The stress tests form the basis of many directions in which the preformulation studies can assist in the final dosage formulation. The common tests include the shake test (agitation), surfactant test, freeze–thaw test, and heating experiments (limited because of denaturation). Each formulation configuration is shaken in a vial to determine whether it forms aggregates. Then, a surfactant (usually a polysorbate detergent, such as Tween 80®) may be selected to prevent the formation of precipitants by making it harder for proteins to aggregate. Human albumin is a frequent additive, but its use is discouraged because of supply constraints and viral clearance requirements. Formulations are checked through multiple freeze–thaw cycles (which can take about a week) to check for the effects of temperature and freezing-process stresses. Most proteins are stable around 2°C–8°C, but few are stable at room temperature. Heating experiments help scientists examine degradation at temperature extremes by heating them to 30°C (about 86°F) and maybe even to 45°C (about 113°F). At high temperatures, different mechanisms of protein denaturation may arise.

Most prominent stability reactions include oxidation, hydrolysis, and disulfide exchange. Stability of protein products can be significantly enhanced if the oxygen is removed from the headspace of the unit pack, as oxygen induces specific degradation reactions, which are complex and difficult to study. In most instances, this will speed up the development time, as fewer matrices would have to be evaluated for factors affecting stability. Certain amino acids (tryptophan, methionine, cysteine, histidine, and tyrosine) are susceptible to oxidation. Metal ions such as copper and iron can accelerate the process of oxidation, as does the higher pH, fluorescent light, and hydrogen peroxide. If the amino acids along a polypeptide chain are deformed by oxidation, the molecule can be irreversibly altered, and the new molecule will likely be inactive. Antioxidants help to protect against oxidation by scavenging oxygen for themselves. Ascorbic acid is used, but citric acid is preferred, and it can be used as a pH adjuster as well.

Hydrolysis of a side-chain amide on a polypeptide's glutamine or asparagine residues can yield a carboxylic acid. The process, called deamidation, is facilitated by elevated temperature and pH, resulting in the loss of activity. The peptide bonds that hold amino acids together in the chain can also be severed by hydrolysis—particularly where aspartic acid residues are located. This effect is usually due to heat or low pH.

Cysteine residues form disulfide bonds, which are important to protein's structural integrity. Shuffling of these bonds, where two sulfur atoms from two different amino acid molecules link up, often changes the 3D structure, causing a loss of activity.

9.8.1.1 Excipients

Salts and nonelectrolytes (such as ammonium sulfate and glycerol) help to stabilize proteins in high temperatures and low pH, when freezing is not an option, but they still require low-temperature storage. Sometimes, they must be removed before the drug is used, which can be inconvenient, time-consuming, and expensive. Also, the active ingredient must be diluted, allowing further waste and variability in the final product, just as in reconstituting freeze-dried products.

The biological drug is not likely to be stable without the addition of additives such as buffers, albumin, and surfactants, if kept in a liquid form; otherwise, an early conversion of the drug to a lyophilized form is completed. Most formulations would not include any preservative. Table 9.3 lists the recombinant products approved by the