Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 4, pp. 395–402, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Short Communication

Deconvolution of ultraviolet absorption spectra of aqueous Diazepam, Flurazepam and Prazepam in acid and alkaline solutions

P. GONZALEZ-HIERRO, M. M. VELAZQUEZ, J. M. CACHAZA and LICESIO J. RODRIGUEZ*

Departamento de Fisicoquímica Aplicada y Técnicas Instrumentales, Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain

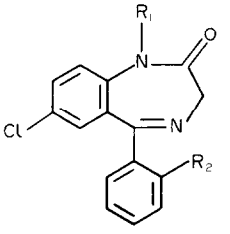
Keywords: *Diazepam; Flurazepam; Prazepam; UV absorption; deconvolution.*

Introduction

It is well known that ultraviolet–visible (UV–V) absorption spectra in solution are commonly made up of a small number of electronic bands. The large half-widths and strong overlapping of the bands blurs the overall appearance of the recorded envelope and makes it difficult to locate the component bands by visual inspection. Recovering the innermost spectral details, i.e. the series of component spectrum bands, is the aim of deconvolution [1, 2]. Deconvolution may give a deeper and more precise qualitative and quantitative description of recorded spectra, revealing new and interesting structural and analytical information. Deconvolution techniques have hitherto mainly been applied to the analysis of IR and Raman spectra [3, 4], due, in part, to the smaller half-width of the vibrational bands, which simplifies the process. Nevertheless, the massive application of computers and microprocessors to spectroscopy has considerably eased the application of deconvolution procedures of spectral bands of any kind [5–7].

In this work, a comparative deconvolution study of the UV absorption spectra of some 1,4 Benzodiazepines in aqueous acid and alkaline solutions has been carried out. The main objective may be considered to be of an analytical rather than a spectroscopic or structural nature. Diazepam, Flurazepam and Prazepam (Fig. 1) were selected for this study. The results obtained express quantitatively the concentration-independent absorption intensity of the studied drugs as a function of wavenumber (wavelength) in a closed mathematical form. The application of this methodology in a routine way can provide a more precise and succinct procedure of reporting electronic absorption spectra, by reducing the information to a small number of parameters instead of the graphical reproduction of a whole spectrum.

*To whom correspondence should be addressed.



Diazepam	$R_1 = CH_3$	$R_2 = H$
Flurazepam	$R_1 = -CH_2-NH-(C_2H_5)_2$	$R_2 = F$
Prazepam	$R_1 = -CH_2-CH(CH_2)(CH_2)$	$R_2 = H$

Figure 1
Chemical structures of Diazepam, Flurazepam and Prazepam.

Experimental

Materials

Drugs were kindly donated by different Laboratories. Diazepam (mp 128°C) was obtained from Prodes and was recrystallized from acetone–petroleum ether (1:1, v/v). Flurazepam (mp 200°C) was obtained from Roche and was recrystallized from methanol–ether (1:1, v/v). Prazepam (mp 144°C) was obtained from Substancia–Parke Davis and was recrystallized from methanol. Hydrochloric acid, sodium hydroxide and ethanol, p.a., were purchased from Merck.

Spectrophotometer

Absorbance values were determined in a single 10.00 mm silica quartz cell, at 25.0 ± 0.1°C, using a Beckmann DU-7 stabilized beam recording spectrophotometer, in the range of 200–400 nm. The spectral slitwidth was 2 nm, the scanning speed was set at 120 nm/min, and the response time was 0.05 s. For preliminary deconvolution purposes, first and second derivative spectra of the samples were also registered. The digitised spectra were collected at 1 nm intervals and subjected to numerical analysis prior to interpretation. A Basic program was written to work in an Olivetti M20 computer.

Stock solutions

All solutions were prepared with deionized and distilled water. Stock aqueous drug solutions were prepared by weight in alkaline medium to prevent slow acid hydrolysis [8, 9] and stirred until their UV absorption spectra showed no variation. In the case of Prazepam the low water solubility of this drug (<1 mg/l), made it impractical to prepare a precise stock solution by weight. Therefore, its concentration was determined by adding enough ethanol to an aliquot as to make an ethanol–water (2:3, v/v) mixture, followed by absorbance assay of the sample at 256 nm. Due to the reasonable solubility of Prazepam in this hydroalcoholic solvent, a molar absorptivity of 11700 ± 200 l mol^{-1} cm^{-1} could previously be determined at this shoulder wavelength.

Standard solutions

Drug standard solutions, used to determine molar absorptivity from absorbance measurements, were prepared by serial dilution of the respective stock solution. In all cases, the pH was adjusted to guarantee that either the non-ionized or ionized drug species was present. As these drugs are slightly unstable in acid solutions, their ionized forms were prepared from the alkaline stock solutions adding enough 1.0 M hydrochloric acid to reach the desired pH, and used the following preparation.

Concentrations of standard solutions of Diazepam and Flurazepam ranged from 5.00×10^{-6} to 3.00×10^{-5}M. Corresponding concentrations of Prazepam solutions ranged from 2.50×10^{-6} to 1.00×10^{-5}M. The spectral behaviour of the non-ionized species were investigated at a pH $\geq$10. The studies on the ionized forms were carried out at a pH $\leq$0.8 for Diazepam and Prazepam, and at a pH $\leq$0.2 for Flurazepam. These pH limits were established according to the pK_a of the drugs at 25.0°C. Reported values are: Diazepam, $pK_a = 3.4$; Flurazepam, $pK_a = 1.9$ [10, 11]. No previous pK_a value seems to have been reported for Prazepam. The value of 2.90 ± 0.05 used in this work was determined spectrophotometrically in this laboratory.

In all cases, five to seven different standard solution concentrations were subjected to absorbance determination.

Results and Discussion

The measured absorbance values of the aqueous solutions of the three studied drugs always showed good behaviour as indicated by the Lambert–Beer law. Common linear least-squares fits were used to determine the molar absorptivities at 1 nm intervals. These results are graphically presented as dots in Figs 2–4 for Diazepam, Flurazepam and Prazepam, respectively. The calculated uncertainties range within 2–5% of the represented value. These molar absorptivities were used as input spectral intensities in the forthcoming deconvolution analysis.

An absorption band can be described by approximation formulae such as Lorentz, Cauchy or Gauss distribution functions. However, Gaussian line shapes are the most suitable to represent electronic absorption bands in solution [12, 13]. A convenient mathematical form to express them is as follows:

$$G(\nu) = G_o(\nu_o) \exp\left[-\ln 2 \frac{(\nu - \nu_o)^2}{\delta^2}\right], \tag{1}$$

where frequency (energy), ν, rather than wavelength is used. G_o is the absorption maximum at the central frequency, ν_o, and δ is the half-width, which means the frequency half-amplitude of the band at the point where absorption is half of the maximum. According to this representation, the whole spectrum contour may then be given by the sum of a number, N, of Gaussians of the type of equation (1):

$$\varepsilon(\nu)_{calc} = \sum^{N} G_i(\nu). \tag{2}$$

It has been made evident from previous deconvolution studies that numerical decomposition of any spectral contour is not necessarily unique [14, 15]. The most important source of error arises from the inaccurate determination of the number of bands within the spectrum envelope. As the number of component bands increases, the

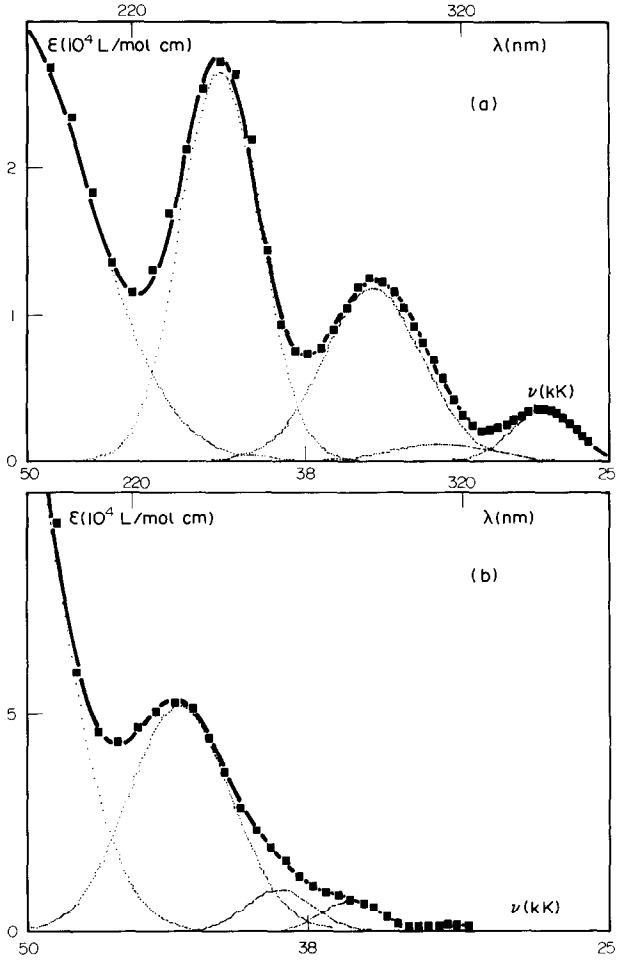


Figure 2
Deconvolution of UV absorption spectrum of aqueous Diazepam: (a) in acid solution, (b) in alkaline solution. Experimental molar absorptivities are represented at 4 nm intervals for clarity. Dotted lines are the Gaussian component bands, and the full line is the calculated whole spectrum contour.

possibility of ambiguous solutions increases as well. Therefore, the sum of a minimum number of Gaussians that can reproduce the spectrum line shape within the error margins, has to be taken as the most adequate. A way to find this minimum number is supplied by simple inspection of the spectrum first and second derivatives. The maxima of the fundamental curve correspond to passages through zero in derivatives of odd order, and in derivatives of even order they correspond to minima. The location and number of minima and inflections in the second derivative can be taken as a first estimate of the deconvolution bands [16].

In the present study, an algorithm has been developed that should be able to produce a meaningful decomposed spectrum, taking as initial parameters the band central frequencies as indicated by the second derivative of the spectra. The curve-resolving

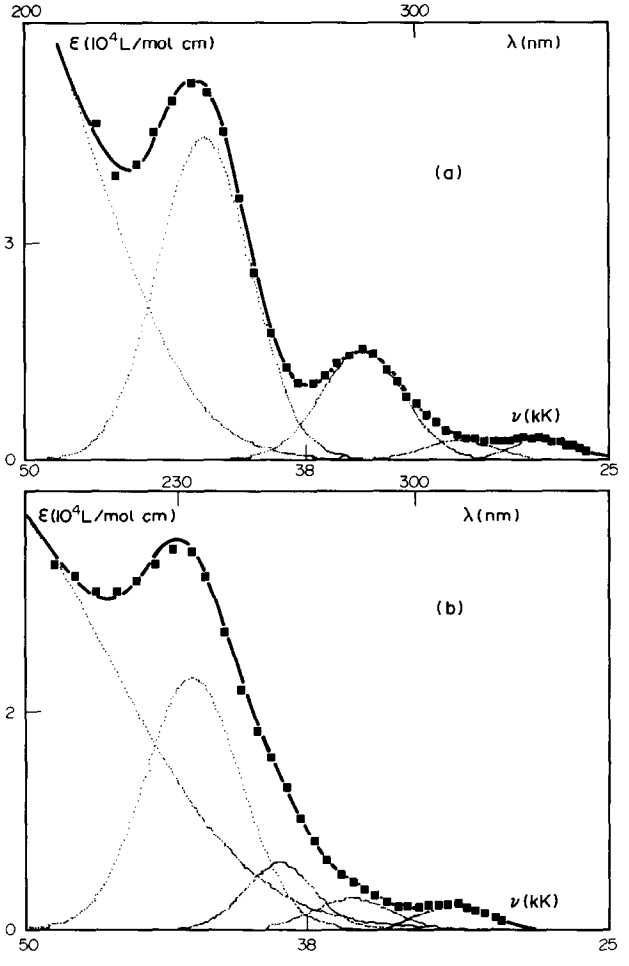


Figure 3
Deconvolution of UV absorption spectrum of aqueous Flurazepam: (a) in acid solution, (b) in alkaline solution. Experimental molar absorptivities are represented at 4 nm intervals for clarity. Dotted lines are the Gaussian component bands, and the full line is the calculated whole spectrum contour.

operation relies on a two-step procedure. In the first step, the computer operates in a conversational mode [15], as the user has complete control of the fitting process. A first approximated solution of the problem can be obtained by sequential modification of the bands parameters as to give the best possible fit. The goodness of the fit is followed using the root mean square (RMS) deviation as indicator. Also, the experimental versus calculated spectra are displayed. Secondly, the completion of the deconvolution process is accomplished by the use of a least-squares routine, based on an iterative grid-search procedure, centered on the previously obtained parameters [17], as to meet the following condition:

$$\sum(\varepsilon(\nu)_{\text{calc}} - \varepsilon(\nu)_{\text{exp}})^2 = \text{minimum.} \tag{3}$$

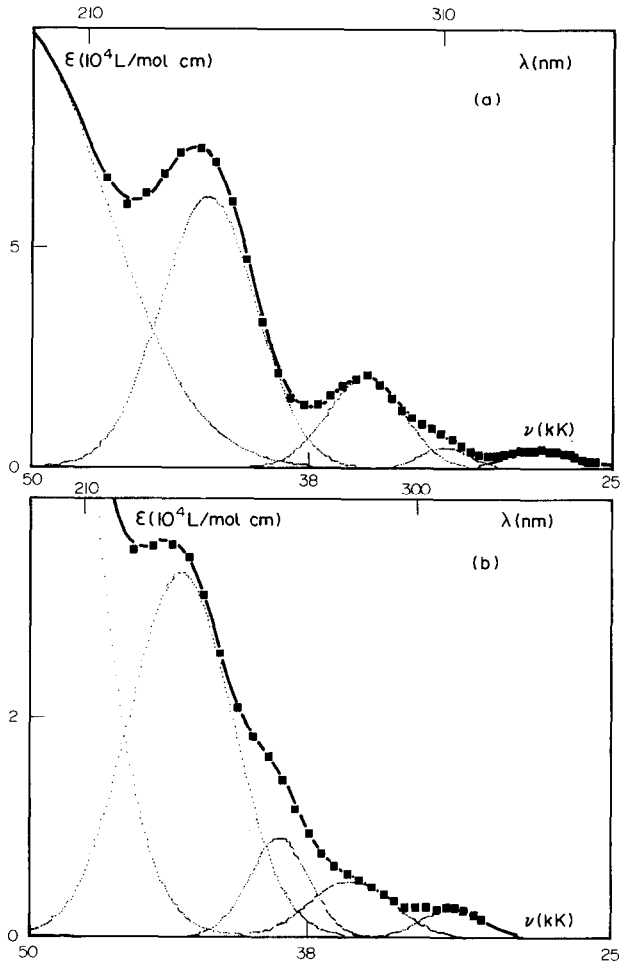


Figure 4
Deconvolution of UV absorption spectrum of aqueous Prazepam: (a) in acid solution, (b) in alkaline solution. Experimental molar absorptivities are represented at 4 nm intervals for clarity. Dotted lines are the Gaussian component bands, and the full line is the calculated whole spectrum contour.

The fitting procedure described above, though more crude and elemental than other powerful operator-independent methods [18], may present the advantage of requiring just a relatively small computer to resolve, at least, up to five bands within a complex spectrum profile.

Results for the resolved spectra are shown along with the experimental data in Figs 2–4. Tables 1–3 present the deconvolution parameters for UV absorption spectra of Diazepam, Flurazepam and Prazepam in acid and alkaline aqueous solutions, respectively.

The short wavelength Gaussian tails, centered around 195 nm have not any significance, and they were introduced only to obtain an adequate fit to the absorption data below 230 nm. In all cases, the presence of the three more intense benzenoid bands

Table 1
Gaussian parameters for deconvolution of UV absorption spectra of aqueous Diazepam

Acid solution			Alkaline solution		
ν_o(kK)(λ_o(nm))	G_o(l mol^{-1} cm^{-1})	δ(kK)	ν_o(kK)(λ_o(nm))	G_o(l mol^{-1} cm^{-1})	δ(kK)
50.8 (197)	29,900	4.08	51.5 (194)	150,000	3.08
41.6 (240)	26,500	2.00	43.5 (230)	51,500	2.58
35.1 (285)	11,800	2.50	39.2 (255)	9500	1.67
32.3 (310)	1100	2.41	36.1 (277)	7000	1.50
27.8 (360)	3600	1.58	31.7 (315)	1200	1.00
% RMS: 4.5*			% RMS: 9.7		

* Percent root mean square deviation.

Table 2
Gaussian parameters for deconvolution of UV absorption spectra of aqueous Flurazepam

Acid solution			Alkaline solution		
ν_o(kK)(λ_o(nm))	G_o(l mol^{-1} cm^{-1})	δ(kK)	ν_o(kK)(λ_o(nm))	G_o(l mol^{-1} cm^{-1})	δ(kK)
50.7 (197)	64,600	4.75	52.1 (192)	40,000	6.83
42.4 (236)	44,200	2.33	42.9 (233)	23,000	2.41
35.6 (281)	14,900	2.08	39.2 (255)	6000	1.75
31.4 (318)	2600	1.75	36.1 (277)	2800	1.91
27.8 (360)	3100	1.58	31.7 (315)	2200	1.58
% RMS: 9.4			% RMS: 3.0		

Table 3
Gaussian parameters for deconvolution of UV absorption spectra of aqueous Prazepam

Acid solution			Alkaline solution		
ν_o(kK)(λ_o(nm))	G_o(l mol^{-1} cm^{-1})	δ(kK)	ν_o(kK)(λ_o(nm))	G_o(l mol^{-1} cm^{-1})	δ(kK)
50.8 (197)	100,000	4.65	51.5 (194)	140,000	3.35
42.4 (236)	61,500	2.42	43.5 (230)	33,100	2.60
35.6 (281)	21,000	1.91	39.2 (255)	9000	1.50
32.1 (311)	4800	1.25	36.1 (277)	5100	2.10
27.8 (360)	4500	1.85	31.7 (315)	2600	1.50
% RMS: 9.0			% RMS: 4.1		

is evident. The two other less energetic bands can be assigned to electronic transitions in the C=N group, as the protonation of the N atom in the ionized species lowers the spectral frequencies.

However, band central frequencies and half-widths do not serve for structural characterization of these drugs. In any case, a rigorous study of the spectroscopic transitions occurring in these systems cannot be undertaken without an elaborate quantum chemical description of the molecules.

The analytical utility of the deconvolution methodology can be made more evident if the results are considered in closed form, i.e. the whole spectrum contour may be written as a linear combination of the component bands in the following way:

$$\varepsilon(\nu) = \sum_{i=1}^{5} G_i(\nu) \exp\left[-\ln 2 \frac{(\nu - \nu_i)^2}{\delta_i^2}\right], \tag{4}$$

where the symbols have the same meaning as in equation (1). This expression for the molar absorptivities of the aqueous acid and alkaline solutions of Diazepam, Flurazepam and Prazepam, according to the parameter values stated in the Tables, seems to achieve a more convenient and concise way to refer to important qualitative and quantitative analytical and spectrophotometric properties of these drugs.

Acknowledgements: The authors express their appreciation to Laboratories Prodes, Laboratorios Substancia-Park Davis, Barcelona, Spain, and to Laboratorios Roche, Madrid, Spain for their generous gifts of samples.

References

[1] P. A. Jansson, R. H. Hunt and E. K. Plyler, *J. Opt. Soc. Am.* **60**, 596–599 (1970).
[2] W. E. Blass and G. W. Halsey, *Deconvolution of Absorption Spectra*. Academic Press, New York (1981).
[3] H. F. Shurvell and J. T. Bulmer, *Vib. Spectra Struct.* **6**, 91–172 (1977).
[4] R. N. Jones, *Pure Appl. Chem.* **18**, 303–321 (1969).
[5] B. E. Barker and M. F. Fox, *Chem. Soc. Rev.* **9**, 143–184 (1980).
[6] W. F. Maddams, *Appl. Spectrosc.* **34**, 245–267 (1980).
[7] E. R. Menzel, W. R. Vincent, D. K. Johnson, E. L. Seebach and J. R. Wasson, *Inorg. Chem.* **13**, 2465–2473 (1974).
[8] H. W. Maulding, J. P. Nazareno, J. E. Pearson and A. F. Michaelis, *J. Pharm. Sci.* **64**, 278–284 (1975).
[9] E. Roets and J. Hoogmartens, *J. Chromatogr.* **194**, 262–269 (1980).
[10] *Analytical Profiles of Drug Substances* (K. Florey, Ed.), Vol. 4. Academic Press, New York (1975).
[11] *Analytical Profiles of Drug Substances* (K. Florey, Ed.), Vol. 3. Academic Press, New York (1974).
[12] W. H. Flygare, *Molecular Structure and Dynamics*, Prentice-Hall, Englewood Cliffs, New Jersey (1978).
[13] H. S. Gold, C. E. Rechsteiner and R. P. Buck, *Anal. Chem.* **48**, 1540–1546 (1976).
[14] J. W. Perram, *J. Chem. Phys.* **49**, 4245–4246 (1968).
[15] A. R. Davis, D. E. Irish, R. B. Roder and A. J. Weerheim, *Appl. Spectrosc.* **26**, 384–389 (1972).
[16] G. Talsky, L. Mayring and H. Kreuzer, *Angew. Chem. Int. Ed. Engl.* **17**, 785–799 (1978).
[17] P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York (1969).
[18] B. E. Barker, *Chem. Soc. Rev.* **9**, 143–184 (1980).

[*First received for review 15 August 1985; revised manuscript received 5 February 1986*]