of Fe^{3+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, or Zn^{2+} does not affect the rate of oxidation for human insulin–like growth factor-1, but when the metal concentration is increased to 1 ppm, a significant increase in oxidation is observed.

Oxidation can be induced during protein processing and storage by peroxide contamination resulting from polysorbates and PEGs, both commonly used as pharmaceutical excipients. A correlation has been observed between the peroxide content in Tween 80 and the degree of oxidation in rhG-CSF, and peroxide-induced oxidation appeared more severe than that from atmospheric oxygen. Peroxide can also leach from plastic or elastomeric materials used in the primary packaging container-closure systems, including prefilled syringes.

The removal of headspace oxygen by degassing may be effective for preventing oxidation in some cases. Filling steps are carried out under nitrogen pressure, and vial headspace oxygen is replaced with an inert gas such as nitrogen to prevent oxidation. With some oxidation-sensitive proteins, processing is carried out in the presence of an inert gas such as nitrogen or argon. For multidose drug preparations, the use of cartridges with negligible headspace overcomes oxidation and related consequences.

Chemical degradation may also result from cross-linking, which may or may not be mediated by the formation of a disulfide bond. When cross-linking is disulfide bond mediated, the reaction occurs either by the formation of a new disulfide bond or by disulfide bond exchange. Intramolecular disulfide linkage may lead to a change in tertiary structure, whereas intermolecular (or interdomain) disulfide linkage may result in change in quaternary structure or formation of covalent aggregates. At higher pH, the formation of reactive thiolate ion (S^-), from the thiol group (–SH) of Cys residue, is favored, which may increase the probability of disulfide linkage formation. There are instances of covalent cross-linking that may occur between different amino acid residues besides Cys, which, unlike disulfide linkage, are nonreducible.

While the specific effects of various chemical reactions remain difficult to predict, the general impact of these reactions on the acidity is universally observed in protein products as shown in Table 8.3.

Table 8.3 Effect of Chemical Modifications of Proteins on Acidity Function and Charge Heterogeneity

Chemical Modification	Acidity/Charge Heterogeneity
Deamidation	More acidic ($z = -1$)
Succinimide formation	More basic or neutral
Glycation	More acidic
Pyroglutamate formation	More acidic ($z = -1$)
Peptide bond hydrolysis	Either acidic or basic