form during purification. However, at pH 6 and above, urea hydrolyses under the formation of cyanate, leading to carbamylation reactive groups in proteins.

The equilibrium $(NH_2)_2CO{=}NH_4CNO$ between an undissociated urea and dissociated cyanate in aqueous urea solutions is the main course of unintended carbamylation of primary amino groups in proteins. As the protein concentration normally is from 0.1 to 30 mg/mL, corresponding to the micromolar range, a considerable part of the protein mass is expected to undergo carbamylation under these conditions. Thus, the exposure of ribonuclease to cyanate in an aqueous solution leads to a considerable loss of enzymatic activity. Formation of cyanate is prevented by storage of neutral urea solutions at 4°C or by buffering the solution at pH 4.7. Thus, acidification of urea solutions just before use will decompose any cyanate present. Cyanate can be removed from urea solutions by mixed IEX. The method of Salinas describes a sensitive and specific method for the quantitative estimation of carbamylation in proteins (see Bibliography).

9.9.4.5 β-Elimination

The β-elimination reaction is caused by the abstraction of a β-hydrogen from cysteinyl, seryl, and threonyl residues under alkaline conditions. The cysteinyl residue decomposes as a result of β-elimination under the formation of HS− and free sulfur, thus affecting the redox potential of the solution. Several studies indicate that the rate of the reaction is proportional to the hydroxide ion concentration, and consequently, pH should be kept low (use dilute NaOH solutions to adjust pH preferably below 0.1 M NaOH). In alkaline solutions, the abstraction of β-hydrogen from cysteinyl, seryl, and threonyl residues results in the formation of a carbanion. Depending on the nature of the side-chain, the carbanion can rearrange to form an unsaturated derivative (dehydroalanine or β-methyl-dehydroalanine) or add a proton to give the L- and D-amino acid residues (racemization). The derivatives formed are reactive with a number of nucleophilic protein groups. The reaction is independent of the primary structure of the protein. The indicators of β-elimination include degradation of the protein, cleavage of disulfide bridges, and smell of sulfur. Preventive actions against β-elimination are listed in Table 9.8.

TABLE 9.8

Preventive Actions Against β-Elimination

Factor	Comment
pH	pH is kept below 10. Do not use NaOH solutions above 0.1 M to adjust pH.
Temperature	High temperature even at pH 4–8 results in β-elimination.
Time	The β-elimination reaction is a function of time.
Conductivity	Increased ionic strength increases the rate of β-elimination.
Redox potential	Cystinyl-rich proteins may decompose under formation of HS−, which will lower the redox potential of the solution. Reduction of disulfide bonds may result.
Co-solvents	Removal of divalent metal ions with EDTA.

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

Abbreviation: EDTA, ethylenediaminetetraacetic acid.