



FIGURE 9.17 *m*-PEGylation using *N*-hydroxysuccinimide ester.

when compared with modification with a linear-PEG counterpart. A branched-PEG reagent was used in the creation of PEGASYS (PEGylated-interferon alpha) by Roche and on aptamers. Substitution values are 95% or greater.

9.9.3 Characterization Methods

9.9.3.1 Spectroscopy

Spectrophotometric analyses are the most common methods to characterize proteins. Ultraviolet-visible (UV-VIS) spectroscopy is typically used for the determination of protein concentration by using either a dye-binding assay (e.g., the Bradford or Lowry method) or by determining the absorption of a solution of protein at one or more wavelengths in the near-UV region (260–280 nm). Another spectroscopic method used in the early-phase characterization of biopharmaceuticals is circular dichroism (CD).

The Bradford method, which is more sensitive and less affected by most common detergents or other common biochemicals than the Lowry method, is the most widely used dye-binding method. There are two common Bradford methods: the standard assay Bradford method, with a range of 10–100 mg, and the microassay method, which is linear between 1 and 10 mg. A standard curve is constructed with a common protein that is readily available in pure form, such as bovine serum albumin and bovine gamma globulin. The standards and the sample are then reacted with a solution of Coomassie Brilliant Blue G250 in an acidic solution, and the absorbance is measured at 595 nm. The protein concentration of the sample is then calculated against the constructed curve. This value is an approximation of the protein concentration, because different proteins react differently with the Bradford reagent. Further on in development, the calibration curve should be determined using the protein of interest.

Direct determination of the absorbance of a protein solution requires no other reagents or standards. Two solutions are prepared, one of the samples and one blank solution of water or containing all the buffer components. After zeroing the