Electrophoresis

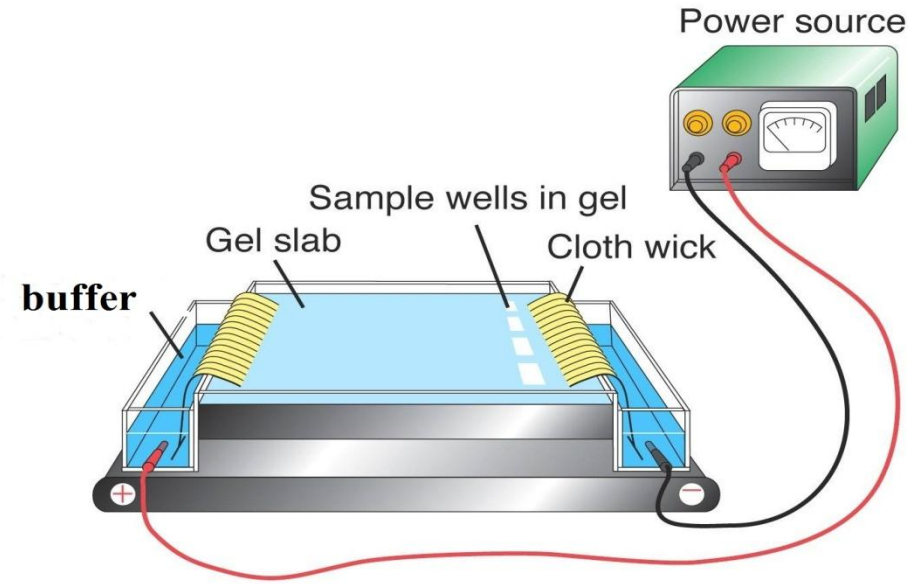
Electrophoresis: is a technique used to separate and sometimes purify charged macromolecules especially proteins and nucleic acids that differ in size, charge or shape by applying an electrical current.

The separation of molecules by electrophoresis is based on the fact that charged molecules migrate through a gel matrix upon application of an electric field. It is one of the most widely-used techniques in **Biochemistry, genetics, microbiology**, **forensics** and **Molecular biology**.

Principal

Biological samples are loaded into a wells cut in jello like gel or paper matrix, after which electrical current is placed across the gel. This current moves the charged molecules toward either the cathode or anode of the electrophoresis apparatus. The speed, direction, and distance that each molecule moves are related to the **charge, shape, and size**.

The apparatus of gel electrophoresis is consists of an electrophoresis **chamber, matrix, buffer, sample and a power supply.**

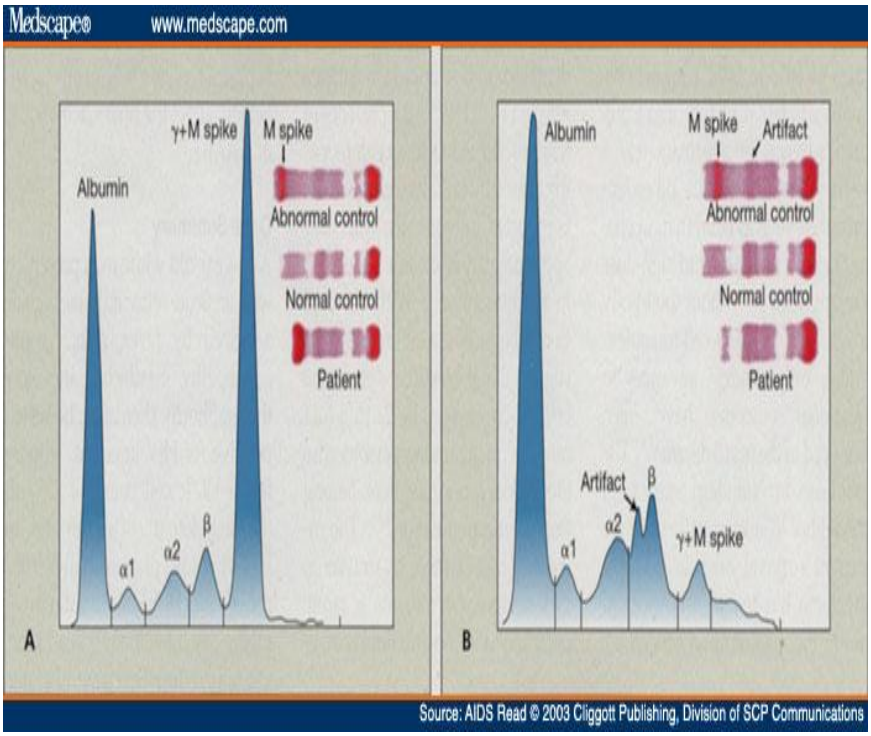


molecules respond to the current by moving from the sample wells into the gel.

Negatively charged molecules move through the gel toward the positive electrode (anode), whereas positively charged molecules move through the gel toward the negative electrode (cathode). The greater the voltage, the faster the molecules move.

The properties of the gel affect the rate of movement of a sample through the gel. Small molecules move easier through the pores than do larger molecules. Consequently, small, compact (e.g. spherical) molecules move faster than do large, rodlike molecules. If molecules have similar shapes and molecular weights, the particles having the greatest charge move fastest, and, therefore, the farthest.

In the end of electrophoresis run, migrated molecules with the same properties will appear as one band; these bands can be visualized by staining them with different stains such as **coomassie blue, cyber green, and ethidium bromide.** The analysis will depend on the number, distance and color intensity of bands.



The direction and speed of Electrophoresis depends upon several factors such as

a. Net charge of the molecule: charged molecules migrate and repel from electrode of a same charge and attract to electrode of opposite charge. **DNA molecules always carry a negative charge**. Proteins, on the other hand, **carry a net charge** that depends **on the isoelectric point** Ip (a specific pH at which the protein molecule will carry neutral net charge) and the **pH of the buffer**. When a pH of a buffer is higher than that of protein Ip, the protein will **carry a negative charge**, and when the buffer pH is lower, the **protein net charge will be positive**.

b. Size and shape of the molecule: small (low molecular weight) and circular molecules migrate faster than large and linear molecules, due to resistance and pores size of the matrix.

c. Electric field strength: increasing the voltage results in faster migration of the molecule.

d. Nature of the supporting matrix: the supporting matrix is a porus material with wells for loading the sample. The gel is immersed within a buffer The matrix can be composed of different materials such as cellulose acetate paper, polyacrylamide, agarose and starch gel each material with different pores size and consequently, different resolution.

f. The temperature of operation: electrophoresis at high voltages produces heat. Additionally, high-conductivity buffers generate more heat than low-conductivity buffers.