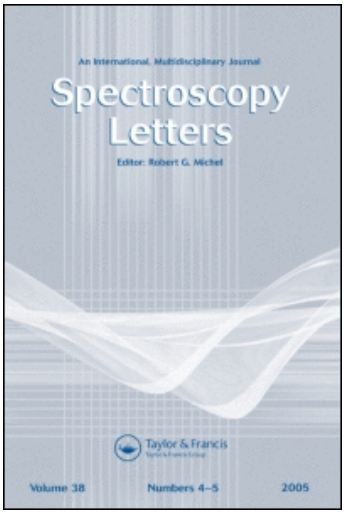

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A NEW MULTIWAVELENGTH SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF THE MOLAR ABSORPTION COEFFICIENTS OF IONIZABLE DRUGS

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A NEW MULTIWAVELENGTH SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF THE MOLAR ABSORPTION COEFFICIENTS OF IONIZABLE DRUGS

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ABSTRACT

A multiwavelength spectrophotometric method, called Dip-Probe Absorption Spectroscopy (D-PAS), developed previously for pK_a determination was applied for the determination of the molar absorption coefficients of ioniz-

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able compounds. Specifically, the spectral data obtained from a spectrophotometric titration are resolved using Target Factor Analysis (TFA) to generate the molar absorption coefficients of each ionizing species. The D-PAS method has been exemplified by the spectral data of p-aminosalicylic acid and nicotinic acid. It has been shown that the molar absorption coefficients as determined by the D-PAS method are comparable to those deduced by manual titration experiment.

Key Words: Molar absorption coefficients; Nicotinic acid; p-Aminosalicylic acid; Target factor analysis; D-PAS

INTRODUCTION

A spectrophotometric method is commonly used to quantify a drug in dosage forms or pharmaceutical formulations[1–3]. This method generally depends on the Beer's law to calculate the concentration of the drug from the absorption spectra. To use this technique, the molar absorption coefficients of the drug are required, which may be determined by independent spectrophotometric measurements using the pure drug compounds. For non-ionizable compounds, the determination of molar absorption coefficients (absorptivity) is relatively straightforward. Within the linear region of the Beer's law, the required molar absorption coefficients can be obtained from the concentration and the absorption data of the pure drug solution. However, this approach is not applicable in the case of an ionizable drug because the absorbance of the drug solution depends on the pH and the ionic strength as well as the drug concentration. Deviation from the Beer's law is expected if the underlying acid-base equilibrium is not taken into consideration.

Consider a diprotic compound, X, the ionization scheme can be described in Eq. (1).

$$\underset{*}{H_2X} \overset{pk_{a,1}}{\longleftrightarrow} \underset{+}{HX} \overset{pk_{a,2}}{\longleftrightarrow} \underset{o}{X} \tag{1}$$

where the symbols $pK_{a,1}$ and $pK_{a,2}$ represent, respectively, the first and second acid dissociation constants. Note that the symbols *, + and o are used to denote the identity of the corresponding species in the figures. In Eq. (1), all free protons and charges are omitted for clarity. At a particular

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pH, the pure sample solution may contain three ionizing species. Thus, the measured absorbance is a composite value of the spectral absorption of all ionizing species. To obtain the molar absorption coefficients of H_2X and X, the sample solution can be buffered at suitably low and high pH values, respectively. This ensures only one species is present at a time. Then, the molar absorption coefficients can be deduced as in the case of non-ionizable compounds. If the pK_a values are known, the molar absorption coefficients of HX can be calculated by fitting the experimental spectral data and the molar absorption coefficients of H_2X and/or X to established formulae[4] and as described in the following text. To this end, the molar absorption coefficients of all ionizing species can be deduced. However, this manual titration procedure is tedious and time-consuming. In addition, the ionic strength may be varied between experiments, which may lead to a slight shift in the pK_a values.

In our previous work, we have developed a multi-wavelength spectrophotometric approach, termed Dip-Probe Absorption Spectroscopy (D-PAS), to measure the pK_a values of monoprotic and multiprotic drug compounds[5,6]. Specifically, we employed a fibre optics dip probe, a UV light source and a photodiode array detector in conjunction with a commercially available titrator to *automatically* capture the absorption spectra in the course of a pH-metric titration. Target factor analysis (TFA) has been applied with success to deduce the pK_a values of ionizable drugs. It has been shown that the D-PAS method can be used to deduce pK_a values with high accuracy, which are consistent with the pK_a values obtained pH-metrically even if the absorption spectra of the reacting species are very similar[5–9].

In this study, we seek to apply the D-PAS method to resolve the molar absorption coefficients of ionizable drugs. The D-PAS approach is fully automated and is the technique of choice compared with the aforementioned manual titration procedure. We note that the molar absorption coefficient provides a valuable handle to quantify the sample of interest, which may find a wider application in physico-chemical characterization of lead compounds in drug discovery and development. For instance, spectrophotometric method can be adopted to determine the solubility of sparingly soluble drugs if the measurement is carried out in saturated drug solution. These led us to initiate this study to see whether it is possible to develop an efficient method to measure the molar absorption coefficient of ionizable compounds. In the subsequent discussion, a brief account on the D-PAS method and the manual titration procedure for measuring the molar absorption coefficients will be given. Spectral data of p-aminosalicylic acid and nicotinic acid will be utilized to illustrate how the methods work.

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METHOD

In spectrophotometric measurement, the absorbance, A, can be related to the concentration of the sample (C) by the Beer's law as shown in Eq. (2).

$$A = CEb \tag{2}$$

where E and b represent, respectively, the molar absorption coefficient and the optical pathlength. In the framework of spectrophotometric titration, Eq. (2) can be expressed in matrix form. $\underline{A}$ represents the absorption spectra obtained at different pH values, with dimensions of N_s (absorption spectra) $\times N_w$ (wavelength). $\underline{C}$ and $\underline{E}$ represent, respectively, the concentration-pH profile ($N_s \times N_c$) and the molar absorptivities matrix ($N_c \times N_w$). 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 the subsequent discussion, we will outline the computation method to process the spectral data obtained from the D-PAS titration.

Target Factor Analysis (TFA)

To resolve the $\underline{C}$ and $\underline{E}$ matrices, it is required to deconvolute the $\underline{A}$ matrix. Specifically, the $\underline{A}$ matrix is first subjected to principal component analysis to deduce an abstract solution $\underline{C}_{abs}$ and $\underline{E}_{abs}$, which contain only the primary eigenvalues ($\underline{\lambda}_r$) and eigenvectors ($\underline{Q}_r$)[5,6,10] . The residual standard deviation[10], IND function[10,11], eigenvalue ratio[12] and reduced eigenvalue ratio[13] are utilized to identify N_c (assuming $N_s > N_w$ and $N_w > N_c$) that are important in describing the absorbance matrix. In TFA treatment, the abstract solution is rotated to the one with relevant physical significant $\underline{C}_p$ and $\underline{E}_p$ by the target transformation procedure as shown in Eqs. (3) through (5)[10,14,15].

$$\underline{T} = \underline{\lambda}_r^{-1}\underline{C}_{abs}^{T}\underline{C}_t \tag{3}$$

$$\underline{A} \approx \underline{C}_{abs}\underline{T}\underline{T}^{-1}\underline{E}_{abs}b \tag{4}$$

$$\approx \underline{C}_p\underline{E}_p b \tag{5}$$

where $\underline{T}$ represents a transformation matrix. The superscripts -1 and T denote, respectively, inverse and transpose operations. Note that $\underline{E}_p$ is the

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molar absorption coefficient matrix, which is readily generated by the target transformation procedure. For chemical systems consisting of an ionizable compound, we have shown that $\underline{C}_t$ is simply a test matrix containing the theoretical distribution-of-species profiles as a function of pH, which can be obtained by solving the mass balance equation of the ionization system[5].

The SPOIL function as proposed by Malinowski[10,15] is utilized to determine whether a test matrix is acceptable or not. In general, a test matrix that minimizes the SPOIL function with a value not greater than 3.0 is considered as the solution for the target transformation procedure[5,10,14,15]. For a particular A matrix, the SPOIL function depends only on $\underline{C}_t$ which in turn is a function of the sought pK_a values. In Eq. (6), we define a cost function, Φ

$$\Phi = \xi + \zeta + \sum_i (\text{SPOIL}_i)^2 \tag{6}$$

where the symbol ξ represents a penalty function for any negative element in the $\underline{E}_p$ matrix. 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 can be used for this purpose[16].

EXPERIMENTAL

D-PAS Titration

All titration experiments were performed by a *GLpKa*™ titrator (Sirius, East Sussex, UK). Absorption spectra were collected using a D-PAS unit[5–9] which consists of a pulsed deuterium lamp, a 256-element photodiode array (PDA) detector and a bifurcated fibre optic dip probe of 1-cm pathlength (Sirius). Digitized spectra were recorded and analyzed using the *pKaUV*™ software (Sirius). The pH change per titrant addition was limited to 0.1–0.2 pH units. The spectral data were acquired after each pH measurement, when the drift was less than 0.01 pH units per minute. About 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 done in aqueous solution with 0.15 M KCl under an argon atmosphere at $25 \pm 0.5\,^{\circ}C$, using standardized 0.5 M HCl or 0.5 M KOH titrants.

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Solutions were made up of deionized water of resistivity $> 10^{14}$ Ω-cm. Sample concentrations of about 10^{-5}–10^{-4} M were employed. Titrations were carried out in the presence of 2.5×10^{-4} M potassium dihydrogen phosphate to allow sufficient spectra to be collected in the un-buffered region of the titration curve[7]. Typically, sample solutions were pre-acidified to a reasonably low pH value (1.8–3.0) then titrated alkalimetrically to an appropriate high pH value (7.0–11.0).

Manual Titration

The sample solution of concentration about 10^{-5}–10^{-4} M was titrated using 1 M HCl and/or 12M NaOH as titrant. This allowed the pH values to span the range from 1.0 to 11.1. All pH measurements were accomplished using a Accumet AR50 pH-meter. For mid-pH range, experiments were performed in the presence of millimolar phosphoric acid. After each aliquot of titrant added, the pH of the solution was measured and a convenient amount of the solution was then pipetted to a quartz cell for spectral measurement. UV absorption spectra were captured using a Philips PU8730 spectrophotometer. The absorbance values were corrected for baseline and dilution effect resulting from the addition of titrant. The corrected absorbance values were then converted to the molar absorption coefficients by using the starting concentration of the sample used. These data were deconvoluted to the molar absorption coefficients of the ionizing species as described in the discussion section.

Materials

Nicotinic acid and p-aminosalicylic acid were of pharmacopoeial grade (Ph. Hg. VII) and were supplied by Reanal Rt. (Budapest, Hungary). All other reagents used were of AR grade.

RESULTS AND DISCUSSION

The D-PAS method was applied to obtain the spectral data of p-aminosalicylic acid and nicotinic acid as a function of pH. The absorption spectra were then subjected to the TFA treatment as described before. In all cases, the pK_as were successfully determined and the values are listed in Table 1. (Note the good agreement with the pH-metric results.) It was found

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Table 1. pK_a Values of p-Aminosalicylic Acid and Nicotinic Acid Determined Using the D-PAS Method at 25°C and an Ionic Strength of 0.15 M

		D-PAS[1]	pH-Metric[1]
p-Aminosalicylic acid	$pK_{a,1}$[2]	1.79 ± 0.02	1.71 ± 0.02
	$pK_{a,2}$	3.58 ± 0.01	3.60 ± 0.01
Nicotinic acid	$pK_{a,1}$	2.10 ± 0.01	2.00 ± 0.01
	$pK_{a,2}$	4.63 ± 0.01	4.63 ± 0.01

[1] Uncertainties equal to the standard deviation of the pK_a values obtained from three experiments.
[2] Protonation scheme is given in Eq. (1).

that the SPOIL function was less than 3.0 for each principal component, suggesting the quality of the spectral data was superb. Figs. 1 and 2 show, respectively, the resolved molar absorption spectra of the ionizing species of p-aminosalicylic acid and nicotinic acid.

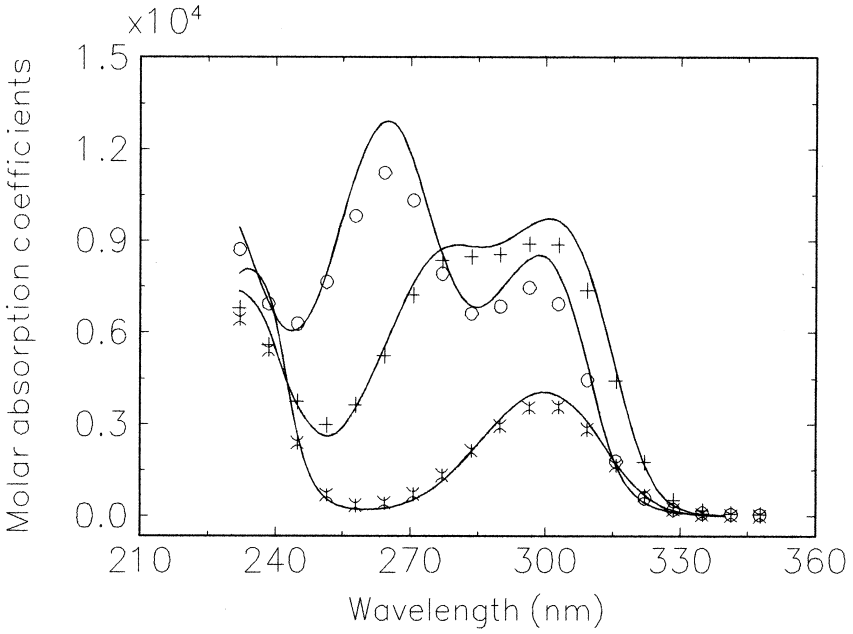

Figure 1. Molar absorption coefficients of p-aminosalicylic acid with the symbols (defined in Eq. (1)) represent the results obtained by the D-PAS method and the lines represent the corresponding values obtained by the manual titration procedure.

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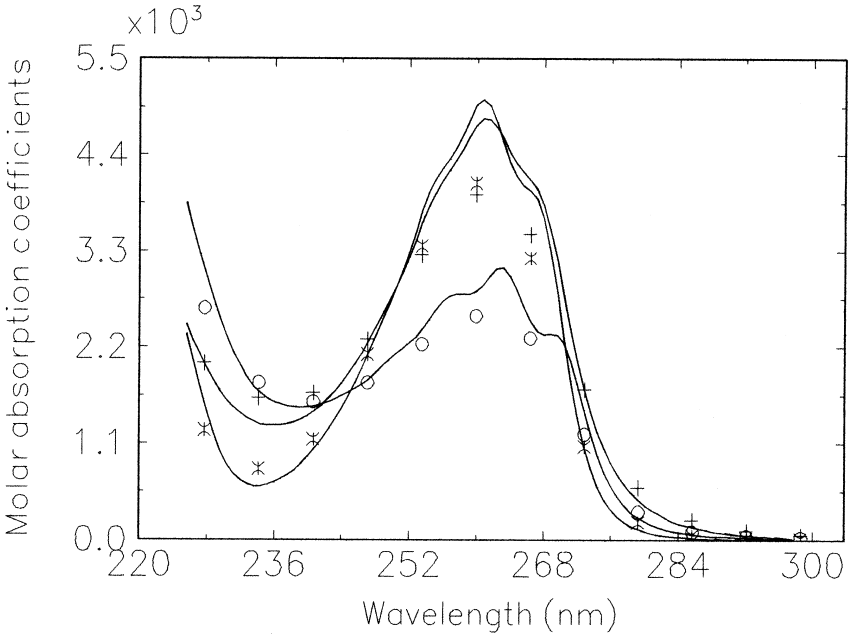

Figure 2. Molar absorption coefficients of nicotinic acid with the symbols (defined in Eq. (1)) represent the results obtained by the D-PAS method and the lines represent the corresponding values obtained by the manual titration procedure.

Next, we turn our attention to the manual titration experiments. The challenge in deconvoluting the experimental spectrum into its component parts divides into two parts. First, the relative concentrations of the species $[H_2X]$ $[HX]$ and $[X]$ in solution as a function of $[H]$ must be calculated. Note that all charges are omitted for clarity. This is accomplished using the pK_a (as K_a) values from Table 1 and Eqs. (7) through (9)[18].

$$[H_2X] = \frac{C_0[H]^2}{[H]^2 + K_{a,1}[H] + K_{a,1}K_{a,2}} \tag{7}$$

$$[HX] = \frac{C_0K_{a,1}[H]}{[H]^2 + K_{a,1}[H] + K_{a,1}K_{a,2}} \tag{8}$$

$$[X] = \frac{C_0K_{a,1}K_{a,2}}{[H]^2 + K_{a,1}[H] + K_{a,1}K_{a,2}} \tag{9}$$

C_0 represents the analytical concentration (sum of all species), but here conveniently normalized to unity to give the relative concentrations. Not surprisingly in view of the pK_a's no higher than 4.63 measured here, deprotonation at high pH's was 100%, whereas protonation at the lowest pH's studied here was by contrast far from complete. This underlines the importance of calculating these values and not making possibly wrong assumptions about completeness.

The second part is to separate the individual molar absorbances of the contributing species. The observed total molar absorbance (A_T) at a given wavelength is a linear combination of the individual molar absorbances (A_1 etc) weighted by concentration, as calculated above.

$$A_T = A_1[H_2X] + A_2[HX] + A_3[X] \tag{10}$$

The molar absorbance of the fully deprotonated species (A_3) was readily obtained for the pH range over which it comprised essentially 100% of the absorbing species. The contribution of this moiety was then subtracted from each individually observed absorbance value, $A_T - A_3[X]$, and this value was scaled up to make $[H_2X] + [HX]$ equal to unity, by dividing by $(1-[X])$. For each wavelength this resulted in a series of absorbances against relative concentration of the remaining two moieties. These were readily solved by linear regression to give the absorbances of each moiety in the pure form.

In most cases, we found that the correlation factor was higher than 0.999, suggesting the spectral data are of excellent quality. Figs. 1 and 2 depict, respectively, the resolved molar absorption spectra of p-aminosalicylic acid and nicotinic (symbols defined in Eq. (1)), which are consistent with the results obtained by the D-PAS technique. This justifies the validity of the D-PAS method to determine the molar absorption coefficients of ionizable compounds. It can be seen that the fine spectral features of nicotinic acid at 250–270 nm cannot be revealed by the D-PAS experiment (see Fig. 2), which is probably due to the poor spectral resolution of about 6 nm (as compared with 0.8 nm for the manual titration experiments). In D-PAS experiment, the pH change per titrant addition was about 0.1 to 0.2 pH units. To use the TFA method, the number of pH points must be greater than the number of wavelength channels in the $\underline{A}$ matrix (see method section). This posts an upper limit for the number of wavelength channels (N_w), which leads to a poor spectral resolution. While both methods are able to obtain comparable molar absorption coefficients, we note that a single optimization calculation is sufficient for the D-PAS method to deconvolute all unknown molar absorption coefficients. In contrast, the manual titration procedure is relatively tedious because independent regression calculations

are required for each wavelength channel to resolve the unknown molar absorption coefficients.

CONCLUSION

We have applied the D-PAS method and a manual titration procedure to determine the molar absorption coefficients of two ionizable drugs, namely, p-aminosalicylic acid and nicotinic acid. Both methods are able to resolve the molar absorption coefficients of each ionizing species and the results obtained are comparable. We have demonstrated that the D-PAS technique is a convenient and effective way to determine the molar absorption coefficients of ionizable compounds and is the method of choice for this purpose. It is envisaged that an efficient method to obtain the molar absorption coefficients of ionizable drugs is indispensable in physico-chemical characterization of drug compounds for drug discovery and development programmes. For instance, the resolved molar absorption coefficients could be useful to quantify each ionizing species of the lead compound in partition, permeability or solubility assays. Further works on these aspects are being carried out in our laboratories and results will be reported in due course.

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