5.2.4 Electrophoresis

5.2.4.1 SDS-PAGE PAGE describes a technique widely used to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Mobility is a function of the length, the conformation, and the charge of the molecule. As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the HOS, or a chemical denaturant may be added to remove this structure and turn the molecule into an unstructured linear chain whose mobility depends only on its length and mass-to-charge ratio. For nucleic acids, urea is the most commonly used denaturant. For proteins, SDS is an anionic detergent applied to the protein sample to linearize proteins and to impart a negative charge to linearize proteins. In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. Proteins that have a greater hydrophobic content, for instance, many membrane proteins and those that interact with surfactants in their native environment, are intrinsically harder to accurately treat using this method, due to the greater variability in the ratio of bound SDS.

5.2.4.2 2D-SDS PAGE Two-dimensional gel electrophoresis, abbreviated as 2DE or 2D electrophoresis, is a form of gel electrophoresis commonly used to analyze proteins. Mixtures of proteins are separated by two properties in two dimensions on 2D gels. 2DE was first independently introduced by O'Farrell and Klose in 1975. 2DE begins with one-dimensional (1D) electrophoresis but then separates the molecules by a second property in a direction 90° from the first. Because it is unlikely that two molecules will be similar in two distinct properties, molecules are more effectively separated in 2DE than in 1D electrophoresis. The two dimensions that proteins are separated by using this technique can be the pI, a protein complex mass in the native state and protein mass.

5.2.4.3 Native electrofocusing Native electrophoresis is one of the electrophoretic methods comprising SDS-PAGE, native electrophoresis, IEF, 2DE, and CE. One-dimensional SDS-PAGE offers separation of proteins according to their molecular weight. Samples run under denaturing, but nonreducing conditions will provide information of presence of other molecular species and of disulfide intermolecular di- and polymers. Samples run under denaturing and reducing conditions will provide information on monomeric compounds. Notice that in the latter procedure, it is common practice to boil the sample in the denaturing and reducing buffers before application. The boiling procedure must not be used if information of aggregates is required (denaturing but non-reducing conditions). Native electrophoresis separates proteins according to charge, molecular weight, shape, and other factors (samples are typically applied under conditions maintaining the tertiary structure).