protein in the folded state has exposed hydrophilic residues which may be hydrogen bonded to specific water molecules (Figure 4.7(b)). Removal of all the water in the system can destabilize the protein conformation by removal of the specific water molecules which hydrogen bond to the polar groups on the protein surface. Thus, over-drying exposes polar groups that can cause opalescence upon reconstitution, while under-drying increases the rates of the hydrolytic reactions, which can cause degradation and potential losses in activity (see Figure 7 in Hsu et al., 1991). An alternative mechanism is that of formation of an amorphous glassy state whereby protein mobility is restricted, rendering a protein more stable because it is less susceptible to degradation mechanisms that require protein flexibility and moisture (Belton & Gil, 1994; Levine & Slade, 1992). To ensure a glassy state, the freeze-dried preparation must be stored at a temperature below the characteristic glass transition temperature, Tg. The Tg value is dependent on the mass percent of each component including water. Water has a very low Tg value (−135 °C), and thus increasing the water content of a freeze-dried formulation will greatly reduce Tg. As an example, increasing the water content of a freeze-dried preparation from 1% to 3–4% (g H_2O/100 g dried powder) results in a Tg value below room temperature. Thus, development of a lyophilization cycle that results in sufficiently dried powder is extremely important to ensure a Tg value that easily accommodates lower storage temperatures. In fact, Tg values above room temperature are advantageous since the product need not necessarily be stored at refrigerated temperatures. However, even if the cycle produces a high Tg value at low moisture content, residual moisture in the vial stoppers can increase water content of the formulation during storage resulting in a decrease of the Tg value. This problem can be mitigated by thoroughly drying the stoppers before use, or using a stopper with a coating, which serves as a barrier for moisture transfer.

It is likely that both the water replacement and glassy state mechanisms are important to ensure a good drying protectant. It has been shown that the glassy state itself is not sufficient to stabilize a protein after freeze-drying (Prestrelski, Arakawa, & Carpenter, 1993; Zhang, Prestralski, Arakawa, & Carpenter, 1993). In particular, it has been shown that storage of a freeze-dried preparation below the Tg does not always result in a stable product, i.e., stabilization of the protein conformation is also required. Thus, dextran has a high Tg value >75 °C, but is unable to conserve protein structure during freeze-drying. Proteins freeze-dried in dextran degrade more rapidly than when formulated with either sucrose or trehalose, which can contribute hydrogen bonding to the protein during removal of water. Generally, disaccharides such as both sucrose and trehalose can function as promoters of the glassy state due to their relatively high Tg values as well as stabilizers of protein structure via their ability to contribute replacement hydrogen bonding. Sucrose and trehalose are also excellent lyoprotectants since they do not crystallize easily during the lyophilization process. If the lyoprotectant were to phase separate due to crystallization, it would not be able to interact with the protein resulting in little drying protection. Such was the case during development of an anti-IgE mAb freeze-dried formulation where stability was assessed at 2–8 °C storage in a 10 mM sodium succinate buffer at pH 5 in the presence of several sugars (Figure 4.8). Of all the sugars tested, mannitol was by far the worst lyoprotectant, mainly due to the ease of crystallization of mannitol during the freezing process.