TABLE 9.7

Preventive Actions Against Oxidation

Factor	Comment
pH	The oxidation rate is assumed low at slightly acidic pH.
Temperature	Working at low temperatures decreases the rate of oxidation.
Time	The oxidation reaction is a function of time.
Conductivity	No data available.
Redox potential	Disulfide bond formation will take place at a redox potential above 0 mV. A high redox potential indicates presence of oxidizing agents.
Co-solvents	Avoid oxidizing agents and protect against light. Addition of chelating agents (EDTA, citric acid, thioglycolic acid), antioxidants (BHT, BHA, propyl gallate, vitamin E), and/or reducing agents (cysteine, DTT, methionine, ascorbic acid, sodium sulfite, thioglycolic acid, thioglycerol) may reduce oxidation.

Source: *Handbook of Biogeneric Therapeutic Proteins: Regulatory, Manufacturing, Testing and Intellectual Property Issues*, Taylor & Francis Group, Boca Raton, FL, 2005.

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid.

9.9.4.4 Carbamylation

Cyanate is able to react with amino, sulfhydryl, carboxyl, phenolic hydroxyl, imidazole, and phosphate groups in proteins according to the general scheme, $RXH + HNCO = RXCONH_2$. Cyanate is easily soluble in water. Most reactions have a pH optimum around seven. Acidic pH should be avoided, as acidic conditions are ideal for modifications of carboxyl groups. For the same reason, reactions with cyanate should not be terminated with acid. At high concentrations, cyanate may react with itself to form cyanuric acid and cyamelide, and it is recommended to work at concentrations of about 0.2 M. Cyanate reacts rapidly with amino groups. At neutral pH and below, the *a*-amino group can be expected to react about 100 times faster than the 1-amino group. The resulting carbamoylamino groups are stable, even in dilute NaOH. Typical reaction conditions are 3 mg/mL protein and 0.1 M cyanate at pH 8, at 25°C, for 1 hour. Cyanate also reacts even more rapidly with sulfhydryl groups than amino groups, resulting in the formation of *S*-carbamylcysteine residues. As cyanate reacts rapidly with sulfhydryl groups, labile disulfide bonds may be ruptured. The resulting carbamylmercaptans decompose readily to free mercaptan and cyanate at alkaline pH. Consequently, cyanate can be used as reversible blocking agent for –SH groups. At acidic pH, cyanate reacts with carboxylic groups, resulting in the formation of a mixed anhydride, which can react with many nucleophiles (e.g., formation of amides). The reaction can be avoided entirely at pH 7–8. Aliphatic hydroxyls are resistant to carbamylation, even at high cyanate concentrations at low pH. However, the reactive hydroxyl groups of chymotrypsin and other proteases react with cyanate to give urethans. Phenolic hydroxyl groups react more readily than aliphatic groups in a reversible reaction that is quite analogous to the one that occurs with –SH groups.

Cyanate present in aqueous urea solutions reacts with the free amino and sulfhydryl groups of proteins. Urea is often tacitly assumed to be a reagent, which alters the structure of the protein and may be used to keep target proteins in their monomeric