SPECTROPHOTOMETRIC DETERMINATION OF DERIVATIVES OF THIOBARBITURIC ACID

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A spectrophotometric method has been elaborated to determine thiobarbituric acid derivatives and their sodium salts, the compounds being measured in absolute ethanol medium in the form of Cu (II) $(TB)_4$ complexes. Measurements were performed with amounts 10 to 70 μg/ml in 1 cm cuvets at 360 nm. The accuracy ot the measurements is within the range of the usual ± 1 per cent.

Introduction

Thiobarbiturates belong to the most widely used intravenous narcotics, thus their analytical determination is very important. A colour-reaction appropriate for spectrophotometric determination is given by their cobalt(II) [6] and copper(II) [8] complexes, as a rule in the form of mixed ligand complexes with amines and pyridine. On this basis a number of determinations have been developed [1, 2, 3, 4, 7]. MORVAY and KÖZÉPESY [5] have determined the composition of some copper(II)-pyridine thiobarbiturate mixed ligand complexes and pointed out that a complex of suitable composition for spectrophotometric determination is only formed in the presence of a certain amount of pyridine, and at a given p_H. Regarding that the obtained colour intensity is markedly influenced by the amount of amines and of pyridine, we have attempted to determine thiobarbiturates without pyridine in the form their copper(II) complex.

Experimental

The sodium salts of the following thiobarbituric acid derivatives (TBNa) have been used for the experiments.

I. 5-allyl-5-(1-cyclohexenyl)-2-thiobarbituric acid (Intranarcon). II. 5-ethyl-5-(1-methyl-propyl)-2-thiobarbituric acid (Narkotion, Venobarbital). III. 5-ethyl-5-(1-methyl-butyl)-2-thiobarbituric acid (Trapanal).

From these derivatives 10^{-2} M stock solutions were prepared in distilled absolute ethanol, since in this medium the precipitation of a complex could not be experienced. The only difference was with the determination of the alcohol content necessary for the reaction. 10^{-2} M $Cu(NO_3)_2 . 3H_2O$ (Reanal p.a.) was dissolved in absolute ethanol and used as reagent. Measurements were carried out in a thermostated Beckman DU and a Spektromom 360 spectrophotometer, respectively.

Results

In order to clear up the most suitable experimental conditions, the following experiments have been carried out: the absorbance of solutions containing copper(II)-nitrate, copper(II)-nitrate and different TBNa (Fig. 1).

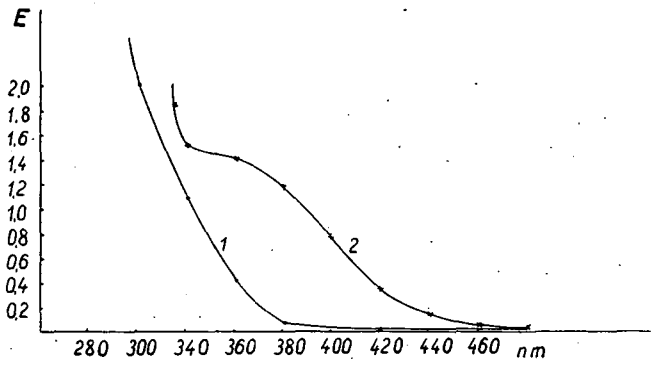

Fig. 1. Light absorption curves of copper(II)-nitrate (1) and copper (II)-nitrate and different TBNa(III) (2). (1) $4 \cdot 10^{-3}$ M $Cu(NO_3)_2.3H_2O$, (2) $4 \cdot 10^{-3}$ M $Cu(NO_3)_2.3H_2O$, $3{,}7 \cdot 10^{-4}$ MTB (III)

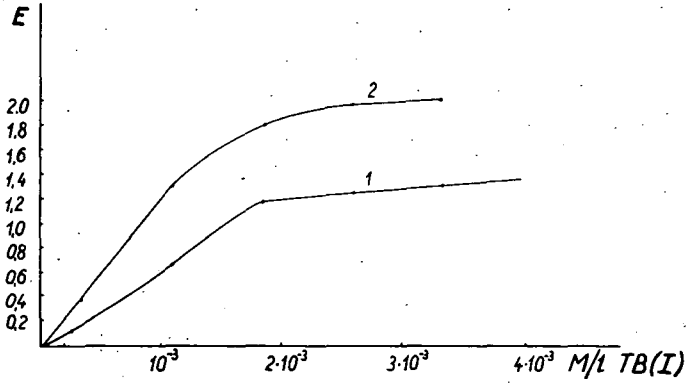

Fig. 2. Determination of the composition of the copper(II)-TBNa(III) complex by the mol-ratio method. $10^{-3}-4 \cdot 10^{-3}$ M/l TB(III). $4 \cdot 10^{-1}$ M/l $Cu(NO_3)_2.3H_2O$ in absolute ethanol (1): $\lambda = 420$nm, (2): $\lambda = 400$ nm

As it appears from the above figure, copper(II) formed a complex with TBNa. Spectra of the solutions were taken after an hour and no change in the light absorption could be found.

The composition of the copper(II)-TBNa complex has been determined by the mol-ratio method; maintaining the metal ion concentration constant, the TBNa concentration was altered and the extinction of the solution measured at different wavelengths (Fig. 2).

Figure 2 shows that in absolute ethanol medium four 5-5-substituted thiobarbituric acid derivatives react with one copper(II) ion, thus the composition of the complex is: $Cu(II)(TB)_4$.

The effect of excess concentration of Cu(II) ions on the light absorption of the $Cu(II)(TB)_4$ complex has also been studied (Fig. 3).

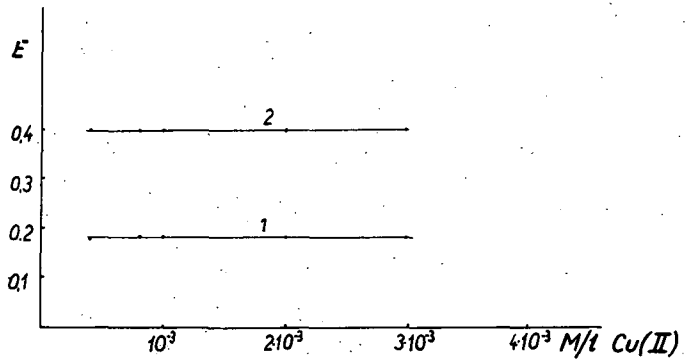

Fig. 3. Influence of the Cu(II) ion concentration on the light absorption of $Cu(II)(TB)_4$ complexes.
(1): $\lambda = 420$ nm $3{,}7 \cdot 10^{-4}$ M/1 TB(I)
(2): $\lambda = 400$ nm $3{,}7 \cdot 10^{-4}$ M/1 TB(I)

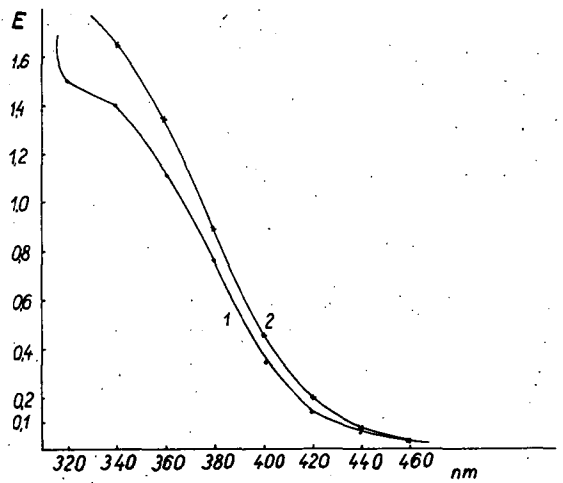

Fig. 4. Effect of water on the light absorption of Cu(II) $(TB)_4$ comple xes in absolute ethanol. $4 \cdot 10^{-3}$M Cu^{2+} $3{,}7 \cdot 10^{-4}$ M TB(I) (1) 100% ethanol (2) 50% ethanol

The above figure shows that the excess concentration of Cu(II) ions does not influence the light absorption of the complexes.

The effect of water on the light absorption of the $Cu(II)(TB)_4$ complexes in absolute ethanol has been investigated as well (Fig. 4).

Itt appears from the figure that in 50 per cent ethanol medium the light absorption changes (there is still no complex precipitation), which means that on applying non-absolute alcohol a separate calibration curve must be prepared. It has also been studied whether or not the light absorption of the $Cu(II)(TB)_4$ complex in absolute alcohol changes proportionally to the concentration, *i.e.* whether it follows the BOUGUER—LAMBERT—BEER rule at 360 nm (Fig. 5). This wavelength was

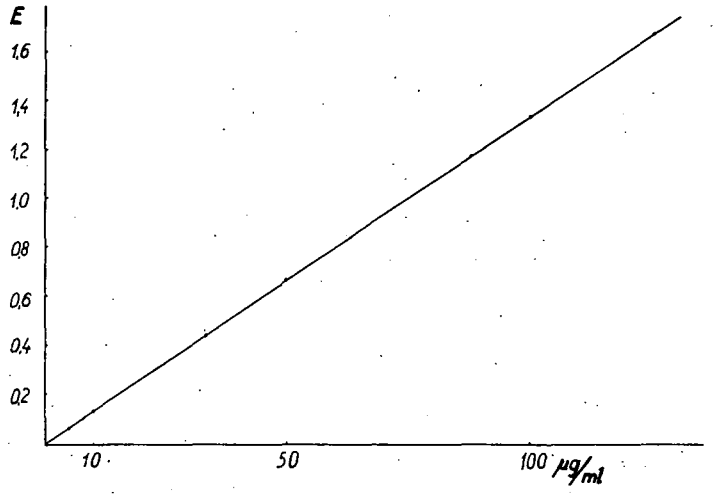

Fig. 5

chosen since it is here that the light absorptions of $Cu(II)(TB)_4$ and Cu(II)-nitrate exhibit the largest deviation. The figure demonstrates well that the concentration curve results to be a straight line, indicating that the $Cu(II)(TB)_4$ complex is suitable for spectrophotometric determination. The best is to apply aliquots of 10^{-5} and $7 \cdot 10^{-5}$ µg/ml thiobarbituric acid derivatives concentrations wherever the extinction is in the measurement range of 0,1—0,9.

Procedure

Measurements were performed in the following manner: 20 mg of the sodium salt of the thiobarbituric acid derivative to be determined was back-measured (hygroscopic compound) into a 100 ml volumetric flask, dissolved in absolute ethanol and filled up to the mark. 2 ml were pipetted into a 25 ml volumetric flask in which previously 10 ml absolute ethanol copper(II)-nitrate solution had been added. Thereafter it was filled up to the mark with absolute ethanol, thoroughly shaken and measured in a 1 cm cuvet in the spectrophotometer at 360 nm wavelength. Results were read from a previously prepared calibration curve. The method has been successfully applied for the determination of sodium salts and powder ampoule injections of thiobarbituric acid derivatives. Our results are listed in Table I.

Table I

Spectrophotometric determination of TBNa in form of $Cu(II)(TB)_4$ *complexes*

TBNa	Taken (μg)	Found (μg)	Difference ($\Delta\mu$g)	Deviation %
No. III.	50	50,2	+0,2	+0,4
	50	50,0	0,0	0,0
	75	74,7	−0,3	−0,4
	75	74,5	−0,5	−0,7
	100	100,1	+0,1	+0,1
	100	99,9	−0,1	−0,1
	110	110,0	0,0	0,0
	110	110,5	0,5	+0,4

* * *

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References

[1] *Awe, W., W. Winkler:* Arzneimittel Forsch. **5**, 578 (1955).
[2] *Gomahr, H., H. Kresbach:* Sci. Pharm. (Wien) **19**, 148, 154. (1951).
[3] *Középesy, Gy.:* Pharm. dissertation, Szeged, (1964).
[4] *Levi, L., C. E. Hubley:* Analyt. Chem. **28**, 1951 (1956).
[5] *Morvay, J., Gy. Középesy:* Acta Pharmaceutica Hung. **36**, 160 (1966).
[6] *Parri, W.:* Boll. Chim. Pharm. **63**, 401 (1924).
[7] *Raventos, J.:* Brit. J. Pharmacol. **1**, 210 (1946).
[8] *Zwicker, J.:* Pharm. Weekbl. **68**, 975 (1931); **69**, 1178 (1932).

СПЕКТРОФОТОМКТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ПРОИЗВОДНЫХ ТИОБАРБИТУРОВОЙ КИСЛОТЫ

И. Морваи, В. Николашев, Ш. Каради и Ф. Е. Сонтаг

Авторами был разработан спектрофотометрический метод определения производных тиобарбитуровой кислоты и их солей натрия. Соединения измерялись в абс. этаноле в форме комплексов $Cu(II)(TB)_4$. Измерения выполнялись пробами 10—70 МГ/МЛ в I см кюветке при 360 нм. Точность измерения не превышает обычный ± 1 процент.