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Spectrophotometric Evaluation of Acidity Constants: Can a Diprotic Acid be Treated as a Monoprotic One?

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Abstract Spectrophotometric methods are both sensitive and suitable for studying acidity constants in solutions. These methods imply the direct determination of the mole ratio of acid-base conjugate pairs through absorbance (A) measurements in a series of solutions of known pH. The evaluation of acid dissociation constants is very simple, if the species involved in the equilibria may be obtained in pure form (i.e. the limit absorbances A_2 and A_0 of H_2R and R species respectively, are known), and do not overlap (i.e. A_1, the limit absorbance of the specie HR is also known). The situation is more complex when the two ionizing groups of a substance lie within three pK_a units of one another; the absorbance of the intermediate specie HR cannot then be determined experimentally and calculations being necessarily involved. Based on the expression of the absorbance as a function of the concentration for a diprotic acid, it is possible to calculate the pH values for which the absorbances coincide with the mean of the limit values of the absorbances corresponding to the different species: $A^*=(A_2+A_1)/2$ and $A^{**}=(A_1+A_0)/2$. Then the limit values of pH^* and pH^{**}, and the parameters $\alpha = pK_{a1}-pH^*$ and $\beta = pH^{**}-pK_{a2}$ are calculated, checking in turn under what conditions a diprotic acid can be treated as a monoprotic one from a spectrophotometric point of view. Nevertheless, in order to apply the above expressions A_1 must be known, which can be made by the Polster method, i.e. by measuring the absorbances of varying pH solutions at two wavelengths λ_1 and λ_2, using orthogonal regression method $A_{\lambda1}$ versus $A_{\lambda2}$ (similar errors are assumed in both axis). In this work, a method of evaluation of acidity constants based on the rearrangement of the A versus pH expression is applied which implies the use of a straight-line ($y=a_0+a_1x$ method) in order to separate the variables K_{a2} ($=1/a_0$) and K_{a1} ($=a_0/a_1$). The method presupposes the prior knowledge of A_1, which may be previously obtained by the Polster method. The theory developed in this paper has been successfully applied to the experimental data reported in the literature for the resorcinol system.

Keywords Acidity constant, Spectrophotometric methods, Polster method, Resorcinol system

1. Introduction

Among the physico-chemical properties of molecules, the acidity constants are of vital importance both in the analysis of drugs as well as in the interpretation of their mechanism of action [1-9]. The solution of many galenical problems requires the knowledge of the acidity constants of compounds [10] having pharmaceutical interest. 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 concentration of protonated and non-protonated forms in various media, the knowledge of acidity constants [11-15] being thus of great worth. Evaluation of acidity constants of organic reagents is also of great value in planning analytical work [16, 17], e.g., the acidity constants can be employed in the design of titration procedures [18] and examining the possibility of separation of mixtures of compounds by extraction. The complexing properties of a molecule depends on the number and steric disposition of is donor centres as well as on its acid-base properties [19-21].

The ionization equilibrium of a monobasic acid

$$HR \rightleftharpoons H + R \tag{1}$$

is characterized by the acidity constant

$$K_a = \frac{[H][R]}{[HR]} = \frac{1}{\beta_1} \tag{2}$$

β_1 is the stability constant of HA, i.e. the constant corresponding to the formation equilibria H + R = HR. The ionic strength and temperature of the solution are assumed to be constant, so that mixed or conditional constants are used in the calculations. Charges are omitted for simplicity.

If A is the measured absorbance (for 1-cm pathlength) of a solution containing a total concentration

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$$C_R = [R] + [HR] \tag{3}$$

of the acid, then assuming that Beer's law holds, we have [22, 23]

$$A = f_0 A_0 + f_1 A_1 \tag{4}$$

$$A = \frac{A_0 + A_1 \frac{[H]}{K_a}}{1 + \frac{[H]}{K_a}} \tag{5}$$

where f_0 and f_1 are the molar fractions of R and HR

$$f_0 = \frac{1}{1+\beta_1[H]} = \frac{1}{1+\frac{[H]}{K_a}}$$
$$f_1 = \frac{\beta_1[H]}{1+\beta_1[H]} = \frac{\frac{[H]}{K_a}}{1+\frac{[H]}{K_a}} \tag{6}$$

and A_0 and A_1 are the limit absorbances of the species R and HR, respectively, i.e. the absorbances of the pure forms of the reagent R and HR, respectively, which have molar absorptivities ε_0 and ε_1; $A_0=\varepsilon_0 C_R$ and $A_1=\varepsilon_1 C_R$. Eqn. (5) on rearrangement gives

$$K_a = [H]\left(\frac{A - A_1}{A_0 - A}\right) \tag{7}$$

The slope of the A-pH curve (Figure 1) is given by

$$\frac{dA}{d(pH)} = \frac{d[H]}{d(pH)} \cdot \frac{dA}{d[H]}$$
$$= \left(-\ln 10[H]\right)\left(\frac{(A_0 - A_1)}{K_a} \cdot \frac{1}{\left(1+\frac{H}{K_a}\right)^2}\right) \tag{8}$$
$$= -\ln 10\left(A_1 - A_0\right)\frac{\frac{[H]}{K_a}}{\left(1+\frac{H}{K_a}\right)^2}$$

Differentiation of Eqn. (8) with respect to pH leads to

$$\frac{d^2A}{d(pH)^2} = \frac{d[H]}{d(pH)} \cdot \frac{d}{d(pH)}\left(\frac{dA}{d(pH)}\right)$$
$$= \left(-\ln 10 \frac{(A_1 - A_0)}{K_a} \cdot \frac{\left(1 - \frac{[H]}{K_a}\right)}{\left(1+\frac{[H]}{K_a}\right)^3}\right.$$
$$= \ln^2 10 \frac{(A_1 - A_0)}{K_a} \cdot \frac{[H]\left(1 - \frac{[H]}{K_a}\right)}{\left(1+\frac{[H]}{K_a}\right)^3} \tag{9}$$
$$= \ln 10\left(f_0 - f_1\right) \cdot \frac{dA}{d(pH)}$$

The condition $d^2A/d(pH)^2$ will locate the point of inflexion (Figure 1) in the graph of A against pH [23-25]. At this point $[H] = K_a$, and then by applying Eqn. (5) we have at this point (A'', pH'')

$$A'' = \frac{A_1 + A_0}{2} \tag{10}$$

Note that the value of dA/d(pH) at this point [26] is given by

$$\left(\frac{dA}{d(pH)}\right)_{pH''} = -\ln 10\left(A_1 - A_0\right) \cdot 0.5^2 = -0.576 \cdot \Delta A \tag{11}$$

where $\Delta A = A_1 - A_0$.

The pK_a value of a monoprotic acid can thus be determined by plotting absorbances as a function of pH for a series of solutions having a constant concentration C_R of reagent. The inflexion point of the curve A versus pH, i.e. the value of pH (equal to pH'') that satisfies the condition (10) coincides exactly with the pK_a value. Although this is strictly true for monoprotic acids, one can wonder if this simple procedure is applicable to diprotic acids. In other words: can a diprotic acid be treated as a monoprotic one? An answer to this question is given in that follows.

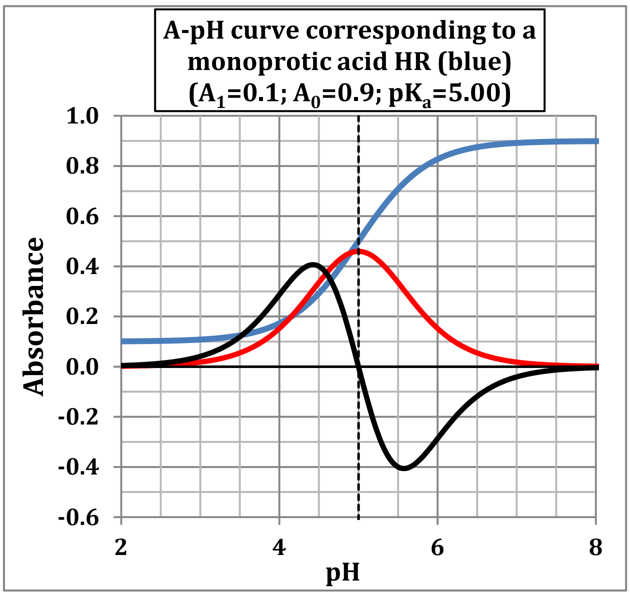

Figure 1. Absorbance-pH curve corresponding to a monoprotic acis HR (blue), and first derivative A-pH curve (red) and second derivative A-pH curve (black)

2. The Diprotic Acid System

For the dissociation of a diprotic acid H_2R we have the equilibria

$$H_2R \rightleftharpoons HR + H \qquad HR \rightleftharpoons R + H \qquad (12a,b)$$

described by the equations

$$K_a = [H]\left(\frac{A - A_1}{A_0 - A}\right) \qquad K_2 = \frac{[R][H]}{[HR]} \qquad (13a,b)$$

where we are specifically neglecting charges for the sake of the generality. The absorbance and the composition of any given solution of a diprotic acid having concentration C_R (Figure 2) is given by [22, 27-30]

$$A = A_2 f_2 + A_1 f_1 + A_0 f_0 \qquad (14)$$

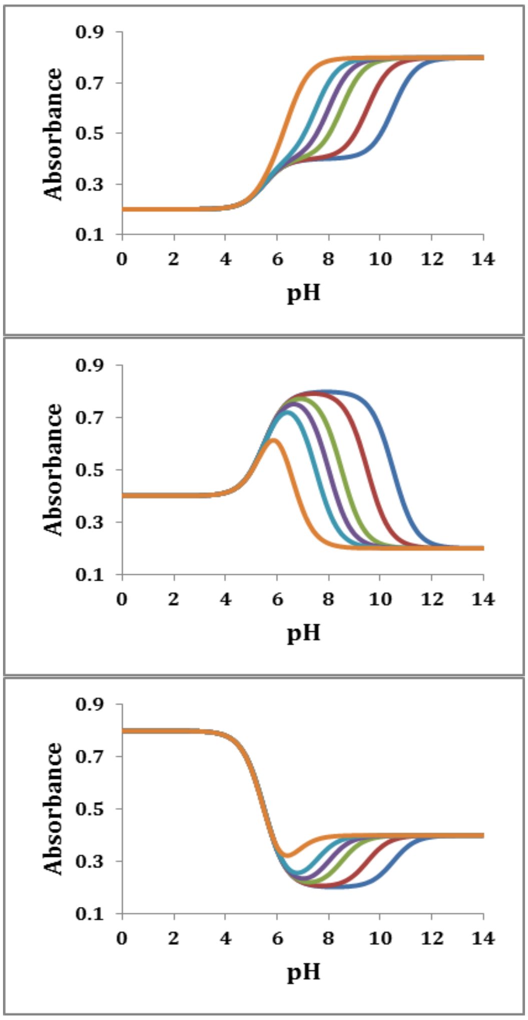

Figure 2. Top: A_2= 0.2; A_1=0.4; A_0=0.8; pK_{a1}=5.5 and varying ΔpK_a. Middle: A_2=0.4; A_1=0.8; A_0=0.2; pK_{a1}=5.5 and varying ΔpK_a. Bottom: A_2=0.8; A_1=0.2; A_0=0.4; pK_{a1}=5.5 and ΔpK_a (ΔpK_a: 1; 2; 2,5; 3, 4; and 5)

$$A = \frac{A_0 + A_1 \frac{[H]}{K_{a2}} + A_2 \frac{[H]^2}{K_{a1}K_{a2}}}{1 + \frac{[H]}{K_{a2}} + \frac{[H]^2}{K_{a1}K_{a2}}} \qquad (15)$$

where A_0, A_1, and A_2 are the limit absorbances of R, HR and H_2R, respectively, and f_2, f_1 and f_0 the molarity fractions ($f_j=[H_jR]/CR$ and $A_j=\varepsilon_j C_R$). Let A^* and A^{**} be the absorbance values of two samples which have pH values pH^* and pH^{**}, respectively, which satisfies the following conditions

$$A^* = \frac{A_2 + A_1}{2} \qquad A^{**} = \frac{A_1 + A_0}{2} \qquad (16a,b)$$

Substituting condition (16a) into Eqn. (15), upon rearrangement and collecting the terms containing identical powers of $[H^*]$ one obtains

$$\left[H^*\right]^2 - K_{a1}\left[H^*\right] + \frac{2A_0 - (A_1 + A_2)}{A_2 - A_1} K_{a1}K_{a2} = 0 \qquad (17)$$

Solving this quadratic equation for the concentration of hydrogen ions gives

$$\left[H^*\right] = \frac{K_{a1}}{2}\left(1 + \sqrt{1 - 4\left(\frac{2A_0 - (A_1 + A_2)}{A_2 - A_1}\right)\frac{K_{a2}}{K_{a1}}}\right) \qquad (18)$$

In this case the negative root has no physical significance. A more compact version of Eqn. (18) can be obtained by using

$$x = pK_{a2} - pK_{a1} \qquad a = 4\left(\frac{2A_0 - (A_1 + A_2)}{A_2 - A_1}\right) \qquad (19a,b)$$

and thus

$$\left[H^*\right] = K_{a1}\left(\frac{1 + \sqrt{1 - a \cdot 10^{-x}}}{2}\right) \qquad (20)$$

From Eqn. (20) it is seen that $[H^*]=K_{a1}$ only if – a 10^{-x} is negligible compared with the unity. Effectively when x is large to unite there is a wide range of concentration of [H] over which $f_1=[HR]/C_R$ is very close to 1, as well as the square root included in the expression (20), and then

$$\lim_{x\to\infty}\left[H^*\right] = K_{a1} \qquad (21)$$

A not unexpected result. The value of the ratio $K_{a1}/K_{a2}=10^x$ is an important property of a diprotic acid, because this relation can be regarded as the equilibrium constant of the following reaction [31]

$$H_2R + R \rightleftharpoons 2HR \qquad (22)$$

$$K_{eq} = \frac{K_{a1}}{K_{a2}} = \frac{[HR]^2}{[H_2R][R]} \qquad (23)$$

On the other hand, Eqns. (16b) and (15) can be combined to give, once upon rearrangement and collecting the terms in powers of [H]

$$\left[H^{**}\right]^2 + \frac{A_1 - A_0}{2A_2 - (A_0 + A_1)} K_{a1}\left[H^{**}\right] + \frac{A_0 - A_1}{2A_2 - (A_0 + A_1)} K_{a1}K_{a2} = 0 \qquad (24)$$

The meaningful solution of this quadratic equation is

$$\left[H^{**}\right] = K_{a1}\left(\frac{1}{2}\left(\frac{A_1 - A_0}{A_0 + A_1 - 2A_2}\right)\left(1 - \sqrt{1 - 4\left(\frac{A_0 + A_1 - 2A_2}{A_1 - A_0}\right)\frac{K_{a2}}{K_{a1}}}\right)\right) \quad (25)$$

The form in which Eqn. (25) is presented is important. In effect, although it is evident from Eqn. (20) that $[H^*]$ tends to K_{a1} when x is large, it is not clear from Eqn. (25) that $[H^{**}]$ tends to K_{a2} when x is large. In order to place the limiting process on a sounder basis we will demonstrate that the limit of the ratio is actually the desired quantity. Taking into account expression (19a) we get for $[H^{**}]$

$$\left[H^{**}\right] = K_{a2}\left(\frac{10^x}{2}\left(\frac{A_1 - A_0}{A_0 - A_1 + 2A_2}\right)\left(1 - \sqrt{1 - 4\left(\frac{A_0 - A_1 + 2A_2}{A_1 - A_0}\right)\cdot 10^{-x}}\right)\right) \quad (26)$$

which can conveniently be re-written as follows

$$\left[H^{**}\right] = K_{a2}\left(\frac{1 - \sqrt{1 - \frac{c}{10^x}}}{\frac{1}{b\cdot 10^x}}\right) \quad (27)$$

where for the sake of brevity

$$b = \frac{1}{2}\left(\frac{A_1 - A_0}{A_0 + A_1 - 2A_2}\right) \qquad c = 4\left(\frac{A_0 + A_1 - 2A_2}{A_1 - A_0}\right) \quad (28a,b)$$

The limit of the term in parenthesis, f(x), in Eqn. (27) when x is very large to the unity is the unity. Effectively, by applying L'Hôpital's rule [32, 33] we get

$$\lim_{x\to\infty} f(x) = \frac{f'\left(1 - \sqrt{1 - \frac{c}{10^x}}\right)}{f'\left(\frac{1}{b\cdot 10^x}\right)} = \frac{bc}{2} = 1 \quad (29)$$

Rewriting Eqns. (20) and (27) in logarithmic form gives

$$\alpha = pK_{a1} - pH^* = \log\left(1 + \sqrt{1 - a\cdot 10^{-x}}\right) - \log 2 \quad (30)$$

$$\beta = pH^{**} - pK_{a2} = \log 2 - x - \log\left(2b\left(1 - \sqrt{1 - c\cdot 10^{-x}}\right)\right) \quad (31)$$

The values of α and β as a function of x for a few systems taken as examples are shown in Table 1, where it is seen that the situation of pK_a values in the pH scale with respect to pH^* and pH^{**} is dependent on the relative values of the limit absorbances A_0, A_1 and A_2, as well as on the value of $x=\Delta pK_a$. However, except for very close pK_a values the acidity constants are easily experimentally obtained making use of expressions (16a,b).

Table 1. Dependence of the α and β values with ΔpK_a

A_2	A_1	A_0	4.00	3.00	2.50	2.00	1.00	← ΔpK_a
0.2	0.4	0.8	0.000	0.002	0.007	0.020	0.135	← α
			0.000	0.001	0.003	0.080	0.068	← β
0.4	0.8	0.2	0.000	-0.001	-0.008	-0.009	-0.140	← α
			0.000	0.000	0.000	-0.001	-0.015	← β
0.8	0.2	0.4	0.000	0.000	0.000	0.001	0.014	← α
			0.000	-0.001	-0.007	-0.024	[a]	← β

[a] Imaginary result

Nevertheless, Eqns. (16a,b) though instructive are not very useful since A_1 must be known. In cases in which equilibria overlap A_1 is usually evaluated together with K_{a1} or K_{a2} by applying graphical methods of evaluation [34], although in some numerical and graphical method of evaluation K_{a1} and K_{a2} are simultaneously evaluated whereas A_1 is not. Thus, at first glance the calculations given above are not only somewhat a waste of time, but are also philosophically unattractive. How can be avoided this logical absurdity? Are the expression derived above merely an academic exercise?. A new method reported by Polster [35-39] and based on the measurements of absorbances at two wavelengths (λ and λ^*) allows to evaluate graphically the limit absorbances A_1 and A_1^* for the intermediate specie HR, thus making the matter presented in this work useful both in research as in the teaching of chemical equilibria methods at all levels.

3. Evaluation of the Limit Absorbance A_1 from Absorbance Measurements at Two Wavelengths

In the evaluation of acidity constants of overlapping equilibria approximations of various sorts are frequently made in order to carry out calculations. Working at low pH values where it is only assumed the presence of the species H_2R and HR, i.e. f_0 is close to zero, and from Eqn. (14), we have for measurements at two wavelengths λ and λ^*

$$A = A_1 f_1 + A_2 f_2 = A_1 f_1 + A_2(1 - f_1) = (A_1 - A_2) f_1 + A_2 \quad (32)$$

$$A^* = \left(A_1^* - A_2^*\right) f_1 + A_2^* \quad (33)$$

since in this specific case $f_2 + f_1 = 1$. Whence it follows that (rearrangement f_1 from Eqns. (32) and (33) and equating the result in both cases)

$$\frac{A-A_2}{A^*-A_2^*}=\frac{A_1-A_2}{A_1^*-A_2^*}=k \tag{34}$$

From which

$$A=kA^*+A_2-kA_2^* \tag{35}$$

and thus plotting A against A^* for a series of solutions we obtain a straight line having a slope equal to k and an intercept on the ordinate axis equal to $A_2 - k A_2^*$. In much the same fashion one can obtain in the pH range in which the species HR and R are present that the absorbance at any particular wavelength is given by

$$A=\left(A_1-A_0\right) f_1+A_0 \tag{36}$$

since in this case $f_2 \simeq 0$, and then from measurements at two wavelengths λ and λ^* we have

$$\frac{A-A_0}{A^*-A_0^*}=\frac{A_1-A_0}{A_1^*-A_0^*}=k' \tag{37}$$

from which

$$A=k'A^*+A_0-k'A_0^* \tag{38}$$

A plot of A against A^* for a series of solutions will be a straight line. Its slope will be equal to *k'* and the point at which $A^*=0$ is equal to $A_0 - k' A_0^*$. It should be noted that Eqns. (32) and (36) are strictly true only when two species are present in solution. However, when equilibria overlaps there is a range of pH in the neighbourhood of ½ ($pK_{a1}+pK_{a2}$) where the three species R, HR and H_2R are present in solution, a curvature being obtained in both representations with these points.

Equations. (34) and (37) are really particular examples of the more general case, easily derivable from Eqns. (33) and (36)

$$\frac{A-A_j}{A^*-A_j^*}=\text{constant} \tag{39}$$

previously used by Coleman et al. [40] in the determination of the number of species, n, in solution. When n=2 the plot of the absorbance minus the absorbance of a solution j taken as reference, at λ, against the absorbance minus the absorbance of the reference solution, at λ^*, for a series of solutions, gives a straight line passing through the origin. Working with different pairs of wavelengths a family of straight lines intersecting in the origin of coordinates is obtained.

It can be easily demonstrated that the point of intersection of the two straight lines is given by

$$\left(\frac{A_0-k'A_0^*-\left(A_2-kA_2^*\right)}{k-k'},\frac{k'\left(A_2-kA_2^*\right)-k\left(A_0-k'A_0^*\right)}{k'-k}\right) \tag{40}$$

On the other hand the required unknowns A_1 and A_1^* can be obtained by solving pairs of simultaneous equations derived from Eqns. (35) and (38)

$$kA_1-A_1=kA_2^*-A_2 \tag{41}$$

$$k'A_1^*-A_1=k'A_0-A_0 \tag{42}$$

which lead to

$$A_1=\frac{k'\left(A_2-kA_2^*\right)-k\left(A_0-k'A_0^*\right)}{k'-k} \tag{43}$$

$$A_1^*=\frac{A_0-k'A_0^*-\left(A_2-kA_2^*\right)}{k-k'} \tag{44}$$

Comparing Eqns. (43) and (44) and Eqn. (40) we see that the coordinates of the point of intersection of both straight lines defined by Eqns. (35) and (38) are given by (A_1^*, A_1). By substituting the expression for k and k' given by Eqns. (34) and (37), respectively, into Eqn. (40) we also get the coordinates of the intersecting point, but much algebra would be needed to achieve the same results.

Plots of A against A^* are easy to construct, especially with the aid of a spreadsheet. It is a simple matter to evaluate A_1 and A_1^* from such a plot; the extended tangents or limiting slopes of straight lines should intersect at the point (A_1^*, A_1). From an experimental point of view the conditions more favourable for obtain A-pH data is spectrophotometric titration, but fairly precise results can be obtained and good accuracy can be achieved obtaining a number of data closely spaced. However, arithmetic calculation in this method is reduced at a minimum, which undoubtedly constitutes an attractive feature with purposes of teaching in the undergraduate analytical laboratory if comparing with other graphical or numerical methods of evaluation.

Nevertheless, although it is true that at the pH values corresponding to the absorbances A^* and A^{**} we have ($\Delta pK_a>2$)

$$pH^*=pK_{a1} \qquad pH^{**}=pK_{a2} \tag{45}$$

there seems little point in measuring the whole absorbance versus pH graphs merely to determine three points. So, the constants obtained are little efficient in terms of return for effort used [41]. Another way, which is more complicated but which uses the data more efficiently and provides a much more reliable value A_1 and A_1^* is described in the following. An orthogonal regression method should be applied to A versus A^* data because the two axes are affected by errors of similar magnitude.

4. Orthogonal Regression

We can apply a least squares method to the experimental data (A^*, A), but single linear regression is not strictly applicable to fitting the best straight line through data points because both variables A and A^* contain analogous random error of measurement [42-45].

The perpendicular distance from the point (x_i, y_i) to the line whose algebraic representation is

$$\hat{y}=a_0+a_1x \tag{46}$$

is given by

$$r_i = \frac{y_i - (a_0 + a_1 x_i)}{\sqrt{1+a_1^2}} \quad (47)$$

We will minimize the sum of the squares of the distance perpendicular to the least squares line

$$Q = \sum r_i^2 = \sum \left(\frac{y_i - a_0 - a_1 x_i}{\sqrt{1+a_1^2}} \right)^2 = \frac{1}{1+a_1^2} \sum (y_i - a_0 - a_1 x_i)^2 \quad (48)$$

Note that there is only to unknown quantities in Q: a_0 and a_1. If Q is to be a minimum the first partial derivatives of Q with respect to a_0 and a_1 must be zero. Then

$$\frac{\partial Q}{\partial a_0} = \frac{2}{1+a_1^2} \sum (y_i - a_0 - a_1 x_i)(-1) = 0 \quad (49)$$

and

$$\sum y_i - N a_0 - a_1 \sum x_i = 0 \qquad \overline{y} = a_0 + a_1 \overline{x} \quad (50a, b)$$

On the other hand by combing Eqns. (48) and (50b) we have

$$\begin{aligned} Q &= \frac{1}{1+a_1^2} \sum \left(y_i - \overline{y} - a_1 (x_i - \overline{x}) \right)^2 \\ &= \frac{1}{1+a_1^2} \left(S_{YY} - 2a_1 S_{XY} + a_1^2 S_{XX} \right) \end{aligned} \quad (51)$$

where S_{XX}, S_{YY} are sums of squares about the mean for two variables (*x* and *y*), and S_{XY} is the corresponding sum of cross-products

$$S_{XX} = \sum (x_i - \overline{x})^2 = \sum x_i^2 - \frac{\left(\sum x_i\right)^2}{N} \quad (52)$$

$$S_{YY} = \sum (y_i - \overline{y})^2 = \sum y_i^2 - \frac{\left(\sum y_i\right)^2}{N} \quad (53)$$

$$S_{XY} = \sum (x_i - \overline{x})(y_i - \overline{y}) = \sum x_i y_i - \frac{\left(\sum x_i\right)\left(\sum y_i\right)}{N} \quad (54)$$

and thus

$$\begin{aligned} \frac{\partial Q}{\partial a_1} &= \frac{1}{1+a_1^2} (-2S_{XY} + 2a_1 S_{XX}) \\ &+ \left(\frac{-2a_1}{\left(1+a_1^2\right)^2} \right) \left(S_{YY} - 2a_1 S_{XY} + a_1^2 S_{XX} \right) \end{aligned} \quad (55)$$

$$\left(1+a_1^2\right)(-2S_{XY} + 2a_1 S_{XX}) - 2a_1 \left(S_{YY} - 2a_1 S_{XY} + a_1^2 S_{XX} \right) = 0 \quad (56)$$

$$S_{XY} a_1^2 + a_1 (S_{XX} - S_{YY}) - S_{XY} = 0 \quad (57)$$

$$a_1 = \frac{S_{YY} - S_{XX} \pm \sqrt{(S_{XX} - S_{YY})^2 + 4S_{XY}^2}}{2S_{XY}} \quad (58)$$

The meaningful solution of the quadratic Eqn. (57) gives the value of a_1. The sign of a_1 is the same as the sign of S_{XY}. Once the value of a_1 is known, the value of a_0 is calculated from Eqn. (50b). The (A_1^*, A_1) point is defined by the intersection of the extrapolated linear portion of the two branches obtained (by the least squares method above described) plotting A against A^*, $y=a_0+a_1x$ and $y = b_0+b_1x$, and then we have

$$\left(\frac{b_0 - a_0}{a_1 - b_1}, \frac{a_1 b_0 - b_1 a_0}{a_1 - b_0} \right) = \left(A_1^*, A_1 \right) \quad (59)$$

In those cases in which measurement errors affect both axes in an unequal way, Boccio et al. [46], and McCartin [47, 48] should be consulted, in addition to the references cited at the beginning of this section. Once the limit absorbance, A_1, of the intermediate specie HR, is known the acidity constants can be evaluated as follows.

5. Spectrophotometric Evaluation of Acidity Constants

From Eqn. (15) we get

$$(A - A_0) + (A - A_1)\frac{[H]}{K_{a2}} + (A - A_2)\frac{[H]^2}{K_{a2}K_{a1}} = 0 \quad (60)$$

which on rearrangement gives

$$\left(\frac{A - A_0}{A_1 - A} \right) \frac{1}{[H]} = \frac{1}{K_{a2}} + \frac{1}{K_{a2}K_{a1}} \left(\frac{A - A_2}{A - A_1} \right) [H] \quad (61)$$

A plot of the left hand against [H] $(A-A_2)/(A-A_1)$ gives a straight line of slope $1/(K_{a2}K_{a1})$ and intercept $1/K_{a2}$ from which

$$K_{a2} = \frac{1}{a_0} \quad (62)$$

$$K_{a1} = \frac{a_0}{a_1} \quad (63)$$

6. Error Analysis

All analytical measurements are random variables and the information they provide is subject to uncertainty. Changes of interest are usually based on a set of observations and we want to know if the mean and variance of these functions are related to the mean, variance and covariance of the original measurements. The relationship for calculating the variance s_R^2 of a continuous arbitrary function of multiple variables, x_1, x_2 ... x_N, which are normally distributed with variances $s_{x_i}^2$, is known as the law of random error propagation [49, 50], which is expressed as

$$R = f(x_1, x_2, \ldots x_N) \quad (64)$$

$$s_R^2 = \sum_i \left(\frac{\partial R}{\partial x_i} \right)^2 s_{x_i}^2 + 2 \sum_{i=j} \left(\frac{\partial R}{\partial x_i} \right) \left(\frac{\partial R}{\partial y_j} \right) \quad (65)$$

The law of error propagation applied to the function R = f (a_0, a_1) leads to

$$s_R^2 = \left(\frac{\partial R}{\partial a_0}\right) s_{a_0}^2 + \left(\frac{\partial R}{\partial a_1}\right) s_{a_1}^2 + 2\left(\frac{\partial R}{\partial a_0}\right)\left(\frac{\partial R}{\partial a_1}\right)\operatorname{cov}(a_0, a_1) \quad (66)$$

s_{a0}^2, s_{a1}^2, and cov(a_0, a_1), are the variance of the intercept, the variance of the slope and the covariance between the intercept and the slope of the regression line using the conventional least squares method in this case

$$a_1 = \frac{S_{XY}}{S_{XX}} \quad (67)$$

$$a_0 = \overline{y} - a_1\overline{x} \quad (68)$$

$$s_{a_1}^2 = \frac{s_{y/x}^2}{S_{XX}} \quad (69)$$

$$s_{a_0}^2 = \left(\frac{1}{n} + \frac{\overline{x}^2}{S_{XX}}\right) s_{y/x}^2 \quad (70)$$

$$\operatorname{cov}(a_0, a_1) = -\overline{x}\frac{s_{y/x}^2}{S_{XX}} \quad (71)$$

where $s_{y/x}^2$ is the variance of the regression line, given by

$$s_{y/x}^2 = \frac{Q}{n-2} = \frac{\sum r_i^2}{n-2} = \frac{\sum\left(y_i - (a_0 + a_1 x_i)\right)^2}{n-2} = \frac{S_{YY} - a_1^2 S_{XX}}{n-2} \quad (72)$$

Note that the $s_{y/x}$ value can be easily get using linear regression (method of the least squares), in EXCEL, with the function LINEST (but no the covariance between the intercept and the slope).

Since K_{a2} is a function of the intercept

$$K_{a2} = f(a_0) \quad (73)$$

the variance of K_{a2} is equal to

$$s_{k_{a2}}^2 = \left(\frac{\partial K_{a2}}{\partial a_0}\right)^2 s_{a_0}^2 = \left(\frac{\partial\left(\frac{1}{a_0}\right)}{\partial a_0}\right)^2 s_{a_0}^2 = \left(\frac{-1}{a_0^2}\right)^2 s_{a_0}^2 = \frac{s_{a_0}^2}{a_0^4} \quad (74)$$

and as pK_{a2}, is a function of K_{a2}

$$pK_{a2} = f(K_{a2}) \quad (75)$$

we can calculate the variance of pK_{a2} as

$$\begin{aligned} s_{pK_{a2}}^2 &= \left(\frac{\partial pK_{a2}}{K_{a2}}\right)^2 s_{K_{a2}}^2 = \left(\frac{-\log K_{a2}}{K_{a2}}\right) s_{K_{a2}}^2 \\ &= \left(\frac{-1}{1n10 K_{a2}}\right)^2 s_{K_{a2}}^2 = \frac{1}{1n10 k_{a2}^2} s_{K_{a2}}^2 \\ &= (\log e)^2 \frac{s_{a_0}^2}{a_0^2} \end{aligned} \quad (76)$$

and its standard deviation as

$$s_{pK_{a2}} = \log e \cdot \left(\frac{s_{a_0}}{a_0}\right) \quad (77)$$

The first acidity constant is function of the intercept and the slope, so the corresponding expressions are more complicated

$$K_{a1} = \frac{a_0}{a_1} = f(a_0, a_1) \quad (78)$$

Thus applying the law of random error propagation

$$\begin{aligned} s_{K_{a1}}^2 &= \left(\frac{\partial K_{a1}}{\partial a_0}\right)^2 s_{a_0}^2 + \left(\frac{\partial K_{a1}}{\partial a_1}\right)^2 s_{a_1}^2 + 2\left(\frac{\partial K_{a1}}{\partial a_1}\right)\left(\frac{\partial K_{a1}}{\partial a_1}\right)\operatorname{cov}(a_0, a_1) \\ &= \left(\frac{\partial\left(\frac{a_0}{a_1}\right)}{\partial a_0}\right)^2 s_{a_0}^2 + \left(\frac{\partial\left(\frac{a_0}{a_1}\right)}{\partial a_1}\right)^2 s_{a_1}^2 + 2\left(\frac{\partial\left(\frac{a_0}{a_1}\right)}{\partial a_0}\right)\left(\frac{\partial\left(\frac{a_0}{a_1}\right)}{\partial a_1}\right)\operatorname{cov}(a_0, a_1) \\ &= \left(\frac{1}{a_1}\right)^2 s_{a_0}^2 + \left(\frac{-a_0}{a_1^2}\right)^2 s_{a_1}^2 + 2\left(\frac{1}{a_1}\right)\left(\frac{-a_0}{a_1^2}\right)\operatorname{cov}(a_0, a_1) \\ &= \frac{1}{a_1^2} s_{a_0}^2 \frac{a_1^2}{a_1^4} s_{a_1}^2 - \frac{2a_0}{a_1^3}\operatorname{cov}(a_0, a_1) \end{aligned} \quad (79)$$

and then, the variance of pK_{a1} is equal to

$$\begin{aligned} s_{pK_{a1}}^2 &= \left(\frac{\partial pK_{a1}}{\partial K_{a1}}\right) s_{pK_{a1}}^2 = \left(\frac{\partial(-\log K_{a1})}{\partial K_{a1}}\right) s_{pK_{a1}}^2 \\ &= (\log e)^2 \frac{s_{pK_{a1}}^2}{K_{a1}^2} \\ &= (\log e)^2 \frac{a_1^2}{a_0^2}\left(\frac{1}{a_1^2} s_{a_0}^2 + \frac{a_0^2}{a_1^4} s_{a1}^2 - \frac{2a_0}{a_1^3}\operatorname{cov}(a_0, a_1)\right) \\ &= (\log e)^2 \left(\frac{s_{a_0}^2}{a_0^2} + \frac{s_{a_1}^2}{a_1^2} - \frac{2\operatorname{cov}(a_0, a_1)}{a_0, a_1}\right) \end{aligned} \quad (80)$$

and as pK_{a1}, is a function of K_{a1}, we can calculate the variance of pK_{a1} as

$$s_{pK_{a1}} = \log e \sqrt{\frac{s_{a_0}^2}{a_0^2} + \frac{s_{a_1}^2}{a_1^2} - \frac{2\operatorname{cov}(a_0, a_1)}{a_0 a_1}} \quad (81)$$

the covariance of measurements can be as important as the variances and both contribute significantly to the total analytical error [51, 52].

7. Evaluation of Acidity Constants in Experimental System: Resorcinol

Resorcinol or 1,3-benzenediol is used in the determination of ascorbic acid in pharmaceuticals and in the synthesis of several organic compounds [53]. However, there are few studies dealing with the molecular structure [54] or the acid–base properties of this compound in solution [10]. Considering that knowledge of the acid dissociation constants (pK_a) becomes essential for the development of new compounds with biological activity [55, 56], in this paper we determine the overlapping pK_a values of resorcinol in water.

In this section, we apply the developed theory to the experimental *A-pH* data (see Table 2) described in the literature for resorcinol published by Blanco et al. [57]. The A-pH curves corresponding to a resorcinol at 368.3 and 293.5 nm are plotted in Figure 3.

Table 2. A-pH data for the resorcinol system published by Blanco et al. [57]

pH	268.3 nm	293.5 nm	pH	268.3 nm	293.5 nm
0.0	0.6990	0.0480	10.5	0.5213	0.9879
2.0	0.6990	0.0480	10.6	0.5155	1.0331
4.0	0.6990	0.0480	10.7	0.5099	1.0795
5.0	0.6990	0.0480	10.8	0.5043	1.1266
6.0	0.6989	0.0484	10.9	0.4988	1.1736
7.0	0.6981	0.0522	11.0	0.4935	1.2196
7.5	0.6962	0.0611	11.1	0.4885	1.2638
7.6	0.6955	0.0644	11.2	0.4839	1.3051
7.7	0.6947	0.0686	11.3	0.4796	1.3431
7.8	0.6936	0.0737	11.4	0.4758	1.3772
7.9	0.6922	0.0801	11.5	0.4725	1.4073
8.0	0.6905	0.0881	11.6	0.4696	1.4334
8.1	0.6885	0.0978	11.7	0.4672	1.4557
8.2	0.6860	0.1097	11.8	0.4651	1.4745
8.3	0.6829	0.1241	11.9	0.4634	1.4902
8.4	0.6793	0.1416	12.0	0.4620	1.5031
8.5	0.6749	0.1624	12.1	0.4608	1.5138
8.6	0.6697	0.1869	12.2	0.4599	1.5225
8.7	0.6637	0.2155	12.3	0.4591	1.5295
8.8	0.6569	0.2482	12.4	0.4585	1.5352
8.9	0.6492	0.2849	12.5	0.4580	1.5398
9.0	0.6407	0.3255	12.6	0.4576	1.5435
9.1	0.6316	0.3693	12.7	0.4573	1.5464
9.2	0.6220	0.4157	12.8	0.4570	1.5488
9.3	0.6122	0.4635	12.9	0.4568	1.5506
9.4	0.6024	0.5120	13.0	0.4566	1.5521
9.5	0.5927	0.5601	13.1	0.4565	1.5533
9.6	0.5834	0.6071	13.2	0.4564	1.5543
9.7	0.5747	0.6526	13.3	0.4563	1.5551
9.8	0.5665	0.6964	13.4	0.4563	1.5557
9.9	0.5588	0.7387	13.5	0.4562	1.5561
10.0	0.5517	0.7798	13.6	0.4562	1.5565
10.1	0.5451	0.8203	13.7	0.4561	1.5568
10.2	0.5389	0.8608	13.8	0.4561	1.5571
10.3	0.5328	0.9019	13.9	0.4561	1.5573
10.4	0.5270	0.9442	14.0	0.4561	1.5574

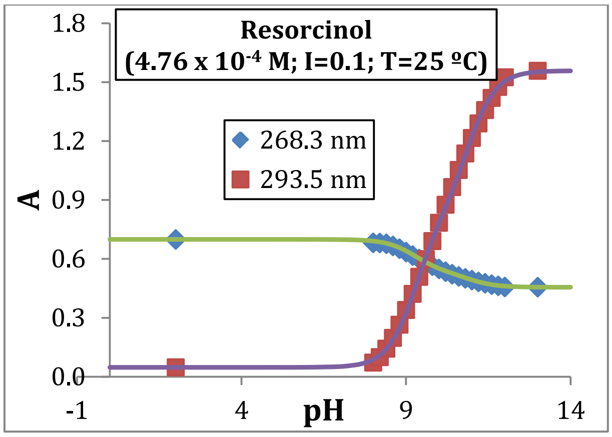

Figure 3. A-pH data at 268.3 and 193.5 nm, I=0.1, T=25°C, C_R=4.76·10^{-4} M for the resorcinol system

Figure 4 shows the application of the Polster method. The orthogonal regression leads to A_1 values of [0.5353; 0.8271]. After the application of Eqn. (61) the pK_a values were obtained from the slope $1/(K_{a2}K_{a1})$ and the intercept $1/K_{a2}$. The best value of the limit absorbance for the intermediate specie *HR* (A_1) was obtained by a trial and error method, i.e. the best value of A_1 is taken as that which minimizes the standard deviation of the corresponding regression line. The value assumed for A_1 was 0.814 (Figure 6).

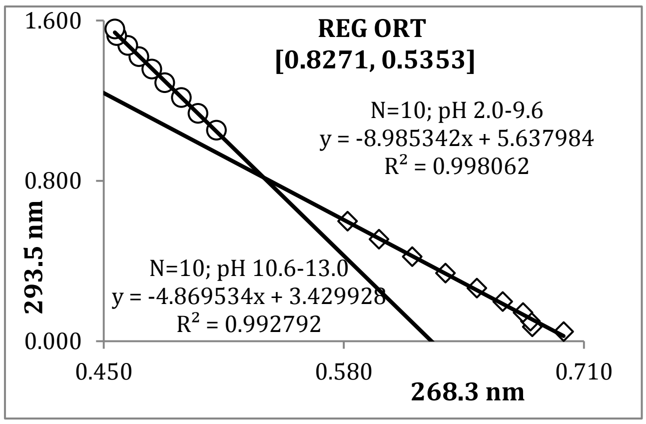

Figure 4. Evaluation of the limit absorbance of the intermediate specie, HR

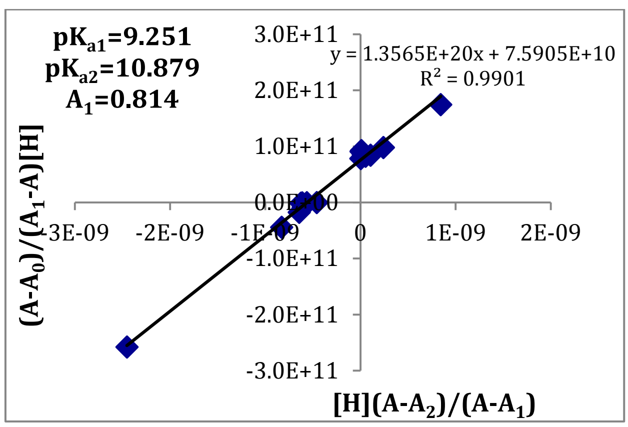

Figure 5. Evaluation of the acidity constants of resorcinol by Eqn. 61

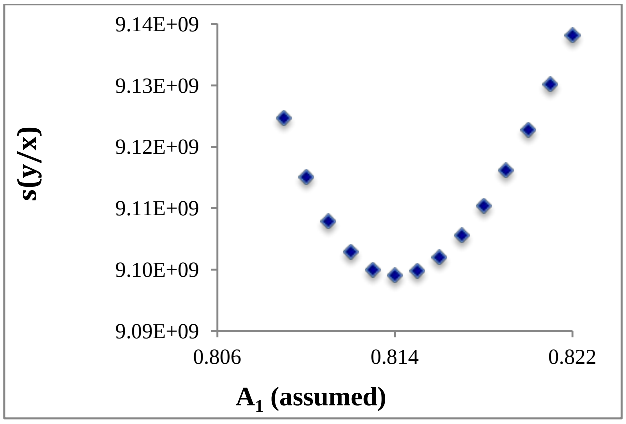

Figure 6. s(y/x) as a function of the A_1 value assumed

The pK_a values obtained are: $pK_{a1} = 9.251 \pm 0.066$ and $pK_{a2} = 10.879 \pm 0.014$. These values are of the same order of magnitude as those published in the original work of Blanco et al. [57], or those described in the literature for resorcinol. Values of estimated pK_a are reported with three digits in all cases, even if they are not significant. In addition, the experimental data considered are of high quality as can be seen from the low standard deviation of the regression lines.

8. Conclusions

It can finally be argued that a diprotic acid with overlapping (simultaneous equilibria) acidity constants may be treated as a monoprotic acid provided that some approximation is made. The simplifying assumption is that the concentrations of the species R or H_2R are small compared with the total concentration C_R at enough low and pH values, respectively. Such approximations are often necessary to have an accurate knowledge of the composition of the solution at a certain pH interval.

A new method reported by Polster and based on the measurements of absorbances at two wavelengths (λ and λ^*) allows to evaluate graphically the limit absorbances A_1 and A_1^* for the intermediate species HR of a diprotic acid H_2R, dealing with overlapping (simultaneous) equilibria. Least squares treatment, which takes into account similar errors in both *x* and *y* variables, i.e. orthogonal regression, is also included in this work. Few attempts to deal with this problem in the evaluation of equilibrium constants have been made.

Except for very close pK_a values the acidity constants are easily experimentally obtained making use of expressions $A_{(pKa1)}=(A_2+A_1)/2$ and $A_{(pKa2)}=(A_1+A_0)/2$. However, there seems little point in measuring a whole A-pH curve to determine one point. The constants so obtained are much less efficient in terms of return for effort used.

The theory developed in this work has been successfully applied to the experimental systems described in the bibliography (resorcinol, with ΔpK_a of about 1.7). A detailed analysis of the errors implied is also made, taking into account the strong correlation existing between the slope and intercept of a straight line obtained by the least squares method. The covariance between two variables is so important as the variances and both contribute significantly to the total analytical error.

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