

Introduction

Electrophoresis is a scientific laboratory technique that is used to separate DNA, RNA, or protein molecules based on their size and electrical charge. An electric current is passed through the molecules to move them so that they can be separated via a gel. The pores present in the gel work like a sieve, allowing smaller molecules to pass through more quickly and easily than the larger molecules. According to the way conditions are adjusted during electrophoresis, the molecules can be separated in the desired size range.

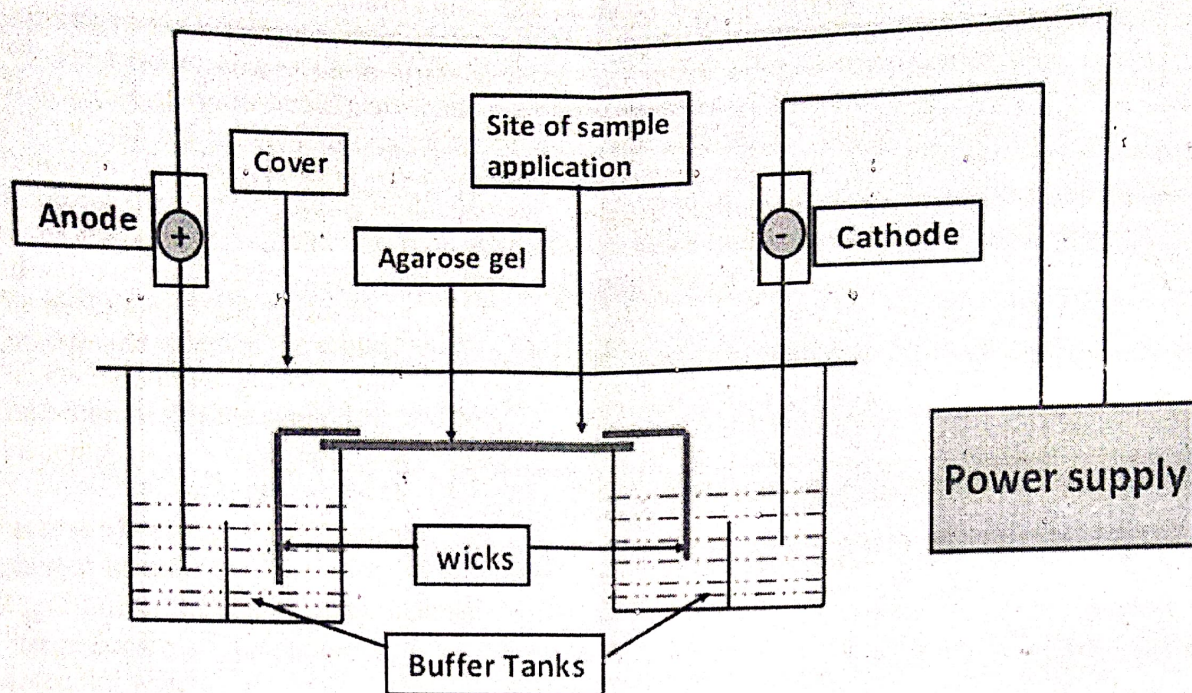
What is electrophoresis and what are its uses?

Electrophoresis is a very broadly used technique that, fundamentally applies electric current to biological molecules they're usually DNA but they can be protein or RNA too and separates these fragments into pieces that are larger or smaller in size. The phenomenon of electrophoresis was first observed by Russian professors Peter Ivanovich Strakhov and Ferdinand Frederic Reuss in 1807 at Moscow University. A constant application of electric field caused the particles of clay dispersed in water to migrate showing an electrokinetic phenomenon. Electrophoresis can be defined as an electrokinetic process that separates charged particles in a fluid using an electrical field of charge. Electrophoresis of cations or positively charged ions is sometimes referred to as cataphoresis. In contrast, sometimes, the electrophoresis of anions or negatively charged ions is referred to as anaphoresis. It's used in a variety of applications. Though it is most often used in life sciences to separate protein molecules or DNA it can be achieved through several different techniques and methods depending upon the type and size of the molecules.

The methods differ in some ways but all we need is a source for the electrical charge, a support medium and a buffer solution. Electrophoresis is also used in laboratories for the separation of molecules based on their size, density and purity.

How Electrophoresis Works

In electrophoresis there are two primary factors that control how quickly a particle can move and in what direction. First the charge on the sample matters. Negatively charged species are attracted to the positive pole of an electric field while positively charged species are attracted to the negative end. A neutral species may be ionized if the field is strong enough. Otherwise, it doesn't tend to be affected. The other factor is particle size. Small ions and molecules can move through a gel or liquid much more quickly than larger ones. While a charged particle is attracted to an opposite charge in an electric field, there are other forces that affect how a molecule moves. Friction and the electrostatic retardation force slow the progress of particles through the fluid or gel. In the case of gel electrophoresis, the concentration of the gel can be controlled to determine the pore size of the gel matrix which influences mobility. A liquid buffer is also present which controls the pH of the environment. As molecules are pulled through a liquid or gel, the medium heats up. This can denature the molecules as well as affect the rate of movement. The voltage is controlled to try to minimize the time required to separate molecules, while maintaining a good separation and keeping the chemical species intact. Sometimes electrophoresis is performed in a refrigerator to help compensate for the heat.



Schematic diagram of an Electrophoresis apparatus

Uses

- Nucleic acid molecule sizing
 - DNA fragmentation for southern blotting
 - RNA fragmentation for northern blotting
 - Protein fragmentation for western blotting
 - Separation of PCR products analysis
 - Detection and analysis of variations or mutations in the sequence
- Its clinical applications involve –
- Serum protein electrophoresis
 - Lipoprotein analysis
 - Diagnosis of haemoglobinopathies and hemoglobin A1c
 - Determination of serum protein Phenotypes and micro heterogenesis
 - Protein genotyping – e.g., ApoE analysis for Alzheimer's disease (polymorphic protein)
 - Monitoring of Small molecules – e.g., drugs and steroids
 - Cerebrospinal fluid analysis
 - Urine analysis – like determination of GNs

Principle of electrophoresis

The fundamental principle of electrophoresis is the existence of charge separation between the surface of a particle and the fluid immediately surrounding it. An applied electric field acts on the resulting charge-density causing the particle to migrate and the fluid around the particle to flow. It is the process of separation or purification of protein molecules, DNA, or RNA that

differ in charge, size and conformation. The charged molecules are placed at one end of the field according to their charge and an electric field is applied. On passing electric current depending upon the kind of charge the molecules carry they move towards the opposite electrodes either cathode (negative electrode) or anode (positive electrode). The size, shape, and charge of the molecule remain constant during electrophoresis and determine ionic particle mobility.

An ampholyte acts as a positively charged particle or ion migrating towards the cathode in acidic conditions. In contrast, it acts as a negatively charged particle or ion in an alkaline condition and moves towards the anode. The rate of migration of an ion in a supporting medium under the influence of an electric field depends upon the following factors –

- The net charge of the molecule
- The size and shape of the molecule
- The strength of the electric field
- The properties of the supporting medium
- The temperature of the procedure

Factors affecting electrophoresis

Inherent factors

- Magnitude of the charge of a molecule
- Charge density of the molecule
- Molecular weight
- Its shape, i.e., whether it's tertiary or quaternary structured

External environment

- The pH of the solution
- Electric field
- The viscosity of the solution or agarose concentration
- The temperature of operation

Types of Electrophoresis

- **affinity electrophoresis** - Affinity electrophoresis is a type of electrophoresis in which particles are separated based on complex formation or biospecific interaction
- **capillary electrophoresis** - Capillary electrophoresis is a type of electrophoresis used to separate ions depending mainly on the atomic radius, charge, and viscosity. As the name suggests, this technique is commonly performed in a glass tube. It yields quick results and a high resolution separation.
- **gel electrophoresis** - Gel electrophoresis is a widely used type of electrophoresis in which molecules are separated by movement through a porous gel under the influence of an electrical field. The two main gel materials are agarose and polyacrylamide. Gel electrophoresis is used to separate nucleic acids (DNA and RNA), nucleic acid fragments, and proteins.
- **immunoelectrophoresis** - Immunoelectrophoresis is the general name given to a variety of electrophoretic techniques used to characterize and separate proteins based on their reaction to antibodies.

- **electroblotting** - Electroblotting is a technique used to recover nucleic acids or proteins following electrophoresis by transferring them onto a membrane. The polymers polyvinylidene fluoride (PVDF) or nitrocellulose are commonly used. Once the specimen has been recovered, it can be further analyzed using stains or probes. A western blot is one form of electroblotting used to detect specific proteins using artificial antibodies.
- **pulsed-field gel electrophoresis** - Pulsed-field electrophoresis is used to separate macromolecules such as DNA by periodically changing the direction of the electric field applied to a gel matrix. The reason the electric field is changed is because traditional gel electrophoresis is unable to efficiently separate very large molecules that all tend to migrate together. Changing the direction of the electric field gives the molecules additional directions to travel so they have a path through the gel. The voltage is generally switched between three directions: one running along the axis of the gel and two at 60 degrees to either side. Although the process takes longer than traditional gel electrophoresis, it's better at separating large pieces of DNA.
- **isoelectric focusing** - Isoelectric focusing (IEF or electrofocusing) is a form of electrophoresis that separates molecules based on different isoelectric points. IEF is most often performed on proteins because their electrical charge depends on pH.

Conclusion

Electrophoresis can be considered as an electrokinetic phenomenon involving an electric field supporting medium and a buffer solution. It has many forensic and clinical applications in our daily life. From using traditional methods of only rectangular gel usage to using enhanced resolution techniques, electrophoresis has come a long way since its discovery in 1807 in Russia. Overall electrophoresis is a separation technique that can single out biomolecules or charged particles of interest based on their mobility in a given electric field. Gel electrophoresis allows scientists to visualise the sizes of DNA segments and aids in the sequencing of lengths of DNA.