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Multiwavelength spectrophotometric determination of acid dissociation constants of ionizable drugs

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Abstract

A multiwavelength spectrophotometric approach has been developed to determine acid dissociation constants (pK_a values) of sparingly soluble drug compounds. UV absorption spectra of the drug solution are acquired using a versatile device based on a fiber optics dip probe, a light source and a photodiode array (PDA) detector while the pH and the ionic strength of the chemical system is manipulated precisely by means of a commercially available titrator. Target factor analysis (TFA) has been applied to deduce the pK_a values from the multiwavelength UV absorption data recorded at different pH values. We have called this multiwavelength approach the WApH technique because the pK_a results are determined from changes in Wavelength and Absorbance as a function of pH (WApH). The WApH technique is exemplified by using several pure drugs, namely, niflumic acid, nitrazepam, pyridoxine, quinine and terbutaline. The pK_a values obtained agree well with those derived from pH-metric titrations. It has been demonstrated that the WApH technique is able to deduce pK_a values with high accuracy even if the absorption spectra of the reacting species are very similar.

Keywords: Acid dissociation constants; pK_a; Multiwavelength spectrophotometric titration; pH-metric titration; Fiber optics; Target factor analysis

1. Introduction

Acid dissociation constant (pK_a value) is an important parameter to estimate the extent of ionization of drug molecules at different pH values, which is of fundamental importance in the consideration of their interaction with biological membranes [1,2]. Many drug compounds are sparingly soluble in water and a precise determination of their pK_a values poses a challenging problem for potentiometric titration since the accuracy of this method is restricted by its detection limit of about 10^{-4} M [3]. Although potentiometric titration of sparingly soluble compounds may be done in the presence of co-solvents such as methanol, the resulting acid dissociation constants (p_sK_a) refer only to the particular solvent medium employed, and extrapolation procedures such as the Yasuda-Shedlovsky method are required to deduce the pK_a values at zero co-solvent [4].

Spectroscopic titration has been utilized as an alternative to determine pK_a values of substances

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with large molar absorptivities because of its high sensitivity at concentrations of substance as low as 10^{-6} M [3]. However, the compound under investigation must possess chromophore(s) in proximity to the ionization center(s) so that the protonated and deprotonated species exhibit sufficient spectral dissimilarity.

Traditionally, spectral data at a single analytical wavelength is acquired from the sample in a series of buffer solutions with known pH values, after which the pK_a is determined by fitting the experimental data to established formulae [3]. To use this method, the absorption spectra of individual species must be characterized beforehand and the molar absorptivities of the protonated and deprotonated species are thus required [3,5–7]. These measurements are non-trivial if the acid-base equilibria comprise more than two ionization steps or if the reacting components are not stable within two pH units of the pK_a value.

Irving et al. suggested the use of a derivative method to locate the pK_a values for processes involving two ionization steps [8]. In particular, they showed how measurement of pH values and absorbances at the points of inflexion in a plot of the absorbance against pH can be used to give the sought pK_a values. However, extension of this method to more than two ionization steps is algebraically complicated. For a one-step ionization process, it has been shown that the pH value at the point of inflexion is equivalent to the pK_a value [8].

Factor analysis methods have been found to be useful to interrogate multivariate data [9–14]. Multiwavelength spectrophotometric titration data can be resolved using this approach to obtain kinetic and spectral information of the chemical system [14–16]. Recently, Tam and Chau have applied principal component analysis (PCA) and target factor analysis (TFA) methods with success to scrutinize two- and three-component kinetic systems [17–19]. Specifically, the absorbance data matrix is decomposed into a linear combination of principal components using the PCA method [20,21]. Based on a suggested reaction model, TFA treatment can be employed to transform the mathematical solution with the components being identified into one with physical significance [21].

In this work, we report a versatile device using an UV light source, a fiber optics dip probe and a diode array detector to capture the spectral changes that arise in the course of a pH-metric titration of an ionizable drug compound. TFA technique is applied to calculate pK_a values from the multiWavelength spectrophotometric Absorption titration data. We refer this multiwavelength approach as the WApH technique. The merits of this approach over the traditional one [3,5–8] are that it enables the pK_a values, the number of independent light absorbing species present in the chemical system and their absorption spectra to be determined from a single titration, without prior knowledge of their optical properties.

In the following discussion, a briefly description on the computation method is given. Attention is then turned to the experimental details and the WApH titration results for niflumic acid (anti-inflammatory drug), nitrazepam (anticonvulsant),

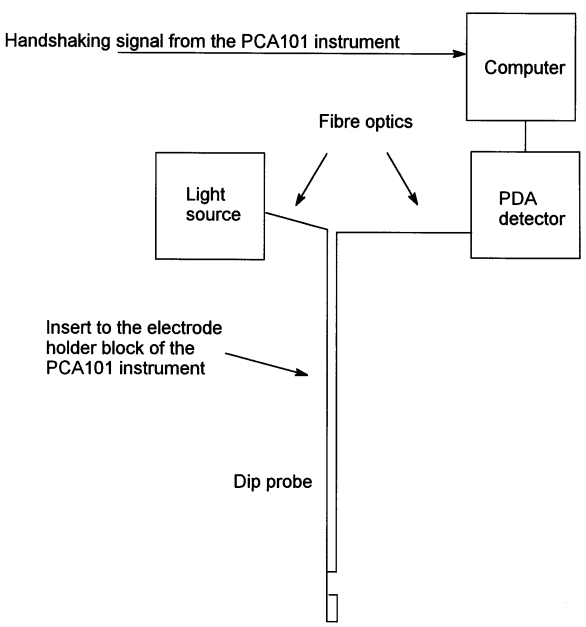

Fig. 1. Schematic for the optical setup utilized in the WApH titration.

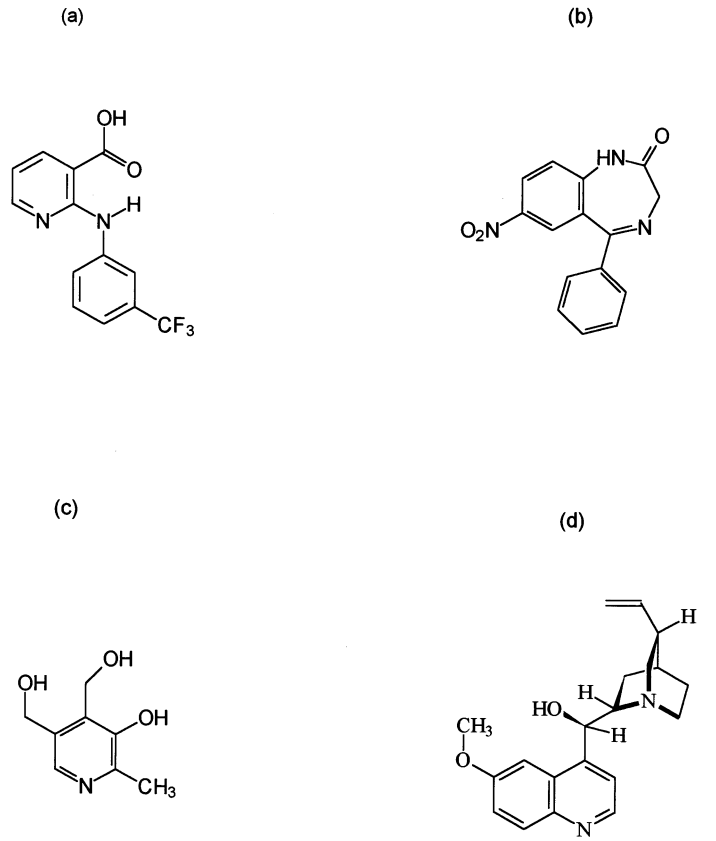

Fig. 2. Structure of the four ionizable drugs: (a) niflumic acid; (b) nitrazepam; (c) pyridoxine; and (d) quinine.

pyridoxine (component of vitamin B6 complex), quinine (antimalarial drug) and terbutaline (bronchodilator). It will be shown that the pK_a values as determined by the proposed method agree well with those derived from pH-metric titration.

2. Method of calculations

2.1. The equilibrium system

Consider a m-step ionization process in which X_i ($i = 1,2....m+1$) represents the individual reacting species (the charge is excluded for clarity). The reactions can be written as follows:

$$X_n \overset{K_{a,n}}{\rightleftharpoons} H^+ + X_{n+1} \quad n = 1...m \tag{1}$$

where $K_{a,n}$ denotes the acid dissociation constant. From the law of mass action we obtain

$$Y = \sum_{n=1}^{m+1} C(n) \tag{2}$$

where Y is the initial concentration and $C(n)$ denotes the concentration of X_n. Eqs. (1) and (2) are readily cast into a system of linear equations with $m+1$ unknown concentrations of X_n. In matrix form, we can write

$$\begin{bmatrix} y \\ 0 \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ \vdots \\ 0 \end{bmatrix} = \begin{bmatrix} 1 & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & 1 \\ K_{a,1} & -H & 0 & \cdots & \cdots & \cdots & \cdots & \cdots & 0 \\ 0 & \ddots & \ddots & 0 & \cdots & \cdots & \cdots & \cdots & 0 \\ \vdots & 0 & \ddots & \ddots & 0 & \cdots & \cdots & \cdots & 0 \\ \vdots & \vdots & 0 & K_{a,n} & -H & 0 & \cdots & \cdots & 0 \\ \vdots & \vdots & \vdots & 0 & \ddots & \ddots & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & 0 & \ddots & \ddots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & 0 & K_{a,m-1} & -H & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & K_{a,m} & -H \end{bmatrix} \begin{bmatrix} C(1) \\ C(2) \\ \vdots \\ \vdots \\ C(n) \\ \vdots \\ \vdots \\ C(m) \\ C(m+1) \end{bmatrix} \tag{3}$$

where H represents proton concentration. Eq. (3) can readily be solved by using Cramer's rule method [22]. In this study, H is related to the operation pH reading by a multi-parametric equation [23].

2.2. The target factor analysis (TFA) method

In a spectrophotometric titration process, the spectral data acquired is a series of absorption spectra measured at different pH values which can be expressed in the form of an absorbance matrix, A, with dimension of N_s (absorption spectra) $\times$ N_w (wavelength). According to Beer's law, A can be written as (Eq. (4)):

$$A = CE \tag{4}$$

where C and E matrices represent, respectively, the concentration-pH profile ($N_s \times N_c$) and the absorptivity matrix ($N_c \times N_w$) with the inclusion of the optical path length. N_c is the number of independent light absorbing species (components) and should be less than or equal to N_s or N_w, whichever is smaller. In applying the principal component analysis (PCA) procedure, the covariance matrix, Z, is first calculated (Eq. (5)):

$$Z = A^T A \tag{5}$$

where the superscript T denotes a transpose operation. Diagonalization of the covariance matrix gives the eigenvector Q and eigenvalue λ (Eq. (6))

$$ZQ = Q\lambda \tag{6}$$

It should be remarked that only those components with large eigenvalues contribute significantly to the observed data while others are regarded as noise and can be discarded. In the present study, residual standard deviation [21], IND function [21,24], eigenvalue ratio [25] and reduced eigenvalue ratio [26,27] were employed to evaluate N_c (assuming $N_s > N_w$ and $N_w > N_c$) that are important in describing the absorbance matrix.

The eigenvector and eigenvalue matrices with selected principal components are symbolized by Q_r and λ_r, respectively. From these, we can compute an abstract solution for the absorptivity matrix (E_{abs}) and concentration-pH profile matrix (C_{abs}) by using the following equations (Eq. (7))

$$E_{abs} = Q_r^T, \quad C_{abs} = AQ_r \tag{7}$$

The abstract solution can be rotated to the one with relevant physical significance, E_p and C_p, through the following target transformation procedure.

$$T = \lambda_r^{-1} C_{abs}^T C_t \tag{8}$$

$$A \approx C_{abs} T T^{-1} E_{abs} \tag{9}$$

$$A \approx C_p E_p \tag{10}$$

with the superscript -1 represents an inverse operation. As shown in Eq. (8), the transformation matrix T is generated from a test matrix, C_t, which can be calculated by solving Eq. (3) with the given initial concentration Y and acid dissociation constants.

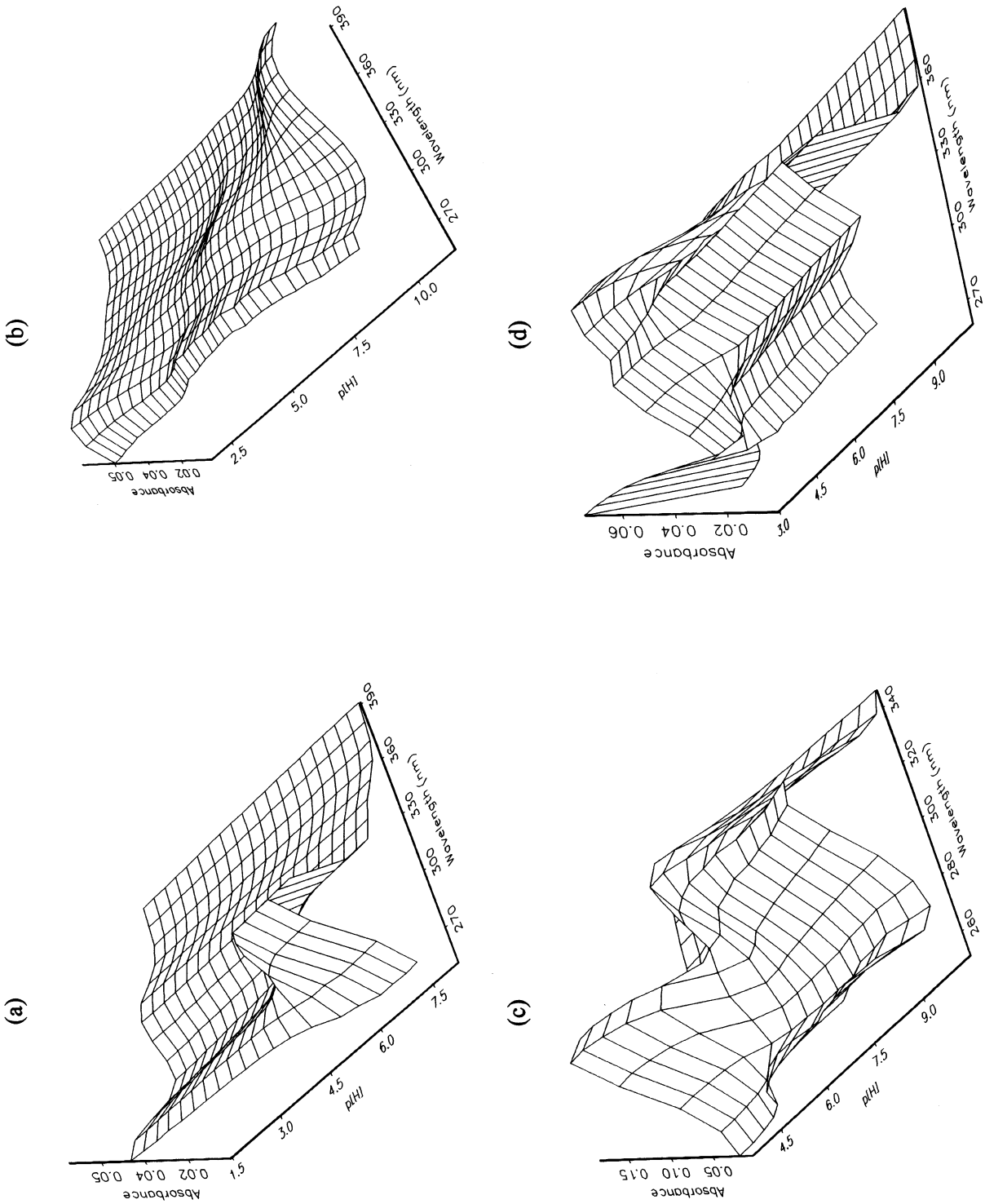

Fig. 3. Absorption spectra of: (a) niflumic acid; (b) nitrazepam; (c) pyridoxine; and (d) quinine.

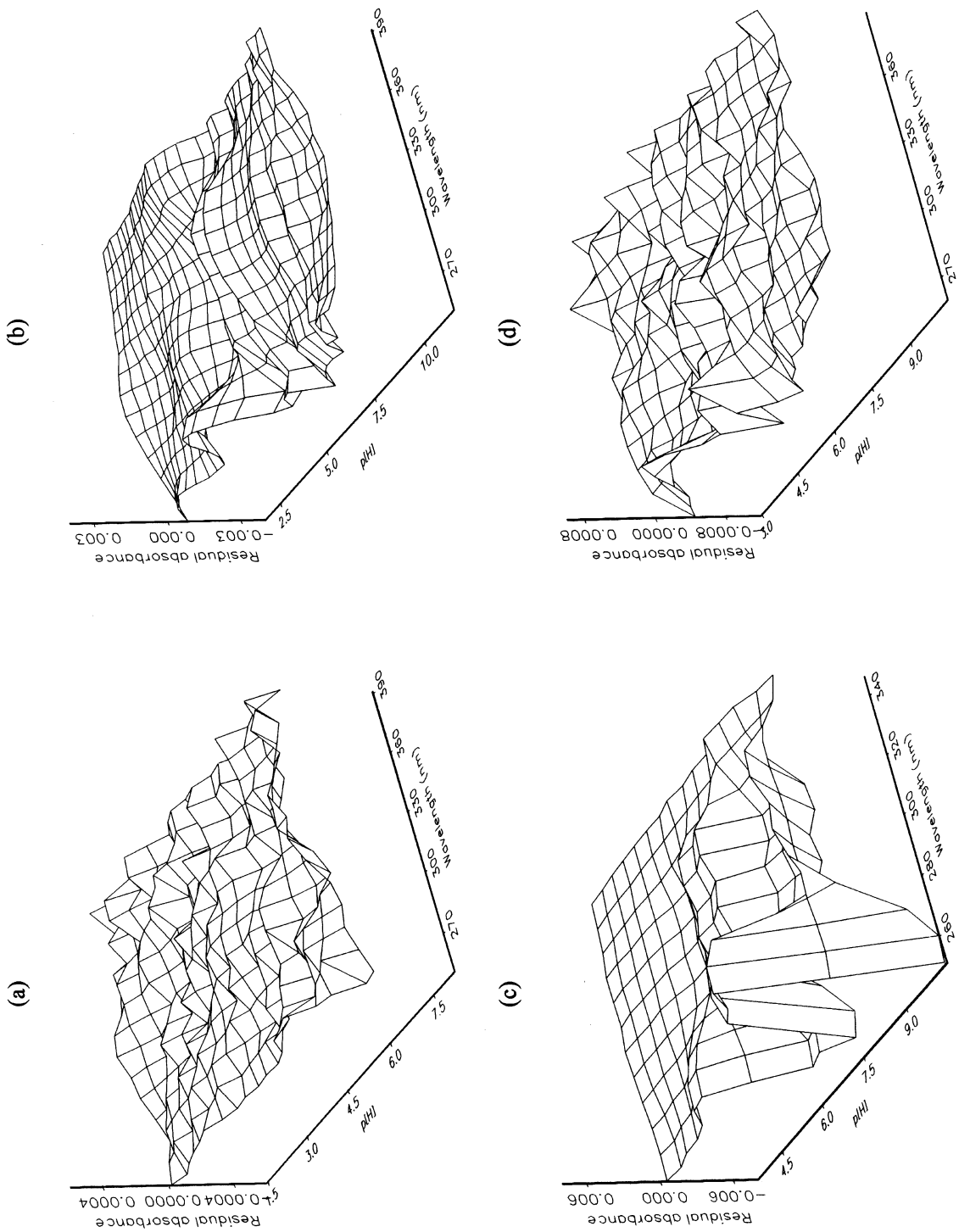

Fig. 4. Residual absorbance values of: (a) niflumic acid; (b) nitrazepam; (c) pyridoxine; and (d) quinine systems generated using a three-component model.

The SPOIL function as derived by Malinowski [21,28] is utilized in this study to determine whether a test matrix is acceptable or not. In general, a test matrix that minimize the SPOIL function with a value not greater than 3.0 is considered as the solution for the target transformation procedure [18,19,21,28,29]. 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 (Eq. (3)). Here, we define a cost function, Φ (Eq. (11))

$$\Phi = \xi + \zeta + \sum_{i=1}^{N_c} (\text{SPOIL}_i)^2 \quad (11)$$

where the symbol ξ represents a penalty function for negative absorption spectra. ξ is assigned to zero if the minimum element in ξ is positive. Otherwise, ξ is proportional to the absolute value of this element. ζ denotes a penalty function for the pK_a values which is activated if the sought values diverge from certain specified feasible ranges. Otherwise, ζ is set to zero. The TFA computation renders to a constrained optimization of the acid dissociation constants for a global minimum of Φ. The SIMPLEX method [30] can be employed for this purpose.

Table 1
pK_a of niflumic acid, nitrazepam, pyridoxine and quinine as determined using the WApH and the pH-metric methods at 25°C and ionic strength of 0.15 M

	WApH[a]	pH-metric[a]
Niflumic acid		
p$K_{a,1}$	2.28 ± 0.08	2.26 ± 0.08
p$K_{a,2}$	4.86 ± 0.05	4.44 ± 0.03
Nitrazepam		
p$K_{a,1}$	2.90 ± 0.05	3.02 ± 0.16
p$K_{a,2}$	10.39 ± 0.04	10.37 ± 0.06
Pyridoxine		
p$K_{a,1}$	4.90 ± 0.05	4.84 ± 0.01[b]
p$K_{a,2}$	8.91 ± 0.04	8.87 ± 0.01[b]
Quinine		
p$K_{a,1}$	4.33 ± 0.01	4.24 ± 0.09
p$K_{a,2}$	8.59 ± 0.01	8.55 ± 0.04

[a] Uncertainties equal to the standard deviation of the pK_a values from at least three experiments.
[b] Determined from aqueous phase titration.

3. Experimental

A schematic diagram of the WApH titration is depicted in Fig. 1. The optical system consists of a continuous deuterium lamp (Cathodeon, Cambridge, UK) with pre-aligned fiber optics output, and an UV-VIS 256-element photodiode array (PDA) detector (Carl Zeiss, Herts, UK). This combination offers a spectral range of 190–735 nm with blaze wavelength at 220 nm. A bifurcated fiber optics dip probe (1/4'' Mini Immersion Probe, Hellma, Essex, UK) with optical path length of 1-cm is connected to the deuterium lamp and the PDA detector.

Titration was carried out by using a PCA101 automatic titrator (Sirius, East Sussex, UK) [31–33]. A 1/4'' hole was drilled through the electrode holder of the PCA101 to accommodate the dip probe such that it could be situated next to the pH electrode. The pH electrode (Orion, Ross™ type, Beverly, MA) was calibrated titrimetrically in the pH range of 1.8–12.2 [23]. 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 greater than 10^{14} Ω-cm. In all titration experiments, sample solutions of 10–20 ml volumes were pre-acidified to a reasonably low pH value (1.8–3.0) using 0.5 M HCl and then titrated alkalimetrically to a suitably high pH value (10.0–12.2). In the WApH technique, spectral data was recorded in the region of 210–400 nm after each pH measurement. The pH change per titrant addition was limited to about 0.2 pH units. Typically, more than 20 pH readings and absorption spectra were collected from each titration.

In the present study, the pK_a values of niflumic acid, nitrazepam, pyridoxine, quinine and terbutaline were determined both spectrophotometrically and pH-metrically. For the WApH technique, sample concentrations from 3.4 to 26 μM were employed for titration. Stock sample solutions of sub-millimolar (or lower) concentrations were prepared. The pH values of the stock solutions were adjusted using 0.5 M HCl or 0.5 M KOH so that the samples were in the most water soluble forms. Then, 0.05–0.25 ml of the stock solution

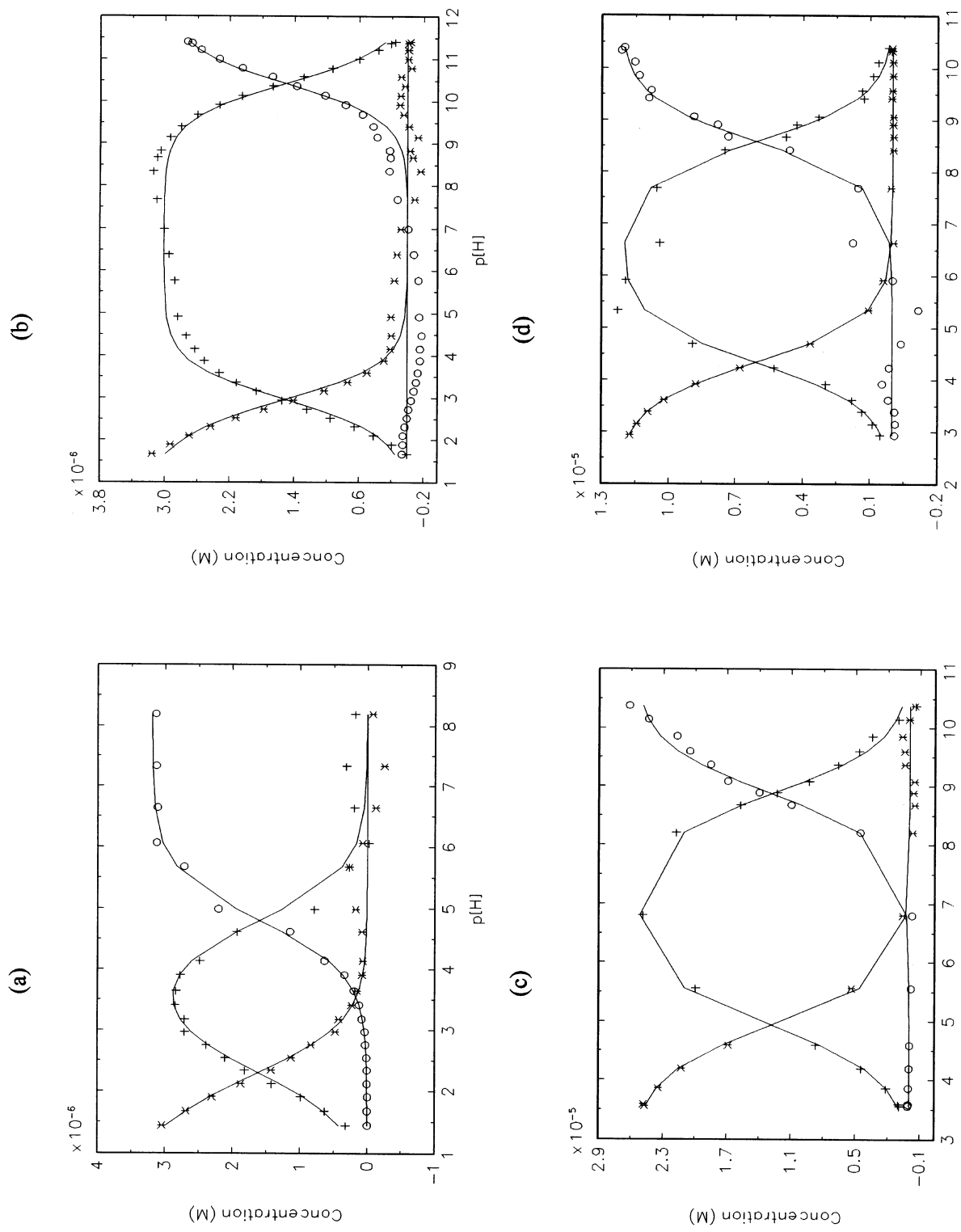

Fig. 5. Distribution of species as a function of pH with the symbols represent the C_p matrix (*, + and ○ indicate, respectively, X_1, X_2 and X_3 as shown in Eq. 12) and solid lines denote the C_t matrix: (a) niflumic acid; (b) nitrazepam; (c) pyridoxine; and (d) quinine.

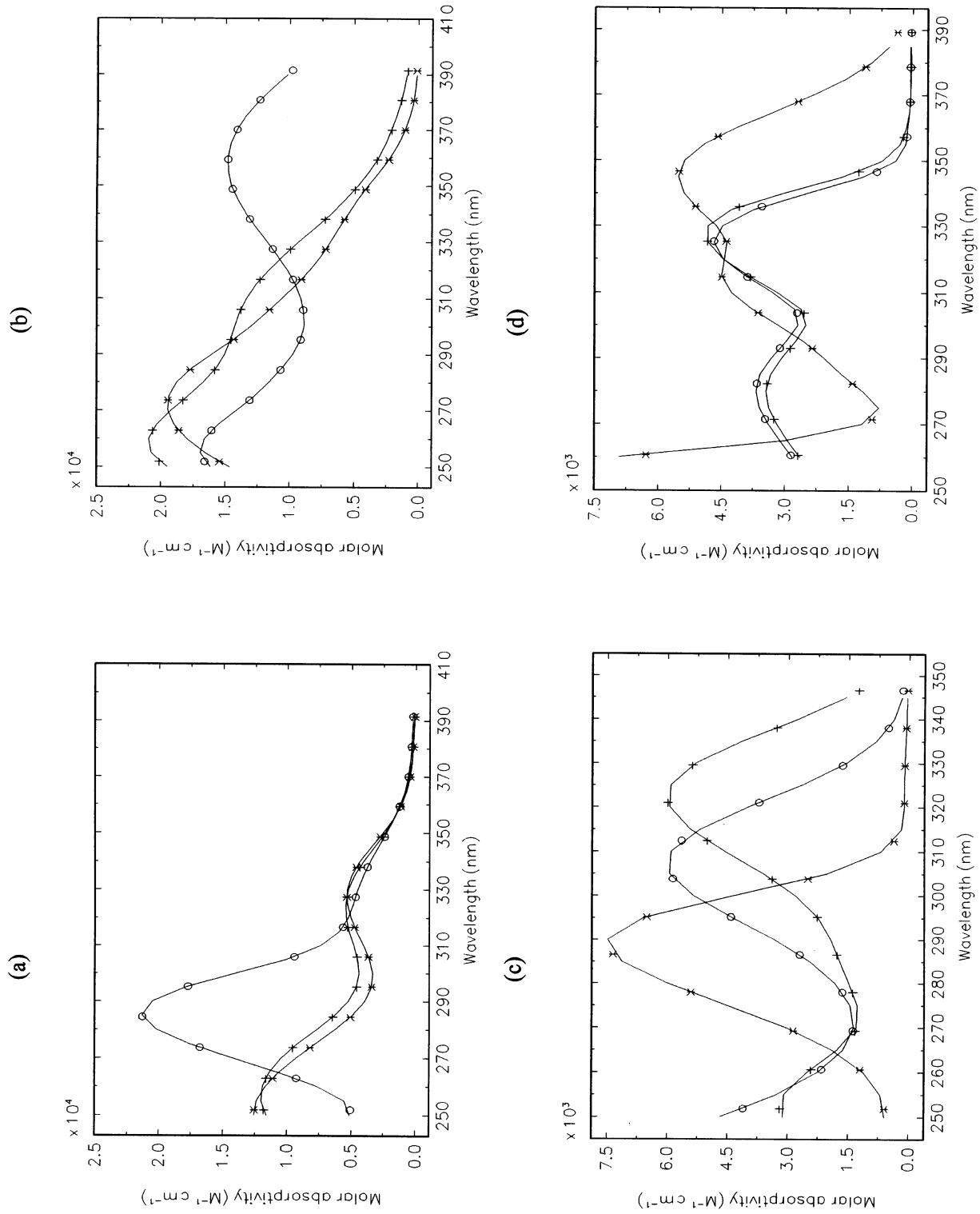

Fig. 6. Molar absorption coefficients of: (a) niflumic acid; (b) nitrazepam; (c) pyridoxine; and (d) quinine systems with the symbols represent the elements in matrix E_p (*, + and ○ indicate, respectively, X_1, X_2 and X_3 as shown in Eq. 12) and solid lines generated using the cubic spline interpolation method.

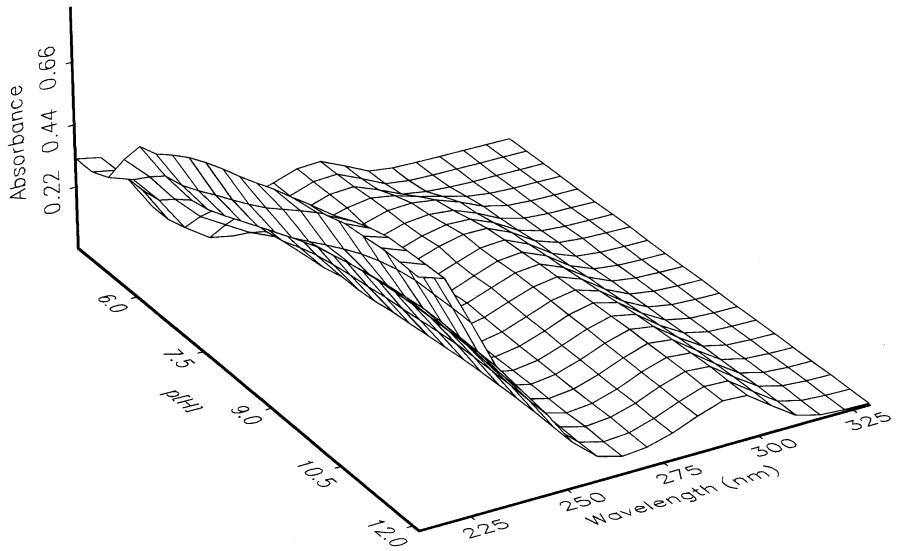

Fig. 7. Absorption spectra of terbutaline.

was pipetted into the sample vial which contained 10–20 ml 0.15 M KCl solution to make up the designated sample concentration. As for the pH-metric method, weighed amount of samples were used to prepare solution concentrations from 0.5 to 15 mM. All experiments were carried out in aqueous solution except for the pH-metric titrations of water-insoluble compounds (niflumic acid, nitrazepam and quinine) in which 15–65 wt.% methanol was utilized. At least three WApH titrations and pH-metric titrations were performed for each drug sample. The formulae for evaluating pK_a values from pH-metric aqueous titration or cosolvent titration data have been reported previously [4,31–33]. No new computation concepts are involved and the reader is directed to the relevant literature for methodological details. Calculations of pK_a values from pH-metric data were performed using *pKaLOGP*™ software (v5.01, Sirius, East Sussex, UK). Programs for PCA-TFA treatment were coded in a Turbo C environment. All numerical routines utilized in the multivariate computations were adopted from an established program library [34].

Samples of niflumic acid, nitrazepam, pyridoxine, quinine and terbutaline were gifts from K. Takacs-Novak (Semmelweis University of Medicine, Budapest, Hungary).

4. Results and discussion

Four drug compounds with two ionization steps (niflumic acid, nitrazepam, pyridoxine and quinine) and a kinetically complicated system (terbutaline) have been examined in this study. The structures of niflumic acid, nitrazepam, pyridoxine and quinine are given in Fig. 2. For the two-ionization step systems, only pyridoxine is water-soluble up to milli-molar level while for the other three samples, methanol is added as co-solvent. The absorption spectra of niflumic acid, nitrazepam, pyridoxine and quinine at different pH values are depicted in Fig. 3. Principal component analysis [21,24–27] on these data matrices indicated that in all cases, three independent components were presented in the equilibrium systems. Fig. 4 exhibits the residual absorbance values ($A - C_p E_p$, Eqs. (9) and (10)), which were generated using a three-component model for the four drug compounds. It can be seen that the noise levels are in line with the standard deviation (S.D.) in absorbance of our optical system which

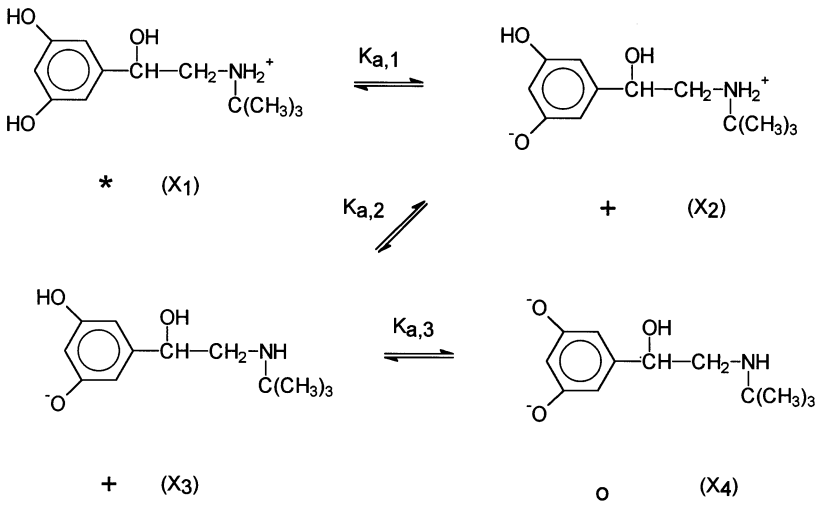

Fig. 8. Suggested protonation scheme of terbutaline.

is varying between 1.5×10^{-4} and 1.5×10^{-3} A.U. The S.D. values were estimated independently in several blank titrations. Here, we suggest a two-step protonation scheme

$$\underset{(*)}{X_1} \overset{K_{a,1}}{\rightleftharpoons} \underset{(+)}{X_2} \overset{K_{a,2}}{\rightleftharpoons} \underset{(\bigcirc)}{X_3} \qquad (12)$$

where the symbols denote different UV active species and the term X_3 represents the fully deprotonated form of the drug. In Eq. (12), all charges and protons are omitted for clarity.

Table 1 lists the pK_a values of niflumic acid, nitrazepam, pyridoxine and quinine determined using the WApH technique and the pH-metric method. Note the good level of agreement between the two techniques. Figs. 5 and 6 depict, respectively, the distribution of species and the molar absorption coefficients for piflumic acid, nitrazepam, pyridoxine and quinine. As shown in

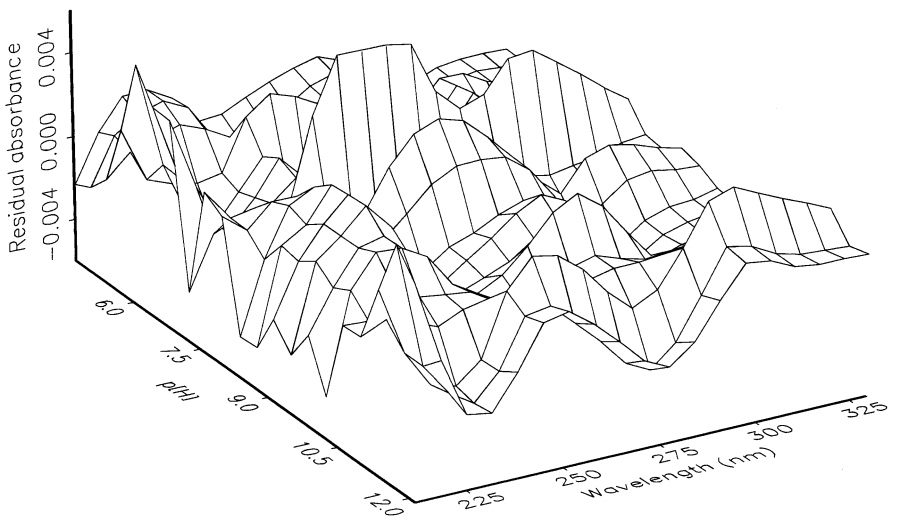

Fig. 9. Residual absorbance values of terbutaline system generated using a three-component model.

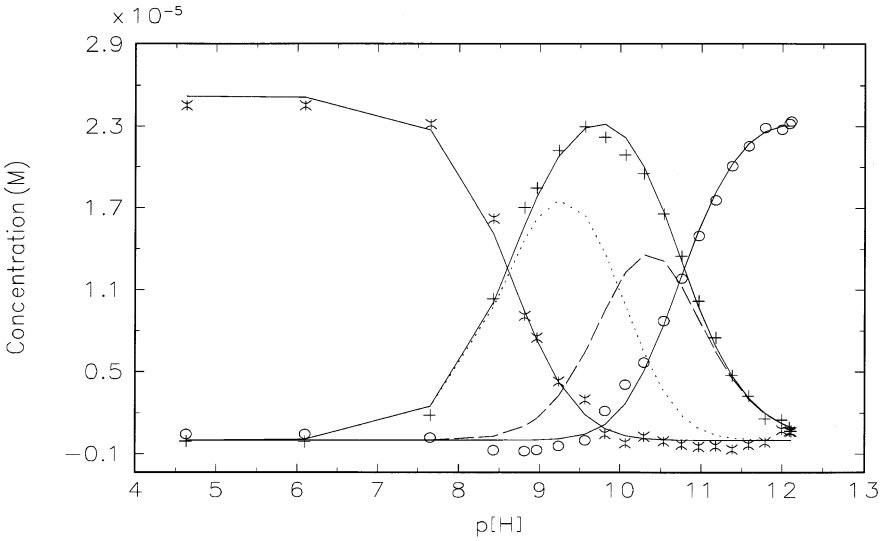

Fig. 10. Terbutaline distribution of species as a function of pH with the symbols represent the C_p matrix (*, + and ○ indicate, respectively, X_1, X_2/X_3 and X_4 as shown in Fig. 8) and solid lines denote the C_t matrix. The dotted and dashed lines designate, respectively, the theoretical concentrations of species X_2 and X_3 as calculated using Eq. 3.

Fig. 6, TFA method is able to resolve the molar absorption data even if the spectra of individual UV active species are highly overlapping, as in the cases of niflumic acid, nitrazepam and quinine. The resolved spectra (Fig. 6) of piflumic acid, nitrazepam and pyridoxine are consistent with literature [35]. This justifies the validity of the WApH approach.

Next, attention is directed to a kinetically complicated system, terbutaline. Typical absorption spectra are given in Fig. 7. Principal component analysis [21,24–27] on this data matrix revealed that only three independent components were present in the chemical system. Based on an earlier work by Takács-Novák et al. [36], we propose a four-step protonation scheme which is shown in Fig. 8 with all free protons omitted for clarity. As we examine the structure of the four reacting species, it is plausible that the absorption spectra of species X_2 and X_3 are almost completely overlapped since the ionization center (amine) is relatively far away from the chromophore (aromatic center). In this manner, the proposed number of UV active species (i.e. X_1, X_2/X_3 and X_4) is in line with our statistical finding. Fig. 9 depicts the residual absorbance values generated using a three-component model. Note that the residuals are pretty random along the pH axis, suggesting that the proposed three-component model is valid.

Since the absorption spectra of species X_2 and X_3 may not be resolved unambiguously, it is difficult to refine $pK_{a,2}$ by using the optical signals as depicted in Fig. 7. In our computation, we assumed a value of 9.97 (as determined pH-metrically, Table 2) for this variable and identical spectral properties for species X_2 and X_3. Table 2 lists the results obtained using the WApH technique and the pH-metric method. Again, reasonable agreement in the pK_a values can be seen between the two techniques and those reported by others [36].

Figs. 10 and 11 give, respectively, the distribution of species and the molar absorption coefficients of the terbutaline system. As shown in Fig. 11, a red shift in absorption spectra can be seen as the phenolic protons are removed from the aromatic center which is probably due to the enhanced π-electron conjugation.

5. Conclusions

We have devised an elegant approach (WApH) based on a fiber optics dip probe, an UV light

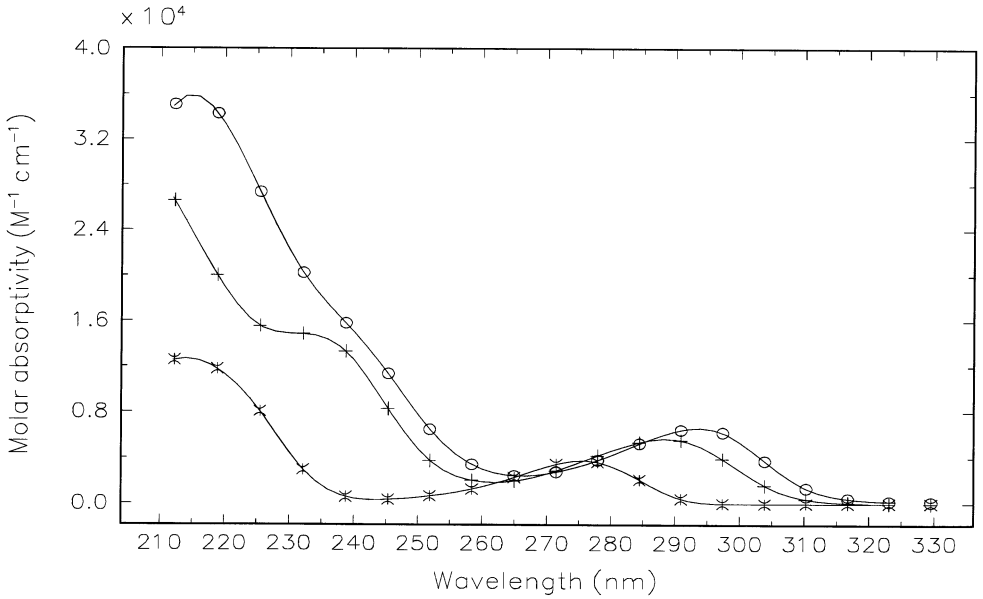

Fig. 11. Molar absorption coefficients of terbutaline system with the symbols represent the elements in matrix E_p (*, + and ○ indicate, respectively, X_1, X_2/X_3 and X_4 as shown in Fig. 8) and solid lines generated using the cubic spline interpolation method.

source and a photodiode array detector in conjunction with a commercially available titrator (Sirius PCA101) to determine pK_a values of ionizable drugs. A multivariate computation method has been adopted to deduce pK_a values from the absorption spectra recorded at different pH values. The WApH technique was found to be very sensitive in drug concentrations about 10^{-5}–10^{-6} M. It has been demonstrated that the sought pK_a values and the molar absorption coefficients of individual light absorbing species at different wavelengths can be derived with high accuracy in a multiwavelength spectrophotometric titration experiment. The WApH technique is exemplified by several pure drugs, namely, quinine, niflumic acid, pyridoxine, nitrazepam and terbutaline. Excellent agreement with pH-metric titration is noted.

Table 2
pK_a of terbutaline as determined using the WApH and the pH-metric methods at 25°C and ionic strength of 0.15 M

	WApH[a]	pH-metric[a]	Lit. data [36][b]
p$K_{a,1}$	8.64 ± 0.06	8.67 ± 0.01	8.57
p$K_{a,2}$	Not refined	9.97 ± 0.01	9.89
p$K_{a,3}$	10.76 ± 0.03	11.02 ± 0.01	11.01

[a] Uncertainties equal to the standard deviation of the pK_a values from at least three experiments.
[b] Determined pH-metrically at 25°C and 0.2 M ionic strength.

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References

[1] J.J. Kautman, N.M. Semo, W.S. Koski, J. Med. Chem. 18 (1975) 647–655.
[2] H.L.J. Fleuren, C.A.M. van Ginneken, J.M. van Rossum, J. Pharm. Sci. 68 (1979) 1056–1058.
[3] A. Albert, E.P. Serjeant, The Determination of Ionization Constants, Chapman and Hall, London, 1984.

[4] A. Avdeef, J.E.A. Comer, S.J. Thomson, Anal. Chem. 65 (1993) 42–49.
[5] P.W. Albro, C.E. Parker, E.O. Abusteit, T.C. Mester, J.R. Hass, Y.S. Sheldon, F.T. Corbin, J. Agric. Food Chem. 32 (1984) 212–217.
[6] I.J. Lee, G.S. Jung, K. Kim, J. Solution Chem. 23 (1994) 1283–1292.
[7] B. Sikorska, E. Danilczuk, Pol. J. Chem. 67 (1993) 791–797.
[8] H. Irving, H.S. Rossotti, G. Harris, Analyst 80 (1955) 83–94.
[9] R.I. Billmers, A.L. Smith, J. Chem. Phys. 95 (1991) 4242–4245.
[10] H. Cartwright, J. Chemometr. 1 (1987) 111–120.
[11] F.G. Halaka, G.T. Babcock, J.L. Dye, Biophys. J. 48 (1985) 209–219.
[12] E.A. Sylvestre, W.H. Lawton, M.S. Maggio, Technometrics 16 (1974) 353–368.
[13] Z.Z. Hugus, A.A. El-Awady, J. Phys. Chem. 75 (1971) 2954–2957.
[14] J.J. Kankare, Anal. Chem. 42 (1970) 1322–1326.
[15] M. Kubista, R. Sjöback, B. Albinsson, Anal. Chem. 65 (1993) 994–998.
[16] S.D. Frans, J.M. Harris, Anal. Chem. 57 (1985) 1718–1721.
[17] K.Y. Tam, F.T. Chau, J. Auto. Chem. 14 (1992) 157–167.
[18] K.Y. Tam, F.T. Chau, Spectrosco. Lett. 26 (1993) 1195–1212.
[19] K.Y. Tam, F.T. Chau, Chemometr. Intell. Lab. Syst. 25 (1994) 25–42.
[20] D. Perez-Bendito, Analyst 115 (1990) 689–698.
[21] E.R. Malinowski, Factor Analysis in Chemistry, 2nd ed., Wiley, New York, 1991.
[22] G. Stephenson, Mathematical Methods for Science Students, Longman, Harlow, 1973.
[23] A. Avdeef, J.J. Bucher, Anal. Chem. 50 (1978) 2137–2142.
[24] E.R. Malinowski, Anal. Chem. 49 (1977) 612–617.
[25] H.B. Woodruff, P.C. Tway, L.J.C. Love, Anal. Chem. 53 (1981) 81–84.
[26] P.J. Gemperline, J.C. Hamilton, in: H.L.C. Meuzelaar (Ed.), Factor Analysis of Spectro-Chromatographic Data, Computer-Enhanced Analytical Spectroscopy, vol. 2, Plenum, New York, 1990, pp. 27–48.
[27] E.R. Malinowski, J. Chemometr. 1 (1987) 33–40.
[28] M. McCue, E.R. Malinowski, Appl. Spectrosc. 37 (1983) 463–469.
[29] M. D'Amboise, B. Lagarde, Comput. Chem. 13 (1989) 39–44.
[30] J.A. Nelder, R. Mead, Comput. J. 7 (1965) 308–313.
[31] A. Avdeef, Quant. Struct.-Act. Relatsh. 11 (1992) 510–517.
[32] A. Avdeef, J. Pharm. Sci. 82 (1993) 183–190.
[33] A. Avdeef, K.J. Box, K. Takács-Novák, J. Pharm. Sci. 84 (1995) 523–529.
[34] W.T. Vetterling, S.A. Teukolsky, W.H. Press, B.P. Flannery, Numerical Recipes, Cambridge University Press, Cambridge, 1988.
[35] K. Takács-Novák, M. Józan, G. Szász, Int. J. Pharm. 113 (1995) 47–55.
[36] K. Takács-Novák, B. Noszál, M. Tökés-Kövesdi, G. Szász, J. Pharm. Pharmacol. 47 (1995) 431–435.