Electrophoresis

Separation of charged particles under electrical field

What is electrophoresis?

- The term electrophoresis describes the migration of a charged particles under the influence of an electric field
- Various essential biological molecules, such as **amino acids, peptides, proteins, nucleic acids, nucleotides**, have ionizable group, which at given pH exist in a solution as electrically charged particled as cation (+ve) or anion (-ve)
- Under the influence of electric field these charged particles migrate either to cathode or anode
- The migration depending on the nature of their net charges
- This is one of the most fundamental processes used in all types of molecular biology and RDT experiments

- **So, Electrophoresis is defined as the migration of charged ions in an electric field in solutions**
- **The ions that migrate towards the anode, are called "anions" and the ions which will migrate to the cathode are called "cations"**
- **The force that cause the molecules to migrate towards either the cathode or the anode, depends on the sign of its charge**
- **Proteins, have either a net positive or net negative charge, migrate towards either cathode or anode**
- **Nucleic acids have a consistent negative charge migrate only toward the anode**

Principle of electrophoresis

- When a potential difference is applied, the molecules with different overall charge will begin to separate owing to their different electrophoretic mobility
- Even the molecules with similar charge will begins to separate if they have different molecular sizes, since they will experience different frictional forces
- Electrophoresis is regarded as incomplete form of electrolysis because the electric field is removed before the molecules in the samples reaches the electrode but the molecules will have been already separated according to their electrophoretic mobilities
- The separated samples are then located by staining with an appropriate dye

The Rate of migration of charged molecules depends upon following factors:

(a) The strength of electric field, size and shape.

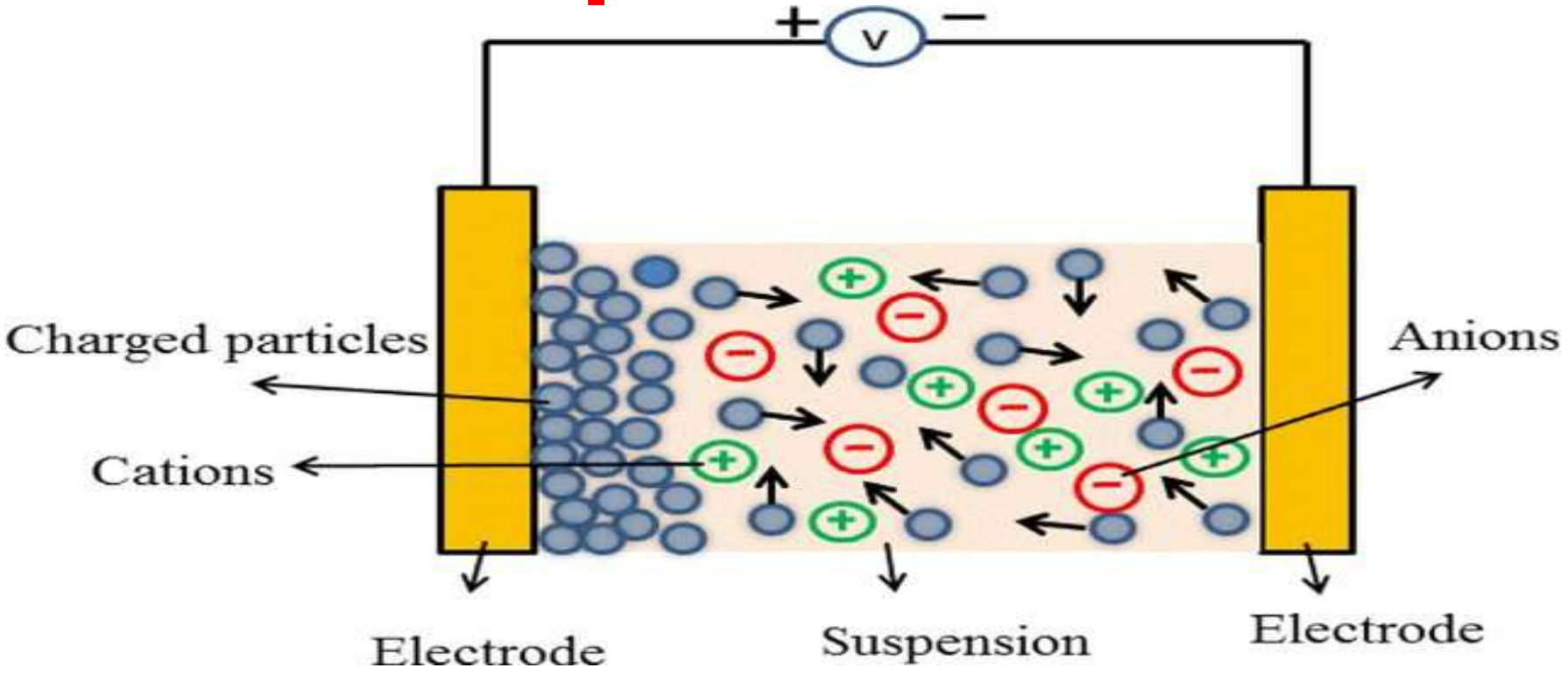
(b) Relative hydrophobicity of the sample.

(c) Ionic strength and temperature of the buffer.

(d) Molecular size of the taken biomolecule.

(e) Net charge density of the taken bio molecule.

Electrophoretic migration process



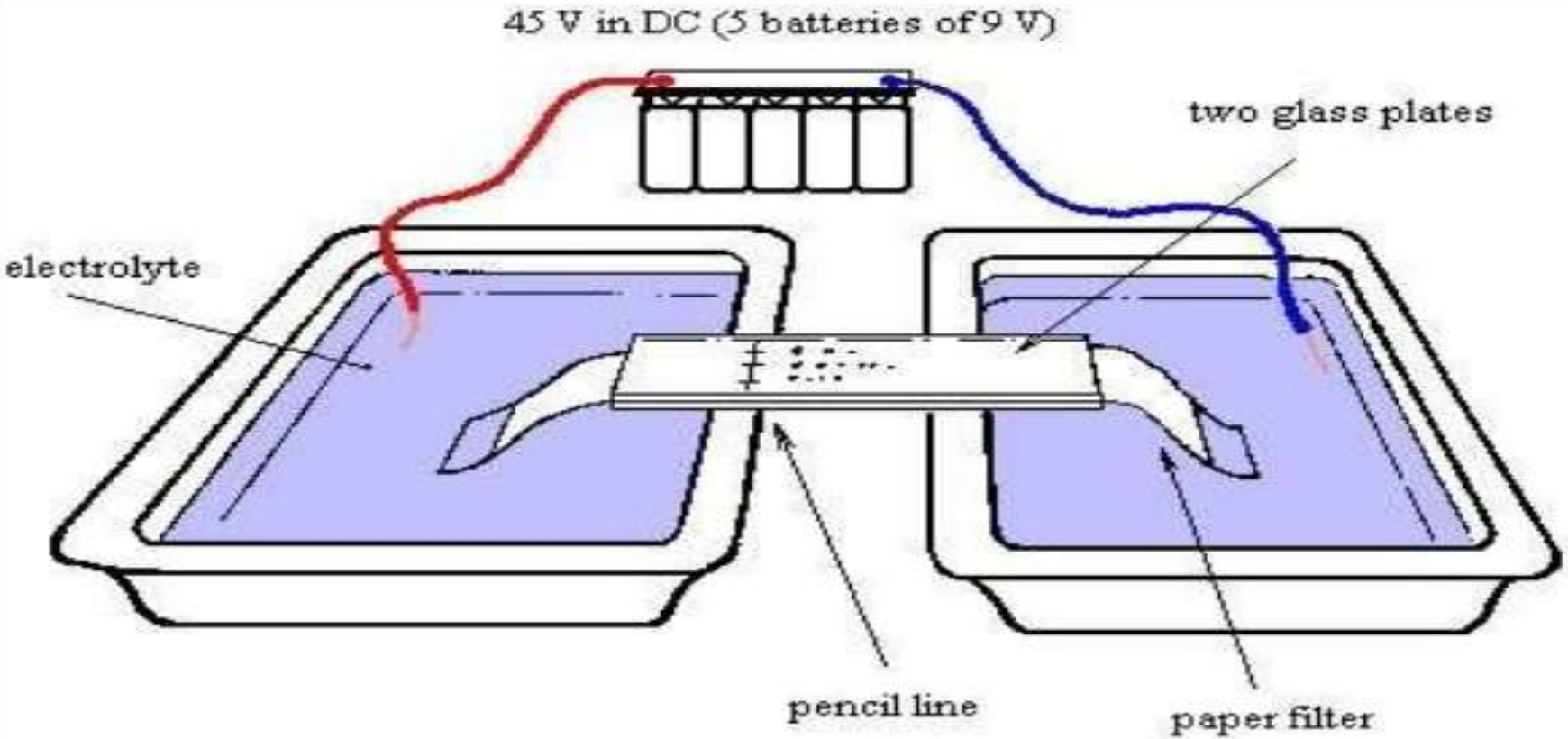
Types of Electrophoresis

This technique is divided into two types

- **Zone Electrophoresis**
  - **Paper Electrophoresis**
  - **Gel Electrophoresis**
- **Moving Boundary Electrophoresis**
  - **Immuno Electrophoresis**
  - **Isoelectrofocusing**
  - **Capillary Electrophoresis**

Paper Electrophoresis

- In this type of electrophoresis a filter paper (like chromatography paper) having slight adsorption capacity and uniform pore size is used as the supporting medium for separation of samples under the influence of an applied electric field
- While carrying out paper electrophoresis, a strip of filter paper is moistened with buffer and ends of the strip are immersed into buffer reservoirs containing the electrodes
- The samples are spotted in the centre of the paper, high voltage is applied, and the spots migrate according to their charges
- After electrophoresis, the separated components can be detected by a variety of staining techniques, depending upon their chemical identity



Apparatus for Paper Electrophoresis

Gel Electrophoresis

- Gel electrophoresis involves the use of gel as supporting media for separation of DNA, RNA or proteins under the influence of electric charge.
- It is usually performed for analytical purposes but may be used as a preparative technique to partially purify molecules prior to use for other methods such as mass spectrometry, PCR, cloning, DNA sequencing and immuno-blotting
- This is the most commonly used electrophoresis in biotechnology laboratories and is used for almost all types of experiments

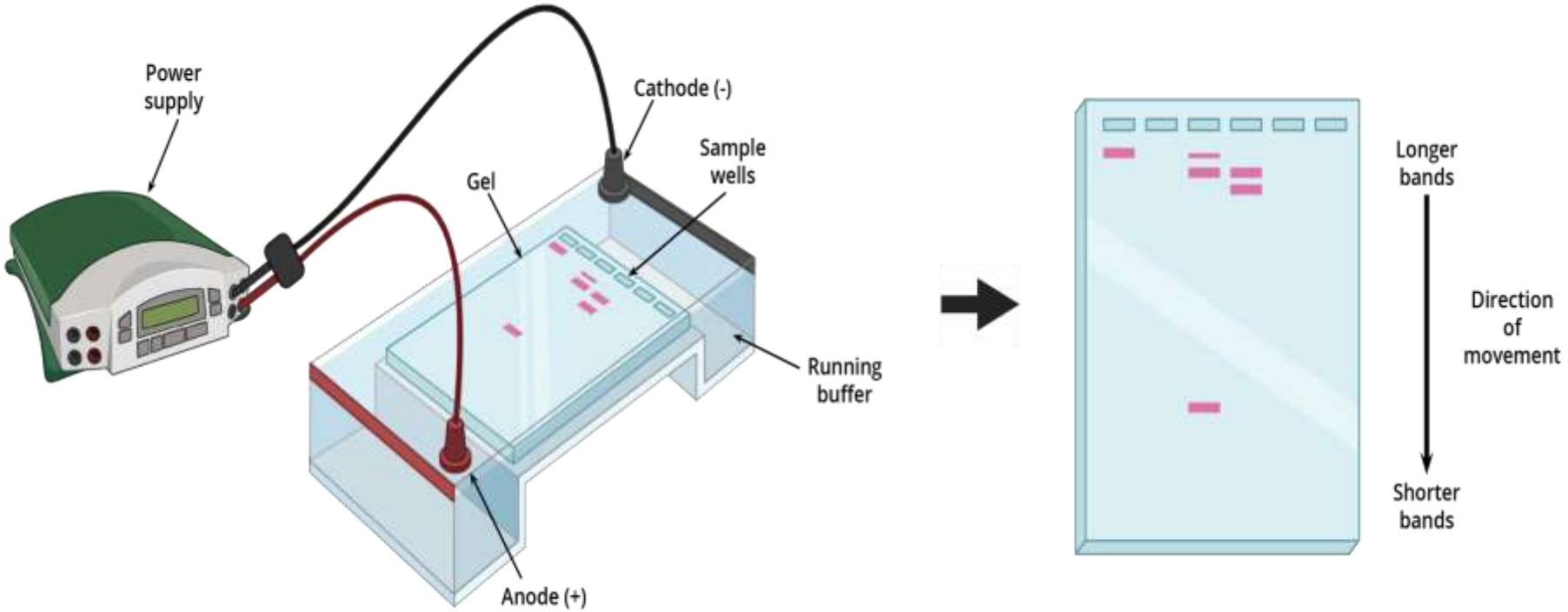
A typical gel electrophoresis apparatus is of two kinds:

(a) Vertical Gel Apparatus:

- It is used for the separation of proteins in SDS-PAGE

(b) Horizontal Gel Apparatus:

- It is used for immune-electrophoresis, isoelectric focusing and electrophoresis of DNA and RNA in the agarose gel


Power supply
Cathode (-)
Sample wells
Gel
Running buffer
Anode (+)
Longer bands
Direction of movement
Shorter bands