spectrophotometer at the wavelengths to be measured, using the blank solution, the analyst measures the absorbance of the protein solution. For relatively pure solutions, measuring the absorbance at 280 nm (A280) is usually sufficient. However, for protein solutions containing significant amounts of nucleic acid (as little as a few percent), it is best also to determine the absorbance at 260 nm (A260), to correct for the presence of nucleic acids. The protein concentration is then determined using the following equation:

$$\text{Protein (mg/mL)} = 1.55(\text{A280}) - 0.76(\text{A260}) \tag{9.1}$$

If the extinction coefficient has not yet been determined, a more absolute concentration is determined using the following equation:

$$\text{A280 (mg/mL)} = (5690 * \text{Trp} + 1280 * \text{Tyr} + 120 * \text{Cys})/\text{protein MW} \tag{9.2}$$

where Trp is the number of tryptophan residues in the protein, and similarly, Tyr is the number of tyrosines, Cys is the number of cysteine residues, and MW is the molecular weight of the protein.

Spectrophotometric methodologies are used less commonly in late-stage development of proteins but are very helpful in the early development of biopharmaceuticals. For example, CD can be used to study the tertiary structure of proteins. Use of CD does not require the highly pure concentrated protein solutions needed to prepare protein crystals for X-ray crystallography. A protein's specific CD spectrum in the near-UV region (250–340 nm) is determined by its regular 3D structure in solution. By comparing the CD spectra of a protein in both a denaturing and nondenaturing solvent, some estimate can be made regarding the conformational stability of the protein. Because the protein concentration needed to perform CD studies is relatively low, these studies can be undertaken early in development with small amounts of manually purified protein. Because interpretation of the spectra is often difficult, in many cases, CD spectroscopy analyses are sent to laboratories experienced in utilizing these techniques.

The FT-IR can also be used to determine the tertiary structure of a protein. It does not require the protein to be in solution, and it can often be used to support early formulation development for either liquid or lyophilized proteins.

9.9.3.2 Electrophoresis

Electrophoresis is the separation of charged molecules in an electric field. In PAGE, the electric field is formed within the pores of a polyacrylamide gel and are filled with a running buffer. The addition of SDS to the sample preparation buffer as well as to the running buffer is often used to pretreat the protein prior to electrophoresis, hence the term SDS-PAGE. In SDS-PAGE, the SDS molecules interact with the protein, unfolding it and adding multiple charges to the molecule from the associated sulfate groups. Complete unfolding of a protein may require the addition of a reducing agent as well as the SDS. Proteins migrate through the polyacrylamide gel and are separated according to their MW in SDS-PAGE.

Another common technique is to run native or nondenaturing PAGE. In native gel electrophoresis, the migration of the protein through the gel is affected by both the