The formulation in sucrose can also increase aggregation over time because of protein glycation when sucrose is hydrolyzed. The presence of certain ligands—including certain ions—may enhance aggregation. Interactions with metal surfaces can lead to epitaxic denaturation, which triggers aggregate formation. Foreign particles from the environment, manufacturing processes, or container-closure systems (e.g., silicone oil) can also induce aggregation. Even handling protein products at compounding pharmacies can induce aggregation 10-fold above initially observed amounts.

8.4 Chemical degradation

Chemical instability refers to the formation or destruction of covalent bonds within a polypeptide or protein structures. Chemical modifications of protein include mainly oxidation, deamidation, reduction, and hydrolysis. Unfolding, dissociation, denaturation, aggregation, and precipitation are known as conformational or physical instabilities. In some cases, protein degradation pathways are synergistic: A chemical event may trigger a physical event, such as when oxidation is followed by aggregation. Generally, physical changes may not bring any significant clinical risk in small molecule drugs, except in the PK profile; in the case of biosimilars, these are crucial in determining the safety of these drugs.

Chemical degradation may occur in a number of different ways. This type of degradation affects the primary sequence and may also lead to significant changes in the HOS. Examples of chemical degradation include deamidation, oxidation, isomerization, clipping/fragmentation, and cross-linking. Deamidation is probably the most common type of chemical degradation encountered in mAb-based biotherapeutics. The reaction is favored at neutral and basic pH. There are examples of deamidation occurring at lower pH. However, that has been reported to occur primarily through a mechanism independent of succinimide formation; for instance, deamidation of Asn in the A chain of insulin is favored at pH <5, which is mediated via the formation of cyclic anhydride intermediate. There are factors other than pH that influence the rate of deamidation, e.g., sequence and local structure (steric effect). Amino acids present at the carboxyl end of Asn have been reported to influence the rate of deamidation; the decrease in rate has been correlated with an increase in size and branching of the side chain.

Isomerization is another common method of chemical degradation that shares the common outcome with deamidation. It can directly occur from Asp residue or from the succinimide intermediate. Similar to deamidation, isomerization is favored at neutral and basic pH, and the rate has been reported to be influenced by the steric effect.

Hydrolysis of a peptide bond leads to fragmentation of the protein. Some peptide bonds, such as Asp–Gly and Asp–Pro, are sensitive to