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Research Paper

Salt Effects on an Ion–Molecule Reaction—Hydroxide-Catalyzed Hydrolysis of Benzocaine

Ahmad Al-Maaieh[1,2,3] and Douglas R. Flanagan[1]

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Purpose. This work investigates the effect of various salts on the rate of a reaction involving a neutral species (benzocaine alkaline hydrolysis).
Methods. Benzocaine hydrolysis kinetics in NaOH solutions in the presence of different salts were studied at 25°C. Benzocaine solubility in salt solutions was also determined. Solubility data were used to estimate salt effects on benzocaine activity coefficients, and pH was used to estimate salt effects on hydroxide activity coefficients.
Results. Salts either increased or decreased benzocaine solubility. For example, solubility increased with 1.0 M tetraethylammonium chloride (TEAC) ~3-fold, whereas solubility decreased ~35% with 0.33 M Na_2SO_4. Salt effects on hydrolysis rates were more complex and depended on the relative magnitudes of the salt effects on the activity coefficients of benzocaine, hydroxide ion, and the transition state. As a result, some salts increased the hydrolysis rate constant, whereas others decreased it. For example, the pseudo-first-order rate constant decreased ~45% (to 0.0584 h^{-1}) with 1 M TEAC, whereas it increased ~8% (to 0.116 h^{-1}) with 0.33 M Na_2SO_4.
Conclusions. Different salt effects on degradation kinetics can be demonstrated for a neutral compound reacting with an ion. These salt effects depend on varying effects on activity coefficients of reacting and intermediate species.

KEY WORDS: activity coefficient; benzocaine hydrolysis; degradation kinetics; nonelectrolyte solubility; transition state.

INTRODUCTION

The majority of kinetic salt effect studies involve reactions between ionic species. In fact, reports of kinetic salt effects involving neutral compounds are rare, especially for compounds of pharmaceutical interest. Although reactions involving neutral compounds are less affected by salts than reactions involving ionic species, nonetheless, salts still show some effects on neutral species reactions. These effects may be small at low ionic strengths; however, the rate effects may become more significant at higher ionic strengths. Different expressions have been utilized to study salt effects on ionic species reactions (1). These expressions utilize the Debye–Hückel or similar equations. Such expressions should not be used to predict salt effects for neutral species because these treatments were originally derived for ionic species. Thus, such treatments will predict no salt effect on reactions involving neutral compounds simply because the charge of a neutral compound is zero. Other expressions should be used to calculate or predict the activity coefficients of neutral compounds as a function of ionic strength. Such expressions (e.g., Setschenow equation) are empirical; nonetheless, they can be used to calculate the effect of cosolutes on activity coefficients of neutral compounds.

Transition-state theory assumes that reactants (i.e., R_1 and R_2) are in equilibrium with a transition state (2), TS, as described in Eq. (1):

$$R_1 + R_2 \Leftrightarrow \mathrm{TS} \tag{1}$$

This equilibrium is characterized by an equilibrium constant $K^{\ddagger}$, where $K^{\ddagger}$ is equal to:

$$K^{\ddagger} = \frac{a_{\mathrm{TS}}}{a_{R_1} a_{R_2}} = \frac{C_{\mathrm{TS}}}{C_{R_1} C_{R_2}} \times \frac{\gamma_{\mathrm{TS}}}{\gamma_{R_1} \gamma_{R_2}} \tag{2}$$

where C_{TS} is the transition-state concentration, C_{R_1} and C_{R_2} are the concentrations of the reactants, and γ_{R_1}, γ_{R_2}, and γ_{TS} are the activity coefficients of the first reactant, second reactant, and the transition-state, respectively. Because Eq. (1) is, in reality, a pseudoequilibrium, the transition state can go back to reactants (R_1 and R_2) or it can proceed in the forward direction to products. Thus, the overall reaction rate (i.e., disappearance of reactants or appearance of products) is

[1] Division of Pharmaceutics, School of Pharmacy, The University of Iowa, Iowa City, Iowa 52242, USA.
[2] Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, Indiana 46285, USA.
[3] To whom correspondence should be addressed. (e-mail: al-maaieh_ahmad@lilly.com)

related to the transition-state concentration C_{TS}. Thus, using Eqs. (1) and (2),

$$Rate \propto C_{TS} = K^{\ddagger} C_{R_1} C_{R_2} \frac{\gamma_{R_1}\gamma_{R_2}}{\gamma_{TS}} \tag{3}$$

Equation (3) shows that although the reaction rate is related to the concentration of TS, it is also affected by the activity coefficients of reactants and the activity coefficient of TS.

If k is the observed rate constant and k_0 is that in a conventional standard state, then (2–5)

$$\log\frac{k}{k_0} = \log\frac{\gamma_{R_1}\gamma_{R_2}}{\gamma_{TS}} \tag{4}$$

For example, if we consider the alkaline hydrolysis of benzocaine, R_1 is benzocaine and R_2 is OH^-, which gives:

$$\log\frac{k}{k_0} = \log\frac{\gamma_{benzocaine}\gamma_{OH^-}}{\gamma_{TS^-}} \tag{5}$$

This equation shows that any observed kinetic salt effects will be due to salt effects on the activity coefficients of the reactants and the transition state. In fact, this is the definition of a primary kinetic salt effect (3). Such an equation has been used to analyze salt effects on the hydrolysis of 2,4-dinitrochlorobenzene (6) and γ-butyrolactone (7).

If benzocaine hydrolysis kinetics are studied with and without added salts, and the corresponding degradation rate constants are determined (i.e., k^{water} in water, k^{salt} with salt), then using Eq. (5), the ratio of k^{salt} to k^{water} will be:

$$\log\frac{k^{salt}}{k^{water}} = \log\left[\frac{\left(\frac{\gamma_{benzocaine}\gamma_{OH^-}}{\gamma_{TS^-}}\right)^{salt}}{\left(\frac{\gamma_{benzocaine}\gamma_{OH^-}}{\gamma_{TS^-}}\right)^{water}}\right] \tag{6}$$

Rearrangement of Eq. (6) yields:

$$\log\frac{k^{salt}}{k^{water}} = \log\left(\frac{\gamma^{salt}_{benzocaine}}{\gamma^{water}_{benzocaine}}\right) + \log\left(\frac{\gamma^{salt}_{OH^-}}{\gamma^{water}_{OH^-}}\right) + \log\left(\frac{\gamma^{water}_{TS^-}}{\gamma^{salt}_{TS^-}}\right) \tag{7}$$

Thus, the overall kinetic salt effect can be divided into three contributions: a salt effect on the activity coefficients of the neutral compound (benzocaine), and contributions related to salt effects on hydroxide and transition state. The transition state is also an ionic species that has the same charge as the ionic reactant (i.e., -1 in this case). However, the transition state is a bigger ion than OH^-.

The first term in Eq. (7), the ratio of the degradation rate constants, can be determined experimentally. The second term refers to salt effect on benzocaine activity coefficients in the aqueous system. This can be estimated from benzocaine solubility data with and without added salts using an expression of Long and McDevit (8) shown below:

$$\log\left(\frac{\gamma}{\gamma^{\circ}}\right) = \log\left(\frac{S^{\circ}}{S}\right) = k_s C_s \tag{8}$$

where γ is the benzocaine activity coefficient in salt solution, γ° is the benzocaine activity coefficient in water, S° is the benzocaine solubility in water, S is its solubility in a salt solution, k_s is the salting parameter, and C_s is the salt concentration.

The third term in Eq. (7) can be calculated using pH data. Because pH values are related to the activity of hydronium ions, which, in turn, are in equilibrium with hydroxide ions, pH values can give a message of the effect of the various salts on hydroxide ion activity. Using the equilibrium of water dissociation,

$$H_2O \Leftrightarrow H_3O^+ + OH^- \tag{9}$$

which is described by the equilibrium constant K_w that is equal to 1×10^{-14} at 25°C. Thus,

$$K_w = (a_{H_3O^+})(a_{OH^-}) = (10^{-pH})((OH^-)(\gamma_{OH^-})) \tag{10}$$

If the concentration of (OH^-) is kept constant, this leads to the following expression for the ratio of hydroxide activity coefficient in salt solutions *vs.* water:

$$\frac{\gamma^{salt}_{OH^-}}{\gamma^{water}_{OH^-}} = \frac{10^{-pH,water}}{10^{-pH,salt}} \tag{11}$$

Thus, the effect of salts on hydroxide ion activity coefficients can be estimated from pH measurements. This provides a means to calculate the effect of salts on the activity coefficients of TS because the other two terms on the right-hand side of Eq. (7) were separately calculated.

Whereas studies of salt effects on stability of various drugs have been abundant in the pharmaceutical literature, these studies have been limited to reactions of charged species. On the other hand, studies of salt effects on reactions involving neutral species have been rare. This work studies salt effects on the alkaline hydrolysis of benzocaine as an example of a reaction involving an uncharged species. Data in the current study show that salt effects do exist when one of the reactants is not ionized. Moreover, solubility and pH data are utilized in this study to allow for the estimation of the various contributions to the overall kinetic salt effects. This treatment uncovers the operative mechanisms for any observed kinetic salt effects. Salts either increased or decreased benzocaine hydrolysis rates depending on their overall effects on neutral and ionic species in solution. Analysis of the data using Eq. (7) shows that although some salts affect benzocaine hydrolysis rates in the same direction (i.e., either increase or decrease), they might do so for different reasons.

MATERIALS AND METHODS

Materials

Benzocaine was obtained from Eastman Kodak Co. (Rochester, NY USA). NaOH (0.1000 N) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Sodium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Fisher Scientific and were of ACS grade. Sodium chloride, sodium sulfate, sodium perchlorate monohydrate, tetramethylammonium chloride (TMAC), and tet-

raethylammonium chloride monohydrate (TEAC) were all of ACS grade.

Methods

Analytical Methods

A Shimadzu high-performance liquid chromatography (HPLC) system was used for solubility/stability analyses, which consisted of LC-6A pump, SPD-6A UV detector, SIL-9A auto injector, SCL-6B system controller, and CR-501 Chromatopac integrator. A Waters μBondapak C18 column (3.9 × 300 mm) was used for the analysis. The mobile phase consisted of methanol/acetic acid/water (40:4:56), which was pumped at a flow rate of 1.0 ml/min. The injection volume was 40 μl. Detection was at λ = 294 nm (AUFS = 0.04). The analysis run time was 10.5 min with a retention time of 3.3 min for *p*-aminobenzoic acid and 6.2 min for benzocaine.

pH Measurements

pH was measured using an Orion Research Expandable IonAnalyzer EA 920. The indicator pH electrode was a glass body universal glass pH electrode (Accumet # 13-620-284). The sodium ion error for this electrode is 0.1 pH units in 1.0 N NaOH at 25°C. The reference electrode was a high flow rate calomel reference electrode with reverse sleeve junction (Accumet # 13-620-61). Electrode response slopes were all 99.3–101% of the ideal Nernst slope.

Solubility Determination

Excess benzocaine was added to ~7 ml of either distilled water or salt solutions in screw-capped glass tubes. The tubes were shaken in a water bath (25 ± 0.1°C) for 2 days to achieve equilibrium. The resulting suspensions were filtered through 0.45-μm Gelman filters (Nylon Acrodisc®). Saturated solutions were then volumetrically diluted in distilled water and assayed by HPLC. Benzocaine standard curves were constructed using peak areas of standard solutions prepared in distilled water over 0.1–10 μg/ml. Solubility studies were done in duplicate.

Hydrolysis Studies

Benzocaine hydrolysis studies were performed at 0.01 N NaOH. A benzocaine stock solution was prepared by dissolving ~0.25 g of benzocaine in 500 ml of distilled water. Different salt stock solutions were prepared by dissolving the particular salt in 100-ml distilled water. Salt concentrations in these stock solutions were 1.667-fold higher than the concentration needed in a particular hydrolysis study. An aliquot (30.0 ml) of either distilled water or a salt stock solution and a 5.0-ml aliquot of 0.1 NaOH were then added into a 50.0-ml volumetric flask. Hydrolysis studies were initiated by adding 5.0 ml of benzocaine stock solution and bringing the volume to 50.0 ml with distilled water. The pH was measured, and these solutions were kept at 25 ± 0.1°C in a water bath throughout the hydrolysis studies. At appropriate time points, samples (2.0 ml) were taken into 10.0-ml volumetric flasks and bringing to volume with phosphate buffer (20 mM, pH 6.5). These samples were then refrigerated (4–6°C) for no longer than 2 days before analysis by HPLC. Hydrolysis studies were done in triplicate.

Benzocaine concentration at the beginning of the hydrolysis studies was about 0.3 mM. Thus, the initial concentration ratio (hydroxide ion-to-benzocaine) is more than 30×. Thus, these reactions are assumed to be under pseudo-first-order conditions because the concentration of one of the reactants, hydroxide ions, remains essentially constant throughout the course of the reaction. The solutions were tightly closed throughout the hydrolysis studies to minimize the effect of atmospheric carbon dioxide.

RESULTS AND DISCUSSION

Salt Effects on Benzocaine Solubility

Benzocaine solubilities in different salt solutions are listed in Table I. Salts affect the solubility of a neutral compound by changing its aqueous activity coefficients (9). Salts such as Na_2SO_4 and NaCl increase the activity coefficient of benzocaine resulting in lower solubility. TMAC and TEAC, on the other hand, have the opposite effect. For example, 0.33 M Na_2SO_4 decreases benzocaine solubility about 40%, whereas 1.0 M TEAC increases benzocaine solubility 3-fold.

Salt Effects on Benzocaine Stability

Benzocaine (ethyl-*p*-aminobenzoate) degrades by ester hydrolysis in the alkaline region to give *p*-aminobenzoate and ethanol. Its pH-rate profile shows specific base catalysis over pH 9–13 (10). The pK_a of benzocaine is 2.8 (11), and thus, it is uncharged at alkaline pH values. Benzocaine reaction with hydroxide is an ion–molecule reaction at high pH.

The natural logarithms of benzocaine concentration remaining *vs.* time were plotted, and the slopes of the resulting lines were determined. These slopes are equal to $-k_{obs}$, where k_{obs} is equal to the product of hydroxide ion concentration and the second-order rate constant of the reaction, i.e., $k_{obs} = (OH^-)k_{OH}$. The standard error of

Table I. Benzocaine Solubility in Water and Aqueous Salt Solutions at 25°C

Solution[a]	Solubility (mg/ml)	Standard deviation
Water	1.03	0.01
0.25 M NaCl	0.91	0.002
0.50 M NaCl	0.83	0.01
0.75 M NaCl	0.72	0.001
1.0 M NaCl	0.66	0.001
0.33 M Na_2SO_4	0.61	0.03
1.0 M $NaClO_4$	1.02	0.002
1.0 M TMAC	1.44	0.01
0.33 M TEAC	1.57	0.02
0.67 M TEAC	2.30	0.06
1.0 M TEAC	3.19	0.04

[a] TMAC: tetramethylammonium chloride; TEAC: tetraethylammonium chloride.

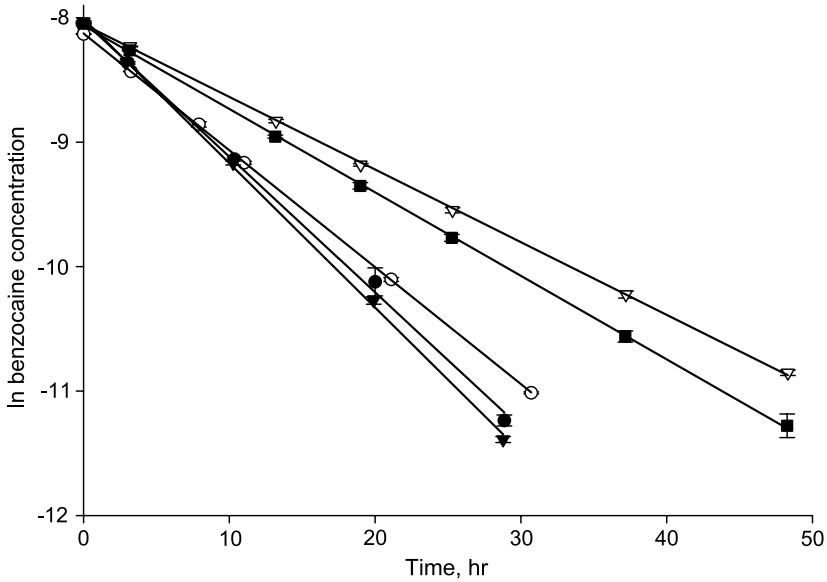

Fig. 1. Pseudo-first-order plots for benzocaine hydrolysis in 0.01 N NaOH at 25°C with different salts. Key: ●, water; ○, 1.0 M NaCl; ▼, 0.33 M Na_2SO_4; ▽, 1.0 M tetraethylammonium chloride; ■, 1.0 M $NaClO_4$.

the slope was $\leq 2.7 \times 10^{-3}$ for all the investigated salts ($R^2 \geq 0.998$). Figure 1 shows a pseudo-first-order plot for benzocaine hydrolysis in different aqueous salt solutions.

It is clear that different salts affect the rate of alkaline benzocaine hydrolysis in different directions and to different degrees. For example, 1.0 M TEAC slows the reaction such that k_{obs} decreases by about 45%, whereas 0.33 M Na_2SO_4 increases k_{obs} by about 8%. Figure 2 shows the effect of increasing concentrations of TEAC on benzocaine hydrolysis kinetics. It can be seen that benzocaine is more stable at higher TEAC concentrations. The observed pseudo-first-order rate constants for benzocaine hydrolysis in 0.01 N NaOH in different salt solutions at 25°C are listed in Table II.

Ratios of the observed benzocaine hydrolysis rate constants (k^{salt}/k^{water}) were calculated according to Eq. (7) and are listed in Table III. As discussed above, changes in the hydrolysis rate constants are caused by changes in the activity coefficients of the reactants or transition state.

Salt effects on benzocaine activity coefficients [second term in Eq. (7)] were estimated from benzocaine solubility in various salt solutions using Eq. (8). For benzocaine, $S°$ is 6.2 mM, and self-interaction terms are ignored (2). Thus, salt effects on benzocaine solubility will provide information about benzocaine activity coefficients in salt solutions. Salts that increased benzocaine activity coefficients (e.g., NaCl and Na_2SO_4) resulted in a lower aqueous solubility. On the other

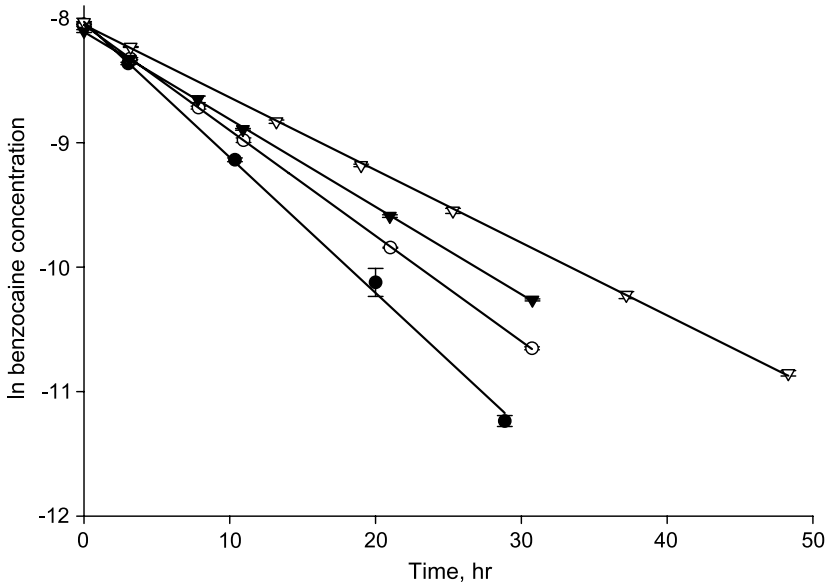

Fig. 2. Pseudo-first-order plots for benzocaine hydrolysis in 0.01 N NaOH at 25°C with different concentrations of tetraethylammonium chloride. Key: ●, no salt added; ○, 0.33 M; ▼, 0.67 M; ▽, 1.0 M.

Table II. Benzocaine Hydrolysis Rate Constants (k_{obs}) and Observed pH Values in 0.01 N NaOH Solutions in the Presence of Different Salts at 25°C

Solution[a]	k_{obs} (h^{-1})[b] ± standard deviation ($n = 3$)	pH[c]
Water	$0.107 \pm 2.34 \times 10^{-3}$	11.97
0.25 M NaCl	$0.0971 \pm 5.51 \times 10^{-4}$	11.78
0.50 M NaCl	$0.0947 \pm 5.13 \times 10^{-4}$	11.73
0.75 M NaCl	$0.0924 \pm 2.65 \times 10^{-4}$	11.69
1.0 M NaCl	$0.0954 \pm 1.48 \times 10^{-3}$	11.69
0.33 M Na_2SO_4	$0.116 \pm 7.81 \times 10^{-4}$	11.70
1.0 M $NaClO_4$	$0.0671 \pm 1.67 \times 10^{-3}$	11.65
1.0 M TMAC	$0.104 \pm 5.69 \times 10^{-4}$	12.06
0.33 M TEAC	$0.0847 \pm 4.00 \times 10^{-4}$	11.96
0.67 M TEAC	$0.0703 \pm 5.77 \times 10^{-5}$	12.05
1.0 M TEAC	$0.0584 \pm 4.62 \times 10^{-4}$	12.14

[a] TMAC: tetramethylammonium chloride; TEAC: tetraethylammonium chloride.
[b] Except for 1 M TMAC, k_{obs} in all salt solutions is statistically different than that in water ($p < 0.025$). For 1 M TMAC, k_{obs} is not statistically different than that in water ($p > 0.025$).
[c] pH values varied by ≤0.01 pH units ($n = 3$).

hand, salts that decreased benzocaine activity coefficients increased its solubility (e.g., TMAC and TEAC). $NaClO_4$ did not change benzocaine solubility significantly (Table I). Ratios of benzocaine activity coefficients (i.e., $\gamma/\gamma°$) in different salt solutions are calculated using Eq. (8) and are listed in Table III.

Although the same NaOH concentration was used for all kinetic studies (0.01 N), different pH values were observed in the reaction media depending on the salt that was used (Table II). NaCl, Na_2SO_4, and $NaClO_4$ decreased the apparent pH compared to that in water, whereas TMAC and TEAC caused an increase in the apparent pH. Thus, the effect of salts on hydroxide ion activity coefficients can be estimated from pH measurements using Eq. (11). This provides a means to calculate the effect of salts on the activity coefficients of TS because the other two terms on the right-hand side of Eq. (7) were separately calculated. This allows the salt effects on the reaction kinetics to be separated into contributions due to benzocaine activity coefficients, hydroxide ion activity coefficients, and transition-state activity coefficients. Equation (7) was used to calculate $\log(\gamma^{salt}/\gamma^{water})$ for TS^- from $\log(k^{salt}/k^{water})$. Table III lists the values of each term in Eq. (7).

For the inorganic salts NaCl, Na_2SO_4, and $NaClO_4$, the values for hydroxide and transition state in Table III are negative. This indicates that the activity coefficients of OH^- and TS^- decreased in the ionic medium. Moreover, these ionic media affected the activity coefficient of OH^- to a higher extent than for TS^-, which is a larger ionic species (compare column 4 in Table III with column 5). This is also expected because larger ions are less affected by ionic strength (2). TMAC and TEAC, on the other hand, generally increased the pH and the activity coefficients of hydroxide ions.

NaCl and Na_2SO_4 solutions increase benzocaine activity coefficient ratios [i.e., positive values for $\log(\gamma^{salt}/\gamma^{water})$; Table III, column 3]. As discussed earlier, this means that the activity coefficient of benzocaine in these salt solutions is higher than that in water, leading to lower benzocaine solubility.

Although the trend of salt effects on $\log(\gamma^{salt}/\gamma^{water})$ for OH^-, TS^-, and benzocaine is similar for both NaCl and Na_2SO_4 solutions, benzocaine is more stable in NaCl solutions and less stable in Na_2SO_4 solutions than in water. This is due to the relative magnitudes of the salt effects on benzocaine and on the ionic activity coefficient ratios [Table III, Eq. (7)].

$NaClO_4$ apparently only affects the activity coefficient of the ionic species (OH^- and TS^-) and not that of benzocaine. Thus, benzocaine hydrolysis rate constant in $NaClO_4$ solutions is less than that in water because $NaClO_4$ decreases the activity coefficient of the ionic reactant (OH^-) more than it decreases the activity coefficient of the larger, ionic transition state.

TEAC solutions also decrease benzocaine hydrolysis rate constants similar to that seen in $NaClO_4$ solutions. However, the decrease in benzocaine reactivity in TEAC solutions is mainly due to the effect on benzocaine activity coefficient. On the other hand, the effect of $NaClO_4$ is mainly on the activity coefficients of the ionic species (OH^- and TS^-). Unlike 1.0 M TEAC, 1.0 M TMAC had a minimal

Table III. Ratios of Benzocaine Hydrolysis Rate Constants, and Activity Coefficients for Benzocaine, Hydroxide Ion, and Transition State in Different Salt Solutions

Solution	$\log(k^{salt}/k^{water})$[a] for k_{obs}	$\log(\gamma^{salt}/\gamma^{water})$[b] for benzocaine	$\log(\gamma^{salt}/\gamma^{water})$[c] for OH^-	$\log(\gamma^{salt}/\gamma^{water})$[d] for TS^-
0.25 M NaCl	−0.042	0.05	−0.19	−0.095
0.50 M NaCl	−0.052	0.09	−0.24	−0.102
0.75 M NaCl	−0.063	0.15	−0.28	−0.064
1.0 M NaCl	−0.050	0.19	−0.28	−0.040
0.33 M Na_2SO_4	0.036	0.22	−0.27	−0.081
1.0 M $NaClO_4$	−0.202	0.00	−0.32	−0.117
1.0 M TMAC	−0.010	−0.15	0.09	−0.046
0.33 M TEAC	−0.101	−0.18	−0.01	−0.095
0.67 M TEAC	−0.182	−0.35	0.08	−0.087
1.0 M TEAC	−0.262	−0.49	0.17	−0.062

[a] Calculated from k_{obs} values.
[b] Calculated from benzocaine solubility.
[c] Calculated from apparent pH values in 0.01 N NaOH.
[d] Calculated with Eq. (7) from values of the other terms.

effect on benzocaine stability. This is due to a predominant effect on benzocaine activity coefficient for 1.0 M TEAC, which offsets its effects on the ionic activity coefficients.

Reaction rates are related to the difference in the free energy between the transition state and the initial reactants (3). Thus, kinetic salt effects will be related to the relative changes the salts cause to the free energies of the reactants and the transition state. If the transition state is stabilized (i.e., its activity coefficient is lowered) to a larger extent than the reactants, the reaction may proceed faster. This was observed for benzocaine hydrolysis in Na_2SO_4 solutions where the salt effect on the transition state was more significant than on the reactants (i.e., combined effect on OH^- and benzocaine). The opposite observation (i.e., reactants are stabilized to a higher extent than the transition state) leads to a decreased reaction rate. This was observed for benzocaine hydrolysis in $NaClO_4$ solutions where the stabilization of the OH^- was more significant than that of TS^- leading to a decrease in the reaction rate constant.

CONCLUSIONS

This study shows that for pharmaceutically significant compounds, there may be kinetic salt effects even if they are uncharged. These salt effects on kinetics could have different origins, which can be related to their effects on activity coefficients of all species involved in the reaction or the transition state. This work also shows that although 1.0 M NaCl, 1.0 M $NaClO_4$, or 0.33 M Na_2SO_4 solutions have the same ionic strength, these solutions have different effects on benzocaine hydrolysis rate constants. This indicates that salt-specific effects may play an important role in drug stability beyond their contribution to ionic strength.

Our analysis of these kinetic salt effects leads one to be able to dissect the overall effect into individual contributions on reactants and transition state. This is the first time that such an analysis has been conducted on kinetics of an ion–molecule reaction to investigate specific contributions.

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