Multiwavelength Spectrophotometric Determination of Acid Dissociation Constants: Part II. First Derivative vs. Target Factor Analysis

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Purpose. Acid dissociation constants (pK_a values) denote the extent of ionization of drug molecules at different pH values, which is important in understanding their penetration through biological membranes and their interaction with the receptors. However, many drug molecules are sparingly soluble in water or contain ionization centres with overlapping pK_a values, making precise pK_a determination difficult using conventional spectrophotometric titration. In this work, we investigate a multiwavelength spectrophotometric titration (WApH) method for the determination of pK_a values.

Methods. Spectral changes which arise during pH-metric titrations of substances with concentration of about 10^{-5} M were captured by means of an optical system developed in this study. All experiments were carried out in 0.15 M KCl solution at 25 ± 0.5°C. Mathematical treatments based on the first derivative spectrophotometry procedure and the target factor analysis method were applied to calculate the pK_a values from the multiwavelength absorption titration data.

Results. pK_a values were determined by the WApH technique for six ionizable substances, namely, benzoic acid, phenol, phthalic acid, nicotinic acid, p-aminosalicylic acid and phenolphthalein.

Conclusions. The pK_a values measured using the WApH technique are in excellent agreement with those measured pH-metrically. We have demonstrated that the first derivative spectrophometry procedure provides a relatively simple way to visualize the pK_a values which are consistent with those determined using the target factor analysis method. However, for ionization systems with insufficient spectral data obtained around the sought pK_a values or with closely overlapping pK_a values, the target factor analysis method outperforms the first derivative procedure in terms of obtaining the results. Using the target factor analysis method, it has been shown that the two-step ionization of phenolphthalein involves a colorless anion intermediate and a red colored di-anion.

KEY WORDS: acid dissociation constants; pK_a; multiwavelength spectrophotometric titration; first derivative spectrophotometry; target factor analysis; fibre optics.

INTRODUCTION

Acid dissociation constants (pK_a values) are key parameters to predict the extent of ionization of functional groups with respect to pH. This information is important in drug discovery and development since the pharmacokinetic and pharmacodynamic properties of different protonation/ionization forms of the drug molecules may vary considerably (1). Spectrophotometric titration is an attractive method to measure the pK_a values at sample concentrations of about 10^{-5} to 10^{-6} M provided that the compound under consideration possesses chromophore(s) in proximity to the ionization centre, but the absorptivity should also change significantly on (de)protonation, indicating that the ionization centre is a part of the chromophore. Traditionally, spectral data at a single analytical wavelength with large changes in absorbances between different species is acquired from the sample in a series of buffer solutions with known pH values. If the molar absorptivities of the reacting species are known, the pK_a value(s) can be computed by fitting the experimental data to established formulae (2). Computer programs used for calculating the acid dissociation constants from multiwavelength spectrophotometric data have been reported (3,4 and the refs. therein). Most of these methods involve a least-squares approach whereby the differences between the theoretical and experimental absorbance values are minimized by means of the Gauss-Newton-Marquardt algorithm (4). In this manner, the unknown pK_a values and/or the molar absorptivity of individual reacting species are treated as adjustable parameters.

Recently, we have devised a multiwavelength spectrophotometric (WApH) titration approach using a fibre optics dip probe, a UV light source and a photodiode array (PDA) detector in combination with a commercially available titrator (Sirius PCA101) to capture the absorption spectra of the sample in the course of a pH-metric titration (5). Since the pH measurement and spectrum acquisition are accomplished almost at the same time, the WApH technique may be regarded as more precise and less time consuming than the conventional spectrophotometric titration. A multivariate computation method based on target factor analysis (TFA) was applied with success to deduce the pK_a values of several sparingly soluble drug compounds and resolve the absorption spectra of the reacting species, without prior knowledge of their optical properties (5,6). It has been shown that the WApH technique used in conjunction with the TFA treatment can be used to deduce pK_a values with high accuracy, which are consistent with pK_a values obtained pH-metrically even if the absorption spectra of the reacting species are very similar.

In some ionization systems, such as those with one pK_a value or two well separated pK_a values, first derivative spectrophotometry (FDS) can be utilized to find the pK_a values from spectrophotometric titration experiments (7). Specifically, at a particular analytical wavelength, the pH value at the point of inflexion in a plot of the absorbance against pH corresponds to the sought pK_a value. Using this approach, the spectral properties of individual reacting species are not required. However, it is desirable to include the spectral data obtained over a range of at least one pH units on either side of the sought pK_a value (7). In contrast to the TFA method, it may be difficult for the FDS procedure to scrutinize ionizable compounds with overlapping pK_a values and/or similar absorption spectra.

In this work, the FDS procedure and the TFA method are applied to process the spectral data obtained by the WApH technique. Several ionizable substances, namely benzoic acid, phenol, phthalic acid, nicotinic acid, p-aminosalicylic acid and phenolphthalein were studied using the WApH technique. We deliberately selected compounds of one ionization step and two ionization steps with well separated or closely overlapping pK_as

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to exemplify the use of the FDS procedure and the TFA method for spectrophotometric pK_a determination. In the following discussion, a brief account on the FDS procedure and the TFA method will be given. It will be shown that, where applicable, the pK_a values obtained using FDS procedure agree with those deduced by the TFA method and pH-metric titration. With the aid of the absorption spectra resolved using the TFA method, it has been confirmed that the two-step ionization of phenolphthalein involves a colorless anion intermediate and a red colored di-anion.

METHOD OF CALCULATIONS

In a WApH titration, the spectral data obtained is a series of spectra acquired at different pH values. According to the Beer's law, the absorbance matrix, A, can be expressed as follows:

$$A = CE \tag{1}$$

where C and E represent, respectively, the concentration-pH profile of the ionization system and the molar absorptivity matrix with the inclusion of the optical path length. The unknown pK_a values are derived from the A matrix using the mathematical treatments as formulated in the following description.

First Derivative Spectrophotometry (FDS) Procedures

For each wavelength channel of A, a smooth absorbance-pH curve is constructed by using the cubic spline interpolation technique (8). The Savitzky-Golay derivative filter (9) is then applied on this smooth curve to produce the first derivative curve. To this end, a multiwavelength first derivative surface (i.e. dA/dpH vs pH and wavelength) is generated. The pK_a value(s) can be identified as the pH value(s) at the mountains (and/or valleys) of the first derivative surface.

Target Factor Analysis (TFA) Methods

The principal component analysis (10–12) is first applied to A to calculate an abstract solution for C and E, namely, C_{abs} and E_{abs}, which contain only the primary eigenvalues (λ_r) and eigenvectors (Q_r). The residual standard deviation (11), IND function (10,11), eigenvalue ratio (13) and reduced eigenvalue ratio (14) are utilized to identify the number of principal components (independent light absorbing species) present in the chemical system. In the TFA treatment, the abstract solution can be rotated to the one with relevant physical significant C_p and E_p by a transformation matrix T (11,15,16) as given below:

$$T = \lambda_r^{-1}\, C_{abs}^{T}\, C_t \tag{2}$$

$$A \approx C_{abs}\, T\, T^{-1}\, E_{abs} \tag{3}$$

$$\approx C_p\, E_p \tag{4}$$

where the superscripts-1 and T denote, respectively, inverse and transpose operations. The test matrix C_t in eqn. 2 contains the concentration-pH profiles of the m-step ionization system which are generated theoretically by solving the following mass balance equations (5).

$$X_n \overset{pK_{a,n}}{\rightleftharpoons} H^+ + X_{n+1} \qquad n = 1 \ldots m \tag{5}$$

$$Y = \sum_{n=1}^{m+1} C(n) \tag{6}$$

where $pK_{a,n}$ and X_n represent, respectively, the acid dissociation constant and the individual reacting species (with charge being excluded for clarity) while Y and C(n) symbolize the initial concentration and concentration of X_n, respectively. In this study, the proton concentration is related to the operational pH reading by a multi-parametric equation (17).

The SPOIL function as derived by Malinowski (11,16) is utilized to determine whether a test matrix is acceptable or not. In general, a test matrix that minimize the SPOIL function with value not greater than 3.0 is considered as the solution for the target transformation procedure (5,6,11,15,16). For a particular A matrix, the SPOIL function depends only on C_t which in turn is a function of the sought pK_a values (see eqns. 5 and 6). Here, we define a cost function, Φ

$$\Phi = \xi + \zeta + \sum_{n=1}^{m+1} (SPOIL_n)^2 \tag{7}$$

where the symbol ξ represents a penalty function for negative absorption spectra. ζ denotes a penalty function for the pK_a values which is activated if the sought values diverge from certain specified feasible ranges. The TFA computation renders to a constrained optimization of the pK_a values for a global minimum of Φ. The SIMPLEX method (18) can be used for this purpose.

EXPERIMENTAL

Equipment

A schematic diagram of the WApH titration is given in Fig. 1. The optical system consists of a pulsed deuterium lamp

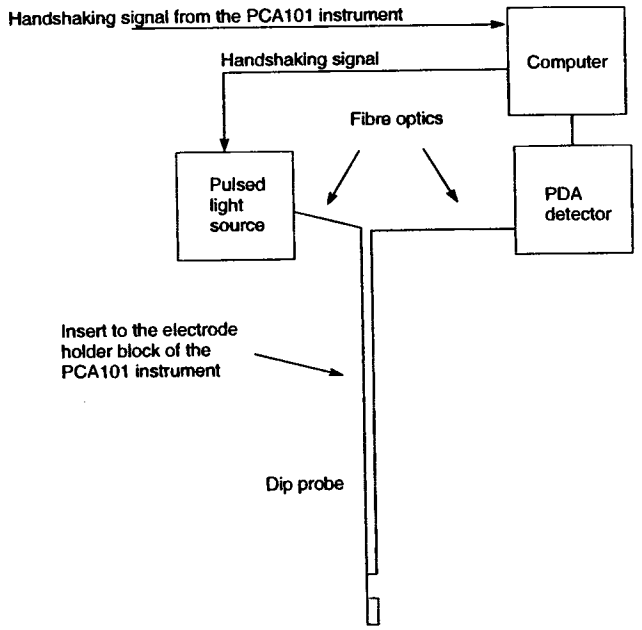

Fig. 1. Schematic for the experimental setup utilized in the WApH titration.

(Cathodeon, Cambridge, UK) with pre-aligned fibre optics output and an UV-VIS 256-element photodiode array (PDA) detector (Carl Zeiss, Herts., UK). This combination offers a spectral range of 200–735 nm with blaze wavelength at 220 nm. A bifurcated fibre optics dip probe (Custom Sensor & Technology, Missouri, USA) with optical path length of 1-cm is connected to the deuterium lamp and the PDA detector.

UV/pH Titrations

All titrations were carried out by using a PCA101 automatic titrator (19,20). A 3-mm hole was drilled through the electrode holder of the titrator to accommodate the dip probe such that it could be situated next to the pH electrode. Synchronization of the titrator, the pulsed deuterium lamp and spectrum acquisition by the PDA detector was accomplished using a terminate-and-stay-resident system (21). In the WApH technique, spectral data was recorded in the region of 200–700 nm after each pH measurement. The pH change per titrant addition was limited to about 0.1 pH units. Data were acquired when the drift was less than 0.01 pH units per minute. Typically, 20 to 30 pH readings and absorption spectra were collected from each titration. The pH electrode (Orion, Ross™ type, Beverly, MA, USA) was calibrated titrimetrically in the pH range of 1.8–12.2 (17). All experiments were performed in solutions of 0.15 M KCl under argon atmosphere at 25 ± 0.5°C using standardized 0.5 M HCl or 0.5 M KOH titrants. Solutions were made up of deionized water of resistivity $> 10^{14}$ Ω-cm.

In the present study, sample concentrations of 1×10^{-5} M to 1×10^{-4} M and 5×10^{-4} M to 5×10^{-2} M were employed, respectively, in WApH and pH-metric titrations. Because of the solubility problem of phenolphthalein in neutral or acidic aqueous solutions, pH of the sample solutions of 10–20 ml volumes were adjusted using 0.5 M KOH to about 11.5 and then titrated with 0.5 M HCl to a suitably low value (about 5). As for other titration experiments, sample solutions were pre-acidified to a reasonably low pH value (2.0–3.0) using 0.5 M HCl then titrated alkalimetrically to an appropriate high pH value (7.0–11.0). At least three WApH and pH-metric titrations were carried out for each sample. Calculations of pK_a values from pH-metric data were performed using *pKaLOGP*™ software (v5.01, Sirius, East Sussex, UK; see also refs. 19 and 20). Programs for the FDS and the TFA treatments were coded in a Turbo C environment. All numerical routines utilized in the multivariate computations were adopted from an established program library (22).

Materials

Benzoic acid (AR grade), potassium hydrogen phthalate (AR grade), Phenol (AR grade) and phenolphthalein (purity > 99.9%) were supplied by Fisher (Loughborough, UK). Sodium-p-aminosalicylate and nicotinic acid were of pharmacopoeial grade (Ph. Hg. VII) and were purchased from Reanal Rt. (Budapest, Hungary).

RESULTS AND DISCUSSION

Two compounds with one ionization step (benzoic acid, phenol) and four compounds with two ionization steps (phthalic acid, nicotinic acid, p-aminosalicylic acid, phenolphthalein) have been examined using the WApH technique. Figure 2 gives the suggested protonation schemes of the compounds utilized for TFA and pH-metric calculations. In the following discussion, we classify these compounds into three types which are listed as follows: I Compounds of one ionization step or two ionization steps with well separated pK_as, such as benzoic acid, phenol and phthalic acid, II Compounds of two ionization steps but some of the absorption spectra were acquired within 0.5 pH units of the sought pK_as, such as nicotinic acid and p-aminosalicylic acid, III Compounds of two ionization steps with closely overlapped pK_as, such as phenolphthalein.

Type I Compounds

Figures 3 shows, the absorption spectra and the multiwavelength first derivative surfaces of benzoic acid, phenol and phthalic acid. As shown in Figs. 3(d-f), the FDS procedure is able to identify all the unknown pK_a values. The TFA method was also applied to the spectra data as given in Figs. 3(a-c), and in all cases, the number of principal components was correctly identified. The unknown pK_a values were successfully determined with the SPOIL function of each component less than 3.0. Table 1 lists the pK_a values of benzoic acid, phenol and phthalic acid obtained by the WApH technique and the pH-metric method. The good agreement between the pK_a values deduced using the pH-metric method and WApH technique justify the validity of using the FDS procedure and TFA method for handling spectral data of Type I compounds.

Type II Compounds

We now turn to nicotinic acid and p-aminosalicylic acid. It is known that the lowest pK_a values of nicotinic acid and p-aminosalicylic acid are both about 2 (23,24). Since the WApH titrations for this two compounds were carried out alkalimetrically from pH 2 to 7, the spectra obtained for the low pH region were within 0.5 pH units of the unknown pK_a values. Here, the phenolic pK_a of p-aminosalicylic acid (about 13) is not considered. The absorption spectra and the multiwavelength first derivative surfaces of nicotinic acid and p-aminosalicylic acid are shown in Fig. 4. It can be seen that a precise determination using the derivative surfaces as given in Figs. 4(c) and 4(d) for the lower pK_a values is difficult since the mountains around pH 2 are not well-defined. However, the TFA treatment revealed three principal components in both chemical systems with the distribution of species and resolved molar absorptivity coefficients shown in Fig 5. Again, the SPOIL function was found to be less than 3.0 for each component. As shown in Figs. 2(d) and 2(e), we propose the zwitterionic forms as the predominant intermediates which was found and published for nicotinic acid (23) and is in line with an earlier observation on the ionization processes of related amphoteric substances in aqueous media (25). Note that the (de)protonation of the acidic and the basic functional groups would introduce significant spectral changes. In this case, it is difficult for the spectrophotometric method to resolve the zwitterionic and the neutral forms since the concentration ratio of these two species is equivalent to the tautomerism k_z (25). Therefore, the spectrophotometric pK_a values reported here refer as the macroconstants. Table 1 lists the pK_a values of nicotinic acid and p-aminosalicylic acid deduced using the WApH technique and the pH-metric method.

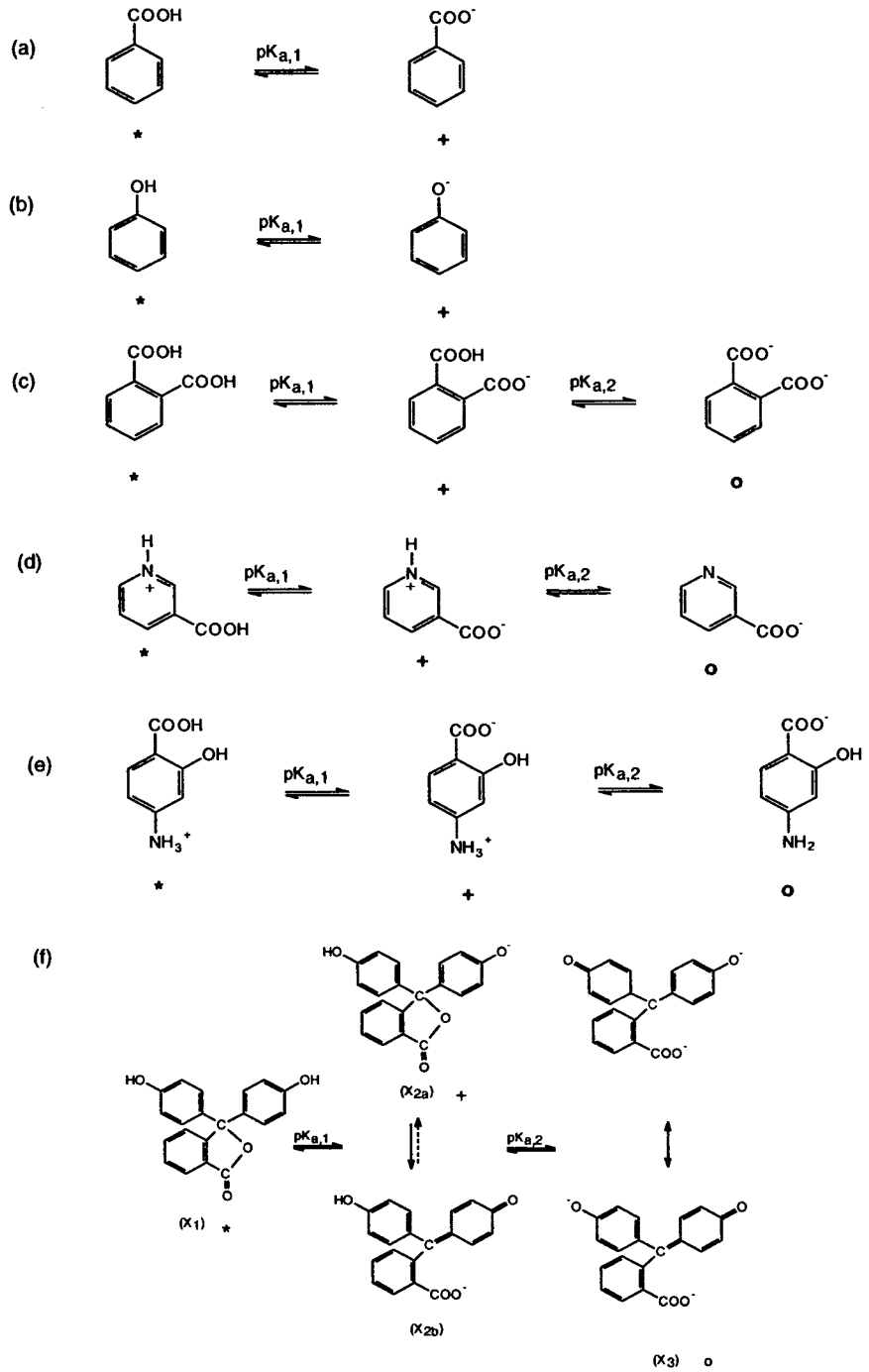

Fig. 2. Suggested protonation schemes (with all free protons omitted) for TFA and pH-metric calculations. (a) benzoic acid, (b) phenol, (c) phthalic acid, (d) nicotinic acid, (e) p-aminosalicylic acid, and (f) phenolphthalein.

The agreement between the pH-metric results and those determined using the WApH technique in conjunction with the TFA method is apparent. However, the FDS procedure fails to locate the $pK_{a,1}$ of these compounds which is probably due to insufficient spectral data acquired at the low pH region. It is interesting to note that interlaboratory reproducibility of our results is superb. Traditional spectrophotometric titrations (23) for nicotinic acid have been performed in the laboratory of one of the authors using a different PDA spectrometer (Hewlett-Packard 8452A), and the pK_a values computed using the TFA method ($pK_{a,1}$ = 1.95, $pK_{a,2}$ = 4.66) are in good agreement with those reported in Table 1.

Type III Compound

Next, attention is directed to phenolphthalein. The ionization and tautomerism of phenolphthalein have been proposed

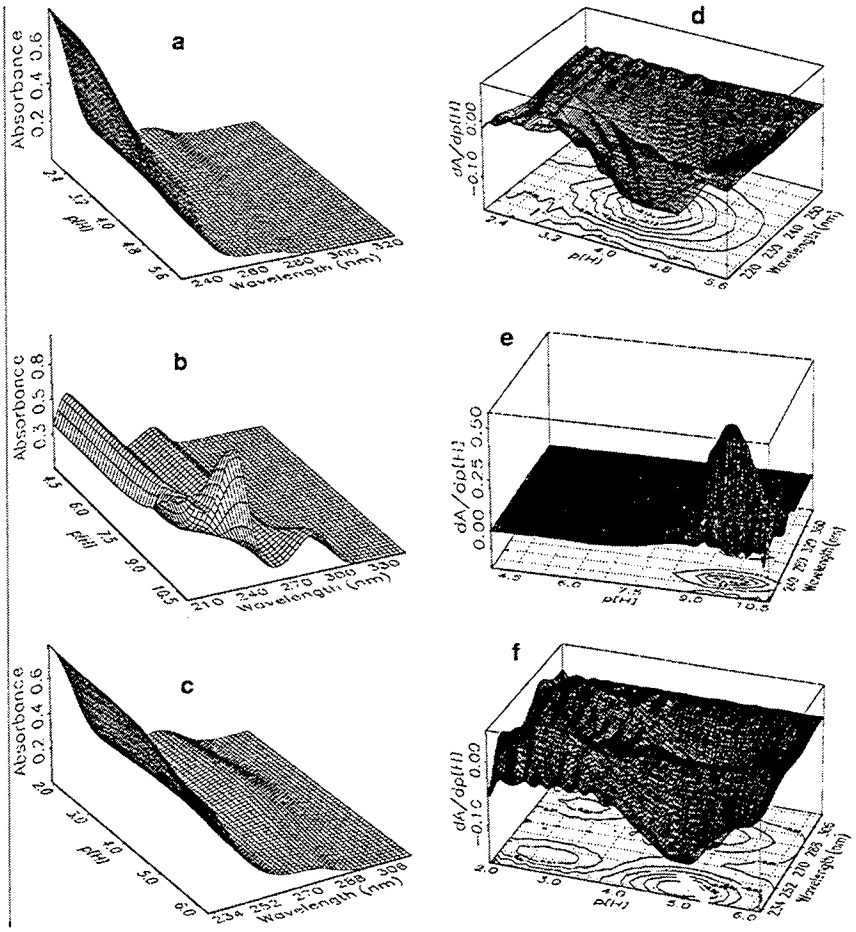

Fig. 3. Absorption spectra of (a) benzoic acid, (b) phenol and (c) phthalic acid. Multiwavelength first derivative surfaces of (d) benzoic acid, (e) phenol, and (f) phthalic acid.

and are summarized in Fig. 2(f) (26). Interestingly, literature information concerning the nature of the predominant intermediate species was somehow contradictory. For instance, some authors concluded that X_{2a} is the key intermediate (27,28) while others preferred X_{2b} (26,29). Despite this discrepancy, it has been reported that the two pK_a values are very close together (26–29). Note that we started our titration experiments from pH 11.5 down to about 5 (see experimental section) such that the sluggish fading reaction of the phenolphthalein di-anion (X_3) with excess hydroxide was not favored (30).

Table 1. pK_a Values of Benzoic Acid, Phenol, Phthalic Acid, Nicotinic Acid, p-Aminosalicylic Acid, and Phenolphthalein as Determined Using the WApH and the pH-metric Methods at 25°C and an Ionic Strength of 0.15 M

			WApH[b]		
Type			FDS	TFA	pH-metric[b]
I	Benzoic acid	$pK_{a,1}$[a]	3.99 ± 0.06	3.98 ± 0.02	3.98 ± 0.02
	Phenol	$pK_{a,1}$	9.73 ± 0.09	9.73 ± 0.02	9.81 ± 0.01
	Phthalic acid	$pK_{a,1}$	2.74 ± 0.06	2.70 ± 0.01	2.74 ± 0.02
		$pK_{a,2}$	4.87 ± 0.03	4.86 ± 0.01	4.84 ± 0.01
II	Nicotinic acid	$pK_{a,1}$	NA[c]	2.10 ± 0.01	2.00 ± 0.01
		$pK_{a,2}$	4.62 ± 0.03	4.63 ± 0.01	4.63 ± 0.01
	p-aminosalicylic acid	$pK_{a,1}$	NA[c]	1.79 ± 0.02	1.71 ± 0.02
		$pK_{a,2}$	3.56 ± 0.04	3.58 ± 0.01	3.60 ± 0.01
III	Phenolphthalein	$pK_{a,1}$	NA[c]	8.87 ± 0.08	8.75 ± 0.01
		$pK_{a,2}$	9.26 ± 0.06	9.35 ± 0.08	9.24 ± 0.01

[a] Protonation schemes are given in Fig. 2.
[b] Uncertainties equal to the standard deviation of the pK_a values from three experiments.
[c] Not available (see text for explanation).

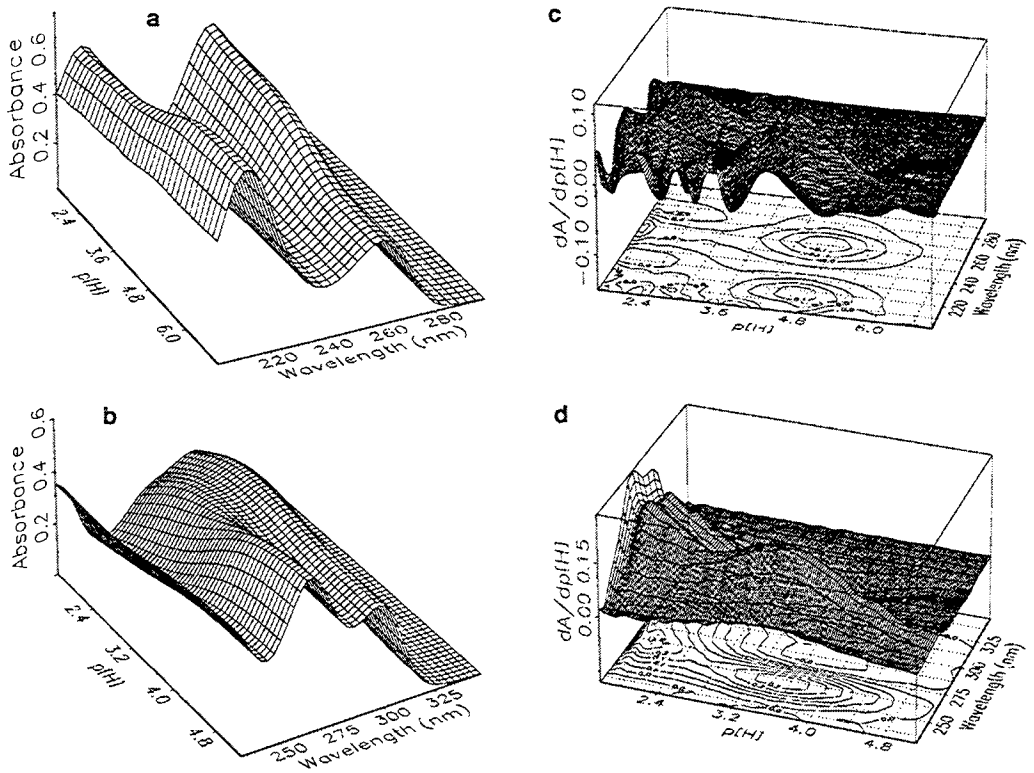

Fig. 4. Absorption spectra of (a) nicotinic acid and (b) p-aminosalicylic acid. Multiwavelength first derivative surfaces of (c) of nicotinic acid, and (d) p-aminosalicylic acid.

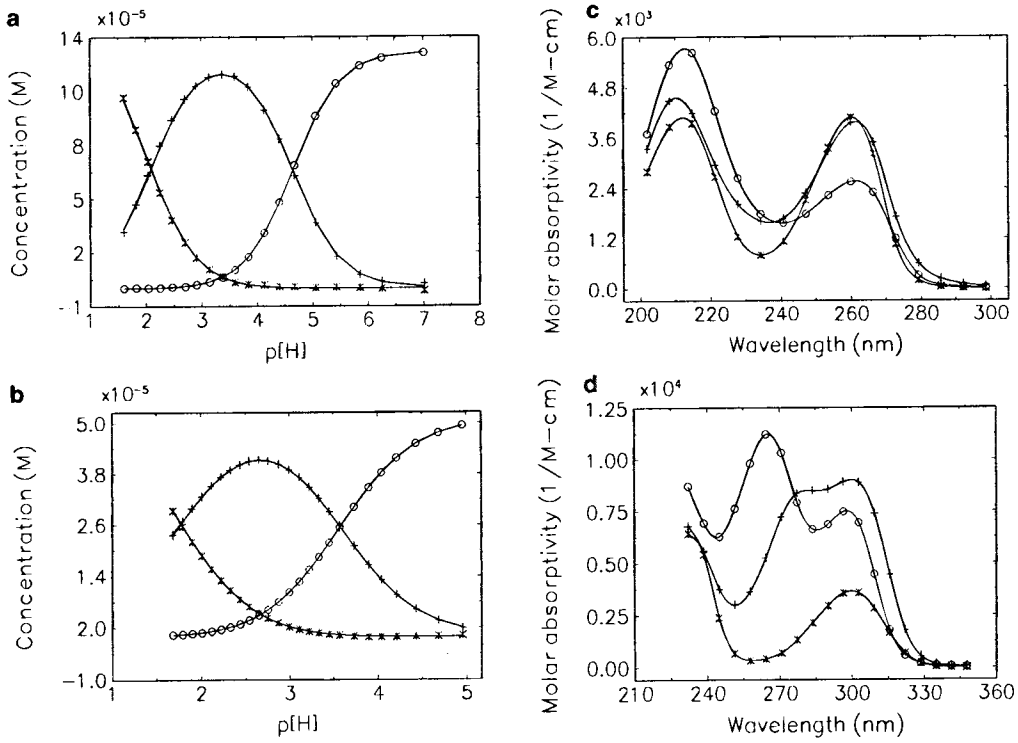

Fig. 5. Distribution of species for (a) nicotinic acid and (b) p-aminosalicylic acid as a function of pH with the symbols (defined in Fig. 2) represent the C_p matrix and solid lines denote the C_t matrix. Molar absorptivity coefficients of (c) nicotinic acid and (d) p-aminosalicylic acid with the symbols (defined in Fig. 2) represent the elements in matrix E_p and solid lines generated using the cubic spline interpolation method.

The absorption spectra and the multiwavelength first derivative surface of phenolphthalein are depicted, respectively, in Figs. 6(a) and 6(b). The FDS procedure finds only one pK_a value of about 9.2 (see Table 1). Although the FDS procedure is not able to deduce both pK_a values successfully, it produces an approximate answer which is comparable to one of the pK_a values as determined using other methods. TFA treatment on the absorption spectra of phenolphthalein (see Fig. 6(a)) statistically confirmed that three principal components are involved in the ionization process. SPOIL function was found to be less than 3.0 for each component. As shown in Table 1, the pK_a values as deduced using the TFA method agree remarkably well with the reported values of 8.83 ± 0.08 and 9.32 ± 0.10 obtained spectrophotometrically at 25°C and an ionic strength of 0.2 M (27). Figures 7(a) and 7(b) portray, respectively, the distribution of species and the resolved molar absorptivity coefficients of phenolphthalein. It can be seen that the intermediate species does not exhibit any significant visible absorption. We realize that X_{2h} (see Fig. 2(f)) is a colored species in the view of the colored compound, phenol red, which contains the analogous aromatic structure (26,27). This suggests that, under our experimental conditions, the intermediate species evolved in the two step ionization of phenolphthalein is mainly present as X_{2a}.

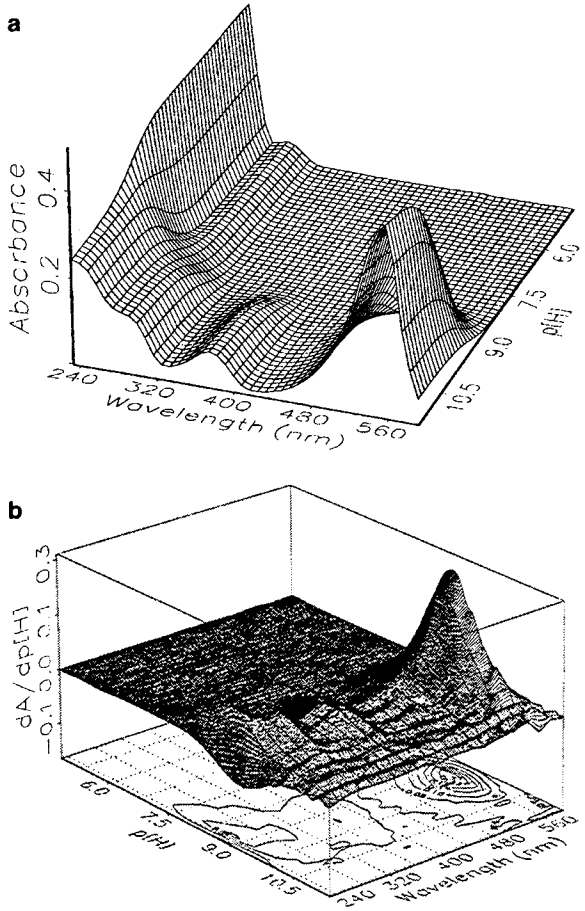

Fig. 6. (a) Absorption spectra and (b) Multiwavelength first derivative surface of phenolphthalein.

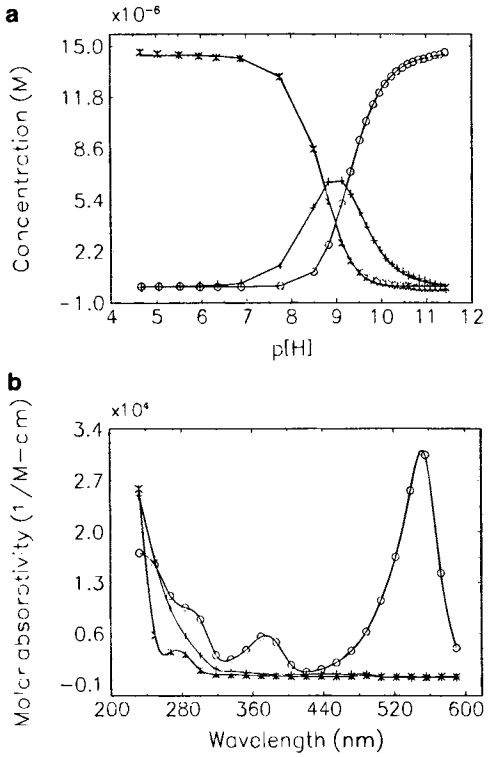

Fig. 7. (a) Distribution of species for phenolphthalein as a function of pH with the symbols (defined in Fig. 2) represent the C_p matrix and solid lines denote the C_t matrix. (b) Molar absorptivity coefficients of phenolphthalein with the symbols (defined in Fig. 2) represent the elements in matrix E_p and solid lines generated using the cubic spline interpolation method.

CONCLUDING REMARKS

We have developed a multiwavelength spectrophotometric titration (WApH) technique based on a fibre optics dip probe, a pulsed UV light source and a photodiode array detector in conjunction with a commercially available titrator (Sirius PCA 101). The first derivative spectrophotometry procedure and the target factor analysis method have been utilized to determine pK_a values from the absorption spectra acquired at different pH values. It was found that the first derivative spectrophotometry procedure provides a relatively simple way to visualize the pK_a values which are consistent with those deduced using the target factor analysis method. However, for ionization systems with insufficient spectral data obtained around the sought pK_a values or with closely overlapping pK_a values, the target factor analysis method outperforms the first derivative procedure in terms of obtaining the results. The WApH technique was exemplified by several ionizable substances, namely, benzoic acid, phenol, phthalic acid, nicotinic acid, p-aminosalicylic acid and phenolphthalein. Excellent agreement with pH-metric titration is noted. Using the target factor analysis method, it has been confirmed that the two step ionization of phenolphthalein involves a colorless anion intermediate and a red colored di-anion.

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