The Mutarotation of Glucose in Frozen Aqueous Solutions[1]

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Abstract: The spontaneous mutarotation of glucose in frozen aqueous solutions occurs with an observed first-order rate constant of 1.6×10^{-5} sec^{-1} at $-4.0°$. The rate constant is independent of glucose concentration (0.01 to 1.4 *M*) or salt concentration (0.05 *M* NaCl) and close to the value extrapolated from rate studies in ordinary aqueous solutions above 0°. In contrast, the catalyzed mutarotation is faster in frozen than in unfrozen solutions at the same temperature. The *rate* of hydrochloric acid catalyzed reaction in frozen solutions (as measured in thawed solutions) is first order in glucose at all concentrations of reactants, first order in acid at low acid concentrations, but near zero order in acid at high concentrations of acid. At constant HCl concentration, the observed first-order *rate constants* also depend on glucose and on NaCl concentrations; k_{obsd} decreases with an increase in total solute concentration (C_s). The catalyzed reaction increases in rate as the temperature is decreased to *ca.* $-7°$, then decreases at lower temperatures. Glucose mutarotation "in ice" is accounted for by concentration of reactants into liquid regions of the frozen system. Changes in observed rates are explained in terms of concentrations and volumes of these reaction regions, while observed rate constants are correlated by the equation $k_{\text{obsd}} = k_1 + k_2C_h[\text{H}^+]_s/C_s$ (where k_{obsd}, k_1, and k_2 are the observed first-order rate constant, the "spontaneous" first-order rate constant, and the second-order rate constant for hydronium ion catalysis, respectively; C_h is the total constant concentration of solute in liquid reaction regions, and $[\text{H}^+]_s$ and C_s are the acid concentration and the total solute concentration in thawed solutions, respectively).

Several studies of reactions in frozen aqueous solutions have recently been reported.[2–14] Among the observed characteristics of these reactions are rate enhancements (relative to the reaction rate in supercooled liquid solutions), changes in kinetic order, and sensitivity to the presence of added solutes. For the acid- and base-catalyzed hydrolysis of acetic anhydride, of β-propiolactone, and of *p*-nitrophenyl acetate in ice,[15] Butler and Bruice[2] have presented evidence that the reactions proceed in liquid regions which contain high concentrations of the reagents. For these second-order reactions the increased concentration of reactants in the liquid part of frozen solutions results in accelerated rates of reaction. This "concentration effect" qualitatively accounted for the dependency of the rate on the initial reactant concentration, as well as the decrease in observed rate caused by addition of a solute such as KCl. Similar rate-depressing effects brought about by added solutes were found by Grant, Clark, and Alburn[5] for the base-catalyzed hydrolysis of penicillin and by Prusoff[9] for the acid-catalyzed dehydration of 5-hydro-6-hydroxydeoxyuridine. Wang[10] has shown that some photochemical reactions in frozen aqueous solutions occur in "puddles" of liquid within the frozen solutions. In the oxidation of iodide ion by arsenic acid[13] and the reaction of ethylene chlorohydrin with hydroxide ion[11] in frozen aqueous solutions the observations are consistent with the concentration effect. Also for two types of reactions in frozen organic solutions, the variations of the observed rate constants are quantitatively correlated by a kinetic treatment based only on the concentration effect.[14]

On the other hand, some of the observed features of reactions in frozen solutions have seemed inconsistent with expectations for the concentration effect.[3–9] Other factors which have been put forward as possible general explanations of reactions in ice involve orientation effects or other participation by the ice crystal surface, and facile proton transfers to or from the reactive substrate.[3–9] While some of the apparently inconsistent features of "frozen reactions" are actually predicted by a general treatment of the concentration effect,[12] the existence of such other effects cannot be discounted. It is clear that, for reactions known to proceed in normal liquid solutions near the freezing point, any discussion of rates in a frozen solution which does not separate the contribution of the concentration effect must be in error. Quantitative separation of the results due to a concentration effect from those arising from any other possibility is not easily carried out when the system contains many solutes, when the reaction is not well known under normal (*i.e.*, unfrozen) conditions, or when the ice–liquid phase relationship is not available.[11–14]

For these reasons, as well as general interest in reactions in ice, we have investigated in frozen aqueous solutions a well-established reaction which follows, in ordinary liquid solutions at least, a simple kinetic equation. The rate constants for "spontaneous" and

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[1] Reactions in Frozen Solutions. VII. Research sponsored by the U. S. Air Force Office of Scientific Research, Grant No. AFOSR 1102-66, and by the National Research Council of Canada.

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

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

[4] T. C. Bruice and A. R. Butler, *Federation Proc. Suppl.*, **15**, s-45 (1965).

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

[6] H. E. Alburn and N. H. Grant, *ibid.*, **87**, 4174 (1965).

[7] N. H. Grant and H. E. Alburn, *Biochemistry*, **4**, 1913 (1965).

[8] N. H. Grant and H. E. Alburn, *Science*, **150**, 1589 (1965).

[9] W. H. Prusoff, *Biochim. Biophys. Acta*, **68**, 302 (1963).

[10] S. Y. Wang, *Nature*, **190**, 690 (1961); *Federation Proc. Suppl.*, **15**, s-71 (1965).

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

[12] R. E. Pincock and T. E. Kiovsky, *J. Chem. Educ.*, **43**, 358 (1966).

[13] T. E. Kiovsky and R. E. Pincock, *ibid.*, **43**, 361 (1966).

[14] (a) R. E. Pincock and T. E. Kiovsky, *J. Am. Chem. Soc.*, **87**, 2072 (1965); (b) *ibid.*, **87**, 4100 (1965); (c) *ibid.*, **88**, 51 (1966).

[15] The use of the word "ice" can be deceptive in that it seems to denote the presence of *only* a solid phase. However, depending on the solute, frozen solutions may contain liquid regions in equilibrium with solid at temperatures considerably below the freezing point. With this important fact in mind, the convenience of the name would seem to make permissible its use to denote not only pure solid water, but also the *apparent* state of a frozen aqueous solution.

for acid-catalyzed mutarotation of glucose in water follow the relation $k_{\mathrm{obsd}} = k_1 + k_2[\mathrm{H}^+]$.[16] The occurrence of the spontaneous, first-order reaction allows the study of glucose mutarotation in ice in the absence of any other solutes. The second-order reaction allows a direct investigation of the catalytic activity of hydronium ion in a frozen aqueous solution.[17] Since the mutarotation is general acid and general base catalyzed[18] and known catalysts include a variety of structures, there would seem to be a greater likelihood of the appearance of any new effects (such as a catalytic effect of the ice surface). In addition, as a convenience in the calculation of the contribution due to the concentration effect, the glucose–H_2O and the HCl–H_2O phase relationships are known[19] and the activation parameters needed to calculate both k_1 and k_2 at low temperatures have been carefully established.[20]

Results

Spontaneous Mutarotation. The first order, uncatalyzed mutarotation in frozen aqueous solutions was studied by measurements of changes in optical rotation of thawed α-D-glucose solutions. The mutarotation was slow enough below 0° ($t_{1/2} > 380$ min) so that any reaction during the time necessary to thaw and analyze an individual sample from a kinetic run was negligible. Runs were followed to about the first half-life and mutarotation rate constants[21] were calculated from the observed rotations by the usual first-order kinetic treatment (see Experimental Section). The observed rate constants for reaction at −4.0°, with the concentration of glucose in initial unfrozen solutions varied from 0.01 to 1.4 *M*, are given in Table I. Over this wide range of concentration, as well as at 0.05 *M* glucose with 0.05 *M* sodium chloride present, the reaction proceeds with the same rate in all frozen solutions (average $k_0 = 1.6 \pm 0.1 \times 10^{-5}$ sec^{-1} at −4.0°). The rate constant calculated from the Arrhenius equation given by Smith and Smith,[20] as obtained from measurements in liquid solutions down to 0°, is 1.9×10^{-5} sec^{-1} at −4.0°.[22]

Table I. Uncatalyzed Mutarotation of Glucose in Frozen Aqueous Solutions at −4.0°

Glucose concn, *M*	$k_{\mathrm{obsd}} \times 10^5$ sec^{-1}	Glucose concn, *M*	$k_{\mathrm{obsd}} \times 10^5$ sec^{-1}
0.011	1.63	0.555	1.73
0.051[a]	1.57	1.39	1.42
0.500	1.38	...	1.92[b]

[a] With 0.051 *M* sodium chloride. [b] Calculated from known activation parameters in water.[20,22]

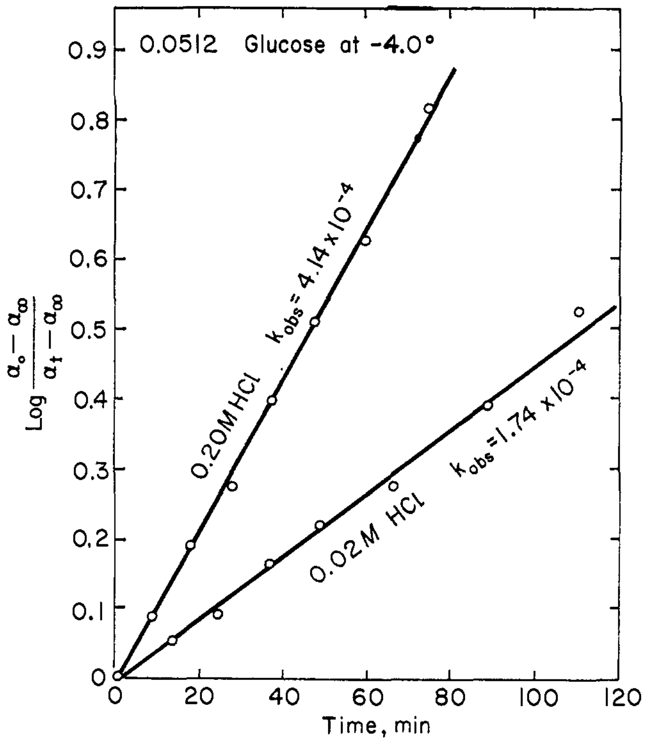

Figure 1. First-order kinetic plots for mutarotation of glucose in frozen aqueous hydrochloric acid solutions at −4.0°. Optical rotations (α) were measured in thawed solutions and rate constants calculated by $\log [(\alpha_0 - \alpha_\infty)/(\alpha_t - \alpha_\infty)] = k_{\mathrm{obsd}}t/2.303$.

Hydrochloric Acid Catalyzed Mutarotation. Unlike the spontaneous reaction, the mutarotation of glucose in aqueous hydrochloric acid is accelerated by freezing the solution. In thawed solutions at low acid concentrations, the reaction is sufficiently slow so that good kinetic plots were obtained simply by thawing and rapidly analyzing samples of a run. Figure 1 shows two "frozen runs," with 0.02 and 0.20 *M* HCl and both with 0.051 *M* glucose at −4.0°, plotted as first-order reactions. The linearity of such plots over several half-lives shows that the mutarotation in ice is first order in glucose, as is the case for the normal reaction in liquid water. However, the kinetic order in acid is not a constant, integral value. This is shown, for example, in Figure 1 where the two observed rate constants (obtained from the slopes of the lines) differ by a factor of only 2.4 for variation of HCl concentration by a factor of 10. When runs are made over a range of HCl concentration from 0.006 to 0.3 *M* at constant glucose concentration, and the values of k_{obsd} plotted against H^+ (see Table II and Figure 2), it is

Table II. Effect of HCl Concentration on Observed First-Order Rate Constants for Mutarotation of Glucose at −4.0° in Frozen Aqueous Solutions.

0.555 *M* glucose		0.0512 *M* glucose	
HCl concn, *M*	$k_{\mathrm{obsd}} \times 10^5$, sec^{-1}	HCl concn, *M*	$k_{\mathrm{obsd}} \times 10^5$ sec^{-1}
None	1.73	None	1.63
0.02	3.82	0.006	8.54
0.05	7.66	0.020	17.4
0.10	12.0	0.052	28.2
0.15	18.0	0.10	30.8
0.20	18.7	0.14	36.0
0.25	22.0	0.20	41.0
		0.30	40.4

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(16) C. S. Hudson, *J. Am. Chem. Soc.*, **29**, 1571 (1907).

(17) In most published studies of reactions in ice, the use of buffered solutions has been a necessary but complicating factor.

(18) J. N. Brønsted and E. A. Guggenheim, *J. Am. Chem. Soc.*, **49**, 2554 (1927); T. M. Lowry and G. F. Smith, *J. Chem. Soc.*, 2539 (1927).

(19) F. E. Young, *J. Phys. Chem.*, **61**, 616 (1957).

(20) G. F. Smith and M. C. Smith, *J. Chem. Soc.*, 1413 (1937).

(21) The observed rate constant, k_1, is the sum of the individual rate constants for mutarotation of α- and β-glucose, *i.e.*, $k_1 = k_\alpha + k_\beta$ where $\alpha \underset{k\beta}{\overset{k\alpha}{\rightleftarrows}} \beta$.

(22) As with the calculated rate constant in ice (see Table I), the rate constant calculated for reaction in ordinary, unfrozen aqueous solutions is slightly higher than reported experimental values (calcd at 0°, 3.05×10^{-5} sec^{-1}; found at 0°, 2.85, 2.80, and 2.82×10^{-5} sec^{-1}; see ref 20).

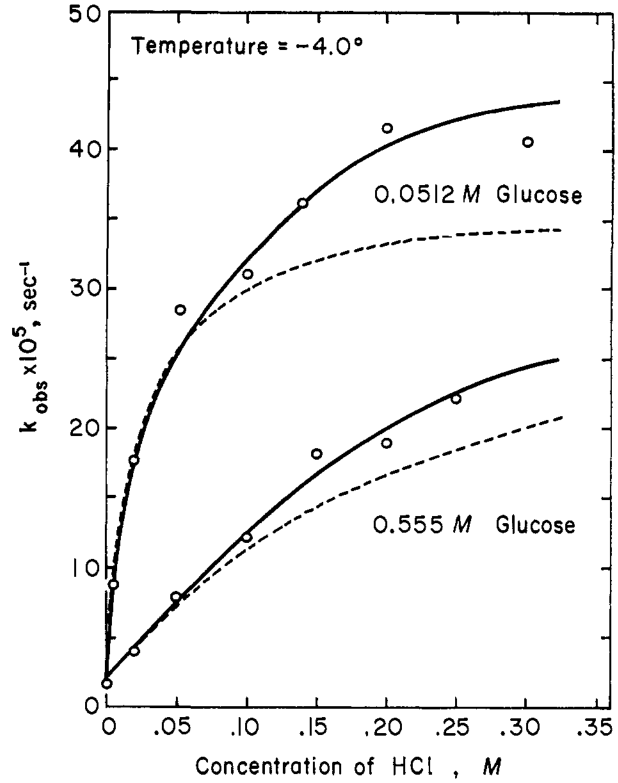

Figure 2. Variation of observed first-order rate constants with hydrochloric acid concentration for mutarotation of glucose in frozen solutions at −4.0°. The solid lines follow the experimental points; the broken lines show the theoretical relation according to eq 1.

seen that the order, x, in the relationship $k_{obsd} = k_1 + k_2[H^+]^x$ is unity at low acid concentrations but near zero at high acid concentrations. A similar variation of kinetic order with catalyst concentration has been observed in the base-catalyzed decomposition of *t*-butylperoxy formate in frozen *p*-xylene solutions.[14b]

The two separate curves in Figure 2 for 0.05 and for 0.5 *M* glucose also show that the observed rate constants depend on the initial concentration of glucose. For the same concentration of acid, the rate constants are greater for 0.05 *M* than for 0.5 *M* glucose. More complete data on this effect are given in Table III and illustrated in Figure 3 where the observed first-order rate constants for reaction in frozen 0.10 *M* HCl solutions are related to glucose concentration over the range 0.05 to 1.10 *M*. In these frozen solutions an inverse dependency of k_{obsd} on glucose concentration is obtained up to a concentration sufficient to prevent freezing at −4°. That a first-order rate constant can depend on *initial* reactant concentration before freezing but not on the *actual* reactant concentration during a run is an interesting characteristic of some reactions in frozen solutions.[14]

Table III. Variation of Observed First-Order Mutarotation Rate Constant with Glucose Concentration for Frozen 0.10 *M* HCl Solutions at −4.0°

Glucose, *M*	k_{obsd} × 10^5, sec^{-1}	Glucose, *M*	k_{obsd} × 10^5 sec^{-1}
0.0512	30.8	0.833	7.21
0.278	18.7	1.10	7.18
0.390	13.8	1.18	4.91[a]
0.555	12.0	. . .	6.17[b]

[a] Supercooled liquid solution. [b] Calculated for liquid solutions at −4° and 0.10 *M* HCl.[20]

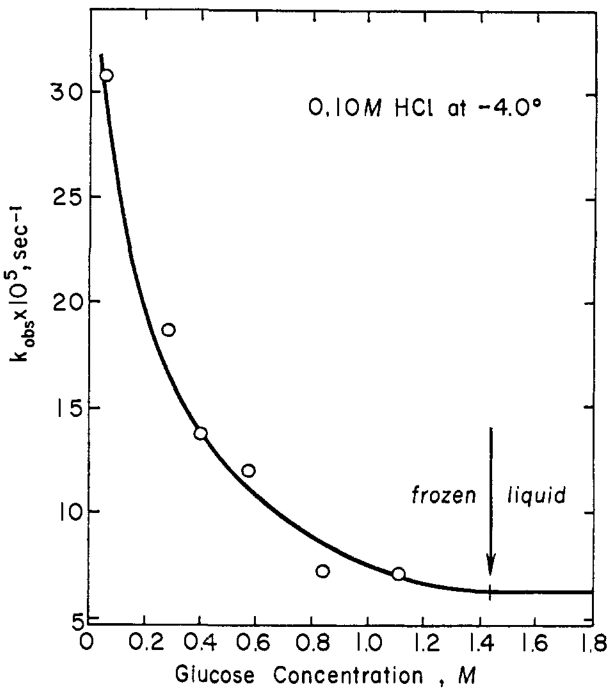

Figure 3. Variation of observed first-order rate constants with glucose concentration for mutarotation in frozen 0.10 *M* HCl solutions at −4.0°. The line shows the theoretical relationship predicted by eq 1.

As previously demonstrated for some bimolecular reactions in frozen organic solvents and in ice,[11–14] a maximum in the rate–temperature relationship is a general feature of the concentration effect for reactions in frozen solutions. The acid-catalyzed mutarotation of glucose shows a rate maximum at about −7°. The observed rate constants at various temperatures for two different sets of initial concentrations are collected in Table IV.

Table IV. Temperature Variation of Observed Rate Constants for Mutarotation of Glucose in Frozen Aqueous Solutions

0.555 *M* glucose, 0.10 *M* HCl		0.0512 *M* glucose, 0.040 *M* HCl	
Temp, °C	k_{obsd} × 10^5 sec^{-1}	Temp, °C	k_{obsd} × 10^5 sec^{-1}
+2.0[a]	12.3	−1.0	12.4
−1.6	7.16	−3.0	23.0
−4.0	12.0	−5.0	29.2
−5.4	14.8	−6.3	30.5
−7.1	14.8	−6.3	20.0[b]
−9.1	13.5	−6.3	14.3[c]
−11.3	8.67	−8.0	30.7
		−9.5	31.0
		−11.0	28.8
		−13.5	24.7
		−14.9	21.8
		−17.0	17.9

[a] Solution not frozen. [b] Solution contained 0.051 *M* sodium chloride. [c] Solution contained 0.097 *M* sodium chloride.

At −6° with 0.05 *M* glucose and 0.04 *M* HCl the observed rate constant is 17 times greater than the calculated rate constant[20] for a supercooled liquid solution at this temperature. With these reactant concentrations the rate in ice is depressed by 33% if 0.051 *M* NaCl is present and by 50% if 0.097 *M* NaCl is present in the initial solutions.[23] Figure 4 illustrates these results; the mutarotation remains first order in glucose and the observed rate constant is less at higher salt concentrations.

Similar to previously studied reactions in frozen systems, experimental observations on the mutarotation of glucose in frozen aqueous solutions then include rate enhancements, changes in kinetic order, a rate maximum at a temperature several degrees below the freezing point, and sensitivity to added solutes.

Discussion

Solutes of an aqueous solution are very rarely incorporated into slowly grown ice crystals.[24] For rapidly frozen solutions the solutes might appear to be in the solid phase, but true solid solutions would not be expected. The formation of ice in an aqueous solution commonly results in an increase in solute concentration in the remaining liquid phase, even if this liquid phase is greatly dispersed among the solid. For a given temperature, the same concentration of solute is finally attained in all the liquid regions of the frozen system. Although the *total* volume of regions would depend on the initial concentration of solute and the individual volumes and distribution of the regions should depend on the rate of freezing, the concentration of the solute in the liquid regions is determined by the phase equilibrium between pure ice and liquid solution.[12]

Spontaneous Reaction. In the case of glucose at −4°, the liquid phase of a frozen solution contains 27% glucose[19] and sufficient water is still present so that essentially the same rate of spontaneous mutarotation is obtained in "ice" as in an ordinary water solution.[25] The results given in Table I show that the reaction rate is not appreciably affected by variation of surface area of the ice in contact with the liquid regions (*i.e.*, the reaction is not sensitive to either the initial concentration of glucose from 0.01 to 1.4 *M*, nor to the rate of freezing of samples). The normal mutarotation rate (at 0.051 *M* glucose) is also not changed by the addition of 0.05 *M* sodium chloride. The actual salt concentration in the reaction regions at −4° would be *ca.* 0.55 *M;* but since it is known that even 1 *M* NaCl or KCl has no effect on the mutarotation rate in liquid water,[26] the absence of a "salt effect" in ice is reasonable. In contrast to results of this type, Butler and Bruice[2] have shown that the spontaneous hydrolyses of acetic anhydride and of β-propiolactone are completely, or at least greatly, depressed in frozen aqueous solutions. In these cases the addition of KCl increased the reaction rates in ice.

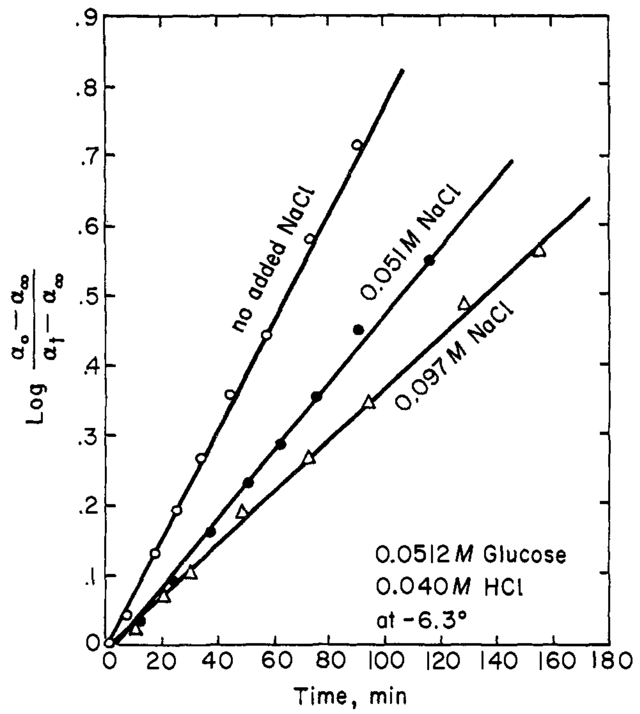

Figure 4. Effect of added NaCl on mutarotation of glucose in frozen solutions at −6.3°. The reactant concentrations were 0.0512 *M* glucose and 0.040 *M* HCl.

Catalyzed Reaction. Unlike the simple first-order mutarotation, the second-order reaction between glucose and acid is promoted by the concentration effect. In order to determine if other effects are present the contribution of the concentration effect must be separated out. To do this it should first be noted that the *observed* rate of reaction depends not only on the concentrations in the liquid "reaction regions" of a frozen solution, but also on the relative volumes of reaction regions and the thawed solution.[12] Rates and rate constants are measured and reported in terms of the volume of thawed solution, whereas the reaction actually occurs in a much smaller volume produced by the freezing process. A rate in the liquid regions of a frozen solution of χ moles/sec-l. will appear as $\chi \times V_h/V_s$ moles/sec-l. when referred to thawed solution.[27] Even if the actual rate does not basically differ from that expected for a normal liquid phase reaction, the observed rate will be modified by any factor affecting the volume of liquid phase V_h.[28] As V_h may vary from run to run,[14a,b] or even during an individual run,[14c] unusual changes in the observed rate may result.[29]

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(23) The acid-catalyzed reaction in ordinary aqueous solutions shows almost negligible salt effect. At pH 1, 1 *M* NaCl causes only an 8% increase in observed rate. See R. Kuhn and P. Jacob, *Z. Physik. Chem.* (Leipzig), **113**, 389 (1924).

(24) A variety of 12 inorganic salts and polar organic compounds were found not to incorporate in slowly formed ice crystals. Only isomorphous NH_4F was incorporated to the extent of *ca.* 0.5%. See S. Zaromb and R. Brill, *J. Chem. Phys.*, **24**, 895 (1956).

(25) However, on long standing at below the ice–α-glucose hydrate eutectic temperature of −4.93°,[19] the α-glucose hydrate should crystallize out and a frozen glucose solution become completely solid.

(26) T. M. Lowry, *J. Chem. Soc.*, **83**, 1314 (1903); J. C. Andrews and F. P. Worley, *J. Phys. Chem.*, **31**, 882 (1927); V. W. Broser and G. Ruecker, *Z. Naturforsch.*, **15b**, 334 (1960).

(27) V_h is the total volume of liquid regions in a frozen solution and V_s is the volume of thawed solution.

(28) For a bimolecular reaction, A + B → products, the observed rate is given by the equation $d[A]_s/dt = -k_2[A]_h[B]_hV_h/V_s$ where subscript s refers to thawed solution and h to the liquid reaction regions of the frozen solution.

(29) Although this volume effect makes no difference in a first-order reaction, for higher order reactions this aspect of frozen solutions may give rise to rather confusing results. For example,[3,4] the rate constants for reaction of two thiolactones with morpholine in supercooled aqueous solutions are proportional to the square of morpholine concentration, *i.e.*, $k_{obsd} = K[N]^2$, while in frozen solutions at the same temperature the rate is first order in morpholine and the observed kinetic equations have the form $k_{obsd} = K'[N]$. Based mainly on this change in kinetic order a different mechanism was suggested for the reaction in ice.[3,4] However, in obtaining these experimental results, the morpholine concentration in initial unfrozen solutions was varied in a series of runs simply by dilution of a standard buffer solution. The volume of the liquid regions of frozen solutions would then be proportional to morpholine concen-

Although the concentrations and volume of the reaction regions are fundamentally important, they are not directly measurable. Instead, the concentrations in the frozen solutions must be related to readily measured concentrations in thawed solutions. For glucose mutarotation in frozen hydrochloric acid solutions, the observed rate constants, according to the concentration effect, are related to hydronium ion concentration of the reaction regions by the equation $k_{\text{obsd}} = k_1 + k_2[\text{H}^+]_{\text{h}}$.[30] When all the acid present in initial (or thawed) solutions is present only in the reaction regions of frozen solutions, then $[\text{H}^+]_{\text{s}}V_{\text{s}} = [\text{H}^+]_{\text{h}}V_{\text{h}}$. If all solutes act similarly, the total number of moles in a thawed solution will be equal to the total number of moles in the liquid part of the frozen solution, *i.e.*, $C_{\text{s}}V_{\text{s}} = C_{\text{h}}V_{\text{h}}$ where C_{s} is the total initial solute concentration of unfrozen solution and C_{h} is the total solute concentration in the liquid regions of the frozen solution. Using these two relationships between concentrations in a frozen and thawed solution, the observed rate constant for mutarotation in ice is given in terms of measureable quantities in thawed solutions as follows.

$$k_{\text{obsd}} = k_1 + k_2V_{\text{s}}\frac{[\text{H}^+]_{\text{s}}}{V_{\text{h}}} = k_1 + k_2C_{\text{h}}\frac{[\text{H}^+]_{\text{s}}}{C_{\text{s}}} \qquad (1)$$

The total concentration in the liquid reaction regions (C_{h}) is constant throughout a run and, like k_2, depends only on temperature.[12] The concentrations $[\text{H}^+]_{\text{s}}$ and C_{s} are known for each run, the value of C_{s} being equal to the total initial solute concentration, $C_{\text{s}} = [\text{glucose}]_{\text{s}} + [\text{H}^+]_{\text{s}} + [\text{Cl}^-]_{\text{s}} + [\text{added solute}]_{\text{s}}$. In these terms the hydronium ion concentration, $[\text{H}^+]_{\text{h}}$, is equal to the total possible concentration, C_{h}, times the ratio of acid to total solute concentration in a thawed solution. Therefore, the hydronium ion concentration in the liquid part of a frozen solution is not always directly proportional to the hydronium ion concentration of thawed solution, but depends also on the concentration of the other solutes.

Qualitative Results. The relationships presented above account for the experimental observations for mutarotation in ice, as well as for some general features of other reactions in frozen systems. For this reason they can first be discussed in a general qualitative manner.

The change in kinetic order in H^+ for glucose mutarotation in ice arises from compensating changes in reaction volume and concentration. At low concentrations of acid the relatively greater amount of glucose present controls the reaction volume in the frozen solutions. The reaction regions remain at nearly constant total volume, while the H^+ concentration and the reaction rate in the regions increase in proportion to the acid concentration in initial solutions; the observed order in H^+ is then unity. On the other hand, at high acid concentrations the solute in the reaction regions is essentially all HCl at the highest possible concentration. Any further increase in acid concentration in initial solutions only increases the volume of liquid regions in the frozen solution. The concentration $[\text{H}^+]_{\text{h}} \cong {}^1/_2C_{\text{h}}$ remains constant, the reaction rate therefore remains constant, and the reaction *appears* to be zero order in acid.

A similar change in kinetic order for glucose does not occur. Although the concentration of glucose in liquid regions is also not directly proportional to initial glucose concentration, and is varied by changes in concentrations of other solutes, the kinetic order is independent of glucose concentration.[31] However, as all solutes are involved in determining the total volume of reaction region, the observed rate constant depends on the initial glucose concentration. A greater number of moles of glucose results in a greater volume, V_{h}, and, for the same number of moles of available acid, the concentration $[\text{H}^+]_{\text{h}}$ must be more dilute. At a constant initial HCl concentration of 0.10 *M*, the decrease in observed rate constant with increase in glucose concentration (see Figure 3) results from such changes in reaction volume. At the lowest glucose concentration a maximum rate constant, corresponding to a maximum acid concentration of $[\text{H}^+]_{\text{h}} = {}^1/_2C_{\text{h}}$, should be obtained. At high concentration of glucose the minimum rate constant is obtained when the solution contains too much glucose to allow freezing at $-4°$ (*i.e.*, when $V_{\text{h}} = V_{\text{s}}$).

The effect of added sodium chloride is similar. A greater concentration of a soluble but inert solute such as NaCl results in a greater volume of total reaction regions, the reactants are made more dilute, and slower rates of reaction are observed (see Figure 4).

The changes in observed rate with temperature may also be explained in terms of concentrations and volumes of liquid reaction regions. With constant initial concentrations of HCl and of glucose, a decrease in temperature results in a decrease in reaction volume as more pure solvent is frozen out. A corresponding increase in concentration, C_{h}, occurs. As long as the increase in concentration outweighs the decrease in second-order rate constant, k_2, an over-all increase in observed rate is obtained (see eq 1). A maximum rate is reached, however, when further decreases in temperature fail to increase C_{h} enough to compensate for decreases in the second-order rate constant. This effect is illustrated in Figure 5.

Quantitative Relation of Kinetic Equation. For quantitative application of eq 1, the rate constant for uncatalyzed mutarotation, k_1, and the second-order rate constant for acid-catalyzed mutarotation, k_2, may be obtained by extrapolation of data obtained in normal solutions down to 0°.[20] The value of C_{h} is

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trations in thawed solutions, *i.e.*, $V_{\text{h}} = K''[\text{N}]_{\text{s}}$, while the actual concentration ($[\text{N}]_{\text{h}}$) in the liquid reaction regions would be the same constant value in all the frozen runs. If the reaction in the liquid regions is normal, then the rate is proportional to $[\text{N}]_{\text{h}}^2$. When measured in thawed solutions the actual rate is modified so that the observed rate constant is $k_{\text{obsd}} = K[\text{N}]_{\text{h}}^2V_{\text{h}}/V_{\text{s}}$ which is equal to $K[\text{N}]_{\text{h}}^2K''[\text{N}]_{\text{s}}/V_{\text{s}}$. As $[\text{N}]_{\text{h}}$ is constant and the volume V_{s} is always in terms of 1 l., the rate constants are proportional to $[\text{N}]_{\text{s}}$, *i.e.*, $k_{\text{obsd}} = K'[\text{N}]_{\text{s}}$ as experimentally observed. The odd change in kinetic order for reaction in ice arises only from the variations in reaction volumes for runs at different initial concentrations. As the reactions also show other features of the concentration effect (such as rate-depressing effects of salts) then it does not seem that any new mechanism occurs in these reactions under frozen conditions.

(30) This relationship may be derived from the general equation for the concentration effect[28] as applied to this second-order reaction. A necessary assumption is that no change in reaction volume occurs *during* a run, or, in other words, that the concentration $[\text{H}^+]_{\text{h}}$ is constant in any run. This is reasonable since no over-all change in number of moles occurs during mutarotation.

(31) For any second-order catalytic reaction, the general equation[28] contains this result. If $[\text{A}]_{\text{s}}V_{\text{s}} = [\text{A}]_{\text{h}}V_{\text{h}}$ then $\text{d}[\text{A}]_{\text{s}}/\text{d}t = -k_2[\text{B}]_{\text{h}}[\text{A}]_{\text{s}}$ and when $[\text{B}]_{\text{h}}$ is constant throughout a run (as in a catalytic reaction), the reaction *order* is seen to be independent of $[\text{A}]_{\text{s}}$. However, the observed rate constant $k_{\text{obsd}} = k_2[\text{B}]_{\text{h}}$ will depend on the initial concentration of all solutes (including A).

obtained from the temperature–composition phase relationship for the glucose–water system.[32]

The curved line of Figure 3 shows the calculated variation of observed rate constant with glucose concentration for runs at constant acid concentration of 0.10 *M*. The values predicted by eq 1 as $k_{\text{obsd}} = (1.92 \times 10^{-5}) + (4.25 \times 10^{-4})(1.64)(0.1)/(0.2 + [\text{G}]_s)$ are very close to the experimentally obtained values. In all supercooled liquid solutions, as in a solution more concentrated than *ca.* 1.4 *M* glucose at −4°, the same rate constant of *ca.* 6.2×10^{-5} sec^{-1} is expected[33] (see Table III). At concentrations lower than 1.4 *M*, the rate constant in frozen solutions increases as predicted by eq 1.

The broken lines in Figure 2 show the theoretical relationship of k_{obsd} to $[\text{H}^+]_s$ (from eq 1 in the form $k_{\text{obsd}} = (1.92 \times 10^{-5}) + (4.25 \times 10^{-4})(1.64)([\text{H}^+]_s)/([\text{G}]_s + 2[\text{H}^+]_s)$ at two different glucose concentrations, $[\text{G}]_s = 0.051$ and 0.555 *M*). At the highest acid concentrations studied, the calculated rate constant is 20% less than observed values. This deviation of predicted and observed lines is small, but apparently real, as such deviation occurs only at concentrations corresponding to high acid concentration in the reaction regions (*i.e.*, large $[\text{H}^+]_h$).[34]

The observation that the rate of hydrochloric acid catalyzed mutarotation of glucose increases faster than the actual acid concentration (for HCl > 0.4 *M*) was reported by Lowry and Smith[18] and discussed in terms of a "catalytic coefficient of undissociated molecules of HCl greater than that of the hydrogen ions derived from them." In ordinary (unfrozen) solutions this greater catalytic effect[35] becomes important only when the mutarotation is too fast for convenient measurement;[36] in frozen solutions the effect, although complicated by the two-phase system, may be more accessible for study. At low initial concentrations, a reaction in a frozen sample can be sufficiently slowed by thawing so that analysis may be carried out at room temperature.[14]

The calculated effect of temperature on the acid-catalyzed mutarotation in ice also shows that the observed rate constants are greater than predicted. The broken lines of Figure 5 show the temperature variation according to eq 1. For these two series of runs at constant reactant concentrations, the rate maximum arises from the decrease in k_2 and an increase in C_h as the temperature is made lower. The experimental points lie above the respective calculated lines and, as discussed above, the difference could arise from more effective acid catalysts at the higher concentrations encountered at lower temperatures or, possibly, from inexact values of C_h (especially at lower temperatures) as obtained from the glucose–water phase diagram.[32]

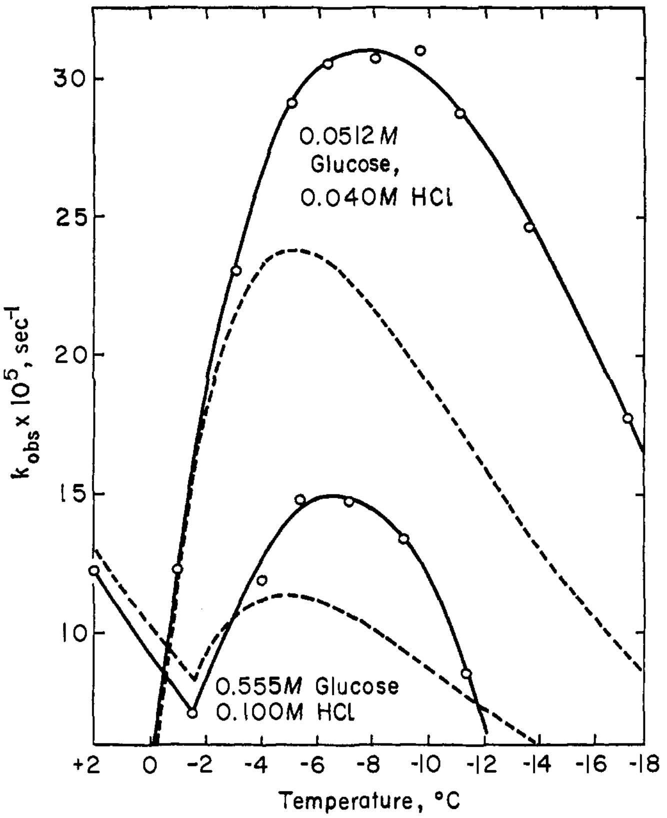

Figure 5. Effect of temperature on mutarotation of glucose in frozen solutions at constant reactant concentrations. The solid lines are experimentally determined; the broken lines are calculated from eq 1.

Summary. It is apparent that the mutarotation of glucose in frozen aqueous solutions is well accounted for by the concentration effect. The kinetic treatment of this effect, as summarized by the equation $k_{\text{obsd}} = k_1 + k_2C_h[\text{H}^+]_s/C_s$, correlates the experimental observations for changes in reactant concentration, in total solute concentration, and in temperature. At the concentrations used in this study, any effect of the solid ice is negligible and the reaction in the remaining liquid regions is normal. It may be that at very low concentrations unusual effects due, say, to the presence of the liquid–solid interface may yet appear. However, no clear evidence for anything other than the concentration effect has so far been presented in any case.

The concentration phenomenon, as well as the rate accelerations it gives rise to, are themselves very interesting. Simply by freezing a solution, the solutes may be concentrated into as little as 0.1% (possibly less) of the original volume.[11] Fast reactions in frozen aqueous solutions occur at temperatures as low as −80°.[13] Although liquid regions in apparently solid systems are rarely discussed, and still then often disregarded, it is clear that many observed results are basically due to their existence. Studies of the type reported here have, so far, only been carried out in frozen systems below room temperature, but, in principle, most solids at high temperatures should also contain liquid regions

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(32) The value of C_h at any temperature was taken from the well-established glucose–water phase diagram[19] rather than from the HCl–water system which differs significantly below −5°. Freezing points of glucose–HCl–water solutions, with the ratio of glucose to HCl near that used in the frozen kinetic runs, showed that the glucose–water phase relationship is similar, down to *ca.* −13°, to that for glucose–HCl–water when related in terms of total solute concentration.

(33) Above a total concentration of 1.6 *M* no ice can form at −4°.[32]

(34) In the relation $k_{\text{obsd}} = k_1 + k_2[\text{H}^+]_h$, at high acid concentration only the $k_2[\text{H}^+]_h$ term is important, and as an error in the value of k_2 would shift *all* the calculated points, then the deviations at high acid concentrations (see Figure 2) must be due to an increase in effective catalyst concentration as HCl concentration is increased.

(35) At −4.0° the rate constants observed at high acid concentrations are not as great as would be the case if the relation $k_{\text{obsd}} = k_1 + k_2(h_0)$ were followed (where log $h_0 = -H_0$, the Hammett acidity function at 25°,[37] and the values of H_0 used correspond to the HCl concentrations of the reaction regions in the frozen system).

(36) T. M. Lowry and G. L. Wilson, *Trans. Faraday Soc.*, **24**, 683 (1928).

(37) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 12 (1957).

where reactions may occur. Kinetic treatments of such systems may be similar to that described here for the case of glucose mutarotation in ice.

Experimental Section

Materials. Glucose used was British Drug Houses Analytical Reagent grade α-D-glucose. Other samples used in some runs gave consistent results. Deionized water was used throughout. Hydrochloric acid solutions were made from British Drug Houses concentrated volumetric solutions. Sodium chloride (U. S. P. grade) was dried at 110° before using.

Kinetics. Solutions were made up by dissolving a weighed amount of α-D-glucose in a volumetric flask, then adding the appropriate amount of HCl solution, and making up to the mark. After thorough mixing the solution was divided into *ca.* 15-ml aliquots held in test tubes which were then supercooled to the temperature of the run. Freezing was initiated by dipping the tubes for a few seconds into a Dry Ice–acetone bath. The first sample was withdrawn from the constant temperature bath approximately 10 min after freezing. Analysis of samples having 0.25 *M* glucose or higher was carried out by first diluting the sample immediately after thawing with an NaOH solution containing buffer (Coleman pH 4 buffer tablets). The resulting solution was about pH 4, and the mutarotation near the slowest possible rate. The angle of rotation (± 0.05°) was then measured with a Bellingham and Stanley Model D polarimeter. Samples having less than 0.25 *M* glucose were analyzed by use of a Bendix Type-143A automatic polarimeter equipped with a mercury filter. The 40-mm cell was fitted with a syphon arrangement so that samples could be quickly exchanged in the cell without removing it from the optical unit. Samples could be thawed and analyzed very quickly; the rotation (±0.001°) was taken when the meter needle was at maximum deflection. Mutarotation in the thawed samples continued as the sample was warmed, of course, but thawing sufficiently slowed the reaction to allow the rapid analysis described above. The rotations of the infinity samples were taken after allowing the solution to stand at room temperature *ca.* 24 hr.

Observed rate constant were obtained from the slope of plots of $\log(\alpha_0 - \alpha_\infty)/(\alpha_t - \alpha_\infty)$ against time (where α is observed rotation). The best straight line was drawn visually through the points. The rate constants for spontaneous mutarotation, k_1, were calculated[20] from −30 to +20° by the equation $\log k_1 = -\log 60 + 10.785 - 16{,}900/2.303RT$ (at −4.0°, $k_1 = 1.92 \times 10^{-5}$ sec^{-1}). The rate constants for second-order acid-catalyzed reactions were calculated[20] from $\log k_2 = -\log 60 - \log 0.04 + 12.111 - 18{,}600/2.303RT$ (at −4.0°, $k_2 = 4.25 \times 10^{-4}$ l. mole^{-1} sec^{-1}).

The values of C_h at various temperatures were taken from the equation for the glucose–H_2O system given by Young.[19] These values in weight per cent were corrected to molarity with the relationship given for glucose–water solutions by Jackson.[38] The freezing points of several glucose–HCl solutions were close to the values expected (after correction to the total number of moles present) for solutions containing only glucose.

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(38) R. F. Jackson in "Polarimetry, Saccharimetry and the Sugars," F. J. Bates and Associates, Ed., United States Government Printing Office, Washington, D. C., 1942, p 652.