A Precise N.M.R. Method for Measuring Isotope Effects on Acid–Base Equilibria: Effects of Solvent, Solvent Isotopic Composition, and Ionic Strength on Hydrogen and Oxygen Isotope Effects in Formic Acid

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Measurement of the isotope effect, Δ, on an n.m.r. chemical shift, as a function of the extent of ionisation, is the basis of a very sensitive method illustrated with formic acid for measuring isotope effects on mobile acid–base equilibria.

The pH dependence of the chemical shift, δ, of a nucleus with $I = 1/2$ at an appropriate site in an acid, HA, has been used[1] as a convenient method for determining K_a for a mobile equilibrium $HA \rightleftharpoons H^+ + A^-$ using equation (1),

$$pK_a = pH + \log(\delta - \delta_0)/(\delta_1 - \delta) \qquad (1)$$

where the chemical shifts in the anion A^- (δ_0) and in the acid HA (δ_1) are measured for solutions with pH much greater (for δ_0) and much less (for δ_1) than the value of pK_a. The accuracy of this method is mainly limited by errors in the measurement of the pH of n.m.r. samples. If two acids HA and HA′ are present in the same solution then pH may be eliminated from equation (1) and its analogue for HA′ to give, after rearrangement, equation (2),

$$\Delta n = \Delta_0 + Rn(\delta_0 - \delta_1 + \Delta_1 - \Delta_0)/(Rn + 1 - n) - n(\delta_0 - \delta_1) \qquad (2)$$

where Δn is a difference in chemical shifts between sites in the two acids in a solution for which $n = (\delta - \delta_0)/(\delta_1 - \delta_0)$, *i.e.*, n is the fraction of the stoicheiometric concentration of the acid HA present as the free acid, and $R (= K_a/K_a')$ is the ratio of dissociation constants. If Δn is measured as a function of n then non-linear curve fitting (varying Δ_0, Δ_1, and R) allows R (and hence ΔpK_a) to be measured with accuracy provided Δ_0 ($= \delta_0 - \delta_0'$) and Δ_1 ($= \delta_1 - \delta_1'$) are effectively constant, *i.e.*, if the solutions are suitably dilute, and R is near unity. In particular, if the two acids differ only isotopically so that Δ is always small (using chemically equivalent sites in HA and HA′) then the use of equation (2) allows many ΔpK_a values to be measured routinely to better than ±0.0001 unit. In the present work, using ^{13}C n.m.r. with isotopically enriched samples, this accuracy is adequate for equilibrium isotope effects (e.i.e.s) to be measured for carbon, nitrogen, and oxygen, as well as for hydrogen. Here, we illustrate this method with a detailed study of hydrogen and oxygen e.i.e.s on the K_a of formic acid (^{13}C, 60 atom % and *either* $^{1/2}H$, *ca.* 2:3, *or* $^{16/18}O$, *ca.* 1:1, randomly distributed). Figure 1 shows the oxygen e.i.e.s on the carbon chemical shift as a function of the extent of ionisation of $H^{13}CO_2{}^2H$ in ^2H_2O at 298 K ($R = 1.0144 \pm 0.0001$, $\Delta pK_a = 0.00623 \pm 0.00005$ for one isotopic substitution by oxygen-18).

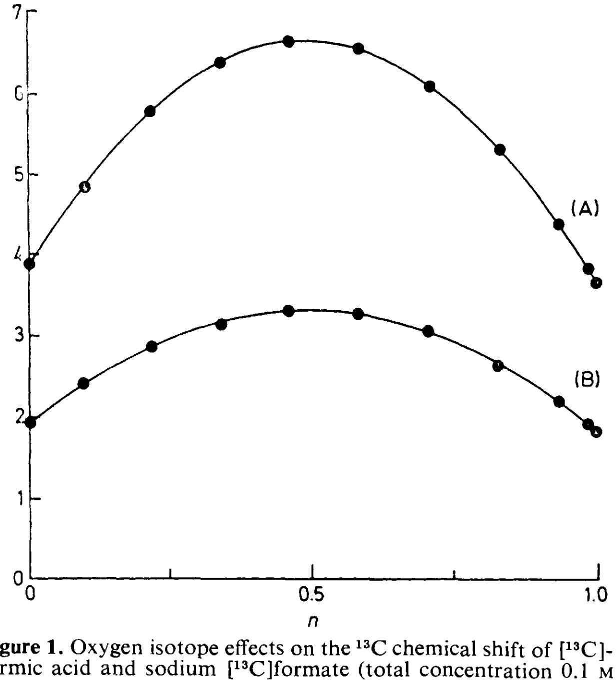

Figure 1. Oxygen isotope effects on the ^{13}C chemical shift of [^{13}C]-formic acid and sodium [^{13}C]formate (total concentration 0.1 M in ^2H_2O at 298 K) as a function of the fraction (n) of free acid. Curve B is for isotopic substitution at one oxygen, and curve A for two oxygens. (The radii of the circles are twice the r.m.s. residual from non-linear least squares curve fitting.)

The published e.i.e.s for hydrogen in formic acid have been measured in ordinary water and we commonly use D_2O as a solvent providing a convenient field-frequency lock. Accordingly we have measured the e.i.e.s as a function of the *isotopic composition of the solvent*, finding that ΔpK_a varies linearly with the mole fraction (n_s) of D in the water, as shown in equations (3) and (4). The hydrogen e.i.e. in ordinary water,

$$\text{E.i.e. for hydrogen: } \Delta pK_a = 0.03276 + 0.00127 n_s \text{ (at 298 K)} \qquad (3)$$

$$\begin{aligned} \text{E.i.e. for one oxygen: } \Delta pK_a &= 0.00476 + 0.00153 n_s \text{ (at 298 K)} \\ \text{two oxygens: } &= 0.00953 + 0.00306 n_s \end{aligned} \qquad (4)$$

Table 1. Equilibrium isotope effects on the ionisation of [^{13}C]-formic acid in deuterium oxide at 298 K and ionic strength 0.1 M for hydrogen (^1H and ^2H) and oxygen (^{16}O and ^{18}O) isotopic substitutions. Values for two oxygen isotopic substitutions are given in parentheses under the corresponding values for a single isotopic substitution.

Species[a]	Cation	R[b]	ΔpK_a[c]
$\overset{*}{H}{}^{13}CO_2H$	Na^+	1.0816	0.03407
	Na^+	1.0768[d]	0.03217[d]
$H^{13}\overset{*}{C}O_2H$	Na^+	1.0144	0.00623
		(1.0291)	(0.01248)
	Na^+(1 M)[e]	1.0146	0.00630
		(1.0295)	(0.01266)
	Li^+	1.0144	0.00624
		(1.0291)	(0.01247)
	Cs^+	1.0141	0.00612
		(1.0290)	(0.01242)

[a] Site of isotopic substitution marked with an asterisk. [b] $R = K_a/K_a'$. [c] $\Delta pK_a = pK_a' - pK_a$; error from non-linear least squares curve fitting ±0.00005 or better: reproducibility ±0.00010 or better. [d] Run at 320 K. [e] Run in 1 M NaCl in D_2O.

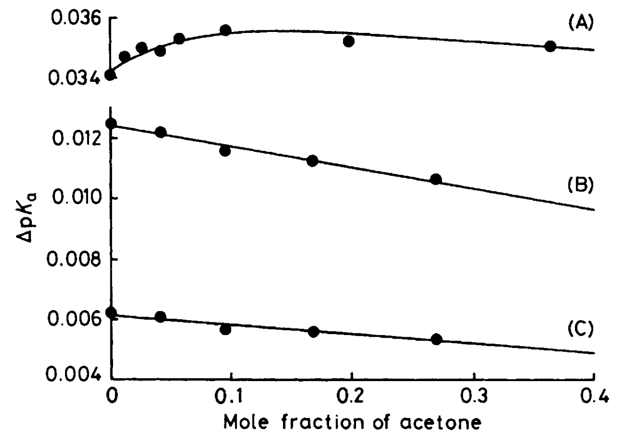

Figure 2. Equilibrium isotope effects (ΔpK_a) for hydrogen and oxygen isotopic substitutions in [^{13}C]formic acid as functions of solvent composition for ^2H_2O–acetone mixtures. The oxygen isotope effects (B and C) are linear functions of the mole fraction of acetone. An arbitrary smooth curve (A) has been drawn through the data for hydrogen isotopic substitution. (The radii of the circles equal twice the standard errors of the data.)

corrected for the small temperature difference, agrees well with the most recent value.[2] The oxygen e.i.e., which is small compared with the hydrogen e.i.e., shows a remarkably large *fractional* change between H_2O and D_2O, increasing by 30% and dramatically demonstrating the importance of solvation of the anion. It is notable that (a) the e.i.e.s for oxygen are practically independent of the metal ion and of ionic strength, (b) the temperature dependence is small so that errors in the measurement of temperature need only be less than *ca.* 0.5 K in order to be negligible, even for 'large' e.i.e.s, (c) the oxygen e.i.e.s for the first and second isotopic substitutions are equal (see Table 1).

The e.i.e.s were also measured as a function of solvent composition for D_2O–acetone mixtures (0—0.40 mole fraction [$^2H_6$]acetone) (Figure 2). The hydrogen e.i.e. rises quite rapidly at low concentrations of acetone, and reaches a maximum at about 20 mole% before falling gradually at higher concentrations. In contrast the oxygen e.i.e. shows a monotonic, nearly linear decrease with increasing concentration of acetone, suggesting a different origin for this solvent effect.

The advantages of the present method of measuring e.i.e.s on acid–base equilibria are as follows. (a) The extent of isotopic substitution need not be high, nor known, nor even constant throughout the experiment. (b) The two isotopic species, being in the same solution, are compared under identical conditions. (c) The potential sensitivity is very high (better than ±0.00005 unit) using ^{13}C chemical shifts for carboxy groups (typically 5 p.p.m.) at 7 Tesla with a 32 K data table and small (*e.g.*, 1 p.p.m.) sweep-widths.

The method, as illustrated here with formic acid, has certain limitations, notably the need for: (a) ^{13}C enrichment if dilute solutions are to be used, as is important if hydrogen decoupling is to be used at high fields; (b) a substantial difference in chemical shift between the acid and the anion; (c) a large enough isotope effect Δ on the chemical shift, in relation to the line-width of the signals at the field strengths available, to give well separated signals; (d) sufficiently rapid proton exchange. The first is a minor problem for many compounds of interest, the second is only important when very high sensitivity is needed, and the third may be circumvented by the use of an auxiliary isotopic substitution, which will be illustrated in a future paper. The fourth limitation is not a problem for carboxylic acids or ammonium ions but is sometimes serious (*e.g.*, for HCN[3]).

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1 See, *e.g.*, J. J. Led and S. B. Petersen, *J. Magn. Reson.*, 1979, **33**, 603.
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