Electrophoresis: the main technique for molecular separation - lab report example...

Science, Biology

Electrophoresis: The Main Technique for Molecular Separation

The paper " Electrophoresis: The Main Technique for Molecular Separation" is an outstanding example of a lab report on formal science and physical science. Electrophoresis may be the main technique for molecular separation in today's cell biology laboratory. Because it is such a powerful technique, and yet reasonably easy and inexpensive, it has become commonplace. In spite of the many physical arrangments for the apparatus, and regardless of the medium through which molecules are allowed to migrate, all electrophoretic separations depend upon the charge distribution of the molecules being separated. Electrophoresis can be one dimensional (i. e. one plane of separation) or two dimensional. One dimensional electrophoresis is used for most routine protein and nucleic acid separations. Two-dimensional separation of proteins is used for fingerprinting, and when properly constructed can be extremely accurate in resolving all of the proteins present within a cell (greater than 1, 500).

The support medium for electrophoresis can be formed into a gel within a tube or it can be layered into flat sheets. The tubes are used for easy one dimensional separations (nearly anyone can make their own apparatus from inexpensive materials found in any lab), while the sheets have a larger surface area and are better for two- dimensional separations. Figure 4. 1 shows a typical slab electrophoresis unit.

When the detergent SDS (sodium dodecyl sulfate) 2 is used with proteins, all of the proteins become negatively charged by their attachment to the SDS anions. When separated on a polyacrylamide gel, the procedure is

abbreviated as SDS--PAGE (for Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis). The technique has become a standard means for molecular weight determination.

Polyacrylamide gels are formed from the polymerization of two compounds, acrylamide, and N, N-methylene- bis-acrylamide (Bis, for short). Bis is a cross-linking agent for the gels. The polymerization is initiated by the addition of ammonium persulfate along with either -dimethyl amino-propionitrile (DMAP) or N, N, N, N,- tetramethylethylenediamine (TEMED). The gels are neutral, hydrophilic, three-dimensional networks of long hydrocarbons crosslinked by methylene groups.

The separation of molecules within a gel is determined by the relative size of the pores formed within the gel. The pore size of a gel is determined by two factors, the total amount of acrylamide present (designated as %T) and the amount of cross-linker (%C). As the total amount of acrylamide increases, the pore size decreases. With cross-linking, 5%C gives the smallest pore size. Any increase or decrease in %C increases the pore size. Gels are designated as percent solutions and will have two necessary parameters. The total acrylamide is given as a % (w/v) of the acrylamide plus the bis-acrylamide. Thus, a 7 1/2 %T would indicate that there is a total of 7. 5 gms of acrylamide and bis per 100 ml of gel. A gel designated as 7. 5%T: 5%C would have a total of 7. 5% (w/v) acrylamide + bis, and the bis would be 5% of the total (with pure acrylamide composing the remaining 2. 5%).

Proteins with molecular weights ranging from 10, 000 to 1, 000, 000 may be separated with 7 1/2% acrylamide gels, while proteins with higher molecular weights require lower acrylamide gel concentrations. Conversely, gels up to

30% have been used to separate small polypeptides. The higher the gel concentration, the smaller the pore size of the gel and the better it will be able to separate smaller molecules. The percent gel to use depends on the molecular weight of the protein to be separated. Use 5% gels for proteins ranging from 60, 000 to 200, 000 daltons, 10% gels for a range of 16, 000 to 70, 000 daltons and 15% gels for a range of 12, 000 to 45, 000 daltons.

Cationic vs anionic systems

In electrophoresis, proteins are separated on the basis of charge, and the charge of a protein can be either + or--, depending upon the pH of the buffer. In normal operation, a column of gel is partitioned into three sections, known as the Separating or Running Gel, the Stacking Gel and the Sample Gel. The sample gel may be eliminated and the sample introduced via a dense non-convective medium such as sucrose. Electrodes are attached to the ends of the column and an electric current passed through the partitioned gels. If the electrodes are arranged in such a way that the upper bath is -- (cathode), while the lower bath is + (anode), and -- anions are allowed to flow toward the anode, the system is known as an anionic system. Flow in the opposite direction, with + cations flowing to the cathode is a cationic system.