charge and the shape of the protein, as well as the size. While SDS-PAGE is commonly used to determine MW of proteins, it would be incorrect to use native gel PAGE for weight determination. Both methods are used to assess the purity of a protein.

Protein is invisible in the gels and must be stained for detection. The most commonly used visualization techniques are silver and Coomassie blue stains. While silver is more sensitive, the intensity of silver stains is affected by the proteins and is not linear with the concentration of protein, as is Coomassie blue staining. If the intention is to quantify the relative amounts of each protein band, Coomassie blue staining should be used.

In addition to the determination of MW, SDS-PAGE is used to examine the presence of aggregates. Samples can be prepared with and without the reducing agent, either mercaptoethanol or dithiothreitol. Comparison of reduced and nonreduced gel patterns allows the analyst to determine whether the higher-MW aggregates seen are due to intermolecular disulfide bridges. In addition, SDS-PAGE provides information about the purity of the protein. After scanning Coomassie blue-stained gels and calculating the area or relative intensity of each band seen in a sample, the percentage of the total protein can be determined. Most laboratories have scanning software capable of performing both image analysis and quantification. Many software programs can also determine the MW by using results from the standards run on the same gels.

The IEF is another electrophoretic separation method. In this method, the polyacrylamide gel or another support layer also contains a pH gradient. This is a powerful method for investigating the charge differences among proteins. In IEF, each protein migrates through the support layer until it is "trapped" at the point where the pI of the protein is the same as the pH gradient formed in the support media. At this point, the charge on the protein is zero, and it no longer migrates but focuses. Separated proteins need to be stained to be visualized. The pI of a protein can be determined in an IEF separation either by comparison with standards run simultaneously or by measuring the pH of the band with a special pH electrode. In proteins with multiple glycosylated forms, it is often difficult to determine the pI, because the multiple forms may run as a smear across the gel. In such cases, the carbohydrates could be enzymatically removed, yielding a single protein form.

An electrophoretic method used increasingly in the early-stage characterization of proteins is 2D electrophoresis. This method separates the proteins in one dimension based solely on charge (IEF) and in the second dimension based on size (SDS-PAGE). This powerful method can determine whether a protein that is a single band on SDS-PAGE comigrates with another protein. A new use of 2D electrophoresis is for the determination of host-cell proteins. This method can often identify host-cell proteins, which comigrate with the protein of interest in SDS-PAGE gels. The protein is separated in a thin IEF gel; the lane is then placed across the top of an SDS-PAGE gel, and a second electrophoresis is run. After staining, the gel contains one or more spots. The gel can be scanned on a densitometer, and the relative intensity of each spot can be used to determine the percentage of protein that is not the product.

9.9.3.3 Chromatography

High-performance liquid chromatography is a core technique in the characterization of proteins. These separations are coupled with detectors that are sensitive to the proteins eluted during chromatographic separation. The most common detector