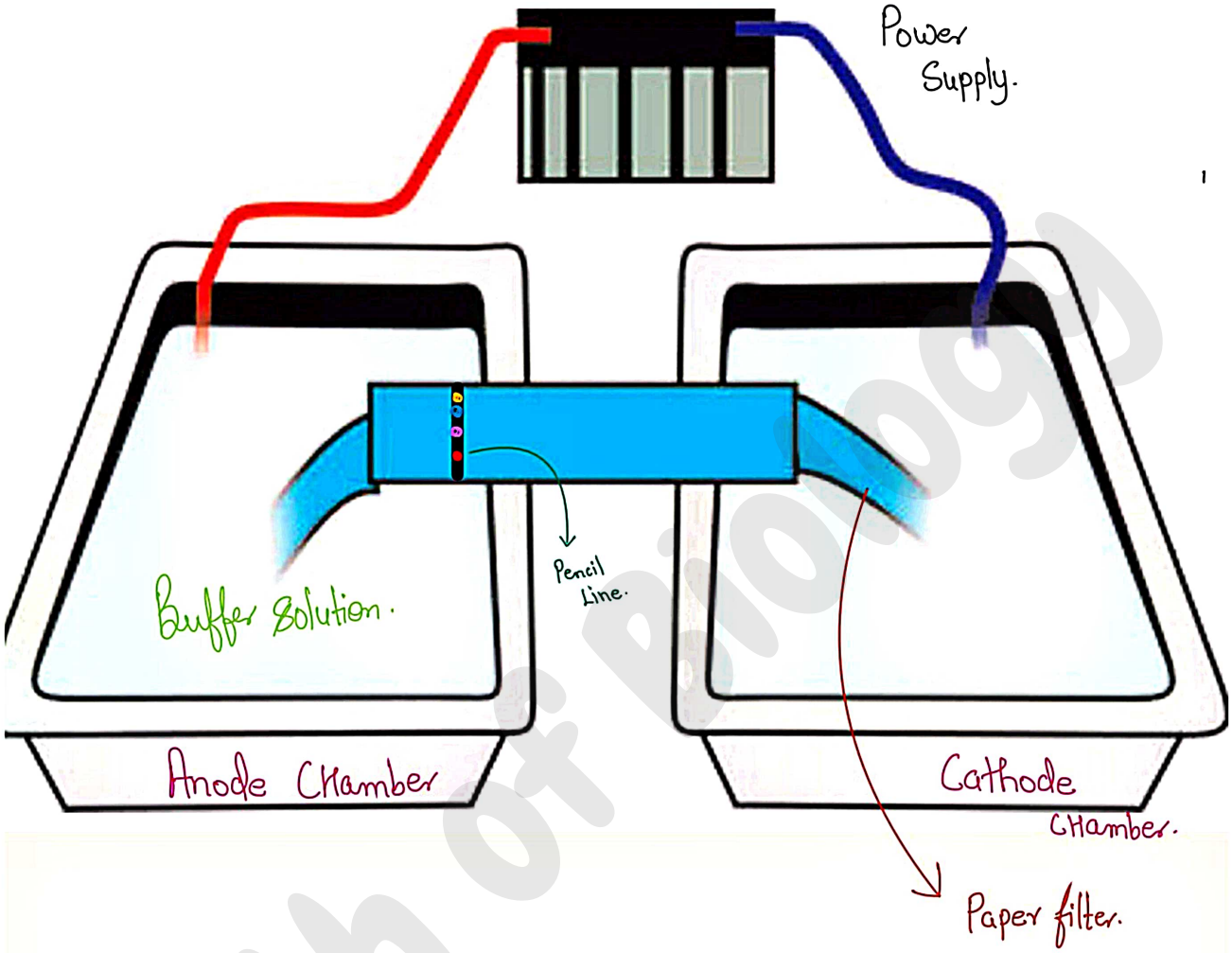
Example → Citrate Buffer, Barbitone Buffer.

Advantages → ① The technique is easy to Handle.

② The Cost of Instrument is low (Because only paper & Buffer is Used)

③ Numbers of sample can be separated on a single paper at a time.

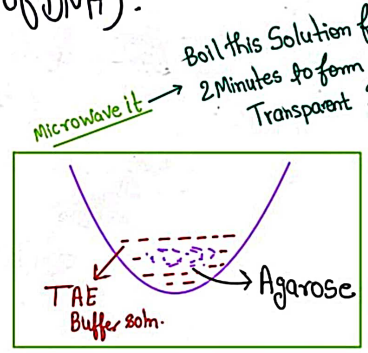
Disadvantages →
- The technique requires more separation time.
- Use of High Voltage may be dangerous so proper precaution should be taken.



2. Gel Electrophoresis

- It is a Separation Technique in which we Separate Molecules (DNA, RNA, Proties) on the basis of their Size.
- Here Gel is Used for Separation of Molecule So, it is Called Gel Electrophoresis.
- Here we apply electric Current on gel So, molecule Separate.
- Gel → Agarose Gel. ⇝ Large pore Size ⇝ So, Used for large molecule Separation (DNA, RNA)
  → Polyacrylamide Gel.
  ↓
  Smaller Pore Size.
  ↓
  Used for Separation of Small molecule (Protein & Small fragment of DNA).

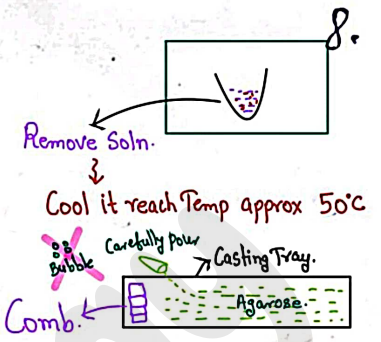
I. We Have Sample
↓
Then IInd step is Agarose gel formation
↓
Agarose weigh & dissolve in TAE buffer Solution & microwave it.
↓
Boil this solution for 2 minutes to form a Transparent Soln.



- Then remove the solution from Microwave

↓

Then Cool it approx when it reaches 50°C



↓

Then transfer this Agarose Soln. to Casting Tray of Electrophoresis Unit.

↓

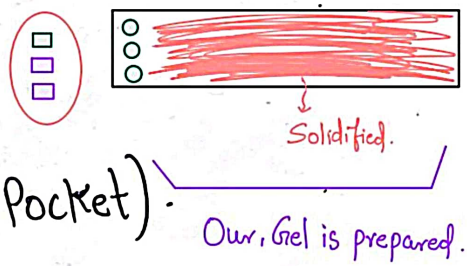
Apply the Comb in Casting Tray the finally you have to pour the Agarose Soln. (Pour Carefully To prevent bubble formation)

↓

Kept it in room Temprature for 15-20 minutes, Untill it has Completely Solidified.

↓

Then remove the Combs, so after removing the Combs well is formed (Hollow Pocket).



↓

Apply the sample in ⟶ (Hollow Pocket).

↓

When we apply sample then finally Our Gel is prepared.

9.

Then placed this Gel into Electrophoresis Unit.

↓

The Well (Hollow Pocket where Sample already Introduced) of Gel must be ⊖nt at Cathode side

↓

Because DNA is ⊖ve Charge & It runs towards Anode when electric field is applied.

↓

Then fill the TAE buffer in Electrophoresis Unit. (to prevent dryness of gel).

✡ Before loading the Sample Sample is prepared Mean We add Loading dye & Glycerol.

- Loading dye is add to know the position (actual) of Sample.
- DNA is lightweight so it can float outside So, we Use Glycerol. DNA is settle down Now because Glycerol is Viscous Solution.

- The Sample loadin Well. ⇝ Then Close the Electrophoresis Unit. 10.

& attach a Voltage meter with Electrophoresis Unit - & with the help of this we provide Current (80-150 Volt).

↓

DNA strand moves in gel.

↓

& then we go for U.V Visulisation (less Intensity of U.V used).

In gel we add EtBr & EtBr bind with DNA & it provide fluorescence in U.V Light.

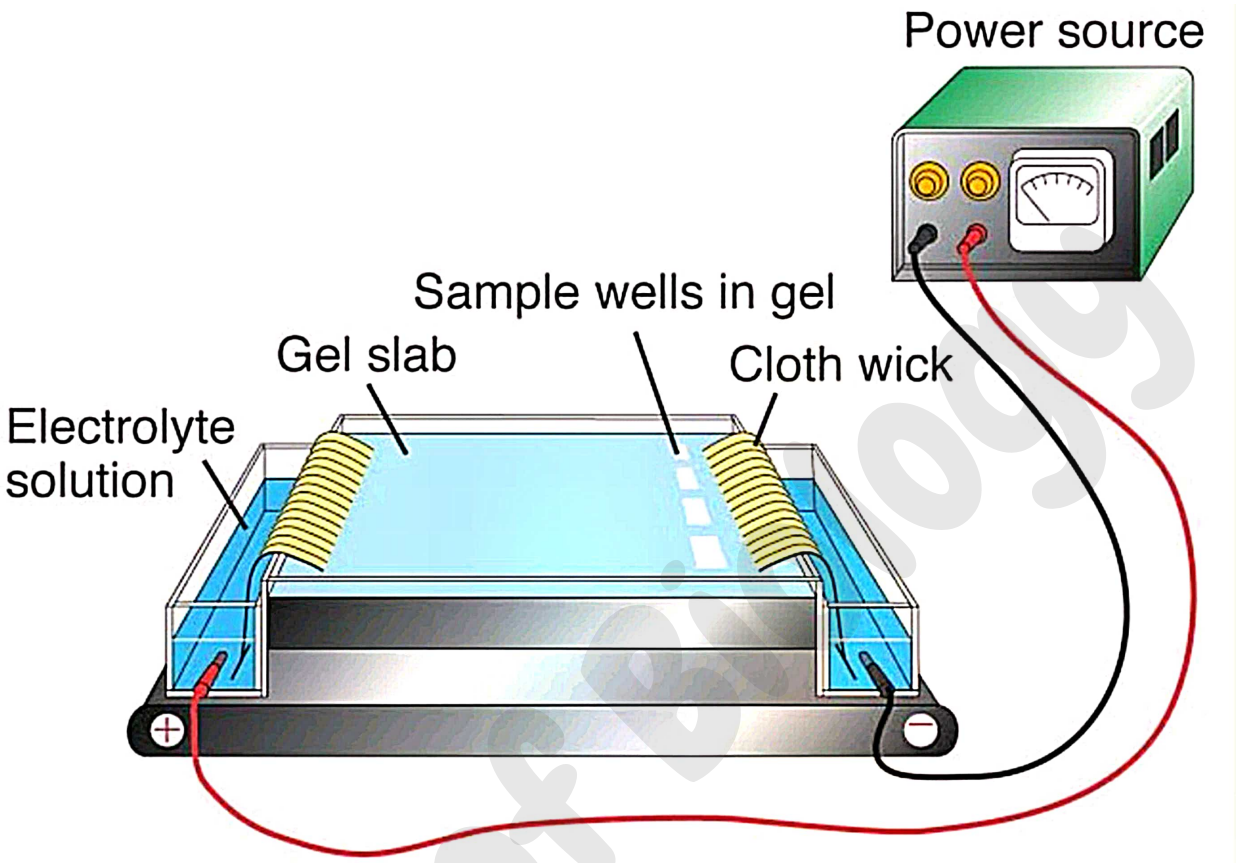
Advantages → ① Separation is more efficient then Paper.

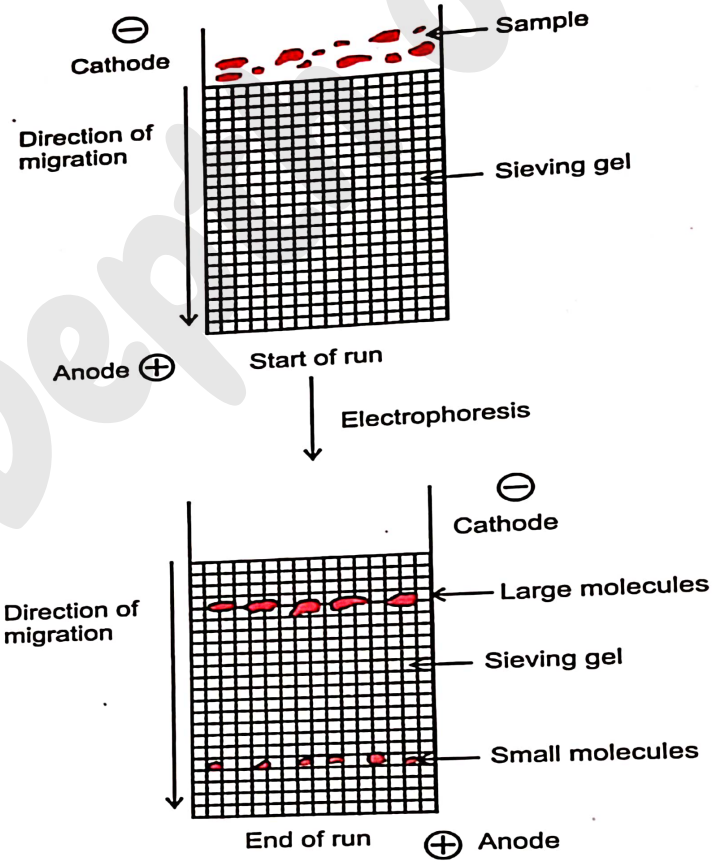
② Resolution of sample is Sharper & Better.

③ Simple to perform

Disadvantages → ① The gel can be altered & can give false results.

② Trained person require to Minimize Manual error.





Capillary electrophoresis

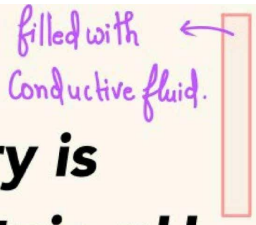
As the name indicates, here the process of separation takes place inside a capillary tube.

Capillary Tube

The capillary electrophoresis is an advanced method of electrophoresis.

In Capilary electrophoresis separation occurs because of differences in rates at which analyte ions move under the influence of an applied electric field and hence only ionic species in the buffered medium are separable.

in capillary electrochromatography both ionic and neutral analyte species may be separated.

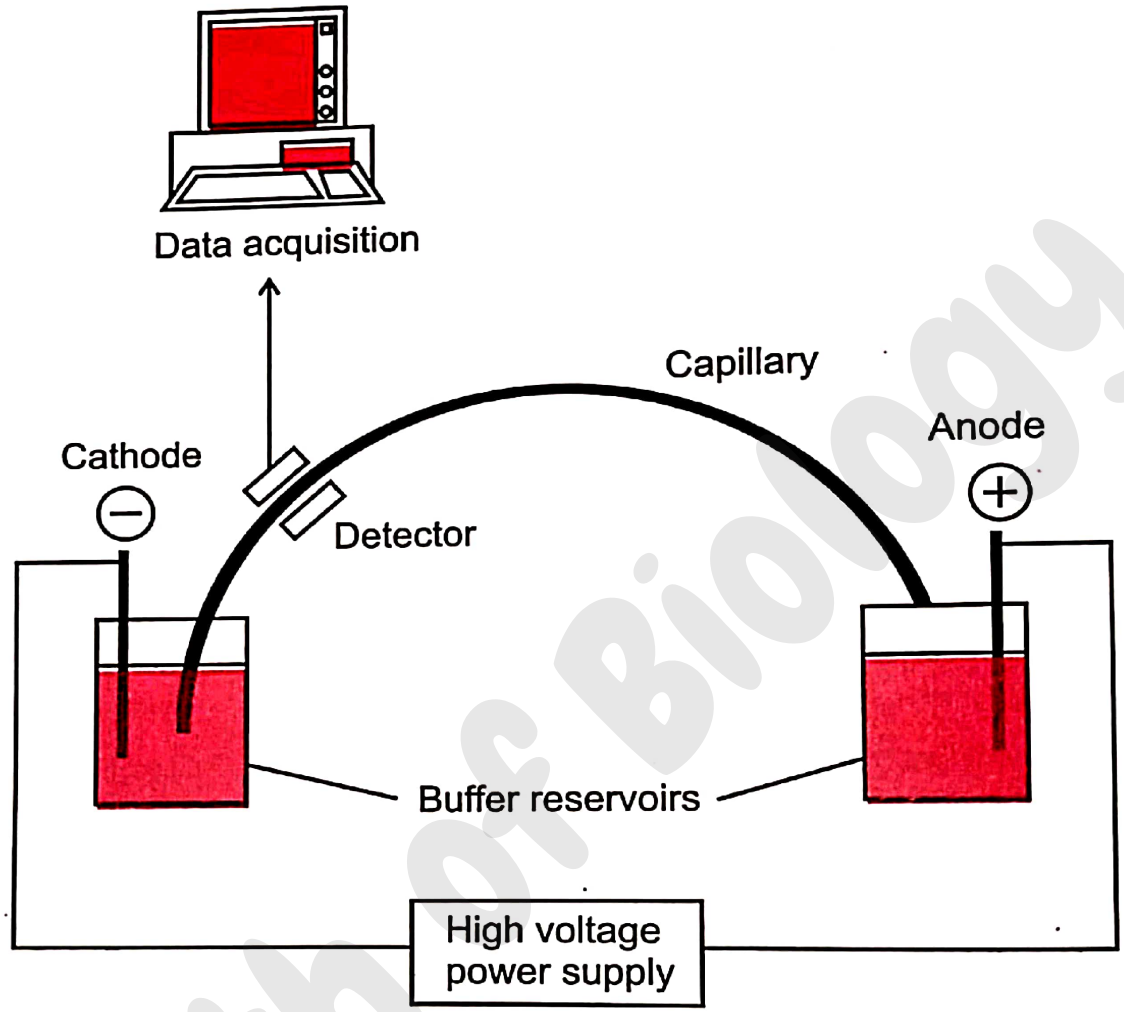


1. Principle

In capillary Electrophoresis a capillary is filed with a conductive fluid at a certain pH value. This is the buffer solution in which the sample will be separated.

A-sample is introduced in to the capillary either by pressure injection or by electro kinetic injection a high voltage is generated over the capilary and due ot this electric field (up ot more then 30 V/cm) the sample components move (migrate) through the capilary at different speeds.

Negative components migrate to The positive electrode. When you look at the capilary at a-certain place with a detector you wil first see the fast components pass, and later on the slower components.



ADVANTAGES

1. Capillary electrohporesis requires small sample in the range if 0.1 to 10 ml while slab method requires in pl range.
2. It yields high speed and high resolution separations.
3. The separated components which exit from one end of capillary, are immediately analysed by detectors fixed at the end of tubes.

DISADVANTAGES

It is time-consuming, expensive and technical skilled procedure required.

Applications of Electrophoresis

- Used to study the properties of a single charged species or mixtures of molecules.
- Used to separate organic bases, acids and inorganic ions.
- Used to identify amino acids, peptides and proteins.
- Used to separate very large proteins, nucleic acids and nucleoproteins etc.
- Used in Clinical Laboratory to separate proteins from each other
  - Proteins analysis in body fluids: Serum, Urine, CSF
  - Proteins in erythrocytes: Hemoglobin
  - Nucleic acids: DNA, RNA