2004). When performing a concentration dependence measurement using UV absorption at ~280 nm, the scattering must be taken into account. If the scattering is sufficiently low a simple subtraction of absorbance at 320 nm will suffice, but larger contributions may need to be corrected using methods such as outlined by Englander (Englander & Epstein, 1957).

Dynamic light scattering can also be performed using the time course of the light scattering signal, which is used to determine the diffusion coefficient by autocorrelation methods (Lorber, Fischer, Bailly, Roy, & Kem, 2012). Independent measurements of shape or approximation of spherical geometry can be used to obtain molecular weights from the diffusion coefficient using the Stokes–Einstein equations. With the advent of computer interfacing and plate readers, DLS has become a higher throughput assay. In addition DLS experiments as a function of concentration can yield an interaction parameter, which is useful in investigations of protein–protein interactions. Much of this will be discussed in a later chapter on high-concentration formulations for subcutaneous delivery of mAbs.

Differential Scanning Calorimetry (DSC)

Microcalorimetry has been used to study thermal transitions in biomolecules and the application of this method in biotechnology has been reviewed (Chowdhry & Cole, 1989). Differential scanning calorimetry (DSC) has been used to aid in formulation development and to characterize the glass transition temperature in freeze-dried solid dosage forms. Although DSC has been used to aid in formulation development, it should not be used in the absence of other assays, especially those that monitor chemical changes. As will be discussed and shown, chemical alterations can occur in natively folded protein, and thus choosing formulation excipients and conditions that show stabilization by DSC does not guarantee a robust formulation. When used in conjunction with other methods to evaluate stability, DSC can be extremely useful. In particular, DSC can give valuable information on which region of a mAb is stabilized by excipients or which regions are prone to physical stability issues (Mehta, Bee, Randolph, & Carpenter, 2014; Oganesyan, Damschroder, Leach, Wu, & Dall'Acqua, 2008).

Also determination of the unfolding temperature for a protein can aid in designing appropriate high-temperature studies where the protein remains essentially in a folded state. A good example of this is a stability study of Pulmozyme where several temperatures were used below the DSC measured unfolding transition (Shire, 1966). This coupled with the use of tentacle ion exchange chromatography as a biomemetic assay (discussed earlier) that specifically monitors the deamidation of an Asn in the binding pocket for the target DNA allowed for successful use of Arrhenius kinetics. This study showed that the extrapolated rate constant from an Arrhenius plot was in very good agreement with that measured in real time (Table 2.2).

DSC has also been used to determine the glass transition temperature, Tg′, for freeze-dried formulations where the matrix becomes more fluid resulting in less stability than when stored at temperatures below Tg′. Formulations that have high sugar content require a modified form of DSC called modulated DSC. In traditional DSC experiments the difference in the heat flow between a sample and a reference (usually the buffer system of the sample) is measured as a result of a linear change in temperature. The heat capacity can also be determined by subjecting two different samples