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Spectrophotometric evaluation of acidity constants of isonicotinic acid

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Summary

The acid–base equilibria of isonicotinic acid in aqueous media with a constant ionic strength 0.1 at $20 \pm 1°C$ have been spectrophotometrically studied in detail. A new numerical method applicable to the evaluation of overlapping acidity constants has been described.

Introduction

Isonicotinic acid (HR) is a compound which is of considerable biological interest (Guzmán et al., 1984). A derivative of isonicotinic acid, the hydrazide, isoniazide, possesses tuberculostatic properties and when it is administered to patients, both isonicotinic acid and isonicotinylglycine are found in the urine as a result of its metabolism in the human body (Cuthbertson, 1953). The elimination either fecal or urinary vitamin B_6 in any of its active forms (pyridoxine, pyridoxamine or pyridoxal) leads to isonicotinic acid as the main metabolite (Rabinowitz and Snell, 1949). Compounds showing the general structure py–CONH–NHR have been used for clinical purposes in the treatment of mental depressions; that they act as monoamine oxidase inhibitors (Biel and Bopp, 1974) and also form free isonicotinic acid as a metabolite.

A search through the literature reveals that a number of investigations dealing with the UV characteristics or/and spectrophotometric evaluation of acidity constants of isonicotinic acid (Evans et al., 1953; Jellinek and Urwin, 1954; Black, 1955; Green and Tong, 1956; Lumme, 1957, Stephenson and Sponer, 1957) have been carried out. However, some criticism can be made on these papers although they are excellent. In particular, the absorbance of the pure species H_2R (A_2) cannot be calculated directly because of the low pK_{a_1} value, without producing a drastic change in the ionic strength of the medium. In addition, the scatter of the experimental points in the plots of absorbance against pH (A–pH) at $pH < 1$ results in a considerable uncertainty in the value of A_2. Other minor criticisms are that tedious iterative calculations are applied (Jellinek and Urwin, 1954), and that few wavelengths are used in the evaluation of pK_a values. A great advantage of

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spectrophotometry is that at different wavelengths, the absorptivities bear quite different relationships to each other. The agreement of pK_a values obtained at different wavelengths gives reliability to results. For that reason a re-investigation of acidity constants of isonicotinic acid might be of some interest. In the present investigation protolytic equilibria of isonicotinic acid have been studied spectrophotometrically in aqueous solution with a constant ionic strength 0.1 M at 20 ± 1°C.

Theory

The following definitions and symbols are used.

(a) Apparent acidity constants: $K_{a_1} = (H^+)[HR]/[H_2R^+]$; $K_{a_2} = (H^+)[R^-]/[HR]$ which differ from the classical and thermodynamic constants because the terms are the hydrogen ion activity and the concentration of two forms of isonicotinic acid.

(b) ϵ_2, ϵ_1, ϵ_0 = molar absorbancy coefficients of H_2R^+, HR and R^-.

(c) C_R = total concentration of reagent; A_2, A_1, A_0 = absorbancy of a solution containing H_2R^+, HR or R^- at a concentration C_R (constant throughout measurements).

The following expression for the absorbance as function of the hydrogen ion activity can be derived (Asuero et al., 1986b).

$$A = \frac{A_0 + A_1\frac{(H)}{K_{a_2}} + A_2\frac{(H)^2}{K_{a_2}\cdot K_{a_1}}}{1 + \frac{(H)}{K_{a_2}} + \frac{(H)^2}{K_{a_2}\cdot K_{a_1}}} \qquad (1)$$

The theoretical background of the methods used in this paper will be outlined in the following.

Evaluation of the second dissociation constant of isonicotinic acid

At high pH values HR and R^- are the only species to take into consideration and the expression for A becomes

$$A = \frac{A_0 + A_1\frac{(H)}{K_{a_2}}}{1 + \frac{(H)}{K_{a_2}}} \qquad (2)$$

As equilibria overlap, there is no pH-range where HR is the only absorbing species. Eqn. 2 can be rearranged into several forms suitable for linear plotting (Navas et al., 1985):

double reciprocal equation:

$$\frac{1}{A - A_0} = \frac{1}{A_1 - A_0} + \frac{1}{A_1 - A_0}\frac{K_{a_2}}{(H)} \qquad (3)$$

parallel straight lines equation:

$$A = A_1 + \frac{A_0 - A}{(H)}K_{a_2} \qquad (4)$$

Agren-Sommer equation:

$$\frac{C_R}{A} = \frac{1}{\epsilon_1} + \frac{A - A_0}{A(H)}\frac{K_{a_2}}{\epsilon_1} \qquad (5)$$

The experimental A–pH data are thus transformed into diagrams of two variables that should give straight lines ($y = a_0 + a_1 \cdot x$).

According to the Eqns. 3 and 5, the ratio of the slope to the y intercept gives K_a, whereas the value of K_a is given in Eqn. 4 by the slope of the corresponding straight line. Taking into account the law of error accumulation for a random variable y which is a function of observation $x_1 \ldots x_N$

$$y = f(x_1, x_2, \ldots, x_N) \qquad (6)$$

we have

$$\mathrm{var}(y) = s^2(y) = \sum_{i=1}\left(\frac{\partial f}{\partial x_i}\right)^2 \cdot s_i^2 + 2\sum_{i\neq j}\left(\frac{\partial f}{\partial x_i}\right)\left(\frac{\partial f}{\partial x_j}\right)\cdot \mathrm{cov}(x_i, x_j) \qquad (7)$$

The application of Eqn. 7 to the double reciprocal and Agren-Sommer equations gives:

$$s^2_{K_a} = \frac{1}{a_0^2} s^2_{a_1} + \frac{a_1^2}{a_0^4} s^2_{a_0} - 2\frac{a_1}{a_0^3} \cdot \mathrm{cov}(a_0,a_1) \qquad (8)$$

whereas in the case of parallel straight lines equation we have

$$s^2_{K_a} = s^2_{a_1} \qquad (9)$$

The variances of slope ($s^2_{a_1}$) and intercept ($s^2_{a_0}$), as well as the covariance of slope and intercept $\mathrm{cov}(a_0,a_1)$, can be easily evaluated from the variance–covariance matrix (Asuero et al., 1986a).

$$s^2_{a_0} = \frac{\sum x_i^2 \cdot s^2_{y/x}}{NS_{XX}}; \qquad s^2_{a_1} = \frac{s^2_{y/x}}{S_{XX}}$$

$$\mathrm{cov}(a_0,a_1) = \mathrm{cov}(a_1,a_0)$$

$$= \frac{\bar{x} \cdot s^2_{y/x}}{S_{XX}} \qquad (10)$$

being $s^2_{y/x}$ the variance of regression line

$$s^2_{y/x} = s_{YY} - b_1^2 S_{XX} \qquad (11)$$

and

$$S_{XX} = \sum x^2 - \left(\sum x\right)^2/N$$

$$S_{YY} = \sum y^2 - \left(\sum y\right)^2/N \qquad (12)$$

(N is the number of pairs of measurements x_i,y_i).

The standard deviation of pK_a is calculated from

$$s^2_{(pK_a)} = \left(\frac{\partial pK_a}{\partial K_a}\right)^2 \cdot s^2_{(K_a)} \qquad (13a)$$

and then

$$s_{(pK_a)} = \frac{1}{K_a} \cdot \log_e s_{(K_a)} \qquad (13b)$$

However, previous to the application of the slope–intercept procedures described by Eqns. 3–5 it is necessary to calculate the pH range in which only the absorbing species R and HR are found in solution from a practical point of view.

Determination of the number of absorbing species in solution

The absorbance of a solution containing n absorbing species at any given wavelength λ is given by

$$A = \sum_0^N \epsilon_n |S_n| \quad (N = 0\text{–}n) \qquad (14)$$

If absorbance is measured at N + 1 wavelengths then according to the Rouche-Frobenius theorem (Rey Pastor and de Castro Brzezicki, 1974)

$$\begin{vmatrix} A_{\lambda_1} & \epsilon_{11} & \epsilon_{21} & \cdots & \epsilon_{n1} \\ A_{\lambda_2} & \epsilon_{12} & \epsilon_{22} & \cdots & \epsilon_{n2} \\ \vdots & \vdots & \vdots & & \vdots \\ A_{\lambda_N} & \epsilon_{1N} & \epsilon_{2N} & \cdots & \epsilon_{nN} \\ A_{\lambda_{N+1}} & \epsilon_{1,N+1} & \epsilon_{2,N+1} & \cdots & \epsilon_{n,N+1} \end{vmatrix} = 0 \qquad (15)$$

and then

$$A_{\lambda_{N+1}} = k_1 A_{\lambda_1} + k_2 A_{\lambda_2} + \ldots + k_N A_{\lambda_N}$$

$$= \sum_1^N k_j A_{\lambda_j} \qquad (16)$$

where $k_1, k_2, \ldots k_N$ are constants (Jimenez et al., in press). From this equation we have the graphical tests included in Table 1 for testing the number of absorbing species in solution. By plotting the ordinate functions against the abscissa functions included in Table 1, a straight line must be obtained if the number of absorbing species assumed are correct (Budesinsky, 1972).

Numerical evaluation of overlapping acidity constants of isonicotinic acid

In the absence of any assumption concerning

TABLE 1

GRAPHICAL TESTS FOR THE DETERMINATION OF THE NUMBER OF ABSORBING SPECIES

Number of species	Wavelengths	Ordinate function	Abscissa function	Comments
One	1 and 2	A_{2g}	A_{1g}	zero intercepts
Two	1, 2 and 3	A_{3g}/A_{1g}	A_{2g}/A_{1g}	
Three	1, 2, 3 and 4	$\frac{A_{4g}A_{1a} - A_{4a}A_{1g}}{A_{2g}A_{1a} - A_{2a}A_{1g}}$	$\frac{A_{3g}A_{1a} - A_{3a}A_{1g}}{A_{2g}A_{1a} - A_{2a}A_{1g}}$	a fixed

the knowledge of absorbances for pure species H_2R, HR and R, Eqn. 1 contains five unknowns and so the measure of absorbances and pH of five different solutions a, b, c, d and e, permit evaluation of the five parameters which determine the form of the graph of A as a function of pH.

Eqn. 1 on rearrangement gives:

$$(A - A_0) + (A - A_1) \cdot \frac{(H)}{K_{a_2}} + (A - A_2) \frac{(H)^2}{K_{a_2} \cdot K_{a_1}} = 0 \qquad (17)$$

which is easily converted into:

$$A \cdot K_{a_2} \cdot K_{a_1} - A_0 \cdot K_{a_2} \cdot K_{a_1} + A \cdot K_{a_1}(H) - A_1 \cdot K_{a_1}(H) - A_2(H)^2 = -A(H)^2 \qquad (18)$$

By eliminating $A_0 \cdot K_{a_2} \cdot K_{a_1}$ between two solutions i and a we have an equation with five terms and four unknowns (between brackets)

$$\left((H)_a^2 - (H)_i^2\right) |A_2 - A_a| + ((H)_a - (H)_i) |K_{a_1}(A_1 - A_a)| + (A_i - A_a) |K_{a_1} \cdot K_{a_2}| + (H)_i(A_i - A_a) |K_{a_1}| = -(H)_i^2(A_i - A_a) \qquad (19)$$

The four unknowns $(A_2 - A_a)$, K_{a_1} $(A_1 - A_a)$, $K_{a_1} \cdot K_{a_2}$ and K_{a_1} can be evaluated by solving a system of four linear equations, which result when in equals successively b, c, d and e, e.g. by Gaussian elimination (Theodor, 1982). The value of A_0 is then calculated from any (pH_j, A_j) point (j = a, b, c, d or e) by applying Eqn. 1:

$$A_0 = A_j + (A_j - A_1)10^{pK_{a_2} - pH_j} + (A_j - A_2)10^{pK_{a_2} + pK_{a_1} - 2pH_j} \qquad (20)$$

Materials and Methods

Apparatus

All photometric measurements were made with a Bausch & Lomb Spectronic 2000 instrument. Matched silica cuvettes were used in all measurements and the light path was 10 mm. The pH was measured with a Crison Model 501 pH meter fitted with a combined glass electrode. The pH meter was standardized against 0.05 M potassium phthalate (pH 4.01 at 25°C).

Reagents

Isonicotinic acid (Sigma), perchloric acid (Merck), sodium hydroxide (Merck) and glass-distilled water.

Procedure

Solutions for absorbance and pH measurements were prepared by mixing 2 ml of 2×10^{-3} M stock solution of isonicotinic acid in water, 2.5 ml of sodium perchlorate and a few drops of sodium hydroxide or perchloric acid at different concentrations. The solutions were then diluted to 25 ml with distilled water. Absorbance was measured against a solvent blank and the pH checked after the absorbance measurements. The temperature was kept at 20 ± 1°C.

Results and Discussion

Absorption spectra with varying pH

The UV-visible absorption spectra of 1.6×10^{-4} M isonicotinic acid in aqueous solutions at various pH values are plotted in Fig. 1. The ionization steps of isonicotinic acid may be represented as $H_2R^+ \overset{K_{a_1}}{\rightleftharpoons} HR \overset{K_{a_2}}{\rightleftharpoons} R^-$. Inspection of the absorbance curves at various pH values shows that suitable wavelengths (250–275 nm) can be selected at which the difference between the absorbances of the H_2R^+ and HR and R^- species are large enough. Absorbance in dependence of the pH for 11 different wavelengths were selected. Typical A–pH curves measured at fixed wavelengths are shown in Fig. 2. Several features of such curves are indicated in the following: (i) at pH > 7 a constant absorbance was obtained and so A_0 is known; (ii) as equilibria overlaps, there is no pH-range where HR is the only absorbing species

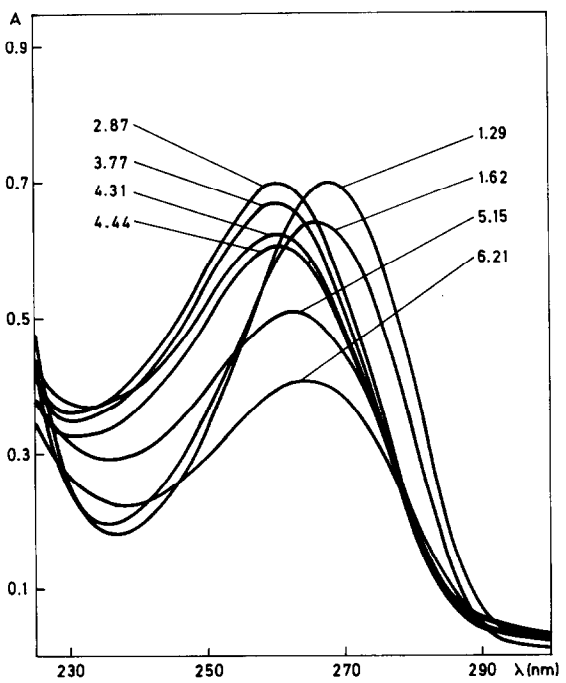

Fig. 1. Absorption curves of isonicotinic acid at different pH values. Dependence of absorption data of isonicotinic acid on pH.

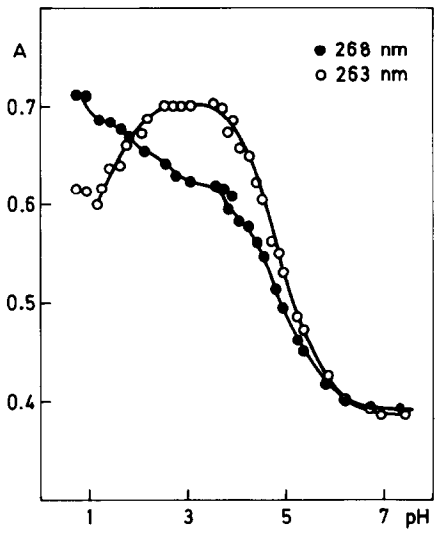

Fig. 2. Absorbance data of isonicotinic acid depending on pH.

and so A_1 is unknown; (iii) the relatively low pK_{a_1} value together with the scatter observed in the measurement of absorbances in acidic medium (pH < 1) precludes the knowledge of A_2; and (iv) in the wavelength range of 267–275 nm, $A_2 > A_1 > A_0$—at 266 nm it follows that $A_2 \simeq A_1 > A_0$, whereas in the 250–265 nm wavelength region, $A_2 < A_1 > A_0$.

Determination of the number of absorbing species

Typical results obtained in the application of graphical procedures for testing the number of absorbing species in solution are shown in Fig. 3. Linear relationships were not obtained assuming three species in the pH range 4–6.5, but instead straight lines were obtained in the two species test. Although straight lines were also obtained in the one species test, the curves did not pass through the origin.

Evaluation of the second dissociation constant of isonicotinic acid

Figs. 4, 5 and 6 show the plots obtained by appling the double reciprocal method, the parallel straight lines method and the method of Agren-Sommer, respectively. As can be seen, straight lines are obtained indicating that the assumption that only two species (R and HR) are present was

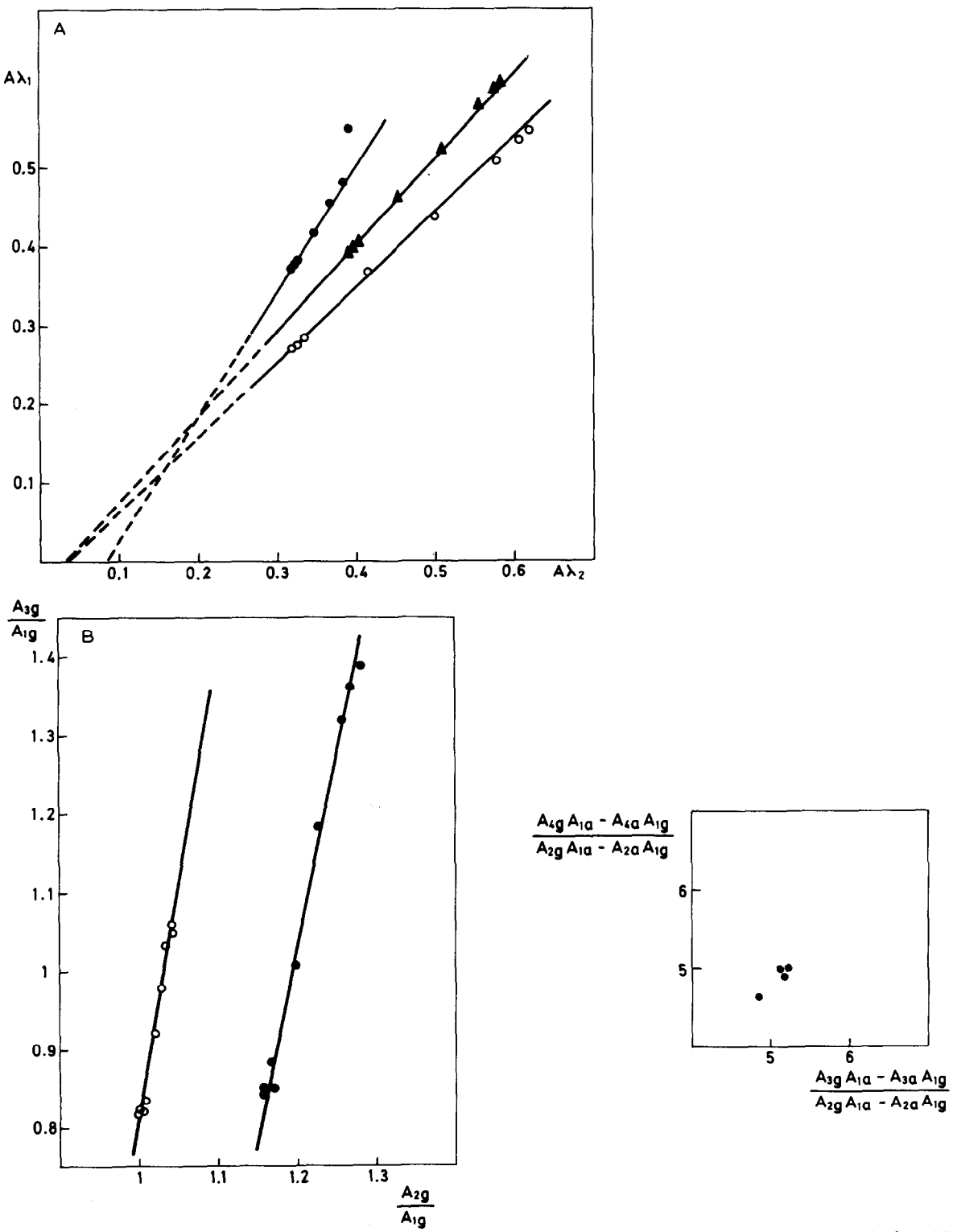

Fig. 3. Determination of number of species at varying pH values. A: one-species. (●) $\lambda_1 = 271$ nm, $\lambda_2 = 275$ nm; (○) $\lambda_1 = 250$ nm, $\lambda_2 = 255$ nm; (▲) $\lambda_1 = 267$ nm, $\lambda_2 = 268$ nm, B: two-species. (●) $\lambda_1 = 275$ nm, $\lambda_2 = 271$ nm, $\lambda_3 = 260$ nm; (○) $\lambda_1 = 268$ nm, $\lambda_2 = 267$ nm, $\lambda_3 = 255$ nm. C: three species. (●) $\lambda_1 = 275$ nm, $\lambda_2 = 271$ nm, $\lambda_3 = 255$ nm, $\lambda_4 = 250$ nm.

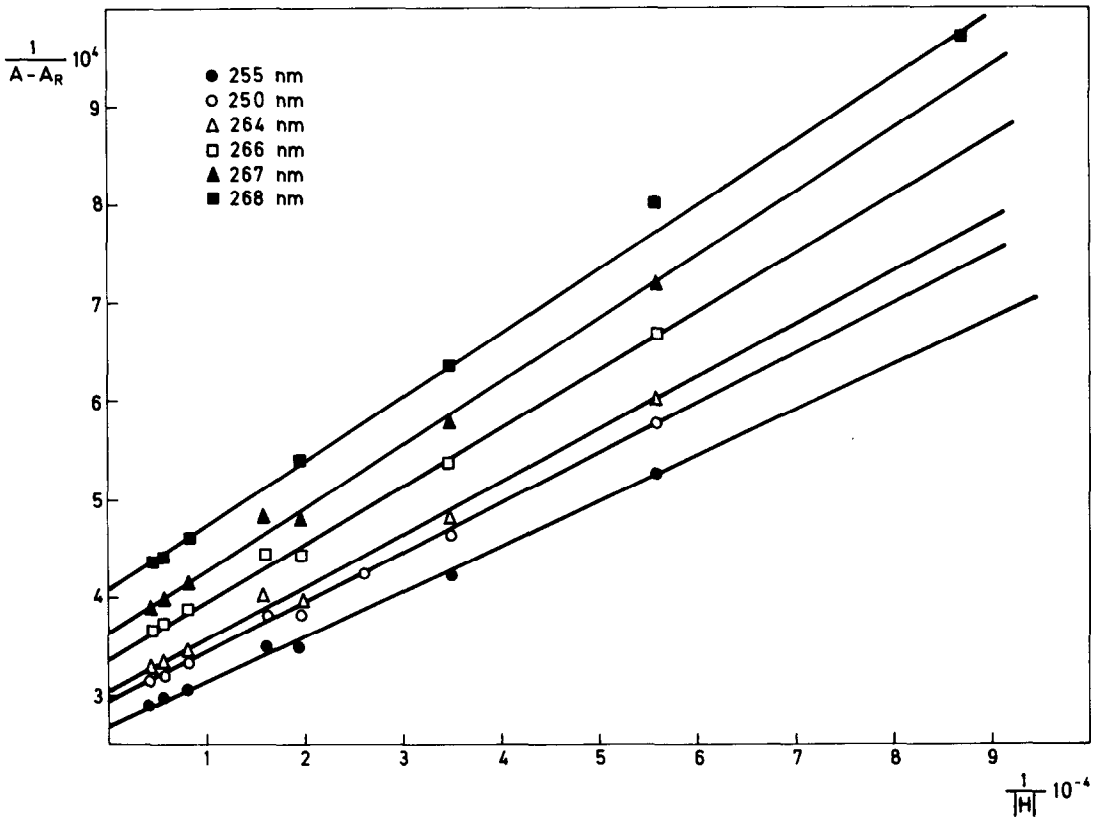

Fig. 4. Typical slope intercept plots: double reciprocal method.

correct (x and y have been multiplied by a power factor to keep them within the range 1 to 10). The results of the least-squares treatment of data, included in the theoretical section, gives the values of $pK_a \pm s_{(pK_a)}$ compiled in Table 2. Excellent agreement was obtained between the pK_a values determined at different wavelengths. The reproducibility of these results illustrated the reliability of the present procedure.

Evaluation of overlapping acidity constants of isonicotinic acid

A number of 5 pairs of points of the A–pH curve were selected in order to evaluate the overlapping pK_a values of isonicotinic acid. The results are given in Table 3 which shows that the mean pK_{a_2} value obtained is about 0.05 units higher than the mean pK_{a_2} value obtained from the slope–intercept procedures. There is some

TABLE 2

SECOND ACIDITY CONSTANT OF ISONICOTINIC ACID EVALUATED FROM SLOPE INTERCEPT PROCEDURES

λ (nm)	Method: Double reciprocal (±S.D.)	Parallel straight lines (±S.D.)	Agren-Sommer (±S.D.)
250	4.771 ± 0.030	4.781 ± 0.017	4.821 ± 0.025
255	4.783 ± 0.029	4.789 ± 0.016	4.804 ± 0.006
260	4.817 ± 0.021	4.794 ± 0.017	4.806 ± 0.015
263	4.771 ± 0.037	4.799 ± 0.016	4.805 ± 0.006
264	4.772 ± 0.040	4.799 ± 0.016	4.804 ± 0.007
265	4.775 ± 0.040	4.804 ± 0.017	4.811 ± 0.012
266	4.775 ± 0.041	4.776 ± 0.019	4.797 ± 0.013
267	4.768 ± 0.050	4.770 ± 0.020	4.812 ± 0.022
268	4.792 ± 0.038	4.793 ± 0.019	4.819 ± 0.026
Mean value	4.78 ± 0.04	4.79 ± 0.02	4.81 ± 0.01

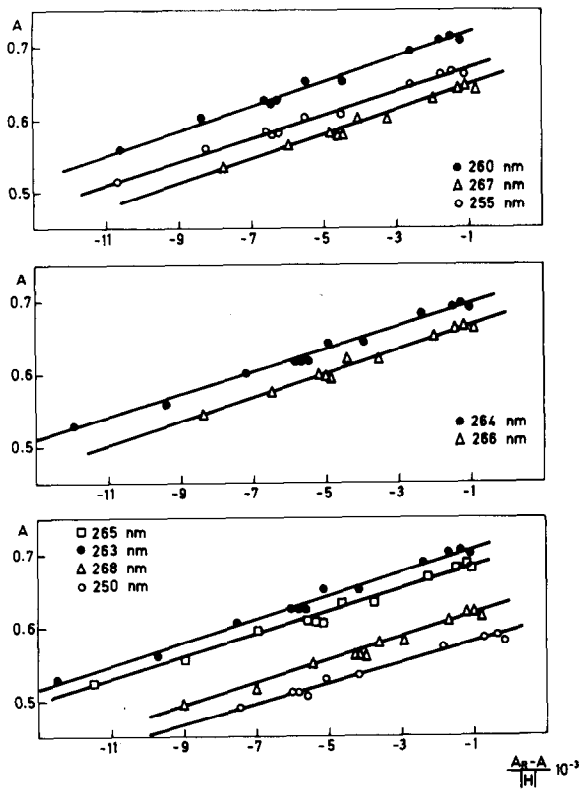

Fig. 5. Typical slope intercept plots: parallel straight lines.

scatter in the absorbance at low pH values and numerical methods are very sensitive to the choice of points used for the calculation. Because graphs have greater impact than values of acidity constants represented as numbers in a table, the results obtained have been graphically represented (Figs. 7 and 8). There is a good agreement between the limit absorbances A_o evaluated in this way and the ones selected from the shape of A–pH graphs in the alkaline range of spectra. Results obtained for the limit absorbances of the HR species, A_1, also agree well with the ones obtained by applying slope–intercept procedures assuming that A_0 is known.

The mean pK_a values obtained by means of slope–intercept procedures and by the numerical method developed in this paper are presented in Table 4. Literature pK_a values of isonicotinic acid are also listed in Table 4 for the sake of comparison. Bearing in mind the difference in temperature and medium, the constants found agree well with some of the previous reported values.

The thermodynamic constants (pK_a^*) of isonicotinic acid are related to the apparent acidity constants (pK_a) by means of:

$$pK^*_{a_2} = pK_{a_2} - \log f_{R^-}$$

$$pK^*_{a_1} = pK_{a_1} + \log f_{H_2R^+}$$

where

$$\log f_{R^-} = \log f_{H_2R^+} = -B\frac{\sqrt{I}}{1+\sqrt{I}} \quad \text{(Bockris and Reddy, 1978)}$$

TABLE 3

NUMERICAL EVALUATION OF THE ACIDITY CONSTANTS OF ISONICOTINIC ACID

λ (nm)	n	$pK_{a_1} \pm$ S.D.	$pK_{a_2} \pm$ S.D.	$A_2 \pm$ S.D.	$A_1 \pm$ S.D.	$A_0 \pm$ S.D.	A_0^*	A_1^{**}
250	6	1.75 ± 0.04	4.87 ± 0.08	0.207 ± 0.020	0.598 ± 0.015	0.272 ± 0.002	0.275	0.609 ± 0.003
255	7	1.79 ± 0.07	4.84 ± 0.05	0.339 ± 0.020	0.682 ± 0.013	0.322 ± 0.002	0.323	0.689 ± 0.002
260	6	1.74 ± 0.08	4.86 ± 0.06	0.491 ± 0.018	0.722 ± 0.014	0.368 ± 0.003	0.368	0.732 ± 0.002
263	6	1.79 ± 0.13	4.84 ± 0.06	0.595 ± 0.018	0.717 ± 0.012	0.388 ± 0.002	0.388	0.725 ± 0.004
264	5	1.73 ± 0.12	4.84 ± 0.06	0.619 ± 0.017	0.706 ± 0.012	0.392 ± 0.002	0.392	0.715 ± 0.004
265	3	1.80 ± 0.10	4.84 ± 0.07	0.641 ± 0.018	0.699 ± 0.007	0.395 ± 0.003	0.394	0.701 ± 0.004
266	2	1.81 ± 0.04	4.80 ± 0.02	0.657 ± 0.008	0.683 ± 0.008	0.396	0.394	0.686 ± 0.002
267	4	1.73 ± 0.10	4.86 ± 0.08	0.692 ± 0.016	0.653 ± 0.013	0.394 ± 0.003	0.393	0.662 ± 0.003
268	6	1.89 ± 0.17	4.87 ± 0.07	0.705 ± 0.014	0.621 ± 0.010	0.392 ± 0.002	0.391	0.634 ± 0.002
271	7	1.79 ± 0.10	4.89 ± 0.08	0.736 ± 0.012	0.526 ± 0.010	0.375 ± 0.002	0.374	–
275	7	1.76 ± 0.03	4.86 ± 0.14	0.698 ± 0.010	0.410 ± 0.008	0.323 ± 0.003	0.321	–

* Selected values from the shape of A–pH graphs.

** As calculated from slope intercept procedure.

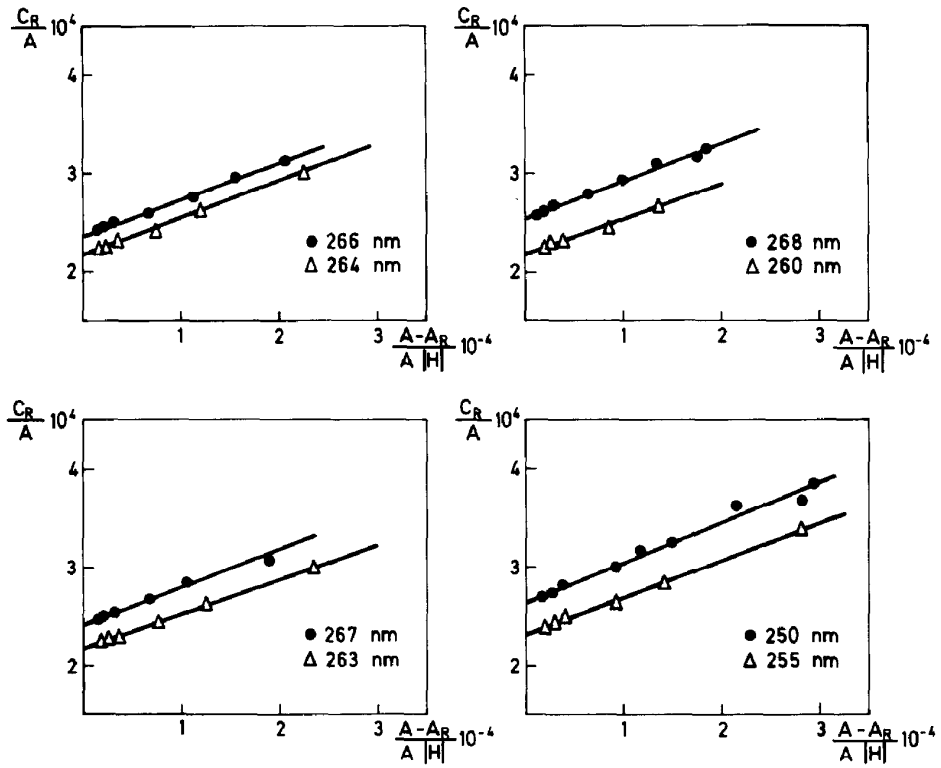

Fig. 6. Typical slope intercept plots: Agren-Sommer method.

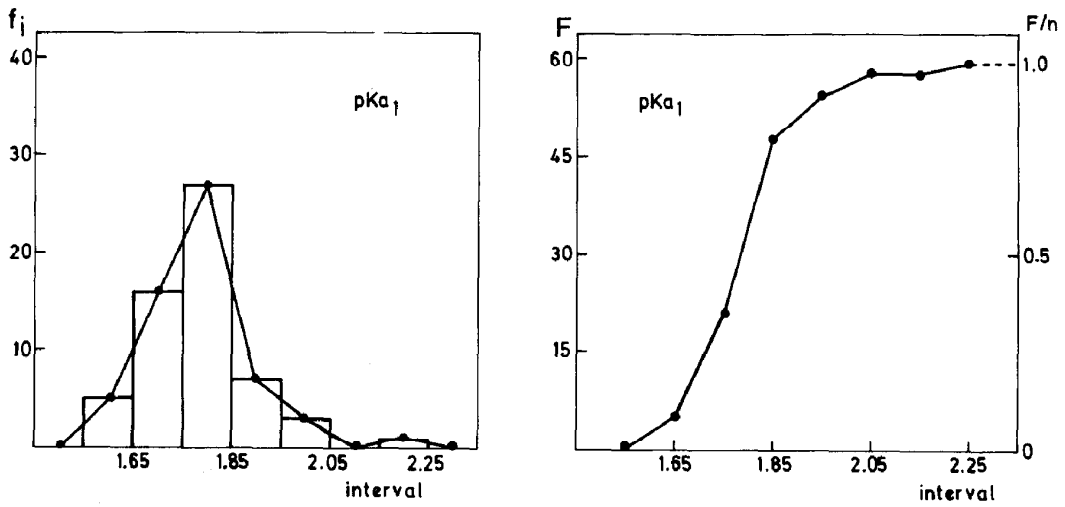

Fig. 7. Histogram, frequency polygon and cumulative frequency, F, and fractional cumulative frequency curve (F/n) for the data of pK_{a_1}.

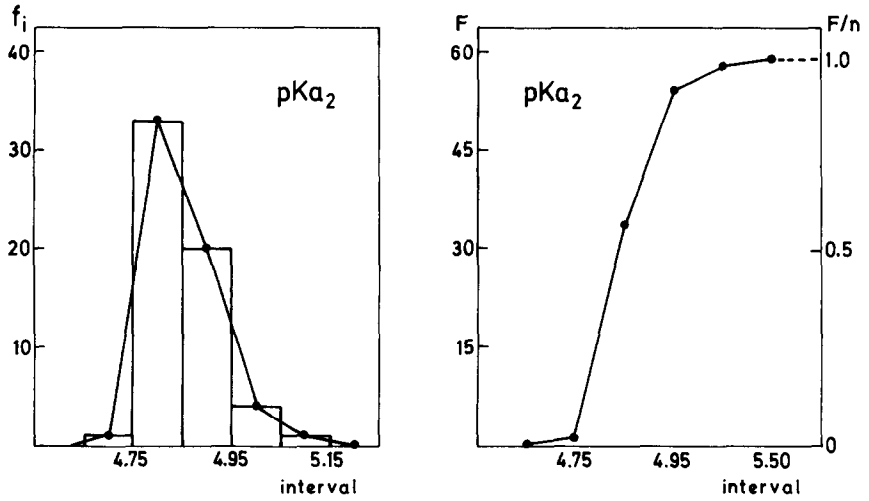

Fig. 8. Histogram, frequency polygon and cumulative frequency, F, and fractional cumulative frequency curve (F/n) for the data of pK_{a_2}.

is calculated from the Debye-Hückel equation.

The value obtained by us for the first thermodynamic acidity constant, 1.66, is in very good agreement with the value reported by Jellinek and Urwin (1954), 1.65, but some differences are encountered if we compare with the values obtained by Evans et al. (1953), 1.77, and Green and Tong (1956), 1.84. The agreement is very good too with

TABLE 4

pK_a VALUES REPORTED FOR ISONICOTINIC ACID

Author(s)	pK_{a_1}	pK_{a_2}	Ionic strength	T (°C)	Method	Comments
Evans et al.	1.79 ± 0.02	4.78 ± 0.02	0.015	25.0 ± 0.1	Spectr.	(Thamer and Voigt, 1952) At 244.4 nm $A_0 \neq A_2$
	1.88 ± 0.03	4.76 ± 0.03	0.015	25.0 ± 0.01	Spectr.	(Thamer and Voigt, 1952) At 258.2 nm $A_0 = A_2$
	–	4.78 ± 0.01	0.015	25.0 ± 0.01	Spectr.	Classical method. At 266.7 nm: $A_2 = A_1$
	1.84 ± 0.04	–	0.015	25.0 ± 0.01	Spectr.	Classical method. At 281 nm: $A_1 = A_0$
Jellinek and Urwin	–	4.95	–	20 ±2	Spectr.	Succesive approximations method
	1.70	–	0.01	20 ±2	Spectr.	
Green and Tong	1.84	4.86	–	22	Pot.	Twenty points distributed over almost the whole course of the titration. Successive approximation method near the isoelectric point; evaluated as a monoprotic acid far from the isoelectric point.
Lumme	1.68	4.91	–	22	Pot.	
Asuero et al.	1.78 ± 0.10	4.85 ± 0.08	0.1	20 ±1	Spectr.	Numerical method
		4.79 ± 0.02	0.1	20 ±1	Spectr.	Slope intercept procedures

the value reported by Lumme (1957), 1.68, from potentiometric measurements.

The graphical slope–intercept procedures lead to a mean $pK^*_{a_2}$ of 4.91; this value is intermediate between those of 4.95 (Jellinek and Urwin, 1954) and 4.84 (Evans et al., 1953). The mean $pK^*_{a_2}$ calculated from the numerical procedure developed in this paper, 4.97, is very close to the one given previously by Jellinek and Urwin (1954), 4.95. Although the latter authors worked at only one wavelength, a trial-and-error curve-fitting procedure was employed to evaluate the unknown parameters, thus giving reliability to the final results obtained. Evans et al. (1953) assumed instead that A_2, the limit absorbance for the species H_2R, is known, but the bad reproducibility of absorbance values at high acidities ($pH < 1$) introduces a source of error in the calculation of A_2. Until now, no satisfactory explanation has been offered for this. On the other hand, as isonicotinic acid is quite a strong acid in its first ionization, in practice the actual value of A_2, may not be determinable by experiment, because the necessary large excess of [H] for which this limiting value is approached may so modify the nature of the solvent (specifically its dielectric constant and solvating powder) as to yield spurious results. It becomes necessary therefore to treat A_2 as an unknown. We have used perchloric acid instead of the previously used sulphuric acid, but this should exhibit no effect upon the pK_a values.

The slope–intercept procedures applied to overlapping equilibria give results less accurate than complete procedures based on the use of Eqn. 1. Nevertheless, some caution must be taken into account as numerical methods are very sensitive to the points used in the calculations (Roth and Bunnett, 1965). However, a good fit of experimental data to the equation relating the absorbance to the composition of solution is found.

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