Stopped-Flow Studies of Carbon Dioxide Hydration and Bicarbonate Dehydration in H_2O and D_2O. Acid–Base and Metal Ion Catalysis

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Abstract: The approach to equilibrium between carbon dioxide and bicarbonate has been followed by zero-order kinetics both from the direction of CO_2 hydration and HCO_3^- dehydration. The rates are monitored at 25.0 °C using the stopped-flow indicator technique in H_2O as well as D_2O. The hydration of CO_2 is subject to catalysis by H_2O ($k_0 = 2.9 \times 10^{-2}\ s^{-1}$) and OH^- ($k_{OH^-} = f_\pm k_{a_{OH^-}} = 6.0 \times 10^3\ M^{-1}\ s^{-1}$). The value of 0.63 for the ratio k_{OH^-}/k_{OD^-} is consistent with a mechanism utilizing a direct nucleophilic attack of OH^- on CO_2. In the reverse direction HCO_3^- dehydration is catalyzed predominantly by H_3O^+ ($k_{H_3O^+} = f_\pm k_{a_{H_3O^+}} = 4.1 \times 10^4\ M^{-1}\ s^{-1}$) and to a much lesser degree by H_2O ($k_0 \cong 2 \times 10^{-4}\ s^{-1}$). The value of 0.56 for the ratio $k_{H_3O^+}/k_{D_3O^+}$ indicates that HCO_3^- may be protonated either in a preequilibrium step or in a rate-determining dehydration step. Both the hydration of CO_2 and the dehydration of bicarbonate are subject to general catalysis. For CO_2, dibasic phosphate, a zinc imidazole complex, and a copper imidazole complex all enhanced the rate of hydration with respective rate coefficients of 3×10^{-1}, 6.0, and 2.5 $M^{-1}\ s^{-1}$. For bicarbonate, monobasic phosphate catalyzed the rate of dehydration ($k_{H_2PO_4^-} = 1 \times 10^{-1}\ M^{-1}\ s^{-1}$). Additionally in going from an ionic strength of 0.1 to 1.0 there was a negligible salt effect for the water-catalyzed hydration of CO_2. However, the rate constant for the hydronium ion catalyzed dehydration of HCO_3^- was reduced from $4.1 \times 10^4\ M^{-1}\ s^{-1}$ to $2.3 \times 10^4\ M^{-1}\ s^{-1}$ for the same change in ionic strength. Finally the rate of CO_2 uptake by the complex $Co(NH_3)_5OH_2^{3+}$ was followed spectrophotometrically both in H_2O and D_2O to determine the solvent isotope effect for a reaction known to involve a nucleophilic attack of a Co(III)-hydroxo complex on CO_2. A value of 1.0 for this isotope effect has interesting implications with respect to the mechanism of action of the metalloenzyme, carbonic anhydrase.

The ubiquity of CO_2 has prompted many researchers to investigate one of life's most fundamental reactions, the reversible hydration of carbon dioxide.[1-8] The results of these studies have clearly illustrated that both H_2O and OH^- are catalysts for the hydration. However, their exact role in the mechanism is still unknown.

Following the kinetic scheme suggested by Eigen et al. the hydration in aqueous solution may proceed either directly to bicarbonate or to bicarbonate via carbonic acid, eq 1.[5] Temperature jump studies have revealed that the rate constant k_{12} is approximately $5 \times 10^{10}\ M^{-1}\ s^{-1}$.[5] Using this value and a value of 1.72×10^{-4} M for the true first dissociation constant of carbonic acid,[9] a value of around $10^7\ s^{-1}$ can be calculated for k_{21}. Because the magnitude of both these rate constants far exceeds the observed rate constant for the hydration and because the kinetics were monitored at a pH much higher than the pK_a for H_2CO_3, the overall reaction can be rewritten as

$$\begin{tikzcd}
H^+ + HCO_3^- \arrow[rr, harpoon, shift left, "k_{12}"] \arrow[dr, harpoon, shift left, "k_{13}"] & & H_2CO_3 \arrow[ll, harpoon, shift left, "k_{21}"] \arrow[dl, harpoon, shift left, "k_{23}"] \\
& CO_2 + H_2O \arrow[ul, harpoon, shift left, "k_{31}"] \arrow[ur, harpoon, shift left, "k_{32}"] &
\end{tikzcd} \tag{1}$$

$$CO_2 + H_2O \underset{k_{H_2CO_3}}{\overset{k_{CO_2}}{\rightleftharpoons}} H^+ + HCO_3^- \tag{2}$$

where $k_{CO_2} = k_{31} + k_{32}$ and $k_{H_2CO_3} = k_{13}K_{H_2CO_3} + k_{23}$. Values for k_{CO_2} have been obtained by a variety of techniques. Generally the rate constant at 25.0 °C falls in the range 2.6×10^{-2} to $4.3 \times 10^{-2}\ s^{-1}$ [1-8] However, recent work has consistently placed the value at $3.7 \pm 0.2 \times 10^{-2}\ s^{-1}$.[3,4,7,8] The rate constant for dehydration of H_2CO_3, $k_{H_2CO_3}$, has a value of $1.8 \pm 0.7 \times 10^1\ s^{-1}$.[3-8,10]

At high pH values the hydration is predominantly catalyzed by hydroxide:

$$CO_2 + OH^- \underset{k_{HCO_3^-}}{\overset{k_{OH^-}}{\rightleftharpoons}} HCO_3^- \tag{3}$$

Sirs has determined k_{OH^-} to be $8.5 \times 10^3\ M^{-1}\ s^{-1}$.[11] The rate constant for the reverse reaction, $k_{HCO_3^-}$, has been calculated[12] and measured[13] to be $2 \times 10^{-4}\ s^{-1}$.

In addition to OH^- other bases will also catalyze the reversible hydration of CO_2.[8,14,15] The most potent of these OH^- (or H_2O) donors is the enzyme carbonic anhydrase. Although the mechanism of action of this enzyme is still being debated, it appears that a metal–hydroxo complex is the basic species required for attack on CO_2.[16,17] It is interesting to note that both a dipeptide Cu(II) complex[18] and hydroxopentaamminecobalt(III) perchlorate[19] have also been demonstrated to be effective catalysts for the uptake of CO_2. The latter of the two metal catalysts is especially interesting because it is thought to function analogously to one of the mechanisms proposed for carbonic anhydrase, eq 4.

$$\begin{tikzcd}
Co(NH_3)_5OH^{2+} + CO_2 \arrow[r, harpoon, shift left, "k_1"] \arrow[d, harpoon, shift left] & Co(NH_3)_5OCO_2H^{2+} \arrow[l, harpoon, shift left, "k_{-1}"] \arrow[d, harpoon, shift left, "K_c"] \\
Co(NH_3)OH_2^{3+} \arrow[u, harpoon, shift left, "K_a"] & Co(NH_3)_5CO_3^+ \arrow[u, harpoon, shift left]
\end{tikzcd} \tag{4}$$

The purpose of the present work is to investigate the reactions described by eq 2–4 both in H_2O and D_2O. Values for the rate constants described in these equations were needed in order to evaluate the contribution by the chemical catalysts to the observed rate in the presence of the enzyme carbonic anhydrase.[20] In addition it was hoped the resulting solvent isotope effects for the reactions described in eq 2-4 would aid in elucidating the mechanism of hydration and dehydration.

Experimental Section

Materials. Saturated solutions of CO_2 (Airco, Research grade 99.99% pure) were prepared by bubbling the gas through deionized, distilled water in a vessel fitted with a stopcock and thermostated at 25.0 °C. Portions of this saturated solution were withdrawn by allowing it to flow by gravity from the vessel into a gas-tight Hamilton syringe. To determine the concentration of CO_2, a known volume of the saturated solution was added to an excess of standardized $Ba(OH)_2$ containing $BaCl_2$. The resulting solution was back-titrated against standardized HCl with phenolphthalein as the indicator. Although this method suffers from having an indecisive end point, it was found that the concentration obtained from the average of many determinations was in excellent agreement with the literature value. The concentration of CO_2 saturated in H_2O at 25.0 °C was found to be 3.38×10^{-2} M, while in D_2O it was 3.81×10^{-2} M. Solutions of $NaHCO_3$ (MCB reagent grade) were prepared by carefully weighing the salt and dissolving it in deionized, distilled water just prior to use.

Carbonatopentaamminecobalt(III) nitrate monohydrate was prepared according to the method of Lamb and Mysels,[21] and the aquopentaamminecobalt(III) perchlorate was prepared from it by acidification in 1 M perchloric acid followed by repeated recrystallizations from 0.1 M perchloric acid. The purity of both complexes was checked by spectral measurement on a Cary 14 spectrophotometer: $Co(NH_3)_5CO_3^+$, λ_{max} 509 nm, ϵ 96 cm^{-1} M^{-1}; $Co(NH_3)_5OH_3^{3+}$, λ_{max} 491 nm, ϵ 47.7 M^{-1} cm^{-1}. In addition, the titration of $[Co(NH_3)_5OH_2](ClO_4)_3$ ($pK_a = 6.22$) with NaOH showed this complex to be pure to within 1%.

Apparatus. The hydration of carbon dioxide and the dehydration of bicarbonate were followed spectrophotometrically by the use of an indicator technique on a Durrum-Gibson stopped-flow spectrophotometer thermostated at 25.0 °C. The instrument was furnished with two equal diameter glass drive syringe barrels to ensure that mixing was 1:1 by volume. For most kinetic determinations of the hydration of carbon dioxide the Kel-F flow system was equipped with a mixing chamber containing a 20-mm cuvette. However, all of the kinetic runs with bicarbonate and the cobalt complexes, and a few with carbon dioxide, were carried out with a new flow system designed for either fluorescence or absorbance measurements. The cuvette on this new flow system had not been manufactured to the specified length of 20 mm. Instead, after performing many experiments utilizing Beer's law, the path length was found to be 16 mm. Furthermore, absorbance measurements had to be kept below 0.3 on this dual purpose cell in order to avoid nonlinearity caused by excessive light piping.

The carboxylation of $Co(NH_3)_5OH_2^{3+}$ and the decarboxylation of $Co(NH_3)_5CO_3^+$ were also followed spectrophotometrically by monitoring the change in absorbance at 508 nm on the stopped-flow instrument.

All pH measurements were recorded on a Beckman Model 101900 research pH meter fitted with a Corning glass electrode (no. 476022) and a Beckman reference electrode (no. 39071). The pD was obtained by adding 0.41 to the observed pH meter reading.[22] If the concentration of hydronium ion was needed, it was calculated from pH meter readings which were corrected for activity effects using eq 5 and 6 where I and Z stand for the ionic strength of the solution and the charge of H_3O^+, respectively.

$$pH = -\log([H_3O^+]f_\pm) \tag{5}$$

$$\log f_\pm = \frac{-0.51 Z^2 I^{0.5}}{1 + 1.5 I^{0.5}} \tag{6}$$

Buffer Components and Solutions. All buffers for the carbon dioxide hydration and bicarbonate dehydration studies were prepared in either deionized, distilled H_2O or in D_2O (Stohler, 99.8% D) and brought to constant ionic strength by the addition of Na_2SO_4 (Baker). The buffer components sodium dihydrogen phosphate, sodium monohydrogen phosphate, sodium sulfate, and sulfuric acid were reagent grade or the equivalent and used without further purification. 3-Picoline (Aldrich) was shown to be 96% pure by titration against HCl and was used as obtained. Imidazole (Eastman Kodak) was recrystallized three times from benzene-Norite, while 1,2-dimethylimidazole (Aldrich) was distilled under reduced pressure. *N,N*-Dimethylglycine was prepared from *N,N*-dimethylglycine hydrochloride (Nutritional Biochemicals Corp.) by a previously published procedure.[23] All indicators were used as obtained except phenol, which was sublimed, and *p*-nitrophenol, which was recrystallized from water made slightly acidic with HCl. Needless to say, in the dehydration study the reactant, $NaHCO_3$, is not neutral and its contribution to the ionic strength must be included. Small amounts of the appropriate indicator were also included in all buffers.

In studying the general-base-catalyzed hydration of carbon dioxide or the general-acid-catalyzed dehydration of bicarbonate in phosphate buffers, the ionic strength was maintained at 1.00. The high ionic strength was desirable in this instance because it allowed greater concentrations of the relatively poor catalytic phosphate species to be used. When metal ions were used as the general catalyst they were introduced as their sulfate salt into imidazole buffers of 0.1 ionic strength. It was always necessary to readjust the pH of these buffers with small amounts of NaOH to compensate for the loss of free imidazole upon its coordination to the metal.

Table I. Selected Physical Properties of the Acid-Base Indicators Used in the Hydration of CO_2 and Dehydration of HCO_3^-

Indicator	λ,[a] nm	$\Delta\epsilon$[b]	pK_{HIn}[c]	K_{HIn}
BCP[d]	588	7.16×10^4	6.20	6.22×10^{-7}
PNP[e]	400	1.79×10^4	7.03	9.31×10^{-8}
MCP[f]	578	3.02×10^4	8.40	3.94×10^{-9}
Phenol	287	2.61×10^3	9.96	1.10×10^{-10}
AYR[g]	494	2.9×10^4	10.86	1.36×10^{-11}

[a] The maximum wavelength of the basic form of the indicator. [b] The difference in molar extinction coefficient of the acidic and basic form of the indicator determined at the maximum wavelength of the basic form. [c] Determined at 25.0 °C and $I = 0.10$. [d] BCP = bromocresol purple. [e] PNP = *p*-nitrophenol. [f] MCP = metacresol purple. [g] AYR = alizarin yellow R.

Only nitrogen buffers were used in studying the carboxylation of $Co(NH_3)_5H_2O^{3+}$ and the decarboxylation of $Co(NH_3)_5CO_3^+$ so that a constant concentration of SO_4^{2-} could be maintained at all pH values. This was accomplished by holding the amount of protonated base constant for each buffer. Then the ionic strength was brought to 0.50 by the addition of Na_2SO_4. Weighed amounts of $[Co(NH_3)_5H_2O](ClO_4)_3$ were added directly to the buffer when it was used as a catalyst. Solutions of $[Co(NH_3)_5CO_3](NO_3)H_2O$ were prepared in 1×10^{-3} N NaOH. This procedure was necessary because the complex decarboxylates noticeably at all pH values below 9[24] and is unstable at all pH values above 12. However, in no instance was the solution used after the complex had been in contact with the base for periods longer than 10 min.

Kinetic Procedure and Calculations. All rates on the stopped-flow spectrophotometer were initiated by rapid 1:1 mixing of the substrate with a buffer containing the desired catalyst and monitored over a very short time interval (ca. 10%) so as to record only the initial linear portion of the reaction. The output on the oscilloscope was adjusted to read 2% transmittance full scale. The data were analyzed by first converting the percent transmittance readings to absorbance and then by plotting absorbance vs. time in the customary zero-order fashion. Usually nine kinetic runs were recorded per buffer. For hydration, the first-order in substrate was confirmed in the range $[CO_2] = 0.0034\text{-}0.017$ M; for dehydration it was confirmed in the range $[HCO_3^-] = 0.005\text{-}0.05$ M.

Since there is no convenient technique available to directly monitor the disappearance of CO_2 or the appearance of HCO_3^-, an indicator technique has been developed which essentially follows changes in the proton concentration.[4,8,25] An expression for the velocity of disappearance of CO_2 can then be written in terms of measureable quantities:

$$V_{CO_2\ \mathrm{hydration}} = \frac{d[CO_2]}{dt} = \frac{d[H^+]}{dt} = \frac{d(OD)}{dt}\frac{d[H^+]}{d(OD)} \tag{7}$$

The first quantity, d(OD)/dt, is simply a term which measures the change in optical density as a function of time. It can be evaluated as the slope of a zero-order rate plot. The second term, $d[H^+]/d(OD)$, which has been called the "buffer factor" and given the symbol Q_0, is simply an expression which relates changes in the optical density of the indicator to the number of protons released in the hydration reaction.

The buffer factor can be determined by one of two methods. First, for small quantities of acid released, the buffer factor equals $\Delta[H^+]/\Delta(OD)$ and it can be experimentally obtained by adding aliquots of standardized HCl to the buffer system and observing the corresponding change in optical density on a spectrophotometer. Second, the buffer factor can be calculated from

$$Q_0 = \frac{C_B\alpha_B(1 - \alpha_B)}{\alpha_{In}(1 - \alpha_{In})bC_{In}\Delta\epsilon} \tag{8}$$

where C_B is the total buffer concentration, α_B is the mole fraction of the basic component of the buffer, α_{In} is the mole fraction of the basic component of the indicator, b is the optical path length, C_{In} is the total indicator concentration, and $\Delta\epsilon$ is the difference in molar extinction coefficient between the acidic and basic components of the indicator at the selected wavelength. Of course, to use this expression an ac-

Table II. Rate Constants, k_b, for the Hydration of CO_2[a]

Buffer	Solvent	pH or pD	k_b, s^{-1}	Log k_b	k_{OL^-}, $[OL^-]$,[b] s^{-1}	k_0,[c] s^{-1}
3-Picoline	H_2O	5.90	0.025	−1.60	0.000	0.025
		6.18	0.026	−1.59	0.000	0.026
Phosphate		6.07	0.026	−1.59	0.000	0.026
		6.37	0.025	−1.60	0.000	0.025
		6.68	0.028	−1.55	0.000	0.028
		6.84	0.033	−1.48	0.001	0.032
		7.02	0.032	−1.49	0.001	0.031
		7.34	0.033	−1.48	0.002	0.031
		7.47	0.038	−1.42	0.002	0.036
1,2-Dimethyl-imidazole		7.77	0.034	−1.47	0.005	0.029
		8.19	0.041	−1.39	0.012	0.029
		8.66	0.064	−1.19	0.035	0.029
N,N-Dimethyl-glycine		9.30	0.19	−0.72		
		9.63	0.38	−0.42		
		9.90	0.64	−0.19		
		10.23	1.34	0.13		
Phosphate	D_2O	6.60	0.012	−1.92	0.000	0.012
		6.91	0.015	−1.82	0.000	0.015
		7.55	0.021	−1.68	0.001	0.020
1,2-Dimethyl-imidazole		8.29	0.018	−1.74	0.003	0.015
		9.18	0.042	− .138	0.025	0.017
N,N-Dimethyl-glycine		10.62	0.71	−0.15		

[a] The concentration of CO_2 after 1:1 mixing was 0.017 M in H_2O and 0.019 M in D_2O, at an ionic strength of 0.10 and t = 25.0 °C. [b] A value of 6.0×10^3 M^{-1} s^{-1} was obtained for k_{OH^-} (Figure 1, insert). A value of 9.6×10^3 for k_{OD^-} was calculated from the data at pD 9.18 and 10.62. [c] $k_0 = k_b - k_{OL^-}[OL^-]$.

curate value for K_{HIn} and $\Delta\epsilon$ is needed. The acid dissociation constant at I = 0.10 and 25.0 °C for each indicator was obtained by a standard, spectrophotometric procedure and these results along with other physical properties are listed in Table I. As pointed out by Khalifah,[8] the primary consideration in selecting an indicator for use with a buffer should go to choosing one whose pK_{HIn} is near the pK_a of the buffer. For this reason bromocresol purple was used with picoline; *p*-nitrophenol with phosphate and imidazole; metacresol purple with 1,2-dimethylimidazole; and phenol with *N,N*-dimethylglycine.

Since the two different methods of determining Q_0 usually agreed to within ±5%, only calculated buffer factors were used in determining initial velocities. These velocities when divided by the initial concentration of CO_2 yield k_{obsd}:

$$k_{obsd} = \frac{V_{CO_2}\text{ hydration}}{[CO_2]_0} = \frac{(d(OD)/dt)(Q_0)}{[CO_2]_0} \qquad (9)$$

This expression is valid only in the pH range where one proton is released per molecule of CO_2 hydrated. Certainly above pH 8.5 this is not the case, because an additional proton is released upon the dissociation of HCO_3^- into CO_3^{2-}, and the appropriate adjustment to eq 9 must be made.

In studying the dehydration of bicarbonate the same considerations hold as for CO_2 hydration. However, kinetic investigations of the two cobalt complexes were handled in a slightly different fashion. The decarboxylation of $Co(NH_3)_5CO_3^+$ was monitored directly by following its disappearance at 508 nm. As with CO_2 and HCO_3^- only the initial velocity was followed. The extinction coefficient for the reactant and the product was determined in order to convert the optical density velocity into the molar velocity, V_{obsd}, where b is the optical path length.

$$V_{obsd} = \frac{d[Co(NH_3)_5CO_3^+]}{dt} = \frac{d(OD)/dt}{(\epsilon_{product} - \epsilon_{reactant})b} \qquad (10)$$

The initial velocity could then be converted into rate constants by dividing V_{obsd} by the initial concentration of the complex. An identical procedure was followed while studying the uptake of CO_2 by $Co(NH_3)_5OH_2^{3+}$.

Results

Hydration of Carbon Dioxide. The hydration of CO_2 can potentially be accelerated by a variety of catalysts. The term k_{obsd}, eq 9, can best be described as the summation of the catalysis afforded to the hydration by the buffering species and any additional catalyst such as a metal complex:

$$k_{obsd} = k_b + k_{metal\ complex}[\text{metal complex}] \qquad (11)$$

The buffer rate, k_b, is a summation of the buffer and water species catalysis:

$$k_b = k_0 + k_{OH^-}[OH^-] + k_{H_3O^+}[H_3O^+] + k_{HB}[HB] + k_B[B] \qquad (12)$$

It was found, however, that so long as the buffer concentration was maintained below 0.05 M, its contribution to the rate was negligible. Consequently the expression for the buffer rate can be further simplified to

$$k_b = k_0 + k_{OH^-}[OH^-] + k_{H_3O^+}[H_3O^+] \qquad (13)$$

The buffer rates obtained at various pH and pD values are listed in Table II. When these results are plotted as log k_b vs. pH, Figure 1, it becomes clear that there are two distinct catalytic regions. First, between pH values 6 and 7.5 water is functioning as the only catalyst and then at pH values above 7.5, hydroxide becomes the dominant catalyst. The term k_{OH^-} was evaluated by plotting the buffer rate, k_b, against the corresponding hydroxide ion concentration at five different pH values, Figure 1 insert. The hydroxide ion concentration was calculated from pH measurements

$$[OH^-] = K_{H_2O}/(a_{H_3O^+})(f_\pm) \qquad (14)$$

where K_{H_2O} was taken as 1.0×10^{-14} at 25.0 °C. The term k_{OD^-} was evaluated from the data collected in the dimethylglycine buffer of pD 10.62 and the 1,2-dimethylimidazole

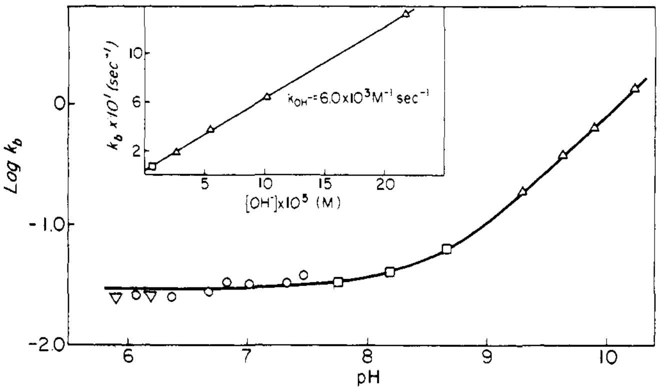


Figure 1. Plot of log k_b against pH and k_b against [OH^-] for the hydration of CO_2 at 25.0 °C and ionic strength 0.10. Data obtained in: ▽, 3-picoline; ○, phosphate; □, 1,2-dimethylimidazole; and △, *N,N*-dimethylglycine buffers.

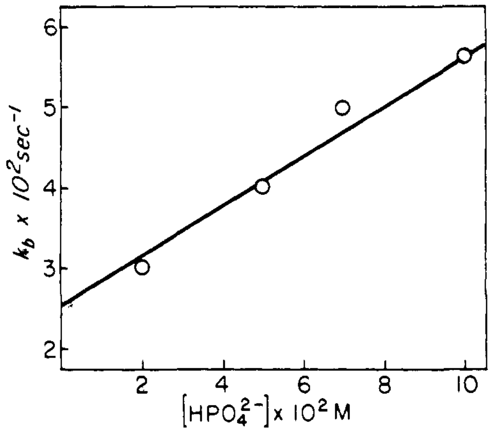


Figure 2. Plot of k_b against [HPO_4^{2-}] for the hydration of CO_2 at 25.0 °C and ionic strength 1.0.

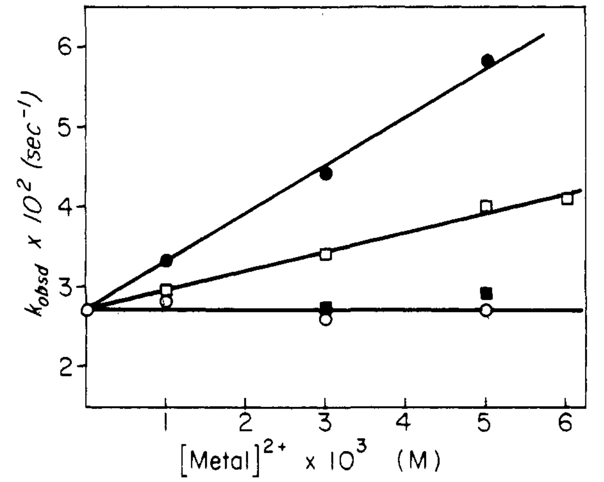


Figure 3. The divalent metal ion catalyzed hydration of carbon dioxide in imidazole buffers at 25.0 °C and ionic strength 0.10. The metal ions employed are ●, Zn^{2+} (pH 6.63); □, Cu^{2+} (pH 6.63); ■, Co^{2+} (pH 6.66); and ○, Ni^{2+} (pH 6.84).

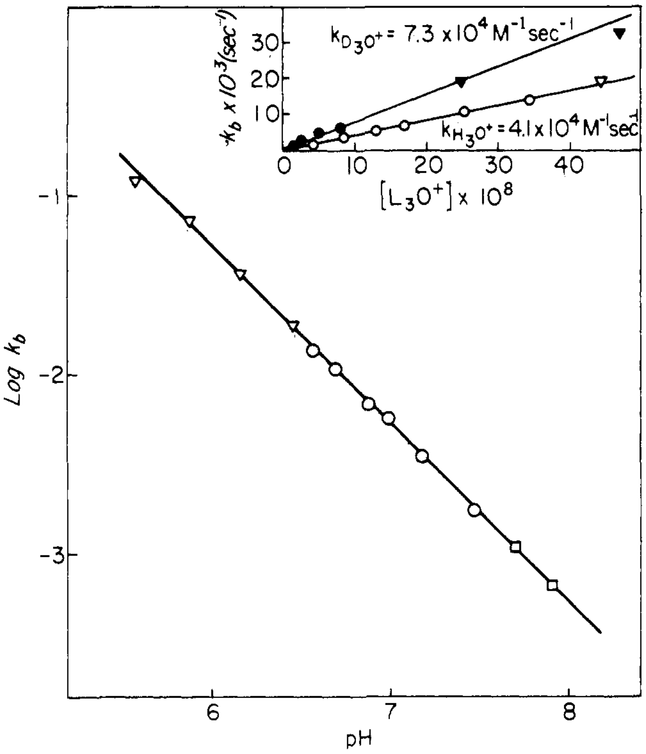


Figure 4. Plot of log k_b against pH and k_b against [L_3O^+] for the dehydration of bicarbonate at 25.0 °C and ionic strength 0.10. Data obtained in: ▽, 3-picoline; ○, imidazole; and □, 1,2-dimethylimidazole. Open symbols correspond to data in H_2O while filled symbols correspond to that in D_2O.

buffer of pD 9.18. The concentration of deuteroxide was calculated from eq 14 using a value of 1.51×10^{-15} for the autoprotolysis constant of D_2O at 25.0 °C.[26]

To determine whether there was general catalysis the hydration rate was followed in phosphate buffers at an ionic strength of 1.0. Figure 2 illustrates that the phosphate buffer is indeed a catalyst, albeit a weak one. The individual rate coefficients for H_2O, $H_2PO_4^-$, and HPO_4^{2-} were separated by the method of Bell and Darwent,[27] and the results from three buffer ratios indicate that $k_{H_2O} = 2.6 \times 10^{-2}$ s^{-1}, $k_{HPO_4^{2-}} = 3 \times 10^{-1}$ s^{-1} M^{-1}, and $k_{H_2PO_4^-} \cong 0$ at $I = 1.0$ and $t = 25.0$ °C. Two divalent metal ions, zinc and copper, were also found to be catalysts for the hydration of CO_2, Figure 3. Since the metal ions were introduced into imidazole buffers at pH 6.6, the exact coordination of the metal ions and the exact identity of the catalytic species are unknown. Assuming that the active catalyst is M(imidazole)$_n$$\cdot$(OH)$^+$, then the lower limits for the catalytic coefficient of the zinc and copper complexes are 6.0 and 2.5 M^{-1} s^{-1}, respectively. Two other divalent metal ions, cobalt and nickel, were not nearly as effective in catalyzing the hydration of CO_2.

Dehydration of Bicarbonate. As in the case of CO_2 hydration the effect of the buffer on the buffer rate was negligible providing that the total concentration was kept below 0.03 M. A plot of log k_b (Table III) vs. pH, Figure 4, indicates that H_3O^+ is the dominant catalyst over the entire pH range investigated. To determine an accurate value for the catalysis by H_3O^+, the buffer rate was plotted against the corresponding hydronium ion concentration, Figure 4 (insert). Also plotted in this figure are the data collected in D_2O. It is clear that the dehydration is catalyzed more by D_3O^+ than H_3O^+. The exact value for the isotope effect, $k_{H_3O^+}/k_{D_3O^+}$, is 0.56. Theoretically the intercept of the plot in Figure 4 (insert) should represent the value for the catalysis by H_2O and D_2O. Unfortunately, the intercept cannot be obtained with much accuracy, but it appears to be 2×10^{-4} s^{-1} in H_2O and 1×10^{-4} s^{-1} in D_2O.

Rates obtained in phosphate buffers of high ionic strength, $I = 1.0$, revealed that the dehydration was catalyzed slightly by dihydrogen phosphate. The catalytic coefficient at 25.0 °C was 1×10^{-1} M^{-1} s^{-1}. Additionally the catalysis by H_3O^+ dropped to 2.3×10^4 s^{-1} M^{-1} at $I = 1.0$.

Carboxylation of $Co(NH_3)_5OH_2^{3+}$ and Decarboxylation of $Co(NH_3)_5CO_3^+$. It has been reported that the pK_a of [$Co(NH_3)_5OH_2$]$^{3+}$ is 6.22 when ClO_4^- is the only anion in

Table III. Rate Constants, k_b, for the Dehydration of HCO_3^- [a]

Buffer	Solvent	pH or pD	$[L_3O^+]$	$k_b \times 10^3$, s^{-1}	$-\log k_b$
3-Picoline	H_2O	5.58	3.33×10^{-6}	124	0.91
		5.87	1.73×10^{-6}	73	1.14
		6.16	8.87×10^{-7}	36	1.44
		6.46	4.45×10^{-7}	19	1.72
Imidazole		6.57	3.45×10^{-7}	13.6	1.87
		6.70	2.56×10^{-7}	10.6	1.97
		6.88	1.69×10^{-7}	6.7	2.17
		6.99	1.31×10^{-7}	5.7	2.24
		7.18	8.47×10^{-8}	3.5	2.46
		7.47	4.34×10^{-8}	1.7	2.77
1,2-Dimethylimidazole		7.70	2.56×10^{-8}	1.08	2.97
		7.91	1.58×10^{-8}	0.65	3.19
3-Picoline	D_2O	6.44	4.70×10^{-7}	32.4	1.49
		6.71	2.51×10^{-7}	18.7	1.73
Imidazole		7.21	7.94×10^{-8}	6.23	2.21
		7.41	4.98×10^{-7}	4.95	2.31
		7.71	2.53×10^{-7}	2.93	2.53
		8.00	1.29×10^{-7}	1.25	2.90

[a] The concentration of HCO_3^- after 1:1 mixing was 0.02 M in both H_2O and D_2O, at an ionic strength of 0.10 and t = 25.0 °C.

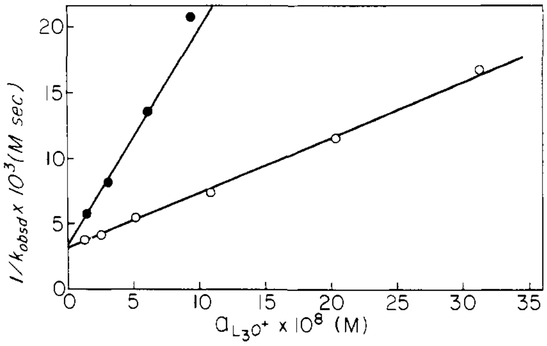


Figure 5. Plot of $1/k_{obsd}$ against $a_{L_3O^+}$ for the uptake of CO_2 by $Co(NL_3)_5OL_2^{3+}$ at 25.0 °C, ionic strength 0.50 and $[SO_4^{2-}]$ = 0.167 M. Filled symbols refer to the data in D_2O and open symbols to the data in H_2O.

Table IV. Effect of SO_4^{2-} on the pK_a of $[Co(NH_3)_5OH_2](ClO_4)_3$

$[Co(NH_3)_5OH_2]_0^{3+} \times 10^3$, M	$[Na_2SO_4] \times 10^2$, M	I	pK_a[a]
20.0	0.00	0.10	6.20
10.0	1.67	0.10	6.68
4.0	2.67	0.10	6.84
2.0	3.00	0.10	6.88
2.0	8.33	0.25	7.06
2.0	16.33	0.50	7.25

[a] Determined by measuring the pH after the complex was half titrated by the addition of standardized NaOH.

Table V. Summary of Some Physical Constants for the Cobalt Complexes

Species	ϵ[a]	pK_a
$[Co(NL_3)_5OL_2]^{3+}$	45.8[b]	7.16[b]
	44.0[c]	7.63[c]
$[Co(NL_3)_5OL]^{2+}$	67.7[b]	
	67.7[c]	
$[Co(NL_3)_5CO_3L]^{2+}$	67.8[b]	6.92[b]
	67.5[c]	7.43[c]
$[Co(NL_3)_5CO_3]^+$	96.0[b]	
	94.5[c]	

[a] λ 508 nm. [b] In H_2O. [c] In D_2O.

solution.[28] We have confirmed this value by half titrating the complex and measuring the pH of the resulting solution, Table IV. In this same table it is important to note that the pK_a of the acid increases upon addition of Na_2SO_4. Since the ionic strength was maintained at 0.1 M, this trend is not an ionic strength effect, but instead seems to suggest that SO_4^{2-} can form a tight ion pair with the complex. Undoubtedly it complexes with both the acid and the conjugate base, but since the acid has charge of +3, the SO_4^{2-} stabilizes it more than the conjugate base whose charge is only +2. Furthermore, as this table illustrates, it is essential to maintain constant both the ionic strength and the concentration of SO_4^{2-} so that the pK_a of the complex remains constant.

In order to accurately determine the pK_a of both complexes at an ionic strength of 0.50 and a SO_4^{2-} concentration of 0.167 M, a spectrophotometric titration was performed in H_2O as well as in D_2O. A summary of the acidity constants for each complex along with the corresponding molar extinction coefficients is found in Table V.

Using the kinetic scheme shown in eq 4, the following rate expression for the uptake of CO_2 by $Co(NH_3)_5OH_2^{3+}$ can be written:

$$-\frac{d[Co(NH_3)_5OH_2^{3+}]}{dt} = k_1[Co(NH_3)_5OH^{2+}][CO_2] - k_{-1}[Co(NH_3)_5CO_3H^{2+}] \quad (15)$$

Since only initial velocities were used this expression reduces to

$$-\frac{d[Co(NH_3)_5OH_2^{3+}]}{dt}$$

$$= k_1[Co(NH_3)_5OH^{2+}]_0[CO_2]_0 \quad (16)$$

$$= \frac{k_1K_a}{[H^+] + K_a}[Co(NH_3)_5OH_2^{3+}]_{stoic}[CO_2]_0 \quad (17)$$

$$= k_{obsd}[Co(NH_3)_5OH_2^{3+}]_{stoic}[CO_2]_0 \quad (18)$$

where $k_{obsd} = k_1K_a/[H^+] + K_a)$. Substituting eq 10 for k_{obsd} gives

$$k_{obsd} = \frac{d(OD)/dt}{(\Delta\epsilon)(b)([Co(NH_3)_5OH_2^{3+}]_{stoic})([CO_2]_0)} \quad (19)$$

where d(OD)/dt is the slope of a zero-order rate plot and $\Delta\epsilon$ can be calculated from the data in Table V. A convenient method to analyze these data for k_1 results from taking the reciprocal of k_{obsd}:

$$1/k_{obsd} = \frac{[H^+]}{k_1K_a} + \frac{1}{k_1} \quad (20)$$

A plot of $1/k_{obsd}$ vs. $[H^+]$, Figure 5, will be a straight line of slope $1/k_1K_a$ and intercept $1/k_1$. The value of k_1 in H_2O is 3.1×10^2 M^{-1} s^{-1} while in D_2O it is 3.0×10^2 M^{-1} s^{-1}.

A similar derivation for the decarboxylation of $Co(NH_3)_5CO_3^+$ gives the following expression for k_{obsd} where $k_{obsd} = k_{-1}[H^+]/([H^+] + K_a)$.

$$k_{obsd} = \frac{d(OD)/dt}{(\Delta\epsilon)(b)[Co(NH_3)_5CO_3^+]_{stoic}} \quad (21)$$

A plot of $1/k_{obsd}$ against $1/[H^+]$ gives a straight line of slope K_a/k_{-1} and an intercept of $1/k_{-1}$, Figure 6. The value of k_{-1} in H_2O is 9.1×10^{-1} s^{-1} while in D_2O it is 8.2×10^{-1} s^{-1}.

Discussion

From the triangular scheme shown in eq 1, it is clear that the chemical hydration of CO_2 in H_2O can occur via one of

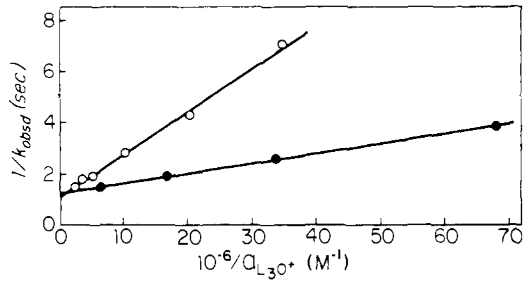
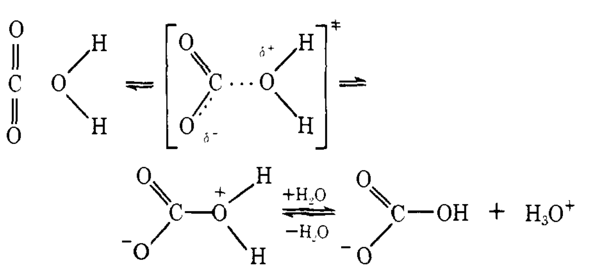
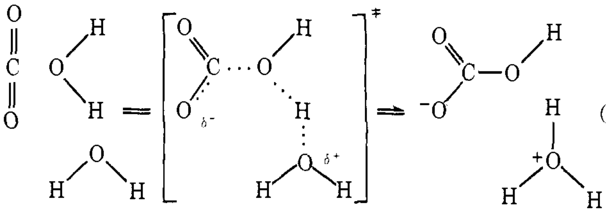


Figure 6. Plot of $1/k_{obsd}$ against $1/a_{L_3O^+}$ for the decarboxylation of $Co(NL_3)_5CO_3^+$ at 25.0 °C, ionic strength 0.50 and $[SO_4^{2-}] = 0.167$ M. Filled symbols refer to the data in D_2O and open symbols to the data in H_2O.

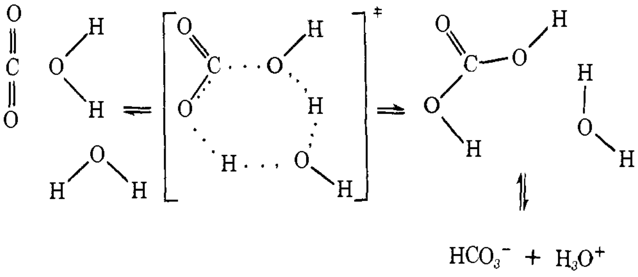
three different routes. First there is a direct route which involves nucleophilic attack by water on CO_2 to form a structural isomer of carbonic acid. This isomer in turn readily loses a proton to form bicarbonate, eq 22. Second, there is a direct route whereby water acts as a general base to facilitate the immediate formation of bicarbonate, eq 23, a cyclic array to form carbonic acid, which in turn readily ionizes to bicarbonate, eq 24.



(22)

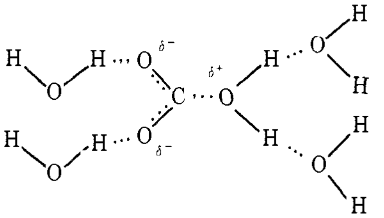


(23)



(24)

Offhand it might be expected that the solvent deuterium isotope effect could distinguish between the former and the latter two mechanisms. In the latter two mechanisms one would expect the ratio, k_{H_2O}/k_{D_2O}, to be greater than unity since a proton is being transferred in the rate-determining step, while in the former mechanism, one would expect the value to lie somewhere around unity which is the value expected for a reaction not involving proton transfer. Furthermore, the hydration mechanism in eq 23 with its one-proton transfer in the transition state would be expected to exhibit a solvent isotope effect, k_{H_2O}/k_{D_2O}, of around 2,[29a] whereas the cyclic mechanism with its two-proton transfer in the transition state, eq 24, would be expected to exhibit a significantly larger solvent isotope effect of ca. 3-4.[29b] However, it must be remembered that secondary isotope effects may make a substantial contribution to the overall value.[29c] Undoubtedly this will be the case for all of the proposed mechanisms. Consider, for example, the likely solvation of the transition state needed in the nucleophilic mechanism, eq 25.



(25)

In going from a neutral ground state to a transition state which possesses separation of charge, the demand for solvation will be enhanced. Clearly the solvent reorganization accompanying the activation process will lead to a kinetic isotope effect larger than unity owing to these increased solute-water interactions. The actual value obtained for k_{H_2O}/k_{D_2O} is 1.8 ± 0.2, a value which is not inconsistent with any of the proposed mechanisms, although, a priori, the cyclic mechanism (eq 24) appears to be less probable.

By the principle of microscopic reversibility the mechanism for the dehydration of bicarbonate must also be the reverse of one of the three hydration mechanisms shown above. All the mechanisms predict that the rate will be proportional to the proton concentration, but they predict different ratios for $k_{H_3O^+}/k_{D_3O^+}$. A priori, the mechanism outlined in eq 23 would appear to require a ratio greater than unity since a proton is being transferred in the rate-determining step. On the other hand, the mechanism outlined in eq 22 requires that the ratio be less than unity since a proton is being transferred in a preequilibrium step. It is difficult to predict the isotope effect for the mechanism in eq 24 because there is both a preequilibrium proton transfer and a proton transfer in the rate-determining step. However, it is not uncommon for primary isotope effects involving two proton transfers, as in the transition state of eq 24, to be as large as 3 or 4.[29b] Therefore the ratio $k_{H_3O^+}/k_{D_3O^+}$ for the mechanism in eq 24 might be expected to be somewhat larger than unity. The observed isotope effect cannot be used to distinguish between the two mechanisms represented by eq 22 and 23, respectively. The $k_{H_3O^+}/k_{D_3O^+}$ value of 0.56 is similar to those ordinarily observed for reactions known to proceed via a specific acid catalyzed mechanism.[31a] According to eq 22 such a mechanism would make the zwitterion H_2O^+-CO_2^- a likely intermediate in the interconversion of CO_2 and HCO_3^-. However, an isotope effect of this magnitude does not exclude mechanisms involving general acid catalysis. Thus, a solvent isotope effect, $k_{H_3O^+}/k_{D_3O^+}$, of 0.7 has been observed in the hydronium ion catalyzed hydrolysis of ethyl orthocarbonate[31a,b] and a value of 0.65 has been recently found for the hydronium ion catalyzed hydration of acetaldehyde at 25.0 °C,[31c] reactions that are also catalyzed by general acids. Clearly then an isotope effect, $k_{H_3O^+}/k_{D_3O^+}$, of 0.56 is also consistent with the dehydration mechanism involving general acid catalysis, eq 23. Furthermore, since no salt effect was observed for k_0 in going from an ionic strength of 0.1 to 1.0 in the hydration of CO_2, this implies that the transition state for hydration must be developed early.

As a check on the validity of the rate constants obtained for the chemical hydration of CO_2 and dehydration of bicarbonate, they can be used to calculate the equilibrium constant for the reaction shown in eq 2. The equilibrium constant, K_{eq}, in 0.1 M NaCl has been measured by Harned et al. and reported to be 7.6×10^{-7} M.[32] As can be seen from

$$K_{eq}^{H_2O} = \frac{k_0^{CO_2}}{k_{H_3O^+}^{HCO_3^-}} = \frac{2.9 \times 10^{-2}\ s^{-1}}{4.1 \times 10^{4}\ M^{-1}\ s^{-1}} = 7.1 \times 10^{-7}\ M \quad (26)$$

the value calculated from the experimental rate data is in excellent agreement with Harned's value. The equilibrium constant in D_2O can also be calculated from the experimentally observed rate data:

$$K_{eq}{}^{D_2O} = \frac{k_0{}^{CO_2}}{k_{D_3O^+}{}^{DCO_3^-}} = \frac{1.6 \times 10^{-2}\ s^{-1}}{7.3 \times 10^{4}\ M^{-1}\ s^{-1}} = 2.2 \times 10^{-7}\ M \qquad (27)$$

A comparison between $pK_{eq}{}^{H_2O}$ and $pK_{eq}{}^{D_2O}$ shows that $pK_{eq}{}^{D_2O}$ is larger by 0.51. This observation is in accord with the empirical finding that acids whose pK is around neutrality are stronger in H_2O than in D_2O by a factor of about 0.55 pK units.[33]

At high pH the hydration of CO_2 is strongly catalyzed by hydroxide:

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad (28)$$

Presumably the reaction involves the direct nucleophilic attack of OH^- as evidenced by a value of 0.63 for the ratio k_{OH^-}/k_{OD^-}. The rates of reaction in which OD^- acts as a nucleophile or a base in D_2O are usually 20-40% higher than the corresponding reactions with OH^- in H_2O.[30a,34-36]

The rate constants obtained for the carboxylation of $Co(NH_3)_5OH_2{}^{3+}$ and decarboxylation of $Co(NH_3)_5CO_3{}^+$ are in good agreement with those reported earlier by Harris using a first-order kinetic analysis. Furthermore, the solvent isotope effects on k_1 and k_{-1} (refer to eq 4) are 1.0 and 1.1, respectively; and these values are precisely in the range expected for a reaction not involving proton transfer in the rate-determining step. Additionally these isotope effects have important implications with regard to the mechanism of action of the metalloenzyme carbonic anhydrase. If the zinc-hydroxo complex is acting as a nucleophile in the hydration of CO_2, then the solvent isotope effect on the turnover number, k_{cat}, must be around unity. If, on the other hand, the zinc-hydroxo complex or any other amino acid is acting as a general base, then the isotope effect should be substantially greater than unity. Current research on the bovine carbonic anhydrase catalyzed hydration of CO_2 has revealed a large solvent isotope effect (around 3) on k_{cat}, even at high buffer concentrations, suggesting that a proton transfer occurs in the rate-determining step because the enzyme either acts as a general base or incorporates a proton relay system.[20]

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