The desire to identify stable solution preparations of insulin for use in novel delivery systems such as continuous infusion pumps, led to the development of test methodology for assessing the impact of various additives on physical stability. Insulin (and many other proteins) physical stability typically is evaluated using thermomechanical procedures involving agitation or rotation of protein solutions at elevated temperature. Turbidity resulting from aggregation is usually determined as a function of time by visual inspection or light scattering analysis. Alternatively, reductions in the soluble protein content due to precipitation can be quantified by HPLC assay as a function of time. Relative stability is defined by the length of time a preparation remains on the test without showing a change in either parameter. It should be noted that the greatest difficulty in applying such testing strategies is interpreting the experimental data and correlating it in a practical way to "real life" conditions that the formulation may actually experience. Nevertheless, regulatory agencies may request data from such testing to support dating periods or other product claims. Physical stress testing, however, is more appropriately used as a development screening tool to identify the capability of various additives to prevent aggregation.

Analytical methods used for determining protein aggregation are listed on pages 177–178 (chapter 11 under "Answers for Case Study 10").

Foreign Particles, Protein Aggregation, and Immunogenicity

The reality of protein aggregation has raised the concerns about such aggregates, even at subvisible levels, leading to an immune response resulting from antibody-mediated neutralization of the protein's activity or alterations in bioavailability (34,35). Among many causes for protein aggregation are protein particles resulting either from the protein alone or resulting from heterogenous nucleation on foreign micro- or nanoparticles originating from the manufacturing process (mixing tanks, process tubing, filter systems, filling machines) and from the container/closure system (36). Silicone oil, used as a lubricant for rubber closures on vials and rubber plungers in prefilled syringes also can induce protein aggregation (37).

Large protein aggregates are subvisible particles (smaller than 10 micrometers) that are not currently monitored and quantified by compendial subvisible particulate matter measurement systems. Carpenter, et al. (34) have questioned this current practice and have proposed that (*i*) scientists from industry and academia work together to define the quantitative capabilities of particle counting instruments for particles as small as 0.1 μm, (*ii*) develop new particle counting instruments for more reliable measurement of particles at sizes approaching 0.1 μm, and (*iii*) more studies be conducted and published on the impact of protein aggregation on immunogenicity including the role of protein class, amount of aggregate, size of aggregates, and protein conformation in aggregates.

Also, the reader is referred to the end of chapter 29 where there is some discussion about the huge variety of biopharmaceutical commercial product package insert language regarding acceptability of visible particulate matter and use of different types of transfer and in-line filters.

ADSORPTION

Proteins exhibit a certain degree of surface activity; that is, they adsorb to surfaces due to their innate nature of being amphiphilic polyelectrolytes. Consequently biological activity may be either reduced or totally lost if such adsorption occurs during manufacturing, storage, or use of the final product. Insulin has been the most studied protein with respect to surface adsorption. Potential problems may be encountered while delivering insulin because of its ability to adsorb onto the surfaces of delivery pumps, glass containers, and to the inside of the intravenous bags. Insulin adsorption usually is finite once binding sites are covered and such adsorption is usually not clinically significant.

Adsorption to surfaces depends on protein–protein interactions, time, temperature, pH, and ionic strength of the medium and the nature of the surface (38). Interactions that determine the overall adsorption process between a protein and a surface include redistribution of charged groups in the interfacial layer, changes in the hydration of the sorbent and the protein surface, and structural rearrangements in the protein molecule. Surface denaturation which commonly takes place at the liquid–solid and liquid–air interface to involve conformational changes such as loss of α-helices to β-sheets and certain random structures (39). These structural changes,