of HPLC detectors to analyze the eluents during the electrophoresis, and the high throughput-rapid turnover for the assay. The capillaries are made from fused silica, and the predominance of negative charges can interfere with electrophoresis of protein samples. Fortunately, appropriate coatings have been developed to mask these charge groups and permit analysis of large protein molecules. CE has been used in several formats including non-gel sieving (Hunt & Nashabeh, 1999) and IEF (Hunt, Hotaling, & Chen, 1998; Hunt, Moorhouse, & Chen, 1996). SDS PAGE analysis of proteins and mAbs with and without reducing agents is very useful for determination of disulfide-linked species. Hunt and Nashabeh (1999) showed that CE non-gel sieving yields similar results to SDS PAGE with silver staining.

Membrane-confined electrophoresis

Many of the charge-based assays such as IEX chromatography and native PAGE do not provide a direct measurement of the effective charge on the protein. Usually the net charge as a function of pH is estimated from a summation of the ionization of the side chain residues in proteins (Shire, 1983; Tanford, 1962). Improvements have been made in such computations by incorporating electrostatic interactions and steric environments of a protein (Olsson, Sondergaard, Rostkowski, & Jensen, 2011). The actual net charge on a protein or mAb can be determined by measuring electrophoretic mobility coupled with an independent determination of the frictional coefficient. Determining the frictional coefficient in an electric field can be difficult. A more direct determination involves electrophoresis in the absence of any sieving mechanism. An apparatus for such a measurement has been developed and described by Laue et al. (Ridgeway et al., 1998). Comparison of charge determined by MCE with predicted theoretical charge using T4 lysozyme charge mutants showed very good agreement (Durant, Chen, Laue, Moody, & Allison, 2002). However, it has been shown that often the determined charge of mAbs is significantly different from the computed values. Some of this difference may be due to selective binding of anions by mAbs (T. Laue, personal communication).

Spectroscopic methods

Spectroscopic assays have been used mainly for analysis of protein conformation and higher order structure. Many of these methods are rapid and can provide valuable stability data to enable formulation development. The most commonly used methods in therapeutic protein formulation development include ultraviolet absorption, circular dichroism, fluorescence, and infrared spectroscopy.

Ultraviolet absorption spectroscopy

Ultraviolet absorption spectra from 240 to 320 nm are due to the aromatic amino acid residues, Tyr, Phe, and Trp, and a spectral scan from 240 to 320 nm provides a fast and reliable way to determine concentration of proteins in solution. Often an independent method such as quantitative amino acid composition is used to obtain concentration of the protein in order to determine absorptivity values. Once