because, in complex solutions, proteins are not the only molecules that absorb UV light. Other compounds can skew the results, but their interference is minimized when the absorbance is measured at the UV level. Note that presence of nucleic acids in the sample will interfere with the absorbance; the extinction coefficient of a protein is pH dependent.

In the past, the extinction coefficient was determined via a number of experimental procedures that tended to be very laborious and error prone. Today, nearly all protein extinction coefficients are assessed using empirical equations that utilize the average molar extinction coefficients determined from a collection of published literature data for the three key chromophores that contribute to the UV absorbance at 280 nm (tyrosine, tryptophan, and disulfide bond), in conjunction with known amino acid sequence of the product drug of interest:

$$\text{Protein} \times \text{molar extinction coefficient at } 280\ \text{nm} = \varepsilon_{tyr}(n_{tyr}) + \varepsilon_{try}(n_{try}) + \varepsilon_{disulf}(n_{disulf}),$$

wherein ε is the extinction coefficient and *n* is the number of moles.

The accuracy of the extinction coefficient generated from this empirical formula has been assessed at about 5%, but could be as great as 10% particularly if the protein has an unusual amino acid composition. The FDA recommends determining the extinction coefficient using an orthogonal measurement such as using analytical centrifuge and SEC with LS, UV, and refractometric detection.

Once the extinction coefficient is determined, it can be combined with other attributes of the protein, such as amino acid composition, to improve the robustness of the measurement of the concentration of protein in the samples.

5.3.1.4 Amino acid analysis (AAA) AAA is done to determine the amino acid composition of a protein without using an external standard. It can be applied in many ways to reveal different aspects of the protein. In combination with UV-absorbance measurements; it can be used directly to determine the extinction coefficient of a protein. By omitting hydrolysis, it can be used to quantify free amino acids, for example, in cell culture media. It can quantify unusual amino acids such as norleucine (encountered in *E. coli* fermentations) or hydroxyproline and lysine. The concentration based on mass can be combined with MALDI-TOF to reveal the intact mass of the protein.

5.3.1.5 Peptide mapping Peptide mapping is commonly undertaken to analyze the protein's primary structure after cleavage of the protein into proteolytic peptides. The power of peptide mapping lies in the large number of site-specific molecular features that can be detected. When using one digestion enzyme (for example, trypsin), peptide mapping is typically carried out for protein identification. The analysis is performed using MALDI-MS/MS or LC-ESI-MS/MS, for example, during protein