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Ultraviolet Differential Spectrophotometry of Thiobarbiturates*

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A special class of barbiturates, the thiobarbiturates, are used as hypnotic agents in anaesthesiology,[1] in obstetrics,[2] and in experimental approaches to the problems of placental physiology.[3] Ultraviolet differential spectrophotometry, an analytical technique which has been used extensively in our laboratories for the past several years for barbiturates, salicylates, or mixtures of the two,[4, 5] could considerably enhance the quantitative determination of thiobarbiturates. This procedure eliminates the use of reference blanks by using the technique of internal self-blanking. It obviates the need for the complete isolation and purification of the desired constituent by chromatography or some other means, since the spectrophotometrically interfering materials co-extracted from the specimen can only interfere if they show similar spectral shifts under the reaction conditions employed.

The present investigation is concerned with automatic recording spectrophotometry for the measurement of thiobarbitals such as thiamylal sodium and thiopental sodium, by virtue of the fact that a change in pH can cause a shift in their absorption spectra. Thus, a regulated decrease in pH for a solution of either compound can effect a hypsochromic shift in its absorption maximum by a change of the chemical state of the compound from the anionic form to the acidic form.[6]

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Method

Treatment of the Specimen

Pipette 3·0 ml of serum, plasma, standard or neutral solution of the pharmaceutical product to be analyzed into a separatory funnel, add 50 ml of chloroform (spectral grade or redistilled analytical reagent) and extract the mixture for 3–5 min. Allow the two layers to separate, and then filter the chloroform layer through Whatman 41-H filter paper into a dry separatory funnel. Next add 7 ml of 0·45 N sodium hydroxide and re-extract the barbiturate in the aqueous layer using an extraction time of 3–5 min. The chloroform layer is discarded and the aqueous phase is centrifuged at 3000 rev/min for several minutes. Three millilitre aliquots of the clear solution are pipetted into two cuvettes. To one cuvette is added 0·5 ml of sodium hydroxide, and 0·5 ml of 16 per cent ammonium chloride is added to the other. Both cuvettes are mixed and then subjected to differential spectrophotometry on a Beckman DK-2 spectrophotometer.

If there is insufficient sample for the determination, one can run the procedure as a micro-technique. That is, reduce the sample size to 0·5–1·0 ml, and extract with 30 ml of chloroform. A retrograde extraction is then carried out on the filtered chloroform with 1·5 ml of 0·45 N sodium hydroxide. A pyrocell silica cuvette measuring 3 mm × 10 mm × 50 mm is substituted for the standard cuvette and two 0·6-ml aliquots of the centrifuged aqueous phase are placed in the cuvettes. Then 0·1 ml of 0·45 N sodium hydroxide is added to one cuvette and 0·1 ml of 16 per cent ammonium hydroxide is added to the other. The well-mixed samples are then subjected to differential spectrophotometry. Standard solutions should be carried through the same macro or micro processes as the samples.

Instrumentation

The initial wavelength of the spectrophotometer at which recording is to begin is adjusted to 360 mμ at a sensitivity of 100 and the absorbance range is set on the differential setting −0·3 to +0·7. The aliquot of the barbiturate in sodium hydroxide to which ammonium chloride has been added is placed in the reference beam and the sodium hydroxide solution of the barbiturate

is placed in the sample beam. The pen is readjusted to 0·3 absorbance on the chart, which is now the zero line, the time is set for a 2-min record, and the ultraviolet differential spectrum is graphed from the initial setting to approximately 220 mμ. The absorbance differences obtained at 283 mμ are used for both the calibration of standards and the determination of samples.

A manual type of instrument could be substituted for the automatic recording spectrophotometer. The instrument would be zeroed at 0·3 absorbance and then the ammonium chloride–sodium hydroxide solution read in the blank position against the sodium hydroxide solution of the barbiturate in the sample position at several wavelengths from 360 mμ and including 283 mμ, in order to define the difference spectrum.

Discussion and Results

Fig. 1 shows the graphical results obtained for both thiamylal sodium and thiopental sodium in an experiment carried out

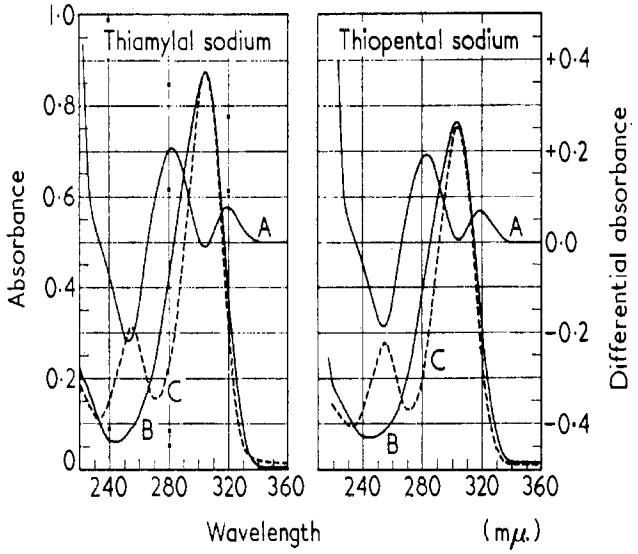

Fig. 1. Normal and differential spectra for thiamylal sodium (Surital®) and thiopental sodium (Pentothal®) at two different pH values.

under two different spectrophotometric conditions. Curve B for either compound represents the spectrum obtained at a pH which shows absorbance intensity for the anionic form of the thiobarbiturate, while the curves C represent the hypsochromic

shifts which take place when the pH is changed to the acid side of the pK value. This pH change causes an increase in the intensity at a lower wavelength, due to the presence of the acidic form of the barbiturate.[6] Manual subtraction of one spectrum from the other, as it is commonly performed,[7] is extremely difficult because the shift is not great and small measurement errors will be greatly magnified. However, if one graphs curve B *versus* curve C using the differential setting of an automatic recording

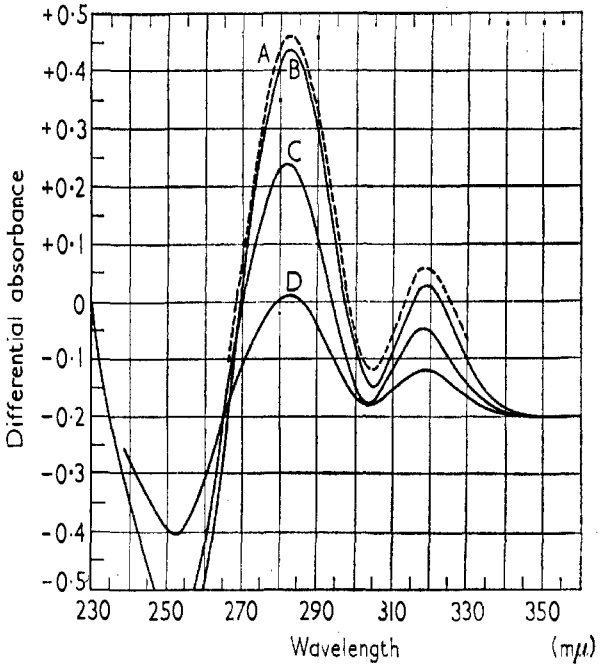

Fig. 2. Calibration spectra, curves B, C, and D; and superimposed serum extract spectrum for thiopental sodium (Pentothal®), curve A.

spectrophotometer, the ultraviolet differential spectrum shown as curve A is obtained. The practical effect involves a simple subtraction of one curve from the other. This is simplified and made more accurate than the manual procedure by continuous beam chopping to achieve a mechanical ratio for sample *versus* reference.

Fig. 2 shows the differential spectra obtained for several concentrations of thiopental sodium in the 0–6 mg/100 ml range. The peak at 283 mμ is the wavelength used for quantitative calibration and the differences obtained at this wavelength are a linear function of concentration. All standards were subjected to the

double extraction process. Curve A represents the composite spectrum of 6 mg/100 ml of thiopental sodium extracted from thiobarbiturate bolstered serum according to the described procedural details. It has been shifted up instrumentally for better delineation, and it can be seen that it would reasonably superimpose on the standard curve of the same concentration, curve B. This is representative of a number of similar experiments also carried out for various concentrations of both compounds and it

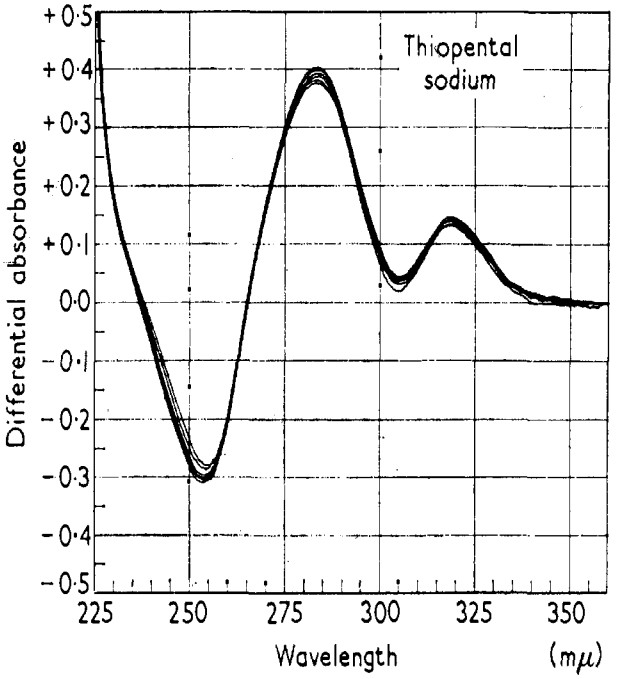

Fig. 3. Precision study carried out for thiopental sodium (Pentothal®) 3 mg/100 ml, showing differential spectra.

reiterates the concept that complete isolation from spectrophotometrically interfering material is unnecessary for good quantitative estimation.

A number of serum samples, artificially prepared to contain the same concentration of thiopental sodium or thiamylal sodium (3 mg/100 ml), were graphically investigated after extraction into chloroform followed by retrograde extraction into sodium hydroxide. The recovery range was 2·88 mg to 3·14 mg per 100 ml which represents a range of 96·2 to 105 per cent of the original amount. An example of this precision is the graph shown as Fig. 3 where 10 samples of thiopental sodium are illustrated as

ultraviolet differential spectrophotometric recoveries. A similar identical graphing, not shown, was also carried out for thiamylal sodium with correspondingly similar results.

The excellent results of an accuracy study are given in Table I. Several absolute concentrations of thiamylal sodium and thiopental sodium either in water solution or added to serum were extracted out, treated chemically and then instrumentally in the manner described. The differential spectra of the compounds

Table I. Recoveries of thiamylal and thiopental sodium from water and serum

Thiamylal sodium, mg/100 ml			Thiopental sodium, mg/100 ml		
Sample	Present	Found	Sample	Present	Found
Water	2·00	1·90	Water	2·00	1·95
Water	2·00	1·90	Serum	2·00	2·00
Serum	2·00	1·75	Serum	2·00	1·95
Serum	2·00	2·10	Water	4·00	4·15
Water	4·00	3·85	Serum	4·00	4·00
Water	4·00	4·00	Serum	4·00	4·15
Serum	4·00	4·00	Water	6·00	6·00
Serum	4·00	4·10	Serum	6·00	5·75
Water	6·00	6·20	Serum	6·00	5·80
Serum	6·00	5·95			
Serum	6·00	6·25			

obtained by the double extraction process from water could be superimposed on those of the same concentration extracted from various serums, indicating once more that no interfering material was present in these randomly selected specimens.

Fig. 4 shows the spectral results one would obtain if the possibility of a common barbiturate being present as a contaminant during the differential scanning is anticipated. Curve A represents the differential scan for thiamylal sodium while curve B represents a similar differential graphing for secobarbital. It can be seen that either of the two positive maxima obtained for thiamylal sodium are free from absorbance additive interference due to secobarbital so that thiobarbiturates can be analyzed with

accuracy at 283 mμ in the presence of significant amounts of regular therapeutic barbiturates. Salicylates do not interfere since they are not extracted from serum at pH 7·4.

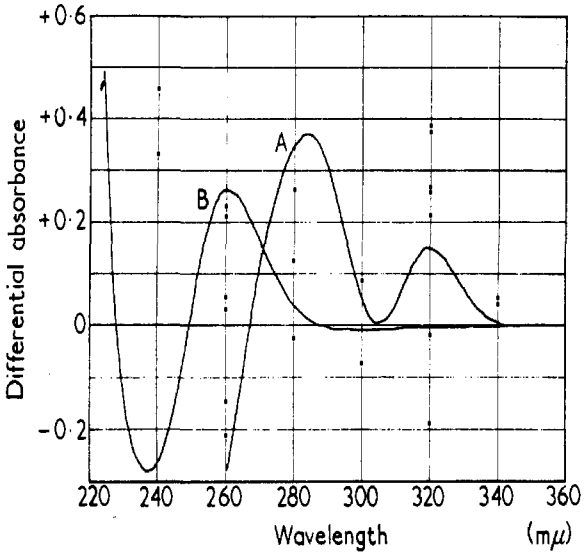

Fig. 4. Differential spectra of thiamylal sodium (Surital®), curve A, and secobarbital (Seconal®), curve B.

Summary. A method has been described for the determination of thiobarbiturates which is applicable to serum. It involves the simultaneous and automatic recording of two spectra of the same material differentiated one from the other only by a spectrum shift due to acidification of an alkaline solution of the thiobarbiturate. It obviates the use of the reference blank, precludes complete isolation of the desired constituents, and it is not interfered with by regular barbiturates or salicylates. The procedure should be useful as an analytical tool in experimental physiology studies or in pharmaceutical control analysis.

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