

charge on the protein will be positive. The net charge on the protein will determine the velocity of the protein. When a protein molecule is placed in a pH gradient, the electrophoretic velocity becomes zero when $\text{pH} = \text{pI}$, since the net charge on the protein is zero at $\text{pH} = \text{pI}$. Precipitation of proteins in a pH gradient at their isoelectric point is known as *isoelectric focusing*. Since the size and charge of protein molecules are different at a given pH, the terminal velocity of the proteins will be different according to eq. 11.96 and, therefore, proteins will be separated from each other in an electric field. Certain gels, such as agar or polyacrylamide, are used for protein separations by gel electrophoresis. Gel electrophoresis is an important analytical separation technique. Scale-up is problematic due to thermal convection resulting from electrical heating.

One analytical and micropreparative version of electrophoresis that has excellent resolution is called two-dimensional protein electrophoresis (2DE). The 2DE procedure is actually the series combination of two electrophoretic separations which resolve protein mixtures based on two independent characteristics—charge and size. Proteins are first separated in a polyacrylamide gel matrix using isoelectric focusing—an equilibrium separation technique that resolves proteins based on their respective isoelectric points. Proteins are subsequently coated with an anionic surfactant and separated by size in another polyacrylamide gel. Finally, proteins are detected using a chemical stain, by autoradiography or by other methods. The result of this two-dimensional separation is a high-resolution fingerprint of protein expression that is characteristic of a particular biological system. This technique has at least two orders of magnitude better resolution than any other analytical tool for protein analysis. Separated protein spots can be cut out of the polyacrylamide gels and subjected to further microchemical analysis to determine the amino acid sequence.

11.4.11. Electrodialysis

Electrodialysis (ED) is a membrane separation method used for the separation of charged molecules from a solution by application of a direct electric current. The membranes contain ion-exchange groups and have a fixed electrical charge. Positively charged membranes (anion membranes) allow the passage of anions and repel cations; negatively charged membranes (cation membranes) allow the passage of cations and repel anions. This method is very effective in the concentration of electrolytes and proteins.

The ED process is driven by electrostatic forces and can be used to transfer salts from low to high concentration. Salt solutions can be concentrated or diluted by this method. The ED method is much faster than dialysis and is a more efficient desalting method.

Ion-exchange membranes (IEM) used in ED units are in essence ion-exchange resins in sheet form. IEMs contain mobile counterions that carry electric current. The cation-exchange membrane consists of polystyrene with negatively charged sulfonate groups bonded to phenyl groups in polystyrene. Anion membranes contain positively charged quaternary ammonium groups ($-\text{N}_3\text{R}^+$) chemically bonded to the phenyl groups in the polystyrene. Mobile counterions account for the low electrical resistance of the membranes. To control swelling of sulfonated polystyrene, some cross-linking agents such as divinyl benzene are included in the polymer. A typical ED membrane has a pore