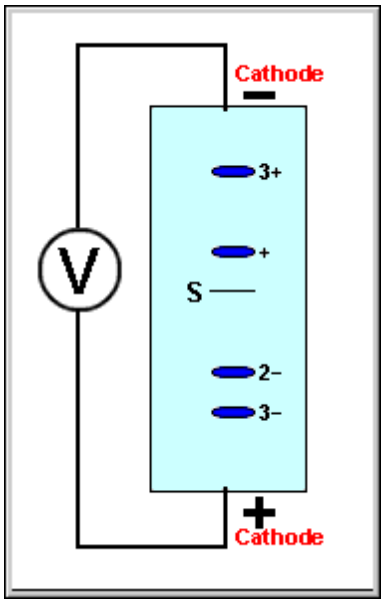
Lecture 2

ELECTROPHORESIS

Electrophoresis is a separation technique that is based on the mobility of the ions in an electric field.. it is a Greek word Electros meaning “energy from an electric field and Phoros meaning to “carry across”. Thus electrophoresis means the techniques in which molecules are forced across a span of gel motivated by an electric field



Electrophoresis is a technique whereby charged molecules are separated on the basis of their speed of migration in an applied electric field. Molecules that are cationic (that is, are positively charged) will migrate towards the negatively charged electrode (the cathode), while those molecules that are anionic (that is, are negatively charged) will migrate towards the positive electrode (the anode). The higher the charge, the faster will a molecule move towards the oppositely charged electrode.

If a mixture of charged molecules are placed in the center of a supporting medium (at the point S in the diagram on the right), and the electric field set up by means of an applied potential, **V**, then, after a while, separation of the molecules will take place. It similar to chromatography in many ways except that separation is due to movement of charged particles through an electrolyte (buffer when subjected to electric current.

Types of Electrophoresis

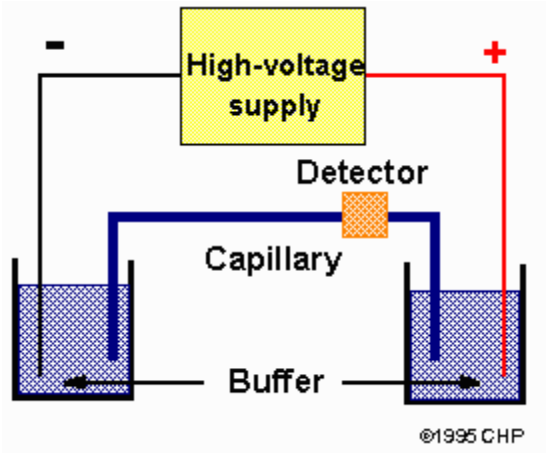
Capillary Electrophoresis

Capillary electrophoresis (CE) encompasses a family of related separation techniques that use narrow-bore fused-silica capillaries to separate a complex array of large and small molecules. High electric field strengths are used to separate molecules based on differences in charge, size and hydrophobicity.

Capillaries are typically of 50 µm inner diameter and 0.5 to 1 m in length. The applied potential is 20 to 30 kV. Due to electroosmotic flow, all sample components migrate towards the negative electrode. A small volume of sample (10 nL) is injected at the positive end of the capillary and the separated components are detected near the negative end of the capillary. CE detection is similar to detectors in HPLC, and includes absorbance, fluorescence, electrochemical, and mass spectrometry.

The capillary can also be filled with a gel, which eliminates the electroosmotic flow. Separation is accomplished as in conventional gel electrophoresis but the capillary allows higher resolution, greater sensitivity, and on-line detection.

Schematic of capillary electrophoresis



Gel Electrophoresis

Gel electrophoresis is a technique used for the separation of deoxyribonucleic acid, ribonucleic acid, or protein molecules using an electric current applied to a gel matrix. It is usually performed for analytical purposes, but may be used as a preparative technique prior to use of other methods such as mass spectrometry. In most cases the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed. When separating proteins or

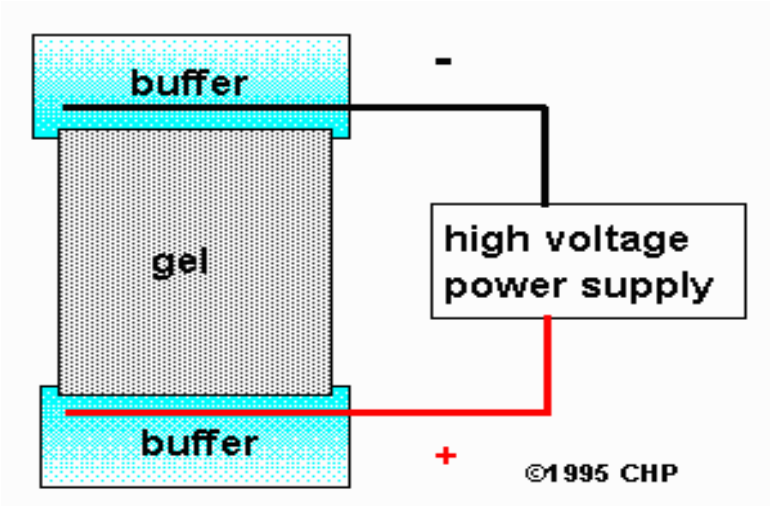
small nucleic acids (DNA, RNA, or oligonucleotides) the gel is usually composed of different concentrations of acrylamide and a cross-linker, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids (greater than a few hundred bases), the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a neurotoxin and must be handled using Good Laboratory Practices to avoid poisoning. By placing the molecules in wells in the gel and applyng an electric current, the molecule will move through the matrix at different at different rates usally determined by mass toward the positive (Anode) if negatively charged or toward the negative (Cathode) if positively charged.

After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. Ethidium bromide, silver, or coomassie blue dye may be used for this process. Other methods may also be used to visualize the separation of the mixture's components on the gel. If the analyte molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions. If the molecules to be separated contain radioactivity added for visibility, an autoradiogram can be recorded of the gel.

If several mixtures have initially been injected next to each other, they will run parallel in individual lanes. Depending on the number of different molecules, each lane shows separation of the components from the original mixture as one or more distinct bands, one band per component. Incomplete separation of the components can lead to overlapping bands, or to indistinguishable smears representing multiple unresolved components.

Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers available that contain a mixture of molecules of known sizes. If such a marker was run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule.

Schematics of Gel Electrophoresis



Application

Gel electrophoresis is used in forensics, molecular biology, genetic and biochemical analysis.