unlike the small-molecule drugs that would be highly stable in most lyophilized formulations.

Table 8.2 summarizes typical stability problems observed during protein formulation development and potential methods to solve each problem. The list does not represent the complexity of multiple problems that can be experienced with a given protein; as a result, the formulation research should be designed to handle each protein based on its unique stability profile.

8.3 Physical degradation

Proteins degrade upon physical stresses of many types including hydrophobic surfaces, heating, lyophilization, reconstitution, contact with organic solvents, shaking and many other permutations, and combinations of physical and chemical factors. The final result of physical stress can be denaturation, adsorption on the container walls, precipitation, or aggregation.

Aggregation is a common problem encountered during manufacture and storage of proteins. The potential for aggregated forms is often enhanced by exposure to a protein to liquid–air, liquid–solid, and even liquid–liquid interfaces. Mechanical stresses of agitation (shaking, stirring, pipetting, or pumping through tubes) can cause protein aggregation. Freezing and thawing can promote it as well. Solution conditions such as temperature, protein concentration, pH, and ionic strength can affect the rate and the amount of aggregates observed.

Aggregated proteins are a significant concern for biopharmaceutical products because they may be associated with decreased bioactivity and increased immunogenicity. Macromolecular protein complexes can trigger a patient's immune system to recognize the protein as nonself and mount an antigenic response.

Protein aggregates are formed by mechanisms such as domain swapping, strand association, edge–edge association, or beta strand stacking. The term *aggregation* usually refers to multimers of proteins, e.g., dimers, trimers, tetramers, all the way to large polymers. The aggregates can be noncovalent or covalent (disulfide-linked), and these can be present as fully soluble in a clear solution, partially insoluble in a turbid solution, or mostly insoluble as a precipitate that collects in the bottom of the container. Nonspecific protein-to-protein association resulting from interactions among solvent-exposed hydrophobic groups can also form aggregates. The covalent aggregation is not reversible. The weakly associated noncovalent aggregate can be reversible, and it usually follows the path of dimers to multimers; the strongly associated noncovalent aggregates are not reversible by dilution and may result in precipitation. Based on the size range, aggregates have been classified into the following categories: (a) submicron (<1 μm in size) particles, which are