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ORIGINAL PAPER

# Kinetics and mechanism of the general base-catalyzed hydrolysis of *N*-hydroxyphthalimide

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**Abstract** The kinetics and mechanism of hydrolysis of *N*-hydroxyphthalimide in the presence of various buffers (dichloroacetic acid, chloroacetic acid, glycine, sodium formate, sodium acetate, *N*-(2-morpholinoethane)sulfonic acid, and tris(hydroxymethyl)aminomethane) were studied at 50 °C and an ionic strength of 1.0 M. The second-order rate constants $k_b$ for the buffer-catalyzed hydrolysis of *N*-hydroxyphthalimide were found to conform to the Brønsted equation $\log k_b = C + \beta\ pK_a$. A plot of $pK_a$ versus $k_b$ for the data obtained in the buffers and $H_2O$ (covering a $pK_a$ range of −1.74 to 8.33) was constructed, and the data were fitted with a straight line that had a slope ($\beta$) of 0.29 ± 0.05 and an intercept ($C$) of −5.19 ± 0.20.

**Keywords** Kinetics · Buffer catalysis · *N*-hydroxyphthalimide · Hydrolysis · Mechanism

## Introduction

Amide (i.e., peptide) bonds are the “glue” that hold proteins—and thus living systems—together. The hydrolysis of imides or amides is therefore frequently used as a model to investigate the cleavage of peptide bonds [1]. Although numerous reports on the hydrolysis of phthalimide and *N*-substituted phthalimides have been published, reports on the hydrolysis of imides in acidic media and at neutral pH are very rare. The water-promoted hydrolysis, or neutral hydrolysis, of unactivated imides and amides is very slow, and in most cases undetectable [2]. The reported pseudo-first-order rate constants ($k_0$) for the uncatalyzed hydrolysis of five-membered ring imides and acyclic amides are on the order of $10^{-6}$ to $10^{-8}$ and $10^{-9}$ to $10^{-12}$ $s^{-1}$, respectively, at 35 °C (which correspond to half-lives ($t_{1/2}$) of 8–800 days and 22–2.2 × $10^4$ years, respectively) [3–5].

The mechanisms of these reactions, especially when general acid and general base (GA-GB) catalysis are involved, are also miraculous inimitable and are not yet fully understood [6–8]. The occurrence of intramolecular GB assistance was noted for the pH-independent hydrolysis of *N*-(*o*-hydroxymethyl)phthalimide [4].

Kinetic studies of such slow reactions ($t_{1/2}$ of more than a few days) have not been attractive to researchers because they are associated with some unavoidable experimental complications [9]. An enzyme’s efficiency can be estimated quantitatively once the rate constant for the analogous uncatalyzed reaction has been determined [10–12]. Recently, it was suggested that the cleavage of a certain atypical peptide bond might occur through the formation of an acyclic imide bond [13]. Thus, studies involving inter- and intramolecular GA-GB-assisted hydrolysis of imides and amides are considered to be very important, given the expectation that the results of such studies could be applied to gain a deeper understanding of enzyme-catalyzed reactions [14]. In work described in the present paper, which is a continuation of our mechanistic studies of inter- and intramolecular GA-GB catalysis in the cleavage of imide and amide bonds, we investigated the effect of [HCl], [NaOH], and [buffers] on the rate of hydrolysis of *N*-hydroxyphthalimide (**1**). The results

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obtained and their probable mechanistic explanation(s) are discussed in this manuscript.

## Results and discussion

### *Effects of [HCl] and [NaOH] on the pseudo-first-order rate constant ($k_{obs}$) for the hydrolysis of **1***

A few kinetic runs were carried out within the [HCl] range 0.01–1.0 M at a constant ionic strength of 1.0 M (maintained by NaCl) at 50 °C. The values of $k_{\mathrm{obs}}$ were found to fit well to Eq. (1), where $k_0$ and $k_{\mathrm{H}}$ represent the pH-independent and $\mathrm{H}^+$-catalyzed rate constants for the hydrolysis of **1**. The values of $k_{\mathrm{obs}}$ at different [HCl] are shown in Table 1. These data were fitted to Eq. (1) with least-squares-calculated values of $k_0$ and $k_{\mathrm{H}}$ of $37.4 \pm 0.9 \times 10^{-6}\ \mathrm{s}^{-1}$ and $98.6 \pm 1.0 \times 10^{-6}\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$, respectively.

$$k_{\mathrm{obs}} = k_0 + k_{\mathrm{H}}\,[\mathrm{HCL}]. \tag{1}$$

Similarly, a few kinetic runs were carried out within the [NaOH] range $1.5 \times 10^{-3}$ to $7.0 \times 10^{-3}$ M at a constant ionic strength of 1.0 M and 50 °C. The $k_{\mathrm{obs}}$ values at different [NaOH], as shown in Table 1, were found to fit to Eq. (1) when $k_0$, $k_{\mathrm{H}}$, and HCl were replaced with $k_0''$, $k_{\mathrm{OH}}''$, and NaOH, respectively. The least-squares calculated values of $k_0''$ and $k_{\mathrm{OH}}''$ were $-19.2 \pm 2.0 \times 10^{-4}\ \mathrm{s}^{-1}$ and $3.30 \pm 0.06\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$, respectively. The symbol $k_{\mathrm{OH}}''$ is the second-order rate constant for the reaction of $\mathrm{HO}^-$ with ionized **1** (i.e., $\mathrm{S}^-$). The rate constant $k_0''$ is for the reaction between either $H_2O$ and $\mathrm{S}^-$ or $\mathrm{HO}^-$ and SH (i.e., nonionized **1**). The calculated negative value of $k_0''$ is physicochemically meaningless. Therefore, a more reliable value of $k_{\mathrm{OH}}''$ of $2.84 \pm 0.50\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ was obtained from Eq. (1) by setting $k_0'' = 0$. The fit of the data to Eq. (1) is evidently reliable, considering the standard deviations of the calculated parameters and the values of $k_{\mathrm{calcd}}$ shown in Table 1.

### *Effect of the total concentration of buffer, $[\mathrm{Buf}]_T$, on $k_{obs}$ at 50 °C*

A series of kinetic runs were carried at different pH values in solutions of various buffer-forming compounds/salts [mono- and dichloroacetic acids (ClAcA and $\mathrm{Cl_2AcA}$), glycine (Gly), sodium formate (ForNa), sodium acetate (AcNa), *N*-(2-morpholinoethane)sulfonic acid (MES), and tris(hydroxymethyl)aminomethane (TRIS)]. The values of $k_{\mathrm{obs}}$ obtained at a constant pH but different values of $[\mathrm{Buf}]_{\mathrm{T}}$ (the total buffer concentration) were found to fit reasonably well to Eq. (2):

$$k_{\mathrm{obs}} = k_0 + k_{\mathrm{b}}^{\mathrm{ap}}\,[\mathrm{Buf}]_{\mathrm{T}}, \tag{2}$$

where $k_0$ is the $[\mathrm{Buf}]_{\mathrm{T}}$-independent first-order rate constant, $k_{\mathrm{b}}^{\mathrm{ap}}$ is the $[\mathrm{Buf}]_{\mathrm{T}}$-dependent second-order rate constant for the hydrolytic cleavage of **1**, and $[\mathrm{Buf}]_{\mathrm{T}} = [\mathrm{B}] + [\mathrm{BH}^+]$, where B and $\mathrm{BH}^+$ represent free base and protonated base (i.e., conjugate acid), respectively. The values of $k_0$ and $k_{\mathrm{b}}^{\mathrm{ap}}$ were calculated from Eq. (2) using the linear least-squares technique. The calculated values of $k_0$ and $k_{\mathrm{b}}^{\mathrm{ap}}$ at different pH values for different buffers are summarized in Table 2. The fit of the observed data ($k_{\mathrm{obs}}$) vs. $[\mathrm{Buf}]_{\mathrm{T}}$ to Eq. (2) is clearly reliable given the standard deviations associated with the calculated parameters, $k_0$ and $k_{\mathrm{b}}^{\mathrm{ap}}$, and given the plots shown in Fig. 1 for typical MES buffers, where solid lines can easily be drawn through the least-squares-calculated rate constants ($k_{\mathrm{calcd}}$).

The values of $k_{\mathrm{obs}}$ were found to be independent of $[\mathrm{Buf}]_{\mathrm{T}}$ at constant pH for the $\mathrm{Cl_2AcA}$ buffer. Similar

**Table 1** Values of $k_{\mathrm{obs}}$ for the hydrolysis of **1** at different [HCl], [NaOH], and at 50 °C

| HCl/M | $10^6 k_{\mathrm{obs}}/s^{-1}$ | $10^6 k^{\mathrm{a}}_{\mathrm{calcd}}/s^{-1}$ | NaOH/M | $10^4 k_{\mathrm{obs}}/s^{-1}$ | $10^4 k^{\mathrm{a}}_{\mathrm{calcd}}/s^{-1}$ |
|---|---|---|---|---|---|
| 0.03 | 39.0 ± 1.0 | 40.0 | $1.5 \times 10^{-3}$ | 29.0 ± 1.0[b] | 30.3 |
| 0.05 | 40.0 ± 0.7 | 42.0 | $2.0 \times 10^{-3}$ | 45.5 ± 1.0 | 46.8 |
| 0.10 | 47.2 ± 1.0 | 46.9 | $2.5 \times 10^{-3}$ | 63.6 ± 1.0 | 63.3 |
| 0.20 | 58.4 ± 1.0 | 56.8 | $3.0 \times 10^{-3}$ | 85.5 ± 2.0 | 80 |
| 0.30 | 68.2 ± 3.0 | 66.7 | $4.0 \times 10^{-3}$ | 109 ± 3.0 | 113 |
| 0.50 | 86.8 ± 3.0 | 86.4 | $5.0 \times 10^{-3}$ | 147 ± 3 | 146 |
| 0.80 | 117 ± 3 | 116 | $6.0 \times 10^{-3}$ | 178 ± 3 | 179 |
| 1.00 | 134 ± 5 | 136 | $7.0 \times 10^{-3}$ | 212 ± 3 | 212 |

Conditions: $[\mathbf{1}]_0 = 2.5 \times 10^{-4}$ M, ionic strength 1.0 M, $\lambda = 300$ nm, 50 °C. For each kinetic run, the solvent contained 1 % $\mathrm{CH_3CN}$ and 99 % $H_2O$. Alkaline hydrolysis of **1** was monitored at 410 nm

[a] Rate constants were calculated using Eq.(1) and the calculated parameters $k_0$ and $k_{\mathrm{H}}$ or $k_0$ and $k_{\mathrm{OH}}$

[b] Error limits are the standard deviations

**Table 2** Values of pH, $k_0$, $k_b^{ap}$, and $k_b$ for the cleavage of **1** at different pH values

| Buffer | pH[a] | $10^6 k_0^b/s^{-1}$ | $10^6 k_b^{apc}/M^{-1}s^{-1}$ | $10^6 k_b^d/M^{-1}s^{-1}$ | $[Buf]_T$ range/M |
|---|---|---|---|---|---|
| $Cl_2AcA$ | 1.14 ± 0.01[e] | (35.8 ± 1.0[e])[f] | | | 0.1–0.75 |
| | 1.47 ± 0.3 | (31.7 ± 1.2) | | | 0.1–0.8 |
| | 1.94 ± 0.2 | (28.0 ± 2.8) | | | 0.1–0.8 |
| ClAcA | 2.35 ± 0.01 | 32.6 ± 1.0 | 4.8 ± 2.0[e] | 16.0 | 0.1–0.75 |
| | 2.46 ± 0.01 | 30.3 ± 0.5 | 13.2 ± 1.0 | 33.0 | 0.1–0.75 |
| | 2.61 ± 0.01 | 33.5 ± 0.5 | 16.0 ± 1.0 | 32.0 | 0.1–0.75 |
| | 2.99 ± 0.03 | 36.3 ± 1.0 | 22.3 ± 3.0 | 31.4 | 0.1–0.8 |
| | 3.26 ± 0.03 | 37.4 ± 2.0 | 26.0 ± 3.0 | 32.5 | 0.1–0.8 |
| Gly | 1.98 ± 0.05 | 32.2 ± 1.0 | 15.3 ± 2.0 | 51.0 | 0.1–0.8 |
| | 2.09 ± 0.04 | 32.2 ± 1.0 | 16.5 ± 2.0 | 41.3 | 0.1–0.8 |
| | 2.33 ± 0.02 | 29.5 ± 0.4 | 22.9 ± 1.0 | 45.8 | 0.1–0.75 |
| | 2.51 ± 0.01 | 28.2 ± 0.8 | 25.8 ± 2.0 | 43.5 | 0.1–0.8 |
| | 2.70 ± 0.01 | 30.7 ± 0.8 | 42.2 ± 2.0 | 60.3 | 0.1–0.8 |
| | 2.95 ± 0.01 | 30.4 ± 1.0 | 59.4 ± 3.0 | 74.3 | 0.1–0.8 |
| ForNa | 3.03 ± 0.01 | 36.8 ± 1.0 | 38.8 ± 3.0 | 129 | 0.1–0.75 |
| | 3.25 ± 0.01 | 32.8 ± 1.0 | 60.5 ± 2.0 | 151 | 0.1–0.8 |
| | 3.42 ± 0.01 | 36.7 ± 4.0 | 87.3 ± 8.0 | 175 | 0.1–0.8 |
| | 3.88 ± 0.03 | 32.6 ± 2.0 | 94.2 ± 3.0 | 135 | 0.1–0.8 |
| | 4.11 ± 0.03 | 34.3 ± 1.0 | 109 ± 2 | 136 | 0.1–0.6 |
| AcNa | 4.25 ± 0.04 | 49.7 ± 3.0 | 78.0 ± 6.0 | 260 | 0.1–0.8 |
| | 4.37 ± 0.03 | 45.2 ± 2.0 | 87.2 ± 4.0 | 218 | 0.1–0.8 |
| | 4.42 ± 0.03 | 37.3 ± 4.0 | 102 ± 8 | 204 | 0.1–0.8 |
| | 4.92 ± 0.07 | 50.7 ± 3.0 | 170 ± 7 | 243 | 0.1–0.75 |
| | 5.17 ± 0.10 | 50.9 ± 4.0 | 158 ± 9 | 200 | 0.1–0.8 |
| MES | 5.35 ± 0.03 | 37.6 ± 1.0 | 38.1 ± 3 | 191 | 0.1–0.8 |
| | 5.51 ± 0.03 | 38.1 ± 1.0 | 43.6 ± 2 | 174 | 0.1–0.8 |
| | 5.60 ± 0.03 | 39.2 ± 2.0 | 49.6 ± 3.0 | 165 | 0.1–0.75 |
| | 5.76 ± 0.03 | 38.4 ± 3.0 | 56.7 ± 7.0 | 142 | 0.1–0.75 |
| | 5.98 ± 0.02 | 40.9 ± 3.0 | 65.6 ± 3.0 | 131 | 0.1–0.8 |
| | 6.12 ± 0.04 | 39.6 ± 3.0 | 77.1 ± 6.0 | 129 | 0.1–0.8 |
| | 6.32 ± 0.03 | 42.3 ± 2.0 | 71.1 ± 5.0 | 102 | 0.1–0.75 |
| | 6.54 ± 0.02 | 14.9 ± 1.0 | 103 ± 2 | 129 | 0.1–0.8 |
| TRIS | 7.48 ± 0.1 | 84.8 ± 6.0 | 160 ± 12 | 527 | 0.1–0.8 |
| | 7.77 ± 0.0 | 134 ± 1 | 260 ± 25 | 520 | 0.1–0.8 |
| | 7.98 ± 0.0 | 202 ± 26 | 299 ± 49 | 497 | 0.1–0.8 |
| | 8.12 ± 0.0 | 138 ± 7 | 322 ± 13 | 459 | 0.1–0.8 |
| | 8.33 ± 0.0 | 109 ± 9 | 365 ± 18 | 457 | 0.1–0.8 |

$[\mathbf{1}]_0 = 2.5 \times 10^{-4}$ M, 50 °C, $\lambda = 300$ nm. For each kinetic run, the aqueous solvent contained 1 % of $CH_3CN$

[a] The pH values were calculated by averaging the pH measurements performed at a constant pH

[b,c] $k_0$ and $k_b^{ap}$ values were obtained from Eq. (2)

[d] The values of $k_b$ were obtained from the relationship $k_b = k_b^{ap}/f_b$

[e] Error limits are the standard deviations

[f] Values in parentheses were obtained using the average values of $k_{obs}$

observations were made at different pH values within the pH range 1.14–1.94. These observations indicate that buffer catalysis did not occur. Thus, the average of the different $k_{obs}$ values obtained at a constant pH was considered to be $k_0$ (Table 2). Similarly, an insignificant contribution (~10 %) from $k_b^{ap}$ $[Buf]_T$ in Eq. (2) was found at the lowest pH (2.35) with ClAcA buffer. As a consequence, the calculated value of $k_b^{ap} = 4.8 \pm 2.0$ $M^{-1}$ $s^{-1}$ (Table 2) at pH 2.35 cannot be considered to be very reliable. The calculated values of $k_b = k_b^{ap}/f_b$ (where $f_b$ represents the fraction of free base), as shown in Table 2, are almost independent of pH for all buffer bases except for MES.

The mean values of $k_b = k_b^{avg}$ for all buffers except for MES are shown in Table 3. The values of $k_b$ for MES buffer exhibit a nonlinear decrease with increasing pH within the pH range 5.35–6.54 (Table 2). These results may be explained as follows. The value of p$K_a$ of **1** at 30 °C is 5.99 [15], which is used to estimate the value of p$K_a^{SH}$ as 5.36 at 50 °C. It is evident from this p$K_a^{SH}$ value of

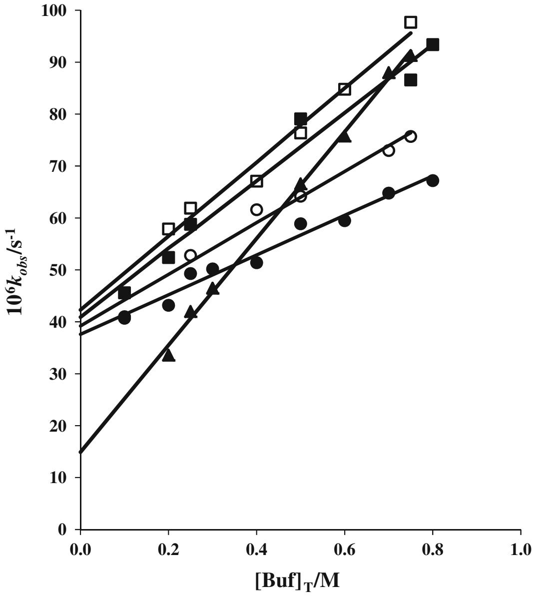


**Fig. 1** Plot of $k_{obs}$ vs. $[Buf]_T$ at different pH values for the hydrolysis of **1** with $[Buf]_T = [MES]_T$ at pH 5.35 (*filled circles*), 5.60 (*open circles*), 5.98 (*filled squares*), 6.32 (*open squares*), and 6.54 (*filled rectangles*), respectively. *Solid lines* representing the fits to Eq. (2) are drawn through the calculated data points, as described in the text

**Table 3** Values of $k_b^{avg}$, $k'_b$ and $k_{b^-}$ for the buffer-catalyzed cleavage of **1**

| Buffer | $pK_a^a$ | $10^6 k_b^{avg b}$/ $M^{-1}$ $s^{-1}$ | $10^6 k_b'^{c}$/ $M^{-1}$ $s^{-1}$ | $10^6 k_{b^-}^{d}$/ $M^{-1}$ $s^{-1}$ |
|---|---|---|---|---|
| $Cl_2AcA$ | 1.47 ± 0.3^e | 31.8 ± 4.0^e | | |
| ClAcA | 2.61 ± 0.01 | 32.2 ± 0.7 | | |
| Gly | 2.35 ± 0.02 | 52.7 ± 12.6 | | |
| ForNa | 3.42 ± 0.01 | 145 ± 18 | | |
| AcNa | 4.42 ± 0.03 | 225 ± 26 | | |
| MES | 5.98 ± 0.02 | | 248.1 | 78.7 ± 6.0 |
| TRIS | 7.77 ± 0.0 | 492 ± 33 | | |
| $H_2O$ | −1.74 | 0.65^f | | |

[a] $pK_a$ values were obtained from the pH values at $f_b = 0.50$

[b] The values of $k_b^{avg}$ are the mean values of $k_b$

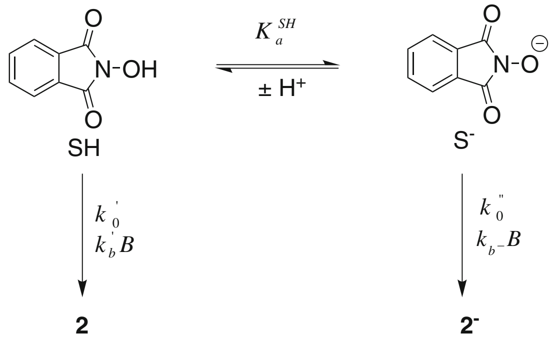
[c] Rate constant for MES-buffer-catalyzed hydrolysis of nonionized **1**

[d] Rate constant for MES-buffer-catalyzed hydrolysis of ionized **1**

[e] Error limits are the standard deviations

[f] The value of $k_b^{avg}$ was obtained from the relationship $k_b^{avg} = k_w/[H_2O]$ with $k_w = 34.2 \times 10^{-6}\ s^{-1}$, as obtained using Eq. (6) and $[H_2O] = 55.5$ M

**Scheme 1**



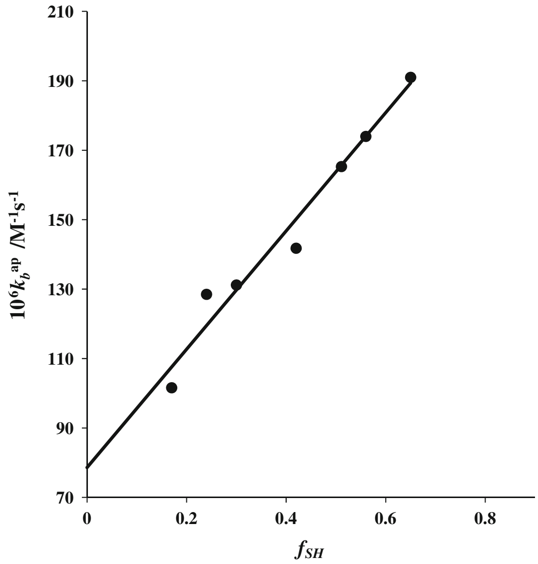


**Fig. 2** Plot of $k_b^{ap}$ vs. $f_{SH}$ for the hydrolysis of **1** with B = MES. A *solid fit line* (based on Eq. 5) has been drawn through the calculated data points, as described in the text

5.36 that the rate of MES buffer-catalyzed hydrolysis of **1** involves both nonionized (SH) and ionized ($S^-$) forms of **1**, as shown in Scheme 1. It can be easily shown that the observed rate law (rate = $k_{obs}[\mathbf{1}]_T$ where $[\mathbf{1}]_T = [SH] + [S^-]$) and Scheme 1 lead to Eq. (3):

$$k_{obs} = k'_0 f_{SH} + k''_0 f_{S^-} + (k'_b f_{SH} + k_{b^-} f_{S^-}) f_B [Buf]_T, \tag{3}$$

where $f_{SH} = [SH]/[\mathbf{1}]_T$, $f_{S^-} = 1 - f_{SH} = [S^-]/[\mathbf{1}]_T$, and $f_B = [B]/[Buf]_T$. A comparison of Eqs. 2 and 3 gives Eqs. 4 and 5:

$$k_0 = k'_0 f_{SH} + k''_0 f_{S^-} \tag{4}$$

$$k_b^{ap} = (k'_b f_{SH} + k_{b^-} f_{S^-}) f_B. \tag{5}$$

Equation 5 predicts that the plot of $k_b (= k_b^{ap}/f_B)$ vs. $f_{SH}$ should be linear with an intercept and slope equal to $k_{b^-}$ and $(k'_b - k_{b^-})$, respectively. Such a plot, as shown in Fig. 2, is linear, with respective least-squares-calculated values of $k_{b^-}$ and $(k'_b - k_{b^-})$ of $78.7 \pm 6.0 \times 10^{-6}\ M^{-1}\ s^{-1}$ and $169.4 \pm 14.0 \times 10^{-6}\ M^{-1}\ s^{-1}$. The calculated values of $k_{\mathrm{b}^-}$ and $(k'_{\mathrm{b}} - k_{\mathrm{b}^-})$ give the value of $k'_{\mathrm{b}}$ as $248.1 \times 10^{-6}\ \mathrm{M^{-1}\ s^{-1}}$. The values of $k'_{\mathrm{b}}$ and $k_{\mathrm{b}^-}$ are also shown in Table 3, where $k'_{\mathrm{b}}$ is equivalent to $k_{\mathrm{b}}$ for other buffers. The values of $k_{\mathrm{b}}$ for MES are almost independent of pH within the pH range 5.3–6.3, which indicates that $k'_0 \approx k''_0$ in Eq. (4).

The rate constant $k'_0$ is equivalent to $k_0$, and was obtained using buffers at pH $\leq 4.3$, where the concentration of ionized **1** (i.e., $[\mathrm{S}^-]$) is negligible compared with [SH] because $\mathrm{p}K_{\mathrm{a}}^{\mathrm{SH}} = 5.36$. The rate of buffer-uncatalyzed hydrolysis of **1** may be expressed as $v = k_{\mathrm{w}}[\mathrm{H_2O}][\mathrm{SH}]$ and consequently $k_0 = k'_0 = k_{\mathrm{w}}[\mathrm{H_2O}]$. The rate constant $k''_0$ represents the pseudo-first-order rate constant for the pH-independent hydrolysis of **1**, where the rate of reaction $v = k''_{\mathrm{w}}[\mathrm{H_2O}][\mathrm{S}^-]$, which is kinetically indistinguishable from $v = k_{\mathrm{OH}}\alpha_{\mathrm{OH}}[\mathrm{SH}]$. Thus, under such conditions, either $k''_0 = k''_{\mathrm{w}}[\mathrm{H_2O}]$ or $k''_0 = k_{\mathrm{OH}}K_{\mathrm{w}}/K_{\mathrm{a}}^{\mathrm{SH}}$. This kinetic indistinguishability was resolved by applying plausible kinetics reasoning, which favors $k''_0 = k_{\mathrm{OH}}K_{\mathrm{w}}/K_{\mathrm{a}}^{\mathrm{SH}}$ [16]. The reported value of $\mathrm{p}K_{\mathrm{w}}$ (= 13.26) was obtained at zero ionic strength [17]. The reported values of the activity coefficients of $\mathrm{H}^+$ and $\mathrm{HO}^-$ at 50 °C and an ionic strength of 1.0 M are 0.77 and 0.67, respectively [18]. The activity-coefficient-corrected value of $\mathrm{p}K_{\mathrm{w}}$ at an ionic strength of 1.0 M and 50 °C is 13.55. The mean value of $k_0 (= k''_0)$ obtained within the pH range >4.3 to $\leq$6.5 (Table 2) is $40.4 \pm 9.20 \times 10^{-6}\ \mathrm{s^{-1}}$. Thus, the relationship $k''_0 = k_{\mathrm{OH}}K_{\mathrm{w}}/K_{\mathrm{a}}^{\mathrm{SH}}$ gives $k_{\mathrm{OH}} = 5.1 \times 10^2\ \mathrm{M^{-1}\ s^{-1}}$.

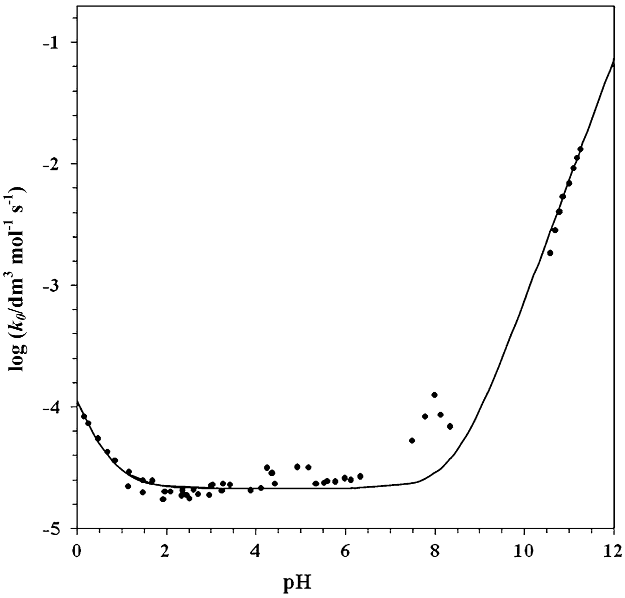


**Fig. 3** Logarithmic plot of $k_0$ vs. pH for the uncatalyzed hydrolysis of **1** at 50 °C. The *solid line* drawn through the values of $k_0$ corresponds to Eq. (6) with $k_{\mathrm{w}} = 34.2 \pm 7.0 \times 10^{-6}\ \mathrm{s^{-1}}$, $k_{\mathrm{H}} = 98.6 \pm 1.0 \times 10^{-6}\ \mathrm{M^{-1}\ s^{-1}}$, and $k_{\mathrm{OH}} = 2.84 \pm 0.50\ \mathrm{M^{-1}\ s^{-1}}$

### *Mechanistic interpretation of* $k_0$ *and* $k_{\mathrm{b}}$

The $[\mathrm{Buf}]_{\mathrm{T}}$-independent rate constant, $k_0$, represents the uncatalyzed, $\mathrm{H^+}$–$\mathrm{HO^-}$ and catalyzed reactions of water with **1**. Thus, $k_0$ may be expressed under such conditions as

$$k_0 = k_{\mathrm{w}} + k_{\mathrm{H}}[\mathrm{H^+}] + k_{\mathrm{OH}}[\mathrm{HO^-}], \tag{6}$$

where $k_{\mathrm{w}}$ represents the pseudo-first-order rate constant for the uncatalyzed reaction of $\mathrm{H_2O}$ with **1**. The values of $k_0$ obtained within the pH range 1.1–8.3 (Table 2) were used to calculate $k_{\mathrm{w}} = 34.2 \pm 7.0 \times 10^{-6}\ \mathrm{s^{-1}}$ from Eq. (6), using the known parameters $k_{\mathrm{H}} = 98.6 \pm 1.0 \times 10^{-6}\ \mathrm{M^{-1}\ s^{-1}}$ and $k_{\mathrm{OH}} = 2.84 \pm 0.50\ \mathrm{M^{-1}\ s^{-1}}$ and the observed data summarized in Table 1. The values of $k_0$ at different pH are shown logarithmically in Fig. 3. It is worth noting that the contribution of the $k_{\mathrm{H}}[\mathrm{H^+}] + k_{\mathrm{OH}}[\mathrm{HO^-}]$ term ($k_0^{\mathrm{min}}$) in Eq. (6) at the pH (= pH$^{\mathrm{min}}$) where the plot of $\log k_{\mathrm{obs}}$ vs. pH exhibits a minimum can be calculated using Eq. (7) [19, 20]:

$$k_0^{\mathrm{min}} = 2(k_{\mathrm{H}}k_{\mathrm{OH}}K_{\mathrm{w}})^{1/2}, \tag{7}$$

where $K_{\mathrm{w}} = [\mathrm{H^+}][\mathrm{HO^-}]$. Thus, the experimentally determined rate constants ($k_0$) for the pH-independent hydrolysis of **1** may be expressed as

$$k_0 = k_{\mathrm{w}} + k_0^{\mathrm{min}}, \tag{8}$$

which predicts the presence of $k_{\mathrm{w}}$ only if $k_0/k_0^{\mathrm{min}} > 1$. The value of $k_0^{\mathrm{min}}$ can be calculated from Eq. 7 using known values of $k_{\mathrm{H}}$, $k_{\mathrm{OH}}$, and $K_{\mathrm{w}}$ The value of $k_0^{\mathrm{min}}$ calculated for **1** at 50 °C in this manner is $= 5.62 \times 10^{-9}\ \mathrm{s^{-1}}$ ($\log k_0^{\mathrm{min}}$ is $-8.25$) with $k_{\mathrm{H}} = 98.6 \pm 1.0 \times 10^{-6}\ \mathrm{M^{-1}\ s^{-1}}$, $k_{\mathrm{OH}} = 2.84 \pm 0.50\ \mathrm{M^{-1}\ s^{-1}}$, and $K_{\mathrm{w}} = 2.82 \times 10^{-14}\ \mathrm{M^2}$. The calculated value of $k_0^{\mathrm{min}}$ is therefore negligible compared with that of $k_{\mathrm{w}}$.

The average values of the general base-catalyzed second-order rate constants ($k_{\mathrm{b}}^{\mathrm{avg}}$) for different buffers (Table 3) were used to construct the Brønsted plot shown in Fig. 4. The least-squares-calculated values of the intercept ($C$) and the Brønsted slope ($\beta$) were found to be $-5.19 \pm 0.20$ and $0.29 \pm 0.05$, respectively, when the values of $k_{\mathrm{b}}^{\mathrm{avg}}$ for all buffers (Table 3) were included. The values of $C$ and $\beta$ become $-5.36 \pm 0.10$ and $0.42 \pm 0.04$ when the data fitted to the Brønsted equation ($\log k_{\mathrm{b}}^{\mathrm{avg}} = C + \beta \mathrm{p}K_{\mathrm{a}}$) include buffers containing carboxylate groups as general base site catalysis.

General acid (GA) and general base (GB) catalysis may be expected to occur in reactions where proton transfer is the rate-determining step [21]. However, GA-GB catalysis has been observed in reactions where the proton transfer step cannot be considered the rate-determining step [6]. Nucleophilic addition as well as addition–elimination reactions involve fast proton transfer, yet many such reactions have been found to involve GA-GB catalysis.

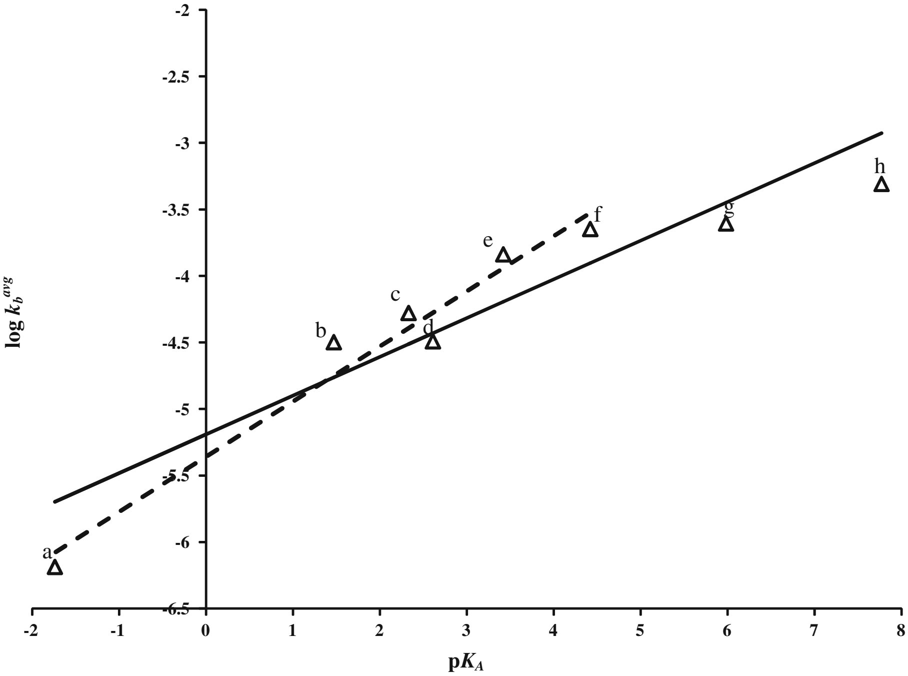
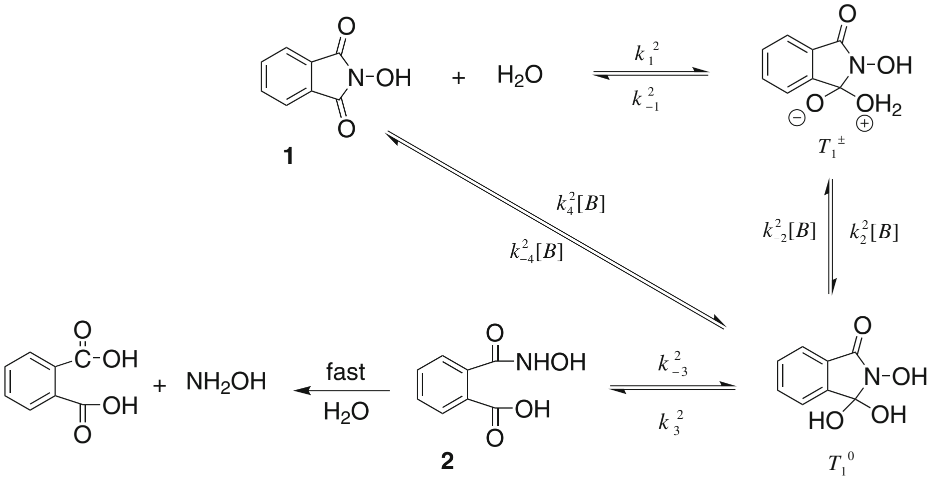


**Fig. 4** The second-order buffer-catalyzed rate constant, $k_b^{avg}$, obtained for the hydrolysis of **1**. The *lines* (*solid* and *dashed*) are *least-squares* fits drawn through the values of $\log k_b^{avg}$ calculated from the Brønsted equation for either (i) all of the buffers [$H_2O$ (*a*), $Cl_2AcA$ (*b*), ClAcA (*c*), Gly (*d*), ForNa (*e*), AcNa (*f*), MES (*g*), and TRIS (*h*); *solid line* with $C = -5.19 \pm 0.20$ and $\beta = 0.29 \pm 0.05$] or (ii) all buffers except for MES and TRIS (*dashed line* with $C = -5.36 \pm 0.10$ and $\beta = 0.42 \pm 0.04$)

**Scheme 2**



GA-GB catalysis occurs in such reactions, including the neutral hydrolysis of **1**, due to the large change in the p$K_a$ of the acidic sites of the reactants during product formation [22, 23].

Plausible reaction mechanisms for the specific acid ($H^+$)-[9] and specific base ($HO^-$)-catalyzed [4, 24] hydrolysis of *N*-substituted phthalimides have been discussed in previous reports. In bimolecular GB-catalyzed reactions, the Brønsted $\beta$ values usually range from 0.2 to 0.5 (see [25] and references cited therein). A small $\beta$ value implies only moderate proton transfer from the water molecule (nucleophile) to the GB catalyst in the transition state. General base-catalyzed hydrolysis of ethyl dichloroacetate (**3**) by a series of different types of GB catalyst resulted in a $\beta$ value of 0.47 [26], which is slightly larger than the $\beta$ value (= 0.3 or 0.4) obtained in the current GB-catalyzed hydrolysis of **1**. The $K_a$ value of the conjugate acid of the leaving group in the GB-catalyzed hydrolysis of **1** is $1.06 \times 10^{-9}$ M [16], and that for **3** is $3.0 \times 10^{-16}$ M. The almost $3 \times 10^6$-fold larger $K_a$ value of the conjugate acid of the leaving group for the hydrolysis of **1** than the corresponding value for **2**, as well as the release of the five-membered ring strain energy in the hydrolytic cleavage of **1**, may be attributed to the difference in the observed $\beta$

values for the GB-catalyzed hydrolysis of **1** and **3**. A plausible reaction mechanism for the GB-catalyzed hydrolysis of **1** is shown in Scheme 2, where the $k_1^2$ and $k_4^2$ steps represent alternative reaction paths for the formation of the highly reactive intermediate $T_1^0$, and the GB catalysts include $H_2O$. If the $k_1^2$ path is energetically favorable compared with the $k_4^2$ path for the formation of $T_1^0$, then it has been concluded elsewhere [16] that the $k_2^2$ step is the rate-determining step for B = $H_2O$. However, if the $k_4^2$ step is energetically favorable for the formation of $T_1^0$, then the $k_4^2$ step should be the rate-determining step. This conclusion is based upon two facts: (1) the p$K_a$ value of the conjugate acid of the leaving group in the $k_3^2$ step is more than 6 p$K$ units lower than that in the $k_{-4}^2$ step, and (2) there is a release of five-membered ring strain energy in the $k_3^2$ step, while there is no such effect in the $k_{-4}^2$ step. These facts suggest that $k_3^2 > k_{-4}^2$, and consequently that the $k_4^2$ step is the rate-determining step.

We can therefore conclude that the water-catalyzed hydrolysis of two substituted acetanilides involves a reaction mechanism similar to that shown in Scheme 2 with B = $H_2O$, where the $k_4^2$ step is the rate-limiting step [27]. Similarly, a theoretical study of the mechanism for the reaction of water with formamide suggests a rate-limiting step similar to the $k_4^2$ step [28].

## Conclusions

The plot of $[\mathrm{Buf}]_T$-independent rate constants $k_0$ for the hydrolysis of **1** versus pH revealed that the values of $k_0$ are independent of pH within the pH range 1.5–6.3. Under such conditions, $k_0 \approx k_w$, where $k_w$ represents the pseudo-first-order rate constant for the reaction of $H_2O$ with **1**. The value of $k_w$ is $35.8 \times 10^{-6}$ $s^{-1}$ at 50 °C. The hydrolysis of **1** within the pH range 1.5–6.3 is catalyzed by buffers. The general base-catalyzed rate constants conform to the Brønsted plot, with a Brønsted slope ($\beta$) of 0.30–0.40.

## Experimental

The rate of hydrolytic cleavage of **1**, under a variety of reaction conditions, was studied spectrophotometrically by monitoring the decrease in the absorbance ($A_{ob}$) as a function of reaction time ($t$) at 300 and 410 nm [16] during the alkaline hydrolysis of **1**. The kinetic study was performed under pseudo-first-order reaction kinetic conditions with a limiting concentration of **1** ($2.5 \times 10^{-4}$ M). Kinetic runs were carried out for >4 half-lives. The immediate hydrolysis product of **1** is *N*-hydroxyphthalamic acid (**2**) which, at pH < 3, converts into phthalic acid and hydroxylamine in a relatively fast step. The details for product characterization and data analysis ($A_{ob}$ vs. $t$) are the same as those described elsewhere [16].

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