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Some Pharmaceutical Applications of Diode Array Spectrophotometers

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SUMMARY

A diode array spectrophotometer with a built-in computer has been used to determine pKas, complexation constants and the content uniformity of tablets containing two drugs. The built-in computation facilitates the acquisition of derivative spectra and the averaging of data obtained at a range of wavelengths. This enables assays of two component mixtures to be accomplished in short periods of time even when one component is present in a large excess.

INTRODUCTION

The introduction of commercial diode array spectrophotometers equipped with sophisticated computation may greatly facilitate the analysis of drugs in dosage forms and the determination of important thermodynamic constants of the drug molecules. The importance of such spectrophotometers in high pressure liquid chromatography has already been well investigated (1). The possibilities of recording the spectrum quickly, computing and averaging the data over a chosen wavelength range offer significant advantages compared with

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conventional spectrophotometer techniques. These preliminary investigations involving measurements of equilibrium constants and the determination of two components mixtures show some of the advantages and limitations of these types of instruments in pharmaceutical research.

EXPERIMENTAL

Materials

Ibuprofen was a gift of Boots Pharmaceuticals Inc., Shreveport, LA. The p-nitrophenol was obtained from Fisher Scientific Co., Fair Lawn, N.J. and recrystallised from acidified water and dried over P_2O_5 *in vacuo*. The α-cyclodextrin was obtained from Nihon Shokukin Kako Co. Ltd., Tokyo, Japan. The acetaminophen and propoxyphene napsylate were U.S.P. standards. The aspirin was obtained from Eastman Kodak Co., Rochester, N.Y. and the codeine phosphate U.S.P. from Merck and Co., Inc., Rahway, N.J. Darvocet N-100 (Eli Lilly Co., Indianapolis, IN) and Empirin with codeine No. 4 (Burroughs Wellcome Co., Research Triangle Park, N.C.) tablets were obtained from a local pharmacy. HPLC grade methanol was obtained from Fisher Scientific Company. All other materials were reagent grade and from various sources. Deionised water was used throughout.

EXPERIMENTAL

All measurements were made in 1 cm quartz cells using a Hewlett Packard 8451A diode array spectrophotometer and recorded on a Hewlett Packard X-Y plotter. Mathematical manipulations of the spectral data were performed using the integrated HP 85 microcomputer and associated software. All derivative spectra were obtained using three smoothing points. The spectral data were stored on 3½" discs using a dual disc drive (HP 7470A). All measurements were made a room temperature (22°C).

pKa Determinations (Ibuprofen)

An ibuprofen concentration of 8×10^{-5} M was used for the measurements in phosphate buffers of ionic strength 0.2. The unionised spectrum was obtained in 0.1 N HCl and the ionised spectrum in 0.01 N NaOH. The spectra were obtained between 210 and 280 nm. The analysis was preformed by the computer assuming a two component mixture.

Complex Formation (α-cyclodextrin, p-nitrophenol)

All measurements were made in a phosphate buffer of ionic strength 0.5 and pH 11.0. The spectrum of 5×10^{-5} M p-nitrophenol was recorded, alone and in the presence of concentrations of α-cyclodextrin from 5×10^{-4} to 1×10^{-2} M over the wavelength range 300-500 nm. The analysis was performed by the computer assuming a two component mixture.

Analysis of Darvocet N-100

Each tablet was washed with water to remove the film coat and dried with tissues. The tablets were then dried further at 60°C for 10 minutes. The crushed tablet was placed in a 100 ml volumetric flask and shaken with about 60 ml of methanol. After adjustment to volume the solution was centrifuged (2500 rpm). 1 ml supernatant was diluted to 100 ml with methanol, and a further 1 to 5 dilution was performed with more methanol. The spectrum was then recorded between 200 and 360 nm, and compared with that of 5.08 µg/ml of propoxyphene napsylate and 10.22 µg/ml of acetaminophen (both in methanol) recorded over the same wavelength range and stored in the memory of the HP 8451A. The data was analysed for a two component mixture with no derivative.

Analysis of Empirin with Codeine No. 4

The crushed tablet was placed in a 200 ml volumetric flask and then shaken with methanol. After sonication for 30 seconds, adjustment to volume and centrifugation, a 2 ml aliquot of supernatant was diluted with deionised water and acidified with hydrochloric acid (pH 2.5) and adjusted to 100 ml. The spectrum was recorded from 220 to 300 nm and compared with that of 31.25 µg per ml codeine phosphate (in 2% methanol) recorded over the same wavelength range and stored in the memory of the HP8451A. The data was analysed for a two component mixture using the zeroth, first and second derivatives.

RESULTS AND DISCUSSION

pKa determination Ibuprofen has been reported to have pKas varying from 4.13 to 5.2 (2,3). The measurements were made in the diode array spectrophotometer in the classical manner, namely spectra were obtained of the unionised and ionised species and then of solutions of accurately known pH at a constant ionic strength of 0.2. Table 1 shows the apparent pKas determined by a) single

Table 1 pKa Determination of Ibuprofen by Hendersen-Hasselbalch Equation at 22°C and ionic strength 0.2.

pKa	nm	derivative	n
4.25 ± 0.03	226	0	12
4.31 ± 0.05	224-234	0	11
4.41 ± 0.02	224-234	1	12

wavelength determinations, b) by analysis of a two component mixture over a wavelength range of ten nanometers and c) by analysis of a two component mixture using the first derivative technique over the same wavelength range. The pKa values of 4.31 ± 0.05 at 22°C (Table 1) seems the most reliable because the data is averaged over the 10 nm wavelength range of maximum difference between the spectra of ionised and unionised forms. The derivative method gives a slighly higher result, which may be due to the increased signal to noise ratio associated with obtaining the derivative spectra (4), although the statistics associated with the measurements are excellent. In any case the literature value (3) of 5.2 seems to be erroneously high.

Drug-Cyclodextrin Complexes

Great interest has recently been afforded to drug-cyclodextrin complexes because of the increased bioavailability of poorly soluble drugs from these complexes (5). Poorly soluble drugs such as non-steroidal antiflammatories (6) and phenothiazines (7) complexes more strongly than most drugs with the cyclodextrins, however the binding constants are still only of the order of 10^3. This means that the spectrum of the fully bound complex is difficult to obtain because of the limited aqueous solubility of either the drug or cyclodextrin. This is illustrated by the data of table 2 which shows the interaction between α-cyclodextrin and p-nitrophenol, and assumes the complexation is complete when the cyclodextrin is in two hundred fold excess. The data of table 2, obtained using first derivatives to obtain concentrations of the 1:1 complex, does not give constant values of the binding constant. This is due

Table 2 Complexation of α-cyclodextrin with p-nitrophenol at 22°C and ionic strength 0.5. The p-nitrophenol concentration is constant and 5×10^{-5} M. The data is obtained by first derivative, two component analysis assuming 1:1 complexation. K^1 uses data over the wavelength range 340-360 nm and K^2 over the range 400-420 nm.

α-CD	K^1	K^2
5×10^{-4} M	1.976×10^3 M^{-1}	1.843×10^3 M^{-1}
1×10^{-3} M	1.916×10^3 M^{-1}	1.990×10^3 M^{-1}
2×10^{-3} M	2.207×10^3 M^{-1}	2.170×10^3 M^{-1}
5×10^{-3} M	2.866×10^3 M^{-1}	2.707×10^3 M^{-1}

to either complex formation other than 1:1 or unsatisfactory reference spectra data due to incomplete complexation. This latter phenomenon can be checked by using the Benisi-Hildebrand (8) treatment (Figure 1) which eliminates the need for obtaining the spectra of the fully complexed p-nitrophenol (8,9). This approach satisfies the equation for a 1:1 equilibrium and gives an equilibrium constant of 1.77×10^3 M^{-1} for p-nitrophenolate ion - α-cyclodextrin complex. This compare with values of 1.94×10^3 M^{-1} (10) reported in the literature. The data shown is obtained by single wavelength determination (354 nm). The advantages of the diode array spectrophotometer for these determinations is in speed of measurement and hence rapid selection of the wavelength of measurement, but a conventional spectrophotometer at the current stage of development will give more accurate absorbances at the single wavelength and so is preferable for this type of measurement.

Two component Mixtures

The integral computation as well as the most instantaneous acquisition of complete UV spectra make diode array spectrophotometers attractive for the analysis of multicomponent mixtures in dosage forms. In the present report two component mixtures are investigated in which all drugs satisifed the Beer Lambert Law over the concentration range of the investigations. The first,

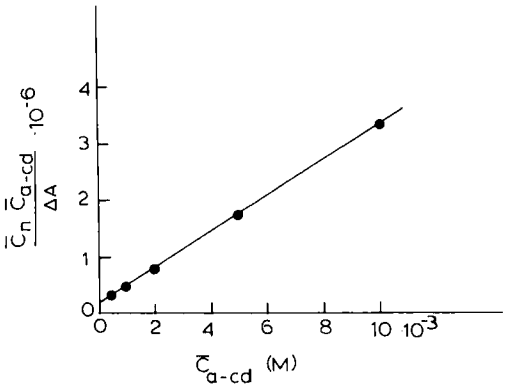

Figure 1 - Benesi-Hildebrand plot (8) for the α-cyclodextrin - p-nitrophenolate ion interaction. The equilibrium constant of the complex (K_D) is given by

$$\frac{C_N \, C_{\alpha\text{-CD}}}{\Delta A} = \frac{1}{K\Delta\varepsilon} + \frac{C\alpha\text{-CD}}{\Delta\varepsilon}$$

$\bar{C}_N$ and $\bar{C}_{\alpha\,CD}$ are the total concentrations of drug and cyclodextrin respectively. $\Delta\varepsilon$ is the difference in molar extinction coefficients for free and bound nitrophenol and ΔA is the change in absorbance of nitrophenol upon the addition of cyclodextrin.

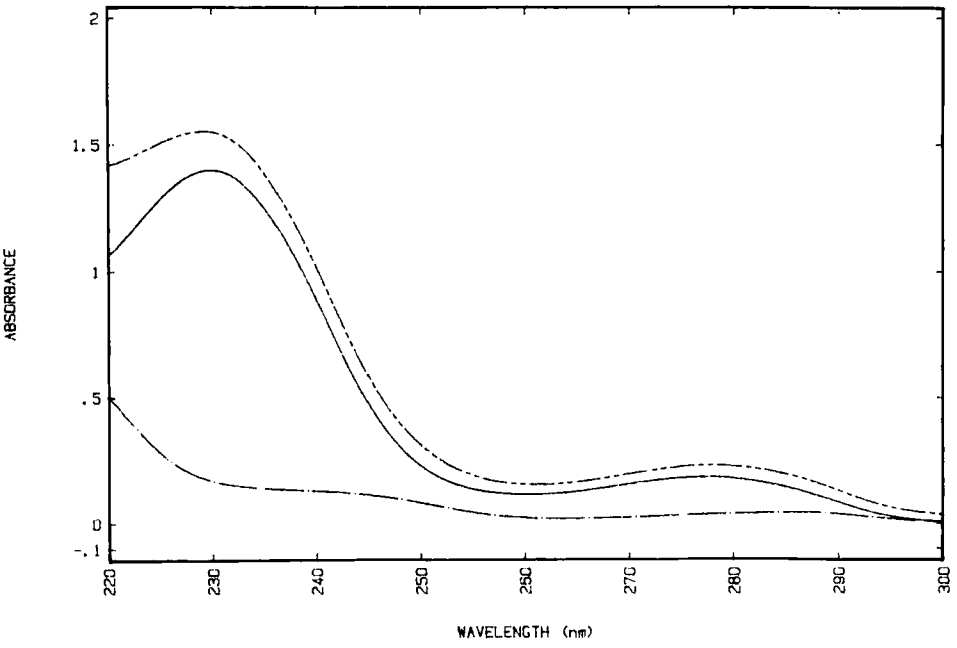

Figure 2 - UV spectra of solutions of aspirin (——), codeine ——— . ——— and, Empirin with Codeine No. 4 (—— - - ——) concentrations as in the text.

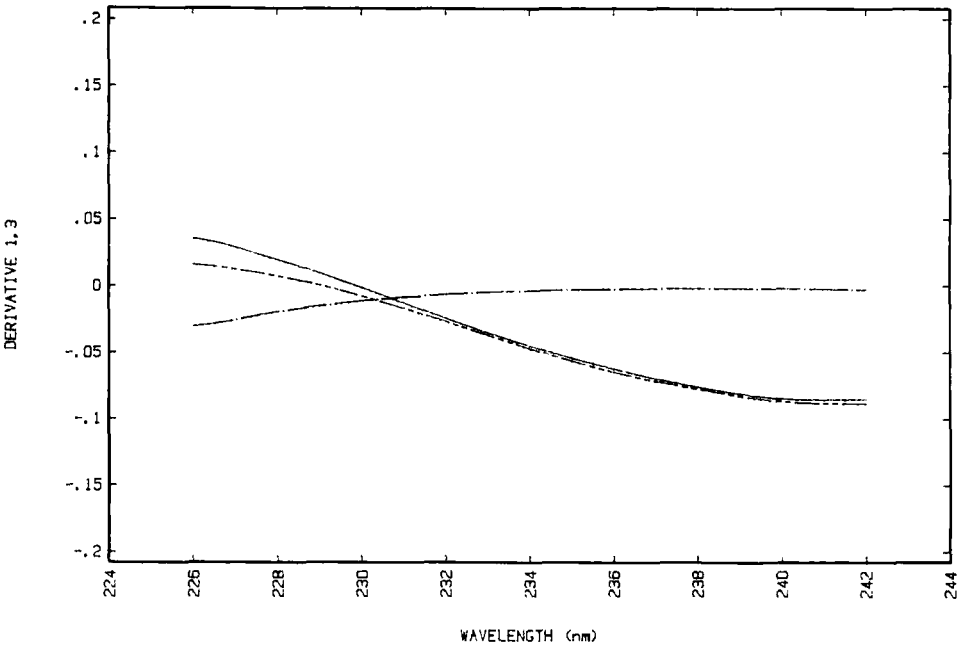

Figure 3 - First derivative spectra with three smoothing points (derivative 1, 3) of the aspirin, codeine and Empirin with Codeine No. 4 solutions of figure 2.

Table 3 Acetaminophen (APAP) and Propoxyphene napsylate (PN) contents of Darvocet N-100 tablets determined by conventional two components analysis over the wavelength range 200-360 nm.

Tablet	APAP (mg)	PN (mg)
1	664.72	99.18
2	667.79	97.57
3	696.45	107.90
4	680.51	103.58
5	675.08	102.79
6	667.82	101.82
7	675.84	104.52
8	665.68	103.13
9	669.83	103.75
10	671.39	104.56
MEAN	673.51	102.88
	(103.61%)	(102.88%)
S.D.	± 9.46	± 2.89

Table 4 Aspirin and Codeine content of Empirin with Codeine No. 4 tablets determined over the wavelength ragne A) 220-240 nm and B) 224-244 nm using zeroth, first and second derivative.

A)

	Zeroeth Derivative		First Derivative		Second Derivative	
Tablet	Aspirin (mg)	Codeine (mg)	Aspirin (mg)	Codeine (mg)	Aspirin (mg)	Codeine (mg)
1	325.6	62.3	313.5	61.8	320.9	69.0
2	340.7	63.4	323.7	63.6	329.8	69.5
3	334.0	63.2	320.8	62.8	325.3	67.2
4	337.5	62.7	321.0	63.2	325.4	66.5
5	337.2	62.7	321.5	63.0	325.3	65.9
6	337.3	62.3	319.8	62.9	325.5	68.2
7	334.7	61.9	322.4	61.6	331.3	69.4
8	338.4	63.3	326.0	63.2	331.7	68.7
9	337.1	62.3	320.7	62.6	323.3	64.4
10	342.1	62.8	328.0	63.9	330.6	66.5
MEAN	336.5	62.7	321.7	62.9	326.9	67.5
	(103.5%)	(104.5%)	(99.0%)	(104.8%)	(100.6%)	(112.5%)
S.D.	± 4.51	± 0.50	± 3.88	± 0.72	± 3.69	± 1.7

B)

1		316.9	86.2	316.5	63.0	328.1	84.6
2		329.6	94.0	329.3	64.0	341.8	87.6
3		324.3	89.5	325.0	63.1	333.0	77.1
4		328.0	89.4	325.3	64.3	337.6	86.6
5		330.5	84.1	325.7	63.1	333.5	77.8
6		327.9	89.3	324.8	61.6	332.8	75.5
7		327.1	83.9	325.8	62.1	336.5	81.0
8		330.0	88.0	329.0	62.4	337.4	78.4
9		331.1	82.0	324.6	64.1	333.9	79.8
10		332.2	89.6	331.7	64.6	342.6	86.2
	MEAN	327.8	87.6	325.8	63.2	335.7	81.5
		(100.9%)	(146%)	(100.2%)	(105.3%)	(103.3%)	(135.8%)
	S.D.	± 4.43	± 3.56	± 4.08	± 1.00	± 4.39	± 4.43

Darvocet N-100 (propoxyphene napsylate 100 mg, acetaminophen 650 mg) is a rather simple one to assay because of the propoxyphene napsylate spectrum is sufficiently different from that of acetaminophen therefore the two components can be assayed by the classical two wavelength method. However, the HP8451A offers significant advantages such as rapid determinations plus the ability to average the data from absorbances obtained over a wide wavelength range. In this work the spectra were obtained over the spectral range of 200-360 nm, and the data analysed for a two component mixture over this entire range. The data shown in table 3 shows that the commercial tablets satisfied the U.S.P. content uniformity test (11) and the analytical method should be readily adaptable, using a flow cell to dissolution tests. A more complicated mixture is Empirin with Codeine #4 (aspirin 325 mg, codeine 60 mg). The drugs are initially extracted with methanol and then an aliquot diluted with water acidified to pH 2.5, a pH near that of maximum stability of aspirin (12). The assay of ten tablets, from the initial extraction to the last spectra measurements can be made in 60-90 minutes. Little, or not deterioration of the aspirin is obtained in this time interval at pH 2.5 (12). Figures 2 and 3 show zeroth and first derivative spectra of the components. Table 3 shows data obtained using, no derivative, first derivative and second derivative over two wavelength ranges. The classical method for two component mixtures using no derivatives gives a satisfactory answer over the wavelength range 220-240 nm but not over the range 224-244 nm. This illustrates that considerable attention to detail is needed in chosing the wavelength range of investigation when overlapping spectra are involved and/or one of the components is present in a large excess. It is unlikely that the non-derivative method could be used for products containing less codeine. Using the first derivative measurement and the equation below for a two component mixture and averaging over the two wavelength ranges consistently, gave good results.

$$\frac{dA}{d\lambda} = \frac{d(\varepsilon_1^{\lambda})C_1}{d\lambda} + \frac{d(\varepsilon_2^{\lambda})C_2}{d\lambda}$$

The second derivative method gave erratic results depending upon the chosen wavelength range. In these laboratories, it has been consistently found in

two component analysis that the first derivative approach gives the best information if one of the components is in large excess, provided that in addition there is significant spectral overlap. Higher order derivatives increase resolution and specificity of spectra but increased signal to noise ratio may hinder quantitative analysis. Quantitative measurements should be made at wavelengths where there is the biggest difference between the non-zero values of the first derivatives of the two components, (figure 3) this frequently means that the $\frac{d\epsilon}{d\lambda}$ values are of opposite sign.

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