

## SPECTROPHOTOMETRIC DETERMINATION OF BARBITURIC ACID WITH NINHYDRIN

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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  $\mu\text{g/ml}$  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 class of drugs used as hypnotics, sedatives and anesthetics. A number of analytical methods such as potentiometric [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  $\lambda_{\text{max}}$  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  $M^{-1} \text{ cm}^{-1}$ . 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  $\text{H}^+$  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 glutacetaldehyde at 502 nm. These workers assumed the formation of glutacetaldehyde 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, instability 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 components has maximum absorbance at 367 nm ( $\epsilon$  416) and is sensitive in a solution containing as low as 4  $\mu\text{g/ml}$  of barbituric acid. The maximum color intensity is obtained in a solution of pH 7.5-8.5. Also, the color produced is stable for more than 48 hr. Beer's law is obeyed in the concentration range of 5-280  $\mu\text{g/ml}$  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 Accumet 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  $\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$  and 3.3921 g  $\text{Na}_2\text{HPO}_4$  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  $\mu\text{g}/\text{ml}$  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.

## 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  $\lambda_{\text{max}}$  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,  $\lambda_{\text{max}}$  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

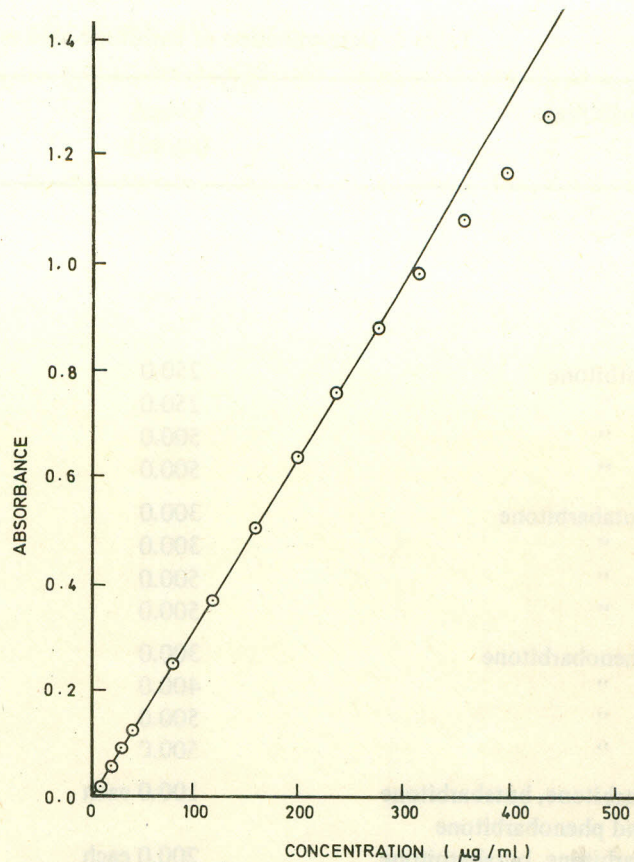


Fig. 1. Calibration curve for barbituric acid.

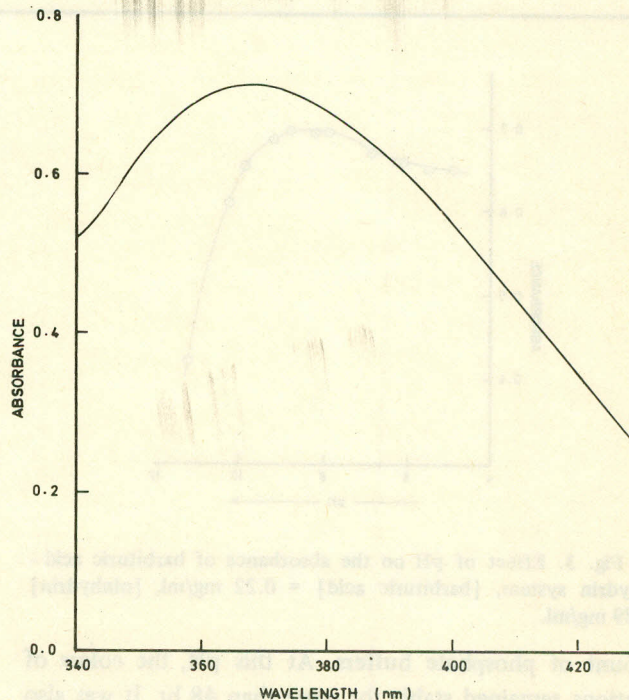


Fig. 2. Absorption spectrum of barbituric acid-ninhydrin complex in 340–420 nm spectral range, [barbituric acid] = 0.22 mg/ml, [ninhydrin] = 0.89 mg/ml, pH = 8.2.

range of 7.7 – 8.7. Thus all subsequent determination of barbituric 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 ( $\mu\text{g/ml}$ )	Barbituric acid		Difference (%)
		added ( $\mu\text{g/ml}$ )	found ( $\mu\text{g/ml}$ )	
—	—	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
Butabarbitione	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, butabarbitione and phenobarbitone	100.0 each	30.0	30.5	+1.67
Barbitone, butabarbitione and phenobarbitone	200.0 each	50.0	50.3	+0.60

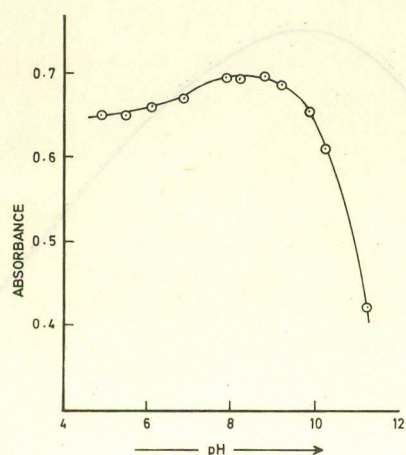


Fig. 3. Effect of pH on the absorbance of barbituric acid-ninhydrin system, [barbituric acid] = 0.22 mg/ml, [ninhydrin] = 0.89 mg/ml.

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 affect the absorbance of the system.

From the calibration plot (Fig. 1) it appears that the Beer-Lambert's law is obeyed within 4–280  $\mu\text{g/ml}$  concentration range of barbituric acid. The calibration plot starts from the origin and negative deviation is observed at concentration higher than 280  $\mu\text{g/ml}$  of barbituric 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 barbiturates. This was experimentally 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 ( $\pm 2\%$ ). Small amounts of barbituric acid in presence of barbiturates 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.

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laboratory alcohol has been recommended by Thompson [14] for the extraction but the colour intensity has been reported to be increasing and decreasing with passage of time.

An improved method of obtaining a stable colour of the thiocyanate complex involves extraction with ethyl ether saturated with sulfur dioxide [12]. Baly [15] used methyl ethyl ketone for the stabilization of the colour. Zsigler [17] extracted the coloured species into organic phase after precipitating it as trithiocyanatomercury thiocyanate (III) since [18] measured the density from the molar concentration of iron (II) over a rather broad range of iron (III) and thiocyanate concentrations using the lithium chloride buffer [19].

Although the equilibrium constants of the extracted complex after its extraction into thiocyanate.

The attempt, however, appears to have been made in extracting the coloured complex species into organic phase containing an amine for the spectrophotometric determination of iron. As the coloured species form with a certain degree, efforts were made to establish the presence of an iron complex towards formation of an extractable species. Investigation towards this had to be discontinued because of the coloured species under optimal conditions in spectrophotometric determination of iron.

EXPERIMENTAL

Standard Thiocyanate Solution: 0.1% solution (w/v) of iron (II) and standardized volumetrically.

Standard Thiocyanate Solution: 0.1% solution (w/v) of iron (II) and standardized volumetrically.

INTRODUCTION

The use of alkali thiocyanates as complexing reagents for the colorimetric estimation of a number of metals has been known for sometime. More recently, however, has been taken to the use of thiocyanate complexing as a method of chemical separation [1]. An intensive study of the distribution of many metal thiocyanates to ethyl ether at various pH, SCN concentration has been made by Baly [2], and it has been shown that a specific number of metal thiocyanates are extractable in the organic solvent. The present author after a study of the extraction of thiocyanate complexes of Fe(III) [3], Mn(IV) [4] and Fe(II) [5] into EMVA, undertook the present work of extraction of Fe(III) - SCN for a spectrophotometric determination of iron in the later work it was reported that Fe(III) also forms various kinds of complexes with thiocyanate which have been studied earlier [6, 7].

Thiocyanate is extensively used for the determination of iron, even though other reagents may give better results in spite of its limitations, which are often too serious, the thiocyanate method is a valuable one in applied analysis [8]. The intensity of the coloured species is dependent on many factors and later mainly in aqueous phase. Arstons [7], 2-methyl ethanol [8] has been recommended for the purpose. The ferric thiocyanate complex can be extracted by methyl alcohol, a mixture of ethyl alcohol and ether [9], a mixture of monochloro ethyl of ethyl ether and ethyl ether [10], ethyl acetate [11] and a butyl phosphate [12]. With some of these solvents, the stability of the colour is greater than it is in water. Ethyl ether (density 0.71) has been often used as an immiscible solvent is used [13].