from the product is nearly balanced by the heat liberated by ice formation. Near the 25-minute mark, as the amount of unfrozen water becomes depleted, the sucrose concentration begins to increase sharply, and the temperature abruptly decreases. At the 30-minute mark, when most of the freezable water has been converted to ice, the sucrose concentration and the viscosity of the freeze concentrate increase very sharply. At about the 45-minute mark, viscosity is about seven orders of magnitude higher than for the starting solution. At this point, the freeze concentrate is nearly a glass.

Since most of the water is removed from the protein phase during freezing, most of the drying is actually accomplished during the freezing process. A number of stresses or perturbations of the free energy of unfolding may develop. First, since it is the unique nature of water that is often credited with stabilization of the native conformation via hydrophobic interactions, one might expect the thermodynamic stability of the native conformation would be decreased as most of the water is transformed to ice. Also, the phenomenon of freeze concentration will increase the protein concentration as well as increase the concentration of any potential reactant, thereby increasing the rate of bimolecular degradation reactions. Thus, as the system freezes, degradation rates may actually increase in spite of the decrease in temperature. Figure 2 gives an illustration of the impact of freeze concentration on degradation rate as well as demonstrates the impact of increasing viscosity on reactivity in the freeze concentrate. The data were calculated for the case of a trace amount of drug in 3% sucrose, using the viscosity and sucrose concentration data from Figure 1 and assuming an Arrhenius activation energy of 20 kcal/mol for the case where rate is independent of viscosity. If one assumes the degradation rate is completely uncoupled from macroscopic viscosity, the rate of degradation is nearly two orders of magnitude higher at the maximum than in the solution at the beginning of the freezing process. Eventually, as the temperature decreases

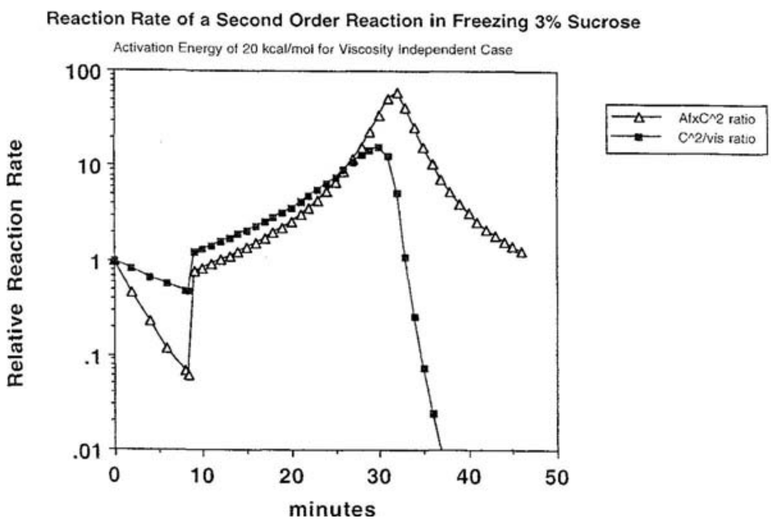

FIGURE 2 Calculated reaction rates for a second-order reaction in freezing 3% sucrose. Key: triangles = rate independent of viscosity with Arrhenius temperature dependence and a 20 kcal/mol activation energy; squares = reaction rate inversely proportional to viscosity.