RESEARCH PAPERS

THE INFLUENCE OF SPECTRAL SLIT WIDTH ON THE ABSORPTION OF VISIBLE OR ULTRA-VIOLET LIGHT BY PHARMACOPOEIAL SUBSTANCES

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The importance of narrow slits in the spectrophotometric determination of extinction coefficients has been demonstrated. Care is needed in the B.P. 1958 spectrophotometric tests or assays of apomorphine hydrochloride, chloroquine phosphate, chloroquine sulphate, diiodohydroxyquinoline, naphazoline nitrate, papaverine hydrochloride and especially procyclidine hydrochloride, to avoid spuriously low results.

WHEN a spectrometer is set to transmit radiation of a certain wavelength, λ, it will actually transmit a wavelength band of finite width. The resolving power of the spectrometer may be expressed in terms of the "half-intensity spectral slit width" h, which is the range of wavelengths over which the intensity of the energy reaching the sample is at least one-half of the intensity at the nominal wavelength setting λ of the monochromator (see Fig. 1). The half-intensity slit width is usually slightly less than half the range of wavelengths transmitted by the monochromator, because aberrations such as diffraction at the edges of the slits permit additional stray light to pass. The width h depends upon the widths of the entrance and exit slits. In grating instruments, it is essentially independent of wavelength; in prism instruments, it increases as the wavelength increases.

It is well known that extinction coefficients in the infra-red vary with the resolving power of the spectrophotometer and that the extent of the variation is greater for the narrower absorption bands[1]. This difficulty has hindered the application of infra-red spectroscopy to quantitative analysis. A method of correcting observed extinction coefficients to "infinite resolving power" has been suggested[2]; such a correction allows results obtained with one instrument to be used in conjunction with measurements on another.

The problem is of less significance in the visible and ultra-violet regions of the spectrum because here the range of wavelengths emerging from the exit slit of the monochromator at a given nominal wavelength setting is in general much smaller in comparison with the width of the absorption bands. However, Hogness, Zscheile and Sidwell[3] and Eberhardt[4] have shown that the effect of change of slit width on the apparent extinction coefficient of such solutes as oxyhaemoglobin and benzene may be considerable. West[5] states that "the possibility of inadequate resolution makes it advisable to include a statement of the spectral slit width in published reports on extinction coefficients along with the other data normally considered relevant to the estimate, such as solvent, temperature,

concentration and cell thickness". At least one manufacturer of spectrophotometers advises analysts to use the narrowest possible slit widths when very reliable extinction estimates are required, and to check that change of slit width at the setting employed is without effect on the observed extinction[6].

The effect of change of half-intensity slit width h on the observed extinction will depend upon (*a*) the shape of the absorption curve of the sample within the waveband being transmitted[7], (*b*) the variation of sensitivity of the photocell with respect to wavelength, and (*c*) the variation of the sensitivity from one part to another of the photosensitive cathode surface. Factor (*b*) is likely to be important only with very wide slits. Factor (*c*) is unlikely to be important unless the slit is not only narrow but also short in height, so that the total area of illuminated photoreceptor surface is very small.

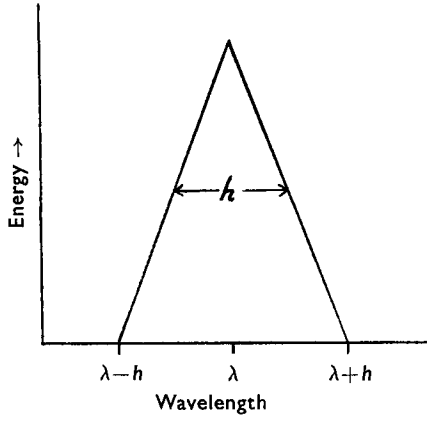

FIG. 1. Idealised energy distribution in the light beam emerging from a monochromator. λ, nominal wavelength setting; h, half-intensity spectral slit width.

It is common for an analyst working in the visible or ultraviolet region of the spectrum to assume that the slit width recommended by the manufacturer is narrow enough for the radiation incident to the sample to be regarded as "monochromatic". Because there is no published survey of the importance of this particular stray-light effect in relation to the absorption of light by pharmaceutical substances, measurements of extinctions of solutions of those substances which are subject to tests of light absorption in the British Pharmacopoeia, 1958, have been made at a variety of half-intensity spectral slit widths, and the results are presented and discussed below.

EXPERIMENTAL

Apparatus. The spectrophotometers were a Beckman model DU, fitted with a photomultiplier; a Unicam SP.500, fitted with a photomultiplier according to the method of Kendall and Smethem[8] by Mr. R. V. Swann and Mr. H. Clements of the Physical Chemistry Laboratory, Allen and Hanburys Ltd.; and a Hilger and Watts Uvispek H.700 Mark VII. The instruments were equipped with quartz prisms, and in each the entrance and exit slits were ganged and equal. The absorption cuvettes were of silica, in matched pairs of optical path length 1 cm. or 0·5 cm. The wavelength calibration was checked daily by means of the hydrogen emission lines at 434·0 mμ, 486·1 mμ and 656·3 mμ; the error was never greater than 0·5 mμ at any of these three wavelengths.

Material. Drugs had been supplied as being of B.P. quality. The solvents complied with the requirement of Appendix IV H of the British Pharmacopoeia, 1958, that in every case "the extinction of the solvent cell and contents shall . . . be less than 0·2, when measured with reference to air at the same wavelength".

Measurements. For each drug, a solution of the approximate concentration specified, within ±10 per cent, was prepared as directed in the appropriate monograph of the B.P., and the extinction was determined at the stated wavelength or wavelengths. Without removal of the solution

TABLE I

THE EFFECT OF CHANGE OF SPECTRAL SLIT WIDTH ON THE SPECTROPHOTOMETRIC DETERMINATION OF EXTINCTION. SOLUTIONS PREPARED AS DIRECTED BY THE B.P. 1958

Substance	λmax. (mμ)	Max. *h*(mμ) for extinction error of < 0·2 per cent	1 per cent	2 per cent	Substance	λmax. (mμ)	Max. *h*(mμ) for extinction error of < 0·2 per cent	1 per cent	2 per cent
Acetazolamide	265	1·9			Mephenesin	270	0·9	1·4	1·8
Adrenaline	280	1·2	1·9	2·5	Methyltestosterone	240	1·3		
Amodiaquine hydrochloride	343	1·7	2·5	3·4	Morphine hydrochloride	285	1·2	1·9	2·5
Antazoline hydrochloride	241	1·4			Nalorphine hydrobromide	285	1·2	1·9	2·5
Apomorphine hydrochloride	273	0·8	1·6		Naphazoline nitrate	280	0·6	1·0	1·2
Azovan blue	612	2·4			Noradrenaline acid tartrate	279	1·2	1·8	2·5
Calciferol	265	1·8			Oxytetracycline dihydrate	353	3·0		
Carbimazole	291	4·0			Papaverine hydrochloride	251	0·8	1·5	
Chloramphenicol	278	2·3			Phenindamine tartrate	259	1·0		
Chlorcyclizine hydrochloride	230	1·1			Phenoxymethylpenicillin	268*	0·9	1·6	
Chloroquine sulphate	343	0·7	1·1	1·3		274			
Chlorpromazine hydrochloride	254	1·2			Phenylephrine hydrochloride	272	1·0	1·6	
Codeine phosphate	284	1·4	2·0		Prednisolone	242	1·2		
Colchicine	350	1·7	4·0		Prednisone	240	1·2		
Cortisone acetate	238	1·2			Procainamide hydrochloride	280	1·6		
Cyanocobalamin	278	1·1	2·2		Procyclidine hydrochloride	257	0·3	0·5	0·7
	361	1·5	1·8	2·3		263·5	0·3	0·4	0·5
	550	1·5			Progesterone	240	1·2		
Deoxycortone acetate	240	1·0			Pyrimethamine	260†	1·0		
Diiodohydroxyquinoline	258	0·7	1·5			272	1·5		
Ethinyloestradiol	281	1·0	1·8	2·4	Reserpine	268‡	2·0		
Ethisterone	240	1·2				268§	2·0		
Folic acid	256	1·8				295	2·8		
	283	2·4			Riboflavine	267	1·0	2·0	
	368	2·5				444	1·0		
Hydrocortisone	242	1·4			Solapsone	306	1·6		
Iopanoic acid	230	1·1			Testosterone propionate	240	1·2		
Isoprenaline sulphate	279	1·2	2·4		Thyroxine sodium	325	1·4	3·5	
					Tubocurarine chloride	280	1·2	1·7	2·2

* E_{268}/E_{274} measured.
† λmin.
‡ Solvent chloroform.
§ Solvent 95 per cent ethanol.

from the cuvette, extinction readings were taken at several slit-width settings and repeated in turn at least once. This procedure increased the precision of the measurements, and served as a check that no change of extinction with time was occuring, due for example to photodecomposition of the sample. With all drugs, extinction measurements were made on two of the spectrophotometers, and with some drugs all three instruments were used.

The range of slit widths which could be used was limited at one extreme by the inability to compensate electronically for high intensity of transmitted radiation with the solvent in the light path, and at the other extreme by the loss of precision of measurement due to the low sensitivity of the photocell or photomultiplier at low intensities of radiation.

RESULTS

At very narrow slit widths, the measured extinction was in every instance independent of the slit width. At very wide slit widths—wider than would commonly be used—almost every solution showed a change of observed extinction with change of slit width. At intermediate slit settings, the change of extinction was significant with about a dozen of the solutions examined.

In order to place the results obtained with the different instruments on the same basis, use was made of the dispersion graphs published by the respective manufacturers to convert the apparent or nominal slit width (in mm.) into the corresponding half-intensity spectral slit width h (in mμ). This procedure is not an exact one, because correction for optical aberrations may not be accurate, or may not be made at all, and because the nominal slit width read from the instrument dials may not be an accurate estimate of the actual distance between the jaws.

Table I lists the drugs examined and shows in column 3 the widest slit width h which may safely be used; the slit width for 1 per cent error (column 4); and the slit width for 2 per cent error (column 5). The slit settings quoted in column 3 are the greatest at which no change of extinction could be detected with certainty. As a guide to the magnitude of these slit widths, it may be noted that the Uvispek spectrophotometer is commonly operated in the ultraviolet region of the spectrum at slit widths corresponding to $h = 0{\cdot}5$ mμ. The spectral slit widths tabulated are average values which have been rounded-off, and may be in error by as much as ± 20 per cent.

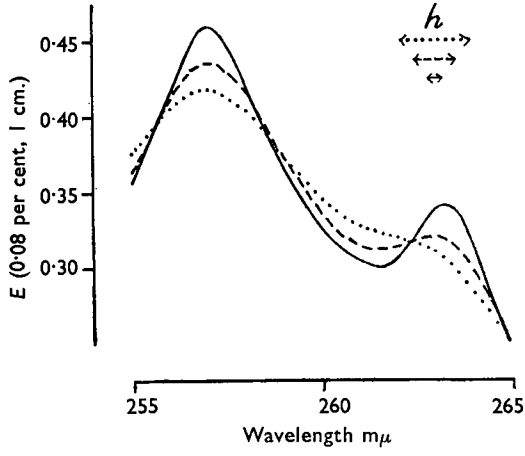

FIG. 2. Absorption spectra of an aqueous solution of procyclidine hydrochloride, and values of h at 263 mμ, obtained with a Uvispek spectrophotometer at nominal slit settings of ———, 0·4 mm.; – – – –, 1·2 mm.; . . . . ., 2·0 mm.

Fish-liver oils and other solutions containing vitamin A have not been included in the Table, because the results would depend upon the nature and amount of the "irrelevant absorption", which would differ from sample to sample. It is unlikely that the slit-width effect would be important in the spectrophotometric assay of vitamin A at values of h less than 1 mμ.

DISCUSSION

In Figure 2 are plotted parts of the absorption spectrum of a 0·08 per cent aqueous solution of procyclidine hydrochloride obtained with the

Uvispek at nominal slit settings of 0·4, 1·2 and 2·0 mm., respectively. The graphs show clearly that opening the slits so as to increase h, decreases the observed extinctions at the maxima and increases the observed extinction at the minimum. The wavelength of maximum absorption for the unsymmetrical band also shifts as h is increased, and indeed at the widest slit setting the 263·25 mμ maximum appears merely as an inflexion in the curve. Values of h corresponding to the three nominal slit widths are also included in the Figure, drawn to the same wavelength scale as for the spectrum; since the relation between h and the nominal slit depends upon the nominal wavelength setting, the values are drawn at a single nominal wavelength only, namely 263 mμ.

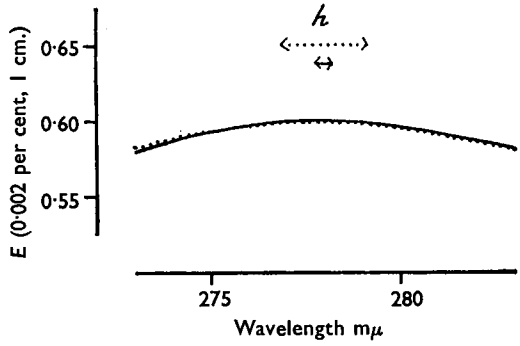

FIG. 3. Absorption spectra of an aqueous solution of chloramphenicol, and values of h at 278 mμ, obtained with a Uvispek spectrophotometer at nominal slit settings of ———, 0·4 mm.; . . . . . 2·0 mm.

Similarly obtained graphs of the absorption spectrum of a 0·002 per cent aqueous solution of chloramphenicol are plotted in Figure 3. Here the absorption peak is so broad and rounded that change of h has no obvious effect on the extinction values. As has been pointed out by Hyde[7], it is the rate of change of the slope of the absorption curve with respect to wavelength, that is $d^2E/d\lambda^2$, that determines the size of the slit-width effect. The sharper the peak or hollow in the adsorption, the greater is the magnitude of $d^2E/d\lambda^2$. The sign of $d^2E/d\lambda^2$ is negative at a maximum and positive at a minimum. If $d^2E/d\lambda^2$ is positive, there is an increase in observed extinction as h is increased (for example, pyrimethamine at 260 mμ); if $d^2E/d\lambda^2$ is negative, there is a decrease.

It seems probable from examination of Table I that analysts are unlikely to use slits wide enough to cause appreciable errors in the spectrophotometric characterisation or assay of B.P. drugs in many cases. However, for a small number of drugs, there is a risk that the analyst would be loath to narrow the slits sufficiently to avoid the stray-light effect discussed in this paper because by so doing the amount of radiant energy reaching the photocell might be too small for adequate precision. This risk will diminish as the number of spectrophotometers which are fitted with photomultipliers increases, and if high-resolution monochromators come into general use for routine pharmaceutical analysis.

Meanwhile, the British Pharmacopoeia Commission may wish to re-examine those tests and assays in the Pharmacopoeia which are based upon spectrophotometric measurements of the absorption of ultra-violet or visible light, and to consider whether any of the tests should be omitted

or amended to include a specification of the maximum half-intensity spectral band width to be tolerated at a stated wavelength.

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