Dissociation Constants of Sparingly Soluble Substances: Nonlogarithmic Linear Titration Curves

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Abstract □ A potentiometric method, employing nonlogarithmic linear titration curves, is proposed for the accurate determination of dissociation constants of acids and bases whose aqueous solubilities are less than 2×10^{-4} mole/l. In this method the substance is allowed to precipitate, and pH measurements are made throughout the titration. An equation is derived to describe the titration of the unknown after precipitation. For a base, the pKa is calculated from the slope (equal to solubility/K_a) of the straight line obtained when the number of millimoles of unknown neutralized is plotted *versus* the hydrogen-ion concentration and the solubility, determined independently of the uncharged species. This method extends the ability to measure aqueous dissociation constants and can avoid the determination of pKa's in nonaqueous solvents. The applicability of the method was demonstrated with lidocaine and two structurally related, sparingly soluble, local anesthetic amines. The limitations of the existing and proposed methods are discussed.

Keyphrases □ Dissociation constants—sparingly soluble substances □ Anesthetics, sparingly soluble—dissociation constants, determination □ Potentiometric titration—dissociation-constant determination □ UV spectrophotometry—analysis

Potentiometric methods are the most generally applicable to the determination of aqueous dissociation constants of compounds. Coupled with the use of nonlogarithmic titration plots (1, 2), which offer a significant advantage over previous techniques for handling the data, accurate dissociation constants are possible using very low concentrations of acids and bases (from 5×10^{-4} *M* to 1×10^{-3} *M*). However, for many compounds the uncharged species is so insoluble that precipitation occurs during the titration even at these concentrations. Generally, at this point the titration is stopped and the method is thought to preclude an accurate pKa determination. This problem of low aqueous solubility is very prevalent among drugs and is attested by the scarcity of reliable pKa values for therapeutic agents. To overcome this problem, pKa determinations based on changes in the spectrum, using concentrations below the solubility of the uncharged moiety, or in the solubility as a function of pH have been very useful; however, these methods either lack general applicability or are very tedious. Several other approaches utilizing potentiometric data have been taken to obtain aqueous dissociation constants for sparingly soluble compounds (3, 4) but they lack adequate accuracy.

To circumvent the problem of low aqueous solubility, Mizutani (5), as early as 1925, originated the practice of determining pKa's in several concentrations of ethanol and water. From a plot of the pKa values *versus* the percent ethanol, the aqueous pKa is taken as the value when the line is extrapolated to 0% ethanol. However, Albert and Serjeant (6) warned that: "This temptation [of extrapolation] should be resisted for reasons which will shortly be discussed." This was further stressed by Benet and Goyan (7) who, after a careful analysis of the theoretical and practical considerations involved in determining the pKa by titration and semiaqueous solvents, concluded that: "There is presently no completely satisfactory method for converting p_sK_a values to pKa values. Therefore, although good accuracy can be realized in determining p_sK_a values, this accuracy cannot be carried over to the aqueous dissociation constants." Nonetheless, several authors (8–10) faced with the need to know the pKa of some sparingly soluble drugs have utilized Mizutani's approach.

In the present study, the authors attempted to extend the applicability of the existing nonlogarithmic titration plots to the determination of dissociation constants of compounds having solubilities less than 5×10^{-4} *M*. In addition, a new method for the determination of dissociation constants of acids and bases is proposed and several of its aspects are discussed. This method involves the titration of the weak acid or base during precipitation, followed by the determination of the solubility of the unionized species. The pKa is then calculated from the slope of a nonlogarithmic plot of the data.

THEORETICAL

Titration during Precipitation: Plot of Z' *versus* $[H^+]$—Consider the titration of a weak acid, like the salt of a strong acid and a weak amine BH^+X^-, with a strong base *M*OH. When the unionized species is sparingly soluble, it precipitates out very early during the titration. From this point, the concentration of the free amine in the solution remains constant. In the experiments to be described, it will be shown that several factors in the experimental design predispose toward maintaining a saturated solution.

At any time after precipitation, three equations have to be simultaneously satisfied:

1. The equilibrium expression:

$$K_a^c = \frac{[B]_{sol}[H^+]}{[BH^+]} \qquad \text{(Eq. 1)}$$

which can be rewritten:

$$K_a^c = \frac{B_{sol}}{BH^+} \cdot [H^+] \qquad \text{(Eq. 2)}$$

using relations adapted from the terminology of Leeson and Brown (2), $[B]_{sol} = B_{sol}(10^3/V)$ and $[BH^+] = BH^+(10^3/V)$, where:

- K_a^c = stoichiometric ionization constant of conjugate acid, BH^+
- $[B]_{sol}$ = molar concentration of free base in saturated solution
- $[H^+]$ = molar concentration of hydrogen ion in saturated solution
- $[BH^+]$ = molar concentration of acid
- V = volume of solution in milliliters
- B_{sol} = absolute number of moles of unionized species (free amine) present in saturated solution
- BH^+ = absolute number of moles of acid present in saturated solution

From Eq. 2, it can be seen that:

$$BH^+ = \frac{B_{sol}}{K_a^c}[H^+] \qquad \text{(Eq. 3)}$$

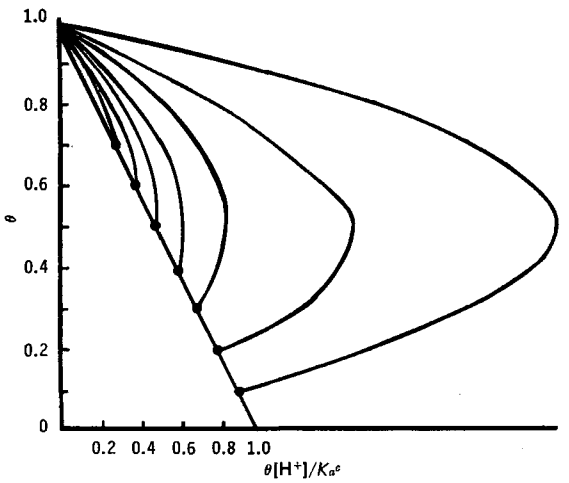

Figure 1—*Analog computer plot of* θ versus $\theta[H^+]/K_a^c$ *for various values of* θ_p.

2. Conservation of the weak acid in solution requires that at any time:

$$X^- = A^0 \qquad \text{(Eq. 4)}$$

where A^0 = absolute number of moles of acid originally added to the solution.

3. Electrical neutrality in the saturated solution requires that:

$$BH^+ + H^+ + M^+ = OH^- + X^- \qquad \text{(Eq. 5)}$$

where:

H^+ = absolute number of moles of hydrogen ion present in solution
M^+ = absolute number of moles of base added to solution
OH^- = absolute number of moles of hydroxyl ion present in solution
X^- = absolute number of moles of acid originally added to solution

The Z' term defined by Leeson and Brown (2) as:

$$Z' = M^+ + H^+ - OH^- \qquad \text{(Eq. 6)}$$

can also be written in terms of Eq. 5:

$$Z' = X^- - BH^+ \qquad \text{(Eq. 7)}$$

Substituting from Eqs. 4 and 3 into Eq. 7 yields:

$$Z' = A^0 - \frac{B_{sol}}{K_a^c} \cdot [H^+] \qquad \text{(Eq. 8)}^1$$

If the concentration of free amine in the solution remains constant throughout the precipitation phase, the B term in Eq. 8 will assume a constant value. Therefore, a plot of Z' *versus* $[H^+]$ should yield a straight line with a slope of B_{sol}/K_a^c and an intercept[2,3] of A^0.

Equation 8 is very similar to the following equation developed by Leeson and Brown (2):

$$Z' = A^0 - \frac{1}{K_a^c} Z'[H^+] \qquad \text{(Eq. 9)}$$

[1] For the titration of the salt of a strong base and a weak acid (A^-M^+), Eq. 8 would read:

$$Z' = A^0 - K_a^c A_{sol} \cdot \frac{1}{[H^+]}$$

[2] When only the amine is available, a slight molar excess of acid (HX) can be added to dissolve the amine. The intercept A^0 is still the number of moles of acid (in this case HX) added but now no longer equals the moles of amine titrated.

[3] The intercept on the $[H^+]$ axis has a value of $A^0K_a^c/B_{sol}$, from which K_a^c could be calculated if B_{sol} is known. However, the accuracy of this method is no greater than determining K_a^c from the slope.

However, the differences are important and warrant some discussion. Equations 8 and 9 apply to two different systems. Equation 8 utilizes only the data obtained during the precipitation phase; when Eq. 9 is applied to these data, it yields a curvilinear function which cannot be used to calculate a pKa value. Conversely, Eq. 8 is inapplicable when no precipitation occurs during a titration.

The B_{sol}/K_a^c term in Eq. 8 is a constant, which is not the case for the Z'/K_a^c term in Eq. 9. The slope of the plot of Z' *versus* $Z'[H^+]$ can be immediately converted into a pKa value. In Eq. 8, two separate determinations are needed before a pKa can be calculated. First, the slope B_{sol}/K_a^c is determined. Then the amount of base in the saturated solution is measured.

Titration before and during Precipitation—As was shown in the preceding section, Eq. 9 yields a linear plot as long as the concentration of the unionized amine is below the saturation point; Eq. 8 applies from the start of precipitation and onward. A more general equation applicable to either phase can be written as:

$$\theta = 1 - \theta' \frac{[H^+]}{K_a^c} \qquad \text{(Eq. 10)}$$

where:

$\theta = \dfrac{Z'}{A^0}$
θ' = θ before precipitation
θ' = θ_p at and after precipitation
$\theta_p = \dfrac{B_{sol}}{A^0}$

By using an analog computer, the characteristics of the plot of θ *versus* $\theta[H^+]/K_a^c$ (analogous to plotting Z' *versus* $Z'[H^+]$) were examined before and during precipitation. To examine these relationships, it was necessary to reexpress Eq. 10. This was accomplished in the following manner.

The ratio θ/θ' can be expressed as:

$$\theta/\theta' = 1 + (\theta - \theta')1/\theta_p \qquad \text{(Eq. 11)}$$

By rearranging Eq. 11,

$$\theta = \left[1 + (\theta - \theta')\frac{1}{\theta_p}\right]\theta' \qquad \text{(Eq. 12)}$$

and, therefore,

$$\theta \frac{[H^+]}{K_a^c} = \left[1 + (\theta - \theta')\frac{1}{\theta_p}\right]\theta' \frac{[H^+]}{K_a^c} \qquad \text{(Eq. 13)}$$

From Eq. 10,

$$\theta' \frac{[H^+]}{K_a^c} = 1 - \theta \qquad \text{(Eq. 14)}$$

Substituting Eq. 14 into Eq. 13 yields:

$$\theta \frac{[H^+]}{K_a^c} = \left[1 + (\theta - \theta')\frac{1}{\theta_p}\right](1 - \theta) \qquad \text{(Eq. 15)}$$

Hence, a plot of θ *versus* the right-hand side of Eq. 15 is equivalent to plotting θ *versus* $\theta[H^+]/K_a^c$. The results are shown in Fig. 1 for various fractions of neutralization at which precipitation occurs. Before precipitation ($\theta < \theta_p$), the plot gives a straight line with a slope of -1 and an intercept of 1. This is analogous to the plot of Z' *versus* $Z'[H^+]$ in Eq. 9, where a straight line with a slope of $1/K_a^c$ is obtained. After precipitation ($\theta > \theta_p$), the plot yields a sickle-shaped curve which eventually intercepts the ordinate axis at a value of 1. It will be shown that the same type of curve is obtained with lidocaine and other sparingly soluble compounds when the data are plotted according to Eq. 9 (Fig. 2). As such, the sickle-shaped curve cannot be used to calculate a pKa unless the data are plotted according to Eq. 8, as shown in Fig. 3.

EXPERIMENTAL

Materials—In the present work, the hydrochloride salts of a homologous series of local anesthetics of the amide type were investigated. Structurally, these are *meta*-substituted 2-diethylamino

acetanilides with solubilities ranging from 1.3×10^{-2} to 6×10^{-5} M.

Lidocaine HCl·H_2O was used[4]. The pH measurements were made on the expanded scale of a Beckman Expandomatic pH meter with an accuracy of ±0.005 pH unit. The meter was equipped with a Beckman glass electrode and a Beckman fiber junction calomel electrode.

Carbonate-free sodium hydroxide was also used[5]. The additions of sodium hydroxide solution were made with a Gilmont micropipet-buret[6].

Ion-free water was prepared using the Crystalab "Deeminizer" water demineralizer[7]. During the titration, the temperature of the solution was maintained constant using a Tamson precision circulation bath[8].

Methods—The sample of base hydrochloride was dissolved in 60 ml. of freshly deionized water or extemporaneously prepared KCl solution. The titration was performed under nitrogen in a water-jacketed thermostated vessel (24 ± 0.1°). Sodium hydroxide was added from a 1-ml. microburet calibrated to 0.0002 ml. In most titrations, the amounts of acid were calculated such that 0.5–1.0 ml. of 0.1 *N* NaOH was consumed. A minimum of 10 additions was made in a single run. During the precipitation phase, 6–10 min. after each addition of base ensured a stable and accurate pH reading.

At the end of those experiments where precipitation occurred during the titration, the solution containing an excess of amine was either filtered or centrifuged at $8000 \times g$ for 10 min. When the filtrate or supernatant was not clear, these operations were repeated. The concentration of the substance in the solution was then determined spectrophotometrically by reference to a previously established calibration curve. All measurements were made using a Beckman DBG spectrophotometer.

Several precautions were taken to avoid changes in the solubility to ensure accuracy in the solubility determination. First, the ultracentrifugation was performed immediately following the titration at a temperature identical to the temperature of the water-jacketed vessel. Next, an appropriate spectroscopic dilution bar, rather than dilution of the solution, was employed with those bases whose saturated solution gave too high an absorbance in the normal 1-cm. cell. Last, all amine solutions were maintained at a pH above 11 because acidification presents the danger of dissolving particles.

Examination of Nonlogarithmic Titration Curves prior to Precipitation—For the most soluble compounds of the series (solubility ranging from 1.3×10^{-2} *M* to 4.6×10^{-4} *M*), the pKa was obtained by using the lowest concentration (5×10^{-4} *M*) recommended by Benet and Goyan (7) and calculating the data according to the method of Leeson and Brown (2). When these same experimental conditions (concentration of drug 5×10^{-4} *M*) were utilized for the rest of the series, precipitation of the unionized species occurred during the titration. In such cases the titration was stopped, and several attempts to use lower concentrations were made. At concentrations below 5×10^{-4} *M*, the pH range covered during the titration is such that unavoidable errors in pH measurements become significant. This represents a serious limitation since the accuracy of the method using nonlogarithmic titration curves depends on the ability to distinguish between small [H^+] differences.

Those compounds with a solubility ranging between 2×10^{-4} *M* and 4×10^{-4} *M* were singled out for further examination. At the concentration used, the fraction neutralized before precipitation (θ_p) ranged between 0.4 and 0.8. The titration was pursued until complete neutralization, *i.e.*, throughout the precipitation phase. The data were treated according to the method of Leeson and Brown (2); the plot Z' *versus* $Z'[H^+]$ presents two distinct regions, the expected linear region (slope $1/K_a^c$), followed by the nonlinear portion of the graph obtained by continuing the titration during precipitation. As previously shown, this sickle-shaped curve cannot be used to calculate the pKa automatically. Therefore, it became necessary to ascertain whether in such cases ($0.4 < \theta_p < 0.8$) the linear portion of the graph could be used to compute the pKa. This was investigated in the following manner.

Lidocaine (2-diethylamino-2′,6′-acetoxylidide) is a fairly water-soluble weak base with an apparent pKa of 7.86 at 25° (11). Using concentrations below the solubility, the pKa was measured in this laboratory and the average of five determinations at 24° was found to be 7.86 (*SD* 0.03). Then duplicate runs were performed at two concentrations of lidocaine which were chosen such that the fraction neutralized before precipitation varied between 0.2 and 0.6. The data from those experiments are summarized in Table I, and Fig. 2 is a plot of Z' *versus* $Z'[H^+]$ for one titration. Note that even at the same concentration (especially at 0.05 *M*), the fraction neutralized at the point of visual precipitation varied between experiments. Also, when $\theta_p > 0.4$, the linear portion of the plot led to a fairly accurate measure of the pKa of lidocaine, and the A^0 value (numbers of moles of acid originally added to the solution) obtained by extrapolation of the linear portion of the graph was within a few percent of the theoretical value. When $\theta_p < 0.3$, the pre-precipitation curve was thereby reduced, and it became difficult to obtain an accurate value of the pKa. In Fig. 2, $\theta_p = 0.24$, and the pKa value was 7.98 instead of 7.85. Furthermore, the extrapolated A^0 value was off the theoretical value (number of moles of acid originally added) by more than 25%.

Table I—Extrapolated pKa of Lidocaine for Different Values of θ_p

Lidocaine Concentration, *M*	Fraction Neutralized at Beginning of Visual Precipitation	A^0 Extrapolated[a]	pKa
0.050	0.520	106.7	7.875
0.050	0.383	105.3	7.871
0.0775	0.258	109.2	7.909
0.0775	0.241	125.8	7.895

[a] Percent of theoretical value.

These inaccuracies would occur for compounds with an unionized species in the solubility range of 5×10^{-4} *M*. In those situations such an extrapolation would be more difficult than has been shown with lidocaine, because the effective pH range of the titration would be very small. Such an example is the 2-diethylamino-3′-phenylacetanilide, which has a water solubility of 7×10^{-5} *M* for the free amine. When the titration was performed at a concentration of 5×10^{-4} *M*, θ_p was approximately 0.1 and almost no linear portion of the plot of Z' *versus* $Z'[H^+]$ was obtained. In an attempt to increase the linear portion, the titration was performed at 1.7×10^{-4} *M*. Even though θ_p was equal to 0.4, it was not possible to obtain a straight line for the pre-precipitation curve because the pH range was too small.

From the foregoing experiments, the authors believe that when $\theta_p < 0.6$ the experimentor should be fully aware of the lack of accuracy inherent in the pKa measurement determined by the procedure of Leeson and Brown (2). Preferably, under these circumstances, Eq. 8 should be utilized as shown in the next section.

EXAMINATION OF NONLOGARITHMIC LINEAR TITRATION CURVES UTILIZING DATA OBTAINED DURING PRECIPITATION

Practical Evaluation of Method Using Lidocaine—Before using Eq. 8 to measure the pKa of the less soluble amines of the series, a preliminary test of that equation and the method was undertaken. Lidocaine was used because its pKa is already known and its solubility can be easily measured by UV spectrophotometry. Figure 3 is a plot of Z' *versus* [H^+] for lidocaine (0.775 *M*, 24°) with a slope of 0.69×10^8. At the end of the experiment, the solution was pipeted through a glass wool plug at the tip of a pipet. Several samples were taken and the absorbance measured. From the solubility obtained with the calibration curve, the B_{sol} term was calculated to be 0.9350 m*M* in the 60 ml. of solution; consequently, the pKa of lidocaine was found to be 7.869. In a duplicate experiment, the following results were obtained: slope = 0.666×10^8, B_{sol} = 0.9184 m*M*, and pKa = 7.861. The pKa values are in good agreement with those obtained in this laboratory using Eq. 9.

Examination of Method Using Less Soluble Compounds (Solubility 7×10^{-5} *M*)—For the foregoing experiments, although the B_{sol} term was constant throughout the titration, it cannot be assumed that this value equals the thermodynamic solubility of the compound. Generally, this will not be so because theoretically in-

[4] Supplied by Astra Pharmaceutical Products Inc.
[5] J. T. Baker Chemical Co.
[6] Scientific Products, Evanston, Ill.
[7] Crystal Research Laboratories, Inc., Hartford, Conn.
[8] Neslab Instruments, Portsmouth, N. H.

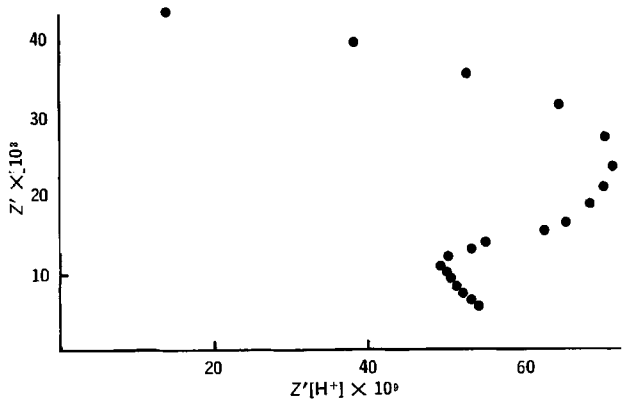

Figure 2—*Plot of* Z′ versus Z′ [H^+] *for lidocaine (0.775* M, *24°) when* $\theta_p = 0.24$.

finite time is required before thermodynamic equilibrium is achieved. However, Eq. 8 only requires that the change in concentration of the unionized species during the titration is nil or negligibly small.

Several factors in the experimental conditions favor constancy of the solubility. First, the solution is maintained at a constant temperature and continually stirred. Next, the unionized species is gradually liberated until the concentration in solution exceeds the saturation point. Finally, as one approaches saturation, there is almost inevitably a local precipitation of unionized species at the tip of the buret caused by the transient high concentration of titrant. This phenomenon tends to prevent supersaturation and favors constancy of the solubility. However, it is useful to know if B_{sol} is reproducible and independent of the concentration of compound. 2-Diethylamino-3′-phenylacetanilide has a water solubility of 7×10^{-5} *M* at 24°. Its absorption at 243 nm. is sufficiently high that it allows direct measurement of the saturated solution. The compound was titrated at three different concentrations (1.67×10^{-4} *M*, 5.22×10^{-4} *M*, and 1.52×10^{-3} *M*) but at constant ionic strength. The plots of *Z′ versus* [H^+] at these concentrations are shown in Fig. 4. The three lines are practically parallel to one another, which implies that the B_{sol} term did not significantly vary between the runs. Furthermore, differences between the absorbances measured at the end of each run were no greater than such variations in repeated runs on the same solution.

From Eq. 8 and a plot of *Z′ versus* [H^+], the dissociation constant is given by:

$$\frac{1}{K_a} = \frac{\text{slope}}{B_{sol}} \qquad \text{(Eq. 16)}$$

This relationship requires that when B_{sol} varies, the slope should vary in the same direction such as to maintain their ratio constant. The extent to which this holds true is a measure of the precision of this method. Twelve consecutive determinations were performed

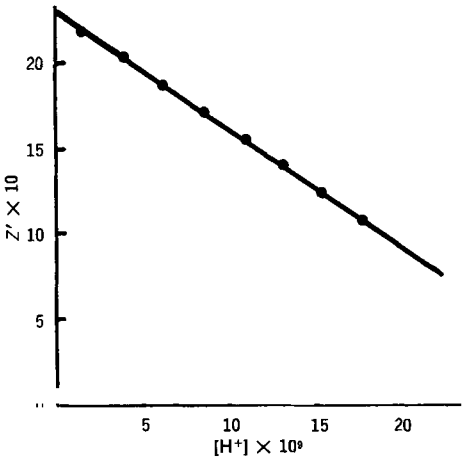

Figure 3—*Plot of Eq. 8* (Z′ versus [H^+]) *for lidocaine (0.775* M, *24°). Intercept* = A^0, *slope* = B_{sol}/K_a^0.

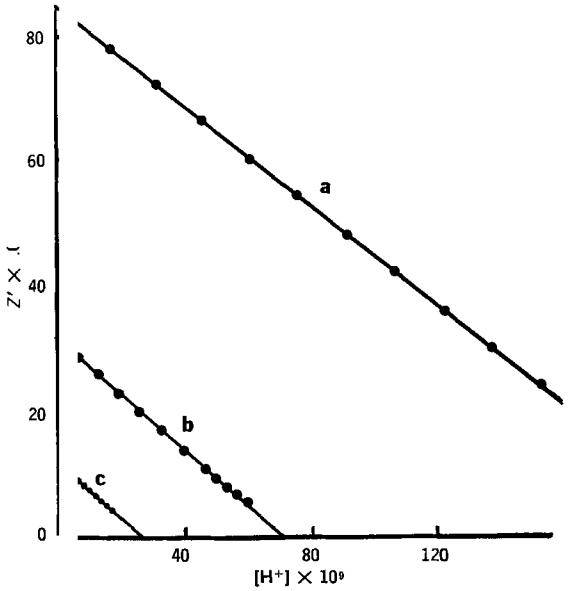

Figure 4—*Plot of* Z′ versus [H^+] *for 2-diethyl-3′-benzyloxyacetanilide showing that* B_{sol} *is not affected by a 10-fold change in concentration:* $a = 1.52 \times 10^{-3}$ M; $b = 5.22 \times 10^{-4}$ M; *and* $c = 1.67 \times 10^{-4}$ M.

with the 2-diethylamino-3′-benzyloxyacetanilide at a concentration of 10^{-3} *M*. At the end of each run, the supernatant was separated by ultracentrifugation and the solubility was measured by UV spectrophotometry. These data are shown in Table II. One can see that the solubility and the slope can vary by as much as 10–15% from run to run, which points out that only the values obtained for B_{sol} and the slope in a particular experiment may be used to calculate the pKa. To evaluate the significance of those variations, the mean and the standard deviation were computed for pKa, solubility, and slope. Table II also shows some data on the precision of the procedure of Leeson and Brown (2) obtained with lidocaine. It is quite common to express the precision of a pKa ±1 *SD*. Although it would be more correct to calculate the standard deviation of K_a's rather than pKa's, experience in this work has shown that the difference between the two is negligible.

It has been estimated that by using Eq. 9, a pKa can be determined with a precision of ±0.03 unit (6). The results with lidocaine agree very closely with those estimations. Table II also shows that this new procedure yields a pKa with a precision of ±0.04 unit. Although (as expected) this standard deviation is larger, it is less than one would anticipate knowing that this procedure has an additional source of error in the solubility measurement. Indeed, the overall precision of the pKa is intimately associated with the precision and, hence, the method of solubility determination of the substance under investigation.

PRACTICAL ASPECTS OF THE METHOD

Solubility Determination—Inherent in this method is the measurement of the concentration of unionized acid or base in a saturated solution of the compound[9]. As previously indicated, the first step involves separation of the solid from the solution; occasionally this presents a serious problem. Several techniques have been tried: centrifugation, filtration through a glass wool plug, filtration using millipore filters, and ultracentrifugation.

In this work, two main criteria, the particle size of the precipitate and the chemistry of the compound, proved useful in choosing a particular procedure. Systems containing large-size crystals afford an easy separation with centrifugation at the usual speeds, although one should watch for temperature changes during centrifugation. However, certain systems will not separate under these conditions. Filtration presents the advantage of simplicity, but adsorption can be a serious limitation. For example, extensive adsorption of 2-di-

[9] Attempts to correlate the fraction of the substance neutralized at the point of visual precipitation with independent solubility measurements proved unsuccessful, probably because of some supersaturation at this point.

Table II—Precision of the Proposed Method Using 2-Diethylamino-3′-benzyloxyacetanilide

2-Diethylamino-3′-benzyloxyacetanilide				Lidocaine	
Experiment	Slope $\times 10^5$	Absorbance	pKa	Experiment	pKa
1	3.36	0.599	7.946	A	7.850
2	3.77	0.540	8.039	B	7.825
3	3.75	0.535	8.041	C	7.907
4	3.87	0.700	7.939	D	7.835
5	3.87	0.710	7.933	E	7.884
6	3.85	0.620	7.990		
7	3.16	0.575	7.998		
8	4.03	0.543	8.061		
9	4.04	0.585	8.034		
10	4.01	0.605	8.016		
11	4.13	0.650	7.999		
12	3.90	0.610	8.001		
Average	3.81	0.606	8.00		7.860
SD	0.29	0.058	0.042		0.034

ethylamino-3′-benzyloxyacetanilide onto a glass wool plug at the tip of a pipet became apparent when successive samples drawn from the same mother solution gave widely different absorbances. Millipore filters did not solve the problem. After one filtration the absorbance of a solution of 2-diethylamino-3′-benzyloxyacetanilide was reduced from 0.577 to 0.385. Ultimately the problem was solved using ultracentrifugation (at $8000 \times g$ for 10 min.). Satisfactory results were consistently achieved with this method. However, a temperature control device is necessary to prevent excessive temperature variations which could affect the solubility.

Calculation of Eq. 8—The $[H^+]$ term is calculated by converting the hydrogen-ion activity (a_{H^+}) to hydrogen-ion concentration. As shown by Leeson and Brown (2), one has the choice between the activity coefficient of the hydrogen ion calculated by Kielland (12) and the experimentally determined $\gamma \pm$ values of Lewis and Randall (13). By knowing a_{H^+}, the K_w at the temperature of the experiment gives the hydroxide-ion activity (a_{OH^-}). The activity coefficient needed to calculate the hydroxide-ion concentration can be either computed from the Guggenheim equation (14) or taken from the tables of Harned and Owen (15). At the concentrations used in this work, all the titration occurred in the pH range 5–10, and H^+ and OH^- became negligible terms in the calculation of Z' in Eq. 6.

Influence of Ionic Strength—Ionic strength had practically no effect on the acidic dissociation constant of lidocaine and 2-diethylamino-3′-benzyloxyacetanilide. Consequently, all the pKa values were pooled together, regardless of the ionic strength at which they were determined (Table II). However, the ionic concentration may affect the solubility (B_{sol}), and careful consideration should be given to this fact. The mechanism involved is the salting-out effect where salt ions withdraw water molecules and cause the unionized species to reach saturation earlier. This would affect the B_{sol} term and the slope without changing the K_a^c. This effect was not noticeable in the case of the local anesthetic amines studied (Fig. 4). However, it should be kept in mind when working with weak acids for which the only way to obtain an accurate thermodynamic pKa is to extrapolate a plot of pK_a^c *versus* $\sqrt{\mu}/(1 + \sqrt{\mu})$.

Limits of Method—For obvious reasons, the proposed method is not suited to the direct titration of an acid by a base and vice versa. Rather, one should use a soluble salt of the sparingly soluble acid or base under investigation. Moreover, in the case of monoprotic species, this system allows the ionic strength to remain constant during all the titration. The concentration at which to run the titration deserves careful consideration. Using very low concentrations (5×10^{-4} *M*) is advantageous when the availability of substance is limited. Also, it circumvents activity effects and enables one to obtain thermodynamic dissociation constants. However, one should also consider the interplay of titration range, pKa, and solubility. In the titration of the salt of weak base $BH^+ X^-$, Eq. 1 can be rearranged to give:

$$\frac{K_a^c}{[H^+]} = \frac{[B]_{sol}}{[BH^+]} \qquad \text{(Eq. 17)}$$

It becomes apparent that for 90% of the titration after precipitation, the pH will change only 1 unit. Moreover, it is desirable that this pH change be within the range 5–9. (In that case, H^+ and OH^- are negligible terms in the calculation of Z'.) What determines the pH at which the base begins to precipitate (pH_p) is the solubility, pKa, and concentration of salt used (A^0), since:

$$pH_p = pKa + \log \frac{[B]_{sol}}{[A^0] - [B]_{sol}} \qquad \text{(Eq. 18)}$$

By choosing $[A^0] = 10^{-3}$ *M* for a base whose solubility is 10^{-5} *M*, it is clear that this method is satisfactory if the pKa is within the range 7–10. Similarly, for a weak acid this corresponds to a pKa range of 4–7. The proposed method would reach its limits of applicability in the case of a base that is very weak and at the same time very insoluble. Then, the unionized species precipitates as soon as the salt is put into water because water is competing with the weak base for the proton. However, most pharmacological agents appear to fall within the practical limits of the method.

In the case of polyprotic species, the relationship between pKa and solubility is even of more consequence. Consider the titration of a dibasic acid where the two pKa's are separated by 2 units. At a pH equal to the second pKa, the unionized species constitute 1% of the total concentration in solution. If the solubility is so small that it is already exceeded at that pH, precipitation with titration of the first hydrogen takes place as the second hydrogen is only half-neutralized. Therefore, the degree of separation between any two pKa's needs to be larger than required for soluble polyprotic species.

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