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A bilogarithmic method for the evaluation of acidity constants of amphoteric substances from solubility measurements

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Summary

A method for the determination of pK_a values of two-step overlapping equilibria from solubility measurements applicable to amphoteric substances is reported in this paper. The method is based on the properties of symmetry of the solubility against pH curves which leads to an hyperbolic cosine relationship, and requires the application of a trial and error procedure, but proves directly the correctness of the assumed equilibria. The bilogarithmic method proposed in this paper has been applied to the evaluation of acidity constants of niflumic acid, sulphadiazine, red fluorescein and oxine. An advantage of the bilogarithmic plot is its capability of presenting data covering several orders of magnitudes in hydrogen ion activities.

Introduction

In spite of the fact that the solubility method for determining aqueous pK_a values data for more than 30–50 years (Hitchcock, 1924; Krebs and Speakman, 1945), a search of the literature reveals that it has only been sporadically applied (Asuero, 1988). Perhaps, the reason for this situation (Peck and Benet, 1978) is the simplicity and ease of performing potentiometric and spectrophotometric methods for evaluating pK_a values. It is well known, however, that drug efficacy can be severely limited by poor aqueous solubility, because only dissolved drugs can be adsorbed. In addition, the side effects of some drugs are also the result of their poor solubility (Yalkowski, 1981). Properties such as ionization and solubility exert and influence over the therapeutic activity of drugs (Crevoisier and Buri, 1971; Bres et al., 1976; Winne, 1987). The solution of many galenical problems requires the knowledge of the acidity constants of compounds having pharmaceutical interest, and so, the solubility of a drug is an extremely important parameter (Zimmermann, 1983) in planning the development of tablets. The purpose of this paper is to elaborate a bilogarithmic method for the evaluation of pK_a values of two-step overlapping equilibria of amphoteric substances from solubility measurements. Attention is directed toward the treatment of experimental data, not how to achieve them. The solubility method is limited in its applicability, and it is laborious compared with potentiometry or spec-

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trophotometry (Rossotti and Rossotti, 1961; Albert and Serjeant, 1984), but for slightly soluble compounds it can be very useful (Connors, 1987) provided an analytical method is available for the solubility measurements. In order to check the usefulness of the method proposed it has been applied to several systems previously reported in the literature: niflumic acid (Bres et al., 1976), sulphadiazine (Krebs and Speakman, 1945), red fluorescein (Diehl and Markuszewski, 1985) and oxine (Irving et al., 1949).

Theory

Basic equations

The solubility of an amphoteric substance, HR, if only R, HR and H_2R are present is given by (charges omitted for simplicity)

$$S = [H_2A] + [HA] + [A]$$

$$= [HA]\left(\frac{[H_2A]}{[HA]} + 1 + \frac{[A]}{[HA]}\right) \qquad (1)$$

Taking into account the definition of apparent acidity constants, $K_{a_1} = (H)\ [HA]/[H_2A]$ and $K_{a_2} = (H)\ [HA]/[H_2A]$, where parentheses indicate activities and braces concentrations, Eqn. (1) becomes

$$S = S_0\left(\frac{(H)}{K_{a_1}} + 1 + \frac{K_{a_2}}{(H)}\right) \qquad (2)$$

where S_0 is the intrinsic solubility of the species HR. At the point of minimum solubility (isoelectric point: (pH′, S')), $dS/d(\mathrm{pH}) = 0$, which implies that

$$(H') = \sqrt{K_{a_1}K_{a_2}} \qquad (3)$$

Hyperbolic cosine relationship

By combining Eqns. 2 and 3 and taking into account the definition of hyperbolic cosine, the following relationship may be established (Asuero, 1988):

$$S = S_0\left(1 + 2\sqrt{\frac{K_{a_2}}{K_{a_1}}}\ \cosh(\ln 10 \cdot \Delta\mathrm{pH})\right) \qquad (4)$$

where

$$\mathrm{pH} = \mathrm{pH}' - \mathrm{pH} \qquad (5)$$

Bilogarithmic method

Eqn. 4 when applied to the point of minimum solubility (pH′, S') affords

$$S' = S_0\left(1 + 2\sqrt{\frac{K_{a_2}}{K_{a_1}}}\right) \qquad (6)$$

By eliminating S_0 between Eqns. 4 and 6, and rearranging, one obtains

$$\sqrt{\frac{K_{a_1}}{K_{a_2}}} = 2\frac{\cosh(\ln 10 \cdot \Delta\mathrm{pH}) - \dfrac{S}{S'}}{\dfrac{S}{S'} - 1} \qquad (7)$$

By taking decadic logarithms of each side and rearranging, Eqn. 7 may be transformed into a form suitable for regression analysis

$$\log\left(\frac{S}{S'} - 1\right) - \log 2$$
$$= \log\sqrt{\frac{K_{a_2}}{K_{a_1}}} + \log\left(\cosh(\ln 10 \cdot \Delta\mathrm{pH}) - \frac{S}{S'}\right) \qquad (8)$$

By plotting the left hand of Eqn. 8 against the second term of the right hand of Eqn. 8, a straight line $y = a_0 + a_1x$ should be obtained. This model, if valid, enables $(\mathrm{p}K_{a_1} - \mathrm{p}K_{a_2})/2$ to be calculated directly from the intercept. A well defined slope of unity should also be obtained. Deviations from this behaviour are diagnostic of more complicated phenomena. This method is based on the properties of symmetry of the solubility against pH curves for amphoteric substances and proves directly the correctness of the assumed equilibria.

It is interesting to note that by applying other least-squares procedures, is not possible to determine whether a given solubility against pH curve is characterized only by the assumed reactions. A model based on a physicochemical concept of wide applicability is more convincing than an empirical curve-fitting result (Connors, 1987). On the other hand, graphs are essential for good statistical analysis (Anscombe, 1973). In this respect, when the independent variable is varied over a number of orders of magnitudes, the points tend usually to be bunched together (Rossotti, 1978). The bilogarithmic method reported here has the dependent variable S on both axes, but it has advantage in that it provides a closed scale representation of y and x, unlike other plots.

The individual pK_a values may be evaluated from

$$\mathrm{p}K_{a_1} = \mathrm{pH}' + \log\sqrt{\frac{K_{a_2}}{K_{a_1}}} \tag{9}$$

$$\mathrm{p}K_{a_2} = \mathrm{pH}' - \log\sqrt{\frac{K_{a_2}}{K_{a_1}}} \tag{10}$$

When the first and second acidity constants differ greatly it is reasonable to assume that the two ionization stages do not overlap to any appreciable extent, methods used for the evaluation of acidity constants of monoprotolytes being applicable (King, 1965; Albert and Serjeant, 1984). Nevertheless, when the acidity constants lie close together, simultaneous adjustment of the data as in the present case is mandatory. The value of the intrinsic solubility is not accessible to direct measurements and its graphical evaluation from solubility data in the alkaline (or acid) range of pH by plotting S against $1/(H)$ (or against (H)) may involve a long and unsatisfactory extrapolation (Irving et al., 1949).

Error analysis

There is no doubt that an estimate of the uncertainty of these calculations would aid in interpreting experimental results. An expression for the variance of pK_a's can be obtained by performing an analysis of variance calculation on Eqns. 9 and 10:

$$\sigma^2_{\mathrm{p}K_{a_i}} = \sigma^2_{\mathrm{pH}'} + \sigma^2_{\log \mathrm{R}} \tag{11}$$

where

$$R = \sqrt{\frac{K_{a_2}}{K_{a_1}}} \tag{12}$$

so that the variance is additive for both a sum and a difference and we assume that pH′ and log R are independent, i.e., uncorrelated (Asuero et al., 1988). The acidity constants are expressed as pK_a since this preserves a more direct relationship with the pH values upon which they depend.

On the other hand, the intrinsic solubility of HR, S_0, may be calculated from Eqn. 6:

$$S_0 = \frac{S'}{1+2R} \tag{13}$$

By applying the random error propagation law (Asuero et al., 1988) to log $R = \mathrm{f}(R)$ and to Eqn. (13) we have, respectively

$$\sigma^2_{\log R} = \left(\frac{\partial \log R}{\partial R}\right)^2 \sigma_R^2 = \frac{\log^2 \mathrm{e}}{R^2}\sigma_R^2 \tag{14}$$

$$\begin{aligned}\sigma^2_{S_0} &= \left(\frac{\partial S_0}{\partial S'}\right)^2 \sigma^2_{S'} + \left(\frac{\partial S_0}{\partial R}\right)^2 \sigma_R^2 \\ &= \frac{1}{(1+2R)^2}\left(\sigma^2_{S'} + \frac{4S'^2\sigma_R^2}{(1+2R)^2}\right)\end{aligned} \tag{15}$$

By combining Eqns. 14 and 15 we get finally

$$\sigma^2_{S_0} = \frac{1}{(1+2R)^2}\left(\sigma^2_{S'} + \left(\frac{2S' \ln 10 R\sigma_{\log R}}{1+2R}\right)^2\right) \tag{16}$$

The variance term depends as expected on the values of R and S' and on the precision with which these values are known or can be measured.

In the ideal case, the slope of the straight line corresponding to Eqn. 8 is the unity, and then

$$\sigma^2_{\log R} = \sigma^2_{a_0} \tag{17}$$

In practice, however, the slopes will differ from the unity (though not significantly) and the uncertainty in calculated values of slope has to be considered in the estimation of $\sigma_{\log \mathrm{R}}$ because a best estimate of log R is then given by

$$\log R = \frac{a_0}{a_1} \tag{18}$$

and so,

$$\sigma^2_{\log R} = \frac{a_0^2}{a_1^4} \sigma^2_{\mathrm{a}_1} + \frac{\sigma^2_{a_0}}{a_1^2} - 2\frac{a_0}{a_1^3} \operatorname{cov}(a_0, a_1) \tag{19}$$

The derivation of Eqn. 8 assumes that the value of S' (and pH$'$) is known, and so, the best experimental approach requires we have at our disposal a number of solubility data at pH values close to the isoelectric point. This poses some restrictions on the application of the method. From a smoothed log S against pH curve the value of S' can be read directly; this does not give rise to serious error. Experimental verification of the accuracy of S' is difficult. In this paper we have taken the inherent uncertainty in analytical concentration measurements of S' as a percentage, e.g. 2%, of its own value. In the extreme case of poor analytical data, uncertainties in the values of S' will be more large.

The crucial point in this procedure is the correct choice of pH$'$. Without microcomputer facilities, the pH$'$ value may be obtained by visual inspection of the smoothed log S–pH curve drawn in a large scale, or averaging a number of pairs of pH values which have corresponding solubilities so that the U-shaped curves of solubility as a function of pH are expected to be symmetrical about the pH of minimum solubility.

If necessary, it is possible to refine and to calculate more precise pK_a values by using a far more sophisticated procedure. First a trial value of pH$'$ is assumed and the entire procedure applied; the pH$'$ value may be progressively modified and the approved value of pH$'$ is taken as that for which the standard deviation, $\sigma_{\mathrm{y/x}}$, of the regression line corresponding to Eqn. 8 is at a minimum

$$\sigma^2_{\mathrm{y/x}} = \frac{\Sigma(y - \hat{y})^2}{N - 2} \tag{20}$$

where $\hat{y}$ is the estimated value of y for a given value of x. A BASIC program for use in a CASIO PB 770 microcomputer with printer FA 10 was written to perform this task. A copy of the program is available on author request. All solubility pH data are used in the calculations except those found to be anomalous by visual inspection of the log S–pH curve and those corresponding to S values close to the value of minimum solubility, S'. σ'_{pH} was assumed to be 0.02.

Applications

In order to check the usefulness of the method, it has been applied to a variety of systems previously described in the literature.

Niflumic acid

The log S–pH data for niflumic acid (Bres et al., 1976) are depicted in Fig. 1. The curve in the figure is calculated with the acidity constants given in the text. The figure suggests that points corresponding to pH values of 3.20 and 3.65 must be discarded in the calculations. The value of minimum solubility was taken as 24.54 mg/liter. A pH$'$ value of 3.622 makes the regression standard deviation corresponding to Eqn. 8 a minimum. The bilogarithmic plot of y against x is shown in the Fig. 2, the points lying very close to the straight line. From this line ($a_1 = 0.9889$, $\sigma_\mathrm{a} = 0.0272$; correlation coefficient = 0.9974). The following values were obtained for the unknowns parameters: p$K_{\mathrm{a}1} = 2.11$, p$K_{\mathrm{a}2} = 5.14$, $\sigma_{\mathrm{pK}_{\mathrm{ai}}} = 0.03$, $S_0 = 23.12$ mg/liter. Fig. 3 is a plot of standardized residual, e_s

$$e_\mathrm{s} = \frac{y - \hat{y}}{\sigma_{\mathrm{y/x}}} \tag{21}$$

vs calculated y, $\hat{y}$, for the data. All points fall

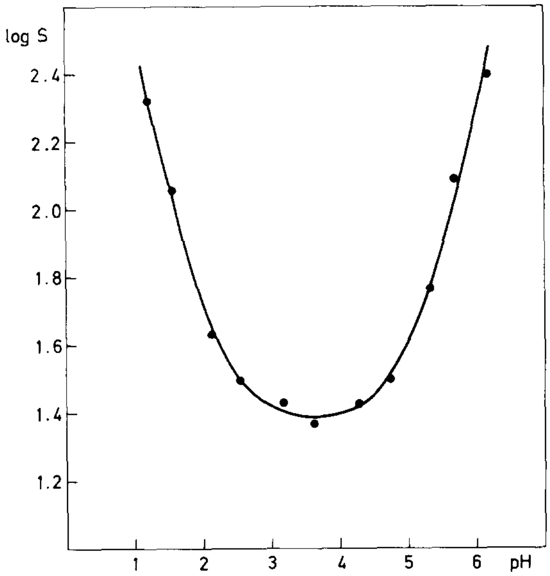

Fig. 1. Solubility data of niflumic acid as a function of pH (*S* in mg/liter).

within −2 and +2 units and are evently distributed about the standardized residual axis, indicating no abnormalities (Draper and Smith, 1981; Anderson, 1987).

Sulphadiazine

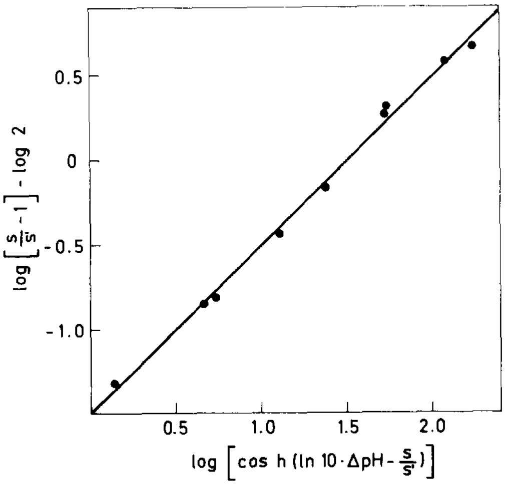

Fig. 2. Bilogarithmic plot for niflumic acid.

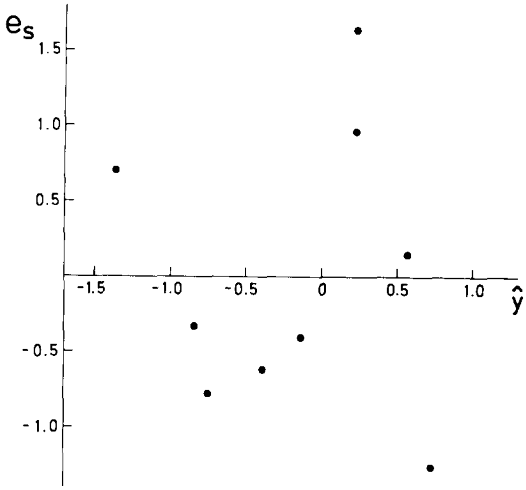

Fig. 3. Plot of standardized residuals against the estimated *y* values for niflumic acid.

Fig. 4 presents data for log *S* against pH for sulphadiazine, representating a system with extremely good analytical data (Krebs and Speakman, 1945). All points were used in the calculations with the exception of those located in the flat portion of the solubility against pH curve (Table 1). For a normal distribution about 95% of all standardized residual will fall between −2 and +2. If a point in the standardized residual plot exceeds ±2 units should be considered suspect as an outlier (Draper and Smith, 1981). The method was repeatedly applied by successively discarding any point whose reduced residual in absolute value was greater than 2. Results obtained are compiled in Table 2. The sequence of signs of the residuals

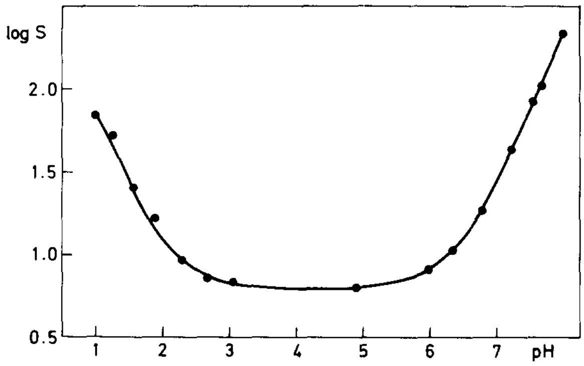

Fig. 4. Solubility data of sulphadiazine as a function of pH (*S* in mg/100 ml; the curve in figure is calculated with the acidity constants given in the text).

TABLE 1

Solubility data (in mg/100 ml) as a function of pH for sulphadiazine

Data point	pH	S	Data point	pH	S
1	1.00	68	8	6.35	11.1
2	1.26	66	9	6.82	19.4
3	1.55	25.2	10	7.23	43.5
4	1.89	16.5	11	7.56	86
5	2.31	9.3	12	7.67	114
6	2.69	7.5	13	8.00	229
7	6.01	8.5			

Data from Krebs and Speakman (1945).

shown in Table 3 suggests that the point corresponding to pH 1.26 may be discarded. In a practical sense, when residuals are small enough to be considered acceptable for a particular application, it is not necessary to test for lack of fit (Deming, 1983). Points number 4 and 9 of Table 1 need not necessarily be discarded in the calculations and so, the following values can be reported for the unknown parameters: $pK_{a1} = 2.04$, $pK_{a2} = 6.45$, $\sigma_{pK_{ai}} = 0.02$, and $S_0 = 6.16$ mg/100 ml.

Red fluorescein

The log S against pH data for red fluorescein (Diehl and Markuszewski, 1985) are plotted in Fig. 5. The minimum solubility, S', of red fluorescein was taken as 1.5×10^{-4} M. The pH′ value which minimizes the regression standard deviation corresponding to Eqn. 8 was equal to 3.404. The bilogarithmic representation obtained is depicted in Fig. 7 ($a_1 = 1.081$, $\sigma_{a_1} = 0.0547$; correlation coefficient = 0.9929). The inclusion of the points corresponding to pH 2.05 and 3.01 in the data treatment led to obtain slopes significantly different from the unity and so were discarded in the calculations. In addition, points corresponding to pH values greater than 4.90 were also discarded because of the existence of an additional equilibrium of deprotonation having a pK_a value of 6.36 (Diehl and Markuszewski, 1985). For this reason, the theoretical curve does not pass through experimental solubility (Fig. 6) at high pH values. Results obtained for the unknown parameters

TABLE 2

Evaluation of acidity constants and intrinsic solubility of sulphadiazine ($S' = 6.24$ mg / 100 ml)

Data points	pH′	Points discarded	Slope, a_1 (σ_{a_1})	Correlation coefficient	pK_{a_1}	pK_{a_2}	$\sigma_{pK_{ai}}$	S_0	$e_s > \|2\|$ (point)
13	4.264	–	1.0202 (0.0269)	0.9962	2.030	6.498	0.069	6.17	−2.57 (2)
12	4.243	2	0.9992 (0.0136)	0.9991	2.041	6.445	0.039	6.16	2.36 (4)
11	4.234	2, 4	0.9993 (0.0080)	0.9997	2.022	6.446	0.028	6.16	−2.20 (9)
10	4.230	2, 4, 9	0.9967 (0.0051)	0.9999	2.028	6.432	0.024	6.16	–

TABLE 3

Sequence of signs of the residuals

Number of data pairs	Points discarded	Sequence of signs of residuals	Number of signs	
			minus	plus
13	–	− + − + − − + + − − − + −	8	5
12	2	− + + − − + + − − − + +	6	6
11	2, 4	− + − + + + − − − + +	5	6
10	2, 4, 9	− + − + + − − − + +	5	5

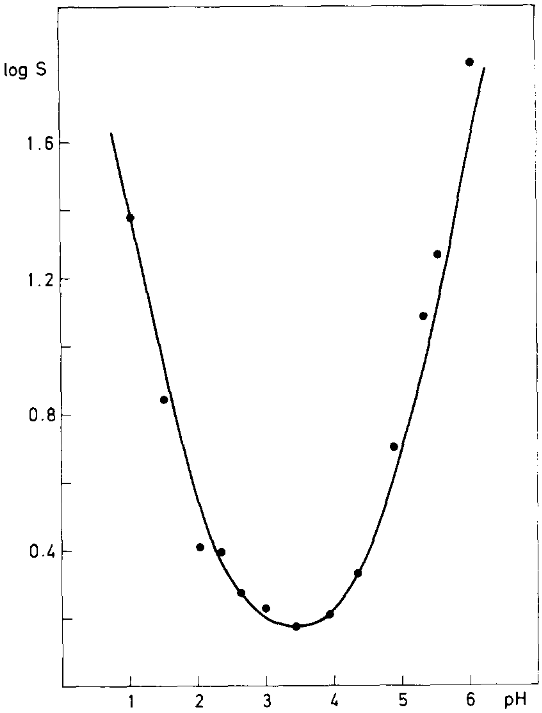

Fig. 5. Solubility data of red fluorescein as a function of pH (S in $M \times 10^4$; the curve in figure is calculated with the acidity constants given in the text).

were: $pK_{a1} = 2.26$, $pK_{a2} = 4.54$, $\sigma_{pK_{ai}} = 0.05$, and $S_0 = 1.31 \times 10^{-4}$ M. All reduced residuals were lower than 1.5.

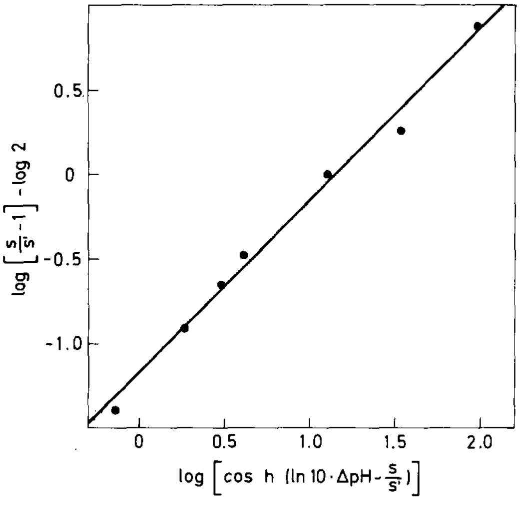

Fig. 6. Bilogarithmic plot for red fluorescein.

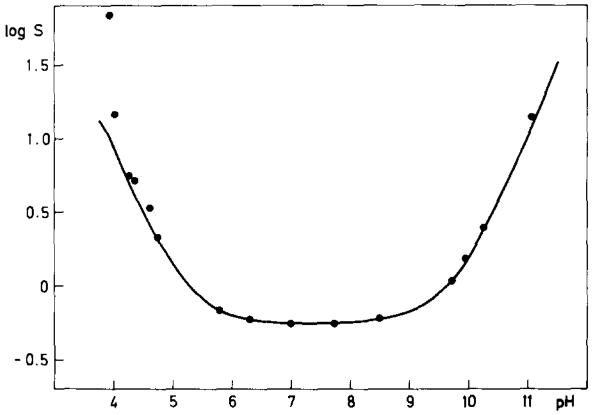

Fig. 7. Solubility data of oxine as a function of pH (S in g/liter; the curve in figure is calculated with the acidity constants given in the text).

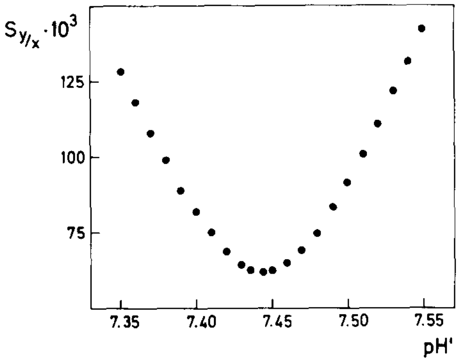

Fig. 8. Regression standard deviation as a function of the pH′ value assumed (oxine system).

Oxine

Fig. 7 presents log S against pH data for oxine (Irving et al., 1949). Solubility data used in the calculations are given in Table 4. Fig. 8 shows

TABLE 4

Solubility data as a function of pH for oxine

pH	S (g/liter)	pH	S (g/liter)
4.29	5.5	8.55	0.594
4.38	5.28	9.76	1.102
4.75	2.093	9.95	1.608
5.83	0.653	10.25	2.525
6.36	0.585	11.07	14.59

S' was taken as 0.555 g/liter. Data from Irving et al. (1949).

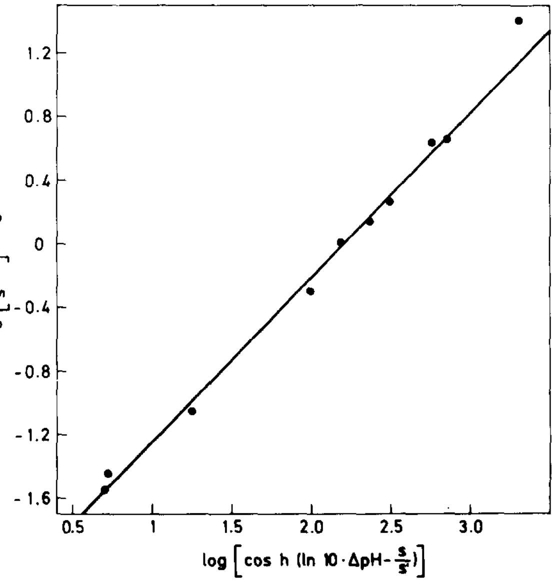

Fig. 9. Bilogarithmic plot for oxine.

data for the standard deviation of regression, $\sigma_{y/x}$, as a function of the assumed pH′ value, which was found to be 7.444. The plot corresponding to the best regression line ($a_1 = 1.0258$, $\sigma_{a_i} = 0.0231$, correlation coefficient = 0.9980) is shown in Fig. 9. The following results were obtained in this case: $pK_{a_1} = 5.21$, $pK_{a_2} = 9.67$, $\sigma_{pK_{a_i}} = 0.03$, and $S = 0.549$ g/liter. Caution should be exercised in interpreting confidence limits about the parameters estimated in this case because data compiled in Table 4, as indicated by Irving et al. (1949), are the mean of at least 3 concordant determinations. Though sound statistics exist to solve this problem, it is necessary, however, to know the number of replicates of each data point (Draper and Smith, 1981) and its standard error. Plot of standardized residuals against the estimated value of y is depicted in Fig. 10.

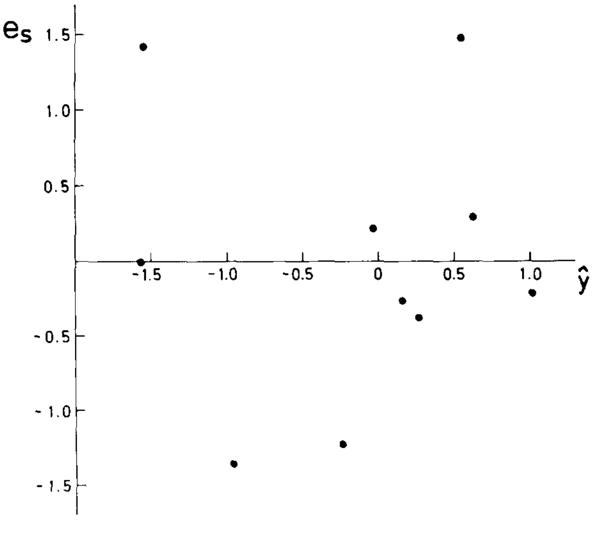

Fig. 10. Plot of standardized residuals against the estimated y values for oxine.

TABLE 5

Results of bilogarithmic method pK_a estimates for the compounds selected

Compound	pK_{a_1}	pK_{a_2}	S_0	Reference
Niflumic acid	2.10	5.00	–	Bres et al. (1976) [a]
	2.11	5.14	23.12 mg/liter	This paper
Sulphadiazine	2.10	6.45	6.16 mg/100 ml	Krebs and Speakman (1945)
	2.04	6.45	6.16 mg/100 ml	This paper
Red fluorescein	2.13	4.44	1.45×10^{-4} M	Diehl and Markuszewski (1985) [b]
	2.26	4.54	1.31×10^{-4} M	This paper
Oxine	5.27	9.68	3.775 M	Irving et al. (1949) [c]
	5.21	9.68	3.79 M	This paper

[a] Krebs and Speakman method.

[b] Modified Krebs and Speakman method.

[c] Successive aproximation method.

The method of this paper has not, apparently, been used in the study of equilibria. The results obtained for the various systems tested are compiled in Table 5, together with the data reported in the original papers. Results agree well if we take into account the differences between the calculation methods used in each case. An advantage of the method is that nearly all data are used in the evaluation of the unknown parameters, permitting statistical treatment and the possibility of tracing systematic errors. No data have been given in the paper for the standard deviation of the intrinsic solubilities, σ_{S_0} because the standard error of S_0 was in all cases equal to the error assumed for the minimum solubility.

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