

On the Existence of Two Species in Solution, Simple or Simultaneous Equilibria?

Julia Martín^{1,*}, Irene Abengózar Suárez², Agustín G. Asuero²

¹Department of Analytical Chemistry, Escuela Politécnica Superior, University of Seville, Seville, Spain

²Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, Seville, Spain

Abstract The aim of this work is to find out in which experimental conditions a diprotic acid H_2R may be treated as a monoprotic one, HR , in order to determining their acidity constants spectrophotometrically, as the relationship between the absorbance (A) and the concentration for a monoprotic acid HR is susceptible of linear transformation. It can be argued that a diprotic acid with overlapping acidity constants may be treated as a monoprotic acid provided that some approximation is made. The simplifying assumption is that the concentration (C_R) at enough low and high pH values equals to $[H_2R] + [HR]$ and $[HR] + [R]$, respectively. A bilogarithmic method for the spectrophotometric evaluation of acidity constants of two-step overlapping equilibria may be applied on this basis. The singular straight line (bilogarithmic) complete (diprotic acid model) or single (monoprotic acid model) version requires for its application the knowledge of the limit absorbance of the intermediate specie HR (A_I). A trial and error method may be applied, i.e. the best value of A_I is taken as that which minimizes the standard deviation of the corresponding regression line. An additional bilogarithmic method is proposed in this work when spectrophotometric measurements are made at the wavelength of an isosbestic point (i.e. a wavelength at which the limit absorbance of two species are equal). The method has been checked with synthetic theoretical data and model systems, and has been applied to the experimental data obtained in the literature for benzidine, vanillyl ammonium ion and isonicotinic acid, and to the A - pH experimental data for isonicotinic acid obtained in this paper.

Keywords Acidity constant, Spectrophotometric measurements, Bilogarithmic Method, Isosbestic point

1. Introduction

The relationship between the absorbance (A) and the composition of any given solution of a diprotic acid having concentration C_R and mixed acidity constants

$$K_{a1} = \frac{[HR](H)}{[H_2R]} \quad (1)$$

and

$$K_{a2} = \frac{[R](H)}{[HR]} \quad (2)$$

corresponding to the dissociation equilibria



where the charges are omitted for the sake of simplicity, and



respectively, is given by [1, 2]

$$A = A_2 f_2 + A_1 f_1 + A_0 f_0 \quad (5)$$

$$A = \frac{A_0 + A_1 \frac{(H)}{K_{a2}} + A_2 \frac{(H)^2}{K_{a2} K_{a1}}}{1 + \frac{(H)}{K_{a2}} + \frac{(H)^2}{K_{a2} K_{a1}}} \quad (6)$$

where A_0 , A_1 and A_2 are the limit absorbance of R , HR and H_2R species, respectively, and f_2 , f_1 and f_0 the molar fractions

$$f_j = \frac{[H_j R]}{C_R} \quad (7)$$

$$A_j = \varepsilon_j C_R \quad (8)$$

$$C_R = [H_2R] + [HR] + [R] \quad (9)$$

(path length one-centimetre); ε_j is the molar absorptivity of the species H_jR , and C_R the analytical (total) concentration of the acid.

* Corresponding author:

jbueno@us.es (Julia Martín)

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At low pH values one would expect the formation of H_2R and HR only and in consequence Eqn. (6) is reduced to

$$A = \frac{A_1 + A_2 \frac{(H)}{K_{a1}}}{1 + \frac{(H)}{K_{a1}}} \quad (10)$$

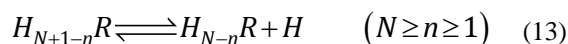
At high enough pH only the species HR and R are found in solution from a practical point of view, and from Eqn. (6) we have instead

$$A = \frac{A_0 + A_1 \frac{(H)}{K_{a2}}}{1 + \frac{(H)}{K_{a2}}} \quad (11)$$

Expressions (10) and (11) have the general form

$$A = \frac{A_{N-n} + A_{N+1-n} \frac{(H)}{K_{an}}}{1 + \frac{(H)}{K_{an}}} \quad (12)$$

corresponding to the single equilibria



and then we may evaluate the acidity constants K_{an} as

$$K_{an} = (H) \frac{A_{N+1-n} - A}{A - A_{N-n}} \quad (14)$$

by measuring absorbances at a given wavelength and pH , once the values of A_{N+1-n} and A_{N-n} are known. Taking decadic logarithms on Eqn. (14) and rearranging terms we get

$$\log \left(\frac{A_{N+1-n} - A}{A - A_{N-n}} \right) = pH - pK_{an} \quad (15)$$

By plotting the left hand of Eqn. (15) against pH we get a straight line (least squares method) with slope unity which cuts the x -axis at $pH = pK_a$. Eqns. (12) or (14) may also be arranged into several forms [3] suitable for linear plotting.

The aim of this work is to find out in which experimental conditions a diprotic acid H_2R may be treated as a monoprotic one, HR , in order to determining their acidity constants spectrophotometrically.

A bilogarithmic method for the spectrophotometric evaluation of acidity constants of two-step overlapping equilibria has been devised on this basis. The method has been checked with synthetic theoretical data. Finally, another non-approximate bilogarithmic isosbestic point method has been devised and applied to A - pH (literature) data for benzidine, vanillyl ammonium ion and isonicotinic acid, being also the A - pH data obtained in this work for isonicotinic acid the subject of study and application.

2. Spectrophotometric Evaluation of Acidity Constants of Diprotic Acids by Monoprotic Acid Methods

From Eqn. (6) we get

$$(A - A_0) + (A - A_1) \frac{(H)}{K_{a2}} + (A - A_2) \frac{(H)^2}{K_{a2}K_{a1}} = 0 \quad (16)$$

which may be rearranged into

$$\frac{A - A_0}{(A_1 - A) \left(1 + \left(\frac{A - A_2}{A - A_1} \right) \frac{[H]}{K_{a1}} \right)} = \frac{[H]}{K_{a2}} \quad (17)$$

Taking decadic logarithms on Eqn. (17) we get

$$\log \left(\frac{A - A_0}{(A_1 - A) \left(1 + \left(\frac{A - A_2}{A - A_1} \right) \frac{[H]}{K_{a1}} \right)} \right) = pK_{a2} - pH \quad (18)$$

which has the form

$$\log \left(\frac{A - A_0}{(A_1 - A)F} \right) = pK_{a2} - pH \quad (19)$$

where

$$F = 1 + \left(\frac{A_2 - A}{A - A_1} \right) 10^{pK_{a1} - pH} \quad (20)$$

A representation of the left hand of Eqn. (18) against the pH gives a straight line (least squares method) of slope -1 which cut the x -axis at a value equals to pK_{a2} .

Similarly, Eqn. (16) on rearrangement gives

$$\left(\frac{A - A_2}{A_1 - A} \right) \frac{1}{\left(1 + \left(\frac{A - A_0}{A - A_1} \right) \frac{K_{a2}}{[H]} \right)} = \frac{K_{a1}}{[H]} \quad (21)$$

Taking decadic logarithms on Eqn. (21) we get

$$\log \left(\frac{A - A_2}{(A_1 - A)G} \right) = pH - pK_{a1} \quad (22)$$

where

$$G = 1 + \left(\frac{A - A_0}{A - A_1} \right) = 10^{pH - pK_{a2}} \quad (23)$$

A representation of the left hand of Eqn. (22) against the pH gives a straight line (least squares method) of slope 1 which cut the x -axis at a value equals to pK_{a1} .

At high enough pH values it is expected that only the two RH and R species will be present in solution; so, from a

practical point of view, since $10^{pK_{a1}-pH} \ll 1$, $F \approx 1$. The equilibrium given by Eqn. (4) is thus obtained, and Eqn. (19) becomes

$$\log \left(\frac{A - A_0}{A_1 - A} \right) = pK_{a2} - pH \quad (24)$$

At low enough pH values it is expected that only the two H_2R and HR species will be present in solution; so, from a practical point of view, since $10^{pH-pK_{a2}} \ll 1$, $G \approx 1$. The equilibrium given by Eqn. (3) is thus obtained, and Eqn. (22) becomes

$$\log \left(\frac{A - A_2}{A_1 - A} \right) = pH - pK_{a1} \quad (25)$$

Both expressions, Eqns. (24) and (25) have the form of Eqn. (14) and are applicable when the limit absorbance of the intermediate specie A_i is known, which can be systematically varied up to minimize a given parameter, such as the standard deviation of the regression line, for example.

3. Spectrophotometric Evaluation of Acidity Constants of Diprotic Acids from Absorbance Measurements at the Isosbestic Point Wavelength

If measurements are made at the wavelength in which $A_1 = A_0 = A_i$, then from Eqn. (16) we get

$$(A - A_i) \left(1 + \frac{(H)}{K_{a2}} \right) + (A - A_2) \frac{(H)^2}{K_{a2}K_{a1}} = 0 \quad (26)$$

which on rearrangement gives

$$\frac{A_2 - A}{A - A_i} = \frac{1 + \frac{(H)}{K_{a2}}}{\frac{(H)^2}{K_{a2}K_{a1}}} \quad (27)$$

Taking into account that

$$\begin{aligned} f_2 &= \frac{[H_2R]}{C_R} = \frac{[H_2R]}{[H_2R] + [HR] + [R]} = \frac{1}{1 + \frac{[HR]}{[H_2R]} + \frac{[R]}{[H_2R]}} \\ &= \frac{1}{1 + \frac{K_{a1}}{(H)} + \frac{K_{a1}K_{a2}}{(H)^2}} = \frac{1}{1 + \frac{K_{a1}}{K_{a2}} \left(\frac{K_{a2}}{(H)} + \left(\frac{K_{a2}}{(H)} \right)^2 \right)} \\ &= \frac{1}{1 + 10^{\Delta pK_a} (10^{-z} + 10^{-2z})} \end{aligned} \quad (28)$$

where

$$\Delta pK_a = pK_{a2} - pK_{a1}; \quad z = pK_{a2} - pH \quad (29)$$

$$f_1 = \frac{[HR]}{[R] + [HR] + [H_2R]} = \frac{\frac{[HR]}{[R]}}{1 + \frac{[HR]}{[R]} + \frac{[H_2R]}{[R]}} \quad (30)$$

$$\begin{aligned} &= \frac{\frac{(H)}{K_{a2}}}{1 + \frac{(H)}{K_{a2}} + \frac{(H)^2}{K_{a1}K_{a2}}} \\ f_0 &= \frac{[R]}{C_R} = \frac{[R]}{[R] + [HR] + [H_2R]} = \frac{1}{1 + \frac{[HR]}{[R]} + \frac{[H_2R]}{[R]}} \\ &= \frac{1}{1 + \frac{(H)}{K_{a2}} + \frac{(H)^2}{K_{a2}K_{a1}}} = \frac{1}{1 + \frac{K_{a1}}{K_{a2}} \left(\frac{(H)}{K_{a1}} + \left(\frac{(H)}{K_{a1}} \right)^2 \right)} \\ &= \frac{1}{1 + 10^{\Delta pK_a} (10^{-y} + 10^{-2y})} \end{aligned} \quad (31)$$

being in this case

$$y = pH - pK_{a1} \quad (32)$$

we get

$$\frac{A_2 - A}{A - A_i} = \frac{f_0 + f_1}{f_2} = \frac{1 - f_2}{f_2} = \frac{1}{f_2} = 1 \quad (33)$$

Then, from Eqns. (33) and (28), after taking decadic logarithms we get

$$\log \left(\frac{A_2 - A}{A - A_i} \right) = \Delta pK_a + \log (10^{-z} + 10^{-2z}) \quad (34)$$

A representation of the left hand of Eqn. (34) against the second term of the right hand gives a straight line (least squares method) of slope unity which cut the x-axis at a value equals to ΔpK_a .

Moreover, from Eqn. (33) we get

$$f_2 = \frac{A - A_i}{A_2 - A} \quad (35)$$

The same considerations can be applied supposing that the measurements are realized at the wavelength of the isosbestic point in which $A_1 = A_2 = A_j$, in order to evaluate pK_{a2} . Then,

$$(A - A_0) + (A - A_j) \left(\frac{[H]}{K_{a2}} + \frac{[H]^2}{K_{a2}K_{a1}} \right) = 0 \quad (36)$$

which on rearrangement gives

$$\frac{A_0 - A}{A_j - A} = \frac{(H)}{K_{a2}} + \frac{(H)^2}{K_{a2}K_{a1}} = \frac{f_1 + f_2}{f_0} = \frac{1 - f_0}{f_0} = \frac{1}{f_0} - 1 \quad (37)$$

Taking reciprocals in Eqn. (31), subtracting 1, and then taking decimal logarithms of both sides we get an expression analogous to Eqn. (34)

$$\log\left(\frac{A_0 - A}{A_j - A}\right) = \Delta pK_a + \log(10^{-y} + 10^{-2y}) \quad (38)$$

As before, a representation of the left hand of Eqn. (38) against the second term of the right hand gives a straight line with unity slope and intercept equals to ΔpK_a .

From Eqn. (37) we get

$$f_0 = \frac{A - A_j}{A_0 - A_j} \quad (39)$$

It can finally be argued that a diprotic acid with overlapping acidity constants may be treated as a monoprotic acid provided that some approximation is made. The simplifying assumption is that the concentration C_R at low enough and high pH values equals to $[H_2R] + [HR]$ and $[HR] + [R]$, respectively.

4. A-pH Curves of Diprotic Acids: Simulated Data

Simulated data have been generated using Eqn. (6). Figure 1 shows the A - pH curves, corresponding to a family of diprotic acids with pK_{a2} equal to 8.000, and pK_{a1} variable (from 5.500 to 7.600). The ΔpK_a studied are: 0.250; 0.200; 0.150; 0.100 and 0.600. This last value is the smallest ΔpK_a difference that a diprotic acid can exhibit, as it is deduced from the analysis of their microconstants of acidity [4].

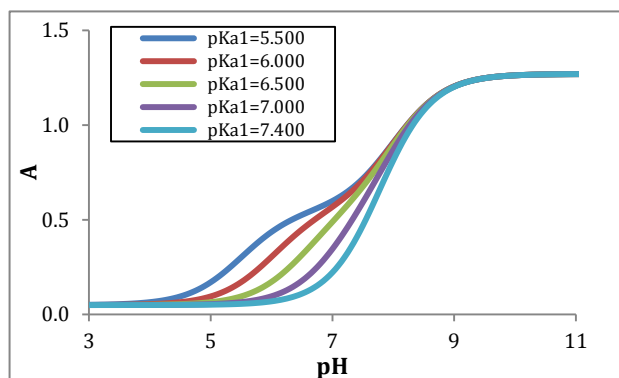


Figure 1. A - pH curves for a diprotic acid H_2R with $pK_{a2} = 8.000$ and variable pK_{a1} (indicated in the legend in each case)

When the two pK_a 's are far away, the two ionization equilibria can be treated independently. If the successive acidity constants are close ($\Delta pK_a < 4.000$) a simultaneous adjustment of the data is necessary [5].

Figure 2 shows the singular straight line (bilogarithmic)

complete (diprotic acid model (RU), Eqn. 19) or single (monoprotic acid model, Eqn. 24) version applied to the calculation of pK_{a2} (assuming known pK_{a1} and the limit absorbance for the intermediate species, A_1). Regardless of the wavelength measurement, which leads to different values for the limit absorbance A_2 , A_1 and A_0 of the species, H_2R , HR and R , respectively, a straight line with slope equal to -1 is obtained. Theoretically, this same line is also obtained in spite of the ΔpK_a value.

When the diprotic acid is treated as monoprotic one (Eqn. 24) an accentuated curvature is observed at lower ΔpK_a values due to the occurrence of the H_2R species involved in the first dissociation equilibrium of the acid.

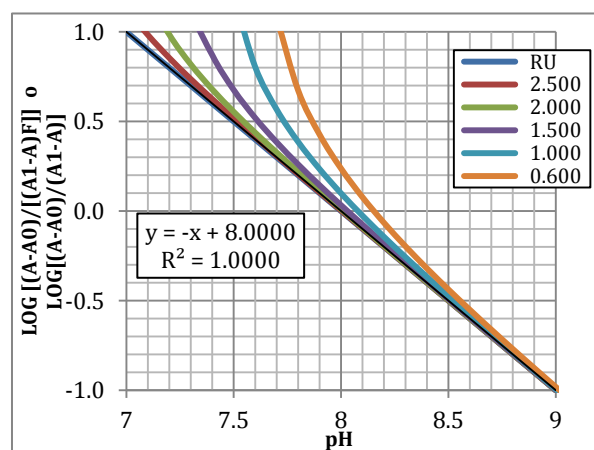


Figure 2. The singular straight line (bilogarithmic) complete (diprotic acid model (RU), Eqn. 19) or single (monoprotic acid model, Eqn. 24) version applied to the family of curves of Figure 1 (assuming known pK_{a1} and the limit absorbance for the intermediate species, A_1)

A graph analogous to Fig. 2, with slope +1.000, is obtained when calculated pK_{a1} from Eqn. (22) assuming pK_{a2} and A_1 known (see Fig. 3). The cut-off point of the straight line with the x -axis is equal to the pK_{a1} value of in each case. If the acid were considered as monoprotic one (Eqn. 25), it would get lines whose curvature is more accentuated when the pH increases and ΔpK_a decreases, due to the occurrence of the R species involved in the second dissociation equilibrium of the acid.

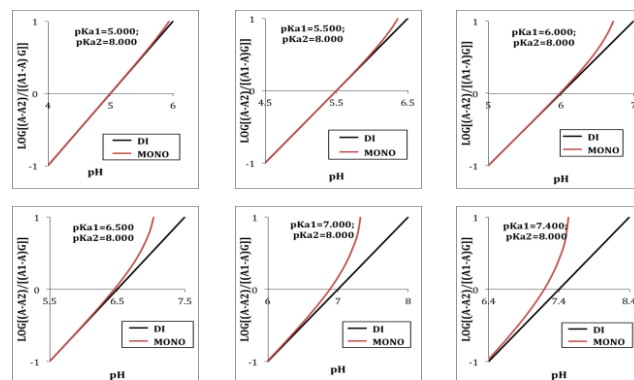


Figure 3. The singular straight line (bilogarithmic) complete (diprotic acid model (RU), Eqn. 22) or single (monoprotic acid model, Eqn. 25) version applied to the family of curves of Figure 1 (assuming known pK_{a2} and the limit absorbance for the intermediate species, A_1)

The application of the complete bilogarithmic method (Eqn. (19)) to the theoretical data generated over the entire range of pH values leads to the exact value of the second dissociation constant, $pK_{a2}=4.761$, assuming a correct value of pK_{a1} equal to 3.602 (straight line with slope -1.000). In the same way, the application of the complete bilogarithmic method based on the use of Eqn. (22) leads to the exact value of the first dissociation constant, $pK_{a1}=3.602$, assuming known the correct value of $pK_{a2}=4.761$ (straight line with slope 1.000).

Additionally, in order to apply the isobestic point bilogarithmic method (Eqn. 34), ten pairs of data $[A, pH]$ were selected in the range of pH from 2.600 to 4.400 (near to pK_{a1}), from 0.200 to 0.200 units of pH. The minimization criterion leads to a pK_{a2} value of 4.763. The bilogarithmic method at the isobestic point wavelength leads to a pK_{a1} value equal to 3.602.

So, it can be concluded that the results obtained are excellent. The value of the selected adjustment parameter is not especially critical on the pK_{a1} calculation, i.e., assuming pK_{a2} values of 4.700 and 4.800 lead to pK_{a1} values equal to

3.619 and 3.592, respectively. If we adopt as the best value of the adjustment parameter those straight line with slope closest to the unit, we get $pK_{a2}=4.761$ and $pK_{a1}=3.602$, coinciding in both cases with the true values.

5.2. Vanillyl Ammonium Ion and Isonicotinic Acid

Analogous good results are obtained with vanillyl ammonium ion system ($pK_{a1}=9.032$; $pK_{a2}=10.460$; $A_2=0.024$; $A_1=A_0=0.787$) and to isonicotinic acid system ($pK_{a1}=1.820$; $pK_{a2}=4.760$; $A_2=A_1=0.702$; $A_0=0.407$).

6. Determination of the Acidity Constants of Diprotic Acids from Absorbance Measurements at the Isobestic Point Wavelength: Experimental Systems

Table 2. Benzidine model system: pK_{a1} evaluation using the bilogarithmic method at the isobestic point, $A_1=A_0$

| | | | | | |
|-------------------------|-------|-----------|-----------------------------|-------------------------------|-----------|
| $A_2=$ | 0.940 | $a_1=$ | 1.0003 | 1.1615 | $=a_0$ |
| $A_1=$ | 0.260 | $s(a_1)=$ | 6.5884E-05 | 8.9999E-05 | $=s(a_0)$ |
| $A_0=$ | 0.260 | $r^2=$ | 1.0000 | 1.2883E-04 | $=s(y/x)$ |
| $pK_{a2}(\text{sup}) =$ | 4.763 | | $pK_{a1}= 3.602$ | | |
| | pH | A | $\text{LOG}(10^Z+10^{-2Z})$ | $\text{LOG}[(A_2-A)/(A-A_1)]$ | |
| | 2.600 | 0.8781 | -2.1600 | -0.9994 | |
| | 2.800 | 0.8465 | -1.9583 | -0.7975 | |
| | 3.000 | 0.8021 | -1.7556 | -0.5945 | |
| | 3.200 | 0.7432 | -1.5513 | -0.3901 | |
| | 3.400 | 0.6708 | -1.3446 | -0.1836 | |
| | 3.600 | 0.5894 | -1.1341 | 0.0271 | |
| | 3.800 | 0.5073 | -0.9181 | 0.2430 | |
| | 4.000 | 0.4329 | -0.6939 | 0.4673 | |
| | 4.200 | 0.3724 | -0.4580 | 0.7033 | |
| | 4.400 | 0.3279 | -0.2066 | 0.9550 | |

6.1. Experimental Systems Described in the Literature

The bilogarithmic method has been applied to the literature data for benzidine [7], vanillyl ammonium ion [8], and isonicotinic acid [9]. Results obtained for benzidine are shown in Fig. 5. The pK_a values obtained were: Benzidine: $pK_{a1}=3.657$ and $pK_{a2}=4.455$, $\Delta pK_a=0.798$; Vanillyl ammonium ion: $pK_{a1}=9.044$ and $pK_{a2}=10.657$, $\Delta pK_a=1.613$; Isonicotinic acid: $pK_{a1}=1.816$ and $pK_{a2}=4.751$, $\Delta pK_a=2.935$.

These values are of the same order of magnitude as those published in their original papers, and of high quality as can be seen from the low $s(y/x)$ of the regression lines, with the

residuals randomly distributed. pK_a are reported with three digits in all cases, even if they are not significant.

6.2. Experimental Data: Isonicotinic Acid

Reagents

Isonicotinic acid ($C_6H_5NO_2$) $M = 123.06$ g/mol (Sigma, > 99%); Sodium hydroxide (NaOH) $M = 40.01$ g/mol (Merck, analytical grade); Perchloric acid ($HClO_4$) $M=100.5$ g/mol (Merck, 72%); sodium perchlorate ($NaClO_4$) $M=122.5$ g/mol (Merck, analytical grade); Water for ACS analysis.

Instruments

Analytical balance (Mettler AE200), Granatario (Mettler PJ 400), pH-meter Crison GLP 21 (3 decimals), with a combined Ag/AgCl glass electrode. pH-meter is calibrated using pH buffers 4.01, 7.00 and 9.21 (setting the pH meter to 4.010, 7.000 and 9.210, respectively). Ultraviolet-visible molecular absorption spectrophotometer (Shimadzu).

Experimental part

Two point five mL of isonicotinic acid solution ($1.750 \cdot 10^{-3}$ M) is pipetted into a 25 mL volumetric flask containing 2.5 mL of a NaClO_4 solution (1 M). Then varying amounts of HClO_4 and NaOH solutions are added in order to adjust the pH, completing to the mark with distilled water. The absorbance is measured at 266.7 nm (quartz cuvettes, pathlength one-centimetre) versus a blank. Finally the pH of the solution is also measured (passed into a 50 mL beaker).

Details of the experimental data (Fig. 6) follows: $A_2 = A_1 = 0.7052$; $A_0 = 0.4028$. [pH, A]: [3.337, 0.6906; 3.962, 0.6793; 4.073, 0.6442; 4.149, 0.6222; 4.230, 0.6277; 4.240, 0.5969; 4.281, 0.6036; 4.383, 0.5756; 4.385, 0.6000; 4.392, 0.5894; 4.429, 0.5817; 4.659, 0.5309; 4.772, 0.5424]. The dispersion of the data is greater than that obtained by Evans et al. [9].

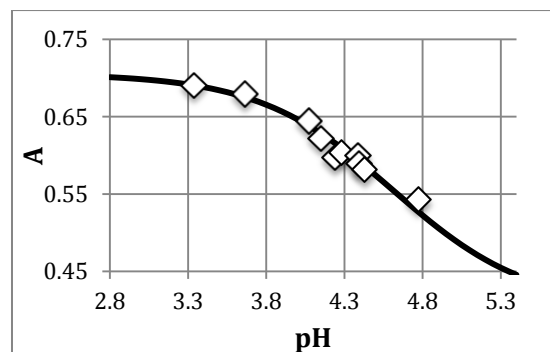


Figure 6. Isonicotinic acid experimental system. A - pH data at 266.7 nm, $I=0.1$ M, $T=25^\circ\text{C}$, $C_R=1.675 \cdot 10^{-4}$ M

The application of the bilogarithmic method from absorbance measurements at the isosbestic point wavelength (Figure 7, top) leads to $pK_{a1}=1.920$ and $pK_{a2}=4.615$, $\Delta pK_a=2.695 \pm 0.125$, not very distant from those previously published by Evans et al. [9], calculated at a different ionic strength. The results are given with three decimal digits in order to show the precision of measurements achieved even if they are not significant.

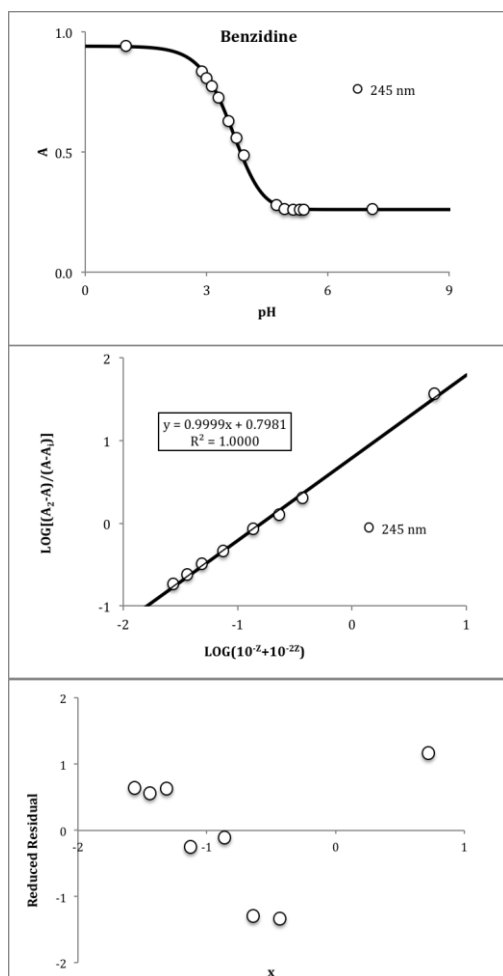


Figure 5. Benzidine test system. Top: A - pH data at 245 nm, $I=0.01$, $T=25^\circ\text{C}$, $C_R=0.5 \cdot 10^{-4}$ M. Middle: pK_a evaluation using the bilogarithmic method at the isosbestic point. Bottom: Reduced residuals (residual divided by $s_{y/x}$)

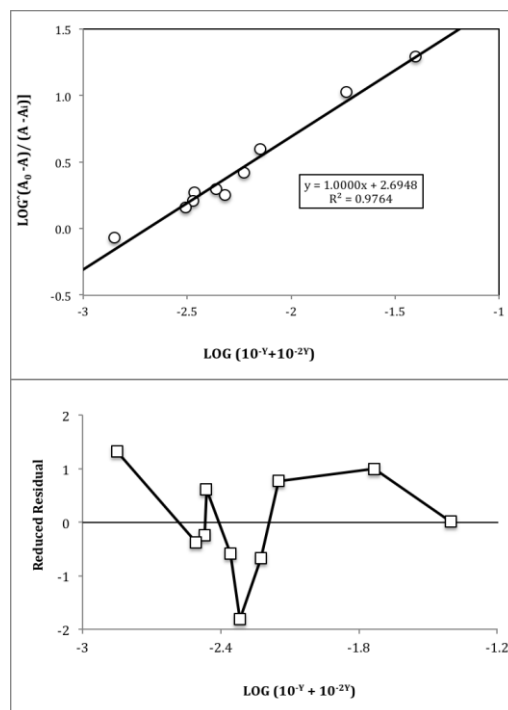


Figure 7. Top: Isonicotinic acid pK_a evaluation by the bilogarithmic method at the isosbestic point. Bottom: Reduced residuals (residual divided by $s_{y/x}$) versus x

The residual analysis (Fig. 7, bottom) indicates a random pattern and values of reduced residuals lower to 2, in absolute value, so that no data was eliminated. The non-linear regression method (CSS Statistica) leads to a pK_{a2} value of 4.611, although the pK_{a1} value by this method is very sensitive to the initial starting value (i.e. Figure 8).

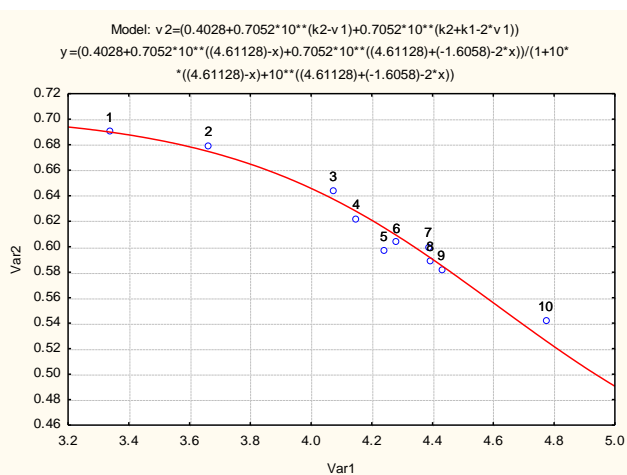


Figure 8. Non-linear regression method applied to the *A-pH* data obtained in this work for isonicotinic acid ($pK_{a2}=4.611$)

7. Conclusions

Many molecules having analytical and biological interest [10] behave as diprotic acids. The bilogarithmic procedures (complete or simplified versions) and isobestic point bilogarithmic method applied in this work have been tested with synthetic data and model and experimental (benzidine, vanillyl ammonium ion, isonicotinic acid) systems and found to be satisfactory, yielding results of noticeable precision. The efficiency of the procedure relies upon having a reasonable a priori estimate of pK_{a1} or pK_{a2} . The efficiency of the procedure relies upon having a reasonable a priori estimate of pK_{a1} or pK_{a2} .

In spite of the modern possibility of calculating acidity constants using black-box computer programs, the determination of these constants by linearized plots seems to be more prevalent, probably owing to the transparency of the method used. Proper nonlinear fitting data is often difficult and non-linear least squares are not always free from problems. Occasionally, problems arise because of the choice of the data, initial estimates, convergence or multiple local minima, all typical of nonlinear regression. Therefore, the importance of transformation stems from the various limitations of nonlinear approaches.

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