Aggregation and Residual Moisture

Katakam and Banga (34) studied moisture-induced aggregation of solid-state albumin and γ-globulin. Moisture-induced (2–10 μL added to 10 mg) aggregation of solid-state albumin and γ-globulin was investigated by incubation at 37°C for 24 hours. Aggregation was observed with increasing moisture content and was especially prominent for bovine serum albumin. When mixed with carbohydrate excipients in a 1:1 ratio, aggregation was reduced for both bovine serum albumin and γ-globulin by Emdex, dextrose, trehalose, and hydroxypropyl β-cyclodextrin excipients. The mechanism of the aggregation was indicated to be covalent linkages formed due to intermolecular thiol disulfide interchange.

Bell et al. (35) studied lyophilized recombinant bovine somatotropin (rbSt) and found an increasingly significant contribution of exothermic aggregation at higher moisture contents. In the presence of moisture they identified hydrophobic aggregation as being most prominent. In the dry state, covalent modifications including polymerization into compounds of higher molecular weight were more prominent, whereas in the presence of moisture, hydrophobic aggregation was most prominent. This can be explained by the increasingly significant contribution of the exothermic aggregation at higher moisture contents.

Hekman et al. (36) linked elevated moisture content to the formation of aggregates for a conjugated I_gG lyophilized with maltose and citrate buffer. The increase in molecular size was a function of both the moisture content in the vial and the amount of time for which the sample was stressed thermally. The data suggest that the increase in molecular size as a function of thermal stress is due to attachment of maltase, which is a glucose disaccharide present in the lyophile as an excipient. This degradation pathway was only observed in the lyophile.

Prestrelski et al. (37) using Fourier transform infrared (FTIR) spectroscopy and accelerated stability studies examined interleukin-2 (IL-2) with respect to pH and stabilizers that provide optimal storage stability for the lyophilized product. IL-2 prepared at pH 5 is approximately an order of magnitude more stable than at pH 7 with regard to formation of soluble and insoluble aggregates.

Pikal et al. (38) also looked at effects of moisture and oxygen on the formulation and stability of freeze-dried human growth hormone evaluating the formation of irreversible aggregates.

Prestrelski et al. (39) demonstrated that FTIR is a rapid and useful method for studying protein conformation in the dried state and can aid in determining the optimum conditions for stabilization of proteins during freeze-drying.

Dong et al. (40) indicated that a successful lyophilized protein formulation is the preservation of the native conformation in the dried solid. They used FTIR as a tool to study lyophilization-induced unfolding and aggregation of proteins, namely, through the bands at l620 cm^{-1} to 1685 cm^{-1}, common IR spectral features indicative of lyophilization- and temperature-induced protein aggregation, used to monitor and quantify aggregation even in the dried solid.

Lueckel et al. (41) assessed the residual moisture, T_g, and excipient physical state of different formulations in relation to the in-process and shelf life stability of freeze-dried IL-6. The amorphous state of the excipients and a high T_g can be considered necessary condition for preventing aggregation of freeze-dried IL-6. Sarciaux et al. (42) compared effects of buffer composition and processing conditions on aggregation of bovine I_gG during freeze-drying. The data were supportive of a mechanism of aggregation formation involving