Adams and Irons (28) freeze-dried the enzyme *Erwinia caratovora's* L-asparaginase assessing criteria of dried cake appearance, moisture content, or ease of reconstitution.

Baffi and Garnick (29) considered

> "the most common modes of degradation to evaluate the stability of" proteins ... to be "oxidation, deamidation, aggregation, disulfide rupture and rearrangement, and fragmentation" ... caused by exposing candidate lyophile to heat, light, agitation, or freeze-thaw cycle.... "Oxidation may be detected by reversed-phase high-performance liquid chromatography (RP-HPLC), hydrophobic interaction chromatography (HIC), or peptide mapping. Deamidation ... detected by isoelectric focusing (IEF) or high-performance ion-exchange chromatography (HPIEC). Aggregation and fragmentation may be detected by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-Page) or high-performance size-exclusion chromatography (HPSEC).... These methods" are "integrated into stability-monitoring protocols used to evaluate multiple batches of product." The residual moisture in the cake must be defined and controlled. "The desired moisture level can be obtained by the development and validation of a reproducible lyophilization cycle. If the moisture level is too high, the cake" might collapse.... "Degradation by deamidation may continue in the presence of small traces of residual moisture. Overdrying of the protein is yet another concern. If the residual moisture is too low aggregation, inadequate reconstitution, and/or loss of activity may occur."

Lai et al. (30) studied the mechanistic role of water in the deamidation of a model asparagine-containing hexapeptide (Val-Tyr-Pro-Asn-Gly-Ala) in freeze-dried formulations containing poly(vinylpyrrolidine) (PVP) and glycerol. Glycerol was used to vary T_g of the formulation without any significant changing water content or activity. Residual water appeared to assist deamidation in the solid PVP formulations investigated by promoting molecular mobility, solvent/medium interaction, and by acting as a chemical reactant in the breakdown of the cyclic imide.

Formulation and Residual Moisture

Pikal et al. (31) studied effects of formulation variables on the in-process and shelf life stability of freeze-dried human growth hormone at low moisture levels. A combination of mannitol and glycine, where the glycine remains amorphous, provided the greatest protection against decomposition via methionine oxidations and asparagines deamidation and aggregation.

Severe aggregation was observed at high pH.

Skrabanja et al. (32) reviewed the lyophilization of biotechnology products and emphasized that the final quality of a protein product is determined by an interplay between the proper choice of excipients and the freeze-drying process; the glass temperature that defines that state of the freeze-dried cake can be influenced by the moisture content and the choice of the excipient.

Mattern et al. (33) investigated sugar-free L–amino acid systems as stabilizers in protein formulations. Increase in moisture content during storage reduced the T_g enhancing crystallization and decreasing protein stability.