interactions that affect the binding of the mAbs to the SC tissue. Thus, appropriate choice of buffer components and alteration of ionic strength may be used to modulate adsorption to SC tissue that could impact the bioavailability of the mAb.

Development of analytical tools for high-concentration formulation development

Many of the analytical methods that are currently used to investigate covalent (chemical) and conformational alterations in proteins are easily adapted to the study of proteins at high concentration (Jones, 1993; Pearlman & Nguyen, 1991). Analytical techniques such as differential scanning calorimetry (DSC), modulated DSC (Breen, Curley, Overcashier, Hsu, & Shire, 2001; McPhillips, Craig, Royall, & Hill, 1999), Fourier transform infrared spectroscopy (Costantino, Chen, Griebenow, Hsu, & Shire, 1998; Prestrelski, Tedeschi, Arakawa, & Carpenter, 1993), Raman (Tuma, 2005), and fluorescence (Sharma & Kalonia, 2003) spectroscopy have been used to characterize solid-state formulations, and can be useful in developing alternatives to TFF for creating concentrated DP subsequent to reconstitution (discussed in more detail in the next chapter). Unfortunately many of the analytical technologies used to characterize proteins require dilution of the protein to lower concentrations or the use of solvents that differ from the initial formulation composition. This may have considerable impact on the results since changes in solvent composition or concentration may change the behavior of the protein that occurs at the higher concentration.

It is especially important to analyze for molecular weight and size in the high-concentration formulation. As discussed previously, SEC has several disadvantages including potential interaction with the chromatographic resin, and impact of hydrodynamic volume on molecular weight determination. The first issue is usually mitigated using organic solvents or salts to minimize the column interactions, but there is then the uncertainty regarding whether there is a perturbation of the protein–protein interactions that results in aggregate formation. The impact of hydrodynamic volume and shape can be addressed using an online static light scattering (SLS) detector to determine molecular weights directly across the chromatographic peaks. However, due to the dilution during the chromatography any reversible self-association that is concentration dependent may not be detected due to the shifting of the equilibrium toward monomer at the lower concentration. As an example, the rapid dissociation of a monoclonal antibody upon dilution yielded varying aggregate levels by SEC, depending on the time and temperature of analysis after sample dilution (Moore, Patapoff, & Cromwell, 1999).

Thus, the characterization and study of protein–protein interactions at high concentration are difficult to assess with those methodologies. Having said that, determinations using DLS at low concentration have proven to be useful in predicting the viscosity behavior and solubility of mAbs. However, in order to investigate the properties of concentrated mAbs requires development of analytics that can be used at high concentrations. Some of the methods, including the DLS technology, will be discussed further in the next chapter.