sharply around 30 minutes, the Arrhenius factor dominates and the rate of reaction decreases. However, since a degradation pathway requires motion or mobility of some kind, most reactions would likely slow as the viscosity increases (7), at least for very high viscosities when the system approaches the glassy state.

The extreme example of coupling is with a diffusion-controlled reaction with the diffusion constant being inversely proportional to viscosity, as in the Stokes–Einstein equation. The curve defined by the square symbols in Figure 2 represents this extreme case. However, even with complete coupling of the reaction rate to viscosity, the degradation rate reaches a maximum of about one order of magnitude higher than in the starting solution. However, once the increase in viscosity dominates, the reaction rate decreases sharply toward extremely low values. While a protein formulation of interest may deviate from the quantitative behavior shown in Figure 2, two generalizations seem valid. Firstly, due to increasing solute concentration, bimolecular processes will be accelerated during the freezing stage in spite of decreasing temperature. Direct experimental observation of this phenomenon has been reported (10). Secondly, at least for reactions that are viscosity dependent, the rates will decrease dramatically near the end of freezing. Indeed, the principle of stabilization by vitrification (7) is based on the concept that viscosity increases near the glass transition temperature depress or eliminate degradation reactions. Experimental studies of three different reactions in frozen maltodextrin systems lend support to this view, though not all reactions are viscosity dependent (11). Rate constants for enzymatic hydrolysis of a substrate, aggregation of a protein, and oxidation of ascorbic acid were obtained in frozen maltodextrin solutions near the T_g' of -10°C. The data show about an order of magnitude reduction in rate constant between -5°C and -10°C for enzymatic hydrolysis and for ascorbic acid oxidation, but the protein aggregation reaction studied shows only a small temperature dependence throughout the range studied. Surprisingly, the rate-determining step for aggregation appears not to be viscosity dependent. However, the possibility of significant dilution with ice melt above T_g' confounds the interpretation of these data.

While sucrose and most excipients fail to crystallize during freezing, mannitol, glycine, sodium chloride, and phosphate buffers will crystallize, if present as the major component, once freeze concentration provides sufficient supersaturation. It should also be noted that very high concentrations may be reached before crystallization occurs (i.e., about 6 molal for NaCl), so the ionic strength environment of the protein during freezing may be quite different than in the starting formulation and could present a "stress" for protein stability (4,5). Crystallization of buffer components, resulting in massive pH shifts, may present an even greater stress for proteins. Under equilibrium conditions attained by seeding, the sodium phosphate buffer system shows a dramatic decrease in pH of about 4 pH units due to crystallization of the basic buffer component, $Na_2HPO_4.2H_2O$ (12). Conversely, the potassium phosphate system shows only a modest increase in pH of about 0.8 pH units. Under non-equilibrium conditions (i.e., no seeding) and with lower buffer concentrations, the degree of crystallization is less and the resulting pH shifts are moderated (13). Table 1 shows data accumulated (14,15) during freezing of phosphate buffer solutions in large volumes at cooling rates intended to mimic freezing in vials. For the concentrated buffer solutions (100 mM), the frozen pH values are