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A hyperbolic cosine method for the spectrophotometric evaluation of acidity constants of two-step overlapping equilibria

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Summary

A method for the determination of pK_a values of two-step overlapping equilibria ($H_2R/HR/R$) from spectrophotometric measurements applicable in those cases in which the absorbance vs pH curve passes through a maximum or a minimum in the region of pH in which the intermediate species predominates is devised in this paper. The method is based on the use of a hyperbolic cosine relationship which allows a number of different transformations to be applied in order to evaluate the acidity constants. The hyperbolic cosine method proposed in this paper has been applied to the evaluation of acidity constants of three systems previously described in the literature.

Introduction

Spectrophotometric determination of acidity constants involves (Connors, 1987) two well differentiated steps: first, measurements of the experimental points in order to obtain the set of values (pH, A) (absorbance, A, vs pH), and then, an appropriate treatment of the data to evaluate the unknown parameters. If the two acidity constants of, e.g., a diprotic acid, are well separated, the two equilibria may be treated successively, the neutralization of the stronger acid species H_2R being completed before ionization of the weaker acid species HR has begun. If, however, the successive acidity constants of the dibasic acid are close (K_{a_1}/K_{a_2} less than 10^4), then it is not an easy task to evaluate the acidity constants, a simultaneous fitting of the data (Asuero et al., 1986a) being necessary.

Many compounds of biological interest have acidity constants which lie close to each other. Their absorption, further transport and effect in the living organism are affected by the ratio of the concentrations of protonated and non-protonated forms in various media (Albert, 1985), the knowledge of acidity constants being thus of great worth. Although potentiometric acid-base titration is the most common and straightforward method for the evaluation of simultaneous step equilibria involving components of similar acidity, the potentiometric method is not usually applicable (Albert and Serjeant, 1984) to compounds which are sparingly soluble in water. In this pa-

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per, a somewhat sophisticated, yet effective technique for solving the problem from the viewpoint of spectrophotometric determination is proposed. Previous general formulae are modified so as to lead to a linear equation, which enables first and second acidity constants to be obtained, and the use of which is very convenient for compounds containing two functional groups whose pK_a values are very similar in magnitude. To perform the calculations, however, the A-pH curve must have a maximum or minimum in the region where the intermediate species HR of a given diabasic acid H_2R predominates, and the limit absorbances for the pure species H_2R and R_2, A_2 and A_0, respectively, need to be known. The efficiency of the method relies upon having a reasonable priori estimate of $pK^* = (pK_{a_1} + pK_{a_2})/2$, which is of course readily available (Asuero et al., 1986b) in such cases.

Theory

For a diprotolyte (diacid base or dibasic acid, or amphoteric substance), the ionization steps are:

$H_2R = HR + H$

$HR = R + H$

(charges has been omitted for the sake of generality) and are described by the apparent acidity constants (working in a medium of fixed ionic strength is assumed)

$$K_{a_1} = \frac{[HR][H]}{[H_2R]} \qquad (1)$$

$$K_{a_2} = \frac{[R][H]}{[HR]} \qquad (2)$$

If A_0, A_1 and A_2 are the limiting absorbances ($A_j = \epsilon_j C_R$, ϵ_j being the molar absorptivity of the species H_jR and C_R the total concentration of diprotolyte) of the species R, HR and H_2R, respectively, it can be shown using procedures outlined elsewhere (Asuero et al., 1986a,b) that at any point of the absorbance vs pH curve, the following expression holds

$$A - A_0 + (A - A_1) \cdot \frac{[H]}{K_{a_2}} + (A - A_2) \cdot \frac{[H]^2}{K_{a_2}K_{a_1}} = 0 \qquad (3)$$

On multiplying Eqn 3 by $K_{a_2}/(H)$ and rearranging, we obtain

$$A = A_1 + \left((A_2 - A) \cdot \frac{[H]}{K_{a_1}} + (A_0 - A) \cdot \frac{K_{a_2}}{[H]} \right) \qquad (4)$$

Then, by multiplying and dividing the term (in parentheses) on the right-hand side of Eqn 4 by

$$\sqrt{\frac{K_{a_1}}{K_{a_2}}} \cdot \frac{1}{\sqrt{(A_2 - A) \cdot (A_0 - A)}} \qquad (5)$$

conversion into the following expression takes place

$$A = A_1 \pm \sqrt{(A_2 - A)(A_0 - A)} \sqrt{\frac{K_{a_2}}{K_{a_1}}} \cdot \left(\sqrt{\frac{A_2 - A}{A_0 - A}}\, 10^{\Delta pH^*} + \sqrt{\frac{A_0 - A}{A_2 - A}}\, 10^{-\Delta pH^*} \right) \qquad (6)$$

The plus sign in Eqn 6 corresponds to the situation where $\{A_2, A_0\} > A_1$, the minus sign being applicable in those cases in which $\{A_2, A_0\} < A_1$ instead. Taking into account the definition of hyperbolic cosine, $\cosh x = (e^x + e^{-x})/2$ and the conversion of natural logarithms to those to base 10, we obtain after some manipulations

$$A = A_1 \pm 2\sqrt{(A_2-A)\cdot(A_0-A)} \times \cosh\left(\ln 10\left(\Delta\mathrm{pH}^* + \log\sqrt{\frac{A_2-A}{A_0-A}}\right)\right) \times \sqrt{\frac{K_{a_2}}{K_{a_1}}} \qquad (7)$$

or

$$A = A_1 \pm G\cdot R \qquad (8)$$

TABLE 1

Linear transformations for the determination of overlapping acidity constants (regression line, $y = a_0 + a_1 x$)

y	x	A_1	R
A	G	a_0	a_1
A/G	$1/G$	a_1	a_0
G	A	$-a_0/a_1$	$1/a_1$
$1/G$	A/G	$1/a_1$	$-a_0/a_1$
$1/A$	G/A	$1/a_0$	$-a_1/a_0$
G/A	$1/A$	$-a_1/a_0$	$1/a_0$

TABLE 2

Propagation of error variance of A_1 and R

Function	Variance of F	Variance of log F
$F = a_0$	$V_F = s_{a_0}^2 = \frac{\sum x^2}{NS_{XX}} s_{y/z}^2$	$V_{\log F} = \log^2 e \frac{s_{a_0}}{a_0^2} = \log^2 e \frac{\sum x^2}{NS_{XX}} s_{y/x}^2$
$F = \frac{1}{a_0}$	$V_F = \frac{s_{a_0}^2}{a_0^4} = \frac{1}{a_0^4}\frac{\sum x^2}{NS_{XX}} s_{y/x}^2$	
$F = a_1$	$V_F = s_{a_1}^2 = \frac{s_{y/x}^2}{S_{XX}}$	$V_{\log F} = \log^2 e \frac{s_{a_1}^2}{a_1^2} = \log^2 e \frac{s_{y/x}^2}{S_{XX}}$
$F = \frac{1}{a_1}$	$V_F = \frac{s_{a_1}^2}{a_1^4} = \frac{1}{a_1^4}\frac{s_{y/x}^2}{S_{XX}}$	
$F = \frac{a_0}{a_1}$	$V_F = \frac{s_{a_0}^2}{a_1^2} + \frac{s_{a_1}^2}{a_1^4} - 2\frac{a_0}{a_1^3}\mathrm{cov}(a_0,a_1)$ $= \frac{1}{a_1^2}\left(\frac{\sum x^2}{N} + \left(\frac{a_0}{a_1}\right)^2 + 2\frac{a_0}{a_1}\bar{x}\right)\frac{s_{y/x}^2}{S_{XX}}$	$V_{\log F} = \log^2 e\left[\left(\frac{s_{a_0}}{a_0}\right)^2 + \left(\frac{s_{a_1}}{a_1}\right)^2 - 2\frac{\mathrm{cov}(a_0,a_1)}{a_0 a_1}\right]$ $= \log^2 e\left[\frac{\sum x^2}{Na_0^2} + \frac{1}{a_1^2} + 2\frac{\bar{x}}{a_0 a_1}\right]\frac{s_{y/x}^2}{S_{XX}}$
$F = \frac{a_1}{a_0}$	$V_F = \frac{a_1^2 s_{a_0}^2}{a_0^4} + \frac{s_{a_1}^2}{a_0^2} - 2\frac{a_1}{a_0^3}\mathrm{cov}(a_0,a_1)$ $= \frac{1}{a_0^2}\left[\left(\frac{a_1}{a_0}\right)^2\frac{\sum x^2}{N} + 1 + 2\frac{a_1}{a_0}\bar{x}\right]\frac{s_{y/x}^2}{S_{XX}}$	

where

$$G = 2\sqrt{(A_2 - A_0)(A_0 - A)}\ \cosh\left(\ln 10\left(\Delta \mathrm{pH}^* + \log\sqrt{\frac{A_2 - A}{A_0 - A}}\right)\right) \tag{9}$$

and

$$R = \sqrt{\frac{K_{\mathrm{a}_2}}{K_{\mathrm{a}_1}}} \tag{10}$$

Eqn 9 can be rearranged into various forms (Table 1) suitable for linear plotting. The values of the unknown parameters R and A_1 can be calculated from the slope (a_1) and intercept (a_0) of these lines which are determined via the least-squares method.

Once the value of R is known, the individual $\mathrm{p}K_{\mathrm{a}}$ values may be calculated from

$$\mathrm{p}K_{\mathrm{a}_1} = \mathrm{p}K^* - \log R \tag{11}$$

$$\mathrm{p}K_{\mathrm{a}_2} = \mathrm{p}K^* + \log R \tag{12}$$

The variance of a function $F = f(a_0,a_1)$ can be expressed (Asuero et al., 1988) as

$$V_{\mathrm{F}} = \left(\frac{\partial F}{\partial a_0}\right)^2 \cdot s^2_{a_0} + \left(\frac{\partial F}{\partial a_1}\right)^2 \cdot s^2_{a_1} + 2\left(\frac{\partial F}{\partial a_0}\right)\cdot\left(\frac{\partial F}{\partial a_1}\right) \cdot \mathrm{cov}(a_0,a_1) \tag{13}$$

where $s^2_{a_0}$ and $s^2_{a_1}$ are the variances of the intercept and slope, respectively, and $\mathrm{cov}(a_0,a_1)$ is the covariance between the slope and intercept

$$s^2_{a_0} = \frac{\sum x^2}{N \cdot S_{XX}} \cdot s^2_{y/x} \tag{14}$$

$$s^2_{a_1} = \frac{s^2_{y/x}}{S_{XX}} \tag{15}$$

$$\mathrm{cov}(a_0,a_1) = \frac{-\bar{x}}{S_{XX}} \cdot s^2_{y/x} \tag{16}$$

where N is the number of pairs of data (x_i,y_i), S_{XX} represents the sum of the squares of deviations from the mean for the variable x, and $s^2_{y/x}$ is the variance of the regression line

$$s^2_{y/x} = \frac{Q}{N-2} = \frac{\sum (y_i - \hat{y}_i)^2}{N-2} = \frac{S_{YY} - a_1^2 \cdot S_{XX}}{N-2} \tag{17}$$

where $\hat{y}$ is the predicted value of y for a given x and S_{YY} denotes the sum of the squares of deviations from the mean value for the variable y (Asuero and Gonzalez, 1989).

The estimates of the uncertainty in the calculations of A_1 and R (and $\log R$) can be made by applying Eqn 14. Examples are listed in Table 2.

Nevertheless, before Eqn 9 or any of its variants can be applied, $\mathrm{p}K^*$ must be known. A procedure for overcoming this difficulty is, however, suggested. The procedure for obtaining the best values of $\mathrm{p}K_{\mathrm{a}_1}$, $\mathrm{p}K_{\mathrm{a}_2}$ and A_1 involves the following steps:

(a) Calculate an estimated value of $\mathrm{p}K^*$ from (Asuero et al., 1986a)

$$\mathrm{p}K^* = pH' + \frac{1}{2}\log\left(\frac{A' - A_0}{A' - A_2}\right) \tag{18}$$

where (pH',A') are the coordinates of the maximum or minimum of the absorbance vs pH graph, which are read off from the smoothed curve, an approximate value for $\mathrm{p}K^*$ being thus obtained.

(b) Vary $\mathrm{p}K^*$ systematically around the estimated value obtained from Eqn. 18 and follow the entire procedure in each case (transformations I–VI).

(c) Take the most probable value for $\mathrm{p}K^*$ as that which yields the lowest standard deviation of the corresponding regression line.

A program has been written in BASIC (for use in a CASIO PB 770 microcomputer with printer FA 10) in order to perform the necessary calculations.

Applications

The procedure devised above was applied to the experimental data reported by Bryson and

TABLE 3

Evaluation of the parameters and their limits of error by the hyperbolic cosine method for the 3-aminobenzoic acid system

Option	pK*	Intercept	Slope	Corr.	pK_{a_1}	pK_{a_2}	A_1	σ_A
		(SD)	(SD)	SD (y/x)	SD (log R)		(SD A_1)	
I	3.937	0.2367	0.1573	0.9972	3.134	4.740	0.237	1.57$E-3$
		(0.0042)	(0.0028)	(0.0038)	(0.050)		(0.004)	
II	3.937	0.1599	0.2331	0.9976	3.141	4.733	0.233	1.49$E-3$
		(0.0029)	(0.0040)	(0.0027)	(0.050)		(0.004)	
III	3.937	−1.4879	6.3206	0.9972	3.136	4.738	0.235	1.54$E-3$
		(0.0532)	(0.1142)	(0.0243)	(0.001)		(0.004)	
IV	3.937	−0.6791	4.2694	0.9976	3.139	4.735	0.234	1.51$E-3$
		(0.0240)	(0.0724)	0.0116)	(0.008)		(0.004)	
V	3.937	4.2339	−0.6675	−0.9894	3.135	4.739	0.236	1.56$E-3$
		(0.0735)	(0.0238)	(0.0351)	(0.008)		(0.004)	
VI	3.937	6.2742	−1.4666	−0.9894	3.139	4.735	0.234	1.50$E-3$
		(0.1145)	(0.0522)	(0.0522)	(0.001)		(0.004)	
					3.137	4.737		
					±0.003	±0.003		

Matthews (1961) for the 3-aminobenzoic acid, 3-amino-1-naphthoic acid and 4-amino-2-naphthoic acid systems.

The A_0 and A_2 values on the two horizontal parts of the absorbance vs pH curves were used for analysis employing the dependences given in Table 1. Only data which have corresponding absorbances should be employed in the calculations in order to obtain a positive value for the product $(A_2-A)\cdot(A_0-A)$ and the quotient $(A_2-A)/(A_0-A)$, respectively. In the case of the 3-aminobenzoic acid system only the same 19 pairs of data as employed by Albert and Serjeant (1984) for evaluating the overlapping ionization constants by using the SPECPK program (written in FORTRAN IV) were handled for comparative purposes. Experimental details and the absorbance vs. pH data employed are given in the following text.

3-Aminobenzoic acid system $C_R = 8.40\times10^{-4}$; wavelength = 280 nm; $I = 0.016$ (ionic strength), $A_2 = 0.573$, $A_0 = 0.683$. Data pairs

TABLE 4

Evaluation of the parameters and their limits of error by the hyperbolic cosine method for the 3-amino-1-naphthoic acid system

Option	pK*	Intercept	Slope	Corr	pK_{a_1}	pK_{a_2}	A_1	σ_A
		(SD)	(SD)	SD (y/x)	SD (log R)		(SD A_1)	
I	3.472	0.1044	0.1308	0.9947	2.588	4.356	0.104	2.68$E-3$
		(0.0040)	(0.0028)	(0.0071)	(0.007)		(0.004)	
II	3.478	0.1345	0.0997	0.9932	2.607	4.349	0.100	2.20$E-3$
		(0.0024)	(0.0024)	(0.0046)	(0.057)		(0.002)	
III	3.472	−0.7761	7.5676	0.9947	2.593	4.351	0.103	2.57$E-3$
		(0.0464)	(0.1626)	(0.0542)	(0.001)		(0.004)	
IV	3.477	−1.3147	9.8724	0.9932	2.601	4.353	0.101	2.30$E-3$
		(0.0546)	(0.2408)	(0.0462)	(0.002)		(0.002)	
V	3.475	9.6565	−1.2673	−0.9747	2.593	4.357	0.104	2.49$E-3$
		(0.2825)	(0.0606)	(0.2235)	(0.008)		(0.003)	
VI	3.475	7.4688	−0.7496	−0.9747	2.601	4.348	0.100	2.33$E-3$
		(0.1719)	(0.1415)	(0.1719)	(0.001)		(0.003)	
					2.601	4.352		
					±0.007	±0.004		

TABLE 5

Evaluation of the parameters and their limits of error by the hyperbolic cosine method for the 4-amino-2-naphthoic acid system

Option	pK*	Intercept (SD)	Slope (SD)	Corr SD (y/x)	pK_{a_1}	pK_{a_2}	A_1 (SD A_1)	σ_A
I	3.667	0.7311	0.2218	0.9929	3.013	4.321	0.731	2.93$E-3$
		(0.0064)	(0.0066)	(0.0084)	(0.059)		(0.006)	
II	3.665	0.2190	0.7284	0.9996	3.005	4.325	0.728	2.85$E-3$
		(0.0065)	(0.0050)	(0.0090)	(0.059)		(0.005)	
III	3.666	−3.2583	4.4382	0.9929	3.019	4.313	0.734	3.01$E-3$
		(0.0071)	(0.1332)	(0.0378)	(0.003)		(0.006)	
IV	3.665	−0.3014	1.3719	0.9996	3.007	4.323	0.729	2.86$E-3$
		(0.0070)	(0.0093)	(0.0124)	(0.013)		(0.005)	
V	3.667	1.3620	−0.3068	−0.9963	3.020	4.314	0.734	3.04$E-3$
		(0.0134)	(0.0066)	(0.0234)	(0.013)		(0.007)	
VI	3.666	4.3854	−3.2306	−0.9963	3.024	4.308	0.737	3.15$E-3$
		(0.1360)	(0.0700)	(0.0759)	(0.003)		(0.007)	
					3.015	4.317		
					±0.008	±0.007		

{pH, A}: 2.57, 0.501, 2.64, 0.490, 2.72, 0.480, 2.77, 0.472, 2.84, 0.462, 2.91, 0.450, 3.00, 0.434, 3.10, 0.413, 3.20, 0.396, 4.42, 0.385, 4.48, 0.398, 4.56, 0.418, 4.66, 0.440, 4.73, 0.461, 4.80, 0.480, 4.90, 0.500, 4.98, 0.521, 5.08, 0.542, 5.19, 0.566.

3-Amino-1-naphthoic acid system $C_R = 2.612 \times 10^{-4}$; wavelength = 328 nm, $A_2 = 0.414$, $A_0 = 0.490$. Data pairs {pH, A}: 1.81, 0.368, 1.94, 0.355, 2.09, 0.340, 2.19, 0.329, 2.38, 0.302, 2.59, 0.266, 2.64, 0.252, 2.74, 0.240, 2.82, 0.224, 2.91, 0.212, 3.02, 0.196, 3.11, 0.186, 3.21, 0.182, 3.31, 0.175, 3.88, 0.205, 4.00, 0.224, 4.11, 0.246, 4.20, 0.263, 4.31, 0.285, 4.37, 0.300, 4.46, 0.321, 4.54, 0.351, 4.64, 0.360, 4.74, 0.380, 4.82, 0.390.

4-Amino-2-naphthoic acid system $C_R = 8.88 \times 10^{-5}$; wavelength = 265 nm, $A_2 = 0.375$, $A_0 = 0.470$. Data pairs {pH, A}: 2.14, 0.417, 2.24, 0.425, 2.45, 0.45, 2.66, 0.485, 2.74, 0.500, 2.82, 0.515, 2.94, 0.540, 3.04, 0.552, 3.17, 0.575, 3.27, 0.600, 3.40, 0.605, 3.52, 0.620, 3.89, 0.618, 4.07, 0.598, 4.26, 0.567, 4.50, 0.531, 4.86, 0.478, 5.27, 0.432.

The results obtained by applying transformations I–VI to the three systems studied (Table 1) were in good agreement as shown in Tables 3–5.

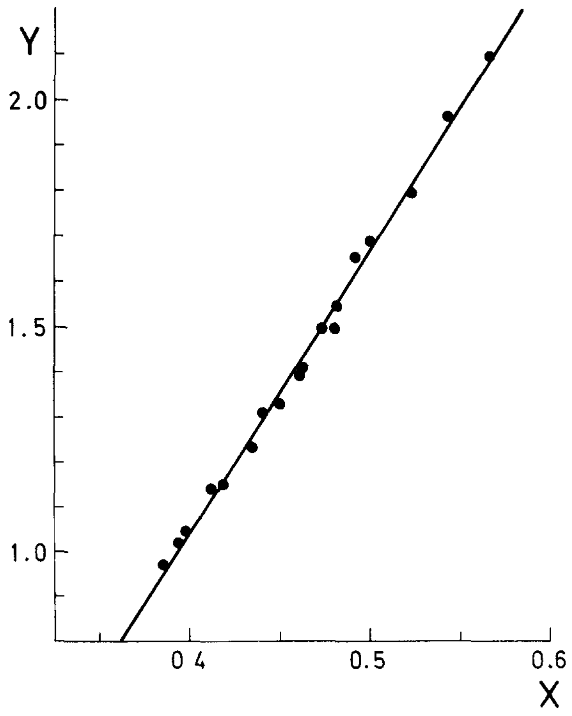

Fig 1 Hyperbolic cosine plot for the 3-aminobenzoic acid system (transformation III)

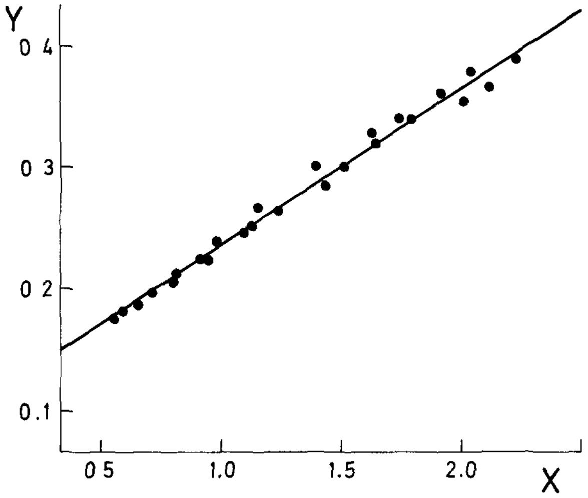

Fig. 2. Hyperbolic cosine plot for the 3-amino-1-naphthoic acid system (transformation I).

The linearization as judged on the basis of the residual-squares sum $\sigma_A = \sum (y_{exp} - y_{calc})^2$ is practically equivalent in all cases.

In general, it is unnecessary to plot the results graphically, since any large experimental errors are exposed by a large residual and bad data points, which are defined as data points with reduced residuals e_s greater than 3 (Asuero, 1989)

$$e_s = \frac{y_{exp} - y_{calc}}{s_{y/x}} \qquad (19)$$

may be eliminated in the calculations.

However, working with transformations I–VI the tedious plotting of previous transformations and graphical determination of spectrophotometric evaluation of acidity constants of simultaneous equilibria were completely eliminated, as can be seen in Figs 1–3. Low reduced residual values and frequent sign changes of the residuals indicate that the fit is good (Fig. 4).

The values obtained for the overlapping acidity constants for the 3-aminobenzoic acid system, corrected to $I = 0$ by using for ionic strength the same expression as that used by Albert and Serjeant (1984)

$$-\log f_1 = 0.512 \frac{\sqrt{I}}{1 + \sqrt{I}} \qquad (20)$$

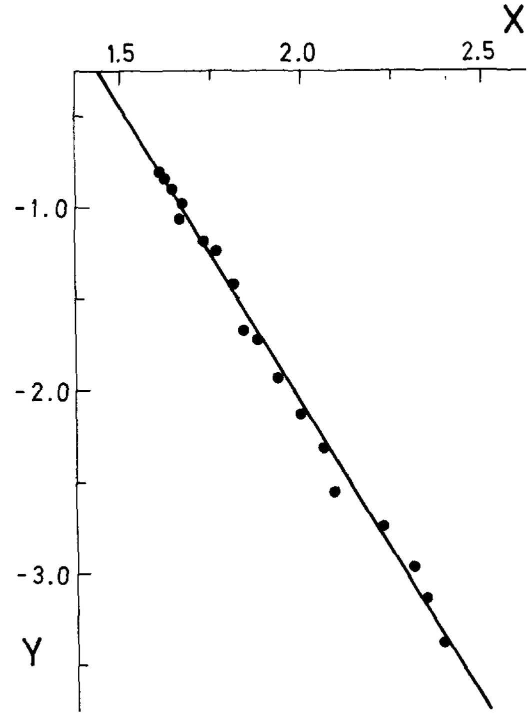

Fig. 3 Hyperbolic cosine plot for the 4-amino-2-naphthoic acid system (transformation VI)

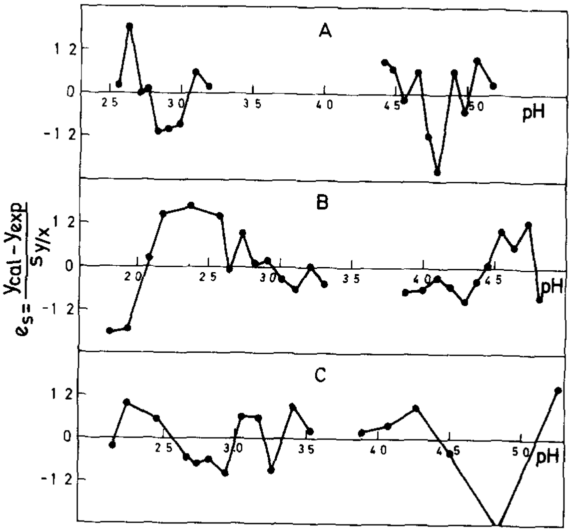

Fig 4. Plots of reduced residuals vs pH (A) 3-aminobenzoic acid system; (B) 3-amino-1-naphthoic acid system, and (C) 4-amino-2-naphthoic acid system

were $pK_{a_1} = 3.083 \pm 0.003$ ($N = 19$), and $pK_{a_2} = 4.791 \pm 0.003$ ($N = 19$) (hyperbolic cosine method), and were in good agreement with the mean values obtained by Albert and Serjeant (1984) by means of the SPECPK program: $pK_{a_1} = 3.075 \pm 0.007$ ($N = 9$), and $pK_{a_2} = 4.798 \pm 0.009$ ($N = 10$). Results obtained for the pK_{a_1} value of the other two systems studied were also in good agreement with those reported by Bryson and Matthews (1961).

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