(6) C. I. Hong, G. L. Tritsch, A. Mittelman, P. Hebborn, and G. B. Chheda, *J. Med. Chem.*, **18**, 465 (1975).
(7) G. B. Chheda and A. Mittelman, *Biochem. Pharmacol.*, **21**, 27 (1972).
(8) C. I. Hong, G. B. Chheda, G. P. Murphy, and A. Mittelman, *ibid.*, **22**, 1927 (1973).
(9) A. Y. Divekar and M. T. Hakala, *Mol. Pharmacol.*, **7**, 663 (1971).
(10) G. A. LePage, S. R. Naik, S. B. Katakkar, and A. Khaliq, *Cancer Res.*, **35**, 3036 (1975).

ACKNOWLEDGMENTS

Supported by U.S. Public Health Service Grant CA 14185.
The authors thank Mr. C. F. Piskorz for technical assistance and Dr. E. D. Holyoke for interest and encouragement.

Differential Potentiometric Method for Determining Dissociation Constants of Very Slightly Water-Soluble Drugs Applied to the Sulfonamide Diuretic Chlorthalidone

H. L. J. FLEUREN ˣ, C. A. M. van GINNEKEN, and J. M. van ROSSUM

Received December 26, 1978, from the *Department of Pharmacology, University of Nijmegen, 6500 HB Nijmegen, The Netherlands.* Accepted for publication February 6, 1979.

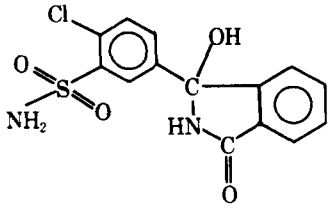
Abstract □ A renewed application of potentiometric acid–base titrations is described, by which dissociation constants of practically water-insoluble drugs can be measured accurately. The method uses the difference in the amount of titrant between a suitable aqueous solvent and a solution of the drug in that solvent. Such potentiometric difference titrations were conducted on a 3.7×10^{-4} *M* solution of chlorthalidone in 0.1 *M* aqueous KCl in the pH 3.5–10.6 range at 25°. Nonlinear least-squares regression analysis was applied to the data. From four determinations, a value of 9.24 ± 0.02 (mean ± *SEM*) resulted for the apparent dissociation constant of the first chlorthalidone acid group. The thermodynamic dissociation constant was calculated at pKa_1 = 9.35 (25°) by using a correction for activity.

Keyphrases □ Chlorthalidone—analysis, differential potentiometry, dissociation constants □ Potentiometry, differential—analysis, chlorthalidone dissociation constants, ibuprofen dissociation constant □ Ibuprofen—analysis, differential potentiometry, dissociation constant

A drug's dissociation constant is an important parameter in pharmacokinetic and pharmacodynamic investigations. Once the pKa is known, the degree of compound dissociation at physiological pH is derived easily; and since only unionized molecules generally pass readily across biological membranes (1), predictions concerning the access to tissue sites of interest can be made. The pKa also has value in the estimation of the lipiphilicity of a compound from its partitioning between an organic solvent and water; if the pKa is known, true partition coefficients can be calculated from the apparent values at any arbitrary pH.

BACKGROUND

Chlorthalidone[1] (I) contains two weakly acidic groups: one sulfonamide and one oxoisoindolin (acid amide) moiety. Although the latter was not expected to contribute to drug dissociation at acid or neutral pH, the former could influence back-resorption of the drug in the renal tubules when pH 8 urine is produced. In this situation, a pH-dependent urinary excretion should become evident. This aspect gains quantitative importance because normally ~70% of an available chlorthalidone dose is excreted unchanged in the urine (2). The present study was undertaken because no chlorthalidone pKa value was available from the literature.



I

Various methods to determine acid and base dissociation constants are used. They were comprehensively evaluated by Albert and Serjeant (3). These authors stated that both potentiometry and UV spectrophotometry produce accurate results, although the latter method is more laborious (3).

One premise for a conventional potentiometric titration is that the weak acid or base concentration must be so high that the amount of titrant needed to titrate the solvent is negligible in comparison with the amount necessary to protonate or deprotonate the dissolved compound. This condition could not be fulfilled at present, because maximum chlorthalidone solubility in neutral aqueous solutions was reported as ~0.12 mg/ml (4), equivalent to 3.45×10^{-4} *M*. In such a case, a difference titration can be conducted, provided that the amounts of strong acid and base, with which the solution of the compound with an unknown pKa and its blank (solvent alone) are titrated, are measured accurately.

Moreover, the pH measurement must be of high precision and very reproducible. To meet this requirement, use was made of automatic potentiometric titration equipment containing a precisely operating, motor-driven microburet and a high-resolution digital voltmeter connected to a low-drift pH meter. The apparatus was used previously to determine pKa values and numbers of titratable groups of purified proteins (5, 6).

EXPERIMENTAL

An aqueous solution of 0.1 *M* KCl served as the blank. A saturated chlorthalidone solution in this solvent was prepared by vigorously stirring ~8 mg of the crystalline drug in 50 ml of solvent at room temperature overnight. After filtration, the concentration, determined in triplicate by GLC (7), was 125.2 ± 0.7 μg/ml (mean ± *SD*), equivalent to 3.695×10^{-4} *M*. Aqueous hydrochloric acid and sodium hydroxide solutions, 0.0604 *M*, were diluted by weighing with four volumes of distilled water and bubbled with nitrogen gas to remove carbon dioxide.

The titrant was added stepwise, using a microburet[2], at 0.01 ml/min with an accuracy of ±0.1%. The titrations were conducted on the chlor-

[1] Hygroton, Ciba-Geigy, Basel, Switzerland.

[2] Metrohm, Zürich, Switzerland.

0022-3549/79/0800-1056$01.00/0

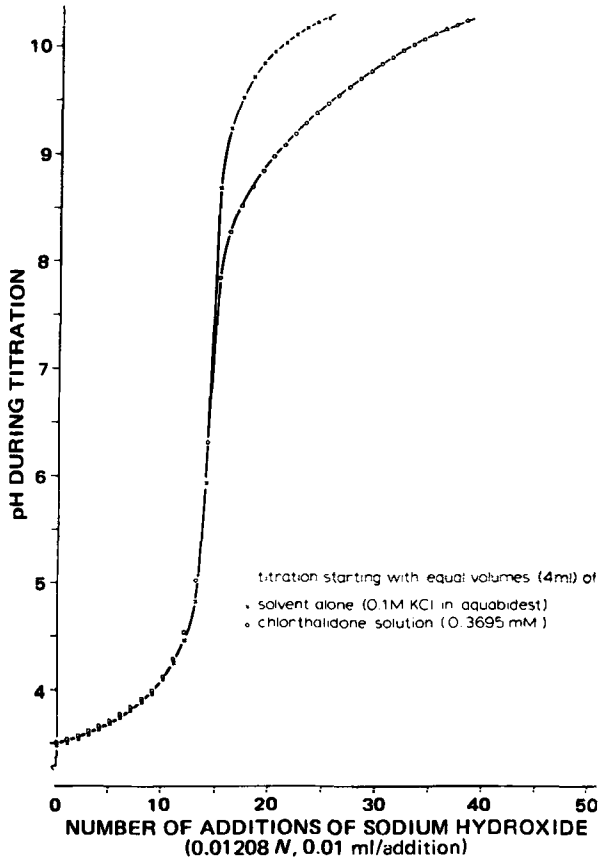


Figure 1—*Acid-base titration of the very slightly water-soluble drug chlorthalidone by measurement of the difference in titrant between samples with and without the substance.*

thalidone solution (4.00 ml) and on the solvent alone (4.00 ml), at 25° and under nitrogen, in both directions of the pH 3.5–10.6 range. Two runs with hydrochloric acid (Experiments B and D) and two with sodium hydroxide (Experiments A and C) were carried out. The actual pH ranges were 3.47–10.28 in A, 8.55–10.58 in B, 8.10–10.50 in C, and 6.61–10.45 in D. A pH meter[3] in combination with a glass[4] and a calomel[5] electrode was used.

A high-resolution digital voltmeter[6] was connected with the pH meter. The pH was measured 10 times after each addition of hydrochloric acid or sodium hydroxide, and the mean of these readings was taken. In this way, very reproducible values, including the third decimal figure, were obtained (8). Detailed descriptions of this automatic potentiometric equipment were published (6, 8). To calibrate the pH meter, phthalate buffer (pH 4.008) and phosphate buffer (pH 6.865) were used.

RESULTS AND DISCUSSION

Typical titration curves are shown in Fig. 1. Equal volumes of 0.1 *M* KCl, with and without chlorthalidone, were titrated with dilute sodium hydroxide from pH 3.5 to 10.3. A larger number of additions of alkali was needed to increase the pH of the drug solution in the region where the drug becomes deprotonated. The difference at each pH value between the number of additions of the two titration runs clearly represents the quantity necessary for titration of the drug itself. When these differences are plotted *versus* the pH, a difference titration curve is obtained. For a weak acid, the titration curve obeys the familiar Henderson–Hasselbalch equation:

$$\mathrm{pH} = \mathrm{pKa}' + \log\frac{\alpha}{1-\alpha} \qquad \text{(Eq. 1)}$$

where α represents the degree of acid group dissociation and pKa′ is its apparent dissociation constant, which becomes equal to the thermodynamic dissociation constant, pKa, at infinite dilution. At higher ionic strength, the following relationship is valid:

$$\mathrm{pKa} = \mathrm{pKa}' - \log\frac{\gamma A^-}{\gamma HA} \qquad \text{(Eq. 2)}$$

in which γA^- and γHA are the activity coefficients of the ionized and unionized forms of the acid, respectively. The activity coefficients can be calculated according to the Debye-Hückel equation (3); for log $\gamma A^-/\gamma HA$, a value of −0.11 was obtained. By using this correction, the pKa_1 is readily obtained if the experimental pKa_1' value is known.

When a strong base is added, an equivalent amount of protons is lost by the weak acid. Then α can be written as $\alpha = Z/A$, where Z represents the amount of strong base added at any moment of the titration and A is the amount added at the titration end-point. Polyacid titration produces the relation:

$$Z = \sum_{i=1}^{n} A_i\alpha_i \qquad \text{(Eq. 3)}$$

where i denotes the identity of a specific weak acid group and n is the total number of classes of weak acid groups.

Chlorthalidone contains two acidic moieties, one sulfonamide and one oxoisoindolin group, potentially dissociating in the pH range under study. Therefore, $A_1 = A_2$ and:

$$Z = A_1(\alpha_1 + \alpha_2) \qquad \text{(Eq. 4)}$$

By applying Eq. 1, one can write:

$$Z = A_1\left(\frac{10^{\mathrm{pH}-\mathrm{pKa}_1'}}{1+10^{\mathrm{pH}-\mathrm{pKa}_1'}} + \frac{10^{\mathrm{pH}-\mathrm{pKa}_2'}}{1+10^{\mathrm{pH}-\mathrm{pKa}_2'}}\right) \qquad \text{(Eq. 5)}$$

The titration data were analyzed by means of a nonlinear least-squares regression analysis program[7]. In every curve-fitting procedure, the correct assignment of independent and dependent variables is essential to obtaining unbiased estimates of the unknown parameters (9). The pH was chosen as the independent variable, because its measurement was highly reproducible (see *Experimental*), such that there existed, apart from a potential calibration error that could influence only the absolute pH scale, a very small error within each experiment. For the dependent variable Z, a constant error of 0.1, expressed as additions of strong base, was taken since this value was the read-off uncertainty of the differences between titration curves of the chlorthalidone solution and the blank (Fig. 1).

At first, the data points were fitted according to the first term of Eq. 5 only, with the assumption that the dissociation of the second acid group was negligible (if pH ≪ pKa_2', the second term approximates zero). Much better fits were obtained when the second term also was considered. For this purpose, the value for pKa_2' was not left free in the least-squares regression analysis, because too few data points were available in the high pH region, only up to pH 10.6, which would introduce large errors in the estimates of the other parameters, A_1 and pKa_1'. Preset values of pKa_2' were systemically changed until the minimum of the sum of weighed

Table I—Superiority of Computer Fits of Difference Titration Curves of Chlorthalidone when the Dissociation of Two Weak Acid Groups Instead of One Is Included[a]

Experiment	Two Titratable Groups Involved in Curve Fitting				One Titratable Group Involved in Curve Fitting		
	pKa_1'	A_1	k_2	χ^2	pKa_1'	A_1	χ^2
A	9.228	12.15	10.94	12.6	9.35	14.43	96.6
B	9.287	12.33	11.11	14.3	9.56	14.16	210
C	9.210	13.27	11.55	8.1	9.27	14.17	67.5
D	9.235	12.67	11.95	6.1	9.26	12.96	11.1
Mean ± *SEM* (n = 4)	9.24 ± 0.02						

[a] pKa_1' = apparent first dissociation constant, A_1 = total amount of base at first titration end-point, k_2 = optimally fixed value for the dissociation of the second acid group, and χ^2 = sum of weighed squared deviations of the experimental data from the fitted curve. The χ^2 (5%) value, the tabulated level of significance at p = 0.05, was 39, 44, 40, and 37 in Experiments A, B, C, and D, respectively. The thermodynamic dissociation constant, pKa_1, is larger than the pKa_1' by a factor of 0.11 due to the contribution of activity coefficients. Therefore, pKa_1 = 9.35 ± 0.02 (mean ± *SEM*).

[3] Type PHM 26, Radiometer, Copenhagen, Denmark.
[4] Type G202B, Radiometer, Copenhagen, Denmark.
[5] Type K401, Radiometer, Copenhagen, Denmark.
[6] Type LM 1867, Solartron, Peekel, Rotterdam, The Netherlands.

[7] Formfit, a digital computer program in use at the Computer Center of the University of Nijmegen. Details are available upon request.

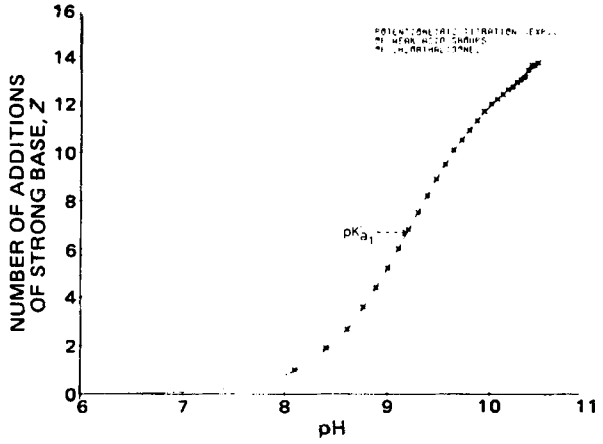


Figure 2—*Computer-fitted difference titration curve of chlorthalidone. From four experiments, the following estimates resulted for* pKa_1': *9.228 (A), 9.287 (B), 9.210 (C), and 9.235 (D), yielding a mean apparent dissociation constant of 9.24 ± 0.02 (±SEM) at 0.1 ionic strength and 25°.*

squared deviations was found. The results of these calculations are included in Table I (pKa_2' has been denoted k_2).

For A_1, the best estimate was sought by the fitting procedure. When this parameter was fixed at its theoretically calculated value (12.24 additions of strong base), it influenced the pKa_1' estimate, with possible errors in the concentration and pipetted volume of the chlorthalidone solution and in the normality and volume of the titrant.

Figure 2 shows a typical difference titration curve, fitted according to Eq. 5 (at fixed optimal pKa_2' values). The decision in favor of such a fit over that based on ionization of only one acid group was made using several criteria: (*a*) visual plot inspection, (*b*) symmetry of the residuals around the fitted curves, and (*c*) calculation of the sum of weighed squared residuals (as χ^2). Table I compares the χ^2 values for both procedures and indicates the superiority of curve fitting according to two titratable groups in all four experiments. In addition, the scatter of the pKa_1' estimates in the left part is much smaller than that in the right part. Also, the A_1 estimates at the left correspond better with the value calculated on the basis of total chlorthalidone in the titration vessel (equivalent to 12.24 additions of strong base), indicating that actually one proton-donor group was dissociated completely at this stage.

The variance of the four pKa_1' estimates exceeds the within-experiment error (the computer-estimated relative errors ranged from 0.0 to 0.1%). This finding points to minor systematic differences between the four experiments caused, for example, by the pH meter calibration. However, the differences are small enough that the pKa_1' (25°) = 9.24 ± 0.02 (mean ± *SEM*) can be treated as a reliable operational ionization constant, suitable for use as a physicochemical parameter in pharmacological studies. When the correction for ionic strength is carried out with Eq. 2, the thermodynamic dissociation constant is calculated at pKa = 9.35 (25°).

Determination of very slightly water-soluble drug dissociation constants appears to be convenient and reproducible by the method described, and no corrections for titrant consumption by the solvent are needed. The technique was applied also to the analgesic ibuprofen[8] in the laboratory (10), indicating that potentiometric difference titrations can be conducted in a wide area of pharmaceutical substances.

REFERENCES

(1) A. Goldstein, L. Aronow, and S. Kalman, "Principles of Drug Action," 2nd ed., Wiley, New York, N.Y., 1974.

(2) H. L. J. Fleuren, T. A. Thien, C. P. W. Verwey-van Wissen, and J. M. van Rossum, *Eur. J. Clin. Pharmacol.*, **15**, 35 (1979).

(3) A. Adrien and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, England, 1962, pp. 10, 58.

(4) E. G. Stenger, H. Wirz, and R. Pulver, *Schweiz. Med. Wochenschr.*, **89**, 1126 (1959).

(5) S. H. de Bruin, L. H. M. Janssen, and G. A. J. van Os, *Biochim. Biophys. Acta*, **188**, 207 (1969).

(6) L. H. M. Janssen, S. H. de Bruin, and G. A. J. van Os, *ibid.*, **221**, 214 (1970).

(7) H. L. J. Fleuren and J. M. van Rossum, *J. Chromatogr.*, **152**, 41 (1978).

(8) G. A. J. van Os, S. H. de Bruin, and L. H. M. Janssen, *J. Electroanal. Chem.*, **37**, 303 (1972).

(9) J. H. Perrin, J. J. Vallner, and S. Wold, *Biochim. Biophys. Acta*, **371**, 482 (1974).

(10) C. A. M. van Ginneken, Ph.D. thesis, University of Nijmegen, Nijmegen, The Netherlands, 1976, p. 93.

ACKNOWLEDGMENTS

Supported by a grant from the Netherlands Organization for the Advancement of Pure Research (ZWO/FUNGO).

The technical assistance of Mr. J. J. M. Joordens, Miss C. W. J. Prinse, and Ir. G. G. M. van Beek as well as the discussion by Dr. S. H. de Bruin (all members of the Department of Biophysical Chemistry, University of Nijmegen, Nijmegen, The Netherlands) is greatly appreciated. The authors also thank Mrs. C. P. W. Verwey-van Wissen for assistance, Mrs. E. L. Klok-Huyser for skillful operation of the Formfit computer program, and Mrs. M. P. M. Klumpkens-Janssen for typing the manuscript.

[8] Preliminary estimate of pKa ~ 4.4.