

# Nonlogarithmic Titration Curves for the Determination of Dissociation Constants and Purity

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Nonlogarithmic titration curves may be used to determine thermodynamic dissociation constants in addition to measuring the purity of the substance titrated and estimating the accuracy of the data. Equations are derived and methods proposed for carrying out these determinations with any monoprotic species (defined here as an acid or base with an independently dissociating hydrogen). A distinct advantage of this method is that dissociation constants may be determined for titrations that begin at a pH past half neutralization. Sample curves are presented to show the effect of incorrect measurement of pH. Methods are also given for the determination of the purity and dissociation constants of polyprotic species with overlapping pK's. The usefulness of the method is demonstrated by the calculated values reported for demethylchlortetracycline, 4-epi-anhydrotetracycline, 6-demethylgriseofulvin, phthalic acid, succinic acid, ethylenediamine, and 1,4-butane-diamine.

IN THE past few years several new pH meters have come into common use. These instruments are considerably more precise than earlier models and consequently make it possible to determine dissociation constants with greater accuracy than previously obtainable. The use of nonlogarithmic titration curves, as proposed here, will give highly accurate results in addition to a measure of the purity of the substance titrated and an estimate of the accuracy of the data.

A survey of the pharmaceutical literature shows that most dissociation constants are determined by methods which are modifications of that described by Parke and Davis (1). 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 (1). In addition to the curve for exactly 1 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 to correct for samples of unknown purity or molecular weight.

Garrett (2) has made an excellent 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 the error in the subtraction of volumes.

The authors would like to carry the argument one step further by proposing that there is little advantage in determining an apparent dissociation constant, when with the same amount of effort it is possible to calculate an accurate stoichiometric dissociation constant at a given ionic strength, or better still, a thermodynamic dissociation constant. In the present work all titrations were run at very low concentrations of titratable species at a relatively high ionic strength. Therefore, estimates of the activity coefficients give only a slight error in the determined thermodynamic dissociation constants.

## EXPERIMENTAL

**Materials.**—Demethylchlortetracycline·HCl and 4-epi-anhydrotetracycline·HCl were donated by Lederle Laboratories. Dr. Sidney Riegelman donated the sample of 6-demethylgriseofulvin. Reagent grade ethylenediamine·diHCl, 1,4-butane-diamine·diHCl, phthalic acid, and succinic acid were used. Carbonate-free potassium hydroxide

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was prepared by the method of Albert and Serjeant (3). Triple-distilled water was boiled for approximately 15 min. to remove carbon dioxide.

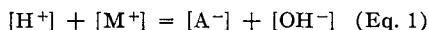
**Titration Procedures.**—One hundred and fifty milliliters of a dilute solution of an acid or a base (approximately  $5 \times 10^{-4} M$ ) was placed in a thermostated vessel ( $25 \pm 0.05^\circ$ ), and nitrogen was bubbled through the solution. The ionic strength was adjusted to 0.05 with KCl. Carbonate-free KOH solution (0.4405  $M$ ) was added from a 1-ml. microburet<sup>1</sup> which was calibrated to 0.002 ml. A Beckman research pH meter,<sup>2</sup> standardized against 0.05  $M$  potassium hydrogen phthalate (pH 4.010) and 0.01  $M$  sodium borate (pH 9.180), was used. In some cases 3 ml. of 0.020  $N$  HCl was added to the solution to increase the titration range.

## RESULTS AND DISCUSSION

**Monoprotic Species.**—Hofstee (4) has demonstrated the use of nonlogarithmic linear titration curves for the determination of the dissociation constant and the titration end point of a monoprotic species. He derived his equation on the basis of the electroneutrality of the solution as shown below. The authors have previously used nonlogarithmic curves for the determination of tetracycline dissociation constants and have derived the equations by using the buffer equation without approximations (5).

In using an electroneutrality equation (Eq. 1) it is necessary to know the ionic strength of the solution so that hydrogen and hydroxide ion activities may be converted to concentrations, since it is the sum of the concentrations of the various charged species which must balance.

Consider the titration of a weak acid HA with a strong base MOH.



where

$[H^+]$  = concentration of the given species

$[M^+]$  = concentration of MOH added

$[A^-]$  = concentration of conjugate base

Let  $Z$  equal the algebraic sum of all the known ionic concentrations with respect to Eq. 1 at any point in a titration:

$$Z = [H^+] + [M^+] - [OH^-] \quad (\text{Eq. 2})$$

Now  $[A^-]$  can be obtained by solving two simultaneous equations: Eq. 3, the definition for the stoichiometric dissociation constant<sup>3</sup> of a weak acid ( $K^c$ ), and Eq. 4, the conservation of mass equation, where  $A^0$  is the stoichiometric amount of acid (in all forms) added to the solution initially.

$$K^c[HA] - [H^+][A^-] = 0 \quad (\text{Eq. 3})$$

$$[HA] + [A^-] = A^0 \quad (\text{Eq. 4})$$

From the above equations

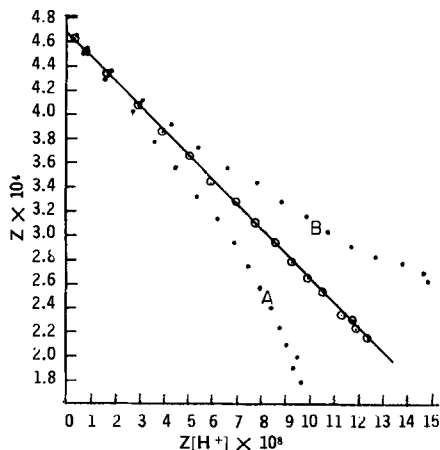


Fig. 1.—Plot of  $Z$  vs.  $Z[H^+]$  for demethylchlorotetracycline. The circled points are calculated from the actual titration.  $A^0 = 4.67 \times 10^{-4} M$ ,  $pK^c_1 = 3.30$ . Dotted curve A is a plot of the DMCT data assuming that the pH meter gave values which were all 0.03 units higher than those values used to calculate the points on the straight line. Dotted curve B is the same data assuming that all pH values were 0.03 units lower.

$$[A^-] = Z = K^c A^0 / [H^+] + K^c \quad (\text{Eq. 5})$$

Rearranging Eq. 5 gives

$$Z = A^0 - (1/K^c)Z[H^+] \quad (\text{Eq. 6})$$

Therefore, a plot of  $Z$  versus  $Z[H^+]$  should give a straight line with a slope of  $-1/K^c$  and an intercept of  $A^0$ .<sup>4</sup> Such a plot is illustrated by the solid line in Fig. 1 for demethylchlorotetracycline (DMCT). Although DMCT has three dissociable hydrogens, the first  $pK$  is sufficiently distant from the second and third so that we may analyze DMCT as if it were a monoprotic acid. From Fig. 1, the actual concentration of the titratable acid can be found from the intercept of the line with the ordinate. This was a valuable point of information since the exact number of waters of hydration attached to the molecule was unknown.

The line drawn in Fig. 1 also serves as an estimate of the accuracy of the data. Any point which deviates from the line immediately shows that the data at this point is invalid. If a calculation were made for the dissociation constant at each point using the buffer equation, all those points on the line would give the same value as would be calculated at half neutralization.

At the low concentrations of titratable species used in this work,  $[H^+]$  or  $[OH^-]$  will be significant values in  $Z$  below a pH of 5 and above a pH of 9. Therefore, a small error in standardization of the meter could affect the results obtained. Rather than being a drawback to the procedure, this is an advantage, since any small error will affect the linearity of the plot and thus draw attention to the fact that an error has been made. Dotted curve A in Fig. 1 is a plot for the DMCT data assuming that the pH meter gave values which were

<sup>1</sup> Gilmont Micropipet-Buret, Scientific Products, Evanston, Ill.

<sup>2</sup> Beckman Instruments, Inc., Fullerton, Calif.

<sup>3</sup> All dissociation constants referred to in this paper are acidic dissociation constants.

<sup>4</sup> If a weak base is titrated with a strong acid HX, Eq. 2 becomes  $Z = [OH^-] + [X^-] - [H^+]$  and Eq. 6 becomes  $Z = B^0 - K^c Z / [H^+]$ .

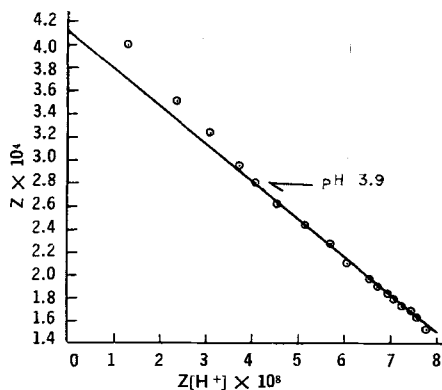


Fig. 2.—Plot of  $Z$  vs.  $Z[\text{H}^+]$  for 4-epi-anhydro-tetracycline.  $A^0 = 4.10 \times 10^{-4} M$ ,  $\text{pK}_1^{\text{T}} = 3.48$ . Deviation from the straight line occurs at pH 3.9 when the second dissociating hydrogen ( $\text{pK}_2^{\text{T}} 5.87$ ) begins to interfere.

all 0.03 units higher than those values used to calculate the points on the straight line. An error of this sort could be due to an incorrect buffer, or an error in standardizing the meter. Curve B is the same data assuming that all pH values were 0.03 units lower. These errors would not affect the calculation of  $A^0$ , however, since it is determined at the point where  $[\text{H}^+]$  approaches zero. If the same errors in standardization occurred in a titration carried out in the pH range 5–9, there would be no curvature in the line since  $[\text{H}^+]$  and  $[\text{OH}^-]$  would be negligible terms in  $Z$ , but the dissociation constant determined from this line would be incorrect by the same amount as the uniform pH error.

A curved line will also result when there are two overlapping dissociation constants. Occasionally a plot of  $Z$  versus  $Z[\text{H}^+]$  will be linear in the early part of the titration, and then begin to curve, as demonstrated for the titration of 4-epi-anhydro-tetracycline in Fig. 2. At low pH values the second hydrogen is not sufficiently dissociated to interfere with the linearity of the plot. However, when the pH approaches within two units of  $\text{pK}_2$ , deviation from linearity occurs, and this deviation increases with decreasing hydrogen ion concentration.

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. Titrations were also run on polyprotic species with overlapping  $\text{pK}$ 's to see if the method would be applicable in these cases.

**Polyprotic Species.**—At a pH which is two units greater than the first  $\text{pK}$  of a diprotic acid, less than 1% of the first dissociating hydrogen will still be bonded to the conjugate base. Therefore, a plot of  $Z$  versus  $Z[\text{H}^+]$  would become linear near this point as shown in Fig. 3, a titration of succinic acid.

The electroneutrality equation for the titration of a diprotic species such as succinic acid is:

$$[\text{M}^+] + [\text{H}^+] = [\text{HA}^-] + 2[\text{A}^{2-}] + [\text{OH}^-] \quad (\text{Eq. 7})$$

In this titration it will be assumed that a pH has

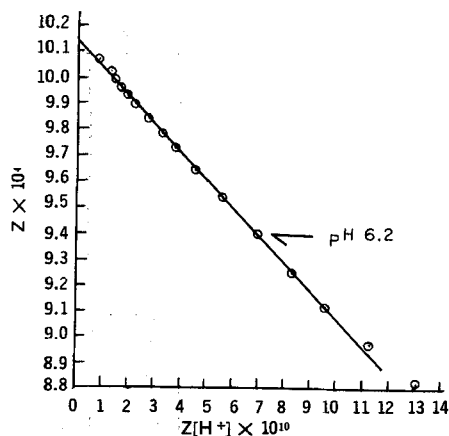


Fig. 3.—Plot of  $Z$  vs.  $Z[\text{H}^+]$  for succinic acid.  $2A^0 = 10.13 \times 10^{-4} M$ ,  $\text{pK}_1^{\text{T}} = 4.21$ ,  $\text{pK}_2 = 5.65$ .

been reached where the concentration of  $\text{H}_2\text{A}$  is negligible compared with  $[\text{HA}^-]$  and  $[\text{A}^{2-}]$ . In this case it is possible to solve two equations similar to Eqs. 3 and 4 for  $[\text{HA}^-]$  and  $[\text{A}^{2-}]$ . Substituting into Eq. 7

$$Z = \frac{[\text{H}^+]A^0}{K_2^c + [\text{H}^+]} + \frac{2K_2^cA^0}{K_2^c + [\text{H}^+]} \quad (\text{Eq. 8})$$

Rearranging

$$Z = 2A^0 + A^0[\text{H}^+]/K_2^c - (1/K_2^c)Z[\text{H}^+] \quad (\text{Eq. 9})$$

A plot of  $Z$  versus  $Z[\text{H}^+]$  is then drawn. As  $[\text{H}^+]$  decreases, the second and third terms on the right hand side of Eq. 9 become negligible, and the intercept of the line with the ordinate will be  $2A^0$ . Repeated titrations were run with succinic acid, phthalic acid, ethylenediamine· $\text{dHCl}$ , and 1,4-butanediamine· $\text{dHCl}$ , where the solutions were accurately prepared by one person, and the titrations and calculations were made by another. In 12 different titrations the values of  $A^0$  determined by graphical extrapolation were found to be on the average  $0.85 \pm 0.3\%$  higher than the measured concentrations. It is reasonable that the graphical  $A^0$  values should be slightly high since a plot of  $Z$  versus  $Z[\text{H}^+]$  would in reality have an intercept of  $(2A^0 + A^0[\text{H}^+]/K_2^c)$ , but in our graphs the second term was neglected by assuming that it will approach zero.

Although  $A^0$  may be obtained from Fig. 3, it is not possible to determine  $K_2$  from the slope of this line since the term  $A^0[\text{H}^+]/K_2^c$  will have a significant value at each point on the line other than the intercept. However, now that  $A^0$  is known, Eq. 9 may be rearranged to

$$Z = 2A^0 - (1/K_2^c)(Z - A^0)[\text{H}^+] \quad (\text{Eq. 10})$$

A plot of  $Z$  versus  $(Z - A^0)[\text{H}^+]$  will now give a straight line with a slope of  $-1/K_2^c$ . However, a graph of these functions would normally be superfluous since  $K_1^c$  cannot be easily determined graphically. Therefore, once  $A^0$  was found,  $K_1^c$  and  $K_2^c$  were determined by the method of Noyes (6) for overlapping dissociation constants.<sup>5</sup>

<sup>5</sup> Graphs of  $Z$  versus  $(Z - A^0)[\text{H}^+]$  were also plotted and  $K_2^c$  values calculated from the slope showed good agreement with values determined by the method of Noyes.

TABLE I.—THERMODYNAMIC DISSOCIATION CONSTANTS DETERMINED BY THE METHOD OF NONLOGARITHMIC TITRATION CURVES

Compd.	Calcd. Values <sup>a</sup>		Lit. Values <sup>b</sup>		Ref.
	pK <sup>T</sup> <sub>1</sub>	pK <sup>T</sup> <sub>2</sub>	pK <sup>T</sup> <sub>1</sub>	pK <sup>T</sup> <sub>2</sub>	
Demethylchlortetracycline·HCl	3.30	...	...	...	(5)
4-epi-Anhydrotetracycline·HCl	3.48	...	...	...	(5)
6-Demethylgriseofulvin	4.27	...	...	...	
Ethylenediamine·diHCl	6.85	9.84	6.79 (30°)	9.81 (30°)	(9, 10)
			7.00 (20°)	10.09 (20°)	
1,4-Butanediamine·diHCl	9.19	10.64	9.04 (30°)	10.50 (30°)	(9)
			9.35 (20°)	10.80 (20°)	
<i>o</i> -Phthalic acid	2.98	5.38	2.96	5.41	(11)
Succinic acid	4.21	5.65	4.207	5.636	(12)

<sup>a</sup> All values at 25°. pK<sup>T</sup> ± 0.03 for singly charged species; ±0.04 for doubly charged species. <sup>b</sup> Literature values reported at infinite dilution. All values at 25° unless noted.

**Activity Coefficients.**—All titrations in this work were run on low concentrations of acids and bases at a constant ionic strength so that an estimate of the activity coefficients of the ionic species could be made, and as a result thermodynamic dissociation constants could be determined. In the titrations reported in Table I the ionic size parameter in the extended Debye-Hückel equation was taken to be 5 Å. for all singly charged ionic species and 6 Å. for all doubly charged species. The activity coefficients used were those tabulated by Butler (7). The activity coefficients for hydrogen and hydroxide ions were calculated from an ion size parameter of 9 Å. for hydrogen ions and 3 Å. for hydroxide ions as estimated by Kielland (8). Using the above ion size parameters at an ionic strength of 0.05 or less, the maximum error due to incorrect activity coefficients will be 0.01 pK units for a singly charged species and about 0.02 for doubly charged organic molecules.

With the strength of titrant used in this work (0.4405 *M*), the effective pH range of the titration was from 2.7 to 11.3. However, at the extremes of the pH titration range [H<sup>+</sup>] and [OH<sup>-</sup>] become the significant terms in *Z*, and the accuracy of the determination depends on the ability of the pH meter to discriminate between very small pH differences. If the molarity of the titrant is increased so that a greater range of pH values may be reached, a concomitant increase is required in the concentration of the acid or base to be titrated. In such cases it would be impossible to report thermodynamic dissociation constants for a new compound unless titrations were run at a variety of ionic strengths and the values obtained extrapolated to infinite dilution.

One of the distinct advantages of nonlogarithmic linear titration curves is that an accurate dissociation constant may be determined, even though the titration may start at a pH beyond the point of half neutralization. In the titrations of the tetracyclines shown in Figs. 1 and 2, hydrochloric acid was added to the solution initially to decrease the pH below the pK. (In these cases *Z* = [H<sup>+</sup>] + [M<sup>+</sup>] - [OH<sup>-</sup>] - [Cl<sup>-</sup>].) Titrations of DMCT were also run without added HCl, in which case the

initial pH was 3.57 (0.27 pH units above the pK). The same results for the dissociation constant and the concentration were obtained.

### SUMMARY

It is proposed that nonlogarithmic titration curves be used for determining the dissociation constants of acids and bases since the method has the advantage of also giving the concentration of the substance being titrated along with a measure of the accuracy of the titration.

Methods and equations are presented for carrying out the titrations on monoprotic species. It is suggested that low concentrations of acids and bases be used at a constant ionic strength so that thermodynamic pK's may be estimated.

Possible variations of the curves from linearity are discussed in view of what situations may cause the deviation.

Methods are introduced for calculating the dissociation constants and concentration for a solution of a polyprotic species with overlapping pK's.

A table of dissociation constants calculated by the above method is presented along with a discussion of the accuracy and limitations of the method.

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