Electrophoresis

By-

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Electrophoresis

- Electrophoresis may be defined as the migration of the charged particle through a solution under the influence of an external electrical field.
- Arne Tiselius (Father of electrophoresis)-Noble price in chemistry in 1948.

Theory of electrophoresis

- The rate of migration of an ion in electrical field depend on factors:
  - 1. Net charge of molecule
  - 2. Size and shape of particle
  - 3. Strength of electrical field
  - 4. Properties of supporting medium
  - 5. Temperature of operation

Theory of electrophoresis

- **Mobility**
- Under the electrical field, the mobility of the particle is determined by two factors:
  - 1- Its charge
  - 2- Frictional coefficient
- Size and shape of the particle decide the velocity with which the particle will migrate under the given electrical field and the medium.

Theory of electrophoresis

- Electrophoretic separations are based upon the fact that the electrical **force** (F) on a charged particle (ion) in an electrical field (E) is proportional to the charge of the particle (q), or $F = qE$ (Eq 1).
- The **migration** of the charged particle in the electric field, called the electrophoretic mobility (μ), is defined as $\mu = v/E = q/f$ (Eq 2),
- where v is the **velocity** of the charged particle and f is a complex term called the frictional **coefficient**. The frictional coefficient relates to the size and the shape of the particle.
- From equation (2) it can be seen that electrophoretic mobility decreases for larger particles and increases with higher charge.

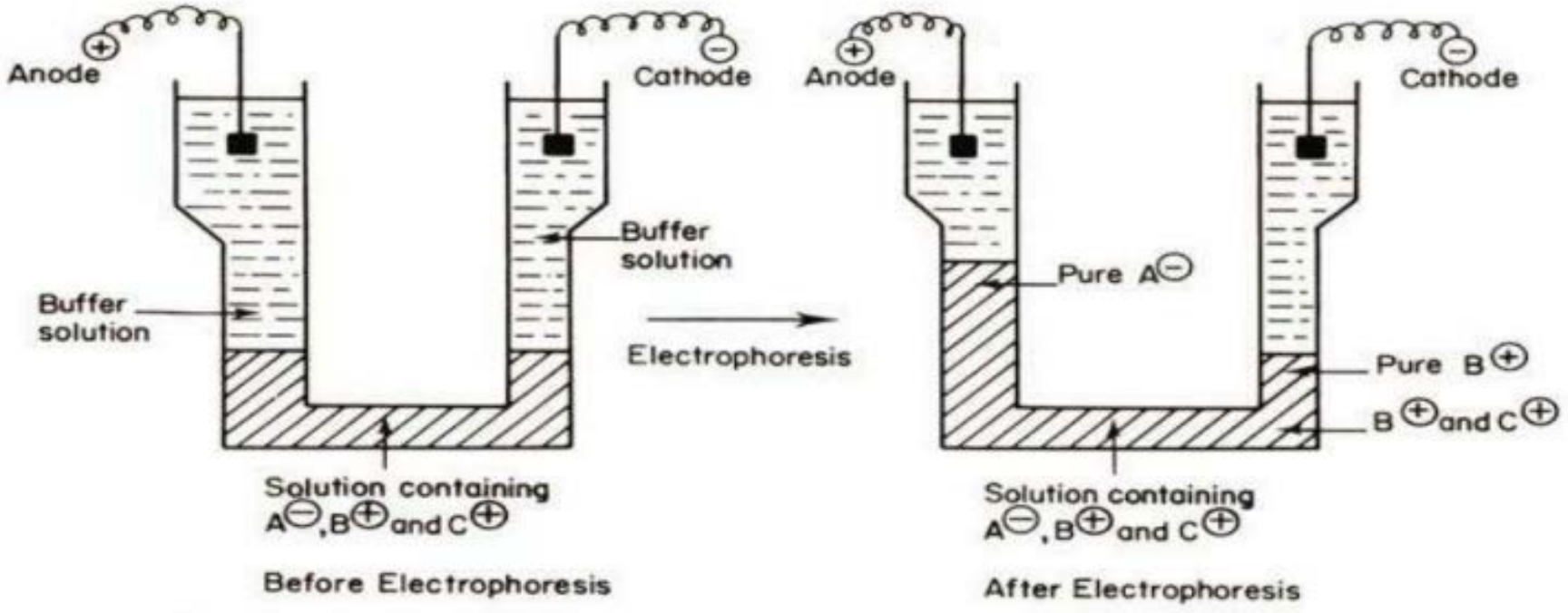
Types of Electrophoresis

- Moving boundary electrophoresis
  - Capillary Electrophoresis
  - Isotachophoresis: separation occurs in a discontinuous system of two electrolytes inside the capillary of suitable dimensions.
  - Isoelectric Focusing
  - Immuno Electrophoresis: biochemical method for separation and characterization of proteins based on electrophoresis and reaction with antibodies.
- Zone electrophoresis
  - Paper electrophoresis
  - Cellulose acetate electrophoresis
  - Gel electrophoresis

Moving boundary electrophoresis

- First used by Sweedish biochemist Arne Tisellus, to separate proteins in 1937.
- In this method, the electrophoresis is carried in solution, without a supporting media.
- The sample is dissolved the buffer and molecules move to their respective counter charge electrodes.
- Moving boundary electrophoresis is carried out in a U shape tube with platinum electrodes attached to the end of both arms.
- At the respective ends, tube has refractometer to measure the change in refractive index of the buffer during electrophoresis due to presence of molecule.
- Sample is loaded in the middle of the U tube and then the apparatus is connected to the external power supply.
- Charged molecule moves to the opposite electrode as they passes through the refractometer, a change can be measured.
- As the desirable molecule passes, sample can be taken out from the apparatus along with the buffer.

Moving boundary electrophoresis



Disadvantages of Moving Boundary electrophoresis

- The resolution of the technique is very low due to the mixing of the sample as well as over-lapping of the sample components.
- The electrophoresis technique is not good to separate and analyze the complex biological sample instead it can be used to study the behavior of the molecule in an electric field.

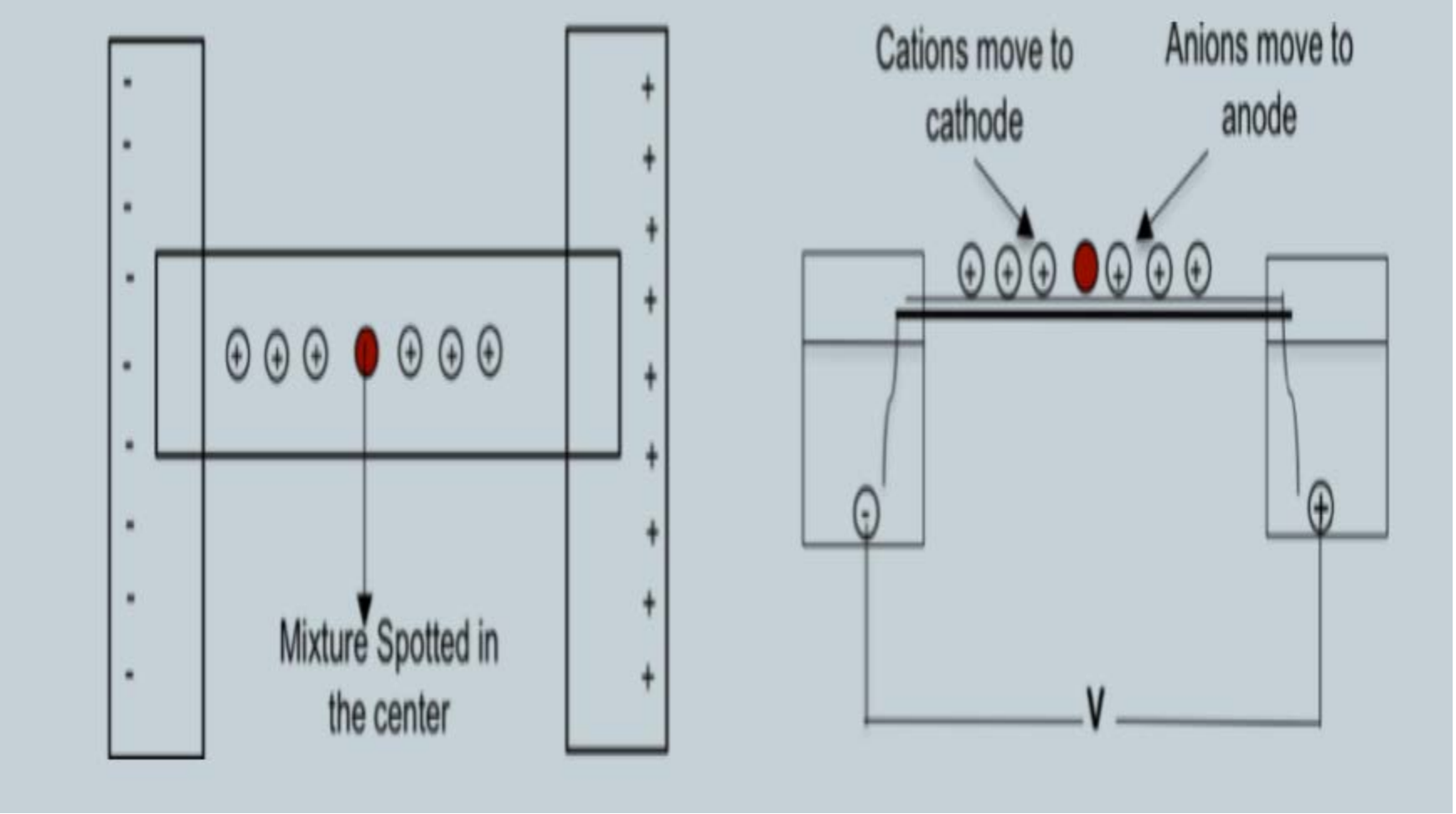
Zone Electrophoresis

- In this method, an inert polymeric supporting media is used between the electrodes to separate and analyze the sample.
- The supporting media used in zone electrophoresis are absorbent paper, gel of starch, agar and polyacrylamide.
- The major advantage of presence of supporting media is that it minimizes mixing of the sample and immobilization of the molecule after electrophoresis.
- It makes the analysis and purification of the molecule from the gel much easier than the moving boundary electrophoresis.
- The gel electrophoresis is the best example of zone electrophoresis.

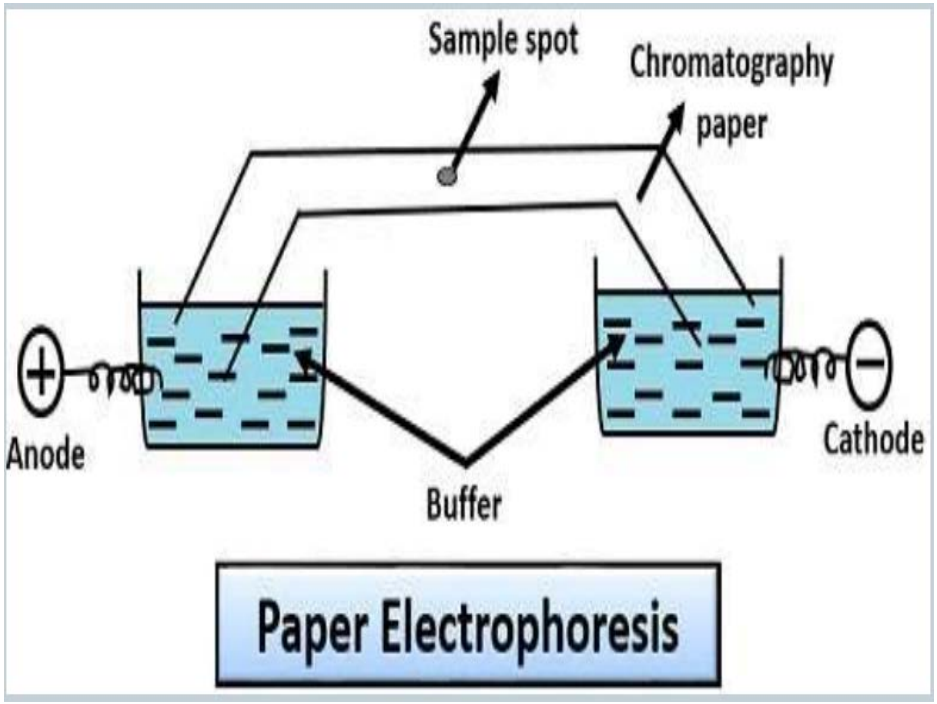
Paper electrophoresis

- Paper electrophoresis (PE) is useful for the separation of small-charged molecules, such as amino acids and small proteins using a strip of paper (chromatography paper).
- In this technique, the motion of colloidal particle of solution occurs leading to subsequent separation along the paper strip.
- PE is easier in comparison to gel electrophoresis.
- It does not require matrix preparation and it does not contain charges that interfere with the separation of compounds.
- A strip of filter paper is moistened with buffer and the ends of the strip are immersed into buffer reservoirs containing the electrodes.
- The samples are spotted in the center of paper and high voltage is applied.
- Application of high voltage causes less diffusion of small molecules giving better resolution and it take less time to complete the process.
- Spots migrate according to their charges.
- After electrophoresis, separated components can be detected by variety of staining techniques, depending upon their chemical composition.

Paper electrophoresis



- The apparatus (Figure 2) consists of two electrode chambers placed 15 cm apart.
- There is also a device which can support up to six (30 cm) filter paper strips between the electrodes.
- A d.c. supply source (0–250 V) is used to apply the desired voltage across the electrodes.
- The two electrode chambers are filled to equal heights with the buffer solution.
- The buffers commonly used for this purpose are (1) Aronsson and Gronwall buffer, i.e. dimethyl barbiturate buffer, and (2) Consden and Powell buffer, i.e. borate buffer, which is a mixture of 1.77 g sodium hydroxide and 9.60 g orthoboric acid with a pH of 8.6.



Applications of Paper Electrophoresis

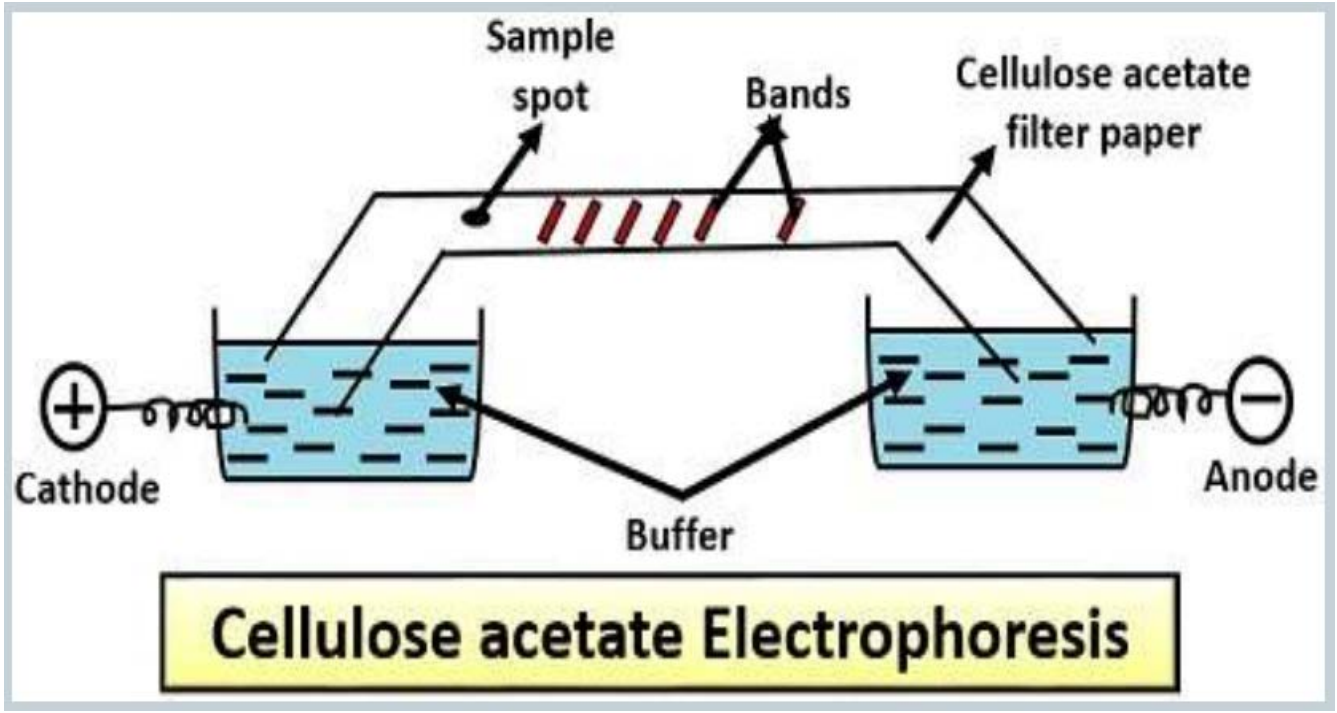
- A simple, inexpensive, and accurate laboratory procedure for various research and clinical studies.
- Easily available and easy to handle, allowing new methodologies to be tried and developed with convenience.
- Clinical applications of PE include study of sickle cell disease, hemoglobin C abnormalities, and separation of blood clotting factors and serum plasma proteins from blood sample.
- Used in separation and identification of alkaloids.
- Used for testing suitability of municipal water supplies, toxicity of water, and other environmental components.
- Drug-testing industry uses paper electrophoresis to determine presence of illegal or recreational drugs in job applicants and crime suspects.
- Since 1950s used by the investigators and in forensics to analyze inks used in currency to check the counterfeiters.
- Lack of sensitivity and reproducibility are limitations of PE.

Cellulose acetate strip electrophoresis

- Many biological samples adsorb on cellulose, that is paper.
- The adsorption is because of hydroxyl groups present in cellulose.
- Adsorption reduces the movement and therefore causes tailing of spots/bands.
- This spreading of spots reduces resolution.
- To solve this problem cellulose acetate membrane is used where most of the hydroxyls have been converted acetate groups.
- Cellulose acetate is preferred because of its simplicity and high resolution at low applied voltage.
- It contains 2-3 acetyl groups per glucose unit and its adsorption capacity is less than that of paper.
- It gives sharper bands.
- Provides a good background for staining glycoproteins.

Cellulose acetate strip electrophoresis

- Application:
  - Widely used in analysis of clinical and biological protein sample (albumin and globulins).
  - Alternative to paper electrophoresis.



Starch

- A suspension of granular starch should be boiled in a buffer to give a clear colloidal suspension.
- The suspension on cooling sets as a semisolid gel due to intertwining of the branched chains of amylopectin.
- In order to avoid swelling and shrinking petroleum jelly is used.
- Advantages
  - High resolving power and sharp zones are obtained.
  - The components resolved can be recovered in reasonable yield especially proteins.
  - Can be used for analytical as well as preparative electrophoresis.
- Disadvantages
  - Electro osmotic effect.
  - Variation in pore size from batch to batch

Gel electrophoresis

- It makes the use of gel as a support matrix.
- Most popular and commonly used method.
- Used for both analytical and preparative processes.
- It is the most common method to carry out the process of electrophoresis.