TABLE 9.6

Preventive Actions Against Deamidation

Factor	Comment
pH	Deamidation is expected above pH 5. The optimal working range in which to avoid deamidation is probably between 3.0 and 5.0.
Temperature	The deamidation rate increases with increasing temperature.
Time	The deamidation rate is a function of time. Presence of des-amido forms is a marker for drug product stability and shelf life.
Conductivity	The ionic strength of the solution is kept low. At high ionic strength, the deamidation reaction can be fast even at neutral pH.
Redox potential	Nonessential parameter.
Co-solvents	In general, the buffer species and the buffer strength will influence the rate of deamidation. High solvent dielectrics favor deamidation. In model peptides, the protein stability was higher in Tris buffer than in phosphate buffer.

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

In many cases, the immunological and/or biological activity was only partially lost. In general, the oxidation of methionyl to methionyl sulfoxide does not affect protein antigenicity, probably because the conformational structure of the oxidized protein is close to the native structure. On the other hand, the oxidation of a single amino acid residue often causes changes in the biological activity, and all efforts should be taken to minimize the oxidation reactions.

The mechanism of oxidation involves methionyl residues, which are converted to methionyl sulfoxide residues under mild oxidizing conditions. The most reactive residues are those exposed to the solvent, while those residues buried within the hydrophobic regions are fairly inert to oxidation (e.g., methionine residues in myoglobin and trypsin). Methionyl residues are susceptible to auto-oxidation, chemical oxidation, and photo-oxidation.

The cysteinyl residues are easily oxidized, and the reaction is usually accelerated at an alkaline pH, where the thiol group is deprotonated. Under mild oxidizing conditions, the reactions are oxidation of cysteinyl residues to sulfenic/sulfonic acid (alkaline conditions), cysteinyl residues to dehydroalanyl residues (alkaline conditions), and cysteinyl to cystine residues (neutral to alkaline conditions). In the absence of a thiol reagent or a nearby thiol, the cysteine may instead oxidize to sulfenic acid.

The oxidation reaction is strongly catalyzed by divalent metal ions (e.g., copper). The indicators of oxidation include extra bands in gel electrophoresis and extra peaks in chromatographic recordings. Preventive actions against oxidation are listed in Table 9.7.

The degradation rate is often governed by trace amounts of peroxides, divalent metal ions, and light, base, and free radicals. There are three classes of antioxidants:

- *Phenolic compounds*: Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, and vitamin E
- *Reducing agents*: Cysteine, dithiothreitol (DTT), methionine, ascorbic acid, sodium sulfite, thioglycolic acid, and thioglycerol
- *Chelating agents*: Ethylenediaminetetraacetic acid (EDTA), citric acid, and thioglycolic acid