

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

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The method of Benet and Goyan for the determination of purity and ionization constant of a weak acid or base was investigated and found to work well. The general equation was modified in order to extend its applicability to titrations involving a volume change. The question of the correct type of activity coefficient to be used for calculating the data was considered, and some experimental evidence presented to indicate that the results are not markedly influenced by the one employed.

IN RECENT publications (1, 2) Benet and Goyan have presented a technique for determining both the purity and the dissociation constant of a weak acid or base. This procedure is based on a non-logarithmic linear plot of titration data. In these laboratories the authors use their method routinely, and have found it to be an excellent one. It is felt, however, that two points in the method warrant further discussion. The first is that the equation presented by Benet and Goyan assumes no volume change occurring during the titration. The second point is concerned with the correct activity coefficient to be used in the computations. Therefore, the basic equation has been rederived to make it generally applicable to all titrations, whether or not a volume change occurs. In addition, studies have been performed in an attempt to decide upon the correct type of activity coefficient to be used in the calculations.

## EXPERIMENTAL

All pH measurements were made on the expanded scale of a Radiometer model 25 SE meter equipped with an A. H. Thomas 4855-B 10 glass electrode and a Corning fiber-type calomel electrode. Samples were prepared by adding to the titration vessel 100 ml. of nitrogen-sparged, triple distilled, pyrogen-free water; 2 ml. of 0.0953 *N* HCl solution (where necessary); sufficient 2 *M* KCl solution to obtain the desired ionic strength; and the sample to be titrated. Blank solutions were prepared identically. Titrations were performed under nitrogen in a water-jacketed vessel, the temperature of which was  $25 \pm 0.1^\circ$ . A 5-ml. semimicro buret was employed; the titrant was 0.1008 *N* KOH solution standardized by titration with potassium acid phthalate, and the 1 *N* acetic acid was Fisher certified reagent. All linear relationships were calculated by the method of least squares.

## DISCUSSION

**Modification of the Equation.**—The following equation was developed by Benet and Goyan (2) for the titration of an acid HA with a strong base MOH:

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

where

$Z$  = concentration of conjugate base formed during titration =  $[\text{H}^+] + [\text{M}^+] - [\text{OH}^-]$ ,

$[\text{M}^+]$  = concentration of  $\text{M}^+$  ion in solution from addition of MOH,  
 $A^0$  = concentration of all acid species in solution =  $[\text{HA}] + [\text{A}^-]$ , and  
 $K^c$  = apparent ionization constant of acid HA.

Upon first examination, Eq. 1 appears to be a linear function. Therefore a plot of  $Z$  versus  $Z/[\text{H}^+]$  should be a straight line with a slope of  $-1/K^c$ , and an intercept of  $A^0$ , from which value the purity may be determined. On further consideration, however, it is apparent that this is true only when the total volume change, which occurs during a titration, is considered negligible. Since  $A^0$  represents the sum of all forms of HA present in solution at any time, a volume increase, which results by addition of titrant, will cause  $A^0$  to vary from the beginning to the end of the titration. Therefore in cases where the titration volume cannot be considered constant, Eq. 1 does not represent a linear function. Although it is recognized that Benet and Goyan were able to consider their volume changes negligible, in this work a volume increase of 2–5% is not uncommon. Therefore, the authors felt it desirable to alter Eq. 1 so as to circumvent any error that may be introduced by a volume change.

If one were to consider the system in terms of absolute amount of material (number of moles) in the titration vessel rather than concentration (moles/liter), the equation can be rederived to make it independent of volume change. For the titration of an acid HA with a strong base MOH, the equation of electroneutrality may be written in terms of absolute molar quantities rather than concentration units.



where

$\text{H}^+$  = absolute numbers of moles of hydrogen ion present in solution,  
 $\text{M}^+$  = absolute number of moles of base added to the solution,  
 $\text{A}^-$  = absolute number of moles of conjugate base present in solution, and  
 $\text{OH}^-$  = absolute number of moles of hydroxyl ion present in solution.

Therefore

$$Z' = \text{A}^- = \text{H}^+ + \text{M}^+ - \text{OH}^- \quad (\text{Eq. 3})$$

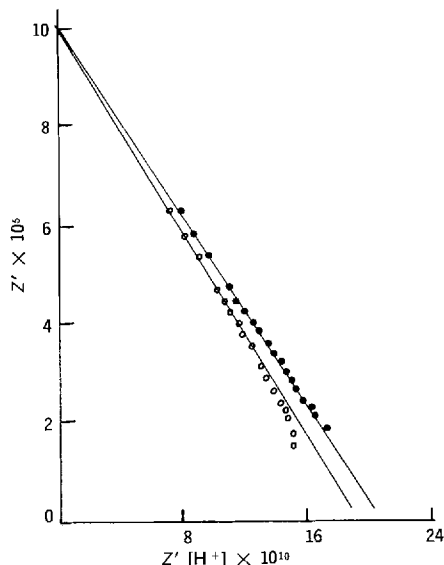
The apparent ionization constant for HA may be written:

$$K^c = \frac{[\text{A}^-][\text{H}^+]}{[\text{HA}]} = \frac{\text{A}^- \left(\frac{10^3}{V}\right) [\text{H}^+]}{\text{HA} \left(\frac{10^3}{V}\right)} = \frac{\text{A}^- [\text{H}^+]}{\text{HA}} = \frac{\text{A}^- [\text{H}^+]}{(A^0 - \text{A}^-)} \quad (\text{Eq. 4})$$

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TABLE I.—COMPARISON OF EXPERIMENTALLY DETERMINED ACTIVITY COEFFICIENTS WITH LITERATURE VALUES

$\mu$	$\gamma_{\pm}$		
	Determined	Lewis and Randall (4)	Kielland (3)
0.09	0.80	0.80	0.83
0.05	0.82	0.83	0.86
0.036	0.83	0.84	0.87
0.018	0.88	0.88	0.89


 Fig. 1.—Plot of  $Z'$  versus  $Z' [H^+]$  calculated at  $\mu = 0.05$  with both the Kielland and  $\gamma_{\pm}$  activity coefficients. Key: ●, data using Kielland values; ○, data using  $\gamma_{\pm}$  values.

where

- $[A^-]$  = concentration of conjugate base =  $A^- (10^3/V)$ ,  
 $A^-$  = absolute number of moles of conjugate base,  
 $V$  = volume of solution in milliliters,  
 $[HA]$  = concentration of acid =  $HA (10^3/V)$ ,  
 $HA$  = absolute number of moles of acid,  
 $[H^+]$  = concentration of hydrogen ion,  
 $A^0$  = absolute number of moles of acid added initially =  $HA + A^-$ .

Substituting from Eq. 3 into Eq. 4 and rearranging,

$$Z' = A^0 - \frac{1}{K^c} Z' [H^+] \quad (\text{Eq. 5})^1$$

Although Eq. 5 is identical in appearance to Eq. 1, there are important differences which make it an exact linear function. The  $A^0$  term in Eq. 5 represents *absolute* number of moles of acid added to the solution. All the terms used to calculate  $Z'$  (including  $H^+$ ) are also *absolute* quantities. The  $[H^+]$  in Eq. 5, however, is a *concentration* term.

The modified equation of Benet and Goyan permits the use of their method without any assump-

<sup>1</sup> For the titration of a base to which a fixed amount of HCl ( $W$ ) has been added,  $Z' = A^0 - K^c \frac{Z'}{[H^+]}$  where  $Z' = W + OH^- - H^+ - M^+$ .

tions as to volume increase during titration. In addition, it also decreases the amount of calculations required, since the only adjustment necessary for volume change is made with the hydrogen ion. In practice, HCl is often added to the solution being titrated,<sup>2</sup> so that calculations in terms of concentration units would require an adjustment for both  $Cl^-$  and  $M^+$  ions at each point in the titration. However, if calculations are made with absolute quantities, the amount of  $Cl^-$  is a constant and is equal to the absolute amount of HCl added, whereas  $M^+$  is merely the volume of MOH added multiplied by its normality.

The authors have found that working in terms of absolute amounts of materials is a good general method for decreasing the computations required in titrimetric techniques. Its use in chelation studies, where numerous species are present (chelating metal,  $M^+$ ,  $Cl^-$ , etc.), has again been found to

TABLE II.—COMPARISON OF RESULTS WITH ACETIC ACID USING BOTH TYPES OF ACTIVITY COEFFICIENTS

% Purity Thermodynamic pKa	Results		
	$\gamma_{\pm}$	Kielland	Theory
	99.96	100.1	100.0
	4.76	4.74	4.75 <sup>a</sup> 4.76 <sup>b,c</sup>

<sup>a</sup> Determined from conductance measurements (5).  
<sup>b</sup> Data reported at an ionic strength of 0.0004  $M$  (6).  
<sup>c</sup> Data reported at infinite dilution (7).

simplify calculations because only one volume adjustment is necessary in most cases.

**Activity Coefficient.**—For evaluating  $Z'$ , it is necessary to convert the hydrogen ion activity, obtained from the pH value, to a concentration term by use of an activity coefficient. In their work, Benet and Goyan used the single ion activity coefficients determined from theoretical considerations by Kielland (3). For the authors' calculations, the more familiar, experimentally obtained,  $\gamma_{\pm}$  values as listed in Lewis and Randall (4) were employed. These two types of activity coefficients differ somewhat.

In an attempt to determine which activity coefficient value is the better for use in these studies, blank solutions were prepared containing all constituents except the material being titrated. Therefore, since the ionic strength and amount of HCl present were known, the apparent activity coefficient could be evaluated by converting the pH of a solution to hydrogen ion activity, and dividing this by the known hydrogen ion concentration. The results of this study (Table I) indicate that the values obtained more closely approximate the  $\gamma_{\pm}$  values than they do the theoretically determined ones.

In addition, the purity and ionization constant of acetic acid were calculated using both the Kielland and  $\gamma_{\pm}$  values (Fig. 1). When  $\gamma_{\pm}$  is employed, the relationship is linear over the entire range studied, whereas with data at higher ionic strengths (0.09 and 0.05) the Kielland single ion coefficient

<sup>2</sup> When an amount of HCl ( $W$ ) is added to the solution,  $Z' = H^+ + M^+ - W - OH^-$ . These studies are often performed in a pH range where the hydroxyl ion is considered negligible, and  $Z' = H^+ + M^+ - W$ .

produces a curved segment at lower pH values.<sup>3</sup> In calculating the purity and ionization constant, only the linear portion of each plot was used. Nevertheless, it may be seen (Table II) that the intercept obtained (and therefore the purity evaluated) is not markedly influenced by the choice of activity coefficient. It is rather the ionization constant that reflects the difference, as may be seen by the two slopes in Fig. 1. A plot of  $pK_a'$  versus  $\sqrt{\mu}$  demonstrated an essentially linear relationship for both sets of data. These were extrapolated to infinite

<sup>3</sup> In this area of the titration, hydrogen ion makes its greatest contribution to  $Z'$  values, so that an error in the activity coefficient would be most evident at these higher acidities. This is comparable to Benet and Goyan's type A curve in Fig. 1 (2) for the case where high erroneous pH values are substituted. In all cases the Kielland values are higher than the  $\gamma_{\pm}$ , and would result in a lower hydrogen ion concentration.

dilution to evaluate a thermodynamic ionization constant. The results (Table II) indicate that the  $pK_a$  obtained with both values are consistent with the literature (5-7). Therefore, since the two sets of data are the same, the type of activity coefficient employed for calculations appears to be a matter of personal choice.

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## Identification of Some Barbiturates by Paper and Thin-Layer Chromatography

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Paper and thin-layer chromatographic procedures are described which can serve to separate a multiple mixture of 12 different barbiturates of toxicological interest.

THE APPLICATION of paper and thin-layer chromatography seems, so far, to be the most promising approach in the identification of barbiturates (1-9). The different procedures adopted leave much to be desired and a simple method for the separation and identification of a multiple mixture of barbiturates is of great value in medico-legal analysis.

A simple, rapid method of separation and identification of 12 barbiturates encountered either alone or in a mixture during the toxicological studies in the National Centre of Criminological Research is reported in this paper. The procedures adopted are based on the application of both paper and thin-layer chromatographic techniques to the following barbiturates: phenobarbital U.S.P., cyclobarbitol,<sup>1</sup> barbital U.S.P., diallylbarbituric acid N.F., allyl-isopropylbarbituric acid,<sup>2</sup> butobarbital,<sup>3</sup> amobarbital U.S.P., secobarbital U.S.P., methylphenobarbital,<sup>4</sup> ethyl-*n*-hexylbarbituric acid,<sup>5</sup> pentobarbital U.S.P., and hexobarbital.<sup>6</sup>

#### EXPERIMENTAL

##### Paper Chromatography

The earlier attempt of Kybing (3) and Ledvina (4) for the chromatographic separation of barbitu-

rates on formamide paper gave promising results. It was, therefore, decided to find out the most appropriate system of formamide and the developing solvent which fulfills speedy and efficient separation.

The following systems were investigated: (A) paper impregnated with formamide, (B) solvent containing formamide, and (C) formamide included in both the paper and the solvent.

**Paper.**—Sheets of Whatman No. 1 filter paper were impregnated with 20-30% formamide in acetone for about 10-15 min. The air-dried sheets were kept in a dark place away from dust. It is recommended that the paper be freshly impregnated.

**Solvents.**—Chloroform-benzene-ammonium hydroxide, concentration 13:3:6, was employed for system A (paper impregnated with formamide). Chloroform-*n*-butanol-formamide-5 *N* ammonium hydroxide, concentration 5:3:1:3, was employed for system B (solvent containing formamide). Chloroform-benzene-formamide-5 *N* ammonium hydroxide, concentration 12:2:1:5, was employed for system C (formamide included in both the paper and the solvent).

**Reagent.**—Silver reagent: (a) Silver nitrate, A. R., 0.5% methanolic solution. (b) Methanol-ammonium hydroxide, concentration 9:1. (c) Sodium hydroxide, A. R., 5% methanolic solution. The reagent is prepared by mixing solutions (a), (b), and (c) in the ratio 5:1:2. The reagent has to be freshly prepared.

**Standard Solution of Barbiturates.**—The above-mentioned barbiturates were used in a chloroform solution of a concentration of 1.5 mcg./ $\mu$ l.

**Procedure.**—The sheets were spotted in duplicate with 3-4  $\mu$ l. of the chloroformic solution of the barbiturates and placed into a chamber previously saturated with the stationary phase. The solvent front de-

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<sup>1</sup> Marketed as Phanodorn by Winthrop Laboratories.

<sup>2</sup> Marketed as Alurate by Roche Laboratories.

<sup>3</sup> Marketed as Soneryl by Specia.

<sup>4</sup> Marketed as Prominal by Winthrop Laboratories.

<sup>5</sup> Marketed as Hebaral by Parke Davis & Co.

<sup>6</sup> Marketed as Evipal by Winthrop Laboratories.