General Method for Determining Macrodissociation Constants of Polyprotic, Amphoteric Compounds from Solubility Measurements

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Abstract □ Equilibrium solubility and pH measurements can be used to determine macrodissociation constants of weak acids and bases of some highly insoluble substances. Equations are derived extending solubility, pH, and pKa (macroscopic) relationships to polyprotic, amphoteric substances. A general method for estimating pKa values, given a set of solubility and pH measurements, is presented. Included in the estimation procedure is a subroutine for approximating thermodynamic pKa values. The method was tested on two data sets (tyrosine and 2,8-dihydroxyadenine) and rendered pKa (thermodynamic) estimates in close agreement with those using other methods.

Keyphrases □ Dissociation constants—polyprotic, amphoteric compounds, determined using solubility and pH measurements □ pKa values—polyprotic, amphoteric compounds, determined using solubility and pH measurements □ Solubility measurements—used to determine dissociation constants for polyprotic, amphoteric compounds □ pH measurements—used to determine dissociation constants for polyprotic, amphoteric compounds

Dissociation behavior of weak acids and bases is unambiguously characterized by determination of the thermodynamic microdissociation constant pertaining to each dissociation reaction. The microdissociation constant of a monoprotic species (or that of an amphoteric compound with widely separated acidity constants) can often be determined directly from potentiometric measurements. Dissociation constants of polyprotic species estimated by this method (macrodissociation constants) generally represent apparent dissociation reactions which do not precisely relate to actual ionic forms present at equilibrium. Nevertheless, macrodissociation constants provide useful estimates of apparent dissociation behavior which can be utilized for characterizing buffer systems, acid strength, and solubility–pH relationships. Thermodynamic macrodissociation constants are preferred over apparent macrodissociation constants since thermodynamic constants are applicable to any aqueous solution in which the activities of participating ions can be estimated.

Several methods exist for determining aqueous macrodissociation constants of weak acids and bases. While potentiometric titrations and conductimetry generally suffice for readily soluble substances, estimating pKa values of poorly soluble compounds is sometimes impossible by these methods (1, 2). UV and fluorescent spectrophotometry can be applied in such cases, provided there is sufficient shift in the spectra with a changing hydrogen-ion concentration (1, 3). Where molecular and ionic spectra are too similar or totally lacking, the solubility method may be uniquely suited (1). This technique takes advantage of the fact that sparingly soluble weak acids become predictably soluble in basic media while poorly soluble weak bases become soluble in acid media. This phenomenon can be modeled mathematically in a form that relates solubility to pH and pKa.

No general treatment extending the solubility method of pKa determination to polyprotic species has been published. Moreover, all previously published pKa determinations by the solubility method have resulted in estimation of apparent pKa values (pKa′). Determination of the thermodynamic pKa by the solubility method has apparently been considered indeterminate (1, 4).

A general method is presented for obtaining estimates of macro pKa values (thermodynamic) of polyprotic, amphoteric compounds of limited solubility from pH and solubility measurements. The method is applied experimentally to two data sets: (*a*) tyrosine solubilities published previously and (*b*) 2,3-dihydroxyadenine solubilities measured in this laboratory. The equivalence of the solubility method is then investigated by comparing resulting pKa estimates to pKa values determined by other methods.

THEORETICAL

Let H_nS be a weak, polyvalent, sparingly soluble amphoteric compound that may acquire m additional hydrogens in its most acidic state $(H_{n+m}S)^{+m}$ and lose n hydrogens in its most basic state $(S)^{-n}$. At any given pH, its *apparent* equilibrium reaction may be as shown in Scheme I, with the net charge of each species indicated above it.

For clarity, the molecular and ionic species of the solute are abbreviated only as $S^{+m} \ldots S^{+1}$ and $S^{-1} \ldots S^{-n}$. The thermodynamic dissociation constant, K_a, for the dissociation of the amphoteric or molecular species, *i.e.*, the $m + 1$th hydrogen dissociation, is given by:

$$K_{a_{m+1}} = \{H\}[S^{-1}]f_{S^{-1}}/[S_0]f_{S_0} \qquad \text{(Eq. 1}a\text{)}$$

where $\{H\}$ is the hydrogen-ion activity; $[S^{-1}]$ is the concentration of S^{-1}; $[S_0]$ is the concentration of S_0, the solubility of the uncharged (or net charge equal to zero) species; and f_{S_0} and $f_{S^{-1}}$ are activity coefficients for S_0 and S^{-1} species, respectively. Solving Eq. 1*a* for $[S^{-1}]$ yields:

$$(H_{n+m}S)^{+m} \cdots \underset{}{\overset{K_{a_1}}{\rightleftharpoons}} (H_{n+2}S)^{+2} \overset{K_{a_{n-1}}}{\rightleftharpoons} (H_{n+1}S)^{+1} \overset{K_{a_m}}{\rightleftharpoons} (H_nS)^{0} \overset{K_{a_{m+1}}}{\rightleftharpoons} (H_{n-1}S)^{-1} \overset{K_{a_{m+2}}}{\rightleftharpoons} (H_{n-2}S)^{-2} \overset{K_{a_{m+n}}}{\rightleftharpoons} \cdots (S)^{-n}$$

$$\longleftarrow \downarrow \text{pH} \uparrow \longrightarrow$$

Scheme I

$$[S^{-1}] = K_{a_{m+1}}[S_0]f_{S_0}/\{H\}f_{S^{-1}} \quad \text{(Eq. 1}b\text{)}$$

The second hydrogen dissociation from the molecular or amphoteric species is given by:

$$K_{a_{m+2}} = \{H\}[S^{-2}]f_{S^{-2}}/[S^{-1}]f_{S^{-1}} \quad \text{(Eq. 2)}$$

Substituting Eq. 1*b* into Eq. 2 and canceling appropriate terms yield:

$$K_{a_{m+2}} = \{H\}^2[S^{-2}]f_{S^{-2}}/K_{a_{m+1}}[S_0]f_{S_0} \quad \text{(Eq. 3}a\text{)}$$

$$[S^{-2}] = K_{a_{m+1}}K_{a_{m+2}}[S_0]f_{S_0}/\{H\}^2f_{S^{-2}} \quad \text{(Eq. 3}b\text{)}$$

In general:

$$K_{a_{m+r}} = \{H\}^r[S^{-r}]f_{S^{-r}}/[S_0]f_{S_0}\prod_{i=m+1}^{m+r-1}(K_{a_i}) \quad \text{(Eq. 4}a\text{)}$$

$$[S^{-r}] = [S_0]f_{S_0}\prod_{i=m+1}^{m+r}(K_{a_i})/\{H\}^rf_{S^{-r}} \quad \text{(Eq. 4}b\text{)}$$

where r is the rth hydrogen dissociation, and numbering begins from the molecular or amphoteric form and increases toward the right in the dissociation scheme.

To convert Eq. 4*b* to contain the frequently used terms pH (= −log {H}) and pKa (= −log K_a), the $\log_{10}$ of each side of Eq. 4*b* is taken and pKa and pH are substituted:

$$\log_{10}[S^{-r}] = \log_{10}(S_0) + r\text{pH} - \sum_{i=m+1}^{m+r}(\text{pKa}_i) + \log_{10}(f_{S_0}/f_{S^{-r}}) \quad \text{(Eq. 4}c\text{)}$$

The antilog$_{10}$ of Eq. 4*c* yields:

$$[S^{-r}] = 10^{[\log_{10}(S_0)+r\text{pH}-\sum_{i=m+1}^{m+r}(\text{pKa}_i)+\log_{10}(f_{S_0}/f_{S^{-r}})]} \quad \text{(Eq. 5)}$$

Making the usual assignment of unity to f_{S_0} and bringing $\log_{10}$ (S_0) down out of the exponent result in:

$$[S^{-r}] = S_0 10^{[r\text{pH}-\sum_{i=m+1}^{m+r}(\text{pKa}_i)-\log_{10}f_{S^{-r}}]} \quad \text{(Eq. 6)}$$

Identical treatment of the dissociation constants for addition of m hydrogens yields:

$$[S^{+\upsilon}] = S_0 10^{[\sum_{i=m-\upsilon+1}^{m}(\text{pKa}_i)-\upsilon\text{pH}-\log_{10}f_{S^{+\upsilon}}]} \quad \text{(Eq. 7)}$$

where υ is the υth dissociation, numbering from the amphoteric form, S_0, to the left in Scheme I.

Now, at any given pH, the total concentration of the compound in solution, S, is given by:

$$S = [S_0] + \sum_{r=1}^{n}[S^{-r}] + \sum_{\upsilon=1}^{m}[S^{+\upsilon}] \quad \text{(Eq. 8)}$$

Inserting Eqs. 6 and 7 into 8, the generalized equation becomes (the brackets about all S species have been removed but *concentration* is understood):

$$S = S_0\left[1 + \sum_{r=1}^{n}10^{(r\text{pH}-\sum_{i=m+1}^{m+r}\text{pKa}_i-\log_{10}f_{S^{-r}})} + \sum_{\upsilon=1}^{m}10^{(\sum_{i=m-\upsilon+1}^{m}\text{pKa}_i-\upsilon\text{pH}-\log_{10}f_{S^{+\upsilon}})}\right] \quad \text{(Eq. 9)}$$

Equation 9 represents the generalized relationship for thermodynamic pKa values. The equivalent equation for apparent pKa′ values in which the activity terms have been set equal to unity is:

$$S = S_0'\left[1 + \sum_{r=1}^{n}10^{(r\text{pH}-\sum_{i=m+1}^{m+r}\text{pKa}_i')} + \sum_{\upsilon=1}^{m}10^{(\sum_{i=m-\upsilon+1}^{m}\text{pKa}_i'-\upsilon\text{pH})}\right] \quad \text{(Eq. 10)}$$

where S_0' is an estimate of the basal solubility when all activity terms are set equal to one.

Faced with Eqs. 9 and 10 and a set of solubilities at given pH values, it may not be intuitively clear which values of n and m specify the proper equations for fitting to get estimates of S_0', S_0, and the various apparent and thermodynamic pKa values. Moreover, it is desirable to use the simplest equation giving the best fit.

An easy solution to the problem involves making a preliminary plot of $\log_{10}$ ($S/S_0'' - 1$) *versus* pH:

$$\log_{10}(S/S_0'' - 1) = \log_{10}\left[\sum_{r=1}^{n}10^{(r\text{pH}-\sum_{i=m+1}^{m+r}\text{pKa}_i')} + \sum_{\upsilon=1}^{m}10^{(\sum_{i=m-\upsilon+1}^{m}\text{pKa}_i'-\upsilon\text{pH})}\right] \quad \text{(Eq. 11)}$$

where S_0'' is the minimum solubility experimentally.

Examination of the plot generally reveals one or several connecting straight lines. The slopes of individual and final segments reveal information relevant to the functional form to be used in the estimation procedures. This approach is equivalent to taking the first derivative of Eq. 11 and evaluating it within given pH intervals. The function (Eq. 11) is such that generally one or, at most, two terms of the equation make the major contribution to the value of $\log_{10}$ ($S/S_0'' - 1$) in any given pH region. Likewise, the straight-line portions result from only the predominant term. The derivatives thus obtained are integer functions of the number of associations or dissociations occurring, and their signs reflect the anionic or cationic nature of the reaction. Depending upon the proximity of pKa′ values, intermediate linear portions of the plot may exhibit slopes that change in serial integer fashion.

Furthermore, a more detailed study of this plot can yield preliminary estimates for apparent pKa values. Solving the terms of Eq. 11 representing each linear portion of the curve for its x-intercept yields expressions for pKa$_i$ values, i = 1 to m:

$$x_{\text{intercept}_j} = \sum_{i=j}^{m}\text{pKa}_i'/(m + 1 - j) \quad \text{(Eq. 12)}$$

and for pKa$_i$ values, $i = m + 1$ to $m + n$:

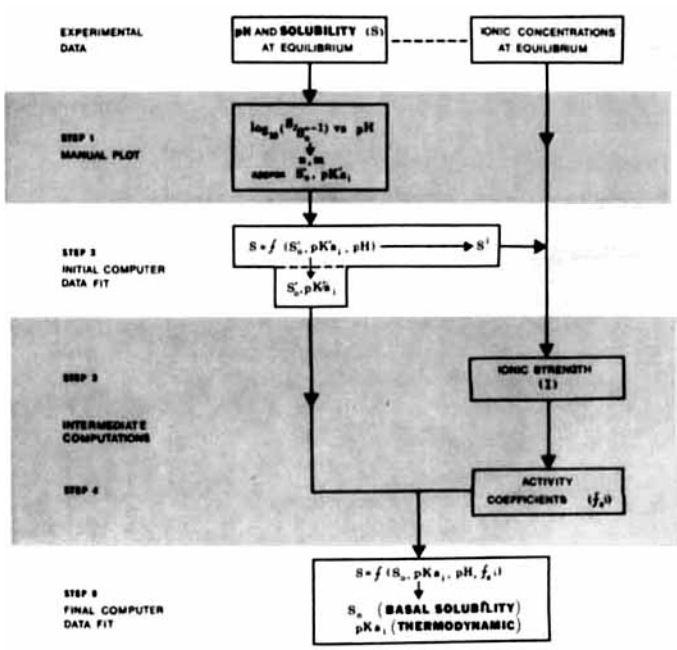
$$x_{\text{intercept}_j} = \sum_{i=m+1}^{j}\text{pKa}_i'/(j - m) \quad \text{(Eq. 13)}$$

where the x-intercept is the intercept on the pH axis of the projection of a given linear portion, numbering left to right from j = 1 to $j = n + m$.

Preliminary pKa$_i$ values are then found by solving the equations serially in order from the simpler to the more complex. Even though the accuracy of this procedure depends upon the pKa values being separated sufficiently to reveal definitive slopes, the general region of the pKa values should be evident even where they lie close together.

GENERAL METHOD AND APPLICATION

A flow diagram outlining the general method is shown in Scheme II. Given a suitable set of pH and solubility measurements, the basic approach is to estimate the preliminary basal solubility, S_0', and apparent pKa′ values from a nonlinear least-squares fit of the data to Eq. 10. The preliminary estimates contribute to the calculation of ionic strengths of the various solutions, which then allow computation of sample-specific



Scheme II

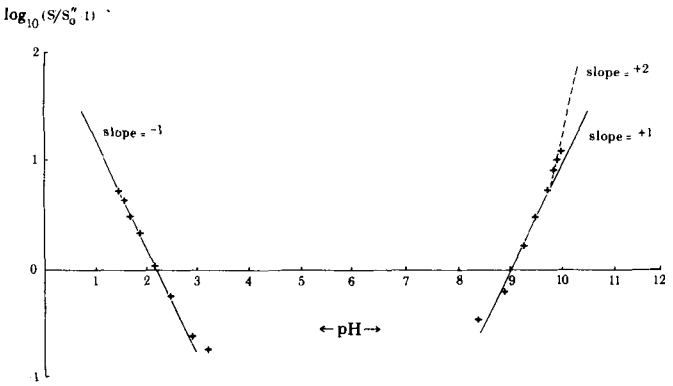


Figure 1—*Plot of* $\log_{10}(S/S_0'' - 1)$ versus *pH [Hitchcock's tyrosine data (5)]. The terminal slopes specify* m *and* n.

activity coefficients for the substance under study. Finally, the activities enter into a final fit of the data to Eq. 9, rendering estimates of the thermodynamic macrodissociation constants.

A step-by-step approach is given here, detailing the general method and applying this method to the tyrosine solubility measurements reported by Hitchcock (5). Briefly, Hitchcock added excess tyrosine to hydrochloric acid or sodium hydroxide solutions of varying starting concentrations, and saturated solutions were obtained by rotation at 25° for 1 or 2 days. After filtration, the concentration of tyrosine was obtained by Kjeldahl determination of nitrogen. The pH was measured with Clark electrodes at 25°. The data appear in Table I.

1. The first step is to specify the values of n and m, the highest valences of anions and cations existent at the upper and lower pH limits, respectively. Since this information may not be reasonably known *a priori*, n and m may be extracted from a visual analysis of the data by plotting $\log_{10}(S/S_0'' - 1)$ *versus* pH, S_0'' being the minimum solubility obtained by inspection of the data. This plot yields a curve from which n and m can be determined by inspection of terminal slopes. The terminal slope in the upper pH region determines n; expected slopes are multiples of 1. If a slope lies between two integers, the higher integer should be chosen. The value of m is determined in an identical fashion. Once n and m are substituted in Eqs. 9 and 10, the functional forms for the data-fitting process are specified.

When this procedure is applied to the tyrosine data, S_0'' is noted to be around 2.62 m*M* (Table I). A plot of $\log_{10}(S/S_0'' - 1)$ *versus* pH is shown in Fig. 1. The terminal slope in the lower pH region is −1, thereby establishing $m = 1$. The terminal slope at higher pH lies between +1 and +2, so $n = 2$.

From the same plot, preliminary estimates of the pKa values can be obtained to provide the nonlinear data-fitting procedure with starting points and limits. Figure 2 illustrates the procedure for gaining preliminary estimates of the apparent pKa′ values.

2. The next step is to estimate S_0' and the apparent pKa′ values. These values result from a nonlinear least-squares fit of the solubility and pH measurements to Eq. 10.

Table I—Tyrosine Data of Hitchcock (5)

$[HCl]_{initial}$, m*M*	$[Tyrosine]_{equilibrium}$, m*M*	$pH_{equilibrium}$
50.00	16.50	1.450
40.00	13.80	1.560
30.00	10.80	1.675
20.00	8.43	1.861
9.99	5.39	2.160
4.99	4.10	2.457
2.00	3.25	2.857
1.00	3.09	3.190
H_2O	2.62	5.300
$[NaOH]_{initial}$, m*M*		
0.98	3.54	8.342
1.95	4.30	8.865
4.88	7.06	9.249
9.76	10.70	9.484
19.50	17.50	9.726
29.90	24.70	9.841
39.80	30.40	9.881
49.80	35.80	9.953

Table II—Results of Solubility Method pKa Estimates for Tyrosine and Values Reported by Other Workers

Methods and Conditions	Apparent pK′ Values	Thermodynamic pK Values (Adjusted to 25° $I \simeq 0.00$)	Reference
Solubility, 25°, $I \leq 0.07$	$pKa_1' = 2.13$ $pKa_2' = 9.21$ $pKa_3' = 9.91$ $S_0' = 2.86\ \mu M$	$pKa_1 = 2.06$ $pKa_2 = 9.18$ $pKa_3 = 10.40$ $S_0 = 2.89\ \mu M$	This work
Potentiometry, 20°, I = 0.005	$pKa_1' = 2.20$ $pKa_2' = 9.19$ $pKa_3' = 10.43$	$pKa_1 = 2.11$ $pKa_2 = 9.12$ $pKa_3 = 10.43$	14
Spectrophotometry, 25°, I = 0.04	$pKa_2' = 9.12$ $pKa_3' = 10.28$	$pKa_2 = 9.19$ $pKa_3 = 10.47$	10
Spectrophotometry, 25°, I = 0.1	$pKa_2' = 8.95$ $pKa_3' = 10.08$	$pKa_2 = 9.04$ $pKa_3 = 10.34$	15

Substituting m and n for tyrosine into Eq. 10 yields:

$$S = S_0' [1 + 10^{(pH - pKa_2')} + 10^{(2pH - pKa_2' - pKa_3')} + 10^{(pKa_1' - pH)}] \quad \text{(Eq. 14)}$$

A nonlinear least-squares fit[1] of the data in Table I to Eq. 14 results in estimates for the preliminary basal solubility, S_0', and apparent pKa′ values shown in the upper part of Table II.

3. The ionic strength at each pH is next computed by summing the ionic concentrations of all ions present as follows (7):

$$I = 0.5 \left[\sum_{|i|=1}^{|p|} a_{|i|} Z_i^2 + \sum_{i=1}^{m} S^{+i} Z_i^2 + \sum_{i=1}^{n} S^{-i} Z_i^2 \right] \quad \text{(Eq. 15)}$$

where I is the ionic strength; $a_{|i|}$ is the ionic concentration of all non-S-ions at equilibrium; $|p|$ is the absolute value of the highest charged species present; $S^{\pm i}$ is the ionic concentration of all S-ions at each pH; m and n are the absolute values of highest cationic and anionic charges, respectively; and Z_i is the net charge.

Computation of ionic strength requires the ionic concentration of all ions present at equilibrium. These ions include the various tyrosine ions, which are computed from Eqs. 6 and 7 by using the S_0' and pKa′ values estimated and setting activity coefficients equal to unity; $[Na^+]$ and $[Cl^-]$ obtained from Table I; and, finally, $[H^+]$ and $[OH^-]$ computed from a mass balance estimation of hydrogen and hydroxide ions, taking into account those lost in forming the various tyrosine ions.

4. The ionic strength computations, I, allow for approximation of the activity coefficient, f_{S^i}, for ionic strengths below 0.1 according to the Debye–Hückel equation (25°) (8):

$$-\log_{10}(f_{S^i}) = \frac{0.5\, Z_i^2 \sqrt{I}}{1 + 0.329\, \mathring{A}_{S^i} \sqrt{I}} \quad \text{(Eq. 16)}$$

where f_{S^i} is the activity coefficient of the S-ion with charge i, $\mathring{A}_{S^i}$ is the

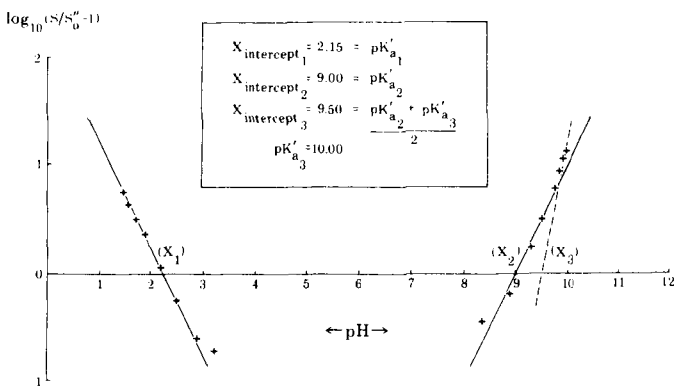


Figure 2—*Plot of* $\log_{10}(S/S_0'' - 1)$ versus *pH [tyrosine data of Hitchcock (5)]. The intercepts allow for preliminary estimates of pKa′ values.*

[1] Nonlinear Regression Program (6), modified by C. C. Peck and B. B. Barrett, Letterman Army Institute of Research, San Francisco, Calif., and implemented on a Hewlett-Packard 9830-A desktop calculator.

Table III—Comparison of Thermodynamic pKa Values for Tyrosine at 25° (Mean ± 1 *SD*)

Source	pKa_1	pKa_2	pKa_3
Published values[a]	2.11 (n = 1)	9.11 ± 0.08 (n = 3)	10.41 ± 0.07 (n = 3)
Solubility method (this paper)	2.06 ± 0.05	9.18 ± 0.06	10.40 ± 0.09

[a] Reported (12, 14, 15) as $-\log_{10}(\overline{X}_{K_{a_i}})$, where $\overline{X}_{K_{a_i}}$ = mean of published dissociation constants (K_{a_i}).

effective ionic radius in angstroms, and all other terms are as defined previously.

Approximate values for $Å_{Si}$ may be obtained from the data of Kielland (9) by choosing an ionic radius corresponding to an ion in his list whose dimensions are similar to those of the compound under study.

The effective ionic radius used in the activity calculations for tyrosine was 7.5 Å. This value is identical to that used by Martin *et al.* (10), who based their choice on Kielland's data (9). The validity of this choice is supported by the study of Edsall *et al.* (11). These investigators estimated the distance between the amino nitrogen and the hydroxyl hydrogen of tyrosine to be 6.95 Å using the Kirkwood–Westheimer equation and experimentally determined microdissociation constants. (Using Å = 6.95 leaves the final estimate for pK_1 and pK_2 unchanged and changes pK_3 to 10.42.)

5. Thermodynamic pKa values can now be estimated by substituting the activity coefficients obtained in Step 4 into Eq. 9 and performing a final nonlinear least-squares fit of the solubility data and pH measurements to Eq. 9.

In practice, Steps 1 and 2 are done as separate procedures; then Steps 3–5 can be performed in a single computer run. Thus, having obtained S_0' and the apparent pKa′ values, a repeat nonlinear least-squares fit of pH, solubility measurements, and activity estimates to Eq. 9 was carried out, resulting in the thermodynamic constants given in Table II.

A consideration of the type of measurement error may be important in the data-fitting process. Since the nonlinear model assumes an additive error in S, one should be confident that the type of measurement error in S emerges as an additive term. Such analytical work, where pipetting errors are carried through, for example, frequently results in a multiplicative type of measurement error. Such an error component can be rendered additive by taking the $\log_{10}$ of each solubility measurement. This approach was shown to be appropriate for a similar exercise by Krüger-Thiemer *et al.* (12). The $\log_{10}$ of both sides of Eqs. 9 and 10 must be taken in Steps 2 and 5 if such a procedure is followed. Alternatively, if the variance of the measurements is known, a weighted analysis can be performed (13). For example, a weight of the reciprocal of the square of the measured solubility is appropriate for a multiplicative error component.

In addition, Table II presents published pKa values for tyrosine that fulfill the criterion of an accurately reported ionic strength of the test solutions in which the total ionic strength was less than 0.1 (10, 14, 15). To exemplify exclusions, close examination of two papers (16, 17) indicated that the ionic strength values were underestimated by virtue of improper assignment of a charge of −1 to tyrosine in the pH region >9.0. Likewise, some pK_1 estimates (10, 17) were made in solutions of $I > 0.15$, rendering the Debye–Hückel approximation for activity in doubt.

Since the published values in Table II were apparent pKa′ values, some at 20°, adjustments were made to obtain thermodynamic pKa values at 25°. The temperature adjustment used was that suggested by Albert and Serjeant (1) based on the work of Hall and Sprinkle (18). Conversion to thermodynamic pKa from apparent pKa′ was made by correcting to zero ionic strength by means of activities computed from Eq. 16 (ionic radius = 7.5 Å), using the ionic strength reported in each paper.

Table III compares the pKa values estimated by the solubility method described here with the means of acceptable published values. The standard deviations about the values calculated by this method are those reported in the computer program. Caution should be exercised in interpreting confidence limits about the parameters (S_0 and pKa values) estimated in Step 5 by the nonlinear fitting procedure. Since the same solubility and pH measurements are used twice in arriving at final estimates of the thermodynamic pKa values, confidence regions would be expected to be broadened by a suitable reduction in the degrees of freedom that contribute to this computation. Apparently, however, no sound theoretical statistical basis currently exists to solve this problem. However, dispersion about the estimated parameters can be established by one or more complete duplications of the experiment.

Table IV—2,8-Dihydroxyadenine Solubility at Various pH Values (37°)

[2,8-Dihydroxyadenine], mg/liter	$pH_{equilibrium}$
603.58	0.018
581.42	0.022
131.60	0.533
137.91	0.535
138.10	0.536
134.09	0.562
129.31	0.573
125.29	0.579
79.55	1.164
80.60	1.175
10.51	2.280
10.40	2.250
1.47	5.08
1.55	5.08
1.43	5.08
1.51	7.06
1.49	7.06
40.49	9.468
46.61	9.615
50.99	9.672
65.80	9.737
68.86	9.748
62.46	9.777
79.55	9.811
76.12	9.825
93.78	9.901
86.33	9.902
117.18	9.971
168.09	10.011
139.43	10.073
140.39	10.087
347.92	10.196
467.29	10.378
3883.34	11.048
4187.80	11.211

EXPERIMENTAL

As a second test of the solubility method, three thermodynamic macrodissociation constants of 2,8-dihydroxyadenine were estimated by the solubility method and by UV spectrophotometry. All measurements relevant to the solubility method were made at 37°. UV absorption was measured at 25 or 37°. The pH was measured with a digital pH meter, and all pH values are reported as the final equilibrium pH. Buffers were made at ionic strengths of 0.1 and were adjusted with hydrochloric acid or sodium hydroxide and citrate and phosphate buffers.

Anhydrous 2,8-dihydroxyadenine was certified[2] to be 98.52% pure. 2,8-Dihydroxyadenine was assayed in aqueous buffer as follows. The final equilibrium sample was filtered through a 0.22-μm filter and diluted as necessary with a solution of the same buffer of similar pH. This prefinal solution was then diluted 1:1 with 6 *N* HCl, and the UV absorbance at 305 nm was read against an identical blank. The 2,8-dihydroxyadenine concentration was computed using the experimentally determined molar absorptivity of 17,130 for 2,8-dihydroxyadenine in 3 *N* HCl at 25°. The quantitative lower limit of sensitivity of this procedure is 0.1 mg/liter, and precision is ±3% for concentrations above 1 mg/liter.

The experimental procedure consisted of massively exceeding the 2,8-dihydroxyadenine solubility in test solutions by heating to 100° for 30 min followed by agitation at 37°. Agitation was continued for no less than 65 hr and for as long as 504 hr. The criterion for attainment of stable solubility for a given sample was a solubility change of less than 3% in three successive 24-hr checks.

RESULTS AND DISCUSSION

The pH and solubility data for 2,8-dihydroxyadenine appear in Table IV. The data analysis procedure for estimating thermodynamic macrodissociation constants by the solubility method was identical to that used for tyrosine, except that the nonlinear regressions were weighted by the reciprocal of the square of the 2,8-dihydroxyadenine concentration because of multiplicative errors due to dilution procedures as discussed previously. The results are presented in Table V.

The solubility method for determining aqueous pKa values was used with some frequency 30–50 years ago (5, 19–24) but only sporadically in

[2] Aldrich Chemical Co., Milwaukee, WI 53233.

Table V—Thermodynamic pKa Values for 2,8-Dihydroxyadenine (37°) (Mean ± 1 *SD*)

Method	pKa_1	pKa_2	pKa_3
Solubility	2.45 ± 0.06	8.12 ± 0.05	11.38 ± 0.16
Spectral	2.49[a]	8.09[a]	11.52[b]

[a] UV absorbance was measured at 25°; resulting pKa values were adjusted to 37° (1, 18). [b] All measurements were performed at 37°.

recent years (4, 12, 25, 26). In part, this change may be accounted for by the relative simplicity and ease of potentiometric and spectrophotometric methods. In addition to the awkward and complicated graphic solutions recommended by early protagonists of the solubility method, it may have been underutilized because it has never been generalized to estimation of thermodynamic pKa values for polyprotic species. Recently, one investigator asserted that a dibasic acid pKa could not be determined by the solubility method (4). Examination of his report, however, suggests no reason why it could not be accomplished using the method described here.

Krebs and Speakman (22) first provided a derivation of the fundamental solubility–dissociation equation in a form relating solubility to pH and pKa′, linearly and separately for monoprotic acids and bases. They correctly predicted the upward change in the terminal slope of the $\log_{10}(S/S_0' - 1)$ *versus* pH plot when a dibasic dissociation becomes operative. However, no generalized derivation was provided, nor was there serious suggestion of extension to dibasic species. They mentioned the problem of correcting the apparent pKa′ to zero ionic strength to obtain the thermodynamic pKa but made no comment on the proper correction for polyprotic species. The Krebs and Speakman approach, however, was useful for studying aqueous pKa′ values of a number of substances (4, 22, 25–27).

Krüger-Thiemer *et al.* (12) published a nonlinear solubility equation for amphoteric substances and suggested the use of a least-squares data fitting procedure for estimating S_0' and pKa′. They applied this approach to a number of monoprotic sulfonamides and found that it yielded pKa′ estimates in close agreement with values obtained by conventional methods. The advantage of this technique over a simple plotting routine is its provision of an automated estimation procedure. It is uniquely suited to cases of amphoteric and/or polyprotic compounds that cannot be fitted by a linear regression.

Krüger-Thiemer *et al.* (12) also reported the $\log_{10}$ form of Eq. 10 to eliminate inaccuracies in the estimation procedures produced by multiplicative errors. Since the tyrosine data of Hitchcock (5) were reported as means of two or more analyses, it was impossible to examine the data for the measurement error type. Therefore, the values from Table I were fit in both the transformed ($\log_{10}$) and untransformed manner. Final estimates by the transformed manner are as follows: S_0 = 2.81 m*M*, pKa_1 = 2.08, pKa_2 = 9.14, and pKa_3 = 10.47. The estimates thus obtained are approximately as close as the untransformed estimates are to the means for the published dissociation values (Table III). However, in this case, there is no compelling argument in favor of the $\log_{10}$ transform approach. Therefore, the values estimated by the untransformed equation remain the best estimates.

An extensive search of the world literature failed to reveal a general treatment of the solubility–dissociation relationships as described here. However, two reports that used solubility–pKa considerations should be mentioned. An equation that can be made equivalent to Eqs. 6 and 7 was included (without derivation) as part of a general discussion of solubility (28); it was not used to provide a basis for the determination of thermodynamic pKa values of polyprotic species. Levy and Rowland (29) proposed a pKa determination method that allows use of potentiometry in pH regions where precipitation of a sparingly soluble substance has occurred.

The validity of the solubility method for determining thermodynamic pKa values described here was tested on tyrosine solubility measurements published by Hitchcock (5) and on 2,8-dihydroxyadenine solubilities measured in this laboratory. Tables III and V indicate substantial agreement between solubility-determined thermodynamic pKa values and those determined by independent methods. All solubility-estimated pKa values fall within ±1 *SD* of the means of acceptable published values for tyrosine and are acceptably close to spectral pKa values in the dihydroxyadenine case.

The maximum number of pKa values of a given compound that can be determined by this method remains unexplored. There appears to be no limitation on the estimation of apparent pKa′ values beyond the requirement of chemical stability at the pH extremes. However, the ionic strength for such apparent pKa′ values would be impossible to summarize in a single number unless a swamping electrolyte was utilized. Accurate estimation of multiple thermodynamic pKa values would be limited by the inaccuracy of the Debye–Hückel activity approximation at ionic strengths greater than 0.1. Although activity approximations exist for ionic strengths greater than 0.1 (30), estimation of thermodynamic pKa values in this circumstance would be tenuous at best. Thus, where the initial pH is ≤1 or ≥13, the contribution of acid or base alone to ionic strength represents a limitation to this method. Moreover, the ionic strength contribution of other ions, especially those of the compound under study, shrinks these limits further.

In summary, a derivation of solubility, pH, and pKa (macroscopic) relationships is presented and is generalized to polyprotic, amphoteric species in aqueous solutions. A general method is provided for determining apparent and thermodynamic macrodissociation constants from a set of solubility and pH measurements using a data-fitting procedure. Finally, this approach is validated by estimating thermodynamic pKa values on two data sets. Solubility-estimated pKa values for tyrosine and 2,8-dihydroxyadenine compare favorably with those using independent methods.

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