**Principle of Electrophoresis**

Electrophoresis is based on the phenomenon that most biomolecules exist as electrically-charged particles, possessing ionizable functional groups. Biomolecules in a solution at a given pH will exist as either positively or negatively charged ions.

When subjected to an electric field, ionized biomolecules will migrate at a different pace, depending on the mass and the net charge of each particle in the solution—negatively-charged particle, *anions*, will migrate towards a positively charged electrode, or *cathode*, and *cations,* or positively-charged particles, will be pulled towards a negatively-charged electrode called *anode*.

The differences in the speed and the direction of each charged particle will result in a migration pattern that is unique to its individual property, leading to the isolation of components of the biomolecules that possess similar characteristics (Andrews, 1986, as cited in Westermeier, *et al*., 2005, p. 3).

## Forms of Electrophoresis

Based on the type of buffer solution and its effect on the mobility of the charged particles, electrophoresis can be broadly divided into four forms as follows (Jorgenson, 1986):

### 1. Moving boundary electrophoresis

Moving boundary electrophoresis is considered the original form of electrophoresis. Samples for separation are performed in free solution, in tubes or capillary tubes, under constant pH value throughout the separation process.

A major advantage of electrophoresis in free solution is the ability to measure the mobilization of separating particles without other intervening factors unrelated to the separating particles. Nonetheless, this electrophoresis format is vulnerable to convection current and the resolution of separation is low due to the mixing of samples in the solution buffer that can result in the overlapping of components or particles that possess similar characteristics (Jorgenson, 1986).

### 2. Zone electrophoresis (ZE)

Zone electrophoresis is similar to moving boundary electrophoresis in that electrophoretic separation takes place in a homogenous buffer system (Westermeier, et al., 2005). This format often makes use of a support medium or a matrix to subdue convection current and prevent uncontrolled sample diffusion. The matrix, in most cases, also provides an additional sieving effect that exerts an influence on electrophoretic separation, as shown in Equation (3) (Jorgenson, 1986; Walker, 2010).

Samples for ZE-separation are surrounded with electrophoretic solution buffer and separated in the matrix for a definite separation time. When the electric current is applied, the samples move at a different velocity, as determined by their mass and charge (q). Once the separation process is terminated, components of the sample possessing similar characteristics are isolated into a distinct zone (Becker, 1973).

Gel electrophoresis is an example of ZE that uses a polymer-sieving matrix as a support media. The technique is widely used in biochemistry and molecular biology research and routine work due to its simplicity and versatility (Westermeier, 2005). Due to the introduction of a support medium, ZE is neither suitable for the analysis of the mobility of charged particles of interest nor the determination of the isoelectric point (pI) of peptides or proteins.

### 3. Isotacho-electrophoresis, or isotachophoresis (ITP)

Isotachophoresis or ITP is a form of electrophoresis, in which all ions migrate at equal velocity (v). In ITP, the samples are placed between two non-homogeneous solution buffer, composing of a leading electrolyte at one electrode, and a terminating electrolyte at the other end (Becker, 1973). Both electrolytes possess the same charge species as that of the particle of interest in the sample. When an electric current is applied, the leading electrolyte will have the highest mobility, followed by the charged particles in the sample and the terminating electrolytes, respectively.

As ITP continues, charged particles in the sample will be displaced, based on its electrical mobility (µ) and concentration, in order of decreasing mobility, resulting in a continuous region of charged particles with similar characteristics, sandwiched by regions where the leading and terminating ions are occupied (Becker, 1973; Jorgenson, 1986).

The information from isotachophoresis is conveyed as graphs of electric field strength over time, which represents the identity of the charged particle, and the length of each region represents the concentration of the charged particle (Jorgenson, 1986).

One major disadvantage of this format is that only one charged species can be determined in one setting, and another round of ITP will be necessary to obtain information of charged particles of the other species.

### 4. Isoelectric focusing (IEF)

Isoelectric focusing or IEF is electrophoresis that is performed in a pH gradient, which runs from low to high – from the anode to cathode. IEF is only applicable to amphoteric molecules because they can donate and receive protons, acting as acid and base. Examples of amphoteric biomolecules are peptides and proteins, which possess the amine and carboxylic acid groups (Walker, 2010).

Once the pH gradient is established, and an electric current is applied, an amphoteric sample will migrate towards either the anode or the cathode, depending on the net charge of the sample. At the isoelectric point (pI), where the net charge of the sample is zero, the velocity (v) and the electrical mobility (µ) of the amphoteric molecule become zero, stopping the migration (Jorgenson, 1986).

All four formats of electrophoresis can be performed in both one- and two-dimensions (2D). Two-dimensional electrophoresis is performed by conducting the first electrophoresis, followed by the second electrophoretic separation in a direction perpendicular to the first dimension. 2D-electrophoresis can offer more information and resolution, which is particularly useful for clinical or field samples, which often requires intensive analysis and characterization but is given only in a limited amount (Jellum & Thorsurd, 1982; Xu, 2008).

## Types of Electrophoresis

The different forms of electrophoresis have been modified to several types of electrophoresis, which are used to separate various types of biomolecules, analyze their characteristics, and study their interaction with a molecule of interest. The following are selected electrophoresis methods, based on different formats of electrophoresis.

### 1. Gel Electrophoresis

Gel electrophoresis is a form of ZE that uses gel, a non-fluid cross-linked polymer network, as a support medium to maintain stable pH value in the solution buffer, acting as an anti-convective stabilizer. It also serves as a separation matrix, due to its porous nature that filters large particles and hinders smaller ones during electrophoretic separation (Jorgenson, 1986).

The gel is cast into strips or slabs with slots or sample wells. Once it is completely polymerized, the gel is submerged in an electrophoresis solution buffer, and samples are loaded into each well before the electric current is applied to initiate electrophoretic separation (Walker, 2010). At the end of the gel electrophoresis, components in the samples will be separated based on their mass (Westermeier, 2005).

As mentioned earlier, gel electrophoresis is one of the most used types of electrophoresis in research and routine diagnosis due to their ease-of-use and their versatility. It can be adapted to separate a variety of biomolecules by changing the type of polymers used to cast the gel and by adjusting the composition of the polymer, altering the pore size of the gel (Xu, 2008; Chung, et al., 2014).

#### Gel types

* [**Polyacrylamide**](https://amzn.to/39xjiV2), a clear and transparent gel made from the co-polymerization of acrylamide monomers in the presence of a crosslinking agent, N,N-methylene-bisacrylamide, commonly referred to as ‘[bis-acrylamide](https://amzn.to/3awedh7)’. The polymerization reaction is catalyzed by [ammonium persulphate](https://amzn.to/2QWKExK) (APS) and N,N,N’,N’-tetramethylenediamine (TEMED). The pore size of polyacrylamide gels is determined by the concentration of acrylamide, which must be in proportion with its crosslinking agent. Generally, a low percentage of acrylamide gel (3%-15%) is used to separate DNA and proteins. Higher percentage acrylamide gel (10%-20%) is commonly used in [sodium dodecyl sulfate (SDS)](https://amzn.to/2QZbUvq)-polyacrylamide gel electrophoresis (SDS-PAGE), in which proteins are separated in denatured condition, according to their size (Walker, 2010; Chung, et al., 2014).
* [**Agarose**](https://amzn.to/2UzS5x7), a natural linear polysaccharide made of galactose and 3, 6-anhydrogalactose chains, extracted from agar isolated from red seaweeds (Westermeier, et al., 2005). Agarose, like agar, is stored as a dry powder. To cast agarose gel, the agarose powder is dissolved in a relevant solution buffer, heated, and allowed to cool to room temperature. Similar to polyacrylamide gel, the concentration of the agarose in the solution buffer determines the pore size of the gel. Agarose gel is commonly used at 0.8% (w/v) to 5% (w/v) to separate DNA and RNA molecules (Walker, 2010). Agarose can be used in combination with SDS to separate high-molecular-weight proteins, which can be problematic when separated using SDS-PAGE (Greaser et al., 2012).

### 2. Pulsed-field Gel Electrophoresis (PFGE)

Pulsed-field Gel Electrophoresis (PFGE) is a variation of gel electrophoresis, in which two electrical fields are periodically applied, in rotation, to the gel electrophoresis at different angles. This type of electrophoresis is specifically designed for the separation of chromosomes, which are high-molecular-weight DNA molecules of over 20 kilobases.

Larger high-molecular-weight molecules will be slower in the process of reorientation and migration renewal, compared to smaller high-molecular-weight molecules due to their longer viscoelastic relaxation time. Repeated application of two alternating electric fields from different angles will eventually reveal further migration distance for smaller high-molecular-weight DNA molecule and shorter migration distance for larger high-molecular-weight DNA (Westermeier, 2005; Westermeier et al., 2005; Walker, 2010).

### 3. Capillary Electrophoresis (CE)

Capillary electrophoresis, also known as High-Performance Capillary Electrophoresis (HPCE), is a type of electrophoresis performed in a narrow capillary immersed in an electrolyte buffer. It is the only type of electrophoresis capable of performing all four types of electrophoreses (Heiger, 2000). The capillary is typically 20-30 centimeters long and possesses a 25-75 micrometer inner diameter.

Electrophoretic separation is initiated when a sample is injected into the capillary, either by high voltage or by pressure, and high electric fields are applied across the capillary (Heiger, 2000; Westermeier, et al, 2005). Components in the sample are separated along the length of the capillary, based on the format of electrophoresis performed. Towards the other end of the capillary, separated components are detected at the detector, where the time of detection or retention time is automatically recorded.

Because CE is performed in a narrow capillary, only a very small sample volume is required for the separation. Another major advantage of CE is the automated instrumentation system, enabling high-throughput analysis (Heiger, 2000).

### 4. Immunoelectrophoresis

Immunoelectrophoresis is a type of electrophoresis that separate antigens, including proteins and peptides, based on their reaction and specificity to antibodies, or immunoglobulins (Ig). The binding of antigen to its corresponding antibody at a specific antigen/antibody ratio, or the equivalent point, will result in the precipitation of antigen-antibody complex. Thus, antigens in a sample of interest can be separated based on their ability to bind to a given antibody (Nowotny, 1979).

Contemporary settings for immunoelectrophoresis are based on modifications of zone electrophoresis and gel electrophoresis. Immunoelectrophoresis can be performed as a one-dimensional or two-dimensional mode (Westermeier et al., 2005; Walker, 2010).

### 5. Affinity Electrophoresis

Affinity Electrophoresis is a type of electrophoresis that separates a biomolecule that interacts with or binds to another molecule for which it has an affinity. It makes use of the phenomenon that the electrical mobility (µ) changes when a biomolecule, including nucleic acids, proteins, peptides, and polysaccharides, binds to another molecule, and this change in the electrical mobility will be reflected in the electrophoretic pattern.

Thus, biomolecules of interest in a sample can be singled out based on their affinity to another molecule—whether or not the biomolecules of interest tend to bind to another molecule more than the other unwanted biomolecule. Contemporary settings for affinity electrophoresis are based on either gel electrophoresis or capillary electrophoresis (Kinoshita et al., 2015).