the value of the absorptivity for a protein in a particular solvent is determined, the concentration can be readily assessed from the UV spectra using Beer's law. UV spectra of known concentrations of N and C terminal-blocked Phe, Tyr, and Trp peptides have also been used to determine the absorptivity contributions from the aromatic side chains in a protein, and then computing the absorptivity value for the protein by summing up the individual contributions from the aromatic amino acid residues. However these computations do not take into account the effect of environment on the UV absorption of the contributing residues due to the folded structure, and therefore usually give concentrations within 10% of the actual value. Difference (Donovan, 1973) and derivative spectroscopy (Balestrieri, Colonna, Giovane, Irace, & Servillo, 1978) can explore the impact of environment due to protein folding. The change from a hydrophobic to a more hydrophilic environment that occurs when a protein unfolds results in a change in polarizability which generally results in a red shift of the UV spectra and an increase in the absorptivity of 10–15% (Donovan, 1973). It has been shown that comparing natively folded protein to enzymatically digested protein using second-derivative UV spectroscopy can assess the magnitude of these effects (Bewley, 1982). Comparison of the second-derivative UV spectra with that computed from blocked Phe, Tyr, and Trp compounds confirms that the protein is fully denatured and the computed absorptivity for unfolded protein is used to determine the absorptivity for native protein (Gray, Stern, Bewley, & Shire, 1995).

Since the UV spectra of a protein are highly dependent on the exposure of the residues during unfolding, this method can be used to assess the impact of formulation and storage conditions on the physical stability of a protein or mAb. For example, stability of the tertiary structure of the mAb Rituxan stored at 0.4 mg/mL for 1 and 6 months at 40 °C was assessed by second-derivative UV spectroscopy (Paul, Vieillard, Jaccoulet, & Astier, 2012). The results showed little change after 1 month but measurable alteration after 6 months.

Circular dichroism

Circular dichroism (CD) measures the difference between right and left circularly polarized light, which provides an indication of the chiral environment around amino acid residues in proteins. CD in the far UV region (~190 to 240 nm) provides information on the secondary structure of a protein due to the arrangement of peptide bonds into well-defined constrained structures. Polylysine was used to show that the main class of structures, i.e., α-helix, β-sheet, and "random" coil, has distinct spectra (Davidson & Fasman, 1967) which can be deconvoluted from the total CD spectra to obtain estimates of secondary structure content (Chen, Yang, & Chau, 1974). Improvements in fitting algorithms coupled with the CD of proteins with known structure determined by NMR or X-ray crystallography have refined the determination of secondary structure by CD (Perczel & Fasman, 1992; Provencher & Glockner, 1981). The modification of CD instruments to allow for reliable CD measurements in a vacuum results in CD spectra as low as 180 nm. As discussed by Johnson (1990) such measurements provide more robust analysis of secondary structure since there are