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^{18}O-Isotope Effect in ^{13}C Nuclear Magnetic Resonance Spectroscopy. 4. Oxygen Exchange of [1-^{13}C,^{18}O$_2$]Acetic Acid in Dilute Acid

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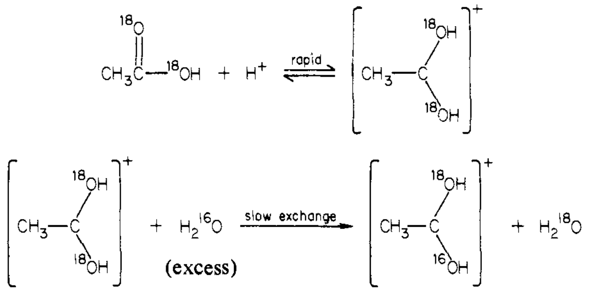
Abstract: The ^{18}O-isotope shift in ^{13}C NMR spectroscopy is shown to provide a widely applicable technique for studying the kinetics of oxygen exchange reactions in a nearly continuous assay mode. Here, the technique is used to study the acid-catalyzed medium carboxyl(oxygen)-water exchange reaction of [1-^{13}C,^{18}O$_2$]acetic acid at 32 °C. The ^{18}O-isotope-induced shift of the ^{13}C NMR signal of the ^{13}C-enriched carboxyl carbon in acetic acid permits a facile and direct measurement of the relative concentrations of the three (oxygen) isotopic species of acetic acid. The pseudo-first-order rate constant is evaluated as a function of pH and added salt for the salts sodium chloride and sodium perchlorate. The pH profile is typical of those observed for related acid-catalyzed oxygen exchange reactions of the carboxyl group. In the dilute-acid region the rate is approximately first order in hydrogen ion. The present results definitively establish the salt effect on the exchange reaction, resolving a conflict between earlier reports. The rate of the exchange reaction is decreased by increasing concentrations of the two salts. The magnitude of the rate decrease depends on the nature as well as the concentration of the salt. Possible interpretations of the results are discussed.

Recently we[1] reported that ^{18}O exerts an isotope effect on ^{13}C NMR signals of directly bonded carbon atoms which results in an upfield shift of the ^{13}C resonance signal relative to the ^{13}C resonance signal of otherwise comparable carbon atoms bearing ^{16}O oxygen. Studies by us and by others demonstrated that the ^{18}O-isotope effect in ^{13}C NMR is a general phenomenon.[2-5] The magnitude of the isotope-induced shift is determined principally by the structure of the carbon-oxygen functional group.[2,4,5] The isotope effect is governed to a lesser extent by the through-bond electronic influences of the substituent groups,[2,3,5] conjugation,[2,5] and hybridization of the carbon atom.[2] Isotope effects are additive,[3] very small solvent effects have so far been detected only in alcohols,[2,5] and bulk steric effects do not affect the magnitude of the isotope effect.[3] At least when different functional groups are compared, there is no apparent correlation between carbon-oxygen bond length and the magnitude of the isotope effect.[2,3] Beyond these physical studies, which define the ^{18}O-isotope effect in ^{13}C NMR, the phenomenon should be applicable as a significant experimental technique to solve problems. In our initial report[1] we demonstrated such a practical application of the isotope effect: the catalytic rate constant for the acid-catalyzed exchange of *tert*-butyl [^{18}O]alcohol in water as measured by ^{13}C NMR agreed with the rate constant measured by the more classical method of mass spectrometry.

The exchange of the oxygen atoms of simple carboxylic acids in aqueous media (medium carboxyl(oxygen)-water exchange) can be followed either by measuring the incorporation of an ^{18}O label from the medium into the acid or by the loss of an ^{18}O label from the acid into the medium. The rate of this exchange reaction has generally been measured by the mass spectrometric analysis of samples of the carboxylic acid collected at specific time intervals during the reaction. The technique employed for analysis by mass spectrometry typically involves isolating the carboxylic acid, usually by precipitation or extraction, often followed by pyrolysis to give CO$_2$ (which may in turn require further purification if a contaminant is present), and subjecting this to mass spectrometric analysis. At best, these sample handling techniques are inconvenient and time consuming; they are also frequent sources of error. In this paper we report the results of a study of the acid-catalyzed medium carboxyl(oxygen)-water exchange of ^{13}C-enriched, ^{18}O-labeled acetic acid in water, using the ^{18}O-isotope shift in ^{13}C NMR. The study was undertaken for three reasons: (1) foremost, to demonstrate a broadly applicable technique for following the kinetics of oxygen exchange reactions in a nearly continuous spectrometric assay mode; (2) to resolve conflicting reports about the salt effect on the exchange reaction of acetic acid; and (3) to explore the advantage of utilizing ^{13}C-enriched compounds in the accumulation of experimental data.

In aqueous media, the acid-catalyzed exchange of the oxygen atoms of labeled acetic acid, and carboxylic acids in general, proceeds by the following overall mechanism:



where the slow-exchange step involves the rate-determining formation of a tetrahedrally coordinated intermediate by nucleophilic attack of solvent water on the protonated carboxylic acid.[6-8] In studying this exchange reaction Llewellyn and O'Connor[6] reported that at constant pH and temperature, the rate of exchange decreased significantly upon the addition of sodium perchlorate. However, when McTigue et al.[8] studied the same reaction in the presence of added sodium chloride they found that their experimental "...results show considerable scatter, but it was clear...that the salt effect is small positive for acetic acid...." Also, "the salt effect studies yielded results of such imprecision that much detailed discussion is pointless. However, it is clear that salt effects are not large...." We were struck by this discrepancy. Because both salts are uni-univalent electrolytes one might expect them to exhibit the same general behavior in solution

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and therefore to exert a similar salt effect on the rate of the oxygen exchange reaction of acetic acid. Thus, in addition to demonstrating the usefulness of the ^{18}O-isotope effect in ^{13}C NMR as providing an important new technique for studying such kinetic systems, we sought to definitively measure the sign and magnitude of some representative salt effects on the acid-catalyzed medium carboxyl(oxygen)–water exchange of acetic acid.

Experimental Section

Synthesis of [1-^{13}C,^{18}O$_2$]Acetic Acid. Sodium [1-^{13}C]acetate (90 atom % excess 1-^{13}C, Merck) (2.0 g, 0.024 mol) and excess phosphorus pentachloride (7.23 g, 0.035 mol) were mixed rapidly in a round-bottomed flask. Most of the [1-^{13}C]acetyl chloride that was produced was distilled at atmospheric pressure. The reaction flask was cooled to room temperature and the receiver flask containing the product was cooled in a dry ice–isopropyl alcohol bath. The remaining [1-^{13}C]acetyl chloride was distilled at room temperature under vacuum (30 mmHg) from the reaction flask. The yield of the [1-^{13}C]acetyl chloride was 1.7 mL (>95%). The flask containing the acid chloride was immersed in an ice bath and 2.0 mL of [^{18}O]water (99 atom % excess ^{18}O, normalized, Norsk Hydro, Oslo) was added slowly to hydrolyze the acid chloride. The solution was refluxed for 7 h. The isotopic composition of the acetic acid was measured on a CEC-21-110B mass spectrometer and was found to contain 90% 1-^{13}C and 88% ^{18}O, specifically 75% ^{18}O$_2$ and 25% ^{18}O$_1$. The compound was also characterized by ^{1}H NMR and ^{13}C NMR.

Kinetics of Oxygen Exchange. Doubly-distilled, deionized water was used to prepare all solutions. The ionic strength of the solution was adjusted with anhydrous sodium perchlorate or sodium chloride. A Corning Model 130 pH meter was used to measure the solution pH, which was carefully adjusted with 1 N HCl, 6 N HCl, 1 M HClO$_4$, or 2 M NaOH. All solutions were pre-equilibrated at the probe temperature for at least 30 min in a circulating water bath. The exchange reactions were followed on a Varian CFT-20 spectrometer or an NTC-200 spectrometer.

The Varian CFT-20 spectrometer was fitted with an 8 mm probe. [1-^{13}C,^{18}O$_2$]Acetic acid (1.87 × 10^{-4} mol) was added to the aqueous solution and a total volume of 1.0 mL was prepared in an 8-mm NMR tube. A 5-mm tube filled with deuterium oxide (99.75 atom % ^{2}H, Baker) was inserted as an external lock. A 300-Hz sweep width, a 45° pulse angle, an 8K data block, and a total of 30 acquisitions per spectrum were used. A line-broadening factor was applied to the accumulated FID and protons were broad-band decoupled. The probe temperature was 32 °C.

The NTC-200 spectrometer was fitted with a 12-mm probe. [1-^{13}C,^{18}O$_2$]Acetic acid (7.48 × 10^{-4} mol) was added to 4.5 mL of deuterium oxide and a total volume of 5.0 mL was prepared in a 12-mm NMR tube. A 400-Hz sweep width, a 90° pulse angle, a 16K data block, and one acquisition per spectrum were used. A line-broadening factor was applied to the accumulated FID and protons were broad-band decoupled. The probe temperature was 18 °C.

Data Analysis. Medium carboxyl(oxygen)–water exchange can be described by the reaction

$$RC^{18}O_2H \xrightarrow{H_2O} RC^{18}O^{16}OH \xrightarrow{H_2O} RC^{16}O_2H \qquad (1)$$

In a large molar excess of unlabeled (^{16}O) water the reaction is assumed to be effectively irreversible. For a given rate constant, k, the probability that an ^{18}O will be lost by an exchange reaction can be expressed as

$$RC^{18}O_2H \xrightarrow{k} RC^{18}O^{16}OH \xrightarrow{k/2} RC^{16}O_2H \qquad (2)$$

The set of linear differential equations which describes the reaction in eq 2 is

$$\begin{gathered} dC_2/dt = -kC_2 \qquad dC_1/dt = -\tfrac{1}{2}kC_1 + kC_2 \\ dC_0/dt = \tfrac{1}{2}kC_1 \end{gathered} \qquad (3)$$

where C_2 represents [RC^{18}O$_2$H], C_1 represents [RC^{18}O^{16}OH], and C_0 represents [RC^{16}O$_2$H], and the concentrations are expressed as fractional proportions. The differential eq 3 may be solved by the variation of parameters method to yield

$$C_2(t) = C_2(0)e^{-kt} \qquad (4a)$$

$$C_1(t) = [C_1(0) + 2C_2(0)]e^{-0.50kt} - 2C_2(0)e^{-kt} \qquad (4b)$$

$$C_0(t) = \\ [C_0(0) + C_1(0) + C_2(0)] - [C_1(0) + 2C_2(0)]e^{-0.50kt} + C_2(0)e^{-kt} \qquad (4c)$$

where $C_n(0)$ for n = 0, 1, 2 is the initial concentration of that isotopic species.

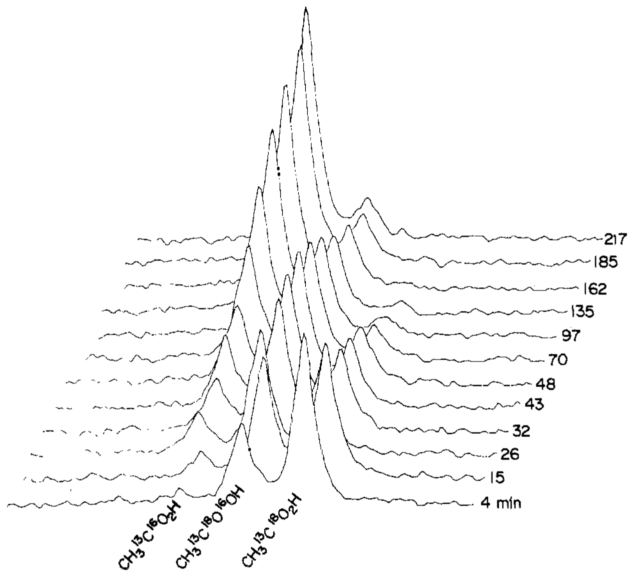

Figure 1. Acid-catalyzed medium carboxyl(oxygen)–water exchange reaction of [1-^{13}C,^{18}O$_2$]acetic acid followed by ^{13}C NMR spectroscopy, using an NTC-200 instrument operating at 50.3 MHz. The solution contained 7.48 × 10^{-4} mol of [1-^{13}C,^{18}O$_2$]acetic acid (90 atom % 1-^{13}C, 88 atom % ^{18}O–75% ^{18}O$_2$ and 25% ^{18}O$_1$), 0.50 M HCl, 4.5 mL of deuterium oxide, and no salt (total volume 5.0 mL). The reaction was followed at 18 °C for 8 half-lives. The stacked plot contains 12 of the 40 spectra taken; the time each spectrum was recorded is given at the side. The carboxyl carbon resonance positions of the three isotopic forms are separated by 0.025 ppm (per ^{18}O).

By definition, the total percentage of oxygen-18 content, C_T, present at any time t is

$$C_T = 100\sum_{n=0}^{2} nC_n(t) = 100[C_1(0) + 2C_2(0)]e^{-0.50kt} \qquad (5)$$

A plot of the natural logarithm of C_T against t will give a slope equal to $-0.50k$, and the pseudo-first-order rate constant, k, may be calculated. The theoretical curves based on this value of k may be calculated for each of the isotopic species present, using eq 4 in order to compare them to the experimental data.

Results

The upfield isotope shift exhibited by acetic acid is 0.025 ± 0.001 ppm (per ^{18}O) at pH 2.0 and 0.027 ± 0.001 ppm (per ^{18}O) at pH 8.0 in deuterium oxide, relative to the resonance position of the unlabeled (^{16}O) species. Figure 1 illustrates the time course of a typical reaction followed on an NTC-200 spectrometer operating at 50.3 MHz. Figure 2 exemplifies the relationship between the experimental data and the theoretical curves calculated for the exchange reaction, using the procedure described in the Experimental Section. The concentrations of the isotopic species are expressed in terms of percentages which are in turn obtained by multiplying the proportions calculated from eq 4 by 100. Table I summarizes the kinetic data obtained for the medium carboxyl(oxygen)–water exchange reaction of [1-^{13}C,^{18}O$_2$]acetic acid. The standard deviation in k from the least-squares analysis of eq 5 was typically ±2% and in no case greater than ±5%. Figure 3 shows the effect of increasing sodium chloride or sodium perchlorate concentrations on the value of the rate constant.

Discussion

The magnitude of the ^{18}O-isotope-induced shift on the ^{13}C resonance position of the carboxyl carbon of acetic acid agrees with the isotope shifts observed previously for saturated carboxylic acids.[2,9] The carboxyl carbons of the 7-carboxynorbornene ep-

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[9] A preliminary observation of an ^{18}O-isotope-induced shift of a carboxyl carbon ^{13}C resonance signal in ^{18}O-labeled glutamic acid was made by Hackney, D. D.; Sleep, J. A.; Rosen, G.; Hutton, R. L.; Boyer, P. D. "NMR and Biochemistry-A Symposium Honoring Mildred Cohn", Opella, S. J.; Lu, P., Eds.; Marcel Dekker: New York, 1979; pp 299–300. They observed that the separation between the ^{18}O$_1$ and ^{18}O$_2$ isotopic species was 0.02 ppm.

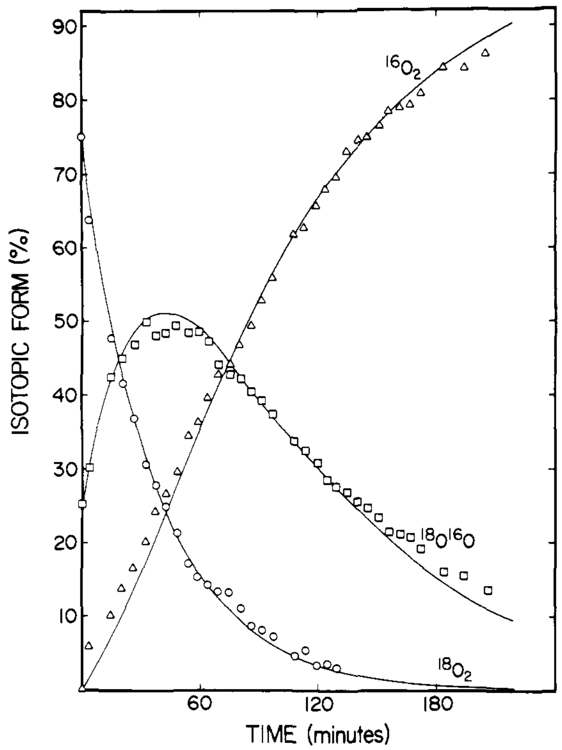

Figure 2. The experimental data points for the reaction illustrated in Figure 1 together with the theoretical curves for the acid-catalyzed medium carboxyl(oxygen)–water exchange reaction (assuming effective irreversibility of the reaction) are presented graphically here and show excellent agreement. The concentrations of the isotopic species are given as percentages. The theoretical curves were calculated from eq 4 (see text) for a pseudo-first-order rate constant of 4.33 × 10^{-4} s^{-1}.

Table I. Acid-Catalyzed Medium Carboxyl(oxygen)–Water Exchange Reaction Data for [1-^{13}C,$^{18}O_2$]Acetic Acid at 32 °C

salt	[salt], M	pH	$10^5 \times k$, s^{-1}
none	0.00	1.60	15.8
NaCl	0.05	1.60	13.8
	0.10	0.90	70.7
		1.37	18.3
		1.60	11.0
	0.50	1.60	9.42
	1.00	0.37	225.
		0.81	60.7
		1.00	52.9
		1.21	25.2
		1.41	15.3
		1.60	8.84
		2.00	3.11
		2.24	1.71
$NaClO_4$	0.05	1.60	15.8
	0.10	1.60	14.0
	0.50	1.60	10.6
	1.00	1.60	7.39
	2.00	1.60	3.75
	2.73	1.60	3.18

imers[2] were shifted upfield the same magnitude as acetic acid at pH 2.0, 0.025 ppm (per ^{18}O). In the case of the carboxylate ion, the carboxyl carbon of sodium formate was shifted upfield 0.025 ppm (per ^{18}O), which compares to the shift observed for acetic acid at pH 8.0 of 0.027 ppm (per ^{18}O). These values are the same within experimental error. The ^{13}C resonance position of the aromatic carboxylic acid benzoic acid was shifted upfield a significantly greater amount, 0.031 ppm (per ^{18}O). The additivity of the isotope effect[3] is demonstrated again by acetic acid. On the NMR time scale the two oxygen atoms of the carboxylic acid are equivalent, whether the acid or the carboxylate ion is being observed. Therefore, substitution by two equivalent isotopic oxygen atoms results in a shift of the carboxyl carbon ^{13}C NMR signal upfield by identical amounts upon each isotopic substitution.

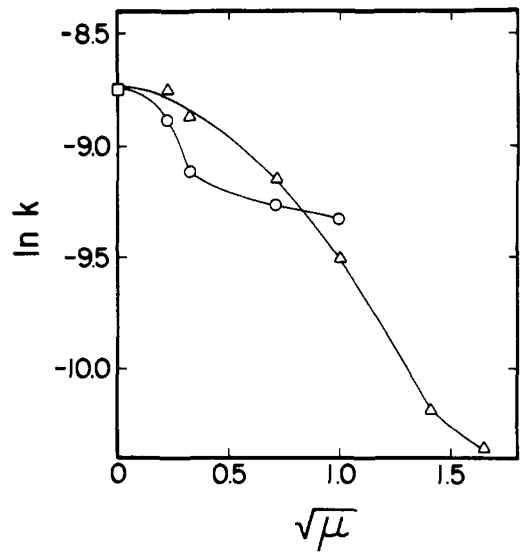

Figure 3. The effect of salt concentration on the rate of medium carboxyl(oxygen)–water exchange for acetic acid is illustrated. The natural logarithm of the pseudo-first-order rate constant is plotted as a function of the square root of the ionic strength due to added salt for the exchange reactions followed at pH 1.60 and 32 °C. Symbols: no salt, □; sodium chloride, ○; sodium perchlorate, △.

The applicability of the ^{18}O-isotope effect in ^{13}C NMR spectroscopy to the study of kinetic reactions is nicely illustrated in Figure 1. Initially the sample of acetic acid contained a total of 88% ^{18}O in the form of 75% $^{18}O_2$ and 25% $^{18}O_1$ species. The rapid disappearance of the $^{18}O_2$ species can be easily followed, while the concentration of the $^{18}O_1$ species increases rapidly at first, and then slowly decreases. Finally, the concentration of the unlabeled ($^{18}O_0$) species can be seen to increase gradually. Figure 2 utilizes the data contained in the spectra of Figure 1 and shows that the theoretical curves calculated for a simple sequential exchange process fit the experimental data closely. The assumption of effective irreversibility of the isotope exchange reaction is justified by the correlation between the theoretical curves and the experimental data points shown in Figure 2. This effective irreversibility is a consequence of the large molar excess of solvent, unlabeled (^{16}O) water, compared to the much smaller molar concentration of labeled (^{18}O) water present during the reaction. The labeled water is effectively diluted out in the reaction such that the probability that labeled water will enter into the exchange reaction to a measurable extent is for practical purposes zero.

Such experimental studies of oxygen exchange reactions do not require high-field NMR instrumentation. The data in Table I were obtained exclusively from measurements on a Varian CFT-20 spectrometer. The relative concentrations of the three isotopic species (as fractional proportions) were calculated directly from the peak-height measurements. This method of measuring the relative concentrations of isotopic species is very convenient and appears to be warranted in the present case. The substitution of ^{18}O for ^{16}O does not measurably change the relaxation times of the ^{13}C nucleus under study and therefore the shape of the lines will be identical for each of the isotopic species. Even on the CFT-20 instrument the peaks are adequately resolved from one another. As a result, the peak heights of the isotopic species are directly proportional to the areas under the peaks and thus to the concentrations of the isotopic species. This mathematical relationship was tested experimentally. Electronic integration of the areas under the peaks is done only with difficulty on this instrument due to the small magnitude of the isotope effect. However, three reactions were electronically integrated and the results obtained were identical with the results calculated from peak-height measurements. The correlation with peak-height measurements was tested further by resolving the spectral data for the same three reactions, using a DuPont 310 curve resolver set for Lorentzian curves; no measurable difference in the kinetic data was apparent when compared with results obtained by peak-height measurements. Finally, electronic integration (as well as spectrum deconvolution) can be done easily on an NTC-200 spectrometer and the kinetic results obtained by integration analysis agreed with the results obtained from the measurement of peak heights.

The pH–rate profile of the data (Table I) at 1.0 M NaCl, 32 °C [log k vs. pH: 8 points, slope −1.13, y intercept −2.23, correlation coefficient 0.998], is a typical linear plot observed for such reactions catalyzed by hydrogen ion. A value for the slope of the log k vs. pH plot which differs significantly from −1.0 is not consistent with a simple first-order dependence on hydrogen ion as would be expected for specific acid catalysis by hydrogen ion. However, such deviations from simple first-order kinetics have been observed for other medium carboxyl(oxygen)–water exchange reactions occurring in salt solutions.[6] The magnitude of the deviation appears to be a function of both salt concentration and temperature and is not yet adequately explained.

The data presented in Table I clearly show that the salt effect of both sodium chloride and sodium perchlorate on the medium carboxyl(oxygen)–water exchange reaction at pH 1.60 is negative. Increasing the salt concentration results in a decrease in the observed rate of exchange. There are definite differences in the effects caused by the two salts, however, which are exemplified (Figure 3) in plots of the natural logarithm of the pseudo-first-order rate constant as a function of the square root of the ionic strength (due to the salt concentration). The rate constant in sodium chloride solution initially decreases more rapidly than it does in solutions of sodium perchlorate, but with the former salt the rate constant begins to level off at a concentration of ca. 0.5 to 1.0 M (Figure 3). On the other hand, the rate constant in sodium perchlorate solution continues to decrease such that even at the highest concentration studied (2.73 M) little or no leveling off can yet be detected (Figure 3).

The physical basis of the salt effect on this reaction is probably complex. The dissociations of acetic acid[10] and of water[11,12] exhibit maxima upon addition of a neutral salt. Analogously the activity coefficient of acetic acid exhibits a minimum,[13] although the activity coefficient of the undissociated part of most weak electrolytes increases as the salt concentration is increased.[14] The activity of water decreases exponentially with increasing concentrations of either acid or salt[15] whereas the activity coefficients of the acids, HCl and $HClO_4$, and the salts, NaCl and $NaClO_4$, exhibit minima with increasing acid or salt concentrations. The concentration of the acid or salt at which the activity coefficient is at a minimum varies with the acid or salt. For example, a minimum value for sodium chloride is reached at ca. 1.3 m while the minimum is not reached until the concentration of sodium perchlorate is ca. 2–2.5 m.

In the present study, we find that the rate constant for exchange is proportional to the square root of the product of the activity of the water and the activity coefficient of the salt. There is some evidence to support this observation. O'Connor and Turney[7] studied the medium carboxyl(oxygen)–water exchange of acetic acid in concentrated mineral acids and found that the rate of exchange could be explained if the activity of the solvent water and the solute acid were taken into account. Also the solvolysis of 1-acetylimidazole[16] was depressed by concentrated solutions of acid or neutral salts. In this latter instance the acidity function was found to increase more rapidly than the concentration of the hydronium ion and this in effect resulted in a decreased availibility of water in the concentrated salt solutions. Therefore, the rate of medium carboxyl(oxygen)–water exchange of acetic acid appears to depend on the activity of the solvent and the salt; the activity of the acetic acid does not significantly affect the rate of exchange.

We have thus demonstrated the applicability of the ^{18}O-isotope effect in ^{13}C NMR spectroscopy to the study of a significant kinetic problem involving an oxygen exchange reaction. The technique offers several distinct advantages over mass spectrometry for following such reactions, primarily with respect to the ease of analysis, accumulation of data, and continuity of assay. The use of ^{13}C-enriched compounds further decreases data acquisition time and reduces the amount of material necessary for study of the reaction. In the present example we studied the medium carboxyl(oxygen)–water exchange reaction of [1-^{13}C,$^{18}O_2$]acetic acid catalyzed by acid as a function of both mineral acid concentration and salt concentration. We found that an increase in salt concentration resulted in a decrease in the rate of the exchange reaction for each of the salts studied. This result conclusively demonstrates the effect that increasing salt concentration exerts on the rate of medium carboxyl(oxygen)–water exchange for acetic acid, an effect which had been reported to be both positive and negative. The magnitude of the decrease in the rate does depend on the nature of the salt and on its concentration, with sodium perchlorate causing a much greater decrease than sodium chloride. The decrease in rate appears to result in part from a decrease in the activity of water.

The ^{18}O-isotope effect in ^{13}C NMR spectroscopy is already proving applicable to a number of areas of investigation. For example, the technique has been applied to the study of phase-transfer-catalyzed nucleophilic reactions at metal carbonyl derivatives.[17] In addition, the origin of the oxygen atoms in averufin synthesized by *Aspergillus parasiticus* was elucidated by using the isotope-induced shifts in the ^{13}C NMR signals of averufin.[18] An interesting example of an application of the technique has been provided by Brandänge et al.[19] The alkaline hydrolysis of an epoxide ring was found to have taken place by nucleophilic attack at the most substituted carbon atom, an unexpected regioselectivity. This allowed Brandänge et al. to ascribe the correct stereostructure to a methylcitric acid, correcting a previously published interpretation. Experimental approaches such as the present one, not requiring elaborate NMR instrumentation but still conveniently providing accurate data, should stimulate further interest in this approach to the study of oxygen exchange reactions. Among the areas where such measurements may be made by NMR spectroscopy might be mentioned many metal-ion-catalyzed oxygen-exchange reactions (depending of course on the magnetic properties of the metal ion), and enzyme-catalyzed oxygen-exchange reactions involving proteases and peptidases such as the serine proteases or carboxypeptidase.

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