role in determining the molecular weight from the reference standards (Andrews, 1970). Thus, molecular weight determinations of highly asymmetric molecules will be in error when using typical calibrated globular protein molecular weight standards. Development of light scattering detection systems has mitigated this problem since actual molecular weight determinations are made on the separated peaks (Wen, Arakawa, & Philo, 1996). Despite this improvement, there remain several difficulties due to nonspecific adsorption of protein molecules onto the column gel matrix. The use of solvents such as isopropyl alcohol (IPA) or salts can decrease these interactions, but may alter the molecular weight distribution that occurs in the aqueous buffer systems (Philo, 2009). Ricker and Sandoval analyzed a number of mAbs at varying ionic strengths (Ricker & Sandoval, 1996). It was shown that the results varied among the antibodies, and that some mAbs showed retention time shifts and poor peak resolution at low ionic strength.

Another complication is that the protein sample dilutes during the chromatography, and if the protein reversibly self-associates as a function of concentration, it may be difficult to determine the actual molecular weight distribution of the original formulated protein at higher concentration (Shire, 1994). Large molecular weight aggregates may also be filtered out during the chromatography, resulting in loss of protein which leads to erroneous determinations of percent aggregate in the protein sample (Philo, 2009). For all of these reasons regulatory agencies have requested the use of other molecular weight determination methods to verify that SEC is measuring the correct molecular weight distribution of the formulated DP. This aspect will be discussed in more detail in descriptions of biophysical assays.

Despite all these disadvantages for actual molecular weight determination, SEC can be a very useful high throughput assay to explore conformational changes in a protein. Thus an independent measurement of molecular weight can lead to determination of effective hydrodynamic radius by SEC (Martenson, 1978). The use of light scattering detectors online facilitates such a determination, and can be useful in assessing conformational changes as a function of formulation conditions (Wen et al., 1996).

Ion exchange chromatography

Ion exchange chromatography (IEX) is a chromatographic separation method essentially based on the net charge of the protein, and is generally used to follow deamidation and succinimide formation. Positive (cationic) or negative (anionic) charge moieties are directly linked to the chromatographic matrix. At any specific pH the protein or mAb will have a net charge that is governed by the different amino acid residues that are capable of hydrogen ion ionization. Acidic residues such as Asp or Glu contribute negative charge when deionized, whereas basic residues such as His, Lys, and Arg contribute positive charge when unionized. The net charge of a protein can be computed from the amino acid composition by summing up the individual side chain ionizations assuming that there are no electrostatic interactions or impact of conformation (Tanford, 1962). The chromatography is usually run at low ionic strength so that the protein can bind to the charged residues on the ion exchange matrix. After binding they can be eluted using either a pH or a salt gradient. Theoretically at any pH value, proteins should elute in order of their net charge, i.e., on an anion exchange