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Evaluation of acidity constants of dibasic acids and diacid bases from liquid-liquid distribution measurements

Agustín G. Asuero

Department of Analytical Chemistry, Faculty of Pharmacy, the University of Seville, 41012 Seville (Spain)

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Summary

The distribution of drugs is of great interest in pharmacological, biological and analytical chemistry, and is strongly dependent on their acid-base characteristics. In this way, the evaluation of acidity constants from liquid-liquid distribution measurements constitutes an interesting topic. In this paper, we formulate the basic algebra concerning the distribution ratio against pH dependences for dibasic acids and diacid bases. A linear bilogarithmic hyperbolic cosine method for evaluating overlapping pK_a values has been devised, which unlike the usual least squares procedure allows checking of the correctness of the assumed equilibria.

Introduction

Distribution of drugs between organic solvents, e.g., octanol, and aqueous media forms the basis of a number of challenging problems in pharmacological and biological chemistry (Austel and Kutler, 1983). Many of these are based on an interest in bioavailability and transport of drugs and in models used to evaluate structure-activity relationships (Austel and Kutler, 1983; Joergensen, 1984; Rigsby and Long, 1987). An understanding of the partition properties of a drug is also important in selecting the appropriate additives such as emulsifiers and oils in preparing formulations (Anderson et al., 1981). On the other hand, the importance of distribution methods in analytical chemistry is well-known (Sucha and Kotrly, 1972). Recognition of these facts had led to the development of methods for calculating acidity constants from liquid-liquid distribution measurements. It will be noted that a principal concern in the context of drug distribution is whether a compound is a base, an acid, a neutral molecule or an ion, as this affects very greatly the solubility, ionization and other chemical properties of the drug (Curry, 1980).

Some of the methods applied for evaluating acidity constants from distribution measurements are sophisticated computer programs based on

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Correspondence to: A.G. Asuero, Department of Analytical Chemistry, Faculty of Pharmacy, The University of Seville, 41012 Seville, Spain.

the use (Shaper, 1979; Kubota et al., 1986) of non-linear regression analysis. Nevertheless, others rely on the use of chemical intuition and valid simplifying assumptions (Ezumi and Kubota, 1980; Dagorn et al., 1985) so that appropriate values may be easily calculated. Two principal approaches to the problem of evaluating acidity constants of dibasic acids, H_2R, and diacid bases, R, from liquid-liquid distribution measurements have been considered in this paper. The first involves the usual least-squares procedure applicable when the distribution constant (partition coefficient) is known. In order to determine the pK_a values of diprotolytes we also have formulated a method of analysis of the liquid-liquid distribution data which permits the evaluation of unknown parameters with perhaps more simplicity than by other methods, allowing one in addition to check the correctness of the assumed equilibria.

It should be noted that if either H_2R or R is the uncharged species which partitions, the plot of distribution ratio vs pH does not pass through a maximum, and so, values of overlapping acidity constants cannot be obtained by methods described previously (Asuero, 1988b, c, 1989b). In this paper, the distribution ratio vs pH dependences for diprotolytes is transformed into a hyperbolic cosine relationship to which a trial and error linear bilogarithmic method may be applied. The pertinent theory is outlined below.

Theory

Dibasic acids

For a neutral dibasic acid, H_2R, if only the undissociated species is able to partition into the organic phase, the distribution constant (partition constant) and the distribution ratio (Irving et al., 1978) are respectively given by (charges omitted for the sake of simplicity):

$$K_D = \frac{[H_2R]_0}{[H_2R]} \qquad (1)$$

and

$$D_C = \frac{[H_2R]_0}{[R] + [HR] + [H_2R]} \qquad (2)$$

because the acid concentration in the aqueous phase represents both ionized and non-ionized forms, K_{a1} and K_{a2} being the apparent acidity constants (Asuero, 1988a, 1989a).

Eqn. 2 can be rearranged to give

$$[H] \cdot \left(\frac{K_D}{D_C} - 1 \right) = K_{a1} + K_{a1}K_{a2} \cdot \frac{1}{[H]} \qquad (3)$$

so that the values of K_D may be obtained from the constant D_C at sufficiently low pH values. When the left-hand term of Eqn 3 is plotted vs the reciprocal of the hydrogen ion activity, a straight line ($y = a_0 + a_1x$; single linear regression) should be obtained. Therefore, it is possible to determine K_{a1} from the intercept and K_{a2} from the ratio of the slope to the intercept if it is assumed that no side reactions occur in the aqueous and organic phase (e.g., self-association).

The standard deviation of pK_i values will be given (Asuero et al., 1988) by

$$s_{pK_{ai}} = \log e \cdot \frac{s_{a_0}}{a_0} \quad (i = 1) \qquad (4)$$

$$s_{pK_{ai}} = \log e \cdot \sqrt{\left(\frac{s_{a_0}}{a_0}\right)^2 + \left(\frac{s_{a_1}}{a_1}\right)^2 - 2 \cdot \frac{\text{cov}(a_0, a_1)}{a_0 \cdot a_1}} \quad (i = 2) \qquad (5)$$

where s_{a_0} and s_{a_1} are the standard deviations of the intercept and slope, respectively, of the regression line, and $\text{cov}(a_0, a_1)$ is the covariance between the intercept and slope.

However, the above-described method has the drawback that it precludes the checking of the assumed equilibria. For this reason, a more sophisticated approach will be described in the following.

From Eqn 3 we have

$$\frac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1 = \frac{K_{\mathrm{a1}}}{[\mathrm{H}]\cdot\sqrt{[\mathrm{H}]}}\cdot\left(\sqrt{[\mathrm{H}]} + \frac{K_{\mathrm{a2}}}{\sqrt{[\mathrm{H}]}}\right) \qquad (6)$$

By multiplying and dividing the right hand of Eqn 6 by $\sqrt{(K_{\mathrm{a2}})}$ we obtain

$$\frac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1 = \frac{K_{\mathrm{a1}}\cdot\sqrt{K_{\mathrm{a2}}}}{[\mathrm{H}]\cdot\sqrt{[\mathrm{H}]}}\cdot\left(\sqrt{\frac{[\mathrm{H}]}{K_{\mathrm{a2}}}} + \sqrt{\frac{K_{\mathrm{a2}}}{[\mathrm{H}]}}\right) \qquad (7)$$

and taking into account the definition of a hyperbolic cosine, cosh $x = (e^{x} + e^{-x})/2$, we obtain

$$\frac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1 = 2\cdot\frac{K_{\mathrm{a1}}\cdot\sqrt{K_{\mathrm{a2}}}}{[\mathrm{H}]\cdot\sqrt{[\mathrm{H}]}} \cdot\cosh\left(\frac{\ln 10}{2}\cdot(\mathrm{p}K_{\mathrm{a2}} - \mathrm{pH})\right) \qquad (8)$$

Taking decadic logarithms on both sides of Eqn 8, a further rearrangement leads to

$$\log\left(\frac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1\right) = -\mathrm{p}K_{\mathrm{a1}} - \frac{\mathrm{p}K_{\mathrm{a2}}}{2} + \left[\log\cosh\left(\frac{\ln 10}{2}\cdot(\mathrm{p}K_{\mathrm{a2}} - \mathrm{pH})\right) + \frac{3}{2}\cdot\mathrm{pH}\right] \qquad (9)$$

Eqn 9, however, is not suitable for direct calculation. The computation method suggested here is based on the iterative refining of $\mathrm{p}K_{\mathrm{a2}}$ and $\mathrm{p}K_{\mathrm{a1}}$ as follows. A representation of the left-hand term of Eqn 9 against the term into brackets should give a straight line – if the value of $\mathrm{p}K_{\mathrm{a2}}$ is known – whose slope is unity and has an intercept with the y-axis equal to $-(\mathrm{p}K_{\mathrm{a1}} + \mathrm{p}K_{\mathrm{a2}}/2)$. Different values of $\mathrm{p}K_{\mathrm{a2}}$ are assumed and Eqn 9 is applied. The best value of $\mathrm{p}K_{\mathrm{a2}}$ may be taken as that which minimizes the standard deviation of y, $s_{y/x}$, estimated by the weighted regression line (Asuero and Gonzalez, 1989)

$$s^2_{y/x} = \frac{\sum w_i\cdot(y_i - a_0 - a_1\cdot x_i)^2}{N-2} \qquad (10)$$

where w_i is the normalized weight of the i-th data point. Without proper weighting this calculation procedure does not yield the best values for the acidity constants unless very reliable data have been used.

In the absence of replication, the transformation-dependent weights will be given (Asuero and González, 1989) by

$$w_i^* = \frac{1}{\left(\dfrac{\partial y}{\partial D_{\mathrm{C}}}\right)^2} = \log^2 e\cdot\frac{(K_{\mathrm{D}} - D_{\mathrm{C}})^4}{K_{\mathrm{D}}^2} \qquad (11)$$

The weights calculated in Eqn 11 may be normalized (Sharaf et al., 1986; Asuero and Gonzalez, 1989) such that the average weight is 1

$$w_i = N\cdot\frac{w_i^*}{\sum w_i^*} = N\cdot\frac{\left(\dfrac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1\right)^4}{\sum\left(\dfrac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1\right)^4} \qquad (12)$$

The straight line intersects the x-axis at the point $\mathrm{p}K_{\mathrm{a1}} + \mathrm{p}K_{\mathrm{a2}}/2$, and then

$$\mathrm{p}K_{\mathrm{a1}} + \frac{\mathrm{p}K_{\mathrm{a2}}}{2} = \frac{a_0}{a_1} \qquad (13)$$

from which we may evaluate the value of $\mathrm{p}K_{\mathrm{a1}}$ once the value of $\mathrm{p}K_{\mathrm{a2}}$ is known.

The acidity constants which satisfy the experimental distribution ratio vs pH curve best may be obtained by applying any of the two procedures described above. Nevertheless, when applying the first method one is not able to determine whether a given D_{c} vs pH curve is characterized only by the assumed equilibria. The alternative bilogarithmic procedure suggested here has the advantage of checking if the slope of the straight line obtained differs significantly from unity by applying the t-test (Kennedy and Neville, 1976). If $t_{\mathrm{exp}} = a_1/s_{a1}$ is lower than t_{tab} for $\nu = N - 2$ degrees of freedom at the stipulated level of significance (e.g., $P = 0.05$), the experimental slope does not differ significantly from the theoretical slope of unity. If $t_{\mathrm{exp}} > t_{\mathrm{tab}}$ the null hypothesis is rejected. In applying the t-test we assume, however, that the weighted residuals

$$r_i = \sqrt{w_i} \cdot (y_i - \hat{y}_i) \tag{14}$$

(where $\hat{y}_i$ is the estimated value of y for a given value of x), follow a normal distribution (Draper and Smith, 1981) if a relatively large number of data points is at our disposal. An additional advantage of the bilogarithmic method reported here is that it provides a closed scale representation of y and x, unlike other plots.

An estimate of the uncertainty of these calculations would aid in interpreting experimental results. By applying the random error propagation law (Asuero et al., 1988) to Eqn 13, and taking into account the expressions for s_{a_0}, s_{a_1} and $\mathrm{cov}(a_0, a_1)$ for weighted regression (Asuero and González, 1989) we obtain

$$s^2_{\mathrm{p}K_{a1}} + \left(\frac{1}{2}\right)^2 \cdot s^2_{\mathrm{p}Ka_2}$$

$$= \left[\frac{\sum w_i x_i^2}{\sum w_i} + \left(\frac{a_0}{a_1}\right)^2 + 2\frac{a_0}{a_1}\bar{x}\right] \frac{s^2_{y/x}}{a_1^2 \cdot s_{xx}} \tag{15}$$

The paper of Asuero and González (1989) should be consulted for details, which are too lengthy to include here. From the expression, Eqn 15, we may obtain the standard deviation of $\mathrm{p}K_{a1}$ by assuming, e.g., that $\mathrm{p}K_{a2}$ is free from error.

Diacid bases

For a neutral diacid base, R, we obtain

$$K_{\mathrm{D}} = \frac{[\mathrm{R}]_0}{[\mathrm{R}]} \tag{16}$$

and from the pH dependence of the experimentally determined distribution ratio we have

$$D_{\mathrm{C}} = \frac{[\mathrm{R}]_0}{[\mathrm{R}] + [\mathrm{HR}] + [\mathrm{H_2R}]} = \frac{K_{\mathrm{D}}}{1 + \dfrac{[\mathrm{H}]}{K_{a2}} + \dfrac{[\mathrm{H}]^2}{K_{a2}K_{a1}}} \tag{17}$$

Rearranging Eqn 17 we obtain

$$\frac{1}{[\mathrm{H}]} \cdot \left(\frac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1\right) = \frac{1}{K_{a2}} + \frac{1}{K_{a2}K_{a1}} \cdot [\mathrm{H}] \tag{18}$$

Thus, a representation of the left-hand term of Eqn 18 against the hydrogen ion activity gives a straight line whose reciprocal slope is equal to K_{a2} and the ratio of the intercept to slope is equal to K_{a1}. The value of K_{D} is calculated from the constant D_{C} in the alkaline range of pH. $s_{\mathrm{p}K_{a1}}$ is now given by Eqn 5 ($i = 1$), and $s_{\mathrm{p}K_{a2}}$ by Eqn 4 ($i = 2$).

Exactly analogous expressions to Eqns 8 and 9 are obtained when dealing with diacid bases. In effect, from Eqn 18, after a rather lengthy but straightforward algebra, we obtain

$$\frac{K_D}{D_{\mathrm{C}}} - 1 = 2\frac{[\mathrm{H}]\sqrt{[\mathrm{H}]}}{K_{a2}\sqrt{K_{a1}}} \cosh\left(\frac{\ln 10}{2}(\mathrm{pH} - \mathrm{p}K_{a1})\right) \tag{19}$$

and

$$\log\left(\frac{K_{\mathrm{D}}}{D_{\mathrm{C}}} - 1\right) = \mathrm{p}K_{a2} + \frac{\mathrm{p}K_{a1}}{2} + \left[\log\cosh\left(\frac{\ln 10}{2}(\mathrm{pH} - \mathrm{p}K_{a1})\right) - \tfrac{3}{2}\mathrm{pH}\right] \tag{20}$$

as forms suitable for regression analysis. A representation of the left-hand term of Eqn 20 against the term in brackets of the right-hand side should give a straight line whose slope is unity and with an intercept with the y-axis equal to pK_{a2} plus $pK_{a1}/2$.

The straight line intersects the x-axis in this case at the point $-(pK_{a2} + pK_{a1}/2)$, and then we obtain

$$pK_{a2} + \frac{pK_{a1}}{2} = -\frac{a_0}{a_1} \tag{21}$$

which allows one to evaluate the value of pK_{a2} once the value of pK_{a1} is known. The standard error of pK_{a2} is evaluated in a similar manner as indicated previously for dibasic acids.

The necessary calculations may be easily accomplished with the aid of a microcomputer. The application of the theory developed above to two real systems will be shown in the following.

Applications

The methods described for dibasic acids have been applied to the distribution data given by Kubota and Ezumi (1980) for the 6059S system [6(*R*),7(*R*)-7-[2(*R*)-carboxy-2-(4-hydroxyphenyl)-acetamido]-7-methoxy-3-{[(1-methyl-1H-tetrazol-5-yl)thio]methyl}-8-oxo-5-oxa-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid disodium salt, a β-lactam antibiotic having strong antibacterial activity, which behaves as a diprotic acid with overlapping acidity constants.

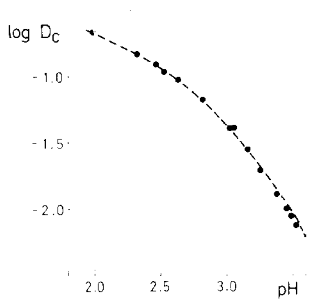

Fig. 1. Distribution ratio data for the 6059S system as a function of pH (the curve in the figure is calculated with the acidity constants given in the text).

The distribution vs pH data of the 6059S system are shown in Fig. 1. The curve is calculated with the acidity constants given in the text (weighted hyperbolic cosine method). The application of Eqn 3 requires a knowledge of the distribution constant K_D. For this reason the value of K_D was successively varied and the procedure applied until the standard deviation of the regression reached a minimum. A poor correlation coefficient was obtained when the usual least-squares procedure based on Eqn 3 (Fig. 2) was applied, but this was improved by discarding points with large reduced residuals.

TABLE 1

Results obtained in the evaluation of overlapping acidity constants and distribution constant of the 6059S system by means of various methods

$\log K_D$	s $\log K_D$	pK_{a1}	$s(pK_{a1})$	pK_{a2}	$s(pK_{a2})$	Calculation method
−0.594	0.122	2.490	0.179	3.239	0.092	parabolic regression (Kubota and Ezumi, 1980)
−0.558	–	2.433	0.020	3.272	0.038	classical least squares based on Eqn 3
−0.538	–	2.439	0.010	3.09	–	unweighted bilogarithmic method
−0.534	–	2.377	0.018	3.377	–	weighted bilogarithmic method
−0.549	0.010	2.409	0.032	3.337	0.084	weighted parabolic regression (Asuero, unpublished data)

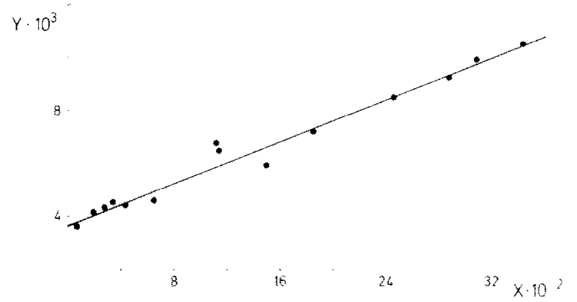

Fig. 2. Evaluation of acidity constants of the 6059S system by means of the usual least squares procedure: $Y = [H](K_D/D_C - 1)$ and $X = 1/[H]$.

Varying values of K_D were also assumed (0.0025 as increment in K_D) when both the unweighted and weighted bilogarithmic hyperbolic cosine method was applied. It is worth noting that the weighted procedure leads to a value of the slope closer to the theoretical value of unity expected as well as to a pK_{a1} lower and a pK_{a2} higher, respectively, than both the unweighted bilogarithmic hyperbolic cosine and the usual least-squares procedures. A summary of the methods applied is collected in Table 1. The bilogarithmic plot for the 6059S system (weighted case) is shown in Fig. 3, leading to pK_a values which are close to those obtained by applying the weighted parabolic regression procedure.

The methods described for diacid bases have been applied to the distribution data given by Kubota and Ezumi (1980) for the *o*-toluidine system as a function of pH, which is depicted in Fig. 4, where the curve is calculated with the acidity constants and distribution constant given in the text (weighted hyperbolic cosine method procedure). Experimental data in the high pH region are some distance from the behaviour of a hypothetical diacid base model as can be seen in the picture. As a matter of fact, the $\{pH, D_C\}$ point $\{5.809, 240.8\}$ must be discarded in the calculations, in order to obtain a sufficiently high correlation coefficient when the classical least squares procedure based on the use of Eqn. 18 is applied. In any case, Fig. 5 reveals a certain dispersion of the corresponding transformed (x, y) data pairs.

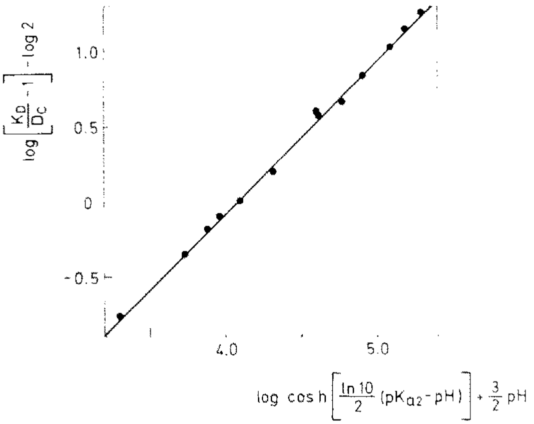

Fig. 3. Bilogarithmic plot for the 6059S system.

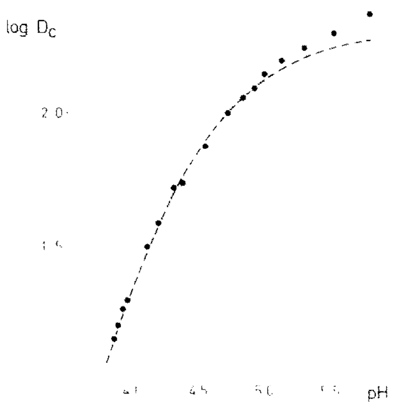

Fig. 4. Distribution ratio data for the *o*-toluidine system as a function of pH (the curve in the figure is calculated with the acidity constants given in the text).

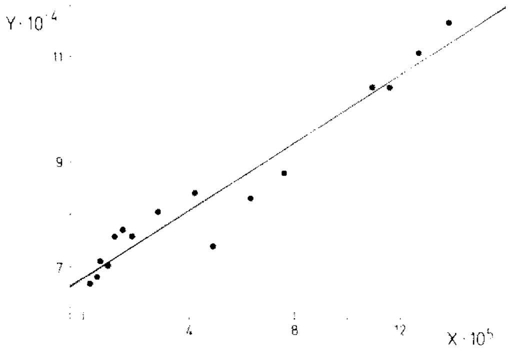

Fig. 5. Evaluation of acidity constants of the *o*-toluidine system by means of the usual least squares procedure: $Y = (K_D/D_C - 1)/[H]$ and $X = [H]$.

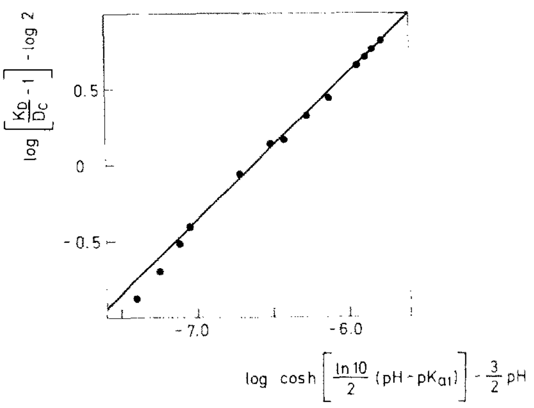

Fig. 6. Bilogarithmic plot for the *o*-toluidine system.

Better correlation coefficients are, undoubtedly, obtained when applying the unweighted and weighted bilogarithmic hyperbolic cosine method assuming a given value of K_D in each case, and varying the pK_{a1} value in order that the standard deviation of the regression line reaches a minimum. However, an optimum in the $s_{y/x}$ value is not obtained with the application of the weighted bilogarithmic hyperbolic cosine method because the lower value admissible for K_D was 204, only one D_C unit higher than the distribution ratio value obtained at pH 5.53. Again, the weighted procedure leads to a value of the slope of the regression line closer to the theoretical value of unity expected.

Results obtained by the application of the various calculation procedures are compiled in Table 2, which reveal that the pK_a values obtained by means of the weighted bilogarithmic hyperbolic cosine method are very similar to those obtained with the parabolic regression procedure with the {pH,D_C} point {5.809,240.8} being discarded. The weighted bilogarithmic plot for *o*-toluidine is shown in Fig. 6.

The pH vs D_C data for the two systems object of study (Kubota and Ezumi (1980)) are given in the following.

6059S system Data pairs (pH,D_C): 1.890,0.2154; 2.321,0.1486; 2.462,0.1233; 2.527,0.1098; 2.638,0.9408$E-1$; 2.821,0.6964$E-1$; 3.060,0.3270$E-1$; 3.053,0.3207$E-1$; 3.178,0.2797$E-1$; 3.267,0.1936$E-1$; 3.390,0.1266$E-1$; 3.460,0.9908$E-2$; 3.490,0.8766$E-2$; 3.537,0.7453$E-2$.

o-Toluidine Data pairs (pH,D_C): 3.860,14.13; 3.893,15.93; 3.934,18.49; 3.960,19.59; 4.120,31.61; 4.200,38.84; 4.310,52.47; 4.379,53,83; 4.550,74.41; 4.730,100.5; 4.830,113.4; 4.911,125.9; 5.018,144.8; 5.140,160.2; 5.290,180.1; 5.530,203.0; 5.809,240.8.

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TABLE 2

Results obtained in the evaluation of overlapping acidity constants and distribution constant of the o-toluidine system

$\log K_D$	s $\log K_D$	pK_{a1}	s(pK_{a1})	pK_{a2}	s(pK_{a2})	Calculation method
2.334	0.031	3.895	0.058	4.890	0.060	parabolic regression (Kubota and Ezumi, 1980)
2.316	0.033	3.919	0.060	4.658	0.063	parabolic regression (point no. 17 discarded)
2.386	–	3.679	0.037	4.832	0.010	classical least squares based on Eqn 18
2.413	–	4.008	–	4.828	0.006	unweighted bilogarithmic method
2.308	–	3.951	–	4.654	0.009	weighted bilogarithmic method
2.396	0.006	3.465	0.110	4.875	0.020	weighted parabolic regression (Asuero, unpublished data)

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