THE METAL ION CATALYSIS OF THE MUTAROTATION OF α-D-GLUCOSE

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By

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ABSTRACT

THE METAL ION CATALYSIS OF THE MUTAROTATION OF α-D-GLUCOSE

by Irene H. Kochevar

Catalysis of the mutarotation of α-D-glucose by metal ions in acetate buffer was studied. The metal ion concentrations varied from 0.025 M to 0.20 M. Polarimetric readings were converted to rates of reaction. Calcium, manganous, nickelous and cupric ions and the monoacetate complex of cupric ion were all found to enhance the rate of mutarotation. The catalyst concentrations were calculated taking into account the formation of metal acetate complexes. Catalytic coefficients were determined for the metal ions and for the cupric monoacetate complex. The order of catalytic efficiency is: cupric monoacetate complex > cupric > nickelous > manganous > calcium. The results were discussed in terms of both stepwise and concerted mechanisms.

The writer gratefully acknowledges the assistance, criticism, and many suggestions given by Professor J. C. Speck, Jr. throughout the course of this study.

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INTRODUCTION

I. Mutarotation of Glucose

The mutarotation of glucose has been known to be a general acid-base catalyzed reaction for almost forty years. Three mechanisms have been proposed to account for the experimental results.

The first of these mechanisms, the stepwise or bimolecular mechanism, requires prior proton donation by an acid to the bridge oxygen of glucose or prior proton abstraction by a base from the C-1 hydroxyl group of glucose before the rate-determining ring opening step. The experimental expression is a sum of second order terms. The general rate expression for the stepwise mechanism is:

$$\text{rate} = \sum_i k_i [A_i][G] + \sum_j k_j [B_j][G]$$

where: k_i = catalytic constant characteristic of A
k_j = catalytic constant characteristic of B
A_i = any Bronsted acid
B_j = any Bronsted base
G = glucose

The second mechanism is called the concerted or termolecular mechanism. In this case proton donation to the bridge oxygen, proton abstraction from the C-1 hydroxyl and ring opening occur simultaneously. The rate expression, as shown below, contains all third order terms.

$$\text{rate} = \left\{\sum_i k_i [A_i]\right\} \left\{\sum_j k_j [B_j]\right\} [G]$$

The notation is the same as the first rate expression that appears on page 1, and it is assumed that the effectiveness of an acid does not depend on the base it acts in concert with.

The development of the first two mechanisms can be followed historically. Brönsted and Guggenheim in 1927 were the first to propose that mutarotation was general acid-base catalyzed.[1] Their experimental expression contained only second order terms. Several years later Pedersen reported that if a concerted mechanism was assumed, discrepancies appeared in the relative catalytic power of various pairs of acid and bases in the experimental expression. He concluded that only the stepwise mechanism was important in aqueous solution.[2]

Swain, in 1950, showed that Pedersen's interpretation was invalid because some of the terms were actually due to a combination of two indistinguishable acid-base pairs.[3] Swain factored the rate expression for the concerted mechanism, inserted experimental values, multiplied, and found that the coefficients for the cross terms agreed well with the coefficients in the original Brönsted expression. In addition, Swain's expression contained a small catalytic term due to the combination of acetic acid and acetate ion. Swain concluded that all catalysis of the mutarotation of glucose in acetate buffer was termolecular with water serving as an acid or a base.

Soon afterwards Hill and Thumm carried out mutarotation experiments with glucose in methanol-water solvent with acetate ion as a catalyst.[4] They varied the concentration of water and looked for third order kinetics; but found that their data indicated second order kinetics.

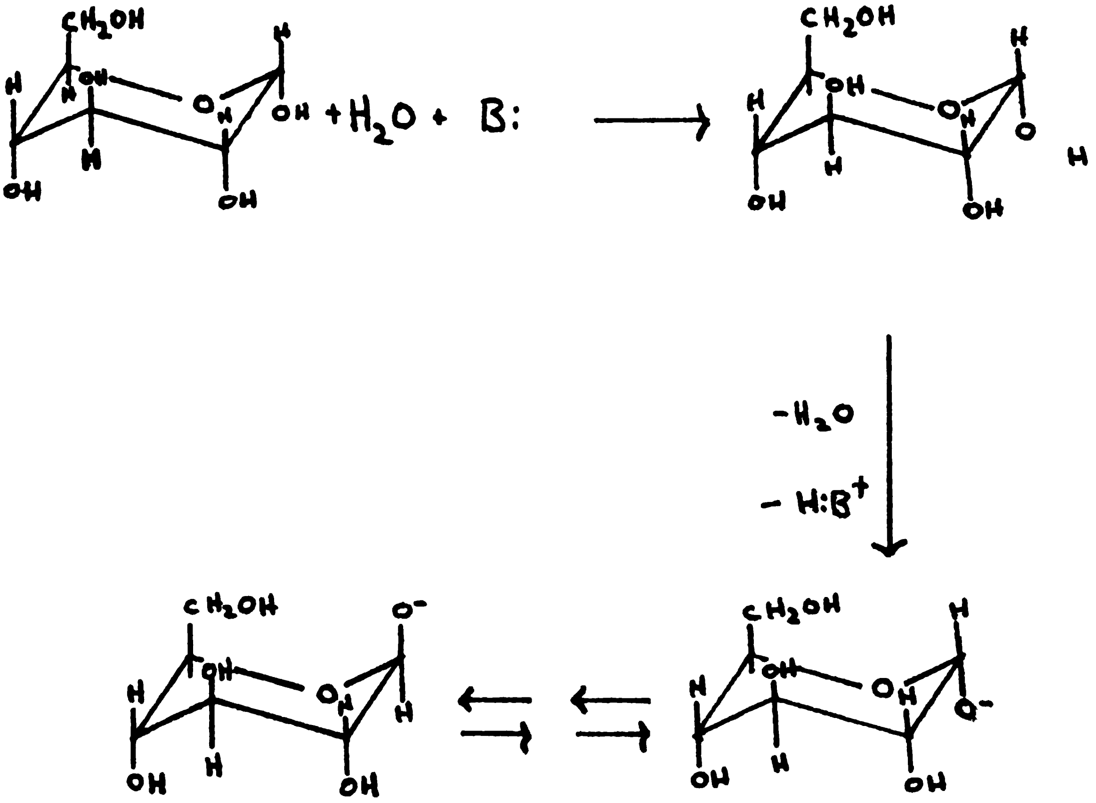
Other arguments against the concerted mechanism in general acid-base catalyzed reactions in aqueous medium were presented by Bell in 1953.[5] He showed that neither the presence of a third order term in a catalytic expression nor its size was sufficient proof that all other terms in the expression were third order. Bell's elegant arguments were accepted as convincing evidence that the reaction in aqueous solution is catalyzed by a stepwise mechanism.

The case for a concerted mechanism has been revived recently by Eigen.[6,7] Using rapid reaction techniques he has experimentally shown that the Brönsted catalysis law, $k_A = G_A K_A^{\alpha}$, is valid only over a limited range of dissociation constants, K_A. The constant α approaches zero as the ΔpK_A is increased for a number of reactions which follow the Brönsted catalysis law in the time range accessable to classical kinetic methods. At $\alpha = 0$ the rate of these reactions is diffusion controlled. Proton switching is no longer the rate determining step and the rate is independent of K_A.

The mutarotation of glucose appeared to be an exception since α remained constant over a considerably larger ΔpK_A

range. To account for this behavior, Eigen proposed a concerted mechanism. The rate of this three centered reaction would be so far below the diffusion controlled rate that even large K_A differences between substrate and catalyst could not make α go to zero.

The third mechanism was proposed by Schmid in 1963.[8] From values for $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ for the formation of the activated complex, he formulated a mechanism which includes a water molecule between the glucose molecule and the catalytic species. He proposed that during base catalysis a water molecule in the solvation shell of the base accepts the C-1 hydroxyl proton from glucose. Simultaneously, the base accepts a proton from the water molecule. This proton switching requires exact positioning of the molecules. Schmid proposed that the process of aligning the molecules is the rate determing step. The mechanism of base catalysis is shown on page 5.



Mutarotation experiments were carried out in aqueous solutions containing various acids and bases as catalysts. Schmid pointed out that the values calculated from the data for $\Delta H^{\ddagger}$ are nearly identical while the values for $\Delta S^{\ddagger}$ vary. From the similarity of the $\Delta H^{\ddagger}$ values he concluded that the same molecule is involved in the proton transfer to or from glucose regardless of the identity of the catalyst. He correlated the values of $\Delta S^{\ddagger}$ with the degree of solvation

of the catalyst. The contribution made to the rate constant ~~made~~ by $\Delta S^{\ddagger}$ in the equation....

$$k = \frac{kT}{h} \exp\left[\frac{-\Delta H^{\ddagger}}{RT}\right] \exp\left[\frac{\Delta S^{\ddagger}}{k}\right]$$

far outweighs that made by $\Delta H^{\ddagger}$.

Schmid's data and mechanism are not consistant with classical general acid-base catalysis theory. First of all, the rate constant in Schmid's treatment is related to the degree of solvation of the catalyst rather than to the acid or base strength as required by the Brönsted catalysis law. Secondly, because the rate determining step postulated for nondiffusion controlled general acid-base catalysis is a proton transfer, the rate constant should vary with $\Delta H^{\ddagger}$ rather than $\Delta S^{\ddagger}$. Finally, Schmid's data gives rates at only two or three temperatures for each catalyst. The largest range is from 5° C. to 25° C. Since the graph of the natural logarithm versus the reciprocal of the absolute temperature required to obtain $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values would have a maximum of three points, the conclusions from this type of data are questionable.

The available evidence does not permit a judgment upon the ~~relative~~ validity of the stepwise and concerted mechanisms. Both mechanisms will be discussed in connection with the present study. The mechanism proposed by Schmid will

not be discussed further because it does not appear to result from reliable experimental findings.

II. Metal Ion Catalysis

A general introduction to metal ion catalysis will be presented first as background for the discussion of metal ion catalysis of the mutarotation of glucose.

The effects of metal ions in aqueous solution upon the rate of a reaction can be grouped as follows:

A. primary salt effects
B. secondary salt effects
C. changes in the catalyst
D. changes in the ground state of the reactants
E. changes in the transition state

This order will be followed in discussing metal ion catalysis.

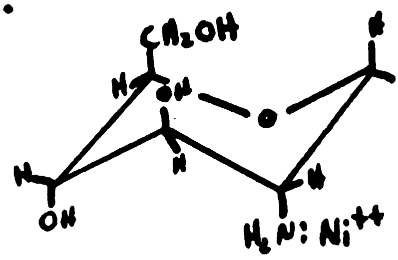
A primary salt effect changes the ionic atmosphere of the reacting species and results in a shift in the equilibrium between reactants and the activated complex. By knowing the charge on each reactant involved in the activated complex, the direction of change produced by the primary salt effect can be predicted.

A secondary salt effect does not change the catalytic constant. Rather, it affects the rate by changing the amount of catalyst present. An increase in the ionic strength changes the activity coefficients thereby disturbing the equilibrium of weekly dissociated species. In order to return to equilibrium the concentrations of the ions and

the undissociated acid must change.

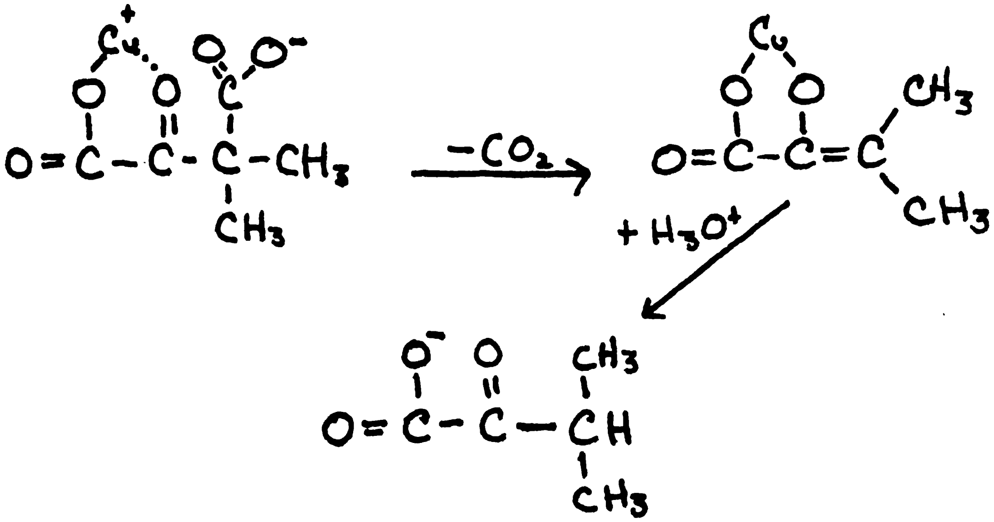
A metal ion can change the rate by acting upon (a) the catalyst, (b) the ground state of the reactants, or (c) the transition state.

(a) The catalyst: If the metal ion merely changes the concentration of the catalyst by associating with a certain number of the catalytic molecules, the rate of the reaction is changed. For example, in the mutarotation of glucosamine the C-2 amino group catalyzes the reaction.[9] Ni^{++} and Cd^{++} slow down the reaction by associating with the free electron pair of the amino group nitrogen thereby destroying its catalytic power.



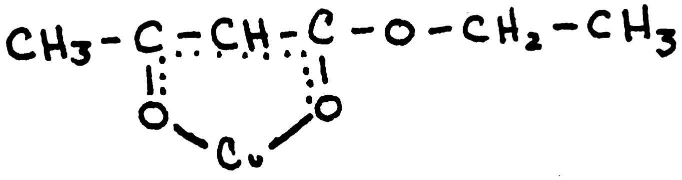
In other cases the metal ion-catalyst complex is a better catalyst than the uncomplexed catalyst.

(b) The ground state of the reactant: Association between metal ion and the ground state of a reactant can increase the rate constant by decreasing the activation energy. An example is the decarboxylation of dimethyloxaloacetic acid.[11] The metal ion associates with two points on the molecule and polarizes the carbonyl bond, thus facilitating the electron shift.

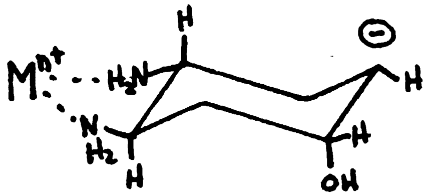


Metal ions could conceivably decrease the rate constant by forming a stable chelate with the ground state in which the electron density is unfavorable for reaction. In effect, formation of the chelate would increase the activation energy.

(c) Transition state: Acceleration due to metal ion association with the transition state has been postulated for several reactions including the bromination of ethyl acetoacetate.[12] The metal ion begins to co-ordinate with the oxygens during the transition state. As the drawing below shows the transition state resembles the final product. This metal-organic complex is more stable than the free transition state; therefore, a lowered activation energy is required and the rate constant is increased.



A reaction which requires a smaller increase in entropy during the formation of the transition state will have a larger rate constant. As shown below, stabilization of a ring into a rigid conformation which has the desired stereochemistry for a 1,2-elimination of water fits this description.



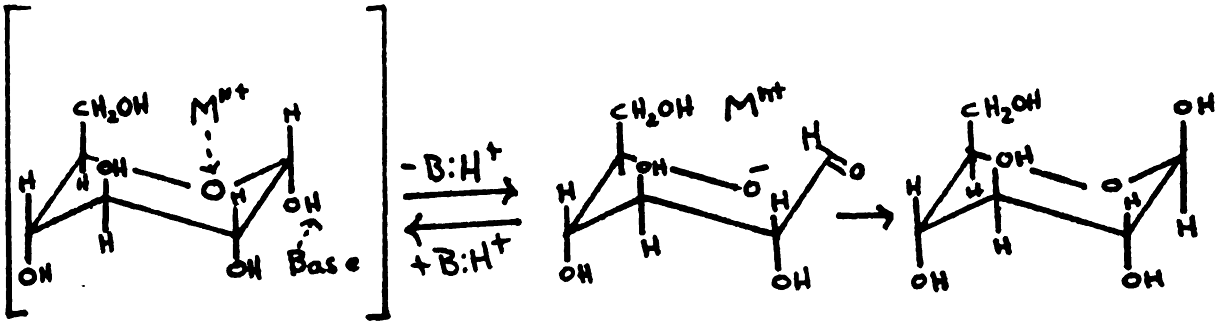
This brief outline attempted to place a certain order upon the many possible ways in which a metal ion could influence the rate of a reaction.

In the particular case of mutarotation of glucose very little investigation of metal ion effects has been carried out. Brönsted and Guggenheim demonstrated the absence of primary salt effects up to an ionic strength of 0.20.[1] Secondary salt effects are expected if a week acid or weak base is used as a catalyst.

The first demonstration of metal ion catalysis was reported in the original article by Brönsted and Guggenheim on the mutarotation of glucose.[1] While studying catalysis by various bases, they found that the basic cations $Co(NH_3)_5OH^{++}$ and $Cr(OH_2)_5OH^{++}$ behaved as general bases in the catalysis of mutarotation. The neutral cation $Co(NH_3)_6^{+++}$ did not catalyze the reaction.

In 1950 Delaney, Kleinberg and Argersinger observed an increase in the rate of mutarotation of α-glucose when $FeCl_3$ was added to the reaction mixture.[13] The authors attributed catalysis to preferential complex formation between β-glucose and Fe^{+++} although the equilibrium rotation was not affected by the presence of $FeCl_3$. Other evidence indicated that complex formation does occur. The rate data could be interpreted as a case of general base catalysis by Fe^{+++}.

Metal ion catalysis was also reported by Neely in 1952.[14] This researcher found increases in the rate constant in the presence of Mg^{++}, and Cu^{++} in unbuffered solutions. Fe^{+++}, Li^+, Mg^{++}, and Be^+ were shown to increase the rate in perchloric acid solution and in acetate buffer, Li^+ and Mg^{++} acted as catalysts. The resultant change in rate in acetate buffer was due to two factors: (1) association between acetate ion and the metal ion which produced a change in catalyst concentration and (2) specific acceleration by the metal ion. At low metal ion concentrations the first factor outweighed the second, resulting in a net decrease in the rate. No evidence was found for the formation of a complex between Li^+ or Mg^{++} and the sugar. Specific acceleration by the metal ion was attributed to stabilization of the transition state by the metal acting as an electrophilic agent. The proposed mechanism is shown on page 12.



The effects of alkali metal and alkaline earth metal ions were investigated by Nicolle and Weisbuch in 1957.[15] These researchers used high concentrations of salts, 1 to 4 M, and unbuffered solutions. They found retardation of the rate of mutarotation by the alkali metal salts and no effect or slight increase in rate by the alkaline earth salts. RbCl had the largest decreasing effect and MgCl had the largest enhancing effect of the salts employed.

At about the same time Grandchamp-Chaudin investigated the effects of 2 M solutions of NaCl, KCl, NH_4Cl and LiCl upon the rate of mutarotation and the equilibrium rotation glucose.[16] Her results correlated well with those of Nicolle and Weishbuch.

Because Nicolle and Weishbuch and Grandchamp-Chaudin used high salt concentrations in unbuffered solutions the reliability of their date makes any interpretation questionable.

In summary, one can see that only indications of the possible role of metal ions in the catalysis of the mutarotation of glucose have been demonstrated. The purpose of this study is to further delineate these effects.

EXPERIMENTAL

Reagents and Solutions: α-D-glucose was obtained from the National Bureau of Standards, Lot numbers 5052 and 5250. The cupric nitrate, nickelous sulfate, manganous sulfate, and calcium nitrate were reagent grade chemicals. The glacial acetic acid from the J. T. Baker Company was 99.9% pure. Stock metal ion solutions were prepared by weighing the anhydrous salt or the hydrate on an analytical balance and then diluting to the correct volume with glass distilled water in a volumetric flask. The correct concentrations were later determined by standard quantitative procedures. The 0.1 M 1:1 stock acetate buffer was prepared from standard sodium hydroxide and glacial acetic acid. The sample solutions were prepared by pipetting the required volume of stock metal ion solution and 10 ml. stock buffer solution into a 50ml. volumetric flask and diluting with glass distilled water.

Equipment: Rotation measurements were taken at 5780 Å with a Zeiss Photoelectric Polarimeter using a mercury lamp. The one decimeter cell was water-jacketed and kept at 18.9°±0.1° C. with a circulating constant terperature water bath.

Procedure: For each run, 1.125 g. (0.00625 mole) of α-D-glucose was washed into a 25 ml. volumetric flask with the appropriate buffer-metal ion solution which had been previously prepared in a 50 ml. volumetric flask and equilibrated

to 19° C. All experiments were run at 18.9° C. The glucose concentration was 0.25 M. The acetic acid and the acetate ion concentrations were both 0.02 M. Elapsed time was measured from the initial mixing of glucose and solvent. The first reading was usually at 250 seconds. Subsequent measurements were made at 250-second intervals.

Data: The equilibrium rotation was 2.465°. Initial rotation was calculated to be 5.049°. The rate was determined by plotting values of log $\frac{\alpha_e - \alpha_o}{\alpha_e - \alpha}$ vs. time (α_e = equilibrium rotation, α_o = initial rotation, and α = rotation at time, t.) The unit of time was the second.

A typical set of data is given in Table I and the corresponding graph in Figure I. Experiments were replicated either once or twice. The rate at each concentration was taken to be the average of the observed values. The observed values were never outside the experimental error, ±2%.

Figure II shows the observed rates versus the concentration of metal ion in the sample solution. The data for this graph is given in Table II.

The metal ions complexed with the acetate ion; therefore, the actual concentrations of metal ion, metal acetate complex, acetate ion and acetic acid were obtained by calculation. The initial metal ion concentration, the dissociation constant for acetic acid, and the association constants for the metal acetate complexes were known.

TABLE I TYPICAL DATA

$[Cu_T^{++}] = 0.175$, $[HOAc_T] = 0.0387$, $[G] = 0.25$, T = 18.9° C

Time, Seconds	Rotation, Degrees	$\log \frac{\alpha_e - \alpha_o}{\alpha_e - \alpha}$
250	4.710	0.0611
500	4.385	0.1290
750	4.100	0.1988
1000	3.860	0.2677
1250	3.660	0.3349
1500	3.480	0.4058
1750	3.335	0.4728
2000	3.200	0.5460
2250	3.100	0.6151
2500	3.005	0.6799
2750	2.920	0.7549
3000	2.855	0.8212

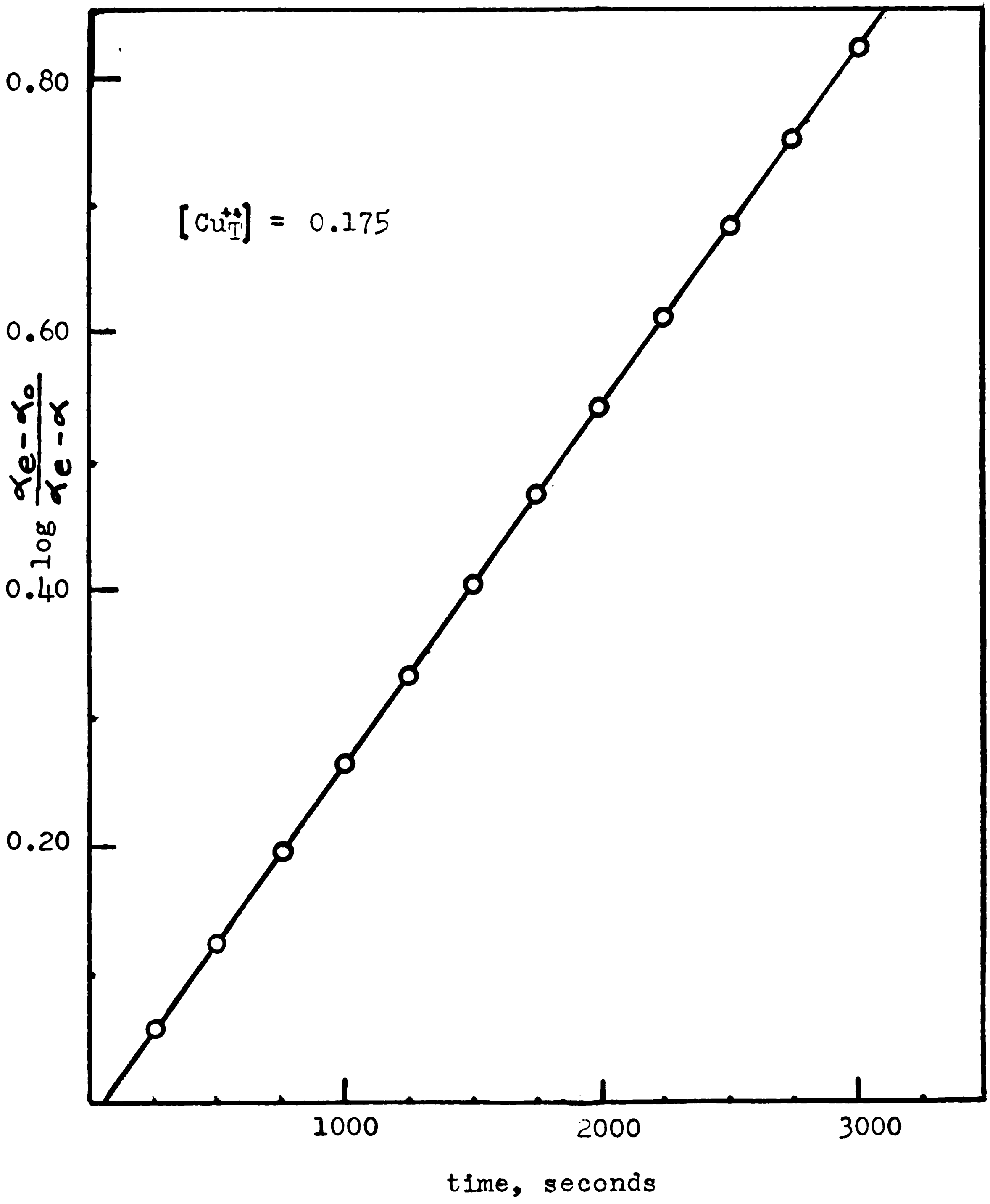


FIGURE I TYPICAL DATA

TABLE II UNCORRECTED DATA ON THE EFFECTS OF METAL IONS

Metal ion	Molarity	Observed rate x 10^4
Ca^{++}*	0.0488	1.08
	0.0732	1.09
	0.0976	1.11
	0.1167	1.11
	0.1404	1.12
Cu^{++}**	0.0258	1.61
	0.0516	1.84
	0.0774	2.07
	0.1032	2.24
	0.1290	2.45
	0.1548	2.61
	0.1806	2.78
Mn^{++}*	0.0242	1.09
	0.0484	1.14
	0.0726	1.17
	0.0968	1.20
	0.1210	1.23
	0.1452	1.25
	0.1694	1.29
Ni^{++}**	0.0240	1.08
	0.0480	1.12
	0.0720	1.14
	0.0960	1.21

*Rates for this ion are the average of three values.
**Rates for this ion are the average of two values.

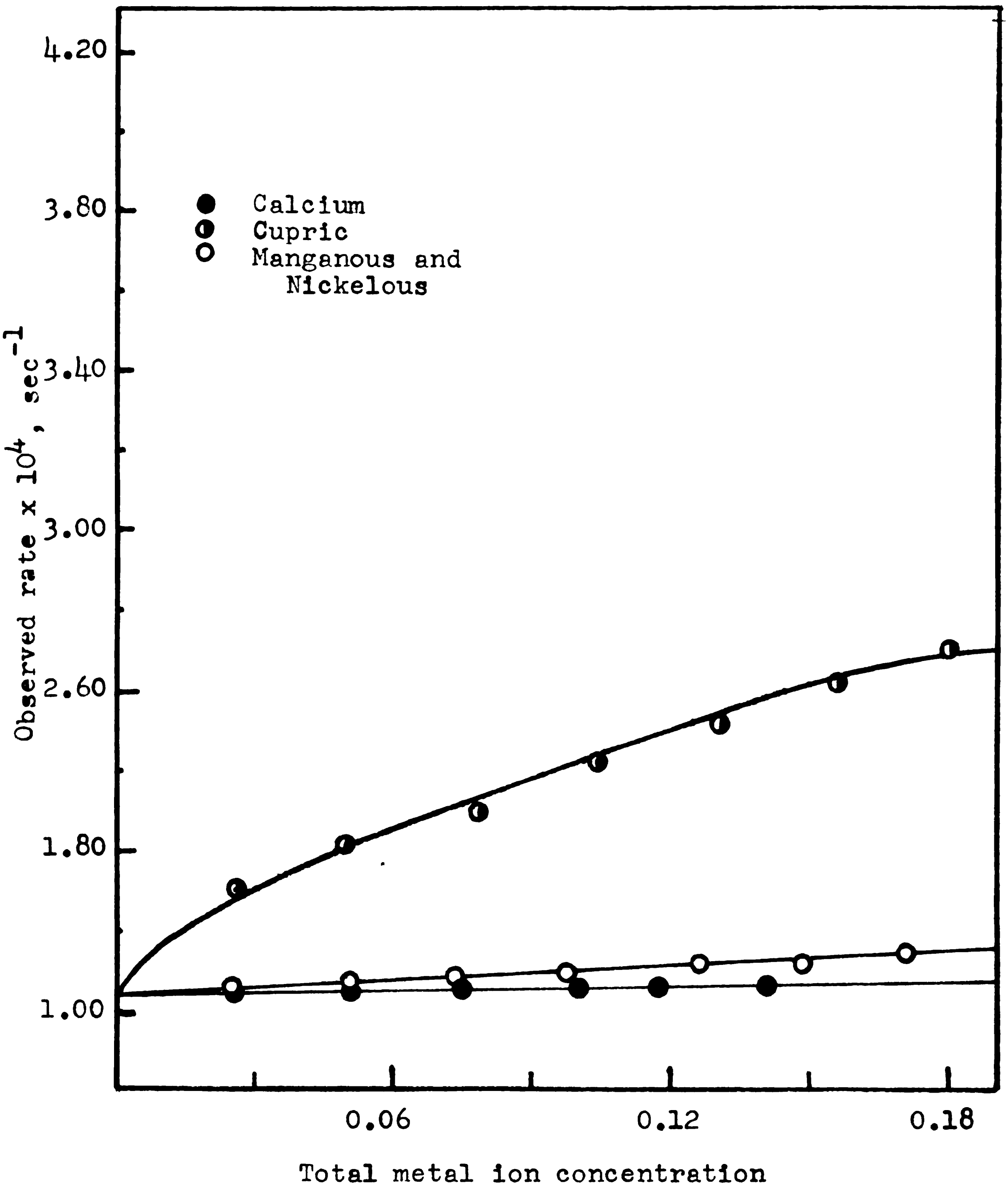


FIGURE II UNCORRECTED DATA ON THE EFFECTS OF METAL IONS

Activity of hydrogen ion as measured by a Beckman pH meter was converted to hydrogen ion concentration.[17] The change in the dissociation constant for acetic acid due to the increase in ionic strength was corrected for.[18]

The association constants used for the metal acetate complexes were:[19]

	log K_1	log K_2
Calcium	0.53	
Cupric	1.67	0.98
Manganous	0.61	
Nickelous	0.67	

$$K_1 = \frac{[MOAc^+]}{[OAc^-][M^{++}]}$$

$$K_2 = \frac{[M(OAc)_2]}{[OAc^-][MOAc^+]}$$

In the following calculations it was assumed that only a monoacetate complex was formed in sufficient quantities to be catalytically important.

$[M_F^{++}]$ = concentration of metal ion free in solution

$[M_T^{++}]$ = total concentration of metal ion added

$[HOAc_T]$ = total concentration of acetic acid and acetate ion added

$[HOAc_F]$ = final concentration of acetic acid

$$K_1 = \frac{[MOAc^+]}{[M^{++}][OAc^-]} \qquad \text{Equation 1}$$

$$K_A = \frac{[H^+]\,[OAc^-]}{[HOAc]} \qquad \text{Equation 2}$$

$$[M_T^{++}] = [M_F^{++}] + [MOAc^+]$$

$$\begin{aligned}[HOAc_T] = {} & [HOAc_F] + [OAc^-] + [MOAc^+] \\ & [HOAc_F] + [OAc^-] + [M_T^{++}] - [M_F^{++}]\end{aligned}$$

$$[HOAc_F] = [HOAc_T] - [OAc^-] - [M_T^{++}] + [M_F^{++}]$$

Substituting for $[MOAc^+]$ into Equation 1 yields:

$$K_1 = \frac{[M_T^{++}] - [M_F^{++}]}{[M_F^{++}]\ [OAc^-]}$$

Rearranging this expression yields:

$$[OAc^-] = \frac{[M_T^{++}] - [M_F^{++}]}{[M_F^{++}]\ K_1}$$

Substituting this value for $[OAc^-]$ and the expression for $[HOAc_F]$ into Equation 2 yields:

$$K_A = \frac{[H^+]\ \dfrac{[M_T^{++}] - [M_F^{++}]}{[M_F^{++}]\ K_1}}{[HOAc_T] - \dfrac{[M_T^{++}] - [M_F^{++}]}{[M_F^{++}]\ K_1} - [M_T^{++}] + [M_F^{++}]}$$

The value of $[M_F^{++}]$ was determined by approximation after substituting values for K_A, K_1, $[M_T^{++}]$, $[HOAc_T]$, and $[H^+]$. The values of $[MOAc^+]$, $[OAc^-]$, and $[HOAc_F]$ were calculated from the above equations. The results of these calculations are tabulated in Table III.

Two possibilities for metal ion catalysis exist: (1) the free (uncomplexed) metal ion, $[M_F^{++}]$, is the only catalytic species or (2) both the metal ion and its acetate complex are catalytically active. If only the uncomplexed metal ion catalyzes the reaction, the experimental rate expression is:

$$\begin{aligned} \text{rate} = {} & 8.8 \times 10^{-5} + 4.4 \times 10^{-4}\ [OAc^-] + 1 \times 10^{2}\ [OH^-] \\ & 4 \times 10^{-5}\ [HOAc_F] + 2.4 \times 10^{-3}\ [H_3O^+] + k_M\ [M_F^{++}] \end{aligned}$$

Table III Caloulated Molarities of Calalytic Species in Reaction Mixtures

Metal ion	M_F	MOAc	OAc	HOAc	$H_3O \times 10^5$
Calcium	0.045	0.004	0.018	0.023	3.10
	0.069	0.005	0.016	0.024	3.74
	0.091	0.007	0.016	0.021	3.96
	0.110	0.012	0.015	0.018	4.29
	0.133	0.013	0.014	0.018	4.24
Cupric	0.018	0.008	0.006	0.012	7.95
	0.039	0.013	0.005	0.021	13.7
	0.064	0.014	0.004	0.021	17.4
	0.089	0.014	0.003	0.022	22.2
	0.114	0.015	0.003	0.021	26.8
	0.140	0.015	0.002	0.021	33.4
	0.165	0.016	0.002	0.020	38.6
Manganous	0.022	0.002	0.018	0.020	3.54
	0.044	0.004	0.016	0.022	4.60
	0.069	0.004	0.013	0.026	6.23
	0.090	0.007	0.010	0.020	6.56
	0.111	0.013	0.008	0.016	6.85
	0.134	0.013	0.007	0.017	8.30
	0.157	0.013	0.006	0.018	10.27
Nickelous	0.023	0.001	0.018	0.021	3.41
	0.045	0.003	0.014	0.022	3.97
	0.068	0.004	0.013	0.022	4.36
	0.091	0.005	0.013	0.022	4.58

This expression is made up of the original Brönsted and Guggenheim expression with a term for metal ion catalysis added. The difference between the observed rate and the rate calculated from the terms of the original Brönsted-Guggenheim expression was attributed to participation of the uncomplexed metal ion. The rate due to metal ion catalysis is plotted versus the uncomplexed metal ion concentration in Figure III. The slopes of the lines were taken to be the catalytic constants for Ca_F^{++}, Mn_F^{++}, and Ni_F^{++}. The catalytic constants are given in Table IV along with the data for Figure III. No value for Cu_F^{++} is given because of the nonlinearity of the plot.

If both the metal ion and its acetate complex are catalytically active the following expression is appropriate.

$$\text{rate} \quad 8.8 \times 10^{-5} + 4.4 \times 10^{-4} [OAc^-] + 1 \times 10^{2} [OH^-] + 4 \times 10^{-5} [HOAc_F] + 2.4 \times 10^{-3} [H_3O^+] + k_M [M_F^{++}] + k_{MOAc^+} [MOAc^+]$$

The combined rate due to M_F^{++} plus $MOAc^+$ is plotted versus the total metal ion concentration, $[M_T^{++}]$, in Figure IV. The lines for Cu_T^{++} are curved indicating that at least two catalytically active species exist. The catalytic coefficients for the two species in Cu^{++} catalysis were determined by substitution of the appropriate rates and concentrations into the following expression at two cupric ion concentrations:

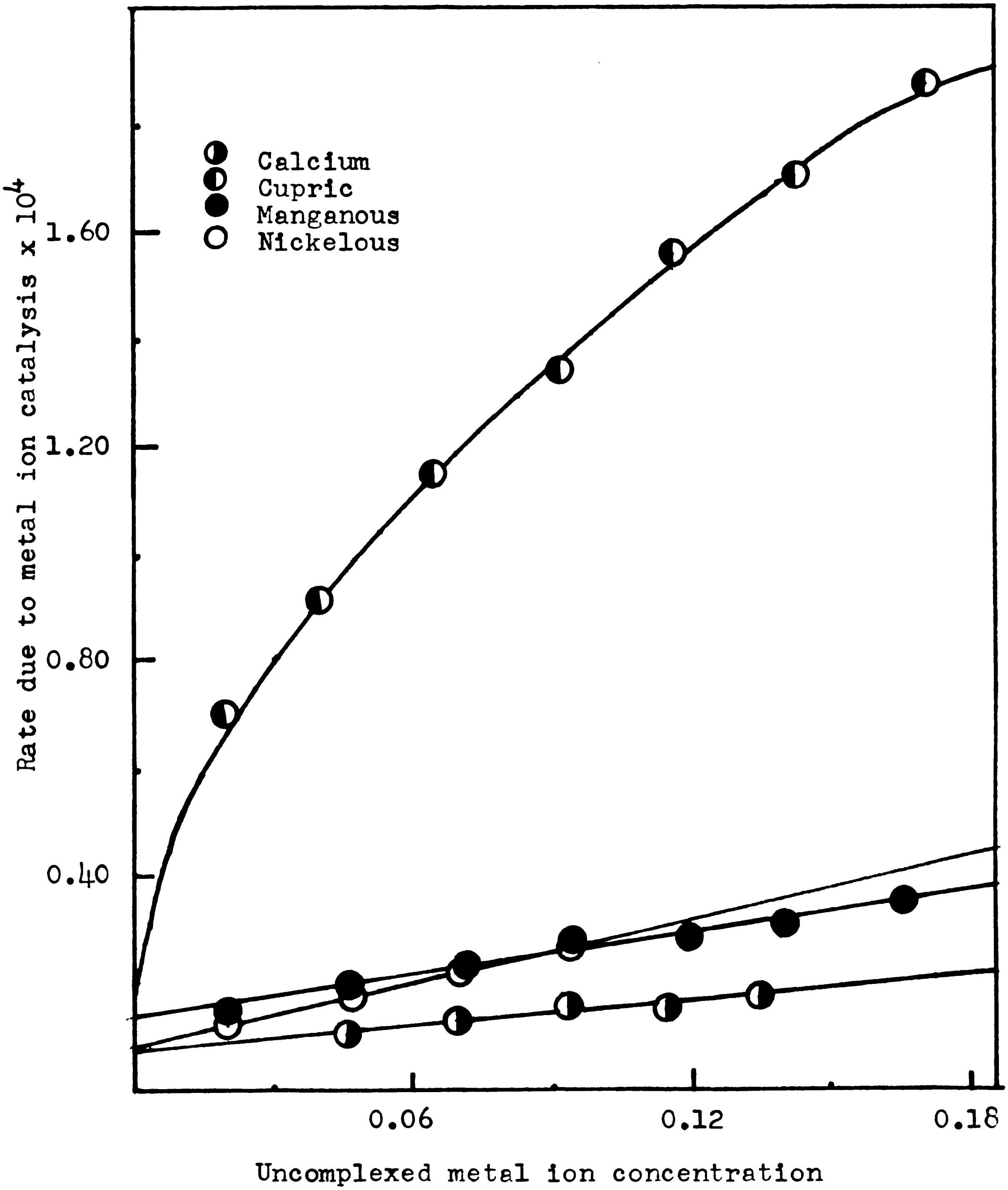


FIGURE III EFFECTS OF UNCOMPLEXED METAL IONS

TABLE IV EFFECTS OF UNCOMPLEXED METAL IONS

Metal ion	Molarity	Rate x 10^4 due to M_F^{++}	k_M x 10^4
Calcium	0.046	0.11	0.59
	0.070	0.13	
	0.093	0.15	
	0.117	0.16	
	0.140	0.17	
Cupric	0.019	0.70	*
	0.043	0.93	
	0.067	1.16	
	0.092	1.33	
	0.117	1.54	
	0.143	1.70	
	0.169	1.87	
Manganous	0.023	0.12	1.47
	0.046	0.18	
	0.070	0.22	
	0.093	0.27	
	0.120	0.31	
	0.143	0.33	
	0.165	0.37	
Nickelous	0.023	0.11	2.32
	0.046	0.17	
	0.070	0.19	
	0.093	0.26	

*No catalytic constant could be determined because of the non-linearity of the curve.

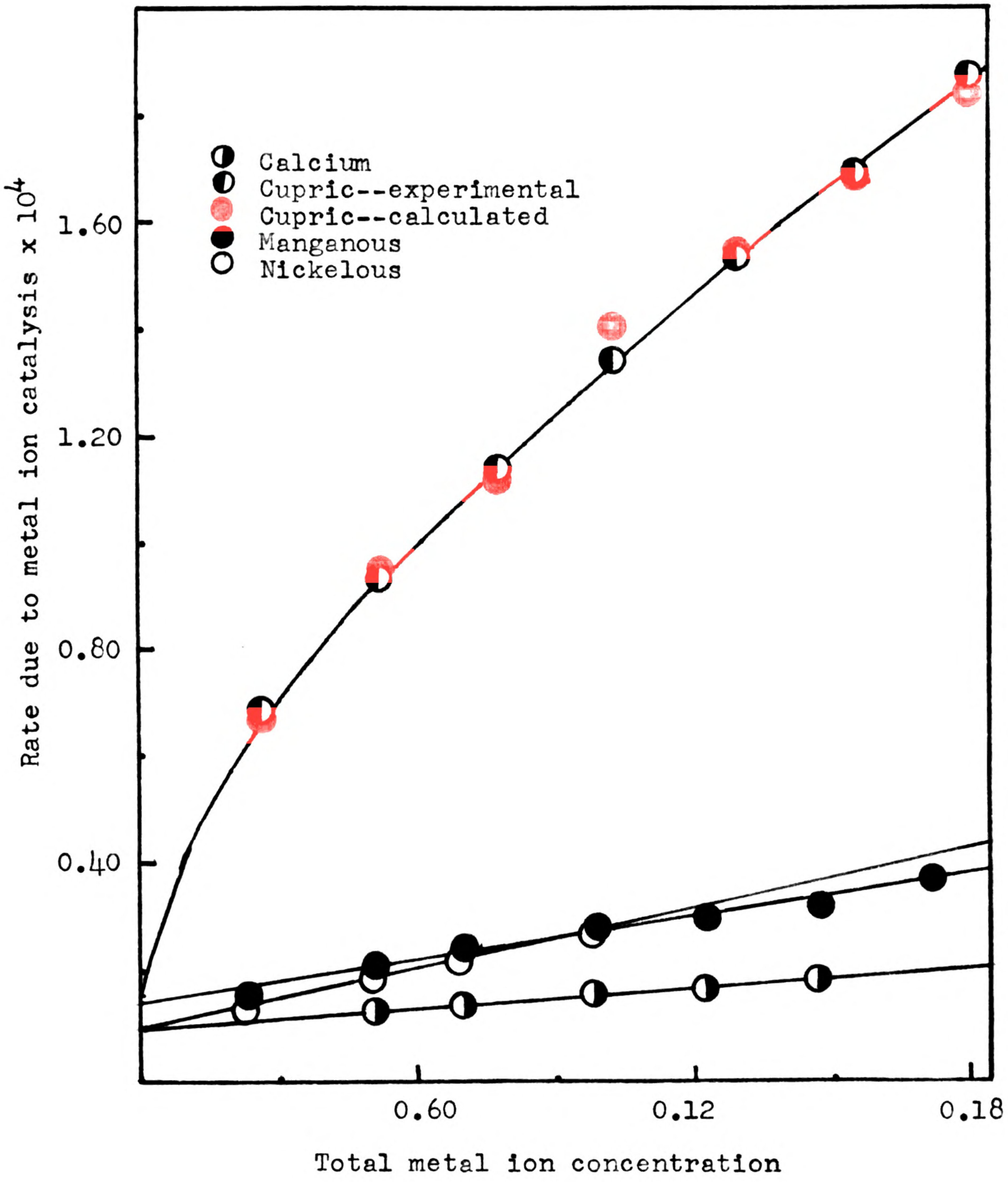


FIGURE IV EFFECTS OF UNCOMPLEXED PLUS COMPLEXED METAL IONS

velocity due to metal ion $= k_M [Cu_F^{++}] + K_{MOAc} [CuOAc^+]$

The two equations were then solved simultaneously for k_M and k_{MOAc}. The rates calaulated using these catalytic coefficients are compared with the experimental values in Figure IV. Table V presents the data for Figure IV and a summary of the catalytic coefficients.

TABLE V EFFECTS OF UNCOMPLEXED PLUS COMPLEXED METAL IONS

Metal ion	Molarity of $M_F^{++} + MOAc^+$	Rate x 10^4 due to $M_F^{++} + MOAc^+$	Calc'd rates	k_M x 10^4	k_{MOAc} x 10^4
Ca^{++}	0.0488	0.113		0.63	
	0.0732	0.131			
	0.0976	0.156			
	0.1220	0.160			
	0.1464	0.173			
Cu^{++}	0.0258	0.69	0.58	5.56	78.4
	0.0516	0.93	0.93		
	0.0774	1.15	1.12		
	0.1032	1.34	1.40		
	0.1290	1.55	1.56		
	0.1548	1.70	1.70		
	0.1806	1.89	1.89		
Mn^{++}	0.0242	0.14		1.40	
	0.0484	0.19			
	0.0726	0.24			
	0.0968	0.27			
	0.1240	0.30			
	0.1470	0.32			
	0.1700	0.36			
Ni^{++}	0.0240	0.12		2.09	
	0.0480	0.17			
	0.0720	0.22			
	0.0960	0.27			

DISCUSSION

Mutarotation of D-glucose in acetate buffer has been studied polarimetrically in the presence of metal ions. An increase in the rate of mutarotation resulted from the addition of $Ca(NO_3)_2$, $Cu(NO_3)_2$, $MnSO_4$ or $NiSO_4$ to the reaction mixture. The total increases cannot be ascribed to primary or secondary salt effects for two reasons. First of all, variation in rate occured between solutions containing different metal ions at identical ionic strengths. Secondly, experiments using salts such as $NaNO_3$ and Na_2SO_4 indicated that only small variations in rate occur with increasing ionic strength. At the maximum ionic strength used in the metal ion catalysis experiments, the variation due to salt effects was less than 4%. For these reasons metal ions appear to be specific catalysts for the mutarotation reaction.

Before an attempt is made to elucidate the mechanism of this catalysis, the catalytic species must be identified. Each of the metal ions forms a complex with acetate ion and the concentrations of the metal ion-acetate ion complexes in the reaction mixtures have been calculated.

The appearance of the plot of total metal ion concentration, $[M_T^{++}]$, versus rate due to metal ion catalysis depends upon which species, the free metal ion (M_F^{++}) or the metal ion-acetate ion complex ($MOAc^+$), is catalytically active.

Three possibilities exist: (1) If both M_F^{++} and $MOAc^+$ are catalysts and have the same catalytic coefficient, the curve would be linear. (2) If only the free metal ion catalyzes the reaction, the slope of the line would decrease at high concentrations due to removal of the metal ion by formation of the acetate complex. (3) If both species are catalysts but have different catalytic coefficients, the shape of the curve would depend on the relative catalytic efficiencies of M_F^{++} and $MOAc^+$.

Ca^{++}, Mn^{++}, and Ni^{++} show a linear relationship between rate and concentration of total metal in Figure IV, indicating that $MOAc^+$ and M_F^{++} have the same catalytic coefficient. However, a linear relationship also exists between M_F^{++} and the rate as shown in Figure III, indicating that only M_F^{++} is catalytically active. Apparently a slight curvature exists in one of these sets of lines which cannot be detected due to the small slopes of the lines. Because of these contradictory pieces of evidence no conclusion can be drawn concerning the identity of the catalytic species for these three metal ions. Catalytic coefficients for Ca_F^{++}, Mn_F^{++}, and Ni_F^{++} were calulated from the slopes of the lines in Figure III. These values must be considered approximations since it could not be determined whether the acetate complex of these metals had any catalytic power.

The plot of Cu_T^{++} versus rate in Figure IV shows a definite decrease in slope with increasing concentrations. The

same is true in Figure III for the concentration of Cu_F^{++} versus rate. Two possible explanations are: (1) more than one species is responsible for the change in rate, or (2) only Cu_F^{++} is active but the reaction is not first order with respect to Cu_F^{++}.

The first explanation requires that the two catalytic species have different catalytic coefficients. If they had had the same catalytic coefficient, the plot of Cu_T^{++} versus rate would have been linear. Catalytic coefficients for Cu_F^{++} and CuOAc have been calculated as shown in the experimental section. The catalytic coefficient for $CuOAc^+$ is larger then that for Cu_F^{++}. The rates determined from these rate constants fit the date very well as shown in Figure IV, indicating that this explanation is consistant with the experimental results.

If Cu_F^{++} is considered the only catalyst as postulated by the second reason above, the rate must be less than first order with respect to Cu_F^{++} because of the shape of the curve in Figure III. A reaction order greater than unity would have led to a curve with increasing slope at high concentrations, and with a reaction order of unity the line would have been straight. A reaction order between zero and unity is also consistant with the date. Only the first explanation was employed in calculating catalytic coefficients.

Three conclusions concerning the identity of the catalytic species can be drawn. First of all, the identity of the

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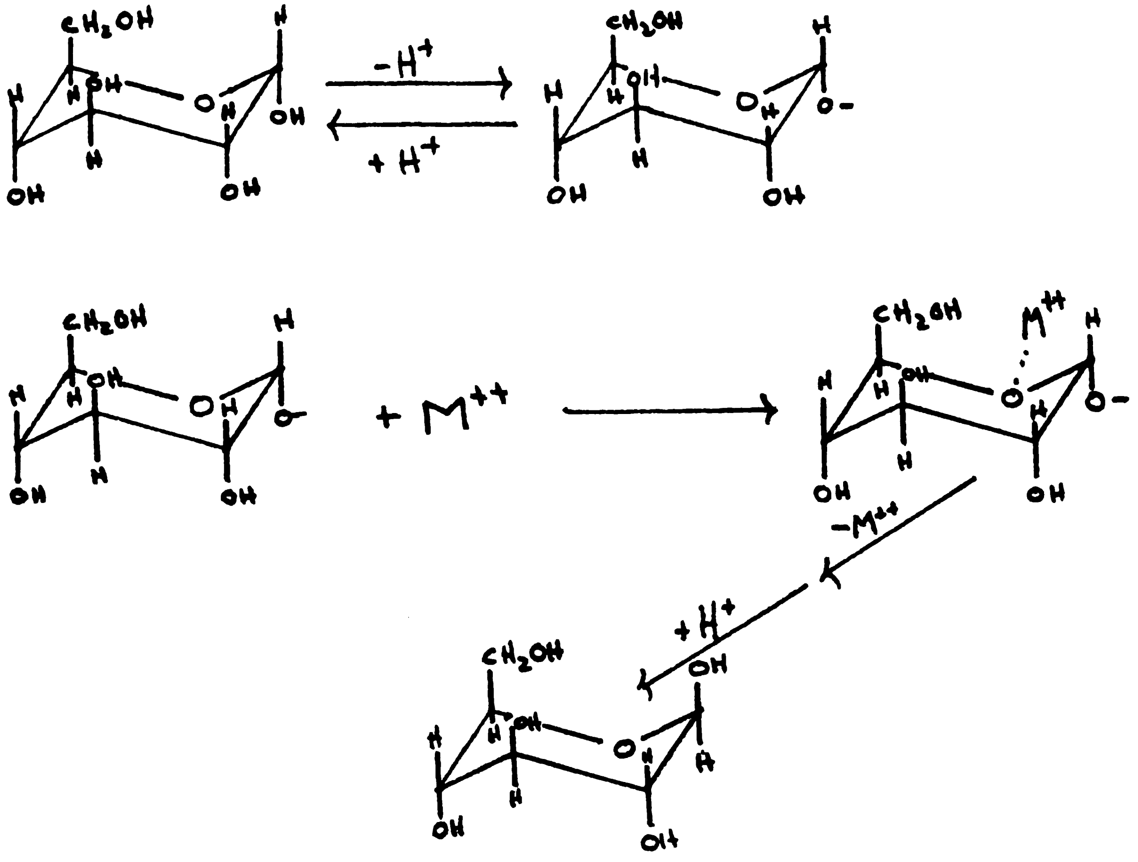
Three conclusions concerning the identity of the catalytic species can be drawn. First of all, the identity of the

catalytic species in solutions of Mn^{++}, Ca^{++} and Ni^{++} in acetate buffer cannot be decided. Secondly, both Cu$_F^{++}$ and CuOAc$^+$ are catalytically active. Thirdly, CuOAc$^+$ is a much better catalyst than Cu$_F^{++}$.

The mechanism of metal ion catalysis will be discussed in terms of both the stepwise and the concerted mechanisms. The stepwise mechanism will be shown first.

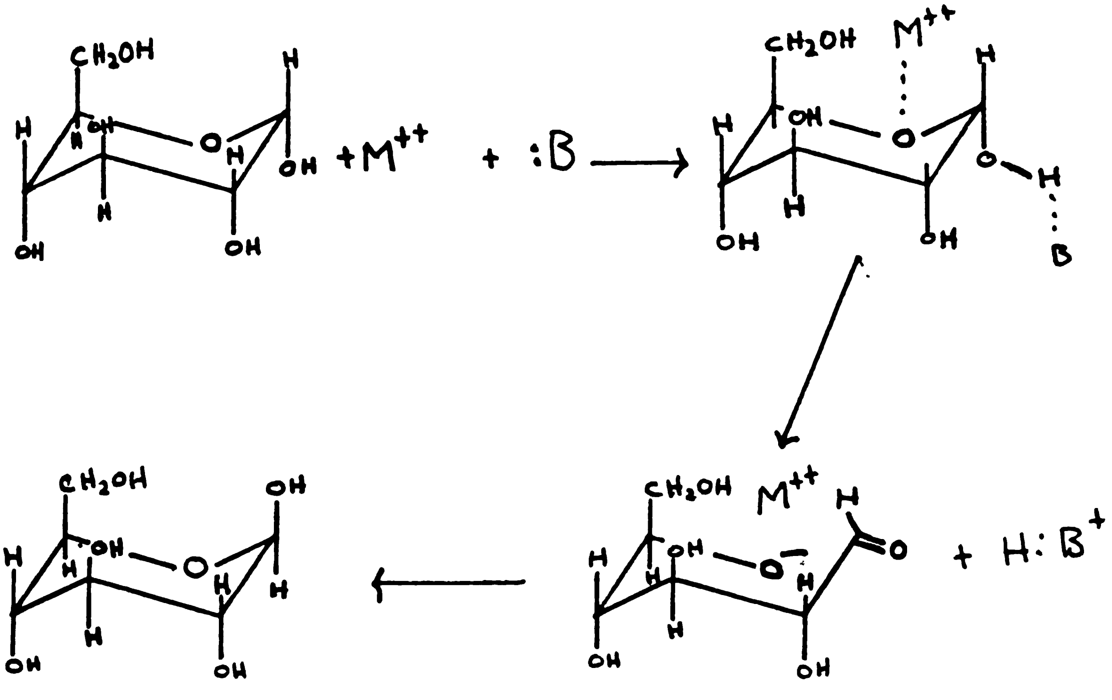
In nondiffusion controlled general acid catalysis, the rate determining step as shown in the following sequence is the complex formation.



A water molecule could be included between the C-5 oxygen and the metal ion without changing this scheme.

The metal ion behaves as a general acid and lowers the activation energy by stabilizing the transition state through binding with the C-5 oxygen. The monoacetate complex of cupric ion could catalyze the reaction by means of this mechanism also. No explanation of its greater catalytic efficiency can be made with this mechanism.

The mechanism for a concerted reaction would be the same as that proposed by Neely.[14] Again a water molecule could be included between the C-5 oxygen and the metal ion.



The proton switching determines the rate of the reaction. proceeding by this mechanism.

The relative catalytic coefficients of the metal ions cannot be quantitatively related using the Bronsted catalysis law. because the dissociation constants of the metal ions acting as acids have not been determined.

In conclusion one can state that metal ions do catalyze the mutarotation of glucose in acetate buffer with characteristic rates. The evidence presented does not allow a decision to be made between the two mechanisms which were discussed.

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