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Review Article

Potentiometric Determination of Dissociation Constants

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AT ONE TIME or another almost every pharmaceutical scientist wants to know the dissociation constant[1] for a particular drug. He might want to know whether his drug is in the ionic or nonionic form, if it will be sufficiently soluble in the gastrointestinal fluid, or how the gastric fluid might affect the rate of dissolution of the drug. He might wish to predict the stability or explain the kinetics of degradation for a dissociable drug in various buffer solutions. He might want to hypothesize a mechanism of action, predict the extent of binding, or examine complex formation. In all of these cases and in many others the pharmaceutical scientist needs to know the dissociation constant in order to solve his problem. His first reaction will be to "look it up" and if he is lucky he will find critically evaluated values in either "Dissociation Constants of Organic Acids in Aqueous Solutions" (1) or the companion volume for organic bases (2). However, in most cases he will be working with a drug for which the dissociation constant is not readily available, and unfortunately when dealing with drugs in biological systems at 37° the reliable data available are quite sparse.[2] He will then have to carry out an experimental determination, and will usually turn to the most readily available and easily operated instrument, the laboratory pH meter and its accompanying electrodes.

Potentiometric pH titration is by far the most convenient method for the determination of dissociation constants and, with care, this method can give the experimenter good reproducible results (pKa ± 0.03 units) for acids and bases having pKa values between 2.5 and 11 (errors due to residual junction potentials increase the error outside these limits).

The techniques used in carrying out the titration are standard and are well covered by Albert and Serjeant (4). The meter should be accurately calibrated with at least two standard buffers, the buffers and the solutions to be titrated should all be kept at a constant temperature, all solutions should be carbon dioxide free, the titration should be carried out under nitrogen, and the pH should be read for a large number of

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The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.

[1] Whenever "dissociation constant" is used, it will refer to the *acid* dissociation constant.

[2] There is no universal relationship which will allow one to predict the dissociation constant of a compound at 37° when the more usually available value at 25° is known. The enthalpy of dissociation is a complex function of temperature and consequently the usual Van't Hoff type of plot is not useful. For example, the pKa (thermodynamic) for salicylic acid shows almost no change with values of 2.97 at 25° and 2.95 at 37°, while the values for barbital are 7.98 at 25° and 7.82 at 37°. Hall and Sprinkle (3) have found that the temperature coefficients for the nitrogenous bases between 0° and 40° follow a general pattern in that all become weaker bases as the temperature increases and that the depression in pKa per degree temperature rise becomes progressively larger as the basicity increases.

titrant additions. Up to this point, the procedure is unambiguous; however, the data must be interpreted and the dissociation constant calculated. There are a large number of possible ways in which the data may be treated; all are useful to some extent, but each method has its limitations. These methods, their usefulness, and their limitations are the subject of this review.

The pharmaceutical scientist and most other biological scientists are particularly interested in the aqueous dissociation constant, and this review will concentrate primarily in this area. However, since a great number of drugs are highly insoluble in the unionized form, dissociation constants are often determined in varying percentages of semiaqueous systems and these values are extrapolated to a value for water. These types of calculations will also be discussed. The sections dealing with aqueous and semiaqueous considerations will be applied to monoprotic species or to those polyprotic acids and bases for which the pKa values differ by at least 3 units. A third section of the review will be devoted to a short discussion of polyprotic species with overlapping pKa values.

AQUEOUS SOLUTIONS

Nonlinear Relationships—Nonlinear plots of pH *versus* titrant added are the most familiar and they may be obtained mechanically, utilizing an automatic titrator and a recorder. These plots are often used to determine the dissociation constant by what we shall call the half-neutralization method. This method is based on a corrupted form of the Henderson-Hasselbalch equation,[3]

$$p[H] = pKa^c + \log \frac{[base]}{[acid]} \qquad \text{(Eq. 1)}$$

where p denotes negative logarithm, [ ] concentration, and Ka^c is the stoichiometric dissociation constant. The equation utilizes the principle that when the concentration of the base (conjugate base if we are titrating an acid) equals the concentration of acid, the substance is half-neutralized. At this point the pH of the solution as read on the pH meter equals the pKa of the drug. However, assuming that the pH is equal to the pKa when an amount of strong titrant equal to one-half the concentration of the pure drug has been added can lead to erroneous results other than that due to assuming that the pH read on the meter is the negative logarithm of the hydrogen-ion concentration.[4]

Meites and Goldman (5–9) have examined the dilution and hydrogen-ion effect in relation to the inflection point of acid-base titration curves and have proposed some general equations to demonstrate the errors inherent in assuming that pH = pKa at half-neutralization. Let f be defined as the degree of neutralization of an acid. Where we are considering the titration of V_a^0 ml. of a C_a^0 solution

$$f = \frac{V_b C_b}{V_a^0 C_a^0} \qquad \text{(Eq. 2)}$$

of a weak monobasic acid with a C_b solution of a completely dissociated monoacidic base. Goldman and Meites (7) have derived the following equation to locate the point at which pH actually does equal pKa during potentiometric titrations:

$$f_{pKa} = \frac{1/2 - \{[K_a - (K_w/K_a)]/C_a^0\}}{1 + r\{[K_a - (K_w/K_a)]/C_a^0\}} \qquad \text{(Eq. 3)}$$

where f_{pKa} is the degree of neutralization when p[H] equals pKa, p[H] = negative logarithm of the hydrogen-ion concentration, pKa = negative logarithm of the apparent dissociation constant, K_w = the ion product of water and,

$$r = C_a^0/C_b \qquad \text{(Eq. 4)}$$

From Eq. 3 it can be seen that f_{pKa} will coincide with half-neutralization only when $K_a = (K_w)^{1/2}$. Substantial errors will result from taking pKa to be equal to pH at $f = 1/2$ if K_a is widely different from $(K_w)^{1/2}$ and this will be especially true if the concentrations of the reagents are small.

As an example, consider the titration of a 0.01 M solution of a weak acid ($K_a = 1 \times 10^{-3}$) by a 1.0 M solution of strong base. Putting these values into Eq. 3, $f_{pKa} = 0.40$. If the initial concentration of the weak acid were 0.001, the titration would begin at a pH higher than the pKa (as indicated by the fact that $f_{pKa} < 0$), and the hydrogen-ion concentration at "half-neutralization" would give a value of 2.8×10^{-4}, which would correspond to an error in the pKa of greater than 0.5 unit. It should be noted in Eq. 3 that r, the ratio of the concentration of solution being titrated to the concentration of titrant, also affects the f_{pKa}. For example, if the

[3] In 1908, Henderson (21) showed that the hydrogen-ion concentration of a solution equals the dissociation constant of a weak acid-base substance times the ratio of the concentration of the acid to the concentration of the base. It was not until the next year that Sørensen (22) introduced the term pH. As best as we can determine, Hasselbalch added his name to the Henderson equation by being the first (23) to combine Sørensen's definition with the equation. In any case, it is clear from the early work that all the values were in terms of concentration units.

[4] The pH meter does not give an exact measure of the hydrogen-ion activity. Instead we have an operational definition of the practical pH value and a standard scale fixed by one or more standard buffer solutions whose *assigned* pH values are at least formally consistent with the thermodynamic properties of a solution and the definition of what the activity of a single ion should be. However, for purposes of our discussion, we shall assume that the pH values read on the pH meter are the negative logarithm of the hydrogen-ion activity. For a detailed discussion of this point, consult the work of Bates (36).

TABLE I—LOCATIONS OF f_{pKa} IN WEAK ACID–STRONG BASE TITRATIONS AFTER GOLDMAN AND MEITES (7)

K_a	f_{pKa}[a]	f_i
1×10^{-2}	0.364	0.111
3×10^{-3}	0.456	0.360
1×10^{-3}	0.485	0.463
1×10^{-4}	0.498	0.496
1×10^{-5}	0.4998	0.499
1×10^{-7}	0.5000	0.5000

[a] The values of f_{pKa} were computed from Eq. 3 for $C_a^0 = 0.1$, $r = 1$, and $K_w = 1 \times 10^{-14}$. The values of f_i were computed as described by Meites and Goldman (5) and correspond to the points where the slopes of the titration curves are at a minimum.

0.01 M solution of acid were titrated with 0.01 M strong base, the f_{pKa} would drop to 0.364. This is due to a greater dilution effect, and gives a good illustration of the principle behind the usual instructions calling for a concentrated solution of titrant.

Very often, a variation of the "half-neutralization" method may be used where the pKa is taken to be equal to the pH at the inflection point of the sigmoid titration curve. Once again, this only gives a correct pKa when $K_a = (K_w)^{1/2}$, otherwise f_i (degree of neutralization at the inflection point) precedes f_{pKa} and the divergence increases as acid strength increases. Table I shows a comparison of some of the data of Goldman and Meites (7). The values of f_i are calculated by an iterative procedure applied to a very complex equation[5] similar to Eq. 16 in *Reference 5*.

In an attempt to overcome some of the errors inherent in utilizing the half-neutralization method, especially those due to the fact that the concentration of hydrogen ions is not negligible, as compared to the concentration of acid or base for fairly strong acids (pKa 2–4) and bases (pKa 10–12), Parke and Davis (11) introduced a different nonlinear method for determining dissociation constants. A survey of the pharmaceutical literature shows that until the past few years most dissociation contants were determined by methods which were modifications of that described by Parke and Davis.

These authors used hydrogen-ion binding curves to determine the apparent dissociation constants for a variety of mono- and polyprotic acids and bases. Identical volumes of sample solution and blank were titrated with a strong titrant over the pH range 2–12. The curves of these two titrations were plotted on a graph having pH as the abscissa and the amount of titrant added as the ordinate. A third curve, the difference curve, was then drawn with the units of the ordinate being the differences in milliliters of titrant required for the sample and blank to reach the same pH. Previously prepared transparent masks on which the standard curve has been drawn and the point of inflection marked are then fitted to the difference curve. Since "any titratable group produces an inflection having the same shape regardless of its position on the pH scale, the apparent dissociation constant will be the pH at the point of inflection" (11). In addition to the curve for exactly one equivalent of hydrogen ion bound per mole of sample, other masks for 1.1, 0.90, 0.75, 0.50, and 0.25 equivalent per mole were also included for use with samples of unknown purity or molecular weight.

Garrett (12) has made an analysis of the theoretical basis for the Parke and Davis method. He has shown that for dissociation constants in the range 4–10, the procedure has little advantage over the traditional technique of determining apparent pK's at half-neutralization. He also notes that for dissociation constants in the range 2–4, the technique is invalid, especially for estimates of stoichiometry, due to errors arising in the subtraction of volumes.

Stokes (13) has developed an ingenious graphical method to determine the pH value at the equivalence point, the pH for the pure weak acid, pure conjugate base, pH at $f = 0.5$, and the pH at the inflection points. His treatment is based upon: (*a*) formulating a degree of neutralization in terms of one parameter which is so defined that a weak acid is first neutralized with excess base, then back titrated with strong acid; (*b*) the use of dimensionless quantities throughout the calculations; (*c*) the use of hyperbolic functions; and (*d*) the use of logarithmic diagrams of the type favored by Sillén (14) and other Scandinavian workers, for the solutions of the equations. His procedures are similar to those proposed by Sillén, with the important difference that the preparation of only two permanent graphs is sufficient for all cases of a single monoprotic weak acid–base substance titrated with strong acid or base, in any solvent and at any temperature for which K_w is known. At high enough concentrations of a weak acid–base substance the titration curve will have three inflection points, one each at the acid and basic equivalence point, and one at half-neutralization. All of these points may be determined graphically, if the "high enough" concentrations are attainable physically.

Stokes further points out that in those cases

[5] In trying to solve Eq. 16 (*Reference 5*) the reader should be aware that the definitions for two of the terms are erroneous. Consult *Reference 10* for the corrections.

where the three inflection points do exist, it is possible to obtain both the pKa and the concentration of the weak acid or base without the use of any standardized strong acid or base solutions. Roe (15), commenting on this point in a review article, suggests that this opens up the possibility for the development of a rather unique automatic titrator. The use of Stokes' method for the determination of pKa values is unfortunately limited by manipulative difficulties.

Linear Relations—In 1958, Joseph (16) described the advantage of bringing the sigmoid form of titration curves based on the mass action law into a linear form by logarithmic transformation. This transformation was applied particularly to the Henderson-Hasselbalch equation and Joseph constructed a semilogarithmic plot as well as a slide rule for estimation of the pK values. Druckrey (17) has pointed out that he had previously proposed a similar logarithmic transformation of the mass action law which allowed linear representation on a prepared log-log paper (18). These methods have the advantage of ease in plotting and in visualizing the ratio of unionized to ionized concentration. Joseph (19) has also pointed out that when linear logarithmic plots are prepared for polyprotic species, the diagram clearly indicates the distribution of electrical charge over the molecule as a function of pH. Although linear logarithmic plots are easy to construct and helpful in visualizing the ratio of acid to base concentrations, they offer no improvement in the accuracy of dissociation constant determinations as compared to the methods listed under *Nonlinear Relationships*. The linear methods listed above are based on the corrupted Henderson-Hasselbalch equation and like the half-neutralization method, do not correct for the concentration of hydrogen and hydroxide ions, the dilution effect, or activities. Setnikar (20) has recently used a variant of the Druckrey method (17, 18) to determine the apparent dissociation constants of bases with limited solubility. For this type of determination, the Druckrey method has a real advantage in that initially a plot of pH *versus* degree of neutralization will be linear until the solubility of the conjugate species is exceeded and then the plot will degenerate from linearity. However, even if the solubility of the conjugate species is exceeded before half-neutralization, the initial linear portion of the plot may be extrapolated to the point where the degree of neutralization equals one-half (*i.e.* [acid] = [conjugate base]), and at that point the apparent pKa is equal to the pH.

Nonlogarithmic Linear Relationships—It should not be construed, from the statements above about the possible inaccuracy of methods based on the Henderson-Hasselbalch equation (Eq. 1), that the equation itself is inaccurate. Equation 1 is accurate, and it is only the substitution of approximations for the concentrations of the base and acid that lead to erroneous pKa^c values. The concentration of base at any time during a titration is given by the following equation:

$$[\text{base}] = C_b^0 - [X] + [M] + [H^+] - [OH^-] \quad \text{(Eq. 5)}$$

where C_b^0 = initial concentration of pure weak base,
$[X]$ = concentration of strong acid added,
$[M]$ = concentration of strong base added.

Likewise, the concentration of acid at any time during a titration is given by:

$$[\text{acid}] = C_a^0 + [X] - [M] - [H^+] + [OH^-] \quad \text{(Eq. 6)}$$

where C_a^0 is the initial concentration of pure weak acid. Substituting Eqs. 5 and 6 into the nonlogarithmic Henderson equation (see *Footnote 3*), we obtain:

$$[H^+] = K_a^c \frac{C_a^0 + [X] - [M] - [H^+] + [OH^-]}{C_b^0 - [X] + [M] + [H^+] - [OH^-]} \quad \text{(Eq. 7)}$$

Equation 7 is the general dissociation equation for a monoprotic species. Usually a titration involves the addition of a strong acid titrant to a solution of pure weak base, in which case C_a^0 and $[M]$ are dropped from Eq. 7, or the addition of a strong base titrant to a pure weak acid, in which case C_b^0 and $[X]$ are dropped from the equation. The derivation of Eq. 7 can be found in a number of publications (25, 43, 44).

Hofstee (26) has shown that titration curves can be based on linear nonlogarithmic forms of the equilibrium equation of a dissociation reaction. He stated that "from such curves, in contrast to those based on logarithmic transformations both the end point of the titration and the dissociation constant can be derived." This can be shown by letting Z equal the sum of all the known concentrations at any point in a titration.

$$Z = [X] - [M] - [H^+] + [OH^-] \quad \text{(Eq. 8)}$$

Therefore substituting Eq. 8 into Eq. 7, we get the general Eq. 9:

$$[H^+] = K_a^c \frac{C_a^0 + Z}{C_b^0 - Z} \quad \text{(Eq. 9)}$$

For the titration of a pure weak acid, $C_b^0 = 0$, Eq. 9 can be rearranged to the form:

$$Z = C_a^0 - (1/K_a^c)\, Z[H^+] \qquad \text{(Eq. 10)}$$

Therefore, a plot of Z *versus* $Z[H^+]$ would be a straight line with a slope which equals the negative reciprocal of the dissociation constant and an intercept which equals C_a^0.

For the titration of a pure weak base, $C_a^0 = 0$, Eq. 9 can be rearranged to the form:

$$Z = C_b^0 - K_a^c(Z/[H^+]) \qquad \text{(Eq. 11)}$$

Therefore, a plot of Z *versus* $(Z/[H^+])$ would be a straight line with a slope equal to minus the dissociation constant and an intercept of C_b^0.

Benet and Goyan (24, 25) have expanded on the advantages of this particular method: (*a*) good reproducible results can be determined using titration data from the pH ranges 1.5–4 and 9–11; (*b*) very weak concentrations of acids and bases which might not give clearly defined inflection points on a pH *versus* ml. titrant plot can give accurate results when [H] and [OH] corrections are included in the Z term for a nonlogarithmic plot. The advantages in being able to use low concentrations are (*i*) less problems will be encountered resulting from the precipitation of the unionized species, and (*ii*) the ionic strength and therefore the activity coefficients can be better controlled; (*c*) for a weak acid the titration may start at a pH already larger than the pKa without affecting the accuracy of the determination; (*d*) the line drawn in the nonlogarithmic plot serves as an estimate of the accuracy of the data. Any point which deviates greatly from the line immediately shows that the data at that point are invalid; and (*e*) one of the great advantages of nonlogarithmic titration curves is in the ability to determine the molarity of the solution being titrated without resorting to elegant drying techniques or an elemental analysis. This makes the method a valuable tool when determining the dissociation constant of a new monoprotic species.

Lanza and Mazzei (27, 28) have used a different form of nonlogarithmic linear plots to determine the end points of titrations. They report that in some cases they may detect end points at about tenfold lower concentrations than those required to get a utilizable inflection in a plot of pH *versus* ml. of titrant.

Solomons (29) has also used a straight-line relationship which is nonlogarithmic. Although his terms are different, he has essentially rearranged Eq. 10 into an equation that resembles the Lineweaver-Burk expression for describing enzyme–substrate dissociation (30).

$$1/Z = \frac{[H^+]}{K_a^c C_a^0} + \frac{1}{C_a^0} \qquad \text{(Eq. 10}a\text{)}$$

Equation 10*a* can be graphically represented as a straight line if $(1/Z)$ is plotted against $[H^+]$. The extrapolated line then cuts the $1/Z$ axis at $1/C_a^0$ and the $[H^+]$ axis at $-K_a^c$.

Not all authors recommend plotting the data even though they are using the general equation (Eq. 7). For example, Albert and Serjeant (31) recommend that the titrant should be added in 10 equal portions, each a tenth of an equivalent. Then using a variant of Eq. 7, the pKa is calculated at each of these points, and the average pKa is determined from the average of the 10 different K_a's. It seems that these authors are not using the equation to the greatest advantage, since they must put a predetermined value of either C_a^0 or C_b^0 into each calculation. This is emphasized by a quote from their book (31):

> One of the commonest errors in titrating with alkali is for the values, in the set of pK_a values, to show an upward trend as the titration progresses. This is usually caused by an impurity in the substance undergoing determination, so that not so much of it is present as had been supposed. By far the commonest and most troublesome impurity is water. To avoid this trouble, every substance submitted for determination of pK_a should be of analytical purity and dried under the same conditions that preceded its analysis.

Of course "this trouble" can easily be avoided by plotting the data according to Eq. 10.

Effects of Dilution—The work of Meites and Goldman (5–10), as described previously, deals with the dilution effects on the correspondence of pKa with the inflection points for sigmoid plots of pH *versus* ml. titrant (see Table I). Le Duigou and Lauer (32) have utilized the formulas given by Meites and Goldman (5, 10) to determine the pKa of the boric acid–mannitol complex. Conventional calculations resulted in a concentration-dependent "constant," but the inclusion of the dilution effect gave a true constant.

Leeson and Brown (33) have pointed out that the nonlogarithmic equations of Benet and Goyan (24, 25), Eqs. 10 and 11, are not truly general since the authors worked with concentrated titrant such that volume changes could be ignored. However, Leeson and Brown wished to work with volume changes of about 2–5% and proposed a variation of Eq. 10 using moles. According to their derivations, they would define a term similar to Eq. 8 as:

$$Z' = X - M - H^+ + OH^- \qquad \text{(Eq. 12)}$$

where H^+ = numbers of moles of hydrogen ion present in the solution,
M = numbers of moles of strong base added to the solution,

X = numbers of moles of strong acid added to the solution,
OH^- = numbers of moles of hydroxide ion present in the solution.

In this form, the numbers of moles of M or X can be determined by multiplying the number of ml. of titrant added times its normality divided by 1000. However, the number of moles of hydrogen and hydroxide ions must be obtained from the experimental pH data. First, the activity of hydrogen ions must be converted to a concentration value by methods described below. Then the numbers of moles of hydrogen ion can be found by Eq. 12*a*:

$$H^+ = \frac{V}{1000}[H^+] \qquad \text{(Eq. 12}a\text{)}$$

where V = volume of solution in ml. at any particular point in the titration (*i.e.*, original volume + volume of titrant added).

Deriving a general equation using moles according to the method of Leeson and Brown (33) results in the following:

$$[H^+] = K_a^c \frac{A^0 - Z'}{B^0 + Z'} \qquad \text{(Eq. 13)}$$

where A^0 and B^0 are the values for the numbers of moles of pure weak acid and base present at the beginning of the titration. Both A^0 and B^0 are constants throughout the titration.

For the titration of a pure weak acid, $B^0 = 0$, and Eq. 13 can be rearranged to the form:

$$Z' = A^0 - (1/K_a^c)\,Z'[H^+] \qquad \text{(Eq. 14)}$$

Therefore a plot of Z' *versus* $Z'[H^+]$ would be a straight line with a slope equal to the negative reciprocal of the dissociation constant and an intercept equal to A^0. The nonlogarithmic titration plot using the Leeson and Brown derivation now allows the investigator to use any concentration of titrant that he wishes. The use of this method limits the accuracy of the determination only to the abilities of the pH meter to read correct values and the investigator to convert activities to concentrations.

Activity Effects—"Although venous blood carries considerably more CO_2 than does the arterial blood, the buffers of the blood are so efficient that the pH of venous blood is more acid than that of arterial blood by only 0.01 to 0.03 unit, i.e., pH 7.40 vs. pH 7.43" (34). This is an interesting fact; the body through its complicated buffer systems is able to maintain the *activity* of hydrogen ions within the blood to a variance of about 0.03 pH units. For how many drugs do we know the dissociation constant at 37° within a variance of even ± 0.04 units?[6] In most cases we know an "apparent dissociation constant" at some particular ionic strength that may or may not be given. An "apparent dissociation constant" is one that is determined at half-neutralization, *i.e.*, when the concentration of titrant added equals one-half the original concentration of the weak acid or basic substance, the pH read on the meter equals the apparent dissociation constant. The apparent pKa is usually designated as pKa′ and is a hybrid of the stoichiometric (concentration) pK_a^c and the thermodynamic (activity) pK_a^T or simply pKa.

For the following dissociation we can define the various dissociation constants:

$$HY + H_2O \rightleftharpoons H_3O^+ + Y \qquad \text{(Eq. 15)}$$

$$K_a^T = \frac{(H_3O^+)(Y)}{(HY)} = \frac{[H_3O^+]\ \gamma_{H^+}[Y]\ \gamma_Y}{[HY]\ \gamma_{HY}} = K_a^c\left(\frac{\gamma_{H^+}\ \gamma_Y}{\gamma_{HY}}\right) \qquad \text{(Eq. 16)}$$

$$K_a' = \frac{(H_3O^+)\,[Y]}{[HY]} = K_a^c\,\gamma_{H^+} = K_a^T\left(\frac{\gamma_{HY}}{\gamma_Y}\right) \qquad \text{(Eq. 17)}$$

where ( ) denotes activity, [ ] concentration, and γ molar activity coefficient.

The charges on Y and HY have been omitted in Eqs. 15–17 so that the definitions may be general. It is usually assumed that the activity coefficient (γ) for an uncharged species is 1, so depending on whether we are titrating an acid or a base, Eqs. 16 and 17 may be simplified accordingly.

It may be seen from Eq. 17 that a correctly determined apparent or stoichiometric dissociation constant could readily be converted to a thermodynamic dissociation constant if the activity coefficient of the charged species can be determined. Unfortunately, it is unlikely that the apparent dissociation constant will be correct, as noted previously. However, assuming that the determination is correct (*i.e.*, that the effect of dilution and hydrogen ion has negligible effect on the divergence of f_{pKa} from f inflection) as for substances with pKa's in the 5–9 range, when and how should the activity correction be applied?

Albert and Serjeant (35) have also asked the rhetorical question: "When is it advisable to use activity corrections in calculating the results from a potentiometric titration?" They rec-

[6] For very few at any temperature. The editors of the two compilations of dissociation constants (1, 2) evaluate all determinations with a variance of greater than ± 0.04 pK units as "uncertain," and it is very difficult to find more than a few drugs without the "uncertain" label.

ommend that activity corrections be made: (*a*) if the pH is measured with an instrument calibrated in 0.01 pH units (or greater), and (*i*) the concentration lies between 0.01 and 0.1 *M*, or (*ii*) the hydrogen-ion or hydroxyl-ion concentration is comparable in magnitude to the stoichiometric concentration of the ionized species, or (*iii*) an extraneous salt is added to achieve "constant ionic strength." Also (*b*) if the pH is measured with an instrument calibrated in 0.005 pH units or less.

These are good rules, with a rationale behind each. For instance, (*b*) can be reread as saying that if you have an instrument that is capable of making good, accurate measurements, you should calculate and report a good, accurate dissociation constant. Criteria (*a*) (*i*) sets the limits for use on a less accurate instrument. A 0.1 *M* solution is taken as the upper limit since that is about the greatest concentration where a general equation describing ion interaction may be used to give valid activity coefficients.[7] The lower limit is included to prevent making calculations to a greater accuracy than the meter can feasibly be read. However, we would tend to be a bit more conservative and set the lower concentration limit at 0.005.

Criteria (*a*) (*ii*) is necessary when the hydrogen ion or hydroxide terms in Eqs. 8 or 12 are significant compared to the sum of other terms. Criteria (*a*) (*iii*) is necessary under the very common circumstances where a swamping concentration of a strong electrolyte, such as potassium chloride, has been added to the solution so that the ionic strength of the solution and the activity coefficients will remain constant throughout the titration. If this is not done, the ionic strength of the solution will change appreciably during the titration as titrant is added. For example, consider the titration of a 0.02 *M* solution of a monoprotic weak acid, HA, with a strong base, MOH. Initially this weak acid will be very slightly dissociated, let us say 3% dissociated; at this point in the titration the ionic strength of the solution would only be 0.0006. However, at half-neutralization, the solution would be 0.01 *M* in M^+ ions and 0.01 *M* in A^- ions. At this point the ionic strength would be 0.01. At the end of titration the ionic strength would have risen to 0.02 and thus, throughout the titration a different activity coefficient would have to be calculated after each addition of titrant. It is easy to see from this example that it would be preferable to add a swamping electrolyte and use a constant activity coefficient. However, since 0.1 *M* solutions are the upper limit for calculating reliable activity coefficients (*vide supra*) only 0.08 *M* KCl may be added, and the activity coefficient will still vary throughout the titration from 0.08 to 0.10. Therefore, in using the "conventional" sigmoid titration curves, we are faced with a paradox. We wish to use a low concentration of drug so that a truly swamping concentration of strong electrolyte may be added, but in using a low concentration of drug, we will have a less sharply defined inflection point and will increase the difference between f_{pKa} and f inflection as was pointed out by Goldman and Meites (*vide supra*). Therefore in order to get an accurate pKa from a nonlinear plot, a large concentration of drug should be used, and the activity coefficient should be calculated at the point of inflection.

Using the nonlogarithmic linear titration plots obtained from general Eq. 13, the problem disappears, since low concentration of drug will not impair the accuracy of the method.

Having decided to use activity coefficients, which value should be used? In order to answer this question we must briefly review the relation of activity coefficients to concentration. It is well known that when the activity coefficients of typical electrolytes are plotted against a function of the concentration (the square root of the ionic strength), the activity coefficients begin at 1, pass through a distinct minima, and then may even become greater than 1, and that these minima occur at different concentrations for each electrolyte.

In a simplified manner this may be explained as follows. At infinite dilution the electrolytes are completely dissociated and free to move; however, as the concentration is increased the ions come closer together and there is interionic attraction, which tends to draw ions together and render them less free to move. Therefore, the effective concentration or activity of ions are less than would be expected if complete dissociation took place.

It is well documented that many ions hydrate in water and that certain ions are hydrated to a greater extent than others (37). These hydrated water molecules are said to enter the coordination spheres of the ion and are essentially removed from the available free solvent. This decrease in available solvent seems to increase the effective concentration of the ions.

Actually both of these effects, the interionic attraction and the removal of solvent molecules, occur simultaneously.

[7] This is an unfortunate upper limit when examining drugs in biological systems, since we would, at least, like to know the activity coefficient for an ionizable drug at a sodium chloride concentration of 0.154 *M* (the isotonic concentration.)

At low concentrations the interionic attraction is the dominating factor affecting the activity coefficient; however, as the concentration of electrolyte is increased, more and more solvent water will be sequestered until this effect overcomes the interionic attraction effect and causes the activity coefficient to increase with increasing concentration. Obviously, the solvent sequestering effect will be different for different ions depending on how much water is coordinated to each ion and, therefore, no general relation can be made between activity coefficient and concentration for this effect.

However, Debye and Hückel (38–41) reasoned that in dilute solutions, only the interionic attraction would affect the activity coefficient and that the ions could be regarded as point charges. Starting with these assumptions, they derived what is known as the Debye-Hückel limiting law:

$$\log \gamma_i = -A(z_i)^2 \sqrt{\mu} \qquad \text{(Eq. 18)}$$

where γ_i = ionic activity coefficient,
A = a term made up of universal constants, the temperature, and the dielectric constant of the solvent,
μ = the ionic strength ($\mu = {}^1/_2 \Sigma c_i z_i^2$),
z_i = the charge of the ion.

According to the Debye-Hückel limiting law, all uni-univalent electrolytes at a given concentration should have the same activity coefficient, and experimental determinations show that this is true up to about 0.01 ionic strength (42). However, at an ionic strength of 0.01, the size of the ions begins to have an effect[8] on the interionic attraction and the limiting law must be extended:

$$\log \gamma_i = \frac{-A(z_i)^2 \sqrt{\mu}}{1 + a_i B \sqrt{\mu}} \qquad \text{(Eq. 19)}$$

where a_i = ion size parameter,
B = a constant dependent on the dielectric constant of the solvent and the temperature.

The above equation is considered accurate up to an ionic strength of 0.1 (45), and this sets the upper limit in concentration for determining accurate pKa values as quoted above from Albert and Serjeant.

Hückel (41) in 1925 extended the theory so as to apply to higher concentrations:

$$\log \gamma_i = \frac{-A z_i^2 \sqrt{\mu}}{1 + a_i B \sqrt{\mu}} + C\mu \qquad \text{(Eq. 20)}$$

where C is a constant dependent on the nature of *all* the ions in the solution. This equation has been shown to fit activity data with high precision[9] to about 1.0 M solutions (46).

However, now there are two unknown constants, a_i and C, which must be "guessed" before a calculation of γ_i can be made. Since it is difficult enough making one guess, Eq. 19 limited to ionic strengths no greater than 0.1 has been the equation of choice (25, 35). Benet and Goyan (24, 25) used the single ion size parameters which Kielland (47) determined from theoretical considerations. Kielland presents ion size parameters and individual activity coefficients for 130 inorganic and organic ions. He estimates a 9-Å. parameter for hydrogen ion, 3.5-Å. for hydroxide ions, and 3-Å. for potassium and chloride ions. The 9-Å. value for hydrogen is especially important, since the conversion of hydrogen-ion activity to concentration is the necessary step before any of the generalized equations presented previously may be used.

Leeson and Brown (33), for their calculations, used "the more familiar, experimentally obtained, $\gamma_\pm$ values for HCl as listed in Lewis and Randall" (48). Leeson and Brown make a comparison between the thermodynamic pKa's calculated using Kielland's activity coefficient and the Lewis and Randall mean ionic activity coefficient. They found that the Kielland activity coefficient for acetic acid gave a pKa value of 4.74, while the Lewis and Randall activity coefficient gave a pKa value of 4.76. The literature value for acetic acid may be taken as 4.76 (49, 50).

Leeson and Brown go on to point out that the intercept of the plot of the data for the two different calculations (using Eq. 14 and determining A^0) is not markedly influenced by the choice of the activity coefficient. It is rather the ionization constant (or slope) that reflects the difference. A plot of pK_a^c *versus* $\sqrt{\mu}$ demonstrates that both sets of data are essentially linear. These latter plots were extrapolated to infinite dilution to evaluate the thermodynamic ionization constants reported above. "These results," Leeson and Brown conclude, "indicate that the pK_a obtained with both values [Kielland, and Lewis and Randall] are consistent with the literature. Therefore, since the two sets of the data are the same, the type of activity coefficient

[8] The size of the ion includes its sequestered water of hydration and is defined as the ion size parameter.

[9] The Debye-Hückel equations (Eqs. 18–20) should actually read $\log f_i = \ldots$ since f_i is the activity coefficient when concentrations in moles per liter are used. The activity coefficient γ_i is associated with molality. However, this difference can be ignored for dilute solutions. For example, the two activity coefficients for univalent electrolytes differ by only 0.0004 for 0.1 M NaCl.

TABLE II—SPECIFIC ION-INTERACTION CONSTANTS AFTER GUGGENHEIM AND TURGEON (54)

Electrolyte	B_{MX} at 25° C.
HCl	0.23
NaCl	0.13
NaI	0.18
NaOAc	0.20
NaOH	0.05
KCl	0.09
KI	0.13
KOAc	0.23
KOH	0.11

employed for calculations appears to be a matter of personal choice."

The authors agree with Leeson and Brown that the Lewis and Randall values may be used for hydrogen ion activity coefficients when either KCl or NaCl is used as a swamping electrolyte and the ionic strength is less than one-tenth. There are sound reasons for this, based on the work of Bronsted and Guggenheim.

In 1922 Bronsted (51) proposed that there be a specific ion interaction term in the Debye-Hückel equation dependent on *which* two ions were involved. In 1935, Guggenheim (52) suggested that a standard value of a_i in the vicinity of 3 Å. would improve the empirical success of the Bronsted type of equation without introducing any serious complications. This yields for a simple M-X electrolyte the following equation:

$$\log \gamma_{\pm} = \frac{-A\,|z_+ z_-|\,\sqrt{I}}{1 + \sqrt{I}} + B_{MX} m \quad \text{(Eq. 21)}$$

where m = molality of MX,
B_{MX} = a constant at a particular temperature which characterizes the interaction between cation M and anion X, a specific ion-interaction constant,
I = ionic strength using molal concentrations.

Values for the coefficient B_{MX} for numerous electrolytes in aqueous solution have been determined (52–54) from experimental data and have been tabulated (54). The values derived from freezing point measurements relate to 0°. Those derived from EMF measurements and isopiestic measurements relate to 25° and other temperatures. A few pertinent values are listed in Table II.

As an example, a 0.05 M solution of HCl calculated by Eq. 21 gives a mean ionic activity coefficient $\gamma_{\pm}$ of 0.829 compared to the Lewis and Randall (48) experimentally determined value of 0.830. Using the specific ion-interaction constants, $\gamma_{\pm}$ values may be computed for solutions up to 0.1 M with an estimated accuracy of the order of 0.5% (55), which is certainly sufficient for most purposes.

The Guggenheim type of equation may also be used to calculate the ionic activity coefficient for a specific ion in a solution containing a mixture of electrolytes (56, 57). The equation for a particular cation M′ of charge $z_{M'}$ is:

$$\log \gamma_{M'} = \frac{-A z_{M'}^2 \sqrt{I}}{1 + \sqrt{I}} + \Sigma_X B_{M'X} m_X \quad \text{(Eq. 22)}$$

where each specific ion-interaction constant is multiplied by the molality of the anion involved (m_X) and summed. No terms are present for other cations.

Now consider the titration of a 1×10^{-3} aqueous solution of acidic drug HD at 25° where the ionic strength has been adjusted to 0.05 by the addition of KCl.[10] Calculating the activity coefficient for hydrogen ions using Eq. 22, gives the following:

$$\log \gamma_{H^+} = \frac{-.509\ (1)^2\ \sqrt{.05}}{1 + \sqrt{.05}} + B_{HCl}\,(.0495) + B_{HD}\,(.0005) \quad \text{(Eq. 23)}$$

The value for B_{HCl} can be obtained from Table II. The value for B_{HD} is unknown, but the third term on the righthand side of the equation is insignificant and can be dropped, so the γ_{H^+} will be identical to the one calculated above for a 0.05 M solution of HCl. Equation 22 should hold for the titration of any acid or base and thus the Lewis and Randall mean activity coefficient for HCl would be the correct value to use for the hydrogen-ion activity coefficient.

However, the Lewis and Randall values would be incorrect for use in calculating γ_{HD^+}, γ_{OH^-}, and γ_{D^-}. The activity coefficients for hydroxide ion could be calculated from the Guggenheim equation for X′, a particular anion (56, 57).

$$\log \gamma_{X'} = \frac{-A z_{X'}^2 \sqrt{I}}{1 + \sqrt{I}} + \Sigma_M B_{MX'} m_M \quad \text{(Eq. 24)}$$

where m_M is the molality of the cation involved. Using a swamping electrolyte, this will be the molality of the cation. We cannot calculate the activity coefficients for D^- or HD^+ (negatively and positively charged drug molecules) since there are no specified interionic–attraction coefficients for drugs. In these cases, we must still make a judicious guess as to which ion-size parameter we should put into the Debye-Hückel equation. However, it seems reasonable to expect that we could make a much better guess of the ion-interac-

[10] The convention usually followed is to add that amount of swamping electrolyte which will give the desired ionic strength at half-neutralization of the drug.

tion coefficient for most drugs using the pharmaceutical data for sodium chloride equivalents and cryoscopic properties (58–60) and isopiestic measurements (61) available for almost all acidic and basic drugs in use. One of us is presently examining the reliability of specific ion-interaction coefficients determined from the above-cited data (62).

Summary

For the potentiometric determination of dissociation constants in aqueous solution, the authors recommend the following on the basis of the foregoing review of the literature. Use the most accurate pH meter available and the techniques suggested by Albert and Serjeant (4). Use a concentration of drug between 5×10^{-4} and 1×10^{-3} M, add a swamping electrolyte such as KCl to adjust the ionic strength to 0.05. Take about 15 readings of pH after addition of titrant. Convert pH as read on the meter to hydrogen ion activity using either the Lewis and Randall values for the activity coefficient (48) or those calculated by Eq. 23. Calculate hydroxide-ion concentration, if necessary, from the K_w for the temperature used (63) and the activity coefficient for hydroxide calculated for Eq. 24, or use the values tabulated by Harned and Owen (64) for the ionic activity function of water. Calculate the data according to the method of Leeson and Brown (33). Plot the data after making the appropriate rearrangement of Eq. 13, and determine the slope of the line and K_a^c. Now determine K_a^T by Eq. 16, using the activity coefficient for hydrogen ion calculated from Eq. 23 and the activity coefficient for the drug ion determined by Eqs. 23 or 24 or some judicious choice of an ion size parameter. At present, however, the most accurate yet burdensome method is to calculate pK_a^c at four or more ionic strengths and then plot pK_a^c *versus* $\sqrt{\mu}/1 + \sqrt{\mu}$ and extrapolate to infinite dilution. Following these procedures, it is estimated that an accurate pK_a^T may be determined within ± 0.03 units.

SEMIAQUEOUS SOLUTIONS

For a large number of pharmaceutical compounds, the uncharged species is so insoluble that it is very difficult to determine accurate dissociation constants by even the most sensitive methods previously described. Albert and Serjeant (65) have said:

> When a substance is poorly soluble in water, but highly soluble in a volatile solvent, it is natural to consider determining the ionization constant in a mixture of the two solvents, e.g., in 50% ethanol. This temptation should be resisted, for reasons which will shortly be discussed.

They go on to point out that Hall and Sprinkle (3) plotted pKa against per cent alcohol concentration (range 10%–97%) for 18 different amines and then extrapolated these "hockey-stick" shaped curves back to zero per cent. Albert and Serjeant correctly point out that these values are highly suspect especially when the amine was too insoluble to give values at 10 and 20%.

They also present a table of pKa values of aniline, methylaniline, and dimethylaniline in various percentages of ethanol (66) showing that in solutions of increasing ethanol percentage, aniline, and its *N*-methyl derivatives become weaker bases. They state, "But the effect of the alcohol is least on the unmethylated substance with this paradoxical result: although methylation increases the basic strength in 0% to 35% alcohol, in more highly alcoholic solutions it decreases the basic strength. Dilute dimethylformamide, methyl cellosolve and other glycol derivatives cause this type of trouble also." Actually, these results may not be troublesome at all, and they can be explained by more recent ideas, as will be discussed.

Finally, Albert and Serjeant comment that ". . . the widespread availability of ultraviolet spectrophotometers makes it possible to obtain the pK_a values of many sparingly soluble substances by special methods." However, there are a large number of substances, particularly amines, which are not suitable for spectrophotometric analysis, and thus we must accept the "temptation" and determine the pKa by titration in semiaqueous solvents.

The practice of determining pKa's in varying concentrations of alcohol and plotting these values *versus* per cent alcohol originated with Mizutani (67), who then extrapolated the plot to zero per cent alcohol to find the aqueous pKa. This procedure has been followed by a number of workers dealing with substances of pharmaceutical interest. Marshall (68) determined the dissociation constants of various antihistamines in ethanol–water mixtures. Edmonson and Goyan (69) used the same solvent combination while studying phenobarbital solubility. A methanol–water solvent was used by Chatten and Harris (70) to study phenothiazine and sympathomimetic amines. Garrett (71) measured the variation of apparent dissociation constants for several tetracycline antibiotics in dimethylformamide–water solvent and used the information in the assignment of pK_a' values to functional groups.

Theoretical Considerations—There is no doubt that an investigator can take a known

amount of a substance in a given mixture of nonaqueous solvent–water containing a known amount of ionic substances and reproduce previously reported pH readings using the usual glass electrode–calomel electrode and a pH meter which has been standardized in a specified manner. The problem is to relate this reading and dissociation constant determinations to values obtained in water.

The numerical values of activity coefficients are commonly assigned with reference to a value of one at infinite dilution of the solute in the particular solvent being studied (72). However, there must be some way of relating an activity coefficient in water to that in a solvent containing alcohol, methanol, dioxane, or other organic liquids miscible with water. The medium effect ${}_m\gamma_i$ is a measure of the free energy change on transfer of 1 mole of a substance i from a standard state in water to the standard state in the mixed solvent SH:

$$\gamma_i = ({}_m\gamma_i)({}_s\gamma_i) \qquad \text{(Eq. 25)}$$

where γ_i = activity coefficient of ion i in water,
${}_s\gamma_i$ = activity coefficient of ion i in solvent SH.

The activity coefficients γ_i and ${}_s\gamma_i$, which become unity at infinite dilution in water and in solvent–water mixtures, respectively, characterize the interionic and ion-molecule forces and other effects dependent on the concentration of the medium. The medium effect ${}_m\gamma_i$ reflects the differences in the electrostatic and chemical interactions of the substance i in the two solvents. Of these interactions, solvation is probably the most important when ions are transferred from one medium to another (72).

The equation for the EMF of a cell with a liquid junction yields the following equation:[11]

$$\text{pH} = -\log a_H + \bar{E} \qquad \text{(Eq. 26)}$$

where $\bar{E}_j$ = the liquid-junction potential expressed in pH units.

Now we may define $a_H{}^*$ as the hydrogen-ion activity referred to the standard state in the mixed solvent SH.

$$pa_H{}^* = -\log (m_H)({}_s\gamma_H) \qquad \text{(Eq. 27)}$$

Now combining Eqs. 25, 26, and 27, we obtain:

$$pa_H{}^* = -\log (m_H) - \log (\gamma_H) + \log ({}_m\gamma_H) = \text{pH} - \bar{E}_j + \log {}_m\gamma_H = \text{pH} - \delta \qquad \text{(Eq. 28)}$$

[11] Equation 26 is actually what we are measuring on the pH meter in aqueous solutions. The pH read equals the negative log of the activity of hydrogen ion plus the residual liquid-junction potential. We attempt to cancel out this last term by standardizing the meter with a buffer solution that has the same $\bar{E}_j$ as the solution we are measuring. In practice, these $\bar{E}_j$'s do cancel out quite well for buffers and solutions between pH 3 and 11 in water (73).

where δ is written for $\bar{E}_j - \log {}_m\gamma_H$ and is a constant for a medium of a given composition. Now, $pa_H{}^*$ can be related to the dissociation equilibrium (Eq. 29) in a semiaqueous medium, by Eq. 30:

$$HA + SH \rightleftharpoons A + SH_2{}^+ \qquad \text{(Eq. 29)}$$

$$\text{p}({}_sK_a) = pa_H{}^* - \log \frac{m_A}{m_{HA}} - \log \frac{{}_s\gamma_A}{{}_s\gamma_{HA}} \qquad \text{(Eq. 30)}$$

where ${}_sK_a$ is the thermodynamic dissociation constant of acid HA in mixed solvent SH. This equation is just the Henderson-Hasselbalch equation using thermodynamic values for solvent SH.

The values of ${}_s\gamma_i$ can be calculated by the extended Debye-Hückel equation allowing for the effect of changes in dielectric constant:

$$-\log {}_s\gamma_i = \frac{(1.82455 \times 10^6)\, z_i{}^2 \sqrt{\mu}}{(DT)^{3/2}\,[1 + 50.2904\,(DT)^{-1/2} a_i \sqrt{\mu}]} \qquad \text{(Eq. 31)}$$

where D = dielectric constant,
T = temperature in degrees Kelvin,
a_i = the ion size parameter.

Determination of Dissociation Constants (${}_sK_a$) in Semiaqueous Solvents—With the foregoing equations, it is now possible to determine $\text{p}({}_sK_a)$ if $pa_H{}^*$ can be determined at a number of points during a titration. The simplest way to determine $pa_H{}^*$ values experimentally would be to apply tabulated corrections to the pH numbers read on a pH meter which had been standardized with aqueous buffer solutions in the usual way (74), *i.e.*, using Eq. 28, $pa_H{}^* = \text{pH} - \delta$. Ong, Robinson, and Bates (75) have carried this correction a step further and applied it to the "seeming" pKa,[12] which is defined as:

$$\text{pKa}'' = \text{pH} - \log \frac{m_A}{m_{HA}} - \log \frac{{}_s\gamma_A}{{}_s\gamma_{HA}} \qquad \text{(Eq. 32)}$$

where pH is the meter reading at any point during the titration, the meter having been standardized with aqueous buffers. Now at any point in the titration we may subtract Eq. 30 from Eq. 32, with the result:

$$\text{pKa}'' - \text{p}({}_sK_a) = \text{pH} - pa_H{}^* = \delta \qquad \text{(Eq. 33)}$$

The term δ (Eq. 28) contains two values, the residual junction potential and the medium effect, and is identical with the quantity derived by Bates, Paabo, and Robinson (76) from a comparison of the EMF of cells with and without liquid junctions.

Ong, Robinson, and Bates (75) have calculated the values for acetic acid and anilinium ions in varying weight percentages of methanol (0–70%)

[12] The authors have chosen the term "seeming" pKa so as not to confuse this term with the apparent pKa defined by Eq. 17.

TABLE III—VALUES OF THE CONSTANT $\delta \equiv (\bar{E} - \log {}_m\gamma_H)$ IN METHANOL–WATER[a] AT 25°C. AFTER ONG *et al.* (75)

Wt. % methanol	0	10	20	30	40	50	60	70
δ	0	0.01	0.02	0.04	0.07	0.11	0.15	0.13

[a] A similar set of values could be compiled for ethanol–water mixtures from the data of Bates *et al.* (76), Butbezahl and Grunwald (80), and Gelsema and de Ligny (81) [cited by Bates, Paabo, and Robinson (76) and Bates (36)] although the agreement is not so good as that for methanol–water solutions. (For tabulation of these data, see *Reference 36*, p. 224.)

by determining the pK″ values and subtracting them from the $p({}_sK_a)$ values of Shedlovksy and Kay (77) for acetic acid and the values of Bacarella *et al.* (78) for anilinium. Ong *et al.* then compared their calculated δ values with those obtained from the EMF of cells with and without liquid junctions (76) and to the values obtained by de Ligny and Rehback (79) who also used cells with and without liquid junctions. The agreement for δ values among these workers is good and the recommended values for each weight percentages of methanol are presented in Table III. The table only contains values up to 70% since ionic association may be considered minimal only up to 68 wt.% methanol.

Thus δ may be subtracted from the "seeming" pK″ value to give the true p_sK_a value since δ is independent of the solute composition, provided that the total ionic strength is not high and the pH is neither very high nor very low (the same conditions which were placed on determinations in aqueous solutions). Finally, Ong *et al.* (75) point out that they and Bacarella *et al.* (78) used glass electrodes, while Bates *et al.* (76) and de Ligny and Rehback (79) used hydrogen electrodes.

> The general correlation which all the authors obtained shows . . . that the standard potential of the glass electrode is independent of solvent composition in water–methanol mixtures as, indeed, it should be if the electrode is behaving as a reversible hydrogen electrode. Furthermore, the general agreement shows that there is little dependence of the liquid junction potential on the type of junction used; de Ligny and Rehback (79) formed the junction at the end of a tube with a capillary opening, Bates, Paabo, and Robinson (76) used sintered disks, while porous fibers were used in this work (75).

Previous to the above work, Bates *et al.* (76) felt that the possibility of a shifting asymmetry potential when the glass electrode was transferred from an aqueous medium to an alcoholic medium was sufficient reason to use standard solutions of known pa_H*, having the same solvent composition as the solution to be titrated. Bates (82) has tabulated the values for some methanol–water (76, 83–85) and ethanol–water (76, 81) buffer systems. Recently pa_H* values have been reported for a number of other buffers in methanol–water solvents at varying temperatures: (*a*) tris(hydroxymethyl)aminomethane and its hydrochloride (Tris buffers) (86); (*b*) ammonia–ammonium chloride (87); and (*c*) 4-aminopyridine and its hydrochloride (88). Paabo *et al.* (89) have also resolved the discrepancies in the standardization of the data obtained with silver–silver chloride electrodes in methanol–water solvents, and were able to report mean ionic activity coefficients for hydrochloric acid to three decimal places in varying percentages of water–methanol solvents at 25°.

Relation of Dissociation Constants in Mixed Solvents to Dissociation Constants in Water—As was stated earlier, Mizutani originated the practice of plotting pKa″ *versus* per cent alcohol and extrapolating back to zero per cent to find pKa′ in water. Although this is a logical approach, there are no theoretical relationships to predict how much the plot will curve at various percentages, and if the titrated substance is not soluble in 10 or 20% alcohol, the extrapolation back to 0% may be highly suspect.

Let us now examine what has to be determined in order to develop a theoretical method for converting $p_sK_a^T$ to pKa^T. Consider the dissociation of acid HA in solvent SH and in water:

$$HA + H_2O \rightleftharpoons A + H_3O^+ \qquad \text{(Eq. 34)}$$

$$HA + SH \rightleftharpoons A + SH_2^+ \qquad \text{(Eq. 29)}[13]$$

$$K_a = \frac{(m_A\gamma_A)(m_{H^+}\gamma_{H^+})}{(m_{HA}\gamma_{HA})} \qquad \text{(Eq. 35)}$$

$${}_sK_a = \frac{(m_{A_s}\gamma_A)(m_{H^+_s}\gamma_{H^+})}{(m_{HA_s}\gamma_{HA})} \qquad \text{(Eq. 36)}$$

Recalling Eq. 25 and dividing Eq. 35 by Eq. 36, we derive Eq. 37:

$$\frac{K_a}{{}_sK_a} = \frac{({}_m\gamma_H)({}_m\gamma_A)}{({}_m\gamma_{HA})} \qquad \text{(Eq. 37)}$$

Taking the log of both sides of Eq. 37, we find that $p({}_sK_a) - pKa$ equals the log of the term on the righthand side of Eq. 37. Therefore, in order to calculate the aqueous dissociation constants from the experimentally determined nonaqueous values, we must be able to determine the medium effect. As stated earlier, the medium effect for an ion reflects the differences in the electrostatic and chemical interactions of the ion with the two solvents (72).

[13] Where we use SH_2^+ to designate the hydronium ion in a mixed solvent.

The standard free energy of transfer of a gram ion of a single ionic species i from water to another solvent SH is given by

$$\Delta G_i^0 = RT \ln {}_m\gamma_i \qquad \text{(Eq. 38}a\text{)}$$

This change in free energy is made up of an electrostatic part, ΔG_{el}, which can be estimated by the Born equation (90) and a nonelectrostatic part which accounts for the specific chemical interactions between the ions and the solvent. At present, there is no known way to characterize all of the nonelectrostatic effects, but since the electrostatic effects will predominate, the predictions of the Born equation are of some interest (91).

The change in free energy arising from charging effects in the process of transferring an ion from water to solvent SH as described by the Born equation is:

$$\Delta G_{el}^0 = \frac{Nz_i^2e^2}{2r}\left(\frac{1}{D_{SH}} - \frac{1}{D_w}\right) \qquad \text{(Eq. 38)}$$

where N = Avogadro's number,
z_i = charge on the ion,
e = the electronic charge,
r = the radius of the ion in the two solvents where the ion is considered a sphere,
D_{SH} = dielectric constant of solvent (SH in water).

Assuming that only electrostatic effects are taking place, substituting Eq. 38a into Eq. 38 and inserting numerical values for the constants at 25° results in Eq. 39:

$$\log {}_m\gamma_i = \frac{121.6z_i^2}{r}\left(\frac{1}{D_{SH}} - 0.0128\right) \qquad \text{(Eq. 39)}$$

Now substituting Eq. 39 into the logarithmic form of Eq. 37, we derive Eq. 40:

$$\mathrm{p}({}_sK_a) - \mathrm{pKa} = 121.6\left(\frac{1}{r_{H^+}} + \frac{z_A^2}{r_A} - \frac{z_{HA}^2}{r_{HA}}\right)\left(\frac{1}{D_{SH}} - 0.0128\right) \qquad \text{(Eq. 40)}$$

Now assuming that the radii of all the ions are equal (*i.e.*, $r_{H^+} = r_A = r_{HA}$), it is possible to make the following predictions. For an acid such as acetic acid (where A is negatively charged and HA has no charge), a plot of $\mathrm{p}({}_sK_a)$ *versus* $1/D_{SH}$ (*e.g.*, dissociation constants determined in solutions of varying alcohol percentages) should be a straight line with a positive slope. For an acid such as anilinium chloride (where HA is positively charged and A has no charge) a plot of $\mathrm{p}({}_sK_a)$ *versus* $1/D_{SH}$ should be a straight line with zero slope. It is found [*e.g.*, (66) and (92)] that p_sK_a values for monoprotic acids do indeed increase markedly as the dielectric constant of the solvent decreases. However, the p_sK_a values for cationic acids (HA^+) do not stay constant as predicted above, but usually decrease, pass through a minimum, and then increase at high concentrations of organic solvent.

Unfortunately, the Born treatment often fails. For example, if the data of Shedlovsky and Kay (77) or Bacarella *et al.* (78) for acetic acid in methanol–water is plotted with the above coordinates, the resulting line will be far from straight. Equation 40 also predicts that an acid should have the same dissociation constant in two different solvent mixtures having the same dielectric constant. However, both Speakman (93) and Dondon (94) observed that aqueous dioxane acted as if it had a higher effective dielectric constant than aqueous alcohol of the same actual dielectric constant. In other words, the values of dissociation constants of several acids in aqueous dioxane were reported to be higher than those determined in aqueous alcohol with the same dielectric constant. Yasuda (95) suggests that the usual way of defining K_a assumes that the activity of water is constant, as in the following equilibrium, K_a is defined according to Eq. 41,

$$HA + H_2O \rightleftharpoons H_3O^+ + A \qquad \text{(Eq. 34)}$$

$$K = \frac{(H_3O^+)(A)}{(HA)} \qquad \text{(Eq. 41)}$$

while he proposes a new dissociation constant dependent on the activity of water, which we shall call K_I.

$$K_I = \frac{(H_3O^+)(A)}{(HA)(H_2O)} \qquad \text{(Eq. 42)}$$

Yasuda reasons thus:

> In aqueous solutions K_a may be used, for (H_2O) is nearly constant. But this is not true in mixed solvents containing a limited amount of water. Due consideration should be paid to the fact that the activity of water in mixed solvents changes with the molar fraction of organic solvents. Therefore, the constant K_I defined by Eq. 42 should be used for the estimation of the dissociation of an acid in mixed solvents, in place of K_a defined by Eq. 41.

Yasuda justified ignoring the activity of the nonaqueous portion of the solvent, by the fact that water has an exceptionally high proton affinity and must be included in the equilibrium calculations, whereas the alcohol or dioxane proton affinities are insignificant. Recalculating the work cited above (77, 78, 94, 95), Yasuda plots pK_I *versus* $1/D$ and finds that the anomalies mentioned above disappear and the plot is linear for values of $1/D$ less than 0.02. It appears that at D values greater than 50, the nonelectrostatic terms in the free energy of transfer are no longer negligible.

One month after the publication of Yasuda's paper (95), Shedlovsky (96) presented a similar equation to the Trieste Symposium on Electrolytes; however, his work had a more complete theoretical derivation. Shedlovsky also considered the data of Grunwald *et al.* (78, 92) and Shedlovsky and Kay (77), particularly the dissociation of acetic acid in water–methanol and water–ethanol solvents.

Shedlovsky points out that on dissolving acetic acid in water or in alcohol, the following sequence of events occurs: first, a "force linkage" forms between the acid and solvent molecules; second, a proton shifts from the acid-carboxyl group to a bound solvent molecule forming an ion pair; third, the ion pair dissociates. These steps are shown in the following equations and the over-all dissociation constant for each solvent, K_H and K_R are derived.

$$HA + H_2O \rightleftharpoons HA \cdot H_2O \qquad \text{(Eq. 43)}$$

$$HA \cdot H_2O \rightleftharpoons H_3O^+ \cdot A^- \qquad \text{(Eq. 44)}$$

$$H_3O^+ \cdot A^- \rightleftharpoons H_3O^+ + A^- \qquad \text{(Eq. 45)}$$

$$HA + ROH \rightleftharpoons HA \cdot ROH \qquad \text{(Eq. 46)}$$

$$HA \cdot ROH \rightleftharpoons RH_2O^+ \cdot A^- \qquad \text{(Eq. 47)}$$

$$RH_2O^+ \cdot A^- \rightleftharpoons RH_2O^+ + A^- \qquad \text{(Eq. 48)}$$

$$K_{43}K_{44}K_{45} = \frac{[H_3O^+][A^-]\,\gamma^2}{[HA][H_2O]} = K_H \qquad \text{(Eq. 49)}$$

$$K_{46}K_{47}K_{48} = \frac{[RH_2O^+][A^-]\,\gamma^2}{[HA][ROH]} = K_R \qquad \text{(Eq. 50)}$$

where it is assumed that the activity coefficients of all charged species are identical and that the activity coefficients of uncharged species are one.

The experimentally determined ionization constant ${}_sK_a$, however, depends on both kinds of hydrogen ions.

$${}_sK_a = \frac{[A^-][H_3O^+ + H_2OR^+]\,\gamma^2}{[HA]} = K_H[H_2O] + K_R[ROH] \qquad \text{(Eq. 51)}$$

Now Shedlovsky employs Bjerrum's theory of ionic association (97–99), to show that $H_2OR^+ \ll H_3O^+$, assuming that Eqs. 45 and 48 are controlled by simple coulombic forces. Therefore:

$$K_H = B_H \exp.(-b) \qquad \text{(Eq. 52)}$$

$$K_R = B_R \exp.(-b) \qquad \text{(Eq. 53)}$$

where $b = \left(\frac{e^2}{DkTa}\right)$ in which e is the electronic charge; D the dielectric constant; k is the Boltzman constant; T is the Kelvin temperature; and a is the average ionic diameter for the anion and cation (the distance of closest approach); B is a constant containing e, D, k, T, N (Avogadro's number), r the actual average distance between ions, and the appropriate K values (*e.g.*, B_H contains $K_{43}K_{44}$).

Shedlovsky further assumes that a is a constant over the entire range of solvent composition within a given solvent system. Then

$${}_sK_a[e^b] = B_H[H_2O] + B_R[HOR]$$

Now Shedlovsky points out that Eqs. 43 and 46 are for dipole–dipole interactions and Eqs. 45 and 48 are for ion pair dissociations. He feels that for acetic acid the magnitude of these interactions and dissociations should be approximately the same in both water and alcohol (*i.e.*, $K_{43} = K_{46}$ and $K_{45} = K_{48}$). However, the proton rearrangements, Eqs. 44 and 47, should take place much more readily in water than in alcohol so that K_{44} should be considerably greater than K_{47} and therefore B_H should be much greater than B_R. Therefore, Eq. 51 will reduce to Eq. 54 when Eqs. 52 and 53 are substituted into Eq. 51, along with the simplifying assumption $B_H \gg B_R$.

$${}_sK_a = B_H e^{-b}[H_2O] \qquad \text{(Eq. 54)}$$

This is essentially the same assumption made by Yasuda (95), except that the Shedlovsky equation can now be rearranged to:

$$p_sK_a + \log[H_2O] = \left(\frac{e^2}{(2.303)akT}\right)(1/D) - \log B_H \qquad \text{(Eq. 55)}$$

so that a plot of $p_sK_a + \log[H_2O]$ *versus* $1/D$ should be linear. But Shedlovsky's derivation shows that the slope of the line may be used to measure a, the average ion size of the cation and anion.

In closing this section, let us return to Albert and Serjeant's comment (65) concerning the variation of p_sK_a for aniline, methylaniline, and dimethylaniline in varying percentages of ethanol (66). A plot of $p_sK_a + \log[H_2O]$ *versus* $1/D$ for each of these three compounds are fairly linear below a value of $1/D = 0.02$. However, the three lines cross each other (*i.e.*, they have different slopes) giving rise to what Albert and Serjeant refer to as "paradoxical results." However, as shown, this may only be a result of different ionic sizes, and further work might elucidate these results.[14]

Summary

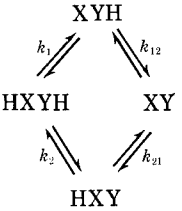
As has been reviewed above, there is presently no completely satisfactory method for converting p_sK_a values to pKa values. Therefore, although good accuracy can be realized in determining p_sK_a

[14] It should be emphasized that a direct correlation with Shedlovsky's equations cannot be derived, since here we are dealing with a protonic acid instead of an uncharged acid. However, an analysis could be made corresponding to the recent work of Paabo, Bates, and Robinson (87), who considered the variation in p_sK_a for ammonia as compared to Tris.

values, this accuracy cannot be carried over to the aqueous dissociation constants. The authors would recommend that semiaqueous titrations be carried out in methanol–water systems if possible, since there is an abundance of data concerning this solvent system. Procedures for determining p_sK_a need not differ from those in aqueous systems, except that Eq. 31 should be used for determining activity coefficients. The values of Ong *et al.* (75) appear to be sufficiently accurate, so that aqueous buffers may be used for standardizing the meter before titrations in a methanol–water system. In extrapolating p_sK_a values back to pKa in water, we recommend plotting $p_sK_a + \log [H_2O]$ *versus* $1/D$, as defined by Shedlovsky (96) and Yasuda (95). As long as p_sK_a values can be determined in solutions with dielectric constants greater than 50, these extrapolations (as per Yasuda and Shedlovsky) seem to give reasonable pKa^T values. Calculations of dissociation constants under these conditions should be accurate to about ± 0.06 pK units.

POLYPROTIC SPECIES

Whenever an acid has more than one ionizable hydrogen, the interpretation of potentiometric titration data becomes more complex. The authors will use the dissociation of a dibasic acid as shown in Scheme I to illustrate the problems,



Scheme I

where the lower case k is used to denote microconstants and the subscripts indicate the order of dissociation (*i.e.*, X is site 1, Y is site 2). Now the titration data will yield the two macroconstants defined as:

$$K_1^T = \frac{[(XYH) + (HXY)]\,(H)}{(HXYH)} = k_1 + k_2 \qquad \text{(Eq. 56)}$$

$$K_2^T = \frac{(XY)\,(H)}{[(XYH) + (HXY)]} = \frac{k_{12}k_{21}}{k_{12} + k_{21}} \qquad \text{(Eq. 57)}$$

If k_1 is much greater than k_2, k_2 and k_{21} will be a negligible pathway and:

$$K_1 = k_1,\ K_2 = k_{12}$$

Thus if K_1 is 1000 times or more larger than K_2, any of the methods outlined earlier may be used. (With polyprotic acids it is often possible to isolate one of the dissociations and determine it independently.)

However, some cases are still bound to occur where K_1 and K_2 overlap and the methods of determining their values differ somewhat from those previously discussed. Probably the oldest method is that due to Noyes (100). The equations are derived by use of the two dissociation equilibria (Eqs. 56 and 57 written in terms of concentration) and the equations for conservation of mass and charge. Where hydroxide-ion concentration may be ignored relative to the other terms in the equation for electroneutrality (*i.e.*, except for extremely weak acids and exp erimental points close to $f = 2$) he obtains:

$$K_1^c = \frac{B_1D_2 - B_2D_1}{A_1B_2 - A_2B_1} \qquad \text{(Eq. 58)}$$

$$K_2^c = \frac{A_1D_2 - A_2D_1}{B_1D_2 - B_2D_1} \qquad \text{(Eq. 59)}$$

Here $A_n = [M_n][H_n] + [H_n]^2 - [H_n][C^0]$

$B_n = 2[C^0] - [M_n] - [H_n]$

$D_n = ([H_n] + [M_n])[H_n]^2$

where [ ] denotes concentration, C^0 is the concentration of the acid being titrated, M is the total concentration of added alkali, and n is used to indicate individual points on the titration curve. Ordinarily, several pairs of data points are taken and the constants averaged to obtain the best estimate of K^c. These values may be converted to K^T values using the activity coefficients due to Kielland (47, 25). For the unusual case where the hydroxide-ion concentration is not negligible, see *Reference 100*. Another method of interest is that of Speakman (101). Using the same basic equations as above, he obtains:

$$\frac{(H)^2Pf_{XY}}{Rf_{HXYH}} = K_1^T\left[\frac{(H)Qf_{XY}}{Rf_{HXY}}\right] + K_1^TK_2^T \qquad \text{(Eq. 60)}$$

or

$$X = K_1^TY + K_1^TK_2^T \qquad \text{(Eq. 61)}$$

where $P = [M] + [H] - [OH]$

$Q = [C^0] - [M] - [H] + [OH]$

$R = 2[C^0] - [M] - [H] + [OH]$

and it is assumed $f_{HXY} = f_{XYH}$.

Thus in this method one uses the experimental data and activity coefficients in obtaining X and Y which are then plotted to obtain K_1^T and K_2^T.

Unfortunately, it is not possible to determine the microconstants from the macroconstants without one additional expression relating the various species (102). This subject is therefore outside the scope of this review, although several interesting papers have been published dealing

with medicinal agents (103–105). In the paper of Riegelman *et al.*, the values obtained for synephrine seem questionable since k_2 is less than k_{21}. Using the same data and equations with the Kielland values for activity coefficient (47), this anomaly disappears (pK_2 9.84 to 9.61, pK_{21} 9.69 to 9.71). This again demonstrates the value of working with reasonable estimates of activity rather than concentrations.

Summary

The determination of macroconstants requires the same degree of experimental care used with monoprotic acids. The data may be manipulated by either of the methods discussed to obtain K^T using the Kielland estimates for activity coefficients.

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