**Electrophorisis**

**Biotechnology**

Biotechnology is an application of the data and techniques of engineering and technology for the study and solution of problems concerning living organisms. One area that is acquiring much acclaim is the identification of unknown proteins through various molecular properties. In most cases, molecules differ with respect to size, weight, configuration, or net electrical charge. Electrophoresis, gel filtration chromatography, and thin-layer chromatography (TLC) are techniques that work by taking advantage of subtle differences in these properties.

**Electrophoresis**

Electrophoresis is the process of measuring the migration of ions in an electric field. This is accomplished by placing a pair of electrodes in an aqueous solution of protein or amino acid. One type of electrophoretic separation is called Isoelectric focusing or IEF. Isoelectric focusing takes place in a pH gradient and can only be used for amphoteric substances such as peptides and proteins. The pH at which an amino acid has a net charge of zero is called the **isoelectric point**. This pH is given the symbol pI. In this cell, anions migrate toward the anode, and cations migrate toward the cathode. At its isoelectric point, an amino acid does not migrate toward either electrode in an electrophoresis cell. The isoelectric point of a given amino acid is a physical constant.

The type of electrophoretic separation that is demonstrated in the module is called Zone Electrophoresis. In this process a homogeneous buffer system is used over the whole separation time and range so as to ensure a constant pH value. The distance covered during a defined time limit are a measure of the electrophoretic mobility of the various substances.

An understanding of the acid-base behavior of amino acids, and also of proteins, is important for two reasons. First, it helps us to understand the solubility of these molecules as a function of pH. While amino acids are generally quite soluble in water, solubility is a minimum at the isoelectric point. To crystallize an amino acid or a protein, the pH of an aqueous solution is adjusted to the pI and the substance is precipitated, filtered, and collected. This process is known as isoelectric precipitation. Second, knowledge of isoelectric points enables us to predict the way components of mixtures of amino acids or proteins migrate in an electric field.

Electrophoretic separations can be carried out using a variety of mediums. In paper electrophoresis, a paper strip saturated with an aqueous buffer of predetermined pH serves as a bridge between the two electrode chambers. A sample of amino acid or protein is applied as a spot. When an electrical potential is applied to the electrode chambers, amino acid or protein molecules migrate toward the electrode carrying the opposite charge. Molecules having a high charge density move more rapidly than those with a low charge density. Any molecule already at the isoelectric point remains at the origin. After separation is complete, the strip is dried. In the case of paper electrophoresis, the paper is sprayed with a dye to make the separated components visible. The dye most commonly used for amino acids is ninhydrin.

Electrophoretic separations can also be done using starch, agar, certain plastics, and cellulose acetate as solid supports. This technique is extremely important in biochemical research, and is also an invaluable tool in the clinical chemistry laboratory. In 1949 Linus Pauling made a discovery that opened the way to an understanding of sickle-cell anemia at the molecular level. He observed that there is a significant difference between normal adult hemoglobin(Hb A) and sickle-cell hemoglobin(Hb S). At pH 6.9, Hb A has a net negative charge and Hb S has a net positive charge and on electrophoresis at this pH, Hb A moves toward the positive electrode and Hb S toward the negative electrode.

Sequencing of DNA, or reading the sequence of DNA bases along its length, can now be accomplished. In DNA sequencing, a radioactive label is added to single-stranded DNA. The DNA is divided into four groups that undergo different chemical treatments. The chemical treatments break the DNA into pieces that when separated reveal the positions of the bases on the original strand. The DNA pieces are separated by the utilization of electrophoretic techniques.

DNA fingerprinting is gaining much acclaim during the last three years. DNA fingerprinting takes advantage of the fact that large portions of the human genome are made up of repeated sequences of varying lengths that do not code for proteins.

DNA fingerprinting works by taking a small sample of human DNA that has been cut with a restriction enzyme. The resulting fragments are separated by size through the process of electrophoresis. The bands that result from using electrophoresis are then compared and measured against any other individual in the world.

## Gel Electrophoresis

### Abstract

Electrophoresis has applications in biology, biochemical research, protein chemistry, pharmacology, forensic medicine, clinical investigations, veterinary science and food quality control. Arne Tiselius received a Nobel Prize in 1948 for development of this moving boundary system technique for separation by electrophoresis in 1939.

Under the influence of an electric field, charged particles migrate to the opposite charged electrode. Because of varying charge and mass different molecules move at different speeds and are separated into fractions. Mobility is a characteristic parameter of each charged molecule and is dependent on the pK value of the charged group and its size. It is influenced by the type of molecule, concentration, pH of the solution, temperature and field strength as well as the nature of the support material during electrophoresis. Buffers are used to guarantee constant pH. The buffer ions are carried just like the other ions so the buffer concentrations are relatively large compared to the materials separated. i.e. Buffers may be 0.1M while only 10 ug protein is used. Because the relative mobility of each substance is dependent upon so many variables, the migration distance is compared to a standard which is used in the same experimental run.

Currently, gels are most commonly used as the support medium for electrophoresis although cellulose fibers or thin layers of silica have been used. They may be used in thin capillary tubes or as a film on glass or plastic plates. There are three major types of gel electrophoresis:

* Zone electrophoresis which uses a homogeneous buffer. Distance traveled depends entirely on the molecular mobility. This module demonstrates this medhcan which can separate dyes, proteins, or peptides, DNA.
* Isotachophoresis (ITP) used a discontinuous buffer system. The ionized sample migrates between a "fast" leading electrolyte and a "slow" terminating one. This method is used in quantitative analysis and will not be considered in this module.
* Isoelectric focusing which uses a pH gradient. This method is only used for amphoteric substances such as peptides and proteins. The molecules move toward electrodes until they reach a portion in the pH gradient where their net charge is zero. This is the isoelectric point and the electric field no longer influences the molecule. This method is most commonly used in qualitative analysis to separate mixtures of substances.

This module incorporates several experiments using zone gel electrophoresis.

* One utilizes a 1% agarose gel because of its simplicity of preparation and nontoxic characteristics. Agarose gels can separate molecules larger than 10nm. the pore size in these gels ranges from 150 nm at 1% to 500 nm at 0.16%. Pore size variation allows separation of a greater range of molecular size. Since agarose becomes cloudy in solutions > 1% it is usually used only in separation of high molecular weight proteins in research or commercial applications. This module introduces gel electrophoresis first with agarose gels and dyes because of its greater ease of preparation and then leads to more sophisticated material using polyacrylamide gels.
* Polyacrylamide gel separations developed by Raymond & Weintraub in 1959 allow greater control of pore size by varying both the cross linking agent between the acrylamide chains and by varying the ratios of catalyst. Larger amounts of acrylamide:cross linking agent produces smaller pore sizes and allows better separation of smaller molecules. Smaller pore size also produces sharper bands of molecules within the gel. Polyacrylamide gels have the advantage of using thin gels which allow faster separations, better defined bands, faster staining after separation and better staining efficiency. However, one possible consideration in use in schools is that acrylamide is listed as a neutotoxin and thus should be handled carefully by the instructor who makes the stock preparations.