promegapoietin, and progenipoietin) using a primary drying product temperature of −10°C. The crystalline mannitol allows primary drying to be performed at temperatures above the T_g' of amorphous sucrose in the formulation.

Maintaining mannitol in the amorphous state during freeze-drying for optimal stabilization can be accomplished (39). All the enzymes studied were protected when mannitol remained amorphous, but become unstable with an increase in mannitol crystallinity. Mannitol in freeze-dried cakes containing enzyme and sodium phosphate buffer remained amorphous at lower concentrations (< 200 mM), although annealing the frozen solution resulted in mannitol crystallization. However, mannitol at higher concentrations (> 250 mM) in this enzyme-phosphate formulation crystallized and had no protective effects on preserving enzyme activity after freeze-drying.

MORE ON STABILIZING EXCIPIENTS IN LYOPHILIZED FORMULATIONS

Plasticizers (examples include glycerol, propylene glycol, ethylene glycol, or DMSO) will modify disaccharide and polymeric lyoprotective glasses (40). The proposed mechanism of protein stabilization was attributed to the following: the plasticizers fill small volumes left open by the larger (or stiffer) host glass-former, restricting motion, and thereby slowing the fast dynamics of the glass and subsequent protein degradation. This approach has narrow application because of the relatively large amounts of plasticizer required, and also because of the suggestion that the lower molecular weight oligomers like ethylene glycol are better stabilizers.

Raffinose will not lyoprotect an unstable drug as well as sucrose or trehalose (41). This observation was in contrast to other studies (42–44) showing raffinose to be as effective as trehalose and superior to lactose, maltose, and sucrose in stabilizing several enzymes. A possible explanation for these differences may involve differences in dehydration conditions and storage temperatures.

Mannitol and glycine in frozen solutions will influence the crystallization of each other (45). Glycine was shown to have a stronger initial tendency to crystallize, while it was easier to influence the crystallization of mannitol. Buffer salts, such as sodium phosphate, inhibited crystallization of both mannitol and glycine. The activity of LDH correlated with the extent of crystallization of these excipients (Fig. 10-4).

LDH formulations containing maltodextrin protected LDH against inactivation during freeze-drying because of the amorphous nature of these partially hydrolyzed starches (46). Maltodextrins were also reported to be better lyoprotectants for LDH than sucrose or maltose, although the mechanism is unknown.

The stabilization effects of amorphous additives on freeze-dried proteins are well accepted on the basis of several publications. β-Galactosidase was stabilized with inositol as long as this excipient stayed amorphous, but if inositol crystallized during storage, the enzyme activity declined (47). Inositol crystallization was prevented by addition of polymers such as dextran, Ficoll, and sodium carboxymethylcellulose.

Stabilization of lyophilized proteins not only depends on the formulation and dehydration process parameters, but also depends sometimes on the formulation of the reconstitution medium (48). Keratinocyte growth factor aggregation upon reconstitution with water can occur readily, but several additives such as sulfated polysaccharides, surfactants, polyphosphates, and amino acids in the reconstitution medium will significantly reduce this aggregation.

The Gordon–Taylor equation: $$T_g = \frac{\left[W_a T_{ga} + k(1 - W_a) T_{gb}\right]}{\left[W_a + k(1 - W_a)\right]}$$

was applied to predict the glass transition temperature of a model tripeptide in the presence of different sugars (sucrose, lactose, trehalose, and maltose) in both frozen solutions and in lyophilized products (49). Correlation was excellent between the predicted and actual T_g for various ratios of tripeptide to sugars. The authors also showed a significant effect of sodium chloride on the T_g' of the tripeptide in frozen solution, but no effect after lyophilization. Figure 10-5 from this paper demonstrates the plasticizing effect of water on decreasing the glass transition temperature.