

## Reactions in Frozen Systems

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*Received October 28, 1968*

Many chemists are acquainted with observations of fast reactions such as may occur during low-temperature storage of frozen solutions. Usually such "frozen-state reactions" are not of primary interest; in fact, they are often taken only as troublesome technical problems. Until recently, therefore, little systematic or quantitative work had been carried out on this interesting phenomenon. But now specific studies on a wide variety of biochemical, organic, and inorganic reactions under frozen conditions have been reported. Such studies have given rise to unusual experimental results and interesting theoretical speculations.

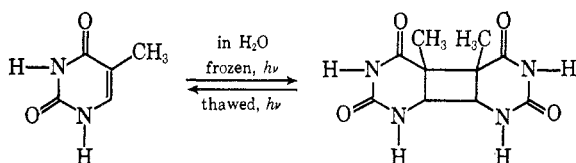
The earliest reports devoted primarily to reactions in frozen aqueous systems were concerned with an important practical problem: the preservation of food-stuffs during frozen storage. It was concluded in the 1930's that enzyme action in foods stored at low temperatures was a very important factor in food preservation.<sup>1-3</sup> For example, it was reported<sup>2b</sup> that "Both protease and esterases are active in artificial systems at low temperatures even when the system is in the solid state" [sic]. Comparisons of frozen and supercooled systems showed that when an enzyme system freezes a sharp discontinuity can occur in the velocity-temperature curve.<sup>3</sup> Most systems showed a rate decrease in the frozen system, but there are recent reports of faster enzymatic reactions in frozen solutions relative to an otherwise identical supercooled system.<sup>4,5</sup> Since the

early studies there has been a great deal of information collected concerning the effects of freezing on systems such as cells, tissues, and even animals,<sup>6</sup> but few modern kinetic studies have been reported on relatively simple enzymatic systems in the frozen state.<sup>7</sup> Lineweaver's 1939 statement<sup>3</sup> that "little work is available on the phenomenon and no predictions seem warranted at present" remains largely true for quantitative studies of enzyme systems. Simpler chemical systems, however, have by now received a good deal more attention.

Much of the impetus for recent studies of reactions in frozen solutions seems due to interesting theoretical suggestions that an ice (rather than liquid water) structure around a solute might distinctly modify its physical (*e.g.*, spectral) and chemical (*e.g.*, kinetic) properties. In 1957 Szent-Györgyi<sup>8</sup> described some spectral changes caused by freezing a number of dyes in dilute aqueous solutions. It was suggested that observed modifications of singlet to triplet electronic transitions by freezing were related to formation of the ice structure. One year later, Beukers, Ijlstra, and Berends<sup>9</sup> first reported the now much discussed photodimerization of thymine in frozen aqueous solutions. This reaction does not occur at all in nonfrozen solutions, but in fact the dimer dissociates on continued irradiation of a thawed solution.<sup>9</sup> Possible model relationships to dimerization of thymine units in DNA and

(1) A. K. Balls and H. Lineweaver, *Food Res.*, **3**, 57 (1938).  
 (2) (a) A. K. Ball, *Ice Cold Storage*, **41**, 101 (1938); (b) *Chem. Abstr.*, **32**, 8011 (1938).  
 (3) H. Lineweaver, *J. Am. Chem. Soc.*, **61**, 403 (1939).  
 (4) N. H. Grant and H. E. Alburn, *Nature*, **212**, 194 (1966); F. Kiermeier, *Biochem. Z.*, **318**, 275 (1948).  
 (5) (a) A. L. Tappell in "Cryobiology," J. C. Meryman, Ed., Academic Press, New York, N. Y., 1966, p 163.

(6) For reviews see (a) "Cryobiology," J. C. Meryman, Ed., Academic Press, New York, N. Y., 1966; (b) *Fed. Proc.*, **24**, Suppl. 15 (1965).  
 (7) See O. P. Chilson, L. A. Costello, and N. O. Kaplan, *ibid.*, **24**, Suppl. 15, S-55 (1965).  
 (8) A. Szent-Györgyi, "Bioenergetics," Academic Press, New York, N. Y., 1957.  
 (9) R. Beukers, J. Ijlstra, and W. Berends, *Rec. Trav. Chim.*, **77**, 729 (1958); **78**, 883 (1959); R. Beukers and W. Berends, *Biochim. Biophys. Acta*, **49**, 181 (1961).



to photodeactivation and reactivation of microorganisms,<sup>10</sup> together with speculation on whether the reaction occurs in the solid state or in "puddles" of liquid in the frozen system, has resulted in a great deal of interest in this type of frozen-state reaction. Dimerization of thymine in ice has recently<sup>11</sup> been quite easily established as a true solid-state reaction (see below).

Several kinetic studies of chemical reactions in frozen systems have been prompted by initial observations that some reactions appear to be catalyzed by the frozen conditions. The increase in rate is often so great that it makes possible the quenching of the reaction simply by thawing a sample; a leisurely analysis of reaction progress can then be ordinarily carried out at room temperature. Such accelerations and other unusual kinetic features of frozen reactions have been interpreted in a great variety of ways.

Grant, Clark, and Alburn<sup>12</sup> described the base-catalyzed hydrolysis of penicillin in frozen systems at  $-5$  to  $-30^\circ$ . Here reaction occurred in frozen samples, but not in identical nonfrozen samples held long periods at  $38^\circ$ . As has been generally found, the reaction was not influenced by the manner in which samples were frozen, but the presence of various solutes (glycerol, ethanol) stopped the reaction in the frozen samples. The authors considered a possible explanation involving concentration of reactants on freezing, but suggested that a favorable substrate-catalyst positional constraint, and possibly the exceptionally high proton mobility in ice, might be factors.<sup>12</sup> They have also suggested that reactant and product diffusion, crystal imperfections, as well as the dielectric properties of ice may play some role in certain reactions in frozen systems.<sup>13</sup> In the hydroxylaminolysis of some amino acid esters in frozen solutions they showed that reactions were often inhibited by addition of compounds structurally analogous to the reactants; the kinetic relationship found was that of a Lineweaver-Burk plot for competitive inhibition. This seemed to suggest the existence of catalytically active sites on the ice surface.<sup>14</sup>

Butler and Bruice<sup>15</sup> compared the kinetics of cata-

lyzed hydrolysis of acetic anhydride,  $\beta$ -propiolactone, and *p*-nitrophenyl acetate in water and in ice and pointed out that a concentration effect was responsible for increased rates of these reactions in ice. The crystallization of water resulted in high concentrations of reactant in liquid regions of the frozen systems, and the rate of any bimolecular reaction was simply increased by this change in concentration. In a later investigation of the reaction of morpholine with two thiolactones<sup>16</sup> they noted that the over-all observed reaction order changes from three in nonfrozen solution to two in frozen solutions. This seemed opposite to results anticipated for a concentration phenomenon, and they suggested that the ice structure itself takes part in a proton transfer reaction in place of one of the morpholine molecules.

Some inorganic reactions in frozen aqueous solutions have also given interesting results and explanations. The autoxidation of iodide ion occurs with enhanced rates in frozen solutions<sup>17</sup> and the oxidation of iodide by arsenic acid proceeds very rapidly even at  $-70^\circ$  in frozen solutions.<sup>18</sup> Perhaps most interesting is the electron exchange between ferrous and ferric ions in  $\text{HClO}_4\text{-H}_2\text{O}$  solutions frozen to  $-78^\circ$ .<sup>19</sup> Under the conditions used it was estimated that the reactants were separated in the solid medium by roughly  $100 \text{ \AA}$ , yet, except for an ordinary decrease due to temperature, the reaction rate was not different from that in liquid solutions at higher temperatures. A facile electron transfer by a water bridging mechanism was suggested for reaction in both liquid and solid systems.<sup>19</sup>

Such interesting possibilities, combined with the fortuitous discovery of a particularly simple reaction in frozen organic solvents,<sup>20</sup> indicated that a systematic investigation of some reactions in frozen solutions would clarify or establish some of the many suggested explanations.<sup>21</sup> Although the projected study did not initially require it, it now seems best to begin a description of the work with definitions of the terms "frozen" and "rate."

**"Frozen State."** Often, in subconscious but mistaken analogy to one-component systems, there is a tendency to think of two- (or more) component systems below their freezing points as solids. The frequent existence of a liquid in equilibrium with a solid at temperatures below the freezing point is often neglected.<sup>18</sup> It is important, therefore, to mention that only below its eutectic point is a system completely solid. It seems best to consider that a "frozen state" exists in the range of temperatures below the freezing point and above the eutectic point, *i.e.*, where solid is

(10) S. Y. Wang, *Fed. Proc.*, **24**, Suppl. 15, S-71 (1965), and references therein.

(11) (a) W. Fuchtbauer and P. Mazur, *Photochem. Photobiol.*, **5**, 323 (1966); (b) S. Y. Wang, *ibid.*, **3**, 395 (1964).

(12) N. H. Grant, D. E. Clark, and H. E. Alburn, *J. Am. Chem. Soc.*, **83**, 4476 (1961).

(13) H. E. Alburn and N. H. Grant, *ibid.*, **87**, 4174 (1965); N. H. Grant, D. E. Clark, and H. E. Alburn, *ibid.*, **88**, 4071 (1966).

(14) N. H. Grant and H. E. Alburn, *Biochemistry*, **4**, 1913 (1965); *Arch. Biochem. Biophys.*, **118**, 292 (1967).

(15) A. R. Butler and T. C. Bruice, *J. Am. Chem. Soc.*, **86**, 313 (1964).

(16) T. C. Bruice and A. R. Butler, *ibid.*, **86**, 4104 (1964).

(17) Y. Eyal, D. Maydan, and A. Treinin, *Israel J. Chem.*, **2**, 133 (1964).

(18) (a) T. E. Kiovsky and R. E. Pincock, *J. Chem. Educ.*, **43**, 361 (1966); (b) R. E. Pincock and T. E. Kiovsky, *ibid.*, **43**, 358 (1966).

(19) R. A. Horne, *J. Inorg. Nucl. Chem.*, **25**, 1139 (1963).

(20) R. E. Pincock and T. E. Kiovsky, *J. Am. Chem. Soc.*, **87**, 2072 (1965).

(21) R. E. Pincock and T. E. Kiovsky, *ibid.*, **87**, 4100 (1965).

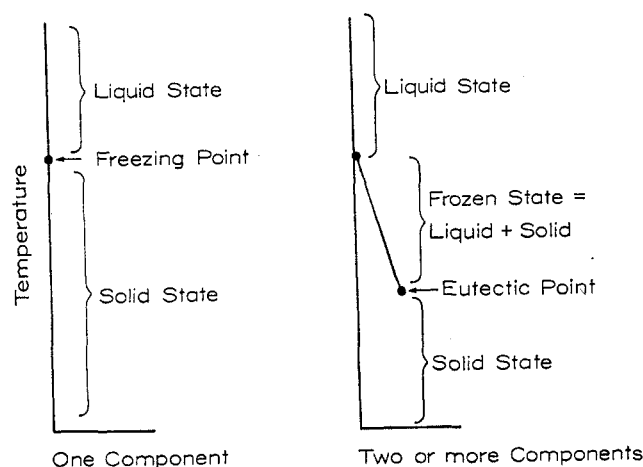


Figure 1. With a pure compound the transition from liquid to solid occurs sharply; in a frozen solution or impure solid a gradual transition from solid to liquid commonly occurs over a range of temperature.

in equilibrium with a liquid phase. This is illustrated in Figure 1.

In addition, use of the word "frozen" often connotes low temperatures (say, less than  $0^\circ$ ), but it should be noted that an apparently solid material at any temperature may contain some liquid phase if the system is above its eutectic temperature. Although a compound macroscopically appears to be a solid, if an impurity is present, the reactive sites in the sample may be liquid phases caused by small amounts of impurities. Any reaction in such liquid regions of the frozen system should be separated out before any true solid-state reaction can be well established.<sup>22</sup> In other words, at any temperature in the frozen state, reactions in the solid, in the liquid, or in both must be considered.<sup>23</sup>

**"Rate."** Interestingly, the meaning of "rate" takes on special significance in considering reactions in frozen, two-phase systems. The usual definition is given as  $\text{rate} = d(\text{concentration})/dt$ . However, when volume changes (caused by freezing or thawing of a liquid phase) occur together with changes in the number of moles of reactant, the above definition is unusable. As illustrated below for several "frozen" reactions, in order to obtain meaningful rate constants from observations of reactions in frozen systems, the following definition of rate must be used:<sup>24</sup>  $\text{rate} = (1/V)(dm/dt)$ , where  $V$  is the volume of the reactive phase and  $m$  is the moles of reactant in that phase. For a bimolecular reaction taking place in the liquid phase of a frozen system the moles converted per second is given by  $dm/dt = -k_2[A_1][B_1]V_1$  where  $k_2$  is the ordinary

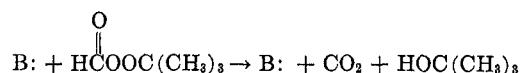
second-order rate constant for liquid-phase reaction,  $[A_1]$  and  $[B_1]$  are concentrations in the liquid phase, and  $V_1$  is the total volume of the liquid phase. When referred (for analytical purposes) to a thawed solution of volume  $V_s$  the observed rate is<sup>18b</sup>

$$\begin{aligned} \text{observed rate} &= (1/V_s)(dm/dt) \\ &= -k_2[A_1][B_1]V_1/V_s \end{aligned} \quad (1)$$

Some applications of this general equation are reviewed below.

### Kinetics of Reactions in Frozen Systems

**Low-Temperature Systems.** We avoided the suggested peculiarities of water and ice by initially applying eq 1 to a frozen-state reaction in organic solvents.<sup>20,21</sup> The base-catalyzed decomposition of *t*-butylperoxy formate (TBF) in frozen *p*-xylene, dioxane, benzene, nitrobenzene, *p*-chlorotoluene, and diphenylmethane



occurs with accelerated rates relative to those in normal (unfrozen) samples.<sup>20</sup> In *p*-xylene with 2,6-lutidine as catalyst the reaction shows the typical observations associated with many reactions in frozen solvents:<sup>21</sup> (1) acceleration; at 0.03 *M* TBF and 0.012 *M* 2,6-lutidine the loss of peroxide is twice as fast at  $0^\circ$  (frozen) than at  $70^\circ$  (nonfrozen); (2) a rate-temperature maximum; solutions at these concentrations have essentially equivalent rates of loss of peroxide at  $65^\circ$ ,  $9^\circ$  (frozen), and  $-20^\circ$  (frozen) (see Figure 2); (3) changes in observed kinetic order; the kinetic order in lutidine changes from unity at low initial concentrations to zero at high concentrations of the catalyst; (4) decreases in rate in the presence of usually inert solutes; in the case of TBF decomposition, addition of impurities (benzene,  $\text{CCl}_4$ , heptane) slows the frozen reaction but does not change the observed kinetic order.

All of these observations are correlated by an application of eq 1. The assumption is first made that no solid-state reaction occurs since none of the reactants form solid solutions with the crystallized solvents. This leaves only liquid pockets of highly concentrated solutes where reaction proceeds at an increased rate. The TBF-base reaction apparently occurs without a change in volume of the liquid holes since two soluble reactants are replaced by two soluble products (*t*-butyl alcohol and the base catalyst). Under these conditions, eq 1 may be integrated to give the expression for the observed rate constants, i.e.,  $k_{\text{obsd}} = k_2 C_1 [B] / ([B] + [P] + [I])$ , where  $k_2$  is the second-order rate constant for liquid-phase reaction,  $C_1$  is the total constant concentration of all solutes in the liquid phase of the frozen system, and  $[B]$ ,  $[P]$ , and  $[I]$  are initial (unfrozen) concentrations of base catalyst, peroxide, and any other solute, respectively. The values of  $k_2$  were obtained from extrapolation of liquid-phase rate con-

(22) R. E. Pincock and T. E. Kiovsky, *Chem. Commun.*, 864 (1966).

(23) R. E. Pincock, K. R. Wilson, and T. E. Kiovsky, *J. Am. Chem. Soc.*, **89**, 6890 (1967).

(24) For a discussion of the definition of "rate" see M. S. Peters and E. J. Skorpinski, *J. Chem. Educ.*, **42**, 329 (1965). The  $dC/dt$  definition normally has utility only because almost all kinetic studies are carried out at constant volume and  $dC/dt$  is then equal to  $d(m/V)/dt = (1/V)dm/dt$ .

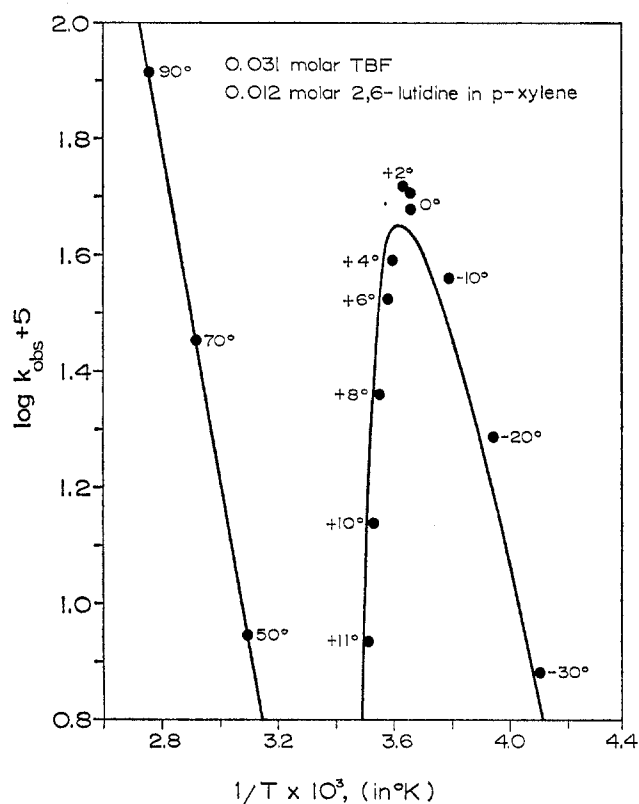


Figure 2. Plot of log of observed rate constants against reciprocal temperature for the decomposition of *t*-butylperoxy formate catalyzed by 2,6-lutidine. The curved line shows the calculated values of  $\log k_{\text{obsd}}$  for reactions in frozen solutions.

stants obtained at higher temperatures;  $C_1$  was obtained from the phase diagram of solutes in *p*-xylene. The above relationship then allowed an independent calculation of  $k_{\text{obsd}}$ , and values obtained were close enough to experimentally observed rate constants so that all of the assumptions were justified. In addition, the maximum in  $k_{\text{obsd}}$  (which is proportional to  $k_2 C_1$ ) is accounted for by decreases in  $k_2$  and increases in  $C_1$  as the temperature is lowered (see Figure 2). Changes in observed kinetic order occur as catalyst concentration becomes greater than peroxide concentration or, in other terms, as the ratio  $[B]/([B] + [P] + [I])$  goes to unity. All of the types of general observation (1 to 4 above) are explained by consideration of the volumes and concentrations of the liquid phase in the frozen system, while quantitative observations are related by use of eq 1.

The bimolecular reaction of equimolar  $\text{CH}_3\text{I}$  and  $(\text{C}_2\text{H}_5)_3\text{N}$  in frozen benzene solutions illustrates the importance of volume changes for reactions in frozen systems.<sup>25</sup> The volume of the liquid phase decreases during a run as the insoluble product, methyltriethylammonium iodide, precipitates out. As viewed from thawed solutions, this results in observed first-order rather than the normally expected second-order kinetics. In this case the addition of "inert" solutes not only depresses the observed rate but changes the observed kinetic order toward second order. Also,

with unequal initial concentrations of reactants, kinetic plots of  $\log ([\text{CH}_3\text{I}][(\text{C}_2\text{H}_5)_3\text{N}])$  against time were straighter lines than those obtained with the conventional second-order plot of  $\log ([\text{CH}_3\text{I}]/[(\text{C}_2\text{H}_5)_3\text{N}])$  against time. Such odd observations largely tend to be confusing until it is realized that integration of eq 1 gives expressions which quantitatively account for all the above results.<sup>25</sup> The change from observed second-order (nonfrozen) to observed first-order (frozen) kinetics is a consequence of constant concentrations of reactants in the liquid holes as they decrease in size during a run.<sup>26</sup>

As especially interesting effects were previously suggested for reactions in frozen aqueous solutions, some reactions in "ice" were investigated. The rate of reaction of ethylene chlorohydrin with sodium hydroxide in frozen solutions was faster by up to 1000 times that found in supercooled solutions at the same temperature.<sup>27</sup> In frozen systems, the rate increase and the rate-temperature maxima, the effects of variation of reactant concentration, and the rate depression caused by addition of soluble solutes were all quantitatively accounted for by integration of eq 1 under the assumption that no effects were present other than the concentration effect. At 0.001 *M* initial reactant concentration, a frozen solution is 99.9% solid at  $-5^\circ$ , but the reaction proceeds at a 1000-fold rate in the 0.1% liquid which is left. No reaction occurs in the solid phase, nor does the presence of ice surface affect the reaction; no phenomena other than the concentration effect was necessary to explain the observed results.<sup>27</sup>

Special results, other than those arising from a concentration effect, might show up in acid-catalyzed reactions in frozen systems. Rapid proton transfer through ice or to the ice surface could increase the observed rate.<sup>12-16</sup> The acid-catalyzed mutarotation of glucose did show quantitative deviations from expectations based on the concentration effect (*i.e.*, the HCl-catalyzed reaction was faster at  $-10^\circ$  than predicted by application of eq 1).<sup>28</sup> However, such variance was small and could be due to the reaction rate following an acidity function at high acid concentrations in the frozen system which is not proportional to HCl molarity itself. Again there was no distinct evidence for special catalytic effects, and the observations were essentially

(25) R. E. Pincock and T. E. Kiovsky, *J. Am. Chem. Soc.*, **88**, 51 (1966).

(26) This decrease in volume of liquid phase may be followed by high-resolution nmr spectroscopy.<sup>25</sup> Analysis by nmr may provide a convenient general way to monitor a reaction which involves a change in volume of liquid phase. A single frozen sample can be analyzed continually without thawing. In the case of triethylamine and methyl iodide in frozen benzene, the first-order rate constant for loss of the liquid benzene signal was found to be the same as the first-order titrimetric rate constant for loss of  $\text{CH}_3\text{I}$  and  $(\text{C}_2\text{H}_5)_3\text{N}$ . This follows from the proportional relationship of moles and volume when reactant concentration is held constant by continuous crystallization of benzene during the reaction (*i.e.*,  $m_1/V_1 = C_1$  and  $d \log V_1/dt = d \log m_1/dt$  when  $C_1$  is constant).

(27) R. E. Pincock and T. E. Kiovsky, *J. Am. Chem. Soc.*, **88**, 4455 (1966).

(28) (a) T. E. Kiovsky and R. E. Pincock, *ibid.*, **88**, 4704 (1966); (b) footnote 29 in ref 28a.

completely accounted for by the concentration effect alone.<sup>28,29</sup>

**Frozen-State vs. Solid-State Reactions.** The above examples of reactions in frozen organic and aqueous systems serve to illustrate how the concentration phenomenon can affect kinetic observations. It is not, of course, always necessary to apply the general form of the rate equation (eq 1); in many cases knowledge of the phase relationships and the kinetic expression for the reaction in liquid allows a simple estimate of the rate increase due to freezing. However, for more detailed comparisons, say of observed rate constants at various temperatures or at various initial concentrations, relationships obtained from the use of eq 1 are very helpful. In some studies of reactions in frozen systems the concentration effect was discounted as a basis of the observations because estimates of its effects did not seem to fit the results. From the background of the frozen-state reactions interpreted with the aid of the general equation, it is now known that observations which arise from the concentration effect can be rather well disguised. For example, observed changes in kinetic order, rather than giving evidence for a new mechanism, can arise directly from the concentration effect itself,<sup>28b</sup> or a kinetic relationship such as a linear Lineweaver-Burk plot for competitive inhibition, rather than an indication of enzyme-like active sites on the ice surface, may only be a manifestation of the concentration effect.<sup>30</sup> It is clear that, if effects other than the kinetic concentration effect are present, they must be established after accounting for any part of the observed rate of reaction which does come from concentration of reactants.<sup>18,27</sup>

In cases where a true solid-state reaction seems possible, it can be distinguished from a liquid-phase reaction by determining if products are formed below the eutectic temperature. The photochemical dimerization of thymine in ice was shown to be a solid-state reaction simply by showing that the thymine-water system has a eutectic point at  $-0.02^\circ$ .<sup>11</sup> Under all conditions normally employed for the reaction the thymine was swept together by the freezing process and crystallized in the solid system in an arrangement suitable for dimerization. On the other hand related

photodimerizations have been shown to occur in the liquid part of a frozen system above the eutectic point.<sup>10</sup>

In complex multicomponent systems the lowest temperature where liquid may exist (and a liquid-state reaction occur) is often far below the freezing point of the major component.<sup>31</sup> The reaction of iodide ion with arsenic acid can occur in frozen solutions down to *ca.*  $-70^\circ$ .<sup>18</sup> Probably a liquid phase still exists at this temperature. In any case, at somewhat higher temperatures the importance of phase properties is illustrated by the precipitation of solid iodine as product. This decreases the volume of liquid phase and pulls the reaction toward completion. Another example of the importance of phase relationships in considering reactions in frozen systems is with the suggested solid-phase electron transfer between ferrous and ferric ions at  $-78^\circ$ .<sup>19</sup> Although the eutectic temperature of a  $\text{HClO}_4\text{-H}_2\text{O}$  solution (the major components of the system studied) is  $-58^\circ$ , the total system contained  $\text{FeCl}_3$  and  $\text{FeCl}_2$ , as well as  $\text{HClO}_4$  and  $\text{H}_2\text{O}$ , and the eutectic point may be less than  $-78^\circ$ . If this is the case, reaction could still occur solely in a liquid phase of a system frozen at  $-78^\circ$  and there would be no necessity for a solid-phase water-bridging electron transfer of the type suggested. Studies of phase relationships are at least as important as kinetic or product studies in establishing the type of reaction occurring.

**High-Temperature Systems.** Some thermal reactions of organic solids have been considered in terms of equations originally developed for decompositions of inorganic solids.<sup>22</sup> For decompositions of *nonmelting* solids, the equations consider the growth of "cracks" or lattice imperfections from which further reaction proceeds, but in cases where melting occurs during reaction, the growth of a liquid phase can also give the kinetic form that is frequently observed for nonmelting systems, *i.e.*, slowly accelerating reaction *vs.* time curves. Many cases of thermal decomposition or rearrangement of solids should be treated as a type of frozen-state reaction; that is, reaction in a minor liquid phase as well as any reaction in a coexisting solid phase should be considered and, if possible, kinetically separated out.<sup>22,23</sup>

For example, the thermal mutarotation of neat, polycrystalline samples of  $\alpha$ -D-glucose begins to occur below its literature melting point of  $146^\circ$ . The reaction accelerates until melting is complete, then it decelerates (see Figure 3, curve C). Certain features of the reaction, such as variation of the observed initial rate constant with temperature (a factor of 7 for a  $4^\circ$  change of temperature), could be accounted for by assuming that reaction occurs only in the growing liquid phase.<sup>22</sup> At higher temperatures the great increase in observed rate was caused not only by a greater absolute rate constant for glucose mutarotation but

(29) Of course, in other reactions, the existence of special catalytic effects such as outlined in the introduction is still possible. An effect due to the solid surface, such as heterogeneous catalytic activity, or a significant influence of the solid on the adjacent liquid structure would show up best in a reaction at very low initial solute concentration (*i.e.*, when the volume of liquid phase in a frozen system is minimized). These effects should also depend on the rate of freezing of samples. Such possibilities might be specifically demonstrated in the future.

(30) Application of eq 1 to a second-order reaction of solutes A and B gives an expression which can be put into the following form:  $1/\text{rate} = K[(1 + [I]/[B])(1/[A]) + 1/[B]]$  where [A] and [B] are reactant concentrations in thawed solutions, [I] is the concentration of any other solute (*i.e.*, an inhibitor), and K is a constant related to the phase properties (K is therefore dependent only on temperature). A plot of  $1/\text{rate}$  against  $1/[A]$  at constant [B] and [I] will give a line whose slope is greater for runs at higher inhibitor concentration. In other words, the frozen-state reaction gives a linear Lineweaver-Burk plot for competitive inhibition. However, the above relationship does not account for observed<sup>14</sup> *specific* inhibition by compounds structurally analogous to reactants.

(31) Interestingly, in the complex case of frozen animal tissue, a liquid phase may exist down to as low as  $-70^\circ$ . See M. V. Sussman and L. Chin, *Science*, **151**, 324 (1966).

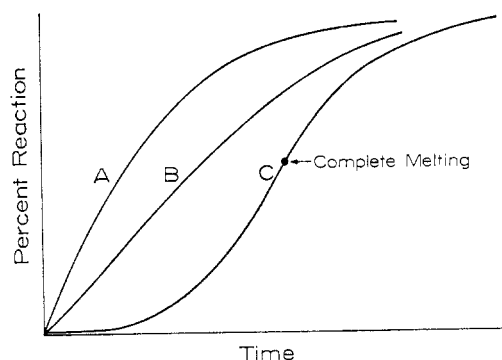
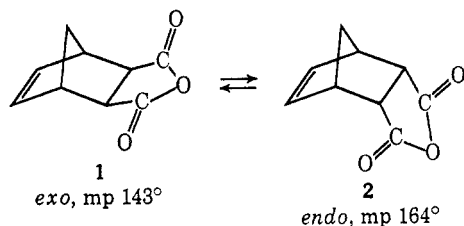


Figure 3. Plots of per cent reaction against time for decomposition or rearrangement of a melting solid. Curve A applies in the case of equal first-order rate constants for the reaction in both liquid and solid phases (*i.e.*,  $k_1 = k_s$ ), curve B is for  $k_1 > k_s > 0$ , and curve C is for the case  $k_1 > 0$ ,  $k_s = 0$ .

also by an increase in the volume of reactive liquid phase in equilibrium with the solid glucose. Although an independent calculation of observed rate constants was not possible, it was unnecessary to assume a solid-state reaction to interpret qualitatively the kinetic characteristics of the mutarotation.

A contrasting case arises with the thermal isomerism of polycrystalline *exo*- and *endo*-5-norbornene-2,3-dicarboxylic anhydrides (**1** and **2**) below their melting points ( $143^\circ$  and  $164^\circ$ , respectively).<sup>23</sup> Here there are concurrent reactions in both the solid and liquid (*i.e.*, melted) parts of the system above the eutectic point.<sup>32</sup>



Surprisingly, the rate of reaction of the solid *endo* compound was the same as in a liquid phase. The observed per cent reaction against time curves were as shown for curve A (Figure 3); the reaction began at its highest rate and decelerated toward completion. No inductive period nor change in rate as the samples became completely melted was observed. There is no noticeable difference between the reactivity of *endo* molecules in a solid or a melted phase; the probability for reaction is the same. This odd behavior was made more understandable when it was realized that the *endo* isomer converts to a liquid-like crystalline state above  $94^\circ$ .<sup>23</sup> The molecules then have gained some rotational freedom, as in a liquid, but lack the translational freedom of a melted phase. The energy separa-

rating liquid from solid phase *endo* at the melting point is relatively small ( $\Delta H_{\text{fusion}} = 5 \text{ cal/g}$ ). As the environments of molecules differ little between the two phases, isomerization proceeds essentially equally well in either phase.

However, with *exo* isomer **1** the rate of isomerization is noticeably slower in the solid relative to the liquid phase. The over-all observed reaction progress follows a curve like that of B in Figure 3, which is intermediate between no reaction in the solid (curve C) and equally facile reaction in the liquid and solid (curve A). The crystalline *exo* compound does not transform into a semiliquid crystalline state as does the *endo* isomer but gains both translational and rotational freedom only at its melting point. Both the structure of solid *exo* and its energy ( $\Delta H_{\text{fusion}} = 32 \text{ cal/g}$ ) differ sufficiently from the melted phase so that the rate of reaction is also different in the two phases.

The kinetic treatment for high-temperature reactions in melting solids is the same as for other frozen systems at low temperatures.<sup>23</sup> For a first-order liquid-phase decomposition or isomerization  $(1/V_1)(dm_1/dt) = -k_1(\text{concentration})$  or  $(dm_1/dt) = -k_1m_1$  where  $m_1$  is the moles of reactant in the liquid,  $V_1$  is the volume of liquid phase, and  $k_1$  is a first-order rate constant. For cases where a reaction also occurs in a solid phase another contribution must be added: the rate in the solid is  $dm_s/dt = -k_sm_s$ , where subscript *s* refers to the solid part of the system. The total observed rate is  $(dm_1/dt) + (dm_s/dt)$ , and, after introduction of a phase relationship governing the relative moles of reactant and product in the liquid and solid phases, three general cases can be considered.<sup>23</sup> If there is no solid-phase reaction, then  $k_s = 0$  and the reaction curve generated is similar to curve C. If the solid phase reacts as well as the liquid phase,  $k_s = k_1$  and curve A is generated by integration of the rate equation. If  $k_1 > k_s > 0$ , then intermediate curves, similar to B (Figure 3), are obtained. These cases are illustrated, respectively, by mutarotation of  $\alpha$ -D-glucose,<sup>22</sup> by isomerization of *endo* adduct **2**, and by isomerization of *exo* adduct **1**.<sup>23</sup> As with low-temperature reactions in biphasic systems, studies of higher temperature reactions of solids require consideration of any part of the reaction which occurs in an initially small amount of coexisting solid phase.

## Conclusions

In order to separate out the respective kinetic contributions of coexisting liquid and solid phases in a frozen system, knowledge of the phase relationships of reactants, products, solvent, and even possible impurities is required. The over-all observed rates or rate constants for reactions in frozen systems must be interpreted on the basis of the relative volumes of solid and liquid, and reactions in both liquid and solid phases may sometimes contribute to total reaction progress. As a further cautionary note it should be pointed out that, for many systems that are offhandedly

(32) Aside from kinetic evidence gathered from the frozen-state reaction, the existence of true solid-state reactions of compounds **1** and **2** was established by showing that isomerization proceeds below the eutectic temperature of *exo-endo* mixtures.

called "solid," both solid and liquid phases may be present (*i.e.*, a "frozen" system). Some observations on such systems may be more related to the liquid part than to the solid part of the total system. For example, nmr signals arising from the minor part of a frozen system have been mistakenly ascribed to the solid phase. Also, interpretations of the decay of esr signals in frozen systems may often require the consideration of liquid-phase free-radical reactions.<sup>33</sup>

The support of the U. S. Air Force Office of Scientific Research, the Petroleum Research Fund, administered by the American Chemical Society, the National Research Council of Canada, and the Alfred P. Sloan Foundation (for a fellowship, 1967-1969) is gratefully acknowledged. The work was greatly aided by A.F.O.S.R. Grant No. 1102-66.

(33) *E.g.*, P. D. Bartlett and G. Guaraldi, *J. Am. Chem. Soc.*, **89**, 4799 (1967); P. D. Bartlett and J. M. McBride, *Pure Appl. Chem.*, **15**, 89 (1967).

## Laser Studies of Vibrational Energy Transfer

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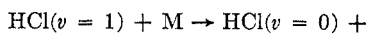
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Received August 12, 1968

Powerful, monochromatic beams of laser light<sup>2,3</sup> are opening many new avenues of experimental investigation in chemistry. The laser has been especially useful in studying the redistribution of energy among the vibrational modes of a single molecule and the exchange of vibrational energy between molecules. When a laser wavelength exactly matches a molecular absorption line, it is possible to excite many molecules into a single vibrational energy state. If the concentrations of vibrationally excited molecules can be measured spectroscopically while the laser is pulsed or modulated, rates of vibrational energy transfer processes may be determined. The relative ease of obtaining vibrational relaxation rates and the rapidly growing variety of systems open to experiment make this a rich field for both experimental and theoretical research.

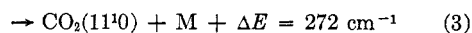
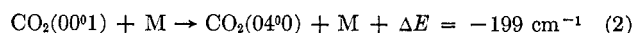
A great variety of vibrational energy transfer processes<sup>4</sup> can occur during bimolecular collisions in gases. A quantum of vibrational energy may be transferred completely into translational and rotational energy of the collision partners (eq 1). Vibrational energy

$V \rightarrow T, R$  transfer

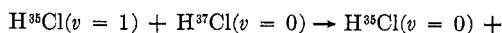


$$\text{M} + \Delta E = 2886 \text{ cm}^{-1} \quad (1)$$

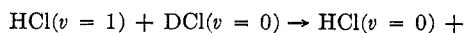
may be transferred from one mode to another within the same molecule with comparatively little energy going into translation and rotation (eq 2 and 3). Intramolecular  $V \rightarrow V$  transfer



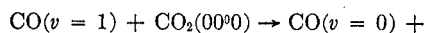
vibrational energy may also be transferred from one molecule into one or more modes of another molecule (eq 4-7). Finally, the quantum transferred may be Intermolecular  $V \rightarrow V$  transfer



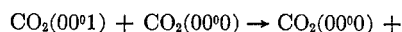
$$\text{H}^{37}\text{Cl}(v = 1) + \Delta E = 2.1 \text{ cm}^{-1} \quad (4)$$



$$\text{DCl}(v = 1) + \Delta E = 775 \text{ cm}^{-1} \quad (5)$$

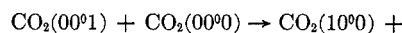


$$\text{CO}_2(11^00) + \Delta E = 65 \text{ cm}^{-1} \quad (6)$$



$$\text{CO}_2(11^00) + \Delta E = 272 \text{ cm}^{-1} \quad (7)$$

shared between the collision partners (eq 8). For each Intermolecular  $V \rightarrow V$  sharing



$$\text{CO}_2(01^00) + \Delta E = 294 \text{ cm}^{-1} \quad (8)$$

type of process there are many different combinations of initial- and final-state quantum numbers. A variety of different intermolecular forces and collision mechanisms may come into play as well.

Two good rules of thumb for vibrational energy transfer may be deduced from theory. They are that

(1) Alfred P. Sloan Fellow.

(2) B. A. Lengyel, "Introduction to Laser Physics," John Wiley & Sons, Inc., New York, N. Y., 1966, provides a good introduction to the properties and uses of lasers.

(3) A. L. Schalow, "Optical Masers," *Sci. Am.*, **204** (6), 52 (1961).

(4) In the energy-transfer reaction equations  $\Delta E$  is the net amount of energy transferred out of vibration into both translation and rotation. When  $\Delta E$  is negative, energy is transferred into vibration. At room temperature  $kT = 200 \text{ cm}^{-1}$ ,  $1 \text{ kcal} = 350 \text{ cm}^{-1}$ . For diatomic molecules the number of vibrational quanta excited is given by  $n$  as in  $\text{HCl}(v = n)$ . The symbol  $\text{CO}_2(X^Y^W)$  denotes a  $\text{CO}_2$  molecule with  $X$  quanta of the symmetric stretch,  $\nu_1$ , excited;  $Y$  quanta of the bending vibrations,  $\nu_2$ , excited with vibrational angular momentum  $z$ ; and  $W$  quanta of the asymmetric stretching vibration,  $\nu_3$ , excited. Figure 3 illustrates this nomenclature clearly. For methane the vibrational quanta excited are indicated in parentheses; *e.g.*,  $\text{CH}_4(\nu_2 + \nu_4)$  is a methane molecule with one quantum each of  $\nu_2$  and  $\nu_4$  excited (Figure 2).