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Determination of thermodynamic dissociation constants of local anaesthetic amines: influence of ionic strength

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Theoretical calculations, based on the Debye-Hückel theory, indicate that for weak monoprotic bases that do not involve a hydroxyl group (B) a stoichiometric pKa determined at any ionic strength compatible with an accurate determination ($\mu < 0{\cdot}10$) should be no different from the thermodynamic pKa. The pKa's of two local anaesthetic amines were determined at several ionic concentrations. The resultant independence of the acidic dissociation constants on ionic concentration supports the theoretical considerations. The implications of these results in local anaesthetic research are discussed.

An increasing body of evidence exists which indicates that the ionized and unionized species of a drug molecule behave differently in a wide variety of physical and biological systems. Hence, it has been found necessary to determine dissociation constants more accurately than previously, and to know the exact relation between thermodynamic, apparent and stoichiometric dissociation constants. In most physical chemistry textbooks (Daniels & Alberty, 1966; Moore, 1962; Martin, Swarbrick & Cammarata, 1969; Robinson & Stokes, 1959); the effect of ionic strength on the acidic dissociation constants of acids and bases appears to be a very well understood phenomenon. Either the example of a weak acid of the HA type is given or a general equation in which charges have been omitted is derived so that it can apply equally to acids and bases. In the present work with local anaesthetic amines, it became apparent that acids and bases exhibit different properties with respect to ionic concentration. Weak bases of the amine type comprise several other pharmacological classes of drugs (antihistamines and sympathomimetics, psychotropic amines), and it is useful to know to what extent, if any, a measured pK_a^c (stoichiometric pKa) differs from the ultimately desirable pK_a^T (thermodynamic pKa). It will be shown that for weak bases that do not involve a hydroxyl group (B), ionic strength does not seem to affect the stoichiometric pKa (pK_a^c). This relation could be predicted by judicious substitutions in the appropriate equations and was borne out by experiments with lignocaine and DABA (2-diethylamino-3′-benzyloxyacetanilide), a chemically related local anaesthetic amine. The implications of these considerations in local anaesthetic research are further discussed.

THEORETICAL

The equilibrium expression for the dissociation of a weak acid $BH^+ X^-$ (salt of a weak base and a strong acid) in water may be written in terms of the following dissociation constants:

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$$K_a^T = \frac{a_B \cdot a_{H^+}}{a_{BH^+}} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$K_a^c = \frac{[B] \cdot [H^+]}{[BH^+]} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$K_a' = \frac{[B] \cdot a_{H^+}}{[BH^+]} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where a and [ ] represent, respectively, activity and concentration; K_a^T, K_a^c, and K_a' are respectively the thermodynamic (activity), stoichiometric (concentration) and apparent (hybrid) acidic dissociation constants. K_a', a hybrid of K_a^T and K_a^c, is the one obtained by the half neutralization method.

K_a^T can be expressed in terms of K_a^c as follows:

$$K_a^T = K_a^c \frac{\gamma_B \cdot \gamma_{H^+}}{\gamma_{BH^+}} = K_a^c \frac{\gamma_{H^+}}{\gamma_{BH^+}} \quad .. \quad .. \quad .. \quad (4)$$

where γ is the molar activity coefficient and γ_B is assumed to be unity on the basis that B is an uncharged molecule and therefore not subject to electrical interactions in dilute solution.

Equation 1 can also be written in terms of K_a':

$$K_a^T = K_a' \frac{\gamma_B}{\gamma_{BH^+}} = \frac{K_a'}{\gamma_{BH^+}} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

Several authors (Garrett, 1963; Goldman & Meites, 1964; Benet & Goyan, 1967) have shown that pK_a' obtained by the half neutralization method will often be in error. This is not so of pK_a^c and pK_a^T obtained by accurate methods of handling the data (Benet & Goyan, 1965; Leeson & Brown, 1966). Since this work is primarily concerned with the accurate determination of dissociation constants, equation 4 expressing the relation between K_a^T and K_a^c will be examined in some detail.

Taking the negative log of both sides of equation 4 yields:

$$pK_a^T = pK_a^c + \log \frac{\gamma_{BH^+}}{\gamma_{H^+}} \quad .. \quad .. \quad .. \quad (6)$$

If one knew how ionic concentration affects the two ions involved, namely the proton and the conjugate acid of the base, the relation between pK_a^T and pK_a^c could be predicted from equation 6. The electrical interaction theory of Debye-Hückel tackles this problem. For very dilute solutions (ionic strength less than 0·01) Debye-Hückel derived the limiting law for the activity coefficient γ_1 of an ion of charge z_1:

$$\text{Log } \gamma_1 = -A\, z_1^2 \sqrt{\mu} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

where A = a factor which depends on the temperature and the dielectric constant of the medium and μ = the ionic strength.

After substitutition of equation 7 for the two ions, equation 6 becomes:

$$pK_a^T = pK_a^c \quad .. \quad .. \quad .. \quad .. \quad (8)$$

According to equation 7, H^+ and BH^+ have the same activity coefficient at any ionic

strength (up to 0·01) simply because they are both univalent, and the result of equation 8 is expected. At higher concentrations the assumptions behind equation 7 no longer hold and Debye-Hückel enlarged the equation to take into account the finite size of the ions:

$$\text{Log } \gamma_1 = \frac{-A\, z_1^2 \sqrt{\mu}}{1 + d_1 B \sqrt{\mu}} \qquad (9)$$

where d_1 = effective ionic diameter or ion size parameter and B = constant which depends on the temperature and the dielectric constant of the medium. This equation holds quite accurately up to an ionic strength of 0·10. For these concentrations no obvious predictions can be made about the relation between pK_a^T and pK_a^c in equation 6. All one can say is that the ratio $\log \gamma_{BH^+}/\gamma_{H^+}$ will differ from 1, only to the extent of the influence of the ion size parameter d_1 in equation 9.

For still higher concentrations, equation 9 can be extended. However, this presents no interest, since it becomes difficult to measure an accurate pK_a^c at ionic strengths higher than 0·10 (Albert & Serjeant, 1962).

MATERIALS AND METHODS

Materials. The materials and instruments used were as previously described (Levy & Rowland, 1971).

Methods. The sample of base hydrochloride was dissolved in 60 ml. of freshly prepared potassium chloride solution. For lignocaine (Table 2), the drug concentration varied in the different runs and the concentration of the KCl solution was adjusted to the desired total ionic strength. In the case of DABA (Table 3), all the determinations were performed at the same drug concentration (10^{-3} M). Consequently no KCl solution was needed in experiments 1 to 6 and KCl 0·049 M was used in experiments 7 to 12. The titration was performed under nitrogen in a water jacketed thermostated vessel (24 ± 0·1°). Sodium hydroxide was added from a 1-ml microburette calibrated to 0·0002 ml. In most titrations the amount of acid was calculated such that 0·5 to 1·0 ml of 0·1N NaOH was consumed. A minimum of ten additions were made in a single run.

RESULTS AND DISCUSSION

Calculation of equation 9 based on theoretical considerations. The activity coefficient for the hydrogen ion can be obtained in several ways. Guggenheim (1935) developed a general equation to calculate the activity coefficient for a specific ion with a specific ion-interaction constant. This equation is accurate ($\mu < 0{\cdot}1$M) for the hydrogen ion. The Tables of Lewis & Randall (1961) give experimentally determined γ^+ values for HCl which have been used quite extensively. Kielland (1937) calculated the ion size parameter of a series of organic and inorganic ions. For the hydrogen ion he estimated the d_1 term in equation 9 to be 9 Å and calculated γ_{H^+} at several ionic strengths. Furthermore, he compared the mean ionic activity coefficient (for HCl) which he calculated to the experimental values of Lewis and Randall and to those obtained from Guggenheim's formula and showed that all three sets of values are in close agreement. Leeson & Brown (1966) conducted a study to determine which of the two sets of activity coefficients, Kielland's or Lewis and Randall's values,

should be used to convert a_{H^+} to $[H^+]$. They concluded that "the type of activity coefficient employed for calculations appears to be a matter of personal choice."

It is much more difficult to obtain an accurate value for γ_{BH^+}, the activity coefficient for the conjugate acid of the base. Guggenheim's equation cannot be used since specific drug ion-interaction constants have not been reported. The solution of choice in this case is to use equation 9. However, one has to guess a value for the ion size parameter of drug ion. Lofgren (1948) determined the thermodynamic acidic dissociation constant of a series of local anaesthetics. After measuring ionic conductances in order to use Brull's empirical formula, he obtained an ion size parameter of 8 Å. However, Kielland's studies are more comprehensive and probably more reliable, since he examined a series of 130 ions (organic and inorganic) and he estimated the ion size parameters by different methods. He found that most of the organic ions had a d_i between 4·5 and 7 Å. More recently, Benet & Goyan (1965) calculated the thermodynamic pKa's of a series of drugs and assumed a value of 5 Å for all singly charged ionic species. It is estimated that 5 Å adequately represents the ion size parameter of most conjugate acids of weak basic drugs. Using this value, the activity coefficients were calculated for equation 9 for ionic strengths varying between 0·010 and 0·10. The A and B terms of the equation are respectively equal to 0·500 and $0{\cdot}330 \times 10^8$ for water at 25°. Table 1 shows the calculated

Table 1. *Activity coefficients for H^+ and BH^+ and calculation of $pK_a^T - pK_a^c$ at various ionic strengths.*

μ	$\gamma_{H^+}^K$	$\gamma_{\pm}^{LR}$	γ_{BH^+}	$pK_a^T - pK_a^c$*	$pK_a^T - pK_a^c$†
0·010	0·914	0·904	0·904	0·0044	−0·0001
0·025	0·88	0·866	0·862	0·0096	0·0019
0·050	0·86	0·830	0·826	0·0147	0·0022
0·100	0·83	0·796	0·784	0·0277	0·0067

* Calculated using $\gamma_{H^+}^K$.

† Calculated using $\gamma_{\pm}^{LR}$.

values for γ_{BH^+} as well as the hydrogen ion activity coefficients of Kielland (γ_H^{K+}) and the $\gamma^{\pm}$ values of Lewis and Randall ($\gamma_{\pm}^{LR}$). Even though the ratio $\gamma_{BH^+}/\gamma_{\pm}^{LR}$ is at all times closer to unity than $\gamma_{BH^+}/\gamma_H^{K+}$, $pK_a^T - pK_a^c$ was calculated for both sets of values, and the results are shown in the last two columns of Table 1. In both cases the difference $pK_a^T - pK_a^c$ increases with the ionic strength. There are sound reasons for this based on the assumptions underlying the Debye-Hückel theory. The size of the ions becomes a more and more significant parameter as the ionic concentration increases. Nevertheless, the difference between pK_a^T and pK_a^c is always insignificant. Even in the worst case, the difference $pK_a^T - pK_a^c$ (0·023) is less than the experimental error of the most sensitive method of pKa determination (±0·03 pKa unit), (Benet & Goyan, 1967).

pKa determinations of lignocaine and DABA at several ionic concentrations

The preceding results, arrived at from theoretical calculations, were experimentally tested in the following manner. The pKa of lignocaine was accurately determined at five different ionic concentrations ($0{\cdot}005 < \mu < 0{\cdot}0775$) using the method of Benet

Table 2. *Dissociation constant of lignocaine determined at various ionic strengths (24°).*

Experiment	Drug concentration (M)	Ionic strength	pKa
A	0·001	0·0050	7·850
B	0·001	0·0100	7·825
C	0·002	0·0240	7·907
D	0·010	0·0300	7·835
E	0·001	0·0775	7·884
			Ave 7·860
			s.d. 0·034

& Goyan (1965) and calculating the data as proposed by Leeson & Brown (1966). The results are shown in Table 2. The average value agrees with the pK'_a of 7·86 (25°) reported by Lofgren (1948). Also there is no trend in the data. Finally, the standard deviation is no larger than would be expected from repeated determinations at the same ionic concentration.

To perform a more thorough analysis of the ionic strength effect, the pKa of DABA was determined six times at two ionic concentrations ($\mu = 0{\cdot}001$ and 0·05). The method used was that of Levy & Rowland (1971) for sparingly soluble substances, and the results are shown in Table 3. The *t*-test applied to this data shows that the difference between the two means is not statistically significant ($P < 0{\cdot}05$). In other words, a fifty-fold change in ionic strength could not be shown to affect the pKa of this compound.

Application to local anaesthetic amines

Most local anaesthetics are secondary or tertiary amines. The determination of dissociation constants is an intrinsic part of a variety of investigations on the relation between physico-chemical (pKa, partition coefficient) and pharmacological (intrinsic activity, blocking potency) parameters (Lofgren, 1948; Truant & Takman, 1959; Ehrenberg, 1948; Skou, 1954a or b). However, studies on the active form of local anaesthetics (base or cation) have been by far the most numerous and also the most controversial (Ehrenberg, 1948; Ritchie & Greengard, 1961, 1966, 1968; Shanes, 1958; Skou, 1954a, b). These are of special interest, since much of the evidence for either

Table 3. *Repeated determinations of the dissociation constant of DABA at two ionic concentrations (24°).*

$\mu = 0{\cdot}001$		$\mu = 0{\cdot}050$	
Experiment	pKa	Experiment	pKa
1	7·946	7	7·998
2	8·039	8	8·061
3	8·041	9	8·0[illegible]
4	7·939	10	8·016
5	7·933	11	7·999
6	7·990	12	8·001
Ave	7·981	Ave	8·018

theory (base or cation) is based on calculations using a more or less corrupted form of the Henderson-Hasselbach equation:

$$pH = pK_a^T + \log \frac{a_B}{a_{BH^+}} \quad .. \quad .. \quad .. \quad .. \quad (10)$$

A review of the literature shows that much confusion exists regarding the relation between the various dissociation constants as well as on activity corrections in calculations of concentrations of charged and uncharged species, utilizing equation 10. Thus, Skou (1954b) defines the minimum blocking concentration of a local anaesthetic, C_B, in terms of what appears to be a K_a^c which is ambiguously called "the dissociation constant." (eqn 11)

$$k = \frac{C_B \times C_{H^+}}{C_{BH^+}} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

However, in calculating C_B for cocaine he utilizes a previously determined apparent dissociation constant $pK_a' = 8{\cdot}70$ (Skou, 1954a). Also, in calculating C_B as a function of pH for several local anaesthetics, Skou used concentrations rather than activities. In further analysing Skou's data, Shanes (1958) pointed out that the discrepancy between theoretical and experimental curves (of blocking potency as a function of pH) for cocaine is eliminated when activities are taken into consideration. As seen from equation 10, activities can be computed directly if one utilizes a thermodynamic dissociation constant. However, when pK_a' is substituted for pK_a^T in equation 10, a_{BH^+} is given by:

$$a_{BH^+} = [BH^+] \cdot \gamma_{BH^+} \quad .. \quad .. \quad .. \quad .. \quad (12)$$

Nevertheless, in several instances various workers (Lofgren, 1948; Truant & Takman, 1959; Skou, 1954b) measured apparent dissociation constants when it was possible, with the same effort, to obtain thermodynamic constants. Finally, it should be noted that most measurements of conduction block are made in isotonic solutions (Ehrenberg, 1948; Skou, 1954b; Ritchie & Greengard, 1961). This further complicates the situation, since there is no general equation describing ion interaction that can be used to calculate activity coefficients at such an ionic strength.

These studies on lignocaine and DABA confirm the previous theoretical calculations. Consequently, for any monoprotic weak base (which does not involve a hydroxyl group) a thermodynamic pKa can be obtained at any ionic strength compatible with an accurate determination ($\mu < 0{\cdot}10$), whereas for acids the most accurate method is still to measure pK_a^c at several ionic concentrations and to extrapolate to infinite dilution a plot of pK_a^c versus $\sqrt{\mu}/1 + \sqrt{\mu}$.

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REFERENCES

ALBERT, A. & SERJEANT, E. P. (1962). *Ionization Constants of Acids and Bases*, p. 60. New York: Wiley.

BENET, L. Z. & GOYAN, J. E. (1965). *J. pharm. Sci.*, **54**, 983–987.

BENET, L. Z. & GOYAN, J. E. (1967). *Ibid.*, **56**, 665–680.

DANIELS, F. & ALBERTY, R. A. (1966). *Physical Chemistry*, 3rd edn, pp. 218–219. New York: Wiley.

EHRENBERG, L. (1948). *Acta chem. scand.*, **2**, 63–81.

GARRETT, E. R. (1963). *J. pharm. Sci.*, **52**, 400–401.

GOLDMAN, J. A. & MEITES, L. (1964). *Anal. Chim. Acta*, **30**, 28–33.

GUGGENHEIM, E. A. (1935). *Phil. Mag.*, **19**, 588–643.

KIELLAND, J. (1937). *J. Am. chem. Soc.*, **59**, 1675–1678.

LEESON, L. J. & BROWN, M. (1966). *J. pharm. Sci.*, **55**, 431–433.

LEVY, R. H. & ROWLAND, M. (1971). *Ibid.*, **60**, 1155–1159.

LEWIS, G. N. & RANDALL, M. (1961). *Thermodynamics*, 2nd edn, p. 317. New York: McGraw-Hill.

LOFGREN, N. (1948). *Dissert.*, Stockholm, p. 84. Worcester, Mass: Reprinted by The Morin Press.

MARTIN, A. N., SWARBRICK, J. & CAMMARATA, A. (1969). *Physical Pharmacy*, 2nd edn, p. 219. Philadelphia: Lea & Febiger.

MOORE, W. J. (1962). *Physical Chemistry*, 3rd edn, pp. 363–364. Englewood Cliffs, N.J.: Prentice-Hall.

RITCHIE, J. M. & GREENGARD, P. (1961). *J. Pharmac. exp. Ther.*, **133**, 241–245.

RITCHIE, J. M. & GREENGARD, P. (1966). *Ann. Rev. Pharmac.*, **6**, 405–430.

RITCHIE, J. M. & GREENGARD, P. (1968). *Science*, **162**, 1394–1395.

ROBINSON, R. A. & STOKES, R. H. (1959). *Electrolyte Solutions*, 2nd edn, pp. 336–344. London: Butterworths.

SHANES, A. M. (1958). *Pharmac. Rev.*, **10**, 59–164.

SKOU, J. C. (1954a). *Acta Pharmac. Tox.*, **10**, 281–291.

SKOU, J. C. (1954b). *Ibid.*, **10**, 297–304.

TRUANT, A. P. & TAKMAN, B. (1959). *Anesth. Analg.*, **38**, 478–484.