International Journal of Pharmaceutics, 72 (1991) 193–197
© 1991 Elsevier Science Publishers B.V. 0378-5173/91/$03.50
ADONIS 037851739100225I

IJP 02412

Research Papers

Spectrophotometric determination of acidity constants of compounds with unsuitable absorption features

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(Received 3 December 1990)
(Accepted 5 February 1991)

Key words: Acidity constant; Spectrophotometry; Derivative spectrophotometry; Absorption band resolution

Summary

Spectrophotometric methods for determining acidity constants of pharmaceutically significant compounds which have unsuitable absorption features have been reviewed. A brief discussion on strategies based on derivative spectrophotometry and computational deconvolution techniques is also included.

Introduction

It is well known that data on proton dissociation of pharmaceutically significant substances are of great interest for a knowledge of the stability and solubility of drugs which are, generally, pH-dependent (Rubino and Berryhill, 1986). Drug stability, drug absorption and drug activity are strongly affected by the degree of ionization and lipid solubility of the drug (Asuero, 1988).

Spectrophotometric techniques for the evaluation of constants are ideal procedures when a substance is too insoluble for potentiometry or when its pK_a value is particularly low or high (Albert and Serjeant, 1971; Cookson, 1974). In the first instance, these techniques are only applicable if:

(i) The compound shows appreciable absorption at the working wavelength;

(ii) The site of protonation or deprotonation is conjugated with or is an inherent part of a chromophoric group in the compound;

(iii) The conjugate acid-base species have different absorption spectra.

Unfortunately, many substances do not exhibit such suitable absorption features and therefore it would be necessary to call on another less straightforward technique. Nevertheless, even if conditions (i–iii) are not fulfilled, it is possible to design procedures that may circumvent the draw-

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backs enabling the realisation of the spectrophotometric evaluation of the ionization constant of the studied substance.

In the present paper, several procedures for evaluating the pK_a of substances which have unsuitable absorption features are reviewed.

In the following, we will consider three typical cases.

Case I

Determination of acidity constants of acids with no appreciable absorbance within the near UV–visible range

A number of substances of analytical and pharmaceutical interest have absorption bands in the vacuum ultraviolet range; it is then necessary to use more complicated instrumentation than the conventional near-ultraviolet–visible spectrophotometer. However, once the acidity constant of an acid with suitable absorbance in the near-ultraviolet–visible range is known, it can be used as an indicator to determine the pK_a of the substances without appreciable absorbance within that more reliable wavelength range.

The acidity constant of the indicator (HIn) is given by Eqn 1 [HIn = H + In (charges are omitted for the sake of generality)]:

$$pK_a(\mathrm{HIn}) = \mathrm{pH} + \log\frac{A_{\mathrm{In}} - A}{A - A_{\mathrm{HIn}}} + \log\frac{f_{\mathrm{HIn}}}{f_{\mathrm{In}}} \qquad (1)$$

where A is the absorbance of a solution of analytical concentration C_{In} at a given pH value; A_{HIn}, A_{In} are the limiting absorbances of the acid and base species, respectively, and f is the activity coefficient for the species considered (King, 1965). These activity coefficients can be estimated by the Davies' approach (Perrin et al., 1981).

The acidity constant of a test compound, that has no appreciable absorbance, is given by (HR = H + R):

$$pK_a(\mathrm{HR}) = \mathrm{pH} + \log\frac{[\mathrm{HR}]}{[\mathrm{R}]} + \log\frac{f_{\mathrm{HR}}}{f_{\mathrm{R}}} \qquad (2)$$

When both HIn and HR are present in the same solution, at concentrations C_{In} and C_{R}, respectively, we have

$$pK_a(\mathrm{HR}) = pK_a(\mathrm{HIn}) + \log\frac{A - A_{\mathrm{HIn}}}{A_{\mathrm{In}} - A} - \log\frac{[\mathrm{R}]}{[\mathrm{HR}]} + \log\frac{f_{\mathrm{In}} f_{\mathrm{HR}}}{f_{\mathrm{HIn}} f_{\mathrm{R}}} \qquad (3)$$

[R] and [HR] can readily be obtained from the mass and charge balance of the species involved in the equilibria, and taking into account Eqn 4 that gives [In] from the absorbance measurements.

$$[\mathrm{In}] = \frac{A - A_{\mathrm{HIn}}}{A_{\mathrm{In}} - A_{\mathrm{HIn}}} C_{\mathrm{In}} \qquad (4)$$

Ang (1959) used *m*-bromophenol to determine the acidity constant of hydrocyanic acid. The ionization constant of the hydroxylammonium ion was determined by Robinson and Bower (1961) by using 3,4-dinitrophenol as an indicator.

Another approach was developed by Halban and Brüll (1944). These authors studied iodic and trichloroacetic acids, which are so strong that they are difficult to investigate by conductance or electromotive force methods. The procedure may be outlined as follows: Consider a solution containing stoichiometric concentrations C_{R} of the acid HR, and C_{In} of the selected indicator HIn. The absorbance of this solution, A_1, is compared with that of another solution, A_2, containing the same concentration of HIn and hydrochloric acid at a variable concentration, C_s, which is adjusted until A_1 and A_2 are equal. The hydrogen ion concentration of the second solution can be found from:

$$[\mathrm{H}] = [\mathrm{Cl}] + [\mathrm{In}] = C_s + [\mathrm{In}] \qquad (5)$$

Both solutions will have the same hydrogen ion concentration if the ionic strength and [In] are identical; hence, neglecting [OH]

$$[\mathrm{R}] = C_s - \frac{A - A_{\mathrm{HIn}}}{A_{\mathrm{In}} - A_{\mathrm{HIn}}} C_{\mathrm{In}} \qquad (6)$$

and

$$[HR] = C_R - C_s + \frac{A - A_{HIn}}{A_{In} - A_{HIn}} C_{In} \quad (7)$$

$pK_a(HR)$ can be calculated by using Eqn 2. Halban and Brüll (1944) used a one-color indicator (2,4-dinitrophenol at 436 nm) with the aim of feasibility ($A_{HIn} \cong 0$).

More recently, some authors have modified this procedure by using a spectrophotometric differential device (Wozniak and Nowogroki, 1978). One cell contains the compound, C_R, and the indicator, C_{In}, in a background of 0.1 M KNO_3. Another cell, specially designed to facilitate the addition of diluting solution (equimolar with respect to the indicator and KNO_3, respectively) contains a volume V_0 of a solution of indicator, C_{In}, and hydrochloric acid, C_s^0, in the same background. Then, the diluting solution is added to the second cell until the absorbances of both cells are equal. If V is the volume of diluting solution that equals those absorbances:

$$C_s = C_s^0 \frac{V_0}{V_0 + V} \quad (8)$$

These authors applied the above procedure to determine the ionization constants of a series of phosphonic and aminoalkylphosphonic acids (Wozniak and Nowogroki, 1978), and *o*-phosphoserine (Verbert et al., 1980).

Case II

Determination of acidity constants of acids whose absorption spectrum is not dependent on pH

When condition (ii) is not fulfilled, one may not expect any change in the absorption spectrum of the compound with pH. In this case, it is advisable to follow the strategies described in case I, but selecting an indicator whose absorption spectrum does not overlap with that of the studied compound.

If this is not possible, there are two strategies for solving the problem:

(a) To select an analytical wavelength where the compound does not contribute to the measured absorbance;

(b) To carry out the absorbance measurements against a blank that contains the compound at the same concentration as in the other cell.

By applying strategy (a), the pK_a of 9-fluoren carboxylic acid has been evaluated using bromocresol green as indicator (Gonzalez and Pablos, 1991).

Case III

Determination of acidity constants of compounds when the spectra of the conjugate acid-base species overlap considerably

A first attempt to solve this problem was developed by Connors and Eboka (1979). When the absorption spectra of the HR and R species do not differ appreciably it may be useful to use the well-known relationship

$$\frac{[R]}{[HR]} = \frac{A - A_{HR}}{A_R - A} \quad (9)$$

rearranged as follows

$$\frac{A}{A_R} = [R] + \frac{A_{HR}}{A_R}[HR] \quad (10)$$

when A/A_R is plotted vs A_{HR}/A_R at a fixed pH for several wavelengths a straight line is obtained. At the pH of the experiment the concentration quotient is readily obtained from the slope and intercept, then $pK_a(HR)$ can be calculated by using Eqn 2. This method has been successfully applied to the evaluation of the acidity constants of benzoic and *trans*-cinnamic acids (Connors and Eboka, 1979).

However, by using the above-described procedure it is difficult to determine the acidity constant when the ionization process yields minor changes in the absorption spectrum.

Basically, two techniques may be applied: derivative spectroscopy and computational deconvolution of the absorption bands.

Derivative spectroscopy

Derivative spectroscopy appears to be suitable for solving this problem as it improves the detect-

ability of small spectral variations, enhances the resolution of multicomponent solutions and allows the determination of two or more species in a mixture, even if their spectra overlap. Therefore, this technique is appropriate to study small spectral variations resulting from acid-base equilibria (Levillain and Fompeydie, 1985).

The first-derivative spectrum is the gradient $dA/d\lambda$ of the absorption envelope and depicts a maximum and a flat base; the vertical distance between them is the amplitude, that is proportional to the concentration of analyte. By contrast, the second derivative spectrum $d^2A/d\lambda^2$ is inverted with respect to the zero-order spectrum. The considerably reduced bandwidth of the second-derivative spectrum is a very important feature because it allows the improvement of the resolution of overlapping bands with increased sensitivity. Hence, the two-order spectrum may lead to the separation of the bands, and then the concentrations [HR] and [R] can be calculated at each pH value. Then, pK_a(HR) can be calculated by using Eqn 2.

Instrumental methods for generating derivative spectra may be classified into two groups. The first includes those methods that operate directly on the radiation beam: modulation techniques, synchronous scanning of two monochromators; currently, these procedures have been neglected. In the second group, the spectrophotometer output is processed to give a higher derivative (algorithmic or electronic differentiation) (Fell, 1978). Nowadays, the derivative spectra are obtained by means of adequate software supplied with the spectrophotometer.

However, when the separation between the maxima of the spectra is very small (less than 5 nm) and its ratio to the half-width of the band is low, it is very difficult to obtain good separation of the compounds by using first or second derivatives (Cahill, 1980). In this case of extreme overlapping of bands, the zero-crossing method is the only one that may solve the problem (O'Haver and Green, 1976). At the wavelength of maximum absorption of one species, for instance, HR, the value of its first derivative is zero at any concentration. Then, the value of the first derivative at this point only depends on the concentration of the species R. Zero crossing is then applied to follow the pH-dependent concentration of R. Once [R] has been obtained at a given pH, [HR] can be readily obtained from $[HR] = C_R - [R]$, and pK_a(HR) can be calculated from Eqn 2. Zero-crossing derivative spectrophotometry has been used to determine the two pK_a values of eosin whose absorption maxima for the mono- and dicationic forms are located at 519 and 516 nm and overlap strongly (Levillain and Fompeydie, 1985).

Computational deconvolution of the absorption bands

The absorption spectrum in terms of the molar absorption coefficient or absorbance as a function of wavelength very often consists of one or more bands. These bands are shown to be approximated by Gaussian curves. Thus, the ultraviolet-visible spectra may be described as an addition of Gaussian curves (Klabuhn et al., 1973). However, in many applications band shapes are intermediate between that of a Gaussian and a Cauchy function (Fraser and Suzuki, 1969). In this case, the asymmetry can be simulated empirically on the basis of a lognormal distribution (Sevilla et al., 1989).

The procedure may be illustrated by considering the overlapping spectra of two species, HR and R, at a given pH. By applying the least-squares resolution technique, the individual bands are obtained and the area, A, under each curve is calculated by integration. A is proportional to the concentration of the species and, then, pK_a(HR) may be obtained from Eqn 11.

$$pK_a(HR) = pH + \log\frac{A_{HR}}{A_R} + \log\frac{f_{HR}}{f_R} \qquad (11)$$

Fraser and Suzuki (1966) were the first to apply least-squares procedures in conjugation with a high-speed digital computer for the resolution of overlapping absorption bands. Nagano and Metzler (1967) calculated the acidity constants of a pyridoxal-alanine system by using this procedure. Others have studied the protonation equilibrium constants of the pyridoxal 5′-phosphate, hexylamine Schiff base, considering the tautomeric equilibria and the microscopic ionization constants (Blazquez et al., 1989).

More recently, Korany et al. (1990) have proposed a computer-assisted technique for the deconvolution of overlapping absorption spectra based on a discrete Fourier transform which gives excellent results in the assay of mixtures of codeine phosphate, phenylephrine hydrochloride, chlorpheniramine maleate and ephedrine hydrochloride in the presence of various additives.

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