branched, or multibranched chains). Often does PEGylation lower the protein's immunogenicity, probably through multiple mechanisms related to blocked antigenic sites, improved solubility, and lower administration frequency of the therapeutic protein. In general, a branched PEG-protein is more efficient than a linear PEG-protein because of improved immunological properties.

7.2.3 Chemical degradation

Chemical modifications of proteins may include deamidation, oxidation, isomerization, hydrolysis, glycation, and C/N terminal heterogeneity of the protein, sometimes leading to aggregation. The susceptibility of an individual amino acid residue to chemical modification is dependent on the neighboring residues, the tertiary structure of the protein, and the solution conditions such as temperature, pH, and ionic strength. Chemical modification may give rise to a less favorable charge of the protein, thus leading to structural changes or even the formation of new covalent cross-links. Covalent aggregation is also a form of chemical degradation.

The deamidation of proteins accelerates at high temperature and high pH, and can occur during bioprocessing and storage. Moreover, deamidation can be accompanied by some degree of oxidation, conformational changes, fragmentation, and aggregation, posing serious risks for immunogenicity. Oxidation, another major chemical modification, can also reduce conformational stability and may cause the protein to aggregate. The oxidation of human serum albumin with hydroxyl radicals resulted in structural alterations and exposure of hydrophobic patches, causing increased immunogenicity.

7.2.3.1 Aggregation Aggregation phenomena may expose new epitopes to the protein's surface for which the immune system is intolerant. That leads to a standard immune response. In other conditions, protein aggregation may lead to presenting a multimeric antibody, which is known for not triggering B lymphocyte tolerance breakdown. This is why, in a therapeutic protein's analysis, it is important to look for the presence of aggregates and to limit their presence to a low level of the formulated drug.

Since aggregation is often accompanied by other structural changes in the protein, it is difficult to distinguish the individual impact of each factor on immunogenicity. Since the information on the nature of immunogenic aggregates from clinical studies is generally limited, animal models are used to provide insight into the link between aggregation and immunogenicity.

Nonnative aggregation can trigger structural changes in the protein leading to the creation of new epitopes or the exposure of existing epitopes. Native-like aggregates, however, are more likely to elicit ADAs that cross-react with the native protein and thus pose a greater risk to the