Multiwavelength spectrophotometric determination of acid dissociation constants
Part IV. Water-insoluble pyridine derivatives

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Abstract

A multiwavelength spectrophotometric (WApH) titration method for determination of acid dissociation constants (pK a values) of ionizable compounds developed previously was applied in the case of pyridine derivatives of pharmaceutical interest. Specifically, UV absorption spectra of the drug solution are acquired in the course of a pH-metric titration using an optical device based on a fibre optics dip probe, a light source and a diode array detector. Target factor analysis was applied to deduce the pK a values from the spectral data recorded at different pH. Using this technique, the pK a values of six pyridine derivatives were determined successfully. It was demonstrated that the WApH technique in this case outperforms conventional pH-metric methods with respect to the measurement of pK a values of the sparingly water soluble samples reported in this study. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Acid dissociation constants (pK a values) are important parameters to denote the extent of ionization of molecules in solution at different pH values. In the context of pharmaceutical drug discovery and development, the aqueous solubility of compounds may not be high enough for precise

\[ pK_a \] determination using conventional pH-metric titration. If the sample is sufficiently soluble in a water-miscible organic solvent, it is possible to determine pH-metrically the apparent pK a (pK a) in co-solvent mixtures [1]. Aqueous pK a values can be determined by extrapolation of the pK a values, using the Yasuda–Shedlovsky method, to zero organic solvent content (a plot of \( pK_a + \log[H_2O] \) vs. \( A/e + B \), where \([H_2O]\), \( e \), \( A \) and \( B \) represent, respectively, the molar concentration of water, the dielectric constant of the co-solvent mixture, the slope and the intercept of the plot)
We have recently reported the use of eight popular organic solvents for this purpose [3]. However, it may even be difficult to apply this technique for water-insoluble samples that are only sparingly soluble in the co-solvent mixtures.

Spectrophotometric pKₐ determination is an alternative method provided the compound is water soluble to the extent of 10⁻⁶ M and it contains chromophore(s) in proximity to the ionization centre(s). Traditionally, spectral data at a single analytical wavelength is acquired from the sample in a series of solutions with known pH values. If the molar absorptivities of the reacting species are known, the pKₐ value(s) can be computed by fitting the experimental data to established formulae [1]. Computer programs used for calculating the acid dissociation constants from multiwavelength spectrophotometric data have been reported [4,5, and the refs. therein]. Most of these methods involve a least-squares procedure whereby the differences between the theoretical and experimental absorbance values are minimized by means of the Gauss–Newton–Marquardt algorithm [5]. To this end, the unknown pKₐ values and/or the molar absorptivity of individual reacting species are treated as adjustable parameters. Chemometric approaches based on factor analysis have been applied to determine acid dissociation constants from spectroscopic titrations [6,7]. In these reported procedures, the molar absorptivities are usually not required for analysis. However, explicit equations for the equilibrium expression are necessary to rotate the eigenvector(s) to give the correct concentration profiles. It may be difficult to generalize these explicit equations for multistep ionization systems. For instance, [6] called for the Henderson–Hasselbalch equation as the parametrized model while [7] needed to solve each element of the transformation matrix algebraically which is obviously tedious for real-life ionizable drug substances with a larger number of unknown pKₐ values than those studied in the paper.

In our previous work [8], we developed a multiwavelength spectrophotometric (WApH) titration approach to interrogate drug compounds with one or two pKₐ values. Specifically, we used a fibre optics dip probe, a UV light source and a photodiode array (PDA) detector in conjunction with a commercially available titrator (Sirius PCA101) to capture the absorption spectra of the sample in the course of a pH-metric titration. Target factor analysis (TFA) was applied with success to deduce the pKₐ values of drug substances and resolve the absorption spectra of the reacting species, without prior knowledge of their optical properties. In another study [9], we have shown that the TFA method outperforms the established first derivative technique in terms of obtaining pKₐ results. Moreover, the WApH technique has been utilized to examine several multi-protic ionization drugs which involved four unknown pKₐ values [10]. In particular, some of these pKₐ values were within mid pH range which were difficult to determine because of insufficient spectra data acquired in the un-buffered region of the titration curve. With the aid of the WApH technique in coupled with an optically transparent buffer, all the unknown pKₐ values have been successfully determined and were in excellent agreement with the pH-metric results. In contrast to the related approaches using factor analysis method [6,7], we generated the target matrices (i.e. the theoretical concentration profiles) using the Cramer’s rule method [8] which is relatively easy to extend to multiprotic ionization systems [10].

In this work, we have examined six pharmaceutical intermediates (pyridine derivatives) with structures given in Fig. 1. We deliberately selected samples that were: I) soluble in water, acetonitrile–water or methanol–water mixtures (SKF-75250, SB-209471 and SB-221789) and II) sparingly soluble in water and other co-solvent mixtures (SB-221787, SB-209247 and SB-234013) to exemplify the use of the WApH technique in measuring the pKₐ values. It is envisaged that the pKₐ values of these substituted pyridine compounds are less than 2 which implies precise potentiometric determination would be difficult [1]. Moreover, the solubilities of type II compounds in water and other co-solvent mixtures are so low such that pH-metric titration is hard to obtain reliable results. In the framework of spectrophotometric titration, the pKₐ values are derived from the spectral changes recorded at the extreme region of pH (< 2) whereby the pH data may be problem-
atic. Here, we employed an established four-parameter equation to calibrate the pH electrode [2,3,11] so giving more reliable measurements as compared with the conventional electrode calibration protocols using two or three buffer solutions [12]. The goal of this study is to apply the WApH technique in conjunction with a robust electrode calibration procedure to determine the pK$a$values which are virtually impossible to measure by means of conventional pH-metric titration. In the following discussion, a brief account on the TFA and WApH methods is presented. It was demonstrated that, when available, the pK$a$values obtained from pH-metric co-solvent titrations agree with those deduced by the WApH technique.

2. Calculations

With WApH titrations, the data obtained consist of a series of spectra acquired at different pH values. According to Beer’s law, the absorbance matrix, A, can be expressed as follows:

\[
A = CE
\]  

(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 treatment as shown below.

Principal component analysis [13–15] 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 ($\lambda_r$) and eigenvectors ($Q_r$). The residual standard deviation [14], IND function [13,14], eigenvalue ratio [16] and reduced eigenvalue ratio [17,18] 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 [14,19,20] as given below:

\[
T = \lambda_1 C_{abs}^T C_t
\]  

(2)

\[
A \approx C_{abs} TT^{-1} E_{abs}
\]  

(3)

\[
A \approx C_p E_p
\]  

(4)

where the superscripts $-1$ and T denote, respectively, inverse and transpose operations. The test matrix C$_t$ in Eq. (2) contains the concentration–pH profiles of the m-step ionization system which are generated theoretically by solving the following mass balance equations [8].

\[
X_n \overset{pK_a}{\rightleftharpoons} H^+ + X_{n+1} \quad n = 1...m
\]  

(5)

\[
Y = \sum_{n=1}^{m+1} C(n)
\]  

(6)

Fig. 1. Structural formula of (a) SKF-75250, (b) SB-209471, (c) SB-221789, (d) SB-221787, (e) SB-209247 and (f) SB-234013.
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 concentration pH value (\( \text{pH} = \log[H^+] \)) is related to the operational pH reading by a multiparametric equation as given below [2,3,11]:

\[
\text{pH} = a + S \text{p}_H + j_H[H^+] + j_{OH} + \frac{K_w}{[H^+]} \tag{7}
\]

The intercept parameter \( a \) corresponds to the negative logarithm of the activity coefficient of \( H^+ \) at working temperature and ionic strength. The \( S \) term denotes the ratio between the actual slope and the Nernst slope. The \( j_H \) term corrects pH readings for the non-linear pH response due to liquid junction and asymmetry potentials in moderately acidic solution (pH 1.2–2.5), while the \( j_{OH} \) term corrects for any high-pH (pH > 11) non-linear effects. These parameters are determined by a weighted non-linear least squares procedure [11]. The ionization constants of water (\( K_w \)) as a function of temperature and ionic strength, were taken from Sweeton et al. [21].

The SPOIL function as derived by Malinowski [14,20] is utilized to determine whether a test matrix is acceptable or not. In general, a test matrix in which the SPOIL function is minimized to a value not greater than 3.0 is considered as the solution for the target transformation procedure [8–10,14,19,20,22]. 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 Eqs. (5) and (6)). The TFA computation optimizes the \( pK_a \) values for a global minimum of the SPOIL function. The SIMPLEX method [23] can be used for this purpose.

3. Experimental

3.1. Reagents and apparatus

Pharmaceutical intermediates SKF-75250, SB-209471, SB-221789 (hydrochloride salt), SB-221787, SB-209247 and SB-234013 (hydrochloride salt) were provided by SmithKline Beecham Pharmaceuticals (Tonbridge, Kent, UK). Acetonitrile (far UV grade) was supplied by Romil (Cambridge, UK). Methanol and potassium chloride (all AR grade) were obtained from Fisher (Loughborough, UK). Solutions were prepared in deionized water of resistivity \( > 10^{14} \, \text{V cm} \). The preparation and standardization of HCl and KOH solutions have been described elsewhere [11]. Potassium chloride was added to standardize the ionic strength of water and solvent–water mixtures. All titrations were performed by using a PCA101 or a GLpK_a automatic titrator (Sirius, Forest Row, UK) [2]. The pH electrode was supplied by Orion (Rossm™ type, Beverly, USA) and was calibrated titrimetrically in the pH range of 1.6–12.2 in the relevant solvent media before use [2,11]. The processing of the pH-metric data, calculations of \( pK_a \) values via a non-linear least square procedure and Yasuda–Shedlovsky extrapolation treatments were carried out using \( pK_a \text{LOGP} \) software (v5.01, Sirius).

For the WApH titration, a schematic diagram of the experimental setup is given in Fig. 2. The optical system consists of a pulsed deuterium
lamp (Cathodeon, Cambridge, UK) and a 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, MO, USA) with optical path length of 1-cm was connected to the deuterium lamp and the PDA detector. Synchronization of the titrator, pulsed deuterium lamp and spectrum acquisition by the PDA detector was accomplished using a terminate-and-stay-resident system [24]. The program for TFA treatment on the WApH data was coded in a Turbo C environment [8–10].

3.2. UV/pH titrations

All titrations 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. In the present study, sample concentrations of $1.6 \times 10^{-3}–4.7 \times 10^{-3}$ M and $5.5 \times 10^{-6}–4.4 \times 10^{-5}$ M were employed, respectively, for pH-metric and WApH titrations. In general, sample solutions of 10–20 ml volumes were pre-acidified to a relatively low pH value (ca. 1.4) and then titrated alkalimetrically to an appropriate high pH value (4.0–8.0). The pH change per titrant addition was limited to ca. 0.1 pH units. pH data were acquired when the drift was less than 0.01 pH units min$^{-1}$. For each co-solvent experiment, a weighed amount of sample was dissolved in 8–40 wt.% of acetonitrile or methanol before titration. For the WApH titration, a stock solution was prepared either by dissolving ca. 1.0 mg of sample in 1.0 ml 80 wt.% methanol or 50.0 ml acidified water. 50-μl or 1.0-ml aliquots of the stock solutions were then transferred into a sample vial containing 10–20 ml 0.15 M KCl solution to produce the required sample concentration. Spectral data were recorded in the region of 200–450 nm after each pH adjustment.

4. Results and discussion

Six pharmaceutical intermediates of pyridine derivative were interrogated in this study. In the Fig. 3. (a) Absorption spectra of SKF-75250, (b) Distribution of species for SKF-75250 as a function of pH with the symbols (• fully protonated species, + mono-protonated species, ○ fully deprotonated species) represent the $C_p$ matrix and solid lines denote the $C_t$ matrix. (c) Molar absorptivity coefficients of SKF-75250. The symbols (see (b)) represent the elements in matrix $E_p$. Solid lines are generated using the cubic spline interpolation method.
subsequent discussion, we classified the compounds into two types:

Type I: Compounds soluble in water, acetonitrile–water or methanol–water mixtures, e.g. SKF-75250, SB-209471 and SB-221789

Type II: Compounds sparingly soluble in water and co-solvent mixtures, e.g. SB-221787, SB-209247 and SB-234013

4.1. Type I compounds

SKF-75250 and SB-209471 were water soluble up to mM concentrations while SB-221789 was soluble in acetonitrile–water and methanol–water mixtures at similar concentrations. This permitted a direct comparison of the $pK_a$ values determined using the WApH technique with pH-metric results. TFA was applied to absorption spectra of these three compounds and in all cases the correct number of light-absorbing species was identified. Fig. 3 shows the absorption spectra, the distribution of species and the resolved molar absorptivity coefficients of SKF-75250. 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 SKF-75250, SB-209471 and SB-221789 obtained by the WApH and the pH-metric techniques. It can be seen that the agreement between the WApH and the pH-metric results is generally good. However, when using pH-metric measurements, accurate determination of the low $pK_a$ values of the 2-bromo-pyridine derivatives such as SKF-75250 as SB-209471 was difficult due to the potentiometric data at the extreme region of pH ($\approx 2$) being relatively noisy compared with the data obtained from the mid-range of pH. It has previously been established that a sample concentration $\leq 15$ mM (or 400 times the buffer capacity of water) should be utilized for measuring $pK_a$ values in the extreme pH region [25]. However, this concentration level was difficult to obtain for the type I compounds. The WApH technique was able to determine the $pK_a$ values of the pyridine derivatives in this extreme region of pH.

4.2. Type II compounds

The solubilities of the type II samples in water, acetonitrile–water and methanol–water mixtures were so low that reliable pH-metric determination was difficult. TFA treatment revealed the correct number of principal components in these chemical systems. Fig. 4 shows the absorption spectra, the distribution of species and the resolved molar absorptivity coefficients of SB-234013. Again, the
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Fig. 4. (a) Absorption spectra of SB-234013, (b) Distribution of species for SB-234013 as a function of pH with the symbols (* fully protonated species, ◦ fully deprotonated species) represent the $C_p$ matrix and solid lines denote the $C_t$ matrix. (c) Molar absorptivity coefficients of SB-234013. The symbols (see (b)) represent the elements in matrix $E_p$. Solid lines are generated using the cubic spline interpolation method.

SPOIL function was found to be less than 3.0 for each component. Table 1 gives the $pK_a$ values of type II compounds derived using the WApH method. By considering the $pK_a$ values of type I and II compounds, it can be seen that the $pK_a$ value of the pyridine group varies with the nature of the substituents in the 2-position which may be summarized as below:

1. Bromo-substituent: $1.2 < pK_a < 1.8$ (SKF-75250, SB-209471, SB-221787)
2. Phenylthio- and propenoic ester-substituent: $1.4 < pK_a < 1.7$ (SB-209247, SB-234013)
3. Propenoic ester-substituent: $2.5 < pK_a < 2.8$ (SB-221789)

The WApH results derived from the analysis of the compounds examined are generally in line with expected values. The presence of the bromo group at the 2-position would be expected to exert a strong negative inductive effect on the pyridine nitrogen, reducing its basicity markedly in comparison to 3-hydroxypyridine ($pK_a$ value ca. 4.8, [26]). Replacement of the bromo group with other less electron-donating substituents eg. propenoic ester or acid groups has the effect of reducing the negative inductive effect and reducing basicity to a lesser extent in comparison to the bromo substituent.

5. Concluding remarks

A multiwavelength spectrophotometric titration (WApH) method was developed for the determination of $pK_a$ values of ionizable compounds. The WApH technique was applied with success to determine the $pK_a$ values of several water-insoluble pyridine derivatives of pharmaceutical interest. Typically, a 10–20 ml sample of concentration in the region of $10^{-6}$ M is sufficient for measurement. It was demonstrated that for water-insoluble samples with low $pK_a$ values, the WApH technique in this case outperforms conventional pH-metric methods. It was also demonstrated that the $pK_a$ value of the pyridine group present in the series of compounds examined depends very much on the nature of the substituents in the 2-position.
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References