Theoretical Basis for the Detection of General-Base Catalysis in the Presence of Predominating Hydroxide Catalysis

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Abstract □ The detection of general-base catalysis in the presence of predominating specific-base catalysis in aqueous buffer solutions is examined for various relationships between k^0_{cat} and k^0_{OH}, the bimolecular rate constants for general-base and hydroxide-ion attack. The three experimental variables that affect the detection of buffer-base catalysis are the type of buffer, conjugate-acid concentration, and ionic strength. Various buffers used in pharmaceutical kinetic studies are considered, and it is concluded that buffers with high K_a values favor detection. Additionally, high conjugate-acid concentrations and ionic strengths appear to optimize the detection of general-base catalysis.

Keyphrases □ Catalysis, general base—detection in the presence of specific-base catalysis, theoretical basis, effect of buffer composition, acid concentration, and ionic strength □ Hydroxide catalysis—detection of coexisting general-base catalysis, theoretical basis, effect of buffer composition, acid concentration, and ionic strength

Aqueous stability studies often attempt to quantitate the dependence of an observed first-order rate constant on the concentrations of potential reactants such as hydroxide ions, hydronium ions, and buffer components (1). Failure to accurately define the rate expression or the reaction pathway can preclude the establishment of a reaction mechanism and limit the accuracy of stability predictions.

The quantitative assessment of buffer catalysis in the presence of predominating hydroxide- or hydronium-ion attack is difficult. The reasons are twofold. First, the pH region of greatest instability is also the region where the efficiency of specific catalysts (*i.e.*, hydroxide or hydronium ions) overshadows that of the buffer catalysts. If one attempts to make use of these faster rates to establish the reaction pathways, then buffer catalysis would probably be overlooked. Subsequent stability predictions would then underestimate the long-term rate of drug loss in buffer solutions in the pH region where buffer catalysis can compete with specific catalysis. The second reason is that in the presence of relatively high ionic strength and buffer concentrations, which are needed to quantitatively determine buffer catalysis in the presence of predominating specific-ion catalysis, secondary-type ionic atmosphere effects can occur even at constant ionic strength. These effects cause small pH changes, which tend to obscure buffer catalysis.

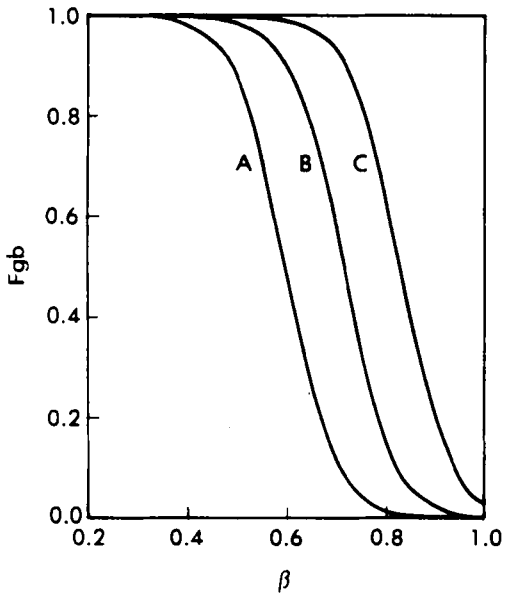

Figure 1—*Fraction of observed general-base catalysis, F_{gb}, as a function of the Brönsted coefficient for a typical buffer (phosphate, pK_a (25°C) = 7.21, p = q = 2). Curves A, B, and C were generated using Eq. 6 and [HB] = 0.01, 0.1, and 1.0, respectively. Ionic atmosphere effects were not included.*

The purpose of this study is to examine the effect of the design of stability studies on the detection of catalysis. A simple rate law was used involving general-base and a related specific-hydroxide degradation pathway. Equations were derived which quantitate the effect of the experimental design on the ability to measure buffer catalysis. Recommendations for detecting buffer-base catalysis in the presence of predominating hydroxide-ion attack are made, and potential problems are examined.

THEORETICAL

Rate Law—When drug degradation is accelerated by general- and specific-base catalysis, the observed first-order rate constant for drug loss, k_{obs}, may be defined:

$$k_{obs} = k^0_{OH}[OH]\frac{\gamma_d\gamma_{OH}}{\gamma_\ddagger} + k^0_{cat}[B]\frac{\gamma_d\gamma_B}{\gamma_{\ddagger'}} \qquad \text{(Eq. 1)}$$

where k^0_{OH} is the bimolecular rate constant for specific-base attack at ionic strength zero, k^0_{cat} is the bimolecular rate constant for general-base attack, [OH] and [B] are specific- and general-base concentrations, γ_d is the activity coefficient of the drug, γ_{OH} and γ_B are the specific- and general-base activity coefficients, and $\gamma_\ddagger$ and $\gamma_{\ddagger'}$ are the transition-state activity coefficients for the specific- and general-base pathways (2). Specific-base attack can involve either nucleophilic hydroxide addition or rate-limiting hydroxide-ion proton abstraction.

General-base catalysis is kinetically indistinguishable from specific-hydroxide and general-acid catalyses (3, 4), which may be defined as:

$$k_{obs(gb)} = \frac{k^0_{cat}K_a}{K_w}[HB][OH^-]\frac{\gamma_{HB}\gamma_{OH}\gamma_d}{\gamma_{\ddagger'}} \qquad \text{(Eq. 2)}$$

where K_a and K_w are the dissociation constants of the buffer and water, and [HB] and γ_{HB} represent the concentration and activity coefficient of the conjugate acid of the base. Division of $k_{obs(gb)}$ by k_{obs} (Eq. 1) yields the fraction due to the general-base pathway, F_{gb}:

$$F_{gb} = \frac{k^0_{cat}[HB]}{\left(\frac{K_w k^0_{OH}\gamma_\ddagger'}{K_a\gamma_{HB}\gamma_\ddagger}\right) + k^0_{cat}[HB]} \qquad \text{(Eq. 3)}$$

The Brönsted relationship may be given by:

$$\log(k/q) = \log GB + \beta(pK_a + \log p/q) \qquad \text{(Eq. 4)}$$

where k is the bimolecular rate constant for the reaction pathway (k^0_{OH} or k^0_{cat}), GB is a constant, β is the Brönsted coefficient, pK_a is the negative logarithm of the dissociation constant for the conjugate acid (and is equal to $pK_w + \log a_{H_2O}$ for hydroxide attack), p is the number of nonequivalent dissociable protons on the conjugate acid base, and q is the number of sites on

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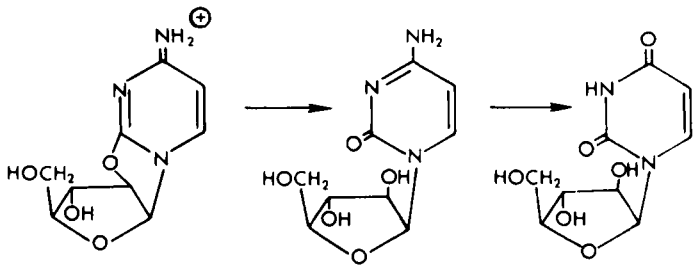

Scheme I

the general base able to accept a proton (3). In practice the Brönsted correlation line may or may not include k^0_{OH}; frequently, specific hydroxide-ion catalysis exhibits negative deviation (3, 4). Assuming that k^0_{OH} is on the Brönsted line made up of k^0_{cat} values allows Eq. 4 to be used to define k^0_{cat} as:

$$k^0_{cat} = k^0_{OH} q \left\{\frac{(p)K_w}{K_a(q)a_{H_2O}}\right\}^{\beta} \qquad \text{(Eq. 5)}$$

and substitution of Eq. 5 into Eq. 3 yields:

$$F_{gb} = \frac{\left\{\frac{(p)K_w}{K_a(q)a_{H_2O}}\right\}^{\beta} K_a[HB]_q}{K_w\left(\frac{\gamma_{t'}}{\gamma_t\gamma_{HB}}\right) + q\left\{\frac{K_w(p)}{K_a(q)a_{H_2O}}\right\}^{\beta} K_a[HB]} \qquad \text{(Eq. 6)}$$

This approach was used to develop the present theory. Where k^0_{OH} values show negative deviation, general-base catalysis will be more readily observed than indicated by this treatment.

DISCUSSION

In Eq. 6, the fraction of general-base attack, F_{gb}, is dependent on the Brönsted coefficient, the activity coefficients for conjugate acid and transition states, the conjugate acid concentration, and the identity of the buffer (*i.e.*, its K_a, q, and p). Initially, for simplicity, it will be assumed that $(\gamma_{t'}/\gamma_t\gamma_{HB}) \cong 1$.

Specific-base attack predominates as the Brönsted coefficient approaches 1. In Fig. 1, F_{gb} is shown as a function of the Brönsted coefficient for a typical general base, dibasic phosphate [$p = q = 2$, pK_a (25°C) = 7.21 (5)], and conjugate acid concentrations equal to 0.01, 0.1, and 1.0 M. The value of F_{gb} decreases sharply when the Brönsted coefficient is between 0.6 and 0.8. A β value of 1.0 is the limiting case, which implies that proton transfer is complete in the transition state (3). Equation 6 then becomes:

$$F_{gb} = \frac{p[HB]}{a_{H_2O} + p[HB]} \qquad \text{(Eq. 7)}$$

Here, except for different p values, all general bases are capable of the same F_{gb} at equal conjugate acid concentrations. Additionally, Eq. 7 shows that for a buffer with $p = 2$, a conjugate acid concentration greater than 9 M is required for 25% buffer-base attack. Clearly this is not experimentally realistic. In contrast, if k^0_{OH} exhibits negative deviation from the Brönsted plot, buffer catalysis may be observed in this limiting case ($\beta = 1.0$). For instance, the log k^0_{OH} value for the conversion of ancitabine to the antileukemic drug cytarabine (Scheme I) was observed to deviate from the Brönsted plot ($\beta \cong 1.0$) by −log 55.

In this case Eq. 6 simplifies to:

$$F_{gb} \cong \frac{p[HB]}{1 + p[HB]} \qquad \text{(Eq. 8)}$$

The prodrug conversion constant in a buffer containing 0.250 M sodium bicarbonate and 0.025 M disodium carbonate ($p = 1$) was observed to be ~24% general-base catalysis (Table I), whereas Eq. 8 predicts 20%.

The value of the Brönsted coefficient will determine whether experimental design is critical in detecting buffer-base attack. The two most important design features are the choice of buffer and concentration of the conjugate acid.

The ability of a buffer to act as a general base is related to the pK_a of its conjugate acid. The larger the pK_a, the better the general base. Therefore, one might expect that general-base attack would be most apparent with an efficient general base such as dibasic carbonate or tribasic phosphate. However, the presence of predominating hydroxide attack, *i.e.*, a Brönsted coefficient of 0.8–1.0, in the high pH range required for the existence of these bases negates the possibility of observing general-base attack. In Fig. 2 the relative amount of general-base attack is shown as a function of the pK_a of the buffer system at three conjugate acid concentrations. Figure 2, generated from Eq. 6 by assuming $p = q = 1$ and $\beta = 0.8$, shows that at a conjugate acid concentration of 0.1 M, general-base catalysis by buffers of p$K_a \geq 7$ cannot be detected. If the conjugate acid concentration is below 0.01 M, it is likely that general-base catalysis will not be detected at $\beta = 0.8$ regardless of the buffer used. In general the lower the pK_a, the more useful the buffer in observing general-base catalysis. By viewing the x-axis in Fig. 2 as a pH scale, the pH region of competitive general-base catalysis can be seen. At lower pH, hydroxide catalysis becomes less effective and, thus, general-base catalysis can become significant.

Figure 3 shows the relationship between F_{gb} and the conjugate acid concentrations for a series of common buffers at $\beta = 0.8$. As the conjugate-acid concentration increases, F_{gb} increases. Thus, doubling the concentration of the general base while maintaining constant conjugate acid concentration will not increase the relative amount of general-base attack. This is due to the fact that such an increase in general-base concentration will also increase the hydroxide-ion concentration.

The kinetics of ancitabine conversion to cytarabine provide an example of the effect of changing conjugate-acid concentrations on F_{gb}. The Brönsted slope for this reaction is approximately one; however, the hydroxide rate constant deviated negatively from the Brönsted plot, allowing general-base catalysis to be observed. The value of k_{obs} is described by Eq. 1 where $k_{OH} = k^0_{OH}\gamma_{OH}\gamma_d/\gamma_t$. The fraction of general-base catalysis, F_{gb}, was determined by estimating k_{OH} from high pH studies and using the following relationship:

$$F_{gb} = (k_{obs} - k_{OH}[OH])/k_{obs} \qquad \text{(Eq. 9)}$$

where [OH] was calculated from measured pH. These values were also approximated using Eq. 8, which allows negative hydroxide rate constant deviation from the Brönsted plot. The agreement between the results (Table I) tends to support the experimental approximations ($\beta \cong 1.0$ and deviation ≅ −log 55) and theoretical considerations (Eq. 6) that were used to derive Eq.

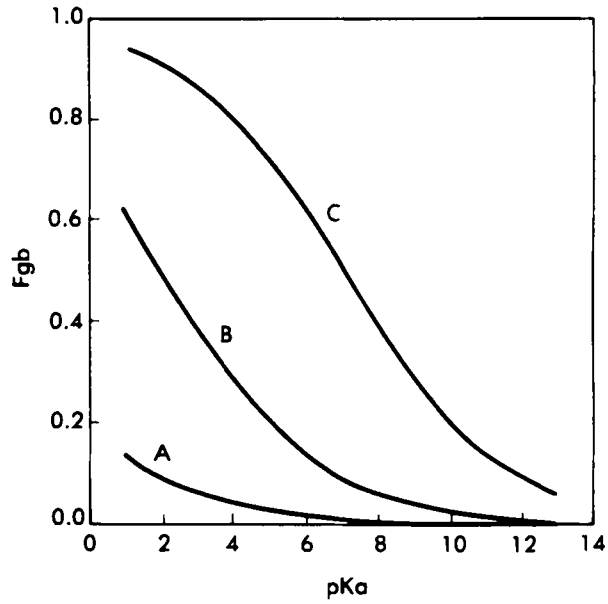

Figure 2—*Fraction of observed general-base catalysis, F_{gb}, as a function of the pK_a of a series of buffer systems. Curves were generated using Eq. 6; p and q were assigned unity and curves A, B, and C correspond to [HB] = 0.01, 0.1, and 1.0, respectively. Ionic atmosphere effects were ignored.*

Table I—Observed First-Order Rate Constants and Fraction of General-Base Catalysis For Ancitabine Prodrug Conversion in Carbonate Buffers at 60°C and μ = 1.0 [a]

Concentration, M				F_{gb}	
HCO_3^-	CO_3^{2-}	pH	k_{obs}, h^{-1}	Obs.[b]	Calc.[c]
0.200	0.025	8.46	13.0	0.20	0.17
0.250	0.025	8.38	11.4	0.24	0.20
0.300	0.025	8.32	10.1	0.25	0.23
0.400	0.025	8.25	9.39	0.31	0.29
0.800	0.025	8.13	9.84	0.50	0.44

[a] Ionic strength adjusted with sodium chloride. [b] Calculated from $k_{OH}(60°C, \mu = 1) = 2.0 \times 10^5\ h^{-1}\ M^{-1}$ and the k_{obs} values using Eq. 9. [c] Calculated from Eq. 8.

8. It is interesting to note that in all of the carbonate buffers used, the concentration of the catalytic buffer base (CO_3^{2-}) was kept constant while the conjugate acid and pH changed (see Table I).

The popular technique of increasing the total buffer concentration while maintaining a constant pH and base-to-acid ratio may or may not allow detection of general-base catalysis. Suppose the Brönsted coefficient is 0.8, and one is attempting to detect base attack using Tris buffers. If constant pH is maintained while the total buffer concentration is increased 10-fold, the conjugate-acid concentration might increase from 0.01 to 0.1 M. The percentage increase in the relative amount of observed general-base catalysis will be only 6% (Fig. 3). Therefore, general-base catalysis would be overlooked. The same technique applied to the monobasic/dibasic phosphate system would result in a 15% increase in k_{obs}, while the formate/formic acid buffer would result in an ~30% increase in k_{obs}.

If one objective in a series of stability studies is to determine buffer-base catalysis, then the approach should be to employ buffers with pK_a values as low as possible using conjugate-acid concentrations as high as is experimentally feasible. Experimental designs which seek to otherwise optimize either the general-base or total buffer concentrations are misdirected.

Activity coefficients are used in Eq. 1 to describe ionic atmosphere effects. Direct extrakinetic measurements of activity coefficients are rare in the pharmaceutical literature. Instead, approximations based on the empirical effect of ionic strength on the logarithm of the activity coefficient of an ion are commonly used (6). Although there are problems associated with these approximations, particularly (as will be discussed later) in solutions of moderately high ionic strength, they conveniently allow the charge of the ion to affect the reaction rate but not its specific chemical identity. By employing ionic strength approximations, salt effects on the two reaction pathways can be evaluated for various combinations of charged and uncharged substrates and buffer components.

As can be seen in Eq. 6, ionic atmosphere effects will influence the relative amount of general-base attack (F_{gb}) to the extent that $\gamma_{\dagger'}/\gamma_{\dagger}\gamma_{HB}$ is affected. Under the ionic strength assumption, ionic atmosphere effects will depend on the charges of the various ions. Since the transition-state charges are equal to the sum of the charges on the reactants (7), the charges on the specific-base ($Z_{\dagger}$) and general-base ($Z_{\dagger'}$) transition states are given by:

$$Z_{\dagger'} = Z_d - 1 \qquad \text{(Eq. 10)}$$

$$Z_{\dagger'} = Z_d + Z_B \qquad \text{(Eq. 11)}$$

where Z_d and Z_B are the charges on the drug and general base and $Z_{OH} = -1$. For a buffer system in which the general base is a monovalent anion and the conjugate acid is neutral (*e.g.*, acetate–acetic acid), $\gamma_{\dagger'}/\gamma_{\dagger}\gamma_{HB} \cong 1$ because $Z_{\dagger'} = Z_{\dagger}$ and $\gamma_{HB} \cong 1$. The value of $\gamma_{\dagger'}/\gamma_{\dagger}\gamma_{HB}$ is also ~1 if the substrate is a monovalent cation, regardless of the charge type of the buffer system. This is because $\gamma_{\dagger'} \cong \gamma_{HB}$ and $\gamma_{\dagger} \cong 1$. In these two situations F_{gb} is unaffected by ionic strength. However, for other combinations of neutral and charged substrates and buffers, the effect of ionic strength can be approximated by using the Güntelberg equation:

$$\log \gamma_i = -Z_i^2 A\sqrt{\mu}/(1 + \sqrt{\mu}) \qquad \text{(Eq. 12)}$$

in which i denotes a central ion, Z_i is the charge on i, A is a function of temperature and solution dielectric constant, and μ is the ionic strength (6).

Table II summarizes the approximated values of $\gamma_{\dagger'}/\gamma_{HB}\gamma_{\dagger}$ for monovalent or neutral substrates and various buffer systems. For neutral or anionic drugs, the effect of increasing ionic strength is to increase F_{gb} if the conjugate buffer acid is anionic and to decrease F_{gb} if the conjugate acid is a cation.

Ionic strength is often maintained constant in pharmaceutical kinetic studies (6). Charged drugs are subject to significant primary and secondary kinetic salt effects which should be quantitated at various fixed ionic strengths or should be normalized by conducting all studies at a constant ionic strength. Uncharged drugs can also be subject to secondary-type salt effects in buffered solutions, and the logarithm of their activity coefficients may display a linear dependence on ionic strength (8).

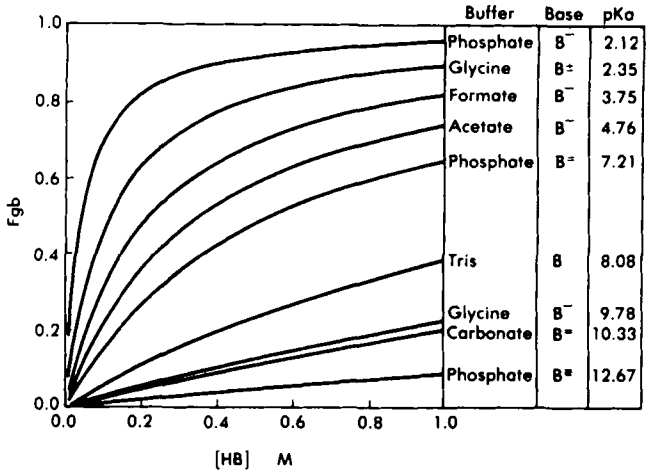

Figure 3—*Fraction of observed general-base catalysis, F_{gb}, as a function of the conjugate-buffer acid concentration for a series of buffers used in pharmaceutical stability studies. Curves were generated using Eq. 6; pK_a (25°C) values were taken from the literature (5). Ionic atmosphere effects were ignored.*

The condition of constant ionic strength sets mathematical limits on the maximum concentration of any given buffer and, thus, sets limits on the maximum F_{gb} value. The maximum concentrations occur in solutions with no added neutral salts. For instance, for a buffer with dibasic and monobasic components such as carbonate or phosphate, the maximum conjugate acid concentration, $[HB]_{max}$, for a fixed ionic strength of μ is given by:

$$[HB]_{max} = \mu - 3[B]_{min} \qquad \text{(Eq. 13)}$$

where $[B]_{min}$ is the minimum concentration of the general base and Eq. 13 was derived using the definition of ionic strength.

To maintain first-order conditions, the attacking species concentrations must be sufficiently higher than the initial substrate concentration to prevent significant loss of buffer catalysts or hydroxide ions. A 5% loss of the attacking species is well within first-order conditions (9). Thus, the minimum general-base concentration should be 20 times that of the initial substrate concentration. Equation 13 can then be written:

$$[HB]_{max} = \mu - 60[D_0] \qquad \text{(Eq. 14)}$$

where $[D_0]$ is the initial drug concentration and is usually selected on the basis of stability-indicating assay detection limits. Since $[HB]_{max}$ can be used in Eq. 6 to calculate the maximum F_{gb} for any given buffer system, a high constant ionic strength chosen by the experimenter will allow for a high maximum F_{gb} value.

CONCLUSIONS

The detection of buffer-base catalysis in the presence of predominating specific-base catalysis is directly influenced by the choice of buffer, conjugate acid concentration, and ionic strength. Two characteristics favor one buffer over another: lower pK_a and, to a lesser extent, the charge types of the buffer base and acid. Despite the fact that buffers with high pK_a values are more efficient general bases, they are unable to compete with hydroxide ions in the pH range where they exist. As can be seen in Fig. 2, it is possible to observe significant buffer-base catalysis in relatively low pK_a buffers.

The ionic charges of the buffer components affect F_{gb} in two ways. First, the maximum conjugate-acid concentration is dependent on the buffer charges, as can be seen in the following equation:

$$[HB]_{max} = \frac{2\mu - \{20(Z_B^2 + |Z_B|)[D_0]\}}{Z_{HB}^2 + |Z_{HB}|} \qquad \text{(Eq. 15)}$$

which is a generalized form of Eq. 14 and was derived from the definitions of ionic strength and first-order conditions. Second, the buffer component charges affect the activity coefficient ratio $\gamma_{\dagger'}/\gamma_{\dagger}\gamma_{HB}$.

The maximum conjugate-acid concentration allows observation of the maximum F_{gb} for any given buffer, and kinetic studies should be conducted near this maximum in order to obtain accurate estimates of k^0_{cat}. Maximum conjugate-acid concentrations are generally ionic strength limited. The higher the ionic strength, the larger the allowable maximum conjugate-acid concentration and, thus, the larger the relative amount of buffer-base catalysis.

Table II—Values for x in the Approximate Equation, $(\gamma_{t^+}/\gamma_t\gamma_{HB}) \cong 10^{x[A\sqrt{\mu}/(1+\sqrt{\mu})]}$, for Monovalent and Neutral Drug in Buffers

Buffer Charges			Values for x Drug Charges		
Base	Acid	Examples (pK_a)	Neutral	Anion	Cation
Monovalent anion	Neutral	Acetate (4.76)	0	0	0
Divalent anion	Monovalent anion	Carbonate (10.33)	−2	−4	0
Trivalent anion	Divalent anion	Phosphate (12.67)	−4	−8	0
Monovalent anion	Zwitterion	Glycine (9.78)	0	0	0
Neutral	Monovalent cation	Tris (8.08)	2	4	0

But, choosing relatively high ionic strength has drawbacks. In particular the ionic atmosphere assumptions used in deriving Eq. 6 tend to fail. In solutions of high ionic strength, the logarithm of an activity coefficient of an ion is not only affected by the ionic strength of the solution, but also by the chemical identities and concentrations of all ions of charges opposite to that of the central ion. This relationship has been empirically described by:

$$\log \gamma_i = \frac{-AZ_i^2\sqrt{\mu}}{1+\sqrt{\mu}} + \sum_j \beta_{ij}C_j \qquad \text{(Eq. 16)}$$

where γ_i is the activity coefficient of an ion i, Z_i is the charge on i, A is a function of the temperature and solution dielectric constant, C_j is the concentration of the jth ion of charge opposite to that of ion i, and β_{ij} is the specific ionic interaction coefficient for ions i and j (7, 10).

The magnitude of the extra-ionic strength salt effects depends on the concentrations of ions and the values of the specific ionic interaction coefficients. One of the major effects of specific ionic interactions in buffer solutions is to cause pH changes at constant ionic strengths. The pH of a buffer solution, such as sodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride, is given by the following equation:

$$pH = pK_a + \log [B]/[HB] + \log (\gamma_B/\gamma_{HB}) \qquad \text{(Eq. 17)}$$

and substitution of Eq. 16 for $\log (\gamma_B/\gamma_{HB})$ yields:

$$pH = pK_a + \log \frac{[B]}{[HB]} - \frac{3A\sqrt{\mu}}{1+\sqrt{\mu}} + \Delta\beta_{Na}[Na^+] \qquad \text{(Eq. 18)}$$

where $[Na^+]$ is the molar concentration of sodium ion and $\Delta\beta_{Na}$ is the difference between the specific interaction coefficient for sodium ion and dibasic and monobasic phosphate ions. For example, the percent change in the hydroxide-ion activity based on measured pH in phosphate buffers at 60°C, wherein the base-acid concentration ratio was kept constant and the ionic strength was kept constant at 1.0, was as much as 80%. The problem of quantitating buffer-base attack in the presence of predominating hydroxide attack is compounded if the hydroxide-ion activity is changing due to specific ionic interactions.

One method of correcting the observed first-order rate constant for the type of secondary ionic atmosphere effect described above has been used in ancitabine hydrolysis studies. It is a general method for cationic substrates that only partly eliminates ionic atmosphere effects at constant ionic strength. It does not correct k_{obs} for specific ionic interactions between the substrate and anions. The approach is to calculate the activity coefficient ratio of the buffer base-acid from experimentally measured pH using:

$$(\gamma_B/\gamma_{HB}) = K_a[HB]/[B]10^{-pH} \qquad \text{(Eq. 19)}$$

Division of k_{obs} as defined in Eq. 1 by Eq. 19 eliminates all activity coefficients except that of the substrate. In the case of ancitabine, it was shown by kinetic measurements in buffers of various ionic strengths that the activity coefficient of the substrate could be approximated by Eq. 12. Similar experimental approaches to the problem of ionic atmosphere effects in solutions of high ionic strength may be necessary to detect buffer catalysis in the presence of predominant specific-acid attack.

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