active, and the magnitude of the decrease in water surface tension has been shown to correlate directly with PEG molecular weight (85). Therefore, the most rational explanation for the results presented in Figure 9 is that PEGs are serving as such potent cryoprotectants because they are operating by the same mechanism(s) as more typical surfactants such as Tweens.

MECHANISMS FOR FAILURE OF DEXTRAN TO PROTECT LYOPHILIZED PROTEINS

In an idealized lyophilization cycle, a solution of proteins, buffers, and excipients is cooled to the solution's freezing point. At this point, it is thermodynamically favorable to form a new solid phase composed of pure ice. Once ice begins to form (not necessarily at the thermodynamic freezing point; substantial supercooling may occur), the remaining components of the solution in the nonfrozen phase become increasingly more concentrated, as shown in Figure 10. The combination of increased concentration and lower temperatures causes the viscosity of the non-ice phase to increase until, at a glass transition point termed T_g', the solution becomes so viscous that further freezing of water is kinetically blocked. Further temperature decreases below T_g' have no additional concentrating effects.

It is important to realize that the preceding is a highly simplified description of the possible phase behaviors that can occur. In actual practice, phase behavior during freezing is rarely so simple. Instead, as the non-ice phase

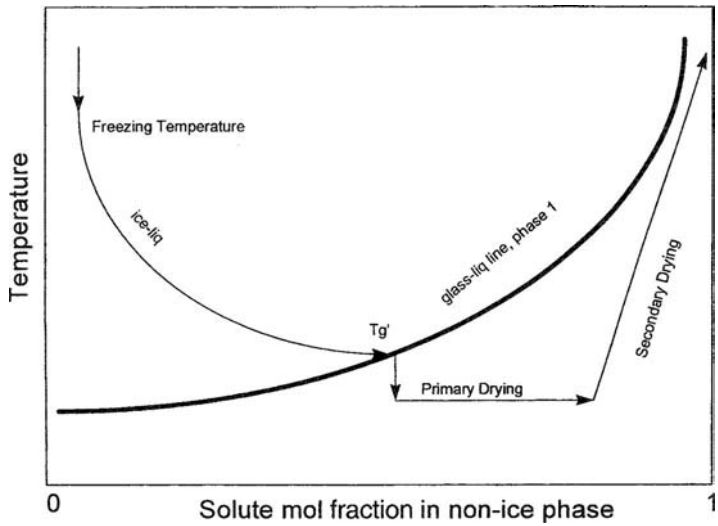

FIGURE 10 Phase diagram for an idealized, simple lyophilization cycle. A liquid sample first is cooled to the freezing temperature. As pure ice is formed, the solute remaining in the non-ice phase is concentrated until the ice-liquid line intersects the glass transition line at T_g'. No further concentration due to cooling occurs. Primary drying occurs under vacuum at a temperature below the glass transition temperature. After primary drying, the temperature is increased to effect secondary drying. Final storage temperature after secondary drying is below the glass transition line.