Table 8-3 Possible Strategies to Overcome Protein Adsorption

- Increase protein concentration during filtration and/or use extra volume to saturate the filter with protein solution
- Modify (e.g., siliconize) the surface of the glass containers, providing a resistant barrier to protein-surface interaction
- Decrease the rate of mixing when it is known that shear will affect protein adsorption
- Add excipients such as surfactants that have higher surface activity
- Add macromolecules such as albumin and gelatin (must be synthetic) to complete for binding sites on the surface.

determined by the nature of the interfaces, are similar to those observed with aggregation caused by heat, high pressure, or chemical denaturants. In the case of proteins, sources such as the polymer of the membrane filter, the administration set, agitation that occurs during the purification process as well as the method of manufacture are known or at least suspected to cause surface denaturation. Strategies often used to overcome protein denaturation due to adsorption are presented in Table 8-3.

SURFACTANTS

Surface-active agents (surfactants) exert their effect at surfaces of solid–solid, solid–liquid, liquid–liquid, and liquid–air because of their chemical composition containing both hydrophilic and hydrophobic groups (see chap. 6). Surfactants effectively compete against proteins for these interfacial hydrophobic locations, thus helping to minimize protein adsorption and potential aggregation.

Generally, ionic surfactants can denature proteins. However, nonionic surfactants usually do not denature proteins even at relatively high concentrations (1% w/v) (40). Most parenterally acceptable nonionic surfactants come from either the polysorbate (sorbitol-polyethylene oxide polymers) or polyether (polyethylene oxide-polypropylene oxide block co-polymers) groups. Polysorbate 20 and 80 and sodium dodecyl sulfate are effective and acceptable surfactant stabilizers in marketed protein formulations (Table 6-6). The chemical structure of polysorbates, factors affecting micelle formation and degradation pathways of polysorbates 20 and 80 are the subject of a review article by Kerwin (41). Effectiveness of polysorbate stabilization is dependent on the structure of polysorbate (monomer or micelle) and polysorbate–protein ratio (42). Other surfactants that have been used in protein formulations for clinical studies and/or found in the patent literature include Pluronic F68, and other polyoxyethylene ethers (e.g., the "Brij" class).

The choice of surfactant and the final concentration optimal for stabilization is quite dependent on a variety of factors including other formulation ingredients, for example, sugars, protein concentration, headspace in the container, the type of container, and test methodology.

Recombinant hGH will aggregate readily under mechanical and thermal stress. Aggregation from mechanical stress can be substantially reduced in the presence of surfactants (43). Mechanical stress may cause proteins to be more exposed to air–water interfaces where denaturation is more likely to occur than in the bulk phase of water. Surfactants will preferentially compete with proteins for accumulation at the air–water interface and keep the protein from undergoing interfacial denaturation resulting from mechanical stress. Pluronic F68 and Brij 35 will stabilize hGH at their critical micelle concentrations (0.1% and 0.013%, respectively), whereas stabilization with polysorbate 80 requires a concentration of 0.1%, higher than the critical micelle concentration value for polysorbate 80 of 0.0013%. The reasons for these differences in stabilizing concentrations are not clear, but simply reflect differences in interactions between different surfactants and proteins. It is interesting to note that these surfactants do not stabilize hGH from aggregation due to high temperature stress.

Surface-active agents, particularly polysorbate 80, protect proteins against surface-induced denaturation during freezing (44). A strong correlation exists between freeze denaturation (quick freezing of the protein) and surface denaturation (shaking the protein in solution). Proteins that tend to denature under these conditions are protected by the addition of polysorbate 80 (0.1%). Other surfactants—Brij 35, Lubrol-px, Triton X-10, and even the ionic surfactant,