

OVERLAPPING EQUILIBRIA: APPLICATIONS TO *m*-AMINOBENZOIC ACID

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Received May 30th, 1986

m-Aminobenzoic acid displays two protonation equilibria which overlap in the pH region, where $pK_2 - pK_1 < 3$. The results show that the equilibria occur between the pH values 6.16–1.77, with the limit of separation of the two equilibria lying between 3.60–3.44. Use has been made of several theoretical methods put forward in the bibliography for calculating pK values of overlapping equilibria and these have been corrected by including the terms of the activity coefficients, by means of the coefficients n_1 and n_2 , which are defined and calculated by the method of separation of equilibria. The values $pK_1 = 3.237 \pm 0.091$ and $pK_2 = 4.767 \pm 0.002$ are proposed.

When a chemical species undergoes two separate ionization equilibria whose pK values lie within three units of each other, there tends to be some interference in the process of determining their respective pK values. The use of the spectrophotometric technique has enabled these problems to be overcome, as well as permitting the number of species in equilibria to be determined¹; *m*-aminobenzoic acid shows two acid–base equilibria when dissolved in aqueous solution, which can be written as:



where AH stands for the neutral species $\text{NH}_2\text{-}\Phi\text{-COOH}$, A^- for the anion corresponding to the ionization of the carboxylic group and AH_2^+ for the cation corresponding to the protonation of the amino group; K_1 and K_2 are the thermodynamic dissociation constants corresponding to Eqs (A) and (B), which can be stated in logarithmic form as:

$$pK_1 = \text{pH} + \log \left(\frac{[\text{AH}_2^+]}{[\text{AH}]} \right) + \log \left(\frac{f_{\text{AH}_2^+}}{f_{\text{AH}}} \right) \quad (1)$$

$$pK_2 = \text{pH} + \log \left(\frac{[\text{AH}]}{[\text{A}^-]} \right) + \log \left(\frac{f_{\text{AH}}}{f_{\text{A}^-}} \right). \quad (2)$$

The value $I = [\text{acid form}]/[\text{basic form}]$ is known as the ionization ratio and, for

an independent equilibrium, can be obtained by means of Gold-Hawes' equation²:

$$I = \sum_{i=1}^n \frac{1}{n} \left(\frac{A - A_B}{A_A - A} \right) \lambda_i, \quad (3)$$

a general equation valid to any equilibrium, where A_B represents the absorbance of the basic species, A_A that of the conjugated acid species and A that of the species at intermediate acidities, all measured at n different wavelengths λ_i ; A_A and A_B correspond to the limits of acidity between which the equilibrium occurs.

In the UV-VIS spectroscopy, the presence of isosbestic points denote the existence of at least two species in equilibrium³; however, when the pK_1 and pK_2 values, in the case of two equilibria, are close to one another, no clearly defined isosbestic point is formed, and there is no general procedure for its resolution. The bibliography makes reference only to a few specific cases, particularly to diprotic acids⁴, beginning with the general statement:

$$[H^+]^2 (A - A_1) + [H^+] K_1 (A - A_2) + K_1 K_2 (A - A_3) = 0, \quad (4)$$

where A_1 , A_2 and A_3 are the absorbances of the diprotonate, monoprotonate and non-protonate forms, respectively. In obtaining this equation, the definitions of K_1 and K_2 have been made without reference to the terms of the activity coefficients, although a mass balance is taken into account:

$$C_t = [AH_2^+] + [AH] + [A^-] \quad (5)$$

as is the additivity of absorbances:

$$A = A_1 + A_2 + A_3, \quad (6)$$

where concentrations are given in mol dm^{-3} .

Among proposal for solving the general Eq. (4) are the following:

a) *Ang's method*⁵. A wavelength λ is chosen close to the isosbestic point, even if this point is not well defined, and the absorbance of the solution is measured; the method can be applied if the plot $A = f(\text{pH})$ shows a maximum or a minimum, that is to say, if the same absorbance is obtained for two solutions at different acid concentration. From Eq. (4) we deduce:

$$A = A_2 - K_2 \cdot P \quad (7)$$

$$P = \frac{(A - A_3) ([H^+]_1 + [H^+]_2)}{[H^+]_1 \cdot [H^+]_2} \quad (8)$$

$$A = A_1 + K_1 \cdot Q \quad (9)$$

$$Q = \frac{(A_2 - A)}{[H^+]_1 + [H^+]_2} \quad (10)$$

Eqs (7) and (9) being the equations for two straight lines from which K_1 and K_2 can be deduced.

b) *Mongay's method*⁶. Eq. (4) can be rearranged as the equation of a plane:

$$Z = a_0 + a_1 \cdot X + a_2 \cdot Y, \quad (11)$$

where a_0 , a_1 and a_2 are related to K_1 , K_2 and A_2 ; X , Y and Z are functions of the parameters A_3 and A_1 and of the variables $[H^+]$ and A . Eq. (11) leads to four adjustable expressions by means of multiple linear regression, from the parameters of which it is possible to obtain the equilibrium constants as well as the intermediary coefficient A_2 . Using the data of Albert⁴ for *m*-aminobenzoic acid at $\lambda = 280$ nm, these authors⁶ reported the values: $pK_1 = 4.788$, $pK_2 = 3.086$ and $\varepsilon = 278$.

In the present paper, the method of separation of overlapping equilibria is proposed; this is a more general method since it may be applied to any wavelength, even outside the triangular isobestic zones, and yields better results because the obtention of the n_1 and n_2 parameters permits an improvement in the pK values as well as wider knowledge of the equilibria studied.

EXPERIMENTAL

Apparatus. Bodenseewerk Perkin-Elmer Spectrophotometer with double beam, slit 1 nm, optic path 1 cm and thermostatable cells. This permits multiple expansion in both absorbances and wavelengths and an accuracy of ± 0.001 in the absorbance readings. Both cells contained throughout an aqueous perchloric acid solution at the same concentration as solvent. Temperature was kept constant at $25 \pm 0.01^\circ\text{C}$ with a P-Selecta thermostat and the pH readings were made with a Crison pH-meter (± 0.01).

Reactants. *m*-Aminobenzoic acid Aldrich 99%, perchloric acid Carlo Erba 70%. Solutions were prepared with twice distilled and deionized water as solvent, over which nitrogen gas was made to flow before being used. In every case the solutions used had been freshly prepared and kept out of the light. It was not necessary to control the ionic strength, since this factor is included in the data treatment; the calculations were carried out with a Sinclair-Spectrum micro-computer.

RESULTS AND DISCUSSION

m-Aminobenzoic acid displays two overlapping acid-base equilibria within the pH range 6.16–1.77 as shown in Fig. 1. The convergence of the spectra at each end shows the beginning and the end of the equilibria⁷. Moreover, use of the calculation

program TRIANG¹ has provided proof that the number of species in the solution is three; this program permits the obtention of the number of species in solution by means of a matrix of absorbances $N \times N$ (N wavelengths, N pH readings); this result points to the existence of two equilibria. The formation of triangular isosbestic zones is of great importance in the calculations based on Ang's method and made necessary to expand the spectral curves in the ranges 260–290 and 220 to 240 nm; Fig. 2 is proposed as an example. All methods described are based on the observance of Lambert–Beer Law; the values of the absorptivities of *m*-aminobenzoic acid, averaged out amongst those obtained at different levels of acidity, are: $\epsilon(206) = 19.570$; $\epsilon(241) = 5.190$; $\epsilon(273) = 784$; $\epsilon(280) = 927$; and $\epsilon(298) = 1.760$.

The methods described for obtaining constants for overlapping equilibria have been developed for low acidity regions and suppose behaviour to be ideal. It has recently been shown⁸ for a number of aniline derivatives, even in low acidity regions,

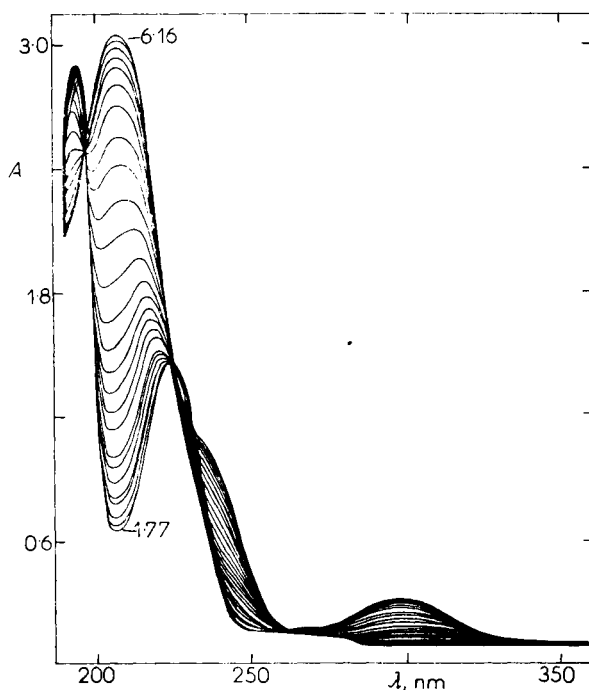


FIG. 1

Absorption UV spectra of the acid-base protonation equilibria of *m*-aminobenzoic acid at 25°C and $1.312 \cdot 10^{-4} \text{ mol dm}^{-3}$ at several acidities: pH = 6.16; 5.94; 5.67; 5.40; 5.27; 5.09; 4.92; 4.75; 4.59; 4.42; 4.29; 4.10; 3.91; 3.75; 3.60; 3.44; 3.29; 3.16; 2.99; 2.83; 2.68; 2.50; 2.40; 2.17; 1.96 and 1.77, consecutively

that $\log(f_{\text{AH}}/f_{\text{A}^-}) \neq 0$ and this behaviour was interpreted in terms of the parameter n , corresponding to an equilibrium, according to the equation:

$$\log I = -n_j \text{pH} + n_j \text{p}K_j \quad (j = 1, 2). \quad (12)$$

The system approaches ideal behaviour in so far as n_j tends towards unity. In this investigation the methods described have been corrected by introducing values for n_1 and n_2 which denote the behaviour of Eqs (A) and (B); the calculation of n_1 and n_2 was carried out by means of the separation method⁹, which also permits the obtention of $\text{p}K_1$ and $\text{p}K_2$.

Method of Separation

The basic Eq. (12) is applied to Eqs (A) and (B) along with a mass balance; this enables us to obtain the C_1 , C_2 and C_3 expressions, which are relative quantities of the species AH_2^+ , AH and A^- , respectively:

$$C_3 = (1 + a_{\text{H}^+}^{n_2} \cdot K_2^{-n_2} + a_{\text{H}^+}^{(n_1+n_2)} \cdot K_1^{-n_1} \cdot K_2^{-n_2})^{-1} \quad (13)$$

$$C_2 = C_3 \cdot a_{\text{H}^+}^{n_2} \cdot K_2^{-n_2} \quad (14)$$

$$C_1 = 1 - (C_2 + C_3). \quad (15)$$

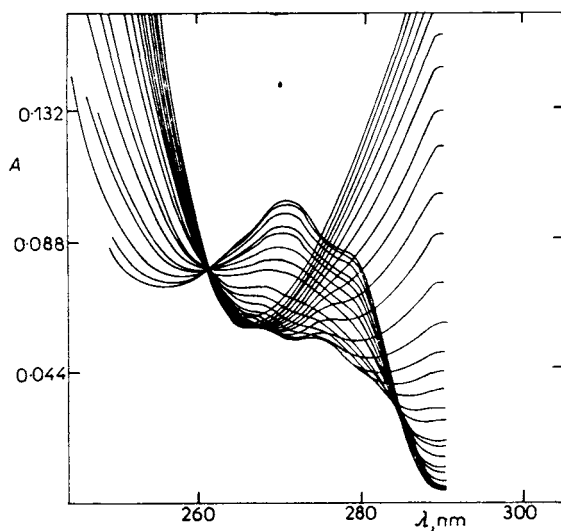


FIG. 2
Expanded spectra in the isosbestic zone 260–290 nm of *m*-aminobenzoic acid

We postulate initial values for pK_1 , pK_2 , n_1 and n_2 ; for each acidity value, the quantities C_1 , C_2 and C_3 are determined. We already know at the same wavelength A_1 (at pH = 1.77), A_3 (at pH = 6.16) and A (at any intermediary level of acidity) and by means of Eq. (16) we can obtain the value for A_2 for each value of the acidity; then, the average can thus be arrived at:

$$A_2 = \frac{A - C_1 \cdot A_1 - C_3 \cdot A_3}{C_2} \quad (16)$$

Once the A_2 value has been obtained it is possible to eliminate the contribution of AH_2^+ to the total absorbance, A , and calculate by means of Eq. (17) values for A_c (corrected absorbance) which would correspond to the system if the only species existing in equilibrium were A^- and AH :

$$A_c = \frac{A - C_1 \cdot A_1}{C_2 + C_3} \quad (17)$$

This process can be applied independently to one wavelength or to several different ones, in such a way that, by means of Eq. (3), the ionization ratio for each level of acidity can be determined starting from the A_c values obtained previously. The linear regression Eq. (12) can be then used to obtain n_2 and pK_2 . In a similar fashion, we then eliminate the contribution made by the A^- species to the total absorption and, by means of Eq. (18), A'_c values can be obtained, which will be those belonging to the system in the case of species AH and AH_2^+ being the only ones in equilibrium:

$$A'_c = \frac{A - C_3 \cdot A_3}{C_1 + C_2} \quad (18)$$

In this way, Eq. (A) can be studied independently and, by means of Eqs (3) and (12), values are obtained for n_1 and pK_1 . If the values now calculated for n_1 , n_2 , pK_1 and pK_2 do not match those postulated initially, the process is repeated until self-consistent results are achieved.

Table I collects the absorbances at different wavelengths used in this work; by taking λ outside the isosbestic zone, which is the case with 206, 241 and 298 nm, and accepting as initial values⁶ $pK_2 = 4.788$ and $pK_1 = 3.086$ and $n_1 = n_2 = 1$, the values of Table II are obtained.

Correction to Ang's Method

Eq. (12) may be written as:

$$\log I = \log [H^+]^{n_j} + \log K_j^{-n_j}$$

and together with Ang's equations, the following equations can be obtained:

$$A = A_1 - K_1^{n_1} \cdot Q \quad (19)$$

$$Q = \frac{(A - A_2) ([H^+]_2^{n_2} - [H^+]_1^{n_2})}{[H^+]_2^{n_1+n_2} - [H^+]_1^{n_1+n_2}} \quad (20)$$

$$A = A_2 + K_2^{n_2} \cdot P \quad (21)$$

TABLE I

Absorbances of *m*-aminobenzoic acid at several wavelengths and different acidity media at 25°C and $1.312 \cdot 10^{-4} \text{ mol dm}^{-3}$

pH	Absorbance				
	206 nm	241 nm	273 nm	298 nm	280 nm
6.16	2.952	0.702	0.080	0.239	0.119
5.94	2.931	0.700	0.078	0.230	0.116
5.67	2.892	0.690	0.075	0.223	0.112
5.40	2.847	0.669	0.072	0.216	0.109
5.27	2.799	0.654	0.070	0.209	0.104
5.09	2.715	0.630	0.068	0.197	0.098
4.92	2.610	0.600	0.065	0.185	0.092
4.75	2.457	0.570	0.063	0.156	0.084
4.59	2.316	0.540	0.061	0.154	0.077
4.42	2.145	0.501	0.058	0.136	0.070
4.29	1.965	0.465	0.045	0.120	0.062
4.10	1.761	0.420	0.044	0.100	0.054
3.91	1.578	0.390	0.044	0.085	0.049
3.75	1.443	0.360	0.044	0.074	0.045
3.60	1.335	0.339	0.057	0.064	0.045
3.44	1.236	0.315	0.059	0.056	0.046
3.29	1.137	0.291	0.062	0.047	0.047
3.16	1.050	0.270	0.066	0.040	0.051
2.99	0.921	0.237	0.073	0.030	0.057
2.83	0.870	0.225	0.077	0.025	0.060
2.68	0.798	0.201	0.081	0.020	0.065
2.50	0.720	0.180	0.086	0.013	0.069
2.40	0.690	0.168	0.088	0.011	0.071
2.17	0.630	0.150	0.092	0.006	0.075
1.96	0.591	0.138	0.095	0.002	0.078
1.77	0.567	0.134	0.096	0.000	0.079

$$P = \frac{(A - A_3) ([H^+]_2^{n_1+n_2} - [H^+]_1^{n_1+n_2})}{[H^+]_2^{n_2} \cdot [H^+]_1^{n_2} ([H^+]_1^{n_1} - [H^+]_2^{n_1})} \quad (22)$$

If n_1 and n_2 are known, then Eqs (19) and (21) are straight line equations which enable K_1 and K_2 to be obtained. The plot $A = f(\text{pH})$ at $\lambda = 280 \text{ nm}$ (the isobestic zone) leads to a curve with a minimum, Fig. 3; an area can be observed where two unequal pH readings coincide for the same absorbance value and this fact allows

TABLE II

Results obtained for *m*-aminobenzoic acid by different methods; r_{xy} represent the linear regression coefficients and $A_2(1)$, $A_2(2)$ and $A_2(3)$ the averaged absorbances at 206, 241 and 298 nm respectively, the parenthesized quantities being the observed deviations

a) Separation method

$$\text{p}K_2 = 4.767 \pm 0.002$$

$$n_2 = 1.244$$

$$r_{xy} = 0.9992$$

$$\text{p}K_1 = 3.237 \pm 0.091$$

$$n_1 = 1.008$$

$$r_{xy} = 0.9820$$

$$A_2(1) = 1.330 (0.1448)$$

$$A_2(2) = 0.327 (0.0448)$$

$$A_2(3) = 0.061 (0.0155)$$

b) Ang's method

$$\text{p}K_2 = 4.874 \pm 0.007 \quad \text{p}K_1 = 3.191 \pm 0.091$$

$$r_{xy} = 0.9927 \quad r_{xy} = 0.9714$$

c) Mongay's method^a

$$\text{p}K_2 = 4.764 \pm 0.004 \quad \text{p}K_1 = 3.176 \pm 0.061$$

^a Obtained as averaged values from planes 1 and 2.

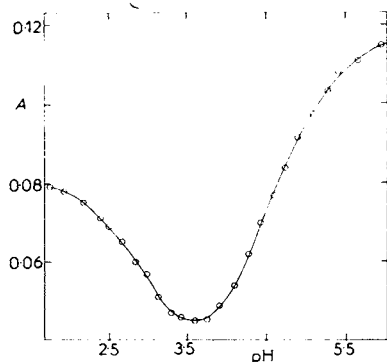


FIG. 3
Plot of the function $A = f(\text{pH})$ at 280 nm

Ang's method to be applied; the results obtained are displayed in Table II. For n_1 and n_2 we used the values calculated in the separation method, and the calculation was then repeated with $n_1 = n_2 = 1$, giving the values $pK_2 = 4.471$ and $pK_1 = 3.090$.

Correction to Mongay's Method

The framework of Mongay's method is used without eliminating the terms relating to the non-ideality of the system. Eq. (12) is applied to Eqs (A) and (B) and the following is obtained:

$$K_1^{n_1} = [\text{AH}] [\text{H}^+]^{n_1} / [\text{AH}_2^+] \quad (23)$$

$$K_2^{n_2} = [\text{A}^-] [\text{H}^+]^{n_2} / [\text{AH}] \quad (24)$$

from which it can be deduced that:

$$[\text{AH}_2^+] = [\text{H}^+]^{n_1+n_2} [\text{A}^-] / K_1^{n_1} \cdot K_2^{n_2} \quad (25)$$

$$[\text{AH}] = [\text{H}^+]^{n_2} [\text{A}^-] / K_2^{n_2} \quad (26)$$

which, bearing in mind Eqs (5) and (6), led in turn to:

$$(A - A_3) + (A - A_2) [\text{H}^+]^{n_2} / K_2^{n_2} + (A - A_1) [\text{H}^+]^{n_1+n_2} / K_1^{n_1} K_2^{n_2} = 0. \quad (27)$$

This equation can be rearranged and set out as the equation of a plane, Eq. (11), where X , Y and Z are functions dependent on known parameters and, then, can be calculated; a_0 , a_1 and a_2 include K_1 , K_2 and A_2 (intermediate absorbance), the problem being solved by a multiple linear regression using the least squares method. Although several planes can be obtained from Eq. (27), for reasons of accuracy⁶ only those of Table III have been selected. Use is made of a set of $A/[\text{H}^+]$ values and n_1 and n_2 are introduced; Z , X and Y are determined for each $A/[\text{H}^+]$ pair and the multilinear regression is carried out. We have chosen the values $n_1 = 1.008$ and

TABLE III

Planes of regression obtained by means of the correction of Mongay's method

Plane	1	2
Equation	$Z = 1/A_2 - K_2^{n_2} \cdot A_2^{-1} \cdot X - (A_2 \cdot K_1^{n_1})^{-1} \cdot Y$	$Z = A_2 - (K_1^{n_1})^{-1} \cdot X - K_2^{n_2} \cdot Y$
X	$(A_3 - A)/A \cdot [\text{H}^+]^{n_2}$	$[\text{H}^+]^{n_1} \cdot (A - A_1)$
Y	$[\text{H}^+]^{n_1} \cdot (A_1 - A)/A$	$(A - A_3)/[\text{H}^+]^{n_2}$
Z	A^{-1}	A

$n_2 = 1.244$, which were obtained in the separation method. By operating with absorbance values at five wavelengths from Table I (273 and 280 nm fall within the isosbestic area and 206, 241 and 298 nm fall outside it) and by taking averaged values from the pK values obtained from planes 1 and 2 as X acts on Y, Z ; Y acts on X, Z ; and Z acts on X, Y , the result is shown in Table III; in every case the coefficients of linear correlation were over 0.99.

The same calculation procedure, taking $n_1 = n_2 = 1$ led to $pK_2 = 4.510 \pm 0.030$ and $pK_1 = 3.036 \pm 0.073$; the intermediate absorbance A_2 corresponds to a pH value in the range 3.60–3.44 at all wavelengths, a result consistent with that obtained by the separation method. Albert⁴ has estimated where equilibria according Eqs (A) and (B) begin, between the pH values 3.20 and 4.42 in agreement with the result obtained in this work; this author points out that the two equilibria considered together occur in the pH region 2.57–5.19, as opposed to the range arrived at in this investigation of 1.77–6.16. This fact suggests that the ϵ_1 and ϵ_2 values used by Albert do not match the diprotonate and non-protonate forms exactly, since the differences $2.57 - 1.77 = 0.80$ and $6.16 - 5.19 = 0.97$ imply that at pH = 5.19 the equilibrium has already begun and that at pH = 2.57 it has not yet finished, where $A_1 = A_{A^-} + A_{AH}$ and $A_3 = A_{AH} + A_{AH_2^+}$. In spite of this, the values arrived at for $I = 0.016$ are $pK_2 = 3.075$ and $pK_1 = 4.798$, in agreement with those obtained in this work.

Of the three methods shown in this work, the method of separation is more accurate since it permits the choice of any wavelength as well as the carrying out of the iterative procedure. Ang's method, however, becomes more limited and applicable only to those compounds showing acceptable $A = f(\text{pH})$ curves, as in Fig. 3; whenever very flat curves are obtained, then, the method proves to be inadequate. In the case of Mongay's method the trouble lies in the need for a proper choice of the planes; for reasons of accuracy, planes 1 and 2 have been selected in this work, but some others could have been used. On the other hand, X, Y and Z depend on the variable A and this fact can lead to the obtention of associated errors and distorted results.

Thanks are due to Prof. P. Mitchell for his contribution to the English version of the text. The financial support of Caja de Ahorros Municipal de Burgos is gratefully acknowledged.

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