are disulfide linked, and thus is a reflection of alterations in oxidation of Cys residues. This method is also useful in quickly ascertaining if there are noncovalent linkages such as those caused by di-Tyr generation as a result of Tyr oxidation. Although apparent molecular sizes can be determined using SDS PAGE with appropriate protein size markers, the actual molecular weight in solution may be dramatically different, as we will show in the physical assay section.

Native PAGE

This method is similar to SDS PAGE but analyzes protein transport in an electric field without the presence of SDS. As might be expected the protein molecules will separate on the basis of both their size and charge. Thus, this can be used as a quick complementary assay for comparison with IEX chromatography.

Isoelectric focusing

As previously mentioned proteins as well as mAbs are multicharged macromolecules. The net charge of the protein is a function of the hydrogen ion equilibria as well as binding of ions. The protein net charge will vary with the pH, whereby the maximum net positive charge is at acidic pH values and the protein progressively becomes less positively charged as the pH increases. At some pH value termed the isoelectric point, pI, the number of positive charges equals the number of negative charges and the protein has a net zero charge. Electrophoresis in an electric field with a pH gradient results in the protein migrating to the pH where the net charge is zero, and the protein no longer migrates in the field. The generation of a pH gradient in the applied electric field results from the use of "carrier ampholytes" of appropriate pI and buffer capacity. When a mixture of these ampholytes is subjected to an electric field they each migrate to a zone determined by their pI resulting in a stable pH gradient. Protein samples run under this gradient then migrate and essentially "focus" at the pI value. Commercially available markers are used to determine the pI for the applied sample. When isoelectric focusing (IEF) is run using large-pore gel systems such as agarose or polyacrylamide (Righetti & Drysdale, 1974) a resolution of as little as 0.02 pH units can be attained (Vesterberg & Svensson, 1966). Although IEF has high resolving power, a significant problem is that many proteins have minimum solubility at their pI and thus surfactants or urea may be required to keep the protein in solution. In this case the determined pI values will not be for the native protein, but for the unfolded protein due to the use of surfactants or a denaturing molecule such as urea. Despite this disadvantage the high resolution that is attained can actually distinguish one single charge difference between proteins. An example of this is shown in Figure 2.2 where the coat protein from a plant virus (TMVP), tobacco mosaic virus, was expressed by rDNA technology in *Escherichia coli* and compared to the wild-type protein from virus isolated from tobacco plant (Shire et al., 1990). In the plant the TMVP is acetylated on the N-terminus, whereas in *E. coli* no such processing occurs resulting in a free amino terminal group. Thus the rDNA-produced TMVP has only one charge difference, and can be resolved by IEF. Another issue when using IEF is that the pH gradient may degenerate over time resulting in a substantial decrease in resolution. This has been overcome by