Pakistan J. Sci. Ind. Res., Vol. 23, Nos. 3–4, June–August 1980

SPECTROPHOTOMETRIC DETERMINATION OF BARBITURIC ACID WITH NINHYDRIN

Rashid Iqbal and Nasir Ahmad

Department of Chemistry, Quaid-i-Azam University, Islamabad

(Received June 25, 1979)

A new and sensitive spectrophotometric method for the determination of micro amounts of barbituric acid with ninhydrin is reported. Working conditions for the method are described. Beer–Lambert's law is obeyed in the concentration range of 4–280 μg/ml of barbituric acid. Also, the method has been found useful for the determination of barbituric acid in presence of different barbiturates which do not interfere.

INTRODUCTION

Barbituric acid and its derivatives (the barbiturates) belong to an important calss of drugs used as hypnotics, sedatives and anesthetics. A number of analytical methods such as potentiometeric [1], volumetric [1–3], refractometric [4] and spectrophotometric [5–8], have been described in the literature for estimation of these compounds from urine, blood and post mortem materials. These methods widely differ in their sensitivity. The spectrophotometric methods are very sensitive and microgram quantities of these drugs can be easily estimated by this method. The spectrophotometric estimation of barbiturates depends upon the measurement of absorbance at the characteristic λ_{max} of their absorption band in the UV region of the spectrum. The compounds usually absorb at 240 nm with extinction coefficients in the range 280-540 M^{-1} cm^{-1}. Further, the wavelength of maximum absorbance is pH-dependent and the absorption band is shifted to longer wavelength with increase in pH and disappears completely at very high H^+ concentration (such as at pH 2). Thus a change in pH introduces an error in determination of the compounds by this method. Unfortunately, very little is published on the spectrophotometric determination of barbituric acid and its derivatives by developing color with other reagents. The only reported method of this kind is due to Asmus and Noack [5] who measured intensity of the color produced by the interaction of barbituric acid with glutaconaldehyde at 502 nm. These workers assumed the formation of glutaconaldehyde in solution by mixing pyridine, chloramine T and potassium cyanide. This method seems to have many disadvantages such as addition of number of reactants specially chloramine T which is an oxidising agent, unstability of the color produced and low extinction coefficient. The presence of an oxidising agent in the system could also decompose barbituric acid which is evident from the variation of the reported error.

We wish to report a new spectrophotometric method for the estimation of barbituric acid with ninhydrin. This method is simple, more sensitive and the color produced is stable for longer period. Moreover, by this method, barbituric acid can easily be estimated in the presence of its derivatives which do not produce color with ninhydrin. A color reaction of barbituric acid with ninhydrin was first reported by Rosenthaler [9] which we have utilized for quantitative determination of barbituric acid. The yellow color produced by mixing the aqueous solutions of the two componen has maximum absorbance at 367 nm (ϵ 416) and is sensitive in a solution containing as low as 4 μg/ml of barbituric acid. The maximum color intensity is obtained in a solution of pH 7.5–8.5. Aslo, the color produced is stable for more than 48 hr. Beer's law is obeyed in the concentration range of 5–280 μg/ml of barbituric acid.

EXPERIMENTAL

Apparatus. The UV spectra were recorded on a Hitachi model 323 recording spectrophotometer. A matched set of two quartz cells of 1-cm thickness was used for these measurements. Fisher model Accument 230 pH-meter with a set of glass and calomel electrodes was used for pH determination.

Reagents. Ninhydrin (Puriss p.a., Fluka), barbituric acid (Fisher, highest purity). All other chemicals used were of reagent grade.

Solutions. (i) Barbituric acid (100 mg) was dissolved in 100 ml distilled water (solution A). This solution contained 1 mg/ml of barbituric acid. 10 ml of solution A was diluted to 100 ml with distilled water in a volumetric flask to yield a solution B which was 0.1 mg/ml with respect to barbituric acid.

(ii) A stock solution of ninhydrin was prepared by

dissolving 890. mg ninhydrin in 100 ml distilled water. This solution contained 8.9 mg/ml of ninhydrin.

(iii) Phosphate buffer solution was prepared by dissolving 0.8268 g $NaH_2PO_4.12H_2O$ and 3.3921 g Na_2HPO_4 in 100 ml distilled water. This solution when diluted ten times gave a solution with pH ranging from 8.2 to 8.3.

Preparation of Calibration Curve. Ninhydrin stock solution (2.5 ml) was pipetted out in different 25-ml volumetric flasks containing 1.0, 2.5, 5.0, 7.5 and 10.0 ml barbituric acid solution B and 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0 ml barbituric acid solution A. Phosphate buffer solution (2.5 ml) was then added in each flask and the volume was made up with distilled water. The pH of each solution was found between 8.2–8.3. These solutions were shaken thoroughly and left for about 1 hr. After, this period, absorbance of each of the yellow solutions was measured at 367 nm using aqueous ninhydrin solution (0.89 mg/ml) as reference. Absorbance was then plotted as a function of concentration of barbituric acid. This resulted in a linear plot (Fig. 1) with deviation starting above 280 μg/ml of barbituric acid. This calibration plot was used in subsequent determinations of barbituric acid alone and in different mixtures containing barbituric acid and various barbiturates.

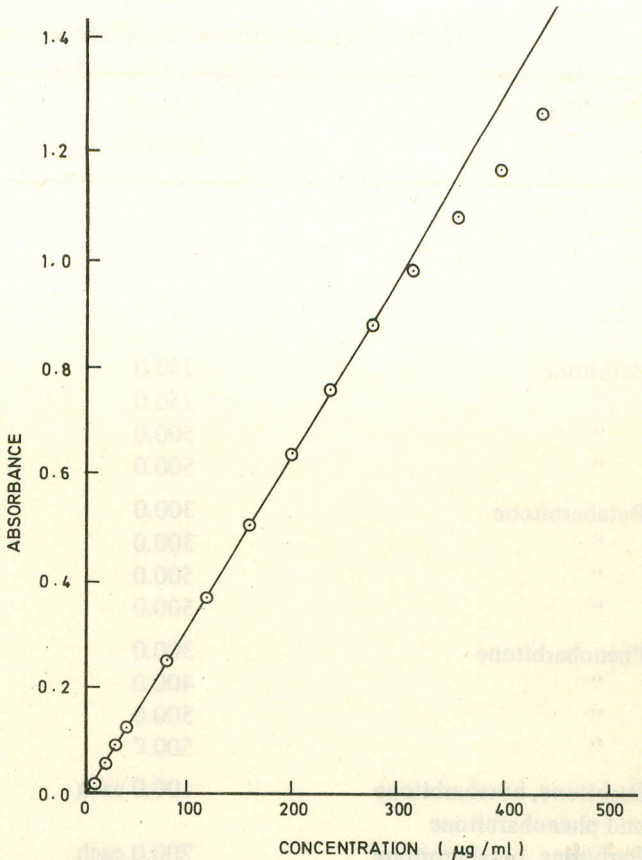

Fig. 1. Calibration curve for barbituric acid.

RESULTS AND DISCUSSION

The absorption of the yellow solution obtained by mixing aqueous solutions of ninhydrin and barbituric acid at pH 8–2 is shown in Fig. 2, from which it is evident that the λ_{max} for this solution is at 367 nm. The molar absorptivity as calculated from the slope of the calibration curve turns out to be 416. Barbituric acid and its derivatives do not absorb at this wavelength. However, ninhydrin has some absorption in this region which is due to the tail of an absorption band in the far UV region of the spectrum. The absorbance due to ninhydrin in the system can be easily eliminated by taking an aqueous solution of ninhydrin of the same concentration in the reference cell as in the sample cell while recording the spectra.

The effect of pH on the absorbance of ninhydrin-barbituric acid system was studied in the pH range of 4.9 to 12.2. Apparently the reaction does not occur and the color is not produced when the pH is below 4.9. The wavelength of absorption maximum remained constant in the pH range of 4.9–9.2. However, at pH 9.8 it started shifting towards longer wavelength. This shifting of absorption maximum continued so that at pH 12.2, λ_{max} was observed at 385 nm. A plot of absorbance at 367 nm as a function of pH is reproduced in Fig. 3. It is evident from the figure that maximum absorbance is obtained in the pH

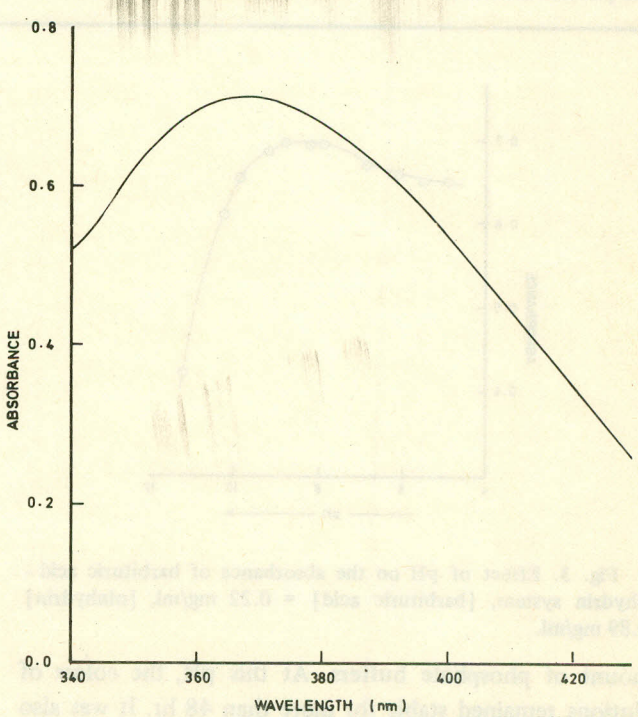

Fig. 2. Absorption spectrum of barbituric acid–ninhydrin complex in 340–420 nm spectral range, [barbituric acid] = 0.22 mg/ml, [ninhydrin] = 0.89 mg/ml, pH = 8.2.

range of 7.7 – 8.7. Thus all subsequent determination of bartituric acid were carried out by maintaining the pH of the measuring solutions at 8.2 by addition of calculated

Table 1. Determination of barbituric acid as such and in mixtures of various barbiturates.

Barbiturate	Concn (μg/ml)	Barbituric acid added (μg/ml)	Barbituric acid found (μg/ml)	Difference (%)
–	–	40.0	40.8	+2.00
–	–	80.0	79.1	–1.13
–	–	160.0	161.0	+0.62
–	–	240.0	238.0	–0.63
Barbitone	250.0	36.0	35.4	–1.67
”	250.0	63.0	62.5	–0.79
”	500.0	40.0	39.5	–1.25
”	500.0	100.0	101.0	+2.00
Butabarbitone	300.0	40.0	40.8	+2.00
”	300.0	60.0	59.7	–0.50
”	500.0	40.0	40.6	+1.50
”	500.0	80.0	80.5	+0.63
Phenobarbitone	300.0	100.0	101.0	+1.00
”	400.0	72.0	71.0	–1.39
”	500.0	91.0	92.0	+1.10
”	500.0	154.0	153.0	–0.67
Barbitone, butabarbitone and phenobarbitone	100.0 each	30.0	30.5	+1.67
Barbitone, butabarbitone and phenobarbitone	200.0 each	50.0	50.3	+0.60

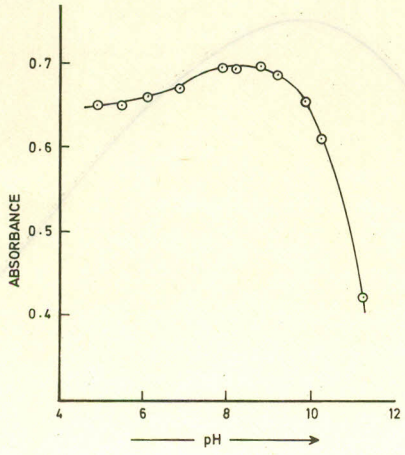

Fig. 3. Effect of pH on the absorbance of barbituric acid–ninhydrin system, [barbituric acid] = 0.22 mg/ml, [ninhydrin] = 0.89 mg/ml.

amount of phosphate buffers. At this pH, the colors of solutions remained stable for more than 48 hr. It was also noted that the intensity of the colors of the solutions at higher pH (11–12) decreased rapidly and the solutions become colorless after about 20 hr. The minimum molar ratio of barbituric acid to ninhydrin for producing maximum absorbance was determined to be 1:1. Excess of ninhydrin did not after the absorbance of the system.

From the calibration plot (Fig. 1) it appears that the Beer–Lambert's law is obeyed within 4–280 μg/ml concentration range of barbituric acid. The calibration plot starts from the origin and negative deviation is observed at concentration higher than 280 μg/ml of bartituric acid. This negative deviation at higher concentration of barbituric acid is mainly due to the intense absorbance of the system which cannot be measured, by the instrument, as accurately as that of the solutions of lower concentrations.

The derivatives of barbituric acid, i.e. the barbiturates, do not produce any color under the specified conditions and hence do not absorb at this wavelength. Therefore, no interference is expected by the presence of different barbiturates in the determination of barbituric acid when present in a mixture of baribturates. This was experimently confirmed by measuring the amount of barbituric acid in presence of a number of barbiturates. Results of the determination of barbituric acid in different mixtures of barbituric acid and barbiturates are reported in Table 1. It is evident from these results that the per cent error is relatively small (± 2%). Small amounts of barbituric acid in presnece of baribturates can be easily measured by this method. However, it is necessary that amino acids and other organic compounds which produce color with ninhydrin should be absent from the system. Also, this method

can be efficiently applied for checking the purity of barbiturate drugs which may contain small amounts of barbituric acid as an impurity.

REFERENCES

1. A. Anastasi, E. McCarelli and L. Novacic, Pharm. Acta. Helv., **30**, 55 (1955).
2. P. Chavanne and H. Marie, Ann. Pharm. (France), **11**, 91 (1953).
3. T. Itai and T. Oka, Bull. Natl. Hyg. Lab. (Tokyo), **73**, 131 (1955).
4. M. Mincsev-Ralcseva, Gyogyszereszet, **5**, 417 (1961).
5. E. Asmus and K. Noack, Z. Anal. Chem., **249**, 122 (1970).
6. A.I. Berdnikov, Aptech. . Delo, **15**, 39 (1966).
7. K. Kigasawa, H. Skimizu, T. Iwata, H. Tanaka and H. Nakaguro, Yakugaku Zasshi, **90**, 257 (1970).
8. A.S. Samarin and N.A. Smarina, Sb. Nauchn. Tr. Permsk. Politekhn. Inst., **14**, 94 (1963).
9. L. Rosenthaler, Pharm. Acta. Helv., **36**, 602 (1961).