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POTENTIOMETRIC DETERMINATION OF THERMODYNAMIC AND APPARENT DISSOCIATION CONSTANTS BY NONLINEAR LEAST SQUARES FITTING

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ABSTRACT

Potentiometric titration curve models have been developed for mono-, di- and triprotic weak electrolytes. Apparent dissociation constants or thermodynamic dissociation constants, corrected for ionic strengths of up to 0.5M, can be determined by nonlinear least squares fitting of potentiometric data to the models. A commercially available program, PCNONLIN (an IBM PC version of NONLIN84), was utilized for the fitting, allowing the models to be portable. The models have been tested with theoretical data containing random or systematic error, and data for several model compounds of various weak electrolyte class.

The method in this report has several advantages over traditional 'half neutralization' or graphical methods. First, the models are based on the analytic solution of the charge balance equation, with no simplifying approximations. Second, the method can determine dissociation constants in cases where no inflection point is present, such as for overlaping ionization processes or low concentration of compound. Finally, alterations in ionic strength during the titration were accounted for in the models.

INTRODUCTION

One of the most common and rapid methods for the determination of dissociation constants is potentiometric titration.[1-4] Alternate methods, such as spectroscopy, are generally less accurate, time consuming, or difficult to use for more complicated equilibria problems. They are usually reserved for cases where potentiometric titration is inappropriate, such as the determination of pKa's above 11 or below 2, or for compounds of low aqueous solubility.[1,3,5] The current availability of commercial automated potentiometric titrators has increased the speed and accuracy of titrations over traditional manual systems using a burette and pH meter. Most automatic titrators report a titration endpoint volume where the slope from a plot of pH versus titrant volume approaches infinity.[6,7] The endpoint represents the point where an equimolar equivalent of titrant has been added to a solution of the compound of interest.[8] This endpoint is typically calculated by numerically determining the point of maximum slope. The pKa is then calculated as the pH at one half of the titration endpoint volume (half neutralization).

Determining the pKa from the pH at half neutralization has three major pitfalls. First, it can be shown on the basis of electrical neutrality that when the pH equals the pKa, the number of equivalents of titrant only approximately equals one half of the number of equivalents of weak electrolyte.[9] This approximation ignores hydrogen ion and hydroxyl ion concentration, which become increasingly important at extremes of pH or low concentration of compound. The second problem involves the inability to detect titration endpoints when there is overlap of the ionization processes in polyprotic compounds. As many compounds of pharmaceutical importance are polyprotic, this is a serious shortcoming. Finally, ionic strength may vary dramatically during the titration due to the change in ionized species concentration if 'swamping electrolyte' is not included.

Analytic solutions which avoid the problems of determination at half neutralization have been derived for ionic equilibria problems in many fields.[2,4,10-12] However, before digital computers were readily

available, the solution of the ionic equilibria problems was limited primarily to data manipulation allowing a linear graphical representation[13-15] or approximation.[16] These methods are often time consuming, imprecise, and subjective.[11,17] Furthermore, no more than three equilibria constants may be solved simultaneously by a two dimensional graphic treatment.[11] Nonlinear least squares fitting of the analytic solution to equilibria problems using digital computers is thus a welcome alternative. A summary of published nonlinear least squares programs for the calculation of stability constants is given by Hartley.[17] However, there is a need for fitting algorithms which utilize commerically available nonlinear least squares fitting programs.

This report details a method for the determination of thermodynamic and apparent dissociation constants of mono- through triprotic weak electrolytes using nonlinear least squares fitting. A commercialy available program, PCNONLIN version V02 (an IBM PC version of NONLIN84, both from Statistical Consultants, Inc.), was utilized for the fitting, allowing the models to be portable. The method is general in that it allows calculation of pKa's for overlapping ionization processes, ampholytes and salts of weak electrolytes in addition to acids and bases, and a wide range of titrant counterion valence. The method is tested using both theoretical and experimental data.

EXPERIMENTAL

Materials

Malonic acid, beta-alanine, diethylenetriamine, piperazine, and 4-hydroxybenzoic acid were obtained from the Aldrich Chemical Company. Benzoic acid and succinic acid were received from the Eastman Kodak Company. Citric acid and sodium chloride were obtained from Mallinckrodt, Inc. Benzylamine was obtained from the Sigma Chemical Company. All solid chemicals were dried overnight in a vacuum oven (Sargent Welch Scientific Co., #1405 Vacuum Pump and Labline Instruments Inc., #3610 Oven) at room temperature prior to use. Titrant solutions were freshly prepared using standard volumetric

solutions (Acculute, Anachemia Chemicals, Inc.) and deionized water under an inert atmosphere. Pottasium hydrogen phthalate and pottasium carbonate were used to standardize (in triplicate) the NaOH and HCl titrant solutions, respectively.

Potentiometric Titration

The titration system consisted of a Metrohm 655 Multi-Dosimat and a 672 Titroprocessor (Metrohm Ltd.) at room temperature (approximately 23°C). Parameter settings on the Titroprocessor which affect the number and spacing of data points are titration rate and anticipation, which were set at 1 ml/min. and 50, respectively. The electrode was calibrated using certified buffer solutions (Fisher Chemical Company). Samples were prepared by adding preweighed (Cahn 31 Microbalance, Cahn Instruments, Inc.) or pipetted (Hamilton Company, 1, 10, and 50µl syringes) compound to purified water in the titration chamber. The solution was stirred by magnetic stirrer and bubbled by a constant flow of inert gas. A Metrohm 3.540.2210 PSM-1 serial interface was installed in the Titroprocessor, allowing the collection of data by an IBM PC. A PCNONLIN execution file containing the titration data was created using a simple BASIC program or a text editor. A typical execution file is shown in Appendix 2.

Theoretical

The development of the titration models used in this report was based on electrical neutrality of the solution, mass balance, and the appropriate equilibrium equations. Davies' empirical modification of the Debye-Huckel Equation[18] was used to estimate ionic strength (I). This approximation is appropriate at room temperature and for ionic strengths of less than 0.5M. Ionic strength corrections often require several iterations since the concentration of all ionic species must be calculated. These species concentrations are then included in the ionic strength calculation, which is used to update the apparent dissociation constant. Then the process continues. The PCNONLIN model used for the fitting of the potentiometric titration data for a triprotic weak electrolyte (model 13) is listed in Appendix 3. The models for mono-

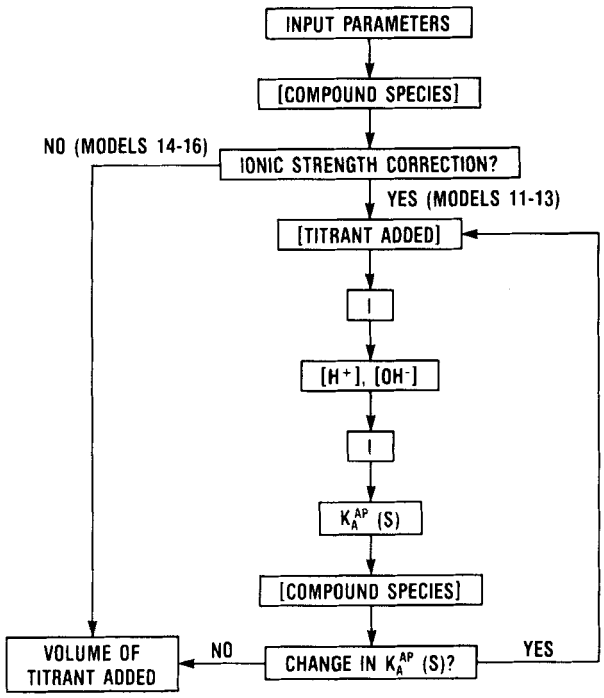

Figure 1. Algorithm of Titration Model Calculation

and diprotic compounds (models 11 and 12) have a similar structure. The models for the determination of apparent dissociation constants (models 14-16) do not include the loop found in models 11-13, and have activity coefficients of unity.

A brief schematic of the algorithm is shown in Figure 1. First, the concentration of compound species is calculated based on the present estimate of the thermodynamic dissociation constants (Ka^T), pH, and total concentration of compound. If no ionic strength correction is desired (Models 14-16), the program proceeds with the calculation of volume of titrant added. For cases with ionic strength corrections, the charge balance equation is rearranged to solve for the concentration of titrant added, [Ta]

Eq. 1 $$[Ta] = ([OH^-] - [H^+] - \Sigma(C_i z_i))/z_t$$

where z_t equals the valence of the titrant counterion (e.g., $z_t = 1$ for NaOH), and z_i and C_i are the valence and concentration of ionic species i. The ionic strength calculation includes compound species, counterions (including those due to added titrant), $[H^+]$, and $[OH^-]$. The first iteration corrects for the activity of H^+ and OH^- for added electrolyte only. $[H^+]$ and $[OH^-]$ are then recalculated using the estimated I from the previous step. Then I is calculated as described before, using the updated hydrogen and hydroxyl ion concentration terms. By calculating ionic strength twice inside the loop, the total number of iterations required can be decreased by up to one half. Apparent ('mixed') acid dissociation constants, $Ka^{Ap}(s)$, are calculated from $Ka^{T}(s)$ and the appropriate activity coefficients. The concentration of compound species are then recalculated based on the $Ka^{Ap}(s)$. The program loops to the step involving Equation 1 if there is a change in any of the Ka's greater than 1% (0.00432 change in pKa). In general, it is probably desirable to control ionic strength with 'swamping electrolyte'. This will allow calculation of Ka^{Ap} as well as Ka^{T}. Furthermore, computation time will decrease since fewer iterations will be necessary.

In the final step, the charge balance equation is rearranged so that all terms except titrant concentration are on one side. Each side of the equation is multiplied by the ratio of total volume (V_{total}) to the product of titrant concentration and titrant counterion valence. Thus, the volume of titrant delivered, Vt, is given by

$$\text{Eq. 2} \quad Vt = V_{total}([OH^-]-[H^+]-\Sigma(C_i z_i))/(C_t z_t)$$

where C_t refers to the concentration of the titrant solution. The models do not account for increases in volume due to the addition of titrant. Therefore, the titrant concentration should be at least 100 times the drug concentration so that the alteration of volume due to titrant addition will be minimal. Furthermore, the error due to dilution may be reduced by entering the volume of solution and compound concentration at 'half neutralization'.

A disadvantage of nonlinear least squares fitting compared to graphical methods is that initial estimates of the pKa's must be given. However, it is possible to obtain initial guesses based on endpoints reported by the Titroprocessor, predictions of pKa based on chemical structure,[19,20] or from graphical representation.[11,17] Once initial estimates are supplied, Equation 2 is fit (with respect to pH) using PCNONLIN, by minimizing the function shown in Equation 3.[21]

Eq. 3 $\Sigma[Vt(observed)-Vt(calculated)]^2$

By defining the sum of the squares of the differences in terms of an observed variable (and not a function of the variable), no weighting is necessary.[17,22]

RESULTS AND DISCUSSION

Theoretical Data

Theoretical data were used in order to test the ability of the models to fit data with overlapping ionization processes, systematic error, and random error. Data were generated using a Pascal program which uses essentially the same algorithm as in the present models. Data points (50) were generated from the theoretical initial pH to the pH after one extra equivalent of titrant was added, using 100 ml of 0.001M drug (acid) and 0.1M NaCl, and 0.1M NaOH as titrant. The relevant PCNONLIN parameters used were method 2 (modified Gauss-Newton algorithm), no parameter limits, convergence criteria of $1x10^{-4}$, and initial estimates of actual pKa minus 1. The maximum number of iterations required by PCNONLIN for this part of the study was 6. The effect of varying the initial guess by ± 1 unit appeared to have little or no difference on the final result (other than altering the required number of iterations).

Dibasic and tribasic acids with the following pKa's were modeled to observe the effect of varying overlap of ionization processes:

Diprotic: 5,9; 5.5,8.5; 6,8; 6.25,7.75; 6.5,7.5; 6.75,7.25
Triprotic: 4,7,10; 6,7,10; 5,7,9; 6,7,8

In all cases, the reported pKa was in agreement (± 0.0005) with the theoretical pKa. The 95% confidence intervals for the reported values were negligible. Thus delta pKa varying from 4 to 0.5 for dibasic acids and from 3 and 3 to 1 and 1 for tribasic acids can be analyzed with the models.

Data were also generated as above for a monobasic acid (pKa 7), a dibasic acid (pKa's 6 and 8), and a tribasic acid (pKa's 5,7 and 9), with the inclusion of up to 1, 5 or 10% random error in the volume of titrant added. The error in the reported pKa and the 95% confidence interval range are listed in Table I. In general, an increase in the random error caused an increase in the error of the reported pKa. The highest percentage of error in the pKa determination due to random error was 1.14%. In addition, there was an increase in the confidence interval range with an increasing percent of random error. The relative range of the confidence interval decreased within a given data set for pKa_1 through pKa_3 (the absolute range for each pKa was approximately the same). A typical plot of data with a 10% random error and the calculated fit is shown in Figure 2.

Systematic error may be introduced through an error in the calculated amount of drug present, the titrant concentration, the volume of titrant added, or the volume of the drug solution. The effect of systematic error was investigated by creating data for a monobasic acid (pKa 7), a dibasic acid (pKa's 6 and 8), and a tribasic acid (pKa's 5, 7 and 9), and entering an incorrect concentration (± 1 or 10%) in the PCNONLIN execution file. The errors in the pKa's for a given data set were found to be greatest for pKa_3, and smallest for pKa_1 (Table I). As the number of parameters being fit was increased, the error was found to increase. Much larger error (over 7% in one case) was observed from systematic error versus random error. Entering an incorrect total volume would also introduce a systematic error. The error introduced by volume error will be quantitatively similar to that from concentration error at intermediate pH, where $[OH^-]$ and $[H^+]$ are negligible compared to the sum (see Equation 2). The direction of deviation in Vt will be dependent on whether the compound is an acid or base. Figures 3 and 4 show the effect of 1 and

TABLE I

Error in Pka and Univariate 95% Confidence Internal (CI)
For Random and Systematic Errors In The Data‡

Theoretical Sample	Error*	Error in pKa (%)	95% CI (± %)
Monobasic acid, pKa = 7	+ 1%C	0.143	0.143
"	+ 10%C	1.86	0.982
"	-1%C	-0.143	0.143
"	-10%C	-1.86	1.31
"	1%R	0	0.143
"	5%R	0	0.429
"	10%R	-0.143	0.715
Dibasic acid, pKa = 6, 8	+ 1%C	0.167, 0.500	0.166, 0.124
"	+ 10%C	1.17, 4.75	1.98, 1.43
"	-1%C	-0.167, -0.500	0.334, 0.251
"	-10%C	-1.00, -4.37	2.69, 2.09
"	1%R	0, 0	0.167, 0.250
"	5%R	0.167, -0.250	0.832, 0.627
"	1%R	-0.500, 0.375	1.51, 1.12
Tribasic acid, pKa = 5, 7, 9	+ 1%C	0.200, 0.286, 0.667	0.399, 0.285, 0.110
"	+ 10%C	1.80, 3.43, 7.22	2.55, 1.80, 1.35
"	-1%C	-0.200, -0.286, -0.667	0.401, 0.143, 0.224
"	-10%C	-1.80, -3.43, -6.11	4.28, 3.11, 2.49
"	1%R	0, 0, 0	0.200, 0.286, 0.111
"	5%R	0, -0.286, 0.333	2.00, 1.29, 1.00
"	10%R	1.00, -1.14, 0.11	2.77, 2.02, 1.44

*C = entered concentration off by indicated percentage
R = up to indicated percentage random error in volume

‡ = listed in the following order: pKa_1, pKa_2, and pKa_3

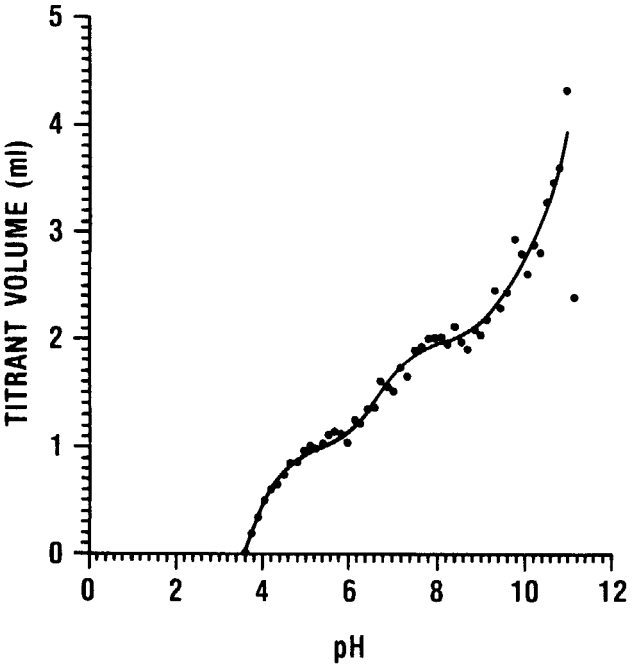

Figure 2. Theoretical titration with a 10% random error for 100ml of a 0.001M solution of a triprotic acid (pKa^T = 4, 7, 10) at an ionic strength of 0.1M. The line represents the nonlinear least squares fit.

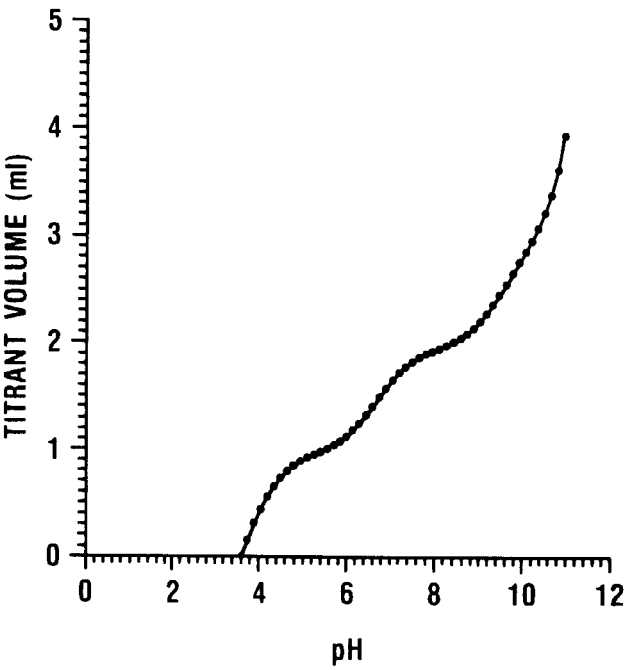

Figure 3. Theoretical titration with a 1% error in volume for 100ml of a 0.001M solution of a triprotic acid (pKa^T = 4, 7, 10) at an ionic strength

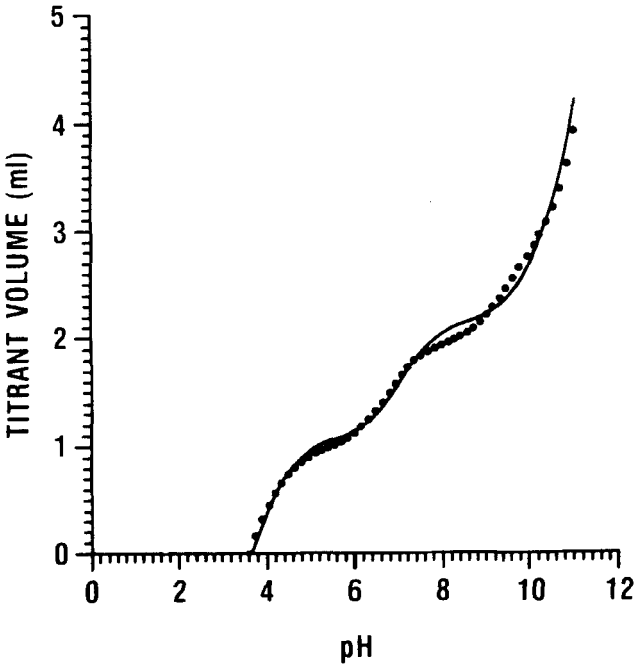

Figure 4. Theoretical titration with a 10% error in volume for 100 ml of a 0.001M solution of a triprotic acid (pKa^T = 4, 7, 10) at an ionic strength of 0.1M. The line represents the nonlinear least squares fit.

10% error in the entered volume for a tribasic acid. The importance of removing systematic errors from experiments can not be overemphasized. Tobias and Yasuda[23] have pointed out that least squares refinement procedures are best applied to systems with errors that are strictly random. Unfortunately, systematic errors are frequently present. They may be introduced by impurities in the compound, sorbed water, improper volumetric procedures, and incorrect weighing procedures. It is therefore important to plot the observed points, versus the calculated points and/or the residuals. Ifsystematic errors are present, a regular wave-like pattern should be observed in the residuals.

TABLE II

Conditions Used in Titration Experiments

Compound	Number of Points	Concentration (M)‡	Titrant Concentration (M)	Initial pKa Estimtes
Benzoic Acid	72	1.004×10^{-3}	9.474×10^{-2}	4
Succinic Acid	125*	3.018×10^{-4}	2.741×10^{-2}	4,5
Malonic Acid	90	9.562×10^{-3}	9.792×10^{-1}	3,5
p-Hydroxybenzoic Acid	62	2.958×10^{-4}	2.741×10^{-2}	4,5
Citric Acid	89	9.655×10^{-3}	9.792×10^{-1}	3,5,6
Benzylamine	123*	9.133×10^{-4}	1.008×10^{-1}	9
Piperazine	125*	3.291×10^{-4}	3.181×10^{-2}	5,10
Diethylenetriamine	117*	8.703×10^{-5}	1.267×10^{-2}	4,9,10
Beta-Alanine (HCl)	125*	3.039×10^{-4}	2.708×10^{-2}	4,10

* Data points from two separate titrations combined.
‡ Concentration at half neutralization.

Models 14-16, which give apparent dissociation constants, were also tested with the above theoretical data (I = 0.1M). The reported pKa^{Ap}'s were in agreement with the pKa^{T}'s (using Davies' Equation to estimate activity coefficients). Thus, it is possible to report apparent dissociation constants at a specified ionic strength.

Model Compounds

Model compounds were chosen to demonstrate the ability of the PCNONLIN models to fit data for mono-, di-, and triprotic acids and bases. Three dibasic acids were included so that the effect of varying the overlap of the two ionization processes (delta pKa from approximately 1.4 to 4.7) could be seen. Finally, the chloride salt of beta-alanine was titrated to show the usefulness of the models for salts or zwitterions. The experimental conditions are shown in Table II. The ionic strength of the solution was held constant using 0.1M NaCl, with

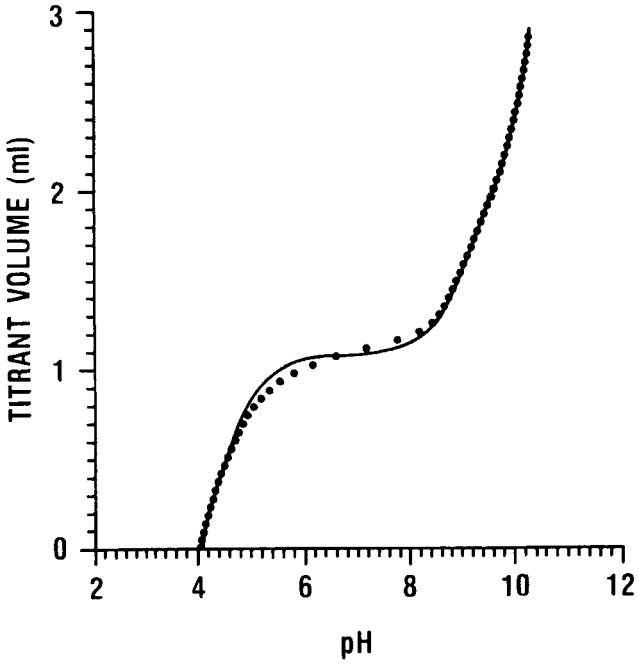

Figure 5. Observed potentiometric data for p-hydroxybenzoic acid (o) and the nonlinear least squares fit.

the exception of malonic acid and citric acid which were not controlled. The initial volume of all solutions was 100 ml, however, the volume at half neutralization was input for the fitting program. Approximately 1 extra equivalent of titrant was added in all experiments. PCNONLIN parameters were the same as those used in the Theoretical Data Section. The maximum number of iterations for this part of the study was 4.

Figure 5 shows a typical potentrometric titration curve and the nonlinear least squares fit. The pKa(s) determined by nonlinear least squares fitting are listed with literature pKa values in Table III. The literature values are taken from Perrin[24] and Kortum, Vogel, and Andrussow[25] for bases (and beta-alanine) and acids at 20-25°C, respectively. These authors critique literature pKa values and suggest

TABLE III

OBSERVED AND LITERATURE pK_a^T VALUES FOR MODEL COMPOUNDS

Compound	Literature $pK_a(s)$*	Observed $pK_a(s)$ (Univariate 95% CI)
Benzoic Acid	4.20 - 4.22	4.073 (0.64%)
Succinic Acid	4.22 5.64	4.012 (1.1%) 5.896 (0.76%)
Malonic Acid	2.85 5.69	2.873 (2.1%) 5.615 (1.2%)
p-Hydroxyben-zoic Acid	4.58 - 4.61 9.31	4.482 (0.83%) 9.575 (0.36%)
Citric Acid	3.14 4.77 6.39	3.166 (2.6%) 4.649 (1.8%) 6.172 (1.4%)
Benzylamine	9.33 - 9.35	9.297 (0.55%)
Piperazine	5.55 - 5.68 9.81 - 9.89	5.474 (0.51%) 9.584 (0.26%)
Diethylene-triamine	3.70 - 4.34 8.88 - 9.13 9.94	4.283 (1.8%) 8.926 (1.5%) 9.405 (1.6%)
Beta-Alanine (HCl)	3.551 10.238	3.259 (1.5%) 10.37 (0.29%)

* See Results and Discussion for source of values.

the 'best' thermodynamic value. These 'best' values are listed in Table III. If different sources are given equal weight, these values are indicated as a range. The difference between the observed and literature data is within 0.3 pH units with the exception of pKa_3 for diethylenetriamine. This agreement appears reasonable considering the fact that the literature values were determined using a broad range of data analysis techniques (including ionic strength corrections) and instrumental methods. 'Best' values from different sources often vary dramatically, as can be seen in Table III.

Ampholytes with overlapping ionization processes are slightly more difficult to titrate. It is necessary to first add one equimolar equivalent of acid (or base) to form the unprotonated species, A - (or fully

protonated species, H_2A^+). Then the titration is carried out in the opposite direction. It is necessary to include the initial addition of the acid (or base) in the various charge balance equations. This procedure was used for beta-alanine, although it is not necessary to do so in this case since delta pKa is approximately 7. With delta pKa of 3 or greater, it is probably simpler to titrate in each direction with two separate solutions.

Preliminary experiments for malonic acid and citric acid used solution concentrations of approximately 1 X 10^{-3}M and 1.0M NaCl to control ionic strength. With these conditions, the estimated pKa_1 for both compounds after 9-10 iterations was negative, with 95% confidence intervals covering several orders of magnitude. Albert and Serjeant[1] have suggested that it is necessary to have a -log concentration which is greater than the pKa for accurate potentiometric determination of pKa. At an ionic strength of 1.0M, the apparent pKa_1 for both compounds is well below 3. When the experiment was repeated at a concentration of approximately 1 x 10^{-2}M (and no added NaCl), the fitting program was able to determine pKa, with 95% confidence intervals of less than 3% (Table III). This example demonstrates the need of reporting confidence intervals for use in determining the reliability of the parameter estimation.

Two major advantages of the nonlinear least squares fitting method are obvious from the data in Table III. The method is able to fit compounds with overlapping ionization processes such as succinic acid, with reasonable 95% confidence intervals (approximately 1%). Furthermore, at concentration ranges used in this study, inflection points in the titration curves are often not detected for high (>10) or low (<4) pKa's. The absence of an obvious second endpoint for p-hydroxybenzoic acid (Figure 5) is such a case. In spite of the lack of an endpoint in the curve, the model was able to determine pKa_2 for p-hydroxybenzoic acid. Potentriometric titration methods which require an inflection point at the endpoint are often limited by the intrinsic solubility of the compound. The fitting method described in the report does not require an inflection point.

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REFERENCES

1. A. Albert and E.P. Serjeant, 'The Determination of Ionization Constants', Metheren, New York, Chapter 1.
2. G.H. Nancollas and M.B. Tomson, Pure and Appl. Chem., 54, 2675-2692 (1982).
3. L.Z. Benet and J.E. Goyan, J. Pharm. Sci., 56, 665-680 (1967).
4. K.A. Connors, 'Binding Constants: The Measurements of Molecular Complex Stability', Wiley, NY, 1987, Chapters 7 and 13.
5. G.A. Lewis, Int. J. Pharm., 18, 207-212 (1984)
6. A. Avdeef and J. Comer, American Laboratory, Feb. 1987, 116-125.
7. Metrohm 672 Titroprocessor Manual, Metrohm Ltd., CH-9100, Herisau, Switzerland.
8. This is an approximation. See L. Meites and J.A. Goldman, Anal. Chim. Acta, 29, 472-479 (1963).
9. Reference 1, Chapter 2.
10. J.N. Butler, 'Ionic Equilibrium: A Mathematical Approach', Addison-Wesley, Reading, Massachusettes, 1964, Chapter 3.
11. F.J.C. Rossotti and H. Rossotti, 'The Determination of Stability Constants and other Equilibrium Constants in Solution', McGraw-Hill, NY, 1961, Chapter 5.
12. F.R. Hartley, C. Burgess and R.M. Alcock, 'Solution Equilibria', Halstead, New York 1980, Chapter 3.
13. B.H.J. Hofstee, Science, 131, 39 (1960).
14. J.C. Speakman, J. Chem Soc., 855-859 (1940).
15. L.Z. Benet and J.E. Goyan, J. Pharm. Sci., 54, 1179-1182 (1965).
16. R.B. Martin, J. Phys. Chem., 75, 2657-2661 (1971).
17. Reference 12, Chapter 4 and 5.
18. Reference 10, Chapter 12.

19. D.D., Perrin, B. Dempsey, and E.P. Serjeant, 'pKa Prediction for Organic Acids and Bases', Chapman and Hall, London, 1981.
20. T.V. Parke and W.W. Davis, Analytical Chem., 26, 642-645 (1954).
21. Reference 4, Chapter 3.
22. I.G. Sayce, Talanta, 15, 1397-1411 (1968).
23. R.S. Tobias and M. Yasuda, Inorg. Chem., 2, 1307-1310 (1963).
24. D.D. Perrin, 'Dissociation Constants of Organic Bases in Aqueous Solution', Butterworths, London, 1965.
25. G. Kortum and W. Vogel, K. Andrussow, 'Dissociation Constants of Organic Acids in Aqueous Solution', Butterworths, London, 1961.

Appendix 1

Definition of the variables used in the models

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REMARK VOL: TOTAL VOLUME
REMARK CONC: CONCENTRATION OF COMPOUND
REMARK CT: CONCENTRATION OF TITRANT
REMARK SUMCZ2: SUM OF CiZi^2 (ADDITIONAL IONS)*
REMARK SUMCZ: SUM OF CiZi (ADDITIONAL IONS)*
REMARK ZA: VALENCE OF UNPROTONATED SPECIES
REMARK ZHA: VALENCE OF MONOPROTONATED SPECIES
REMARK ZH2A: VALENCE OF DIPROTONATED SPECIES
REMARK ZH3A: VALENCE OF TRIPROTONATED SPECIES
REMARK ZT: VALENCE OF TITRANT COUNTERION (E.G., NAOH = 1, HCL = -1, ETC.)
REMARK CTA: CONCENTRATION OF TITRANT ADDED
REMARK I: IONIC STRENGTH
REMARK Yi: ACTIVITY COEFFICIENT FOR SPECIES i
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* Additional ions refer to ions other than H^+, OH^-, or the compound of interest (e.g., sodium from NaA, NaOH, or NaCl).

Appendix 2

Typical PCNONLIN execution file for a triprotic acid.

```
REMA ----------
REMA SAMPLE.DAT
REMA ----------
MODEL 13,'TRI.LIB'
REMA Initial estimate for pKa1, pKa2, and pKa3
INIT  5.0 7.0 9.0
REMA Number of data points
NOBS 10
REMA CONSTANTS ARE VOL, CONC, CT, ZT, SUMCZ^2, SUMCZ, ZA, ZHA, ZH2A, ZH3A
CONS  100.0 0.001 0.1 1 0.2 0.0 -3  -2  -1  0
OUTPUT NAME IS 'PCN.FIT'
DATA
REMA pH Volume(ml) of titrant
 4.030  0.000
    .
    .
    .
10.890  4.008
BEGIN
FINISH
```

Appendix 3

Model for triprotic compounds, with ionic strength correction.

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MODEL 13
REMA ************************************************
REMA
REMA TRI.LIB
REMA W.J. LAMBERT 1987
REMA
REMA ************************************************
COMM
TITLE
TITRATION OF A TRIPROTIC COMPOUND
REMA 3 pKa's
NPAR 3
REMA 10 experimental constants
NCON 10
PNAMES 'PKA1' 'PKA2' 'PKA3'
END
TEMP
PH = X
VOL = CON(1)
CONC = CON(2)
CT = CON(3)
ZT = CON(4)
SUMCZ2 = CON(5)
SUMCZ = CON(6)
ZA = CON(7)
ZHA = CON(8)
ZH2A = CON(9)
ZH3A = CON(10)
PKA1 = P(1)
PKA2 = P(2)
PKA3 = P(3)
END
FUNC 1
I = SUMCZ2*0.5
YH = 10**(-0.51*(SQRT(I)/(1 + SQRT(I))-0.2*I))
KW = 1E-14
H = 10**(-PH)
KA1 = 10**(-PKA1)
KA2 = 10**(-PKA2)
KA3 = 10**(-PKA3)
DEN = KA1*KA2*KA3 + KA1*KA2*H + KA1*H*H + H*H*H
A = CONC*KA1*KA2*KA3/DEN
HA = CONC*H*KA1*KA2/DEN
H2A = CONC*H*H*KA1/DEN
H3A = CONC*H*H*H/DEN
LOOP:
OLDKA1 = KA1
OLDKA2 = KA2
OLDKA3 = KA3
CTA = (KW/(H*YH)-H/YH-SUMCZ-(A*ZA + HA*ZHA + H2A*ZH2A + H3A*ZH3A))/ZT
TEMP = (A*ZA*ZA + HA*ZHA*ZHA + H2A*ZH2A*ZH2A + H3A*ZH3A*ZH3A + SUMCZ2 + &
CTA* ZT*ZT)*0.5
I = TEMP + 0.5*(H/YH + KW/(H*YH))
YH = 10**(-0.51*(SQRT(I)/(1 + SQRT(I))-0.2*I))
```

Appendix 3 - Continued

```
I = TEMP + 0.5*(H/YH + KW/(H*YH))
YH3A = 10**(-0.51*ZH3A*ZH3A*(SQRT(I)/(1 + SQRT(I))-0.2*I))
YH2A = 10**(-0.51*ZH2A*ZH2A*(SQRT(I)/(1 + SQRT(I))-0.2*I))
YHA = 10**(-0.51*ZHA*ZHA*(SQRT(I)/(1 + SQRT(I))-0.2*I))
YA = 10**(-0.51*ZA*ZA*(SQRT(I)/(1 + SQRT(I))-0.2*I))
KA1 = 10**(-PKA1)*YH3A/YH2A
KA2 = 10**(-PKA2)*YH2A/YHA
KA3 = 10**(-PKA3)*YHA/YA
DEN = KA1*KA2*KA3 + KA1*KA2*H + KA1*H*H + H*H*H
A = CONC*KA1*KA2*KA3/DEN
HA = CONC*H*KA1*KA2/DEN
H2A = CONC*H*H*KA1/DEN
H3A = CONC*H*H*H/DEN
IF (ABS(KA2-OLDKA2) >OLDKA2*1E-2) THEN
GOTO LOOP
ENDIF
IF (ABS(KA1-OLDKA1) >OLDKA1*1E-2) OR (ABS(KA3-OLDKA3) >OLDKA3*1E-2) THEN
GOTO LOOP
ENDIF
F = VOL/(CT*ZT)*(KW/(H*YH)-H/YH-SUMCZ-(A*ZA + HA*ZHA + H2A*ZH2A + H3A*ZH 3A))
END
EOM
```