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Proton Inventory Study of the Base-Catalyzed Hydrolysis of Formamide. Consideration of the Nucleophilic and General Base Mechanisms

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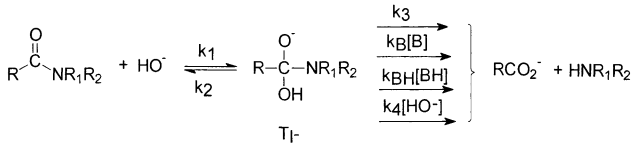
Abstract: An NMR study of the rates of hydroxide-promoted hydrolysis of formamide in aqueous media of varying mole fraction D_2O (n) was performed at $[LO^-] = 1.42$ M, $T = 25$ °C, to shed light on whether the mechanism involves a nucleophilic attack of HO^- on the C=O or HO^- acting as a general base to remove a proton from an attacking water. The solvent deuterium kinetic isotope effect under these conditions is inverse, $k_{OH}/k_{OD} = 0.77 \pm 0.02$ or $k_{OD}/k_{OH} = 1.30 \pm 0.03$. Proton inventory analysis of the k_n versus n data was undertaken through NLLSQ fits to equations representing four possible mechanisms encompassing nucleophilic and general base ones with waters of solvation on the attacking hydroxide, and with or without waters of solvation on the developing amide hydrate oxyanion. Both nucleophilic and general base mechanisms can be accommodated, but there are restraints on each that are discussed. The preferred mechanism is a nucleophilic one proceeding through a transition state having two solvating waters remaining on the attacking hydroxide and three additional waters attached to the developing amide hydrate oxyanion.

Introduction

The mechanisms for acid- and base-catalyzed hydrolysis of simple amides and esters have received much attention due to their relevance to various biological processes, and in general it can be said that these mechanisms are among the best understood of any chemical process.[1] The generally accepted mechanism for the base-catalyzed hydrolysis involves the process depicted in Scheme 1, wherein a hydroxide nucleophile reversibly adds to the C=O to produce an anionic tetrahedral intermediate (T_I^-). This can decompose to products via at least four pathways involving a water-promoted spontaneous reaction, and those promoted by acidic and basic components of buffers, or a second hydroxide. This mechanism is supported by ^{18}O=C exchange experiments, which show the reversible formation of T_I^- and solvent deuterium kinetic isotope experiments (dkie) of various amides, which indicate that k_{OH}/k_{OD} is generally unity or slightly inverse, consistent with a direct nucleophilic attack of hydroxide.[1] A number of studies of ester saponification also demonstrated inverse dkie values[1d,2] in the range of $k_{OD}/k_{OH} = 1.3-1.4$, consistent with the widely held view that DO^- is a better nucleophile in D_2O than is HO^- in H_2O.

Marlier and co-workers[3] recently reported an interesting study of the heavy atom kinetic isotope effects for the hydrolysis of formamide. They concluded that the ^{18}O-isotope effects observed for basic hydrolysis in a medium containing ^{18}O-labeled water are nicely accommodated by a mechanism that involves the HO^- acting as a general base (GB) to remove a proton from one of its waters of solvation during formation of T_I^-. A GB mechanism was also proposed in Marlier's earlier report of the heavy atom isotope effects for the alkaline hydrolysis of methyl formate[4] and was supported by a more recent analysis of a proton inventory study of the hydrolysis of ethyl acetate reported by Mata-Segreda.[5] Interestingly, a very recent heavy atom isotope effect study of the base-catalyzed hydrolysis of the phosphodiester, thymidine-5′-*p*-nitrophenyl phosphate, indicates a nucleophilic role for the hydroxide and that the dkie is slightly inverse at $k_{OD}/k_{OH} = 1.11$, suggesting a different behavior toward HO^- from formamide and ethyl acetate.[6] Nevertheless, the GB mechanism seems attractive in that it does not require desolvation of $HO^-(H_2O)_3$ to form $HO^-(H_2O)_2$ with an available pair of electrons on the hydroxide which would be required for direct nucleophilic attack. Such a mechanism could be a potentially important process and certainly deserves additional

Scheme 1



(1) (a) Brown, R. S.; Bennet, A. J.; Ślebocka-Tilk, H. *Acc. Chem. Res.* **1992**, *25*, 481. (b) Bennet, A. J.; Brown, R. S. Physical Organic Chemistry of Acyl Transfer Reactions. In *Comprehensive Biological Catalysis: A Mechanistic Reference*; Sinnott, M., Ed.; Academic Press: New York, 1997; Vol. 1, pp 293−326. (c) Brown, R. S. Studies of the Hydrolysis of Amides. In *The Amide Linkage: Selected Structural Aspects in Chemistry, Biochemistry, and Materials Science*; Greenberg, A., Brenneman, C. A., Liebman, J. F., Eds.; Wiley-Interscience: New York, 1999; pp 85−114. (d) Kirby, A. J. In *Comprehensive Chemical Kinetics*; Bamford, C. H., Tipper, C. F. H., Eds.; Elsevier: Amsterdam, 1972; Vol. 10, pp 57−207.
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(3) Marlier, J. F.; Dopke, N. C.; Johnstone, K. R.; Wirdzig, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 4356.
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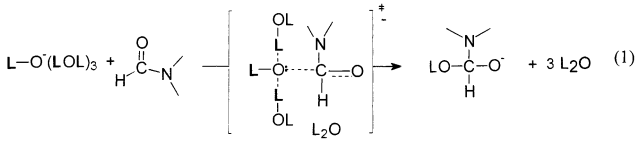
investigation because it is phenomenologically quite different from the normal direct nucleophilic attack mechanism.

Recently we reported some studies on the hydrolysis of formamide wherein we determined the activation parameters for the acid and base processes[7] as well as the experimental values for the activation parameters and rate constant for the water reaction ($k_w = 1.1 \times 10^{-10}$ s^{-1} ($t_{1/2} = 199$ years) at 25 °C), which was previously reported as being important at 80 °C by Hine and co-workers.[8] As part of that study,[7] we also determined an inverse dkie for the hydroxide reaction, $k_{OH}/k_{OD} = 0.77 \pm 0.06$ at $[OL^-] = 1.47$ M, the inverse value being consistent with what is observed for other simple amides and esters.[1] Thus, our preferred direct nucleophilic mechanism for the base-catalyzed hydrolysis of formamide seems at variance with the Marlier[3,4] and Mata-Segreda[5] analyses and suggested to us that further studies with formamide were required to resolve this important dilemma.

The main difference between the nucleophilic and general base-catalyzed processes stems from consideration of the respective simplified transition states (TS **1** and **2**) shown in eqs 1 and 2 (L = H, D). In either mechanism, the reactant state



is the same, a hydroxide with three solvating waters plus formamide. In eq 1, the TS incorporates a hydroxide having one of its solvating waters removed to expose the nucleophile lone pair, while the TS shown in eq 2 incorporates a proton in flight between hydroxide and the attacking water. The bold **L** protons are those undergoing changes in their bonding. Each process explicitly shows the solvating waters of the hydroxide, but for the moment ignores the solvation of the developing (−) on the carbonyl oxygen which, in either mechanism, should be the same. The proton in flight in TS **2** should be subject to a primary dkie, but there is no equivalent in TS **1**, so it seems likely that these two processes should be distinguishable on the basis of detailed dkie experiments, a distinction that was noted by Mata-Segreda for his proton inventory studies with ethyl acetate.[5] In an attempt to determine which of these two mechanisms applies to formamide, we have conducted proton inventory analyses of a series of kinetic experiments on its base-catalyzed hydrolysis in media of varying mole fraction of D_2O. The following describes our findings.

Experimental Section

(a) Materials. H_2O was made free from dissolved CO_2 and stored under Ar. D_2O (CDN Isotopes, 99.9 atom % D) was used as supplied, as was formamide (99.5+%, A.C.S. reagent grade, Aldrich).

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Table 1. Second-Order Rate Constants (k_n) for Base-Catalyzed Hydrolysis of Formamide in Aqueous Media of Different Mole Fraction (n) D, $T = 25$ °C, $[LO^-] = 1.42$ M

n	$10^3 \times k_n$ (M^{-1} s^{-1})[a]	n	$10^3 \times k_n$ (M^{-1} s^{-1})[a]
0.03	3.17 ± 0.05	0.62	3.68 ± 0.05
0.103	3.18 ± 0.04	0.72	3.85 ± 0.03
0.21	3.22 ± 0.04	0.83	4.00 ± 0.16
0.31	3.48 ± 0.04	0.93	3.99 ± 0.04
0.41	3.54 ± 0.03	1.0	4.13 ± 0.03
0.465	3.57 ± 0.03	0.03[b]	(3.07 ± 0.1)
0.52	3.55 ± 0.04	1.0[b]	(4.03 ± 0.1)
0.52	3.67 ± 0.05		

[a] Errors determined from the standard deviations of the linear regressions of the plots of the integrated intensities of the H−C(=O)−X NMR versus time data (see Experimental Section). [b] Data at 27 °C as determined from ref 7.

(b) Kinetic Experiments. The rates of hydrolysis of formamide were determined by ^{1}H NMR analyses at 25 ± 0.2 °C using a Bruker DMX−Avance 500 spectrometer equipped with a broadband inverse probe. The ^{1}H NMR spectra in water were accumulated using a standard presaturation water suppression technique. Solutions of base (1.47 M) in either H_2O or D_2O were prepared under CO_2-free conditions and stored under Ar. Base concentrations were determined by titration with standardized 1.0 and 0.5 N HCl, phenolphthalein indicator. In pure water, the formamide H−C(=O) signal at δ 7.55 appears as a doublet ($J = 14.9$ Hz) coupled to one of the NH protons, while the two NH protons appear as two broad triplets coupled to ^{14}N. The one coupled to the formamide proton appears at δ 7.17 ($J_{N-H} \approx 60$ Hz), while the other NH appears as a sharper triplet at δ 7.55 ($J_{N-H} \approx 62$ Hz).

Solvent deuterium kinetic isotope experiments were undertaken in D_2O/H_2O mixtures, the mole fraction D varying from ~0 to 1. The solutions for individual runs were prepared by adding precise volumes of base ($[NaOL]_i = 1.47$ M, total volume 0.60 mL) and were thermostated in the spectrometer probe at 298 K for ~10 min after which 20 μL of a 10^{-2} M formamide/D_2O solution was added, $[NaOL]_{final} = 1.42$ M. The pseudo-first-order rate constants (k_{obs}) for formamide hydrolysis were obtained by observing the rate of increase of the intensity of the signal at δ 8.46 attributable to the O=C−H proton of the formic acid formed (A) and the decrease of the intensity of the signal at δ 7.55 for that of formamide (B). NMR data were acquired continuously, and every 16 scans were summed separately (the time being recorded as the midpoint of the number of scans utilized). This process was repeated up to at least two hydrolysis half times. Pseudo-first-order rate constants (k_{obs}) were evaluated from the slopes of the $\ln(A/(A + B))$ versus time plots with the errors in k_{obs} being determined as the standard deviation of the linear regression lines. Between 15 and 20 experimental points were used for each plot.

Results and Discussion

Shown in Table 1 are the second-order rate constants for lyoxide-catalyzed hydrolysis of formamide at 25 ± 0.2 °C ($k_n = k_{obs}/[LO^-]$, $[LO^-]$ is 1.42 M, corrected for the additional 20 μL of D_2O in which the formamide is added) at different mole (n) fractions D. Also included in the table are two previous values for the hydrolysis in pure H_2O and D_2O at 27 °C reported in our preliminary work.[7] Error limits presented in the table are derived from the standard deviations of the linear regressions of the $\ln(A/(A + B))$ versus time plots to determine k_{obs}, where A and B, respectively, are the integrated NMR intensities of the formate and formamide H−C(=O)− protons. The 25 °C data are graphically presented in Figure 1, which, upon cursory inspection, form a rather featureless straight line relationship without a definite curvature upward or downward. The best-fit linear regression has a slope of $(1.02 \pm 0.05) \times 10^{-3}$, $r^2 =$

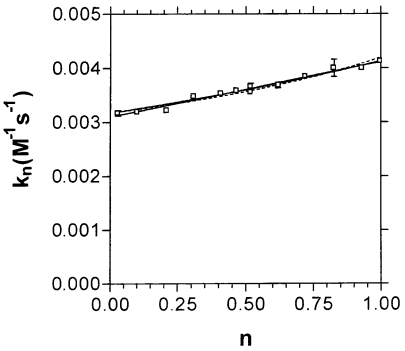
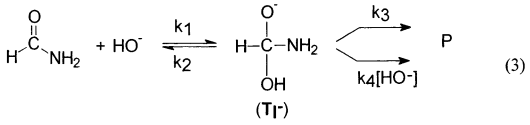


Figure 1. Plot of the second-order rate constants for base-catalyzed hydrolysis of formamide as a function of mole fraction of D_2O (n). Dashed line, NLLSQ fit of the data to eq 15; solid line, fit of the data to eq 16. Best fit parameters are given in Table 3.

0.9724. Also included in the figure are two lines relating to NLLSQ[9] fits to eqs 15 and 16 described below.

Rate Constants at $n \approx 0$ and 1.0. Marlier[3] and, earlier, Kirsch[10] have shown that the rate law for alkaline hydrolysis of formamide contains both first- and second-order terms in hydroxide, which is analyzed in terms of the mechanism presented in eq 3 for which steady-state analysis gives the expression in eq 4. Kirsch's analysis[10] of the secondary DKIE



(3)

for hydroxide attack on $HC(O)NH_2$ and $DC(O)NH_2$ further indicates that the transition state for the addition of hydroxide must be very late.

$$k_{hyd} = \frac{k_1[HO^-](k_3 + k_4[HO^-])}{k_2 + k_3 + k_4[HO^-]} \qquad (4)$$

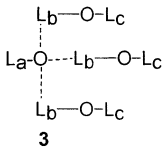
The mechanism shown in eq 3 is a simplified version of the general one for amide hydrolysis presented in Scheme 1, but without buffer components. Graphical analysis[3,10] of the partitioning of the tetrahedral intermediate (T_{I^-}, eq 3) indicates that the k_3/k_2 ratio is 1.05, while the k_4/k_2 ratio is 2.15 M^{-1}. At low $[HO^-]$ (<0.1 M), the pathway second order in $[HO^-]$ is relatively unimportant, and T_{I^-} undergoes substantial reversal, so k_{hyd} is approximated as $k_1[HO^-]k_3/(k_2 + k_3)$. However, at high $[OH^-]$, the tetrahedral intermediate is driven forward via rapid capture by the second hydroxide, so reversal becomes less important, and attack ultimately becomes the rate-limiting step with k_{hyd} being approximated as $k_1[HO^-]$.[11] Under the present conditions at $[LO^-] = 1.42$ M, there is a significant inverse kinetic isotope effect, $k_{OH}/k_{OD} = 0.77 \pm 0.02$, which can thus be taken as the solvent dkie largely on k_1. We note that these conditions are substantially the same as those used previously for determination of the solvent oxygen nucleophile isotope effect (1.7 M) where "the attack of nucleophile is largely rate-determining".[3]

Proton Inventory Analysis. The proton inventory technique[12] provides information about the number of protons undergoing a significant change in bonding in the transition state (TS) relative to the ground state. Equation 5, sometimes referred to as the Gross−Butler equation, expresses the relationship between the rate constant observed in mixtures of L_2O (L = H, D) with known isotopic composition and the fractionation factors (ϕ) for the exchangeable protons; n is the atom fraction of D in the medium, while i and j represent the contributing hydrogens in the transition and reactant states.

$$k_n = k_o \prod_i^{TS} (1 - n + n\Phi_i) / \prod_j^{RS} (1 - n + n\Phi_j) \qquad (5)$$

The fractionation factors for hydrogens refer to the tightness of their bonding and are significantly less than unity for H's being transferred or "in flight" between O and N, or O and O, as part of the rate-limiting step. In these cases, normal primary dkie's of $k_H/k_D > 1$ are expected unless other compensating factors, such as changes in solvation, are at play. In hydrogen-bonding situations where the overall bonding is loose, the fractionation factors are less than unity, also giving rise to normal dkie's of $k_H/k_D > 1$. The latter contribute secondary effects of solvation and can significantly alter the overall dkie.[13] Generally speaking, plots of k_n versus n have one of three shapes: bowed upward, linear, and bowed downward. The latter two scenarios are analyzed in terms of a single proton, or two or more protons undergoing significant changes in bonding in passing from reactant to transition state.[12]

In analyzing the reactant state, we employ procedures we used before,[1] which follow the suggestions of Gold and Grist[14] for hydroxide solvated by three waters (**3**). All conceivable mech-



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anisms must employ a common reactant state of $L_aO^-(L_b-OL_c)_3$, where L_a, L_b, and L_c have assumed ϕ values of 1.22, 0.7, and 1.0, respectively. We consider the two possible mechanisms shown in eqs 1 and 2 for which the nucleophilic and general base modes of action for hydroxide, in fact, have been considered earlier by Gold and Grist[14] in analyzing various hydroxide destroying reactions. Cursory analysis of the overall inverse dkie for the hydrolysis indicates that at $n = 1.0$:

$$\frac{k_{OD}}{k_{OH}} = \prod_i^{TS} \phi_i/(1.22)(0.7)^3 = \prod_i^{TS} \phi_i/0.42 \qquad (6)$$

Because the observed $k_{OD}/k_{OH} = 1.30$, the product of the fractionation factors in any appropriate TS must be ~0.56. As a starting point for the analysis of the TS fractionation factors for the nucleophilic process, we assume that the initially desolvated hydroxide ($H-O^-(H-OH)_2$) has fractionation factors

(9) Nonlinear least-squares fitting was done using GraphPad Prism Version 2.01, GraphPad Software Inc.

(10) Kirsch, J. F. In *Isotope Effects on Enzyme Catalyzed Reactions*; Cleland, W. W., O'Leary, M. H., Northrup, D. B., Eds.; University Park Press: Baltimore, 1977; pp 100−121.

(11) This analysis is identical to that proposed in ref 3 and is substantially correct. However, at $[HO^-] = 1.42$ M, the partitioning of the tetrahedral intermediate is ~4.1 in favor of product formation relative to reversal. Of that ratio, some 3.1 parts are attributed to the second-order pathway (k_4), and 1 part is attributed to the spontaneous pathway, k_3. Strictly speaking, while the dkie is substantially attributable to the attack step, k_1, there is a small additional component attributable to k_4 which should be inverse given OD^- in D_2O is a stronger base than is HO^- in H_2O.

of 1.22 and 0.7 for the lyoxide and solvating waters, respectively. In principle, if removal of the single water of hydration had led to stronger H-bonding to HO^-, the fractionation factors of the two remaining waters of solvation could have values slightly lower than 0.7, while the value for $\mathbf{H}-O^-$ could be larger than 1.22.[15] However, as discussed by Huskey and Schowen in their analysis of the proton inventory for methoxide attack on phenyl acetate,[16] it seems likely that incipient association of the oxyanion with the polarized >C═O can partially offset the effect of the removal of the solvating water in exposing the lone pair for attack so that the fractionation factors of $H-O^-(H-OH)_2$ should not change as much as if there were no electrophilic stabilization at all. For the intermediate amide hydrate oxyanion, $HC(O^-)(O\mathbf{L})(NL_2)$, we assume the $\mathbf{L}-O$ fractionation factor is 1.0 as is the case for a *gem* diol or hemiacetal,[17] the fractionation factor for the N−L is 1.0, and those for the three waters of solvation of the oxyanion (if present) are 0.7. Chemical intuition assists in assessing acceptable computed values for the various TS fractionation factors because appropriate mechanisms require that the L_a hydroxide proton and the remaining L_b H-bonding protons should assume respective ϕ_{TS} values somewhere between those of the reactant and intermediate states, for example, 1.22 → 1.0 and 0.7 → 1.0, during their transitions to the intermediate $HC(O^-)(O\mathbf{L})(NL_2)$ and L_2O, respectively. For the proton in flight in the GB mechanism, a fractionation factor in the range of 0.5 or less[5,12] is anticipated, suggesting it contributes a primary isotope effect of $k_H/k_D \geq 2$. Inclusion of the 0.5 number in the numerator of eq 6 requires that the product of all remaining ϕ_{TS} values be ~1.1, which places an important limitation on this mechanism because any remaining or additionally acquired waters of solvation must have ϕ_{TS} values intermediate between 0.7 and 1.0. Clearly, values of the ϕ_{TS} for the proton in flight <0.5 require the product of the remaining fractionation factors to be >1.1, creating an even larger constraint on the GB mechanism.

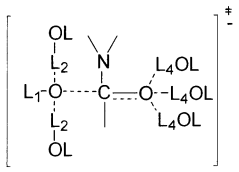
We consider below four mechanistic possibilities. Case a refers to the minimal nucleophilic mechanism of eq 1, while case b refers to the minimal GB mechanism of eq 2, the appropriate formulas for transition states (**1** and **2**) being shown in each equation.

$$k_n = k_o(1 - n + \phi_1 n)(1 - n + \phi_2 n)^2/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (7)$$

$$k_n = k_o\,(1 - n + \phi_1 n)(1 - n + \phi_2 n)^2(1 - n + \phi_3 n)/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (8)$$

In the corresponding proton inventory equations, eqs 7 and 8, ϕ_1 and ϕ_2 refer to the lyoxide $L-O^-$ and its two H-bonding waters, while ϕ_3 in eq 8 refers to the proton in flight.

Case c refers to the nucleophilic mechanism with three solvating waters on the developing alkoxide, while case d is the GB mechanism with additional solvating waters. The proton inventory analyses for these are given in eqs 9 and 10, respectively, corresponding to transition states **4** and **5**. In those equations, the fractionation factors for the H-bonding waters of solvation on the developing alkoxide are given as ϕ_4.



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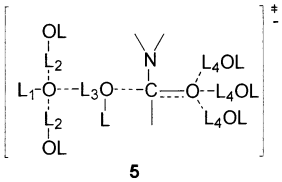

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$$k_n = k_o(1 - n + \phi_1 n)(1 - n + \phi_2 n)^2(1 - n + \phi_4 n)^3/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (9)$$

$$k_n = k_o(1 - n + \phi_1 n)(1 - n + \phi_2 n)^2(1 - n + \phi_3 n)(1 - n + \phi_4 n)^3/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (10)$$

Unrestricted NLLSQ fitting of the data in Table 1 to each equation generates the various parameters listed in Table 2, from which it can be concluded that each has some deficiency. In case a, the computed fractionation factors of $\phi_1 = 1.35 \pm 0.10$ and $\phi_2 = 0.64 \pm 0.02$ seem, respectively, too high and too low to support the simple nucleophilic mechanism of eq 1, and in the most generous analysis these would imply an extremely early TS. For the GB mechanism in case b, the computed $\phi_1 = 1.12 \pm 2.72$ and $\phi_3 = 0.50 \pm 0.21$ are appropriate for the lyoxide L−O and the proton in flight, but the ϕ_2 of 0.99 ± 1.4 implies that the solvating waters are essentially lost in the TS. Additionally, the large uncertainty in all of the computed values suggests that these are heavily correlated. If we accept, for the moment, that the solvating waters have fixed ϕ_2 values of unity, implying complete release in the TS, the mechanism is identical to what Mata-Segreda assumed for the saponification of ethyl acetate.[5] While the ϕ_1 and ϕ_3 values are well within what one anticipates for a good fit to the general base mechanism, we see no good reason to expect that the attacking hydroxide would completely shed all of its solvating waters in what must surely be a very endothermic process (vide infra).

Our recent study[7] provided the activation parameters $\Delta H^{\ddagger}$ (17.9 ± 0.2) kcal/mol and $\Delta S^{\ddagger}$ (-11.1 ± 0.5) cal/mol K for the base-promoted hydrolysis of formamide. The entropy term implies some restriction of waters of solvation in the transition state relative to the ground state.[18] A more complete proton inventory analysis therefore requires consideration of additional

Table 2. NLLSQ Generated Parameters Obtained from Fitting Table 1 Data to Eqs 7−10[a]

	parameter				
eq	$10^3 k_o$	ϕ_1	ϕ_2	ϕ_3	ϕ_4
7	3.10 ± 0.04	1.35 ± 0.10	0.64 ± 0.02		
8	3.10 ± 0.04	1.12 ± 2.72	0.99 ± 1.4	0.50 ± 0.21	
9	3.10 ± 0.04	1.20 ± 21.6	1.18 ± 10.8		0.69 ± 0.14
10	3.10 ± 0.04	1.14 ± 73	0.97 ± 132	0.55 ± 1.5	1.08 ± 112

[a] Unconstrained fittings.[9]

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(16) Huskey, W. P.; Schowen, R. L. *Gazz. Chim. Ital.* **1987**, *117*, 409.
(17) Bone, R.; Wolfenden, R. *J. Am. Chem. Soc.* **1985**, *107*, 4772. This work specifically refers to the fractionation factor for ($R_2C(O\mathbf{H})OR$) ($\phi_H = 1.0$), which we take as a reasonable approximation for the fractionation factor in the amide hydrate anion.

solvating waters on the developing alkoxide oxygen in the transition states shown in **4** and **5** for the nucleophilic and GB mechanisms. Inclusion of the additional solvating waters seems appropriate from a chemical standpoint, but does not lead to more satisfactory results because all of the computed ϕ values in Table 2 for cases c and d are heavily correlated with large uncertainties and are therefore meaningless in the present form.

Reduction of the Number of Variables in Fitting. While transition states **4** and **5** contain a more complete account of possible exchangeable hydrogens, the increased number of parameters introduces large errors in the fits and therefore limits our ability to make meaningful conclusions. It is generally considered that the magnitudes of the various fractionation factors are related in some way to progress along the reaction coordinate.[5,12,19] In previous works concerning the acid-[20] and base-catalyzed hydrolyses of amides[21] and the base-catalyzed hydrolysis of esters,[22] we have considered that solvent dkie data can be treated in terms of fractionation factors derived on the basis of a percent of progress along the reaction coordinate. Such an approach reduces the number of independent variables in transition states **4** and **5** because if all exchangeable hydrogens respond to approximately the same extent in passing from reactants to transition state, the main variable becomes a parameter related to the extent of progress along the reaction coordinate. Schowen[19c] has presented a treatment based on a free energy relationship where some weighting factor, x, describes the structure of the transition state in terms of its progress from reactants ($x = 0$) to product or intermediate ($x = 1$). If some measure of the transition state structure in the vicinity of the isotopic site is available, and if the fractionation factors for the ground (ϕ_{gs}) and intermediate (ϕ_{int}) states are known, ϕ_{TS} for a given H can be calculated as

$$\phi_{TS} = \phi_{gs}^{(1-x)}\phi_{int}^{x} \qquad (11)$$

By combining eq 11 with eq 5, one obtains an expression:

$$k_n = k_o\prod_{i}^{TS}(1 - n + n(\phi_{gs}^{(1-x)}\phi_{int}^{x}))_i(1 - n + n\phi_3)/\prod_{j}^{RS}(1 - n + n\phi_j) \qquad (12)$$

in which the fractionation factors for all exchangeable protons in the TS can be described in terms of known ones for the reactants and intermediates. The ϕ_3 for the proton in flight cannot be derived in such a way and is treated as an independent parameter. In applying eq 12 to the hydrolytic mechanisms proceeding through TS **1**, **2**, **4**, and **5**, we assume that the ground-state fractionation factors (ϕ_{gs}) are those presented by Gold and Grist[14] and that the intermediate is an amide hydrate alkoxide with waters of solvation having ϕ_{int} values similar to those of hydroxide ($\phi = 0.7$). Further, we assume that, with the exception of the proton in flight, the various ϕ_{TS} values for protons undergoing changes in bonding in the TS respond in a similar way to progress along the reaction coordinate. This assumption is clearly an oversimplification because in the rather extended transition states shown in **4** or **5**, the degrees of change in bonding and solvation at the attacking site and remote solvating site are probably not the same.[23] Nevertheless, this treatment, for the moment, has overall merit because any acceptable mechanism must have positive x values, meaning that the various contributing ϕ_{TS}'s will not be random values produced to satisfy solely mathematical fitting criteria, but rather have chemical meaning because acceptable values must be between reactant and intermediate states. Conversely, negative x values, even if these satisfy the mathematical fitting criteria, cannot be chemically acceptable because the computed ϕ_{TS} will not be between reactant and intermediate states. In what follows, we will start from the simplest assumption that all exchangeable protons respond to the progress along the reaction coordinate according to x, and we will subsequently consider other possibilities.

Cases a′ and b′. Minimal Nucleophilic and General Base Mechanisms. From the relationship given in eq 12, the various ϕ_{TS} values in eqs 7 and 8 are recast in eqs 13 and 14 as

$$k_n = k_o(1 - n + 1.22^{(1-x)}n)(1 - n + 0.7^{(1-x)}n)^2/(1 - n + 1.22n)(1 - n + 0.7n)^3 \qquad (13)$$

$$k_n = k_o(1 - n + 1.22^{(1-x)}n)(1 - n + 0.7^{(1-x)}n)^2(1 - n + \phi_3 n)/(1 - n + 1.22n)(1 - n + 0.7n)^3 \qquad (14)$$

NLLSQ fitting of the Table 1 data to these gives the parameters listed in Table 3. The negative computed value for x in both cases is unacceptable because it requires that the ϕ_{TS}'s for the lyoxide protons are unrealistically high ($\phi_1 = 1.26$; 1.29), while the solvating ones are unrealistically low ($\phi_2 = 0.66$; 0.69).[24] Further, for the general base mechanism, the computed value for ϕ_3 is 1.07, an unrealistic value for a proton in flight. Interestingly, NLLSQ fitting of the GB mechanism to eq 14 gives two minima as shown in Table 3,[25] each being statistically

(18) The $\Delta S^{\ddagger}$ of (-11.1 ± 0.5) cal/mol K looks to be rather low for a simple process involving two species going to one in the transition state. However, if the nucleophilic process is correct and one of the waters of solvation is released in reaching the transition state, one would expect that the overall translational entropy change should be close to zero in the absence of additional solvation. The fact that there is a small negative observed value is consistent with the developing solvation on the alkoxy C−O⁻. If the general base mechanism applies, having all three solvating waters released at the TS, then the $\Delta S^{\ddagger}$ is predicted to be substantially positive contrary to the observed value.

(19) See, for example: (a) Hogg, J. L.; Phillips, M. K. *Tetrahedron Lett.* **1977**, 3011. (b) Kershner, L. D.; Schowen, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 2014. (c) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275.

(20) (a) Bennet, A. J.; Slebocka-Tilk, H.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. *J. Am. Chem. Soc.* **1990**, *112*, 8497. (b) Bennet, A. J.; Slebocka-Tilk, H.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. *J. Am. Chem. Soc.* **1990**, *112*, 8497.

(21) (a) Slebocka-Tilk, H.; Bennet, A. J.; Keillor, J. W.; Brown, R. S.; Guthrie, J. P.; Jodhan, A. *J. Am. Chem. Soc.* **1990**, *112*, 8507−8514. (b) Slebocka-Tilk, H.; Bennet, A. J.; Hogg, H. J.; Brown, R. S. *J. Am. Chem. Soc.* **1991**, *113*, 1288. (c) Brown, R. S.; Bennet, A. J.; Slebocka-Tilk, H.; Jodhan, A. *J. Am. Chem. Soc.* **1992**, *114*, 3092.

(22) (a) Kellogg, B. A.; Brown, R. S.; MacDonald, R. S. *J. Org. Chem.* **1994**, *59*, 4652. (b) Kellogg, B. E.; Tse, J. E.; Brown, R. S. *J. Am. Chem. Soc.* **1995**, *117*, 1731.

(23) Huskey and Schowen[16] have demonstrated a substantial imbalance between solvent reorganization and heavy atom reorganization for the attack of methoxide on phenyl acetate. The methoxide:>C═O attack has progressed to the extent of about 15%, while the two residual methanols of solvation have reorganized to the extent of 55−60%. Their analysis does not incorporate any additional solvation of the developing (−)-charge on the carbonyl, which may be slight given the apparent early nature of the methoxide- -C═O bonding.

(24) Even if one assumes for the nucleophilic mechanism that the partially desolvated hydroxide ($HO^-(H_2O)_2$) has fractionation factors slightly different from those in the ground state, the computed ϕ_{TS} implies a very early transition state which is unreasonable for hydroxide attack on formamide.[10] However, for the general base mechanism, where no partially desolvated hydroxide is required, the situation is clearer because any acceptable x value must be positive.

Table 3. NLLSQ Generated Parameters Obtained by Fitting the Table 1 Data to Eqs 12−15[a]

	parameter					
eq	$10^3\,k_o$	x	ϕ_1[b]	ϕ_2[b]	ϕ_3[c]	ϕ_4[b]
13	3.14 ± 0.02	−0.15 ± 0.01	1.26	0.66		
14[d]	3.10 ± 0.04	−0.28 ± 0.09	1.29	0.64	1.07 ± 0.04	
	3.20 ± 0.04	1.19 ± 0.13	0.96	1.07	0.50 ± 0.03	
15	3.17 ± 0.01	0.141 ± 0.001	1.19	0.74		0.95
16[d]	3.10 ± 0.04	−0.22 ± 0.09	1.27	0.65	0.82 ± 0.04	1.08
	3.10 ± 0.04	0.90 ± 0.09	1.02	0.96	1.53 ± 0.09	.73

[a] Unrestricted fits. [b] Calculated from $\phi_1 = (1.22)^{(1-x)}$, $\phi_2 = (0.7)^{(1-x)}$, and $\phi_4 = (0.7)^x$. [c] Computed as independent parameters in fits. [d] Fitting provides two local minima.

equivalent, but the second computed minimum is also unsatisfactory because the x value of 1.19 is greater than the theoretical maximum of unity.

Cases c′ and d′. Nucleophilic and General Base Mechanisms with Solvating Waters on Developing Alkoxide. Equations 9 and 10 are recast as eqs 15 and 16, which yield, after NLLSQ fitting, the various values given in Table 3:

$$k_n = k_o(1 - n + 1.22^{(1-x)}n)(1 - n + 0.7^{(1-x)}n)^2(1 - n + 0.7^x n)^3/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (15)$$

$$k_n = k_o(1 - n + 1.22^{(1-x)}n)(1 - n + 0.7^{(1-x)}n)^2(1 - n + \phi_3 n)(1 - n + 0.7^x n)^3/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (16)$$

The two fits are shown as the dashed and solid lines in Figure 1, which fit the data acceptably and with nearly identical statistics, but only the fit to eq 15 gives chemically acceptable fractionation factors between the reactants and intermediate state values. The negative computed value of x found for the first local minimum for the fit to eq 16 is unrealistic, and the computed fractionation factor for the proton in flight is high, that is, ∼0.82. This fit too has a second local minimum as shown in Table 3, and although the x-value is well within acceptable values for a late TS, the proton in flight has a computed unrealistic fractionation factor of 1.53.

Refining the Mechanism by Uncoupling Attack and Resolvation. Only the nucleophilic mechanism (case c′ above) gives an acceptable fit to the data under the assumed requirement that all components of the nucleophilic reaction progress to the same extent in the transition state. However, the computed value of x for the preferred mechanism (eq 15, $x = 0.14$) is suspiciously low and if interpreted literally would imply a remarkably early transition state, contradicting Kirsch's results[10] that indicate that hydroxide attack on formamide has a very late transition state. Various studies have suggested that the solvent isotope effects for hydroxide and water attack on carbonyls are correlated with the bond order of the nucleophile- - - -C═O bond[19a] and Brønsted parameters describing the sensitivity of the nucleophilic addition to structural variations.[12,19c] The latter parameters are commonly interpreted as indices of progress of bond changes for the heavy atoms at the transition state. Numerous cases are known where there are imbalances of the progress of reacting components along the reaction coordinate.[16,26] We concur with Gold and Grist[14] who state that "it seems doubtful...that the kinetic solvent isotope effects for hydroxide destroying reactions can be formulated on the basis of a transition state which is simply characterized by partial progress from reactants to products in a single process." This suggests that refinement of the mechanism might be accomplished by uncoupling the attack of hydroxide from the resolvation of the developing oxyanion with a late transition state somewhere between 50 and 90% along the reaction coordinate as suggested by Kirsch.[10] Thus, in transition states **4** and **5**, above, the fractionation factors associated with the L_1 and L_2 protons are assumed to be correlated to the same extent with a fixed progress along the reaction coordinate (x), while the L_4 solvating protons are treated as an independent variable (y). The appropriate equations for the nucleophilic and general base mechanisms are given in eqs 17 and 18. Given in Tables 4 and 5 are the best fit k_o and ϕ_4 values for x, five assumed values corresponding to progress of the TS for the HO^- attack of 50−90%.

$$k_n = k_o(1 - n + 1.22^{(1-x)}n)(1 - n + 0.7^{(1-x)}n)^2(1 - n + 0.7^y n)^3/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (17)$$

$$k_n = k_o(1 - n + 1.22^{(1-x)}n)(1 - n + 0.7^{(1-x)}n)^2(1 - n + \phi_3 n)(1 - n + 0.7^y n)^3/(1 - n + 1.22n)(1 - n + 0.7n)^3 \quad (18)$$

Table 4. NLLSQ Fit Values of the Table 1 Data to Nucleophilic Mechanism According to Eq 17

$10^3\,k_o$	x	ϕ_1[a]	ϕ_2[a]	ϕ_4[b]	r^2
3.23 ± 0.04	0.9	1.02	0.96	0.83	0.9330
3.23 ± 0.04	0.8	1.04	0.93	0.85	0.9315
3.23 ± 0.04	0.7	1.06	0.90	0.86	0.9312
3.23 ± 0.04	0.6	1.09	0.87	0.88	0.9324
3.22 ± 0.04	0.5	1.10	0.84	0.89	0.9352

[a] Calculated as $\phi_1 = (1.22)^{(1-x)}$, $\phi_2 = (0.7)^{(1-x)}$. [b] Calculated as $\phi_4 = (0.7)^y$.

Table 5. NLLSQ Fits of Table 1 Data to General Base Mechanism According to Eq 18

$10^3\,k_o$	x	ϕ_1[a]	ϕ_2[a]	ϕ_3[c]	ϕ_4[b]	r^2
3.11 ± 0.03	0.9	1.02	0.96	0.5	1.05	0.9728
3.10 ± 0.03	0.8	1.04	0.93	0.5	1.07	0.9727
3.10 ± 0.03	0.7	1.06	0.90	0.5	1.09	0.9723
3.09 ± 0.03	0.6	1.09	0.87	0.5	1.10	0.9714
3.08 ± 0.03	0.5	1.10	0.84	0.5	1.12	0.9696

[a] Calculated as $\phi_1 = (1.22)^{(1-x)}$, $\phi_2 = (0.7)^{(1-x)}$. [b] Calculated as $\phi_4 = (0.7)^y$. [c] Set value at 0.5 for a primary dkie of 2.0 for the proton in flight; values lower than 0.5 do not lead to acceptable fits, see text.

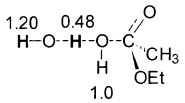
Nucleophilic Mechanism with Solvation. Inspection of the data in Table 4 indicates that fits with very nearly the same correlation coefficients can be obtained when x varies between 0.5 and 0.9. As expected, the latest TS requires more resolvation of the developing $C-O^-$ charge than the earlier ones. When x is set at 0.9, ϕ_4 assumes a value of 0.83, implying that the three resolvating waters have reorganized to the extent of ∼50%, thus lagging behind the attack of HO^- and loosening its two attendant waters of solvation. We tried to fit the data to a model where ϕ_1 has assumed values corresponding to $0.5 \le x \le 0.9$, while ϕ_2 and ϕ_4 are treated as variables, but ϕ_2 in that treatment always assumes unacceptably high values of ∼1.06, while ϕ_4 adopts values of essentially unity (from 1.02 to 1.0, respectively). We conclude that the nucleophilic mechanism can be fit only if the

(25) For each fitting to eqs 13−16, the NLLSQ procedure involved selecting different initial estimates of the variables to probe for additional local minima. Only in the GB cases were two minima found.
(26) Bernasconi, C. F. *Adv. Phys. Org. Chem.* **1992**, *27*, 119.

L_1 and L_2 protons (TS **4**) are assumed to vary to the same extent with x with the L_4 protons lagging behind but nevertheless producing an essential resolvation of the developing oxyanion.

General Base Mechanism with Solvation. We have assumed a value of 0.5 for ϕ_3 of the proton in flight for all of our analyses of the GB mechanism. This is in accordance with the generally accepted notion that protons in flight should contribute primary kinetic isotope effects of 2 or larger and because we find that any smaller value for ϕ_3 leads to fits which are markedly bowed upward with poor statistics. The data presented in Table 5 clearly indicate that with assumed values of $0.5 \leq x \leq 0.9$ fixed for the attacking hydroxide and its solvating waters, the value of ϕ_4 for the three resolvating waters varies between 1.12 and 1.07, values which are too high to be acceptable. We have also tried to fit the data to a GB model, where $\phi_3 = 0.5$, and only ϕ_1 has assumed values corresponding to $0.5 \leq x \leq 0.9$, while ϕ_2 and ϕ_4 are treated as variables. Interestingly, ϕ_2 in that treatment assumes high values of 1.0−1.15, while ϕ_4 adopts values of ∼1.0, and when x is 0.5−0.55, the $\phi_{2,4}$ values are both ∼1.0. This circumstance is essentially identical to the results of the unrestricted fit for the GB process in case b (eq 8), where resolvation is ignored, and the two waters of solvation on the attacking hydroxide are completely removed in the TS. We conclude that the general base mechanism can only have mathematically acceptable fits in any of our treatments under the chemically unlikely scenario, where all waters of solvation on the attacking hydroxide and developing $C-O^-$ have ϕ-values of unity, with the isotope effect being dictated by ϕ_1 and ϕ_3.

Saponification of Ethyl Acetate. The recent report[5] that the proton inventory analysis of the saponification of ethyl acetate fits a highly simplified general base mechanism proceeding through TS **6** having only two contributing fractionation factors and no solvating waters demands that we consider reanalysis of that data within the framework of the models described in eqs 15−18. In the original analysis,[5] the TS was suggested to be about 40% along the reaction coordinate, far earlier than that for hydroxide attack on formamide,[10] but consistent with what Kirsch and co-workers reported on the basis of the formyl hydrogen kinetic isotope effect for the saponification of methyl formate.[27]



6

Hydroxide ion is well known to possess a large hydration enthalpy of 101.3 kcal/mol,[28] and it is difficult to envision any method of recouping the energy lost through desolvating the hydroxide in TS **6** without some additional resolvation of the developing (−)-charge. Fitting of the ethyl acetate data[5] to our eq 15 describing a nucleophilic process with a TS analogous to **4** gives $k_o = 0.124 \pm 0.001$ and $x = 0.16 \pm 0.03$, the best fit being shown as the dotted line in Figure 2. On the other hand, analysis of the data according to eq 16 describing a general base TS analogous to **5** gives $k_o = 0.122 \pm 0.0003$, an unacceptable x of -0.16 ± 0.02, and a fractionation factor for the proton in flight of 0.84 ± 0.01. That fit is shown as the solid line in Figure 2. The small positive computed value for x is for the nucleophilic process consistent with what we have derived above for the nucleophilic attack on formamide with a suspiciously early TS. However, this treatment, requiring that all protons respond to the measure of progress along the reaction coordinate to the same extent, is probably too simplistic in view of the discussion presented above.

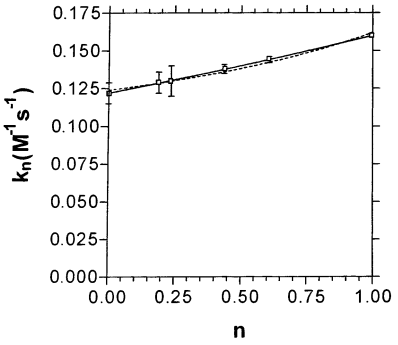


Figure 2. Plot of the second-order rate constants for saponification of ethyl acetate as a function of mole fraction of D_2O (n). Original data from ref 5. Dashed line, NLLSQ fit to eq 15; solid line, NLLSQ fit to eq 16. Best fit parameters are given in the text.

Fitting of the ester saponification data[5] to the uncoupled nucleophilic mechanism of eq 17 provides acceptable fits with nearly identical correlation coefficients for $0.4 \leq x \leq 0.9$, with the ϕ_4 value for the three solvating waters on the developing $C-O^-$ being between 0.92 and 0.83 ($y = 0.27-0.51$), indicating that the resolvation of the oxyanion lags behind the nucleophilic attack. If the value of x is varied for only the L_1 hydroxide proton, and all others are allowed to fit as independent variables, no acceptable values for ϕ_2 and ϕ_4 can be computed as these are all >1.0. In the case of the GB mechanism, when the data[5] are fit to eq 18 with $\phi_3 = 0.5$ for the proton in flight and $0.4 \leq x \leq 0.9$ pertaining to both the L_1 and the L_2 protons of TS **5**, the computed fractionation factors for the resolvating waters, ϕ_4, are invariably greater than 1.0. Interestingly, if only the L_1 is assumed to correspond to a fixed x value between 0.4 and 0.9, and the ϕ_3 and ϕ_4 values are treated as variables, the only acceptable fit comes at $x \approx 0.4-0.5$, but in this case the fractionation factors for all of the solvating waters are 1.0. In the limit, the analysis is the same as that of Mata-Segreda,[5] suggesting that all solvation is lost in the transition state corresponding to TS **6** above.

Conclusion

From the above discussion, a nucleophilic mechanism involving resolvating waters can be fit to the available data for both ethyl acetate and formamide hydrolysis. A simple model satisfying the data considers that all fractionation factors, including the ones for the resolvating waters, respond to the same extent with progress (x) along the reaction coordinate. However, in this case, the computed x values, if taken literally, correspond to much earlier progress along the reaction coordinate than the accepted transition state positions[10,27] of about 0.4 for ester saponification or 0.7−0.9 for amide hydrolysis. A refined nucleophilic mechanism giving good fits to the data is suggested where the attack of hydroxide and resolvation of the developing oxyanion are uncoupled with the resolvation lagging behind the attack. However, these fits, in our hands, are only mathematically and chemically reasonable if the attacking

(27) Bilkadi, Z.; de Lorimer, R.; Kirsch, J. F. *J. Am. Chem. Soc.* **1975**, *97*, 4317.
(28) Friedman, H. L.; Krishnan, C. V. In *Water. A Comprehensive Treatise*; Franks, F., Ed.; Plenum: New York, 1973; Vol. 3, p 56.

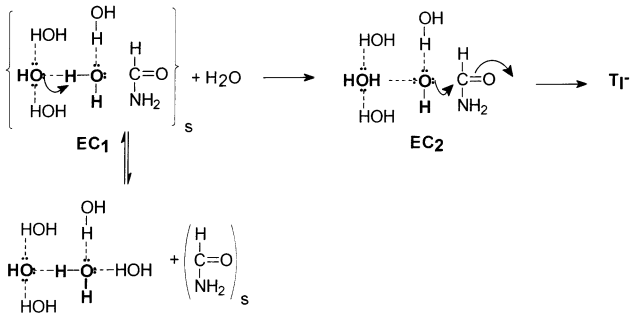
hydroxide and its waters of solvation are assumed to respond to the same extent to progress along the reaction coordinate.

A general base mechanism can also be accommodated, but any successful fits for the ester and amide require that all waters of solvation have fractionation factors of unity, while that for the proton in flight can be no smaller than 0.5.

Analysis of the proton inventory data for the base-catalyzed hydrolysis of formamide suggests that, within the fitting constraints delineated above, one cannot unambiguously rule out either the nucleophilic mechanism or the general base mechanism. In our opinion, the same situation also applies for the analysis of the data for saponification of ethyl acetate.[5] For formamide, we believe this to be a consequence of the low overall inverse dkie of $k_{OH}/k_{OD} = 0.77 \pm 0.02$ at $[LO^-] = 1.42$ M, as well as the rather featureless and nearly linear appearance of the second-order rate constant versus *n* plot (Figure 1), which does not lend itself to unique fitting with multiparameter equations pertaining to different mechanisms. There is a possible additional complication that the kinetic data are substantially, but not entirely, devoted to the single step of hydroxide attack. In our opinion, any successful analysis of these nearly linear proton inventory data in terms of a suite of plausible mechanisms cannot rely exclusively on the goodness of mathematical fit to one model or another but rather must take cognizance of chemical reality. The clearest indication of this is the fact that a straight line accommodates the data with a correlation coefficient which is as good or better than most of the more complex fits, but there is no reasonable hydrolytic mechanism involving bonding changes of a single proton.

The nucleophilic mechanism for hydroxide addition to esters and amides has been widely accepted in explaining all extant data prior to 1994. The main reason for now invoking the GB mechanism stems from Marlier's heavy atom isotope effects for the base-catalyzed hydrolysis of methyl formate[4] and formamide,[3] which are consistent with the attacking oxygen being derived from H_2O and not hydroxide. Those reports prompted Mata-Segreda[5] and us to conduct proton inventory studies in the hopes of supporting one mechanism or the other. From our current study, it seems unlikely that fractionation factor analysis alone will allow one to distinguish between these two mechanisms, but chemical intuition suggests that any acceptable mechanism must incorporate solvent molecules. Solvation must be an important component in hydroxide attack on formamide because in the gas phase, where there is no additional solvent, the attack is barrierless,[29] but the experimental $\Delta H^{\ddagger}$ and $\Delta G_{25}^{\ddagger}$ values for base-promoted hydrolysis of formamide in solution are 17.9 and 21.2 kcal/mol.[7] Further, it seems reasonable that the 101.3 kcal/mol enthalpy of hydration[28] of hydroxide in forming $(HO^-(H_2O)_3)$ is too large to have all of the stabilizing waters lost in any conceivable transition state for a nucleophilic or general base process without compensating stabilization of the negative charge in the transition state through H-bonding interactions with additional solvent molecules.

Scheme 2



Therefore, our currently favored mechanism for the basic hydrolysis of formamide is a nucleophilic one, which we present in Scheme 2 and suggest is consistent with the heavy atom isotope effects. The first step results in the formation of an encounter complex (**EC_1**) where a formamide appears next to one of the solvating waters of hydroxide. This can occur either by diffusion of formamide or by chainlike proton transfer for the hydroxide through the solvent, which is consistent with the high ionic mobility of hydroxide in water.[14] In either event, formation of **EC_1** requires that the C═O of the formamide is exposed to a lone pair of electrons on one of the waters of hydration of the HO^-. In the next step, proton transfer occurs from this water of solvation to the hydroxide, generating **EC_2** with a partially solvated or "hot" hydroxide poised for attack on the carbonyl. Part of the activation energy for the attack must result from this desolvation, but, following Huskey and Schowen,[16] we suggest that this is offset by electrophilic stabilization of the hot hydroxide by its association with the C═O dipole. Because this hot hydroxide is immediately derived from water, the heavy atom distribution must be similar to that of the bulk water, thus accounting for Marlier's observations.[3] In essence, this mechanism is reminiscent of the GB mechanism, but the requisite proton transfer is completed prior to the attack of the hot hydroxide on the formamide, making it a nucleophilic mechanism consistent with the slightly inverse dkie observed.

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