

Spectrophotometric Determination of Barbituric Acid with Sodium Nitrite

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Summary: A new and sensitive spectrophotometric method for determination of micro amounts of barbituric acid with sodium nitrite is described. The method is applicable in acidic medium (pH 4.7) in the concentration range of 4-1600 $\mu\text{g/ml}$ of barbituric acid. The reported method can be used for determination of barbituric acid in presence of various barbiturates which do not interfere.

Introduction

Various analytical methods for determination of barbiturates are reported [1-7]. However, only two methods have been published on spectrophotometric determination of barbituric acid itself. One of these methods is based on measuring the colour intensity of the colour produced by reaction of barbituric acid with glutacetaldehyde [8]. The colour producing reagent, glutacetaldehyde, was assumed to be formed in a mixture of chloramine T, pyridine and potassium cyanide. The presence of an oxidizing agent like chloramine T could decompose barbituric acid which is evident from the error in the reported results. The other spectrophotometric method [9] is based on measuring the absorbance of the yellow colour produced by reaction of barbituric acid with ninhydrin. Both these reported methods are useful in basic medium only. It was, therefore, found desirable to develop a method which could be used in acidic medium.

We now wish to report a new simple and convenient spectrophotometric method for determination of barbituric acid in acidic medium. A common and relatively inexpensive reagent, sodium nitrite, has been used for producing purple color by its reaction with barbituric acid. The color reaction is a characteristic qualitative test for barbituric acid [10]. During the present work this color reaction has been utilized for quantitative determination of barbituric acid. The present spectrophotometric method consists of mixing the aqueous solutions of sodium nitrite and barbituric acid (in aqueous sodium carbonate solution), adjusting the pH to 4.7 using acetic acid sodium acetate buffer solution, warming the mixture on a water bath (60°C) for fifteen minutes and recording the absorbance of the

solution at 545 nm. Both barbituric acid and sodium nitrite as well as the buffer solution used do not absorb at the working wavelength. Furthermore, the purple color produced is stable for at least twenty hours. The Beer-Lambert's law is obeyed in the concentration range of 4-1600 $\mu\text{g/ml}$ of barbituric acid.

Results and Discussion

A dilute solution of barbituric acid in aqueous sodium carbonate solution when mixed with a dilute aqueous solution of sodium nitrite and acidified with acetic acid remains colorless for about eight minutes at room temperature (16-18°C). After this period of time a light purple color starts appearing in the mixture. The intensity of the color goes on increasing gradually over a period of two hours. However, when the reaction mixture is warmed at 60°C, the maximum color intensity is developed within fifteen minutes and remains stable for more than twenty hours. The absorption spectrum of the purple colored solution shows a single band in the visible region with λ_{max} at 545 nm (Fig. 2). Neither barbituric acid nor sodium nitrite absorbs at this wavelength. The extinction coefficient of absorbance at 545 nm, as calculated from the slope of the calibration curve is 48.

Effect of heat and time on absorbance

Fig. 3 illustrates the effect of heat and time on the absorbance at 545nm. It is evident from the Fig. that when the acidic aqueous mixtures of barbituric acid and sodium nitrite is allowed to stand at room temperature (16-18°C), the absorbance at 545 nm

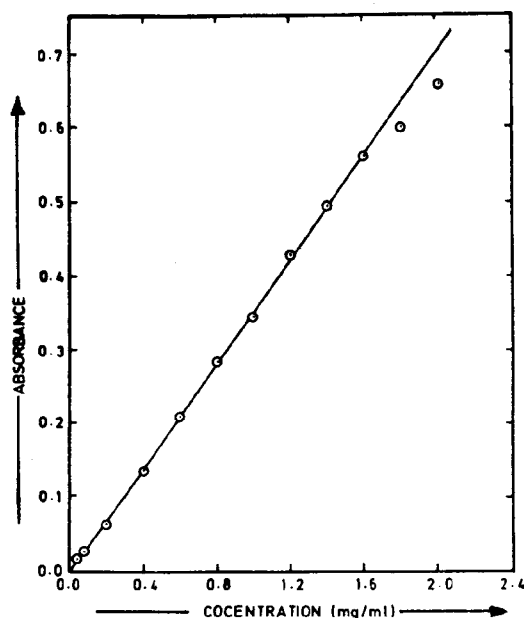


Fig. 1: Calibration curve for barbituric acid.

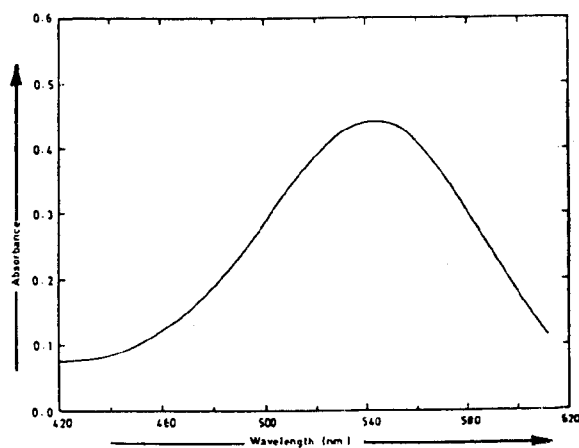


Fig. 2: Absorption spectrum of aqueous barbituric acid-sodium nitrite mixture in 420-600 nm spectral range; [barbituric acid] = 1.20 mg/ml. [sodium nitrite] = 3.45 mg/ml, pH = 4.70.

continues to increase gradually till 115 minutes after mixing the reagents (Fig. 3A). The increase was not followed any further. However, when the reaction mixture is warmed at 60°C (just after mixing the reagents) for 15 minutes a much higher absorbance is observed at 545 nm (Fig. 3B). This observed absorbance remains constant on further warming (or cooling) the reaction mixture.

Effect of pH on absorbance

The effect of variation of pH on the absorbance of barbituric acid sodium nitrite system was studied in the pH range of 3.0- 8.0. No significant absorbance at 545 nm was observed in the pH ranges of 3.0 to 3.8 and 6.5-8.0. The slight absorbance appearing at pH 4.0 increases with the increase in pH and becomes maximum in the pH range of 4.65-4.80 and then decreases with further increase in the pH. No change in the λ_{\max} was observed with change in pH of the reaction mixture from pH 4.00 to 6.50. At pH 4.65-4.80, the purple color was found stable for more than twenty hours. Thus all subsequent determination of barbituric acid were carried out by maintaining the pH of solution at 4.70.

Minimum molar ratio of sodium nitrite to barbituric acid

The minimum molar ratio of sodium nitrite to barbituric acid for producing maximum absorbance, at the specified conditions, was determined by Job's method [12]. The ratio was established to be 2:1. Excess of sodium nitrite had no effect either on the λ_{\max} or on absorbance of the system.

Sensitivity and accuracy of the method

From the calibration plot (Fig. 1) it appears that the Beer- Lambert's law is obeyed within 0.02-1.60 mg/ml concentration range of barbituric acid. The calibration plot starts from the origin and negative deviation is observed at concentration of barbituric acid higher than 1.60 mg/ml. The negative deviation at higher concentrations is apparently due to the intense absorbance that can not be measured as accurately as that of the solutions containing lower concentrations of barbituric acid. This type of negative deviation has previously been observed in other spectrophotometric methods of determination of barbituric acid [8,9].

An independent check on the accuracy of the present method was undertaken by determining the amount of barbituric acid in solutions containing weighed amounts of the latter. Furthermore, this spectrophotometric method was also used to determine the amount of barbituric acid in solutions containing known amount of barbituric acid and various barbiturates. The results of these determinations, reported in Table 1, illustrate an excel-

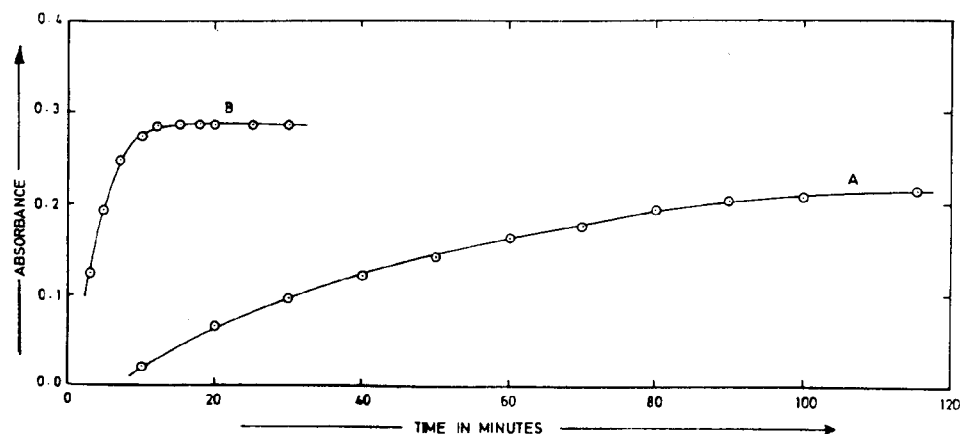


Fig. 3: Effect of heat and time on absorbance at 545 nm; A at 16-18°C, B at 60°C; [barbituric acid] = 0.80 mg/ml, [sodium nitrite] = 3.45 mg/ml, pH = 4.70.

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

Barbiturate	added ($\mu\text{g/ml}$)	Barbituric acid added ($\mu\text{g/ml}$)	found	Difference %
-	-	100	101	+1.0
-	-	100	99.5	-0.5
-	-	200	201	+0.5
-	-	200	201	+0.5
-	-	500	498	-0.4
-	-	500	499	-0.2
Barbitone	300	100	101	+1.0
Barbitone	500	200	202	+1.0
Buta-barbitone	300	100	99.5	-0.5
Buta-barbitone	500	200	202	+1.0
Pheno-barbitone	300	100	99.0	-1.0
Pheno-barbitone	500	200	199	-0.5

lent agreement in the added and found amounts of barbituric acid. The present method appears to be quite precise and accurate and the results of determinations of barbituric acid itself and in mixtures agree within $\pm 1\%$.

Experimental

Reagents

Barbituric acid (GPR) BDH, sodium nitrite cryst. (extra pure) Merck, sodium carbonate anhydrous (GR) Merck, acetic acid (98%, 1.06 g/ml)

Merck and sodium acetate anhydrous (GR) Merck. Barbital, butabarbital and phenobarbital used were Sigma Chemicals.

Apparatus and instruments

The visible spectra were recorded on a Hitachi model 323 recording spectrophotometer. A matched set of two glass cells of 10 mm path length was used for measuring the absorbance. Fischer model Accumet 230 pH-meter with a set of glass and calomel electrodes was used for pH measurements.

Stock solutions

(i) A stock solution of barbituric acid was prepared by dissolving 1.000 g of the compound in 250 ml of 0.10M aqueous sodium carbonate solution. This solution thus contained 4.000 mg/ml of barbituric acid.

(ii) 0.50 M aqueous sodium nitrite solution was prepared by dissolving 6.900 g of sodium nitrite in 200 ml distilled water.

(iii) 1.0 ml of glacial acetic acid (density 1.06 g/ml, 96%) was diluted to 100 ml by addition of distilled water. The resulting solution was found to be $1.695 \times 10^{-2}\text{M}$.

(iv) Sodium acetate-acetic acid buffer solution was prepared [11] by mixing 26.5 ml of 2M

aqueous sodium acetate solution with 23.5 ml of 2M aqueous acetic acid solution. This solution when diluted ten times gave a solution with pH 4.69 to 4.71.

Preparation of the calibration curve

0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5 and 25.0 ml of stock solution of barbituric acid was taken in different 50-ml volumetric flask. In each of these flask was then added 5.0 ml of 0.50 M aqueous sodium nitrite solution, 10 ml of 0.0169 M aqueous acetic acid and 5.0 ml of sodium acetate acetic acid buffer solution. The volume of each mixture was raised to 50 ml by addition of distilled water. The flasks were thoroughly shaken and pH of each solution was checked. It was observed that pH of each of these solution was fairly constant ranging from 4.69 to 4.71. All these solutions were then heated at 60°C (using a hot water-bath) for 15 minutes. The solutions were then allowed to cool to room temperature (16-18°C) and absorbance of each solution was measured at 545 nm using an aqueous solution of sodium nitrite as reference. However, it was pre-established that the addition of the acetate buffer did not affect either the absorbance or the wavelength of the maximum absorption. The calibration curve was then made by plotting the absorbance as a function of concentration of barbituric acid. This gave rise to a linear plot (Fig. 1) with negative deviation starting at concentration of barbituric acid higher than 1.6 mg/ml. The calibration plot was subsequently used for

determination of barbituric acid itself as well as in mixtures containing barbituric acid and barbiturates.

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