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Spectrophotometric reaction rate method for determination of barbituric acid by inhibition of the hydrochloric acid–bromate reaction

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Abstract

A new kinetic-spectrophotometric method was developed for the determination of barbituric acid. The method is based on its inhibition effect on the reaction between hydrochloric acid and bromate. The decolorization of methyl orange by the reaction products was used to monitor the reaction spectrophotometrically at 510 nm. The variable affecting the rate of the reaction was investigated. The method is simple, rapid, relatively sensitive and precise. The limit of detection is 7.9×10^{-7} M and calibration rang is 1×10^{-6}–6.0×10^{-4} M barbituric acid. The linearity range of the calibration graph is depends on bromate concentration. The relative standard deviation of seven replication determination of 5.6×10^{-6} M barbituric acid was 1.8%. The influence of potential interfering substance was studied.

Keywords: Barbituric acid; Bromate; Methyl orange; Spectrophotometry; Inhibition

1. Introduction

Barbituric acid {2,4,6(1H,3H,5H)-pyrimidinetrione} is widely used in preparation of barbiturates, dyes and polymerization catalysts [1], pharmaceutical preparation and indicators [2], textile [3] and also been identified as an intermediate in many processes. It is known that barbituric acid itself has no affect on the central nervous system [4], however it is a precursor to medical barbiturates which can be lethal in excessive amounts [5]. Other work has shown that in mice, barbituric acid will cause liver and kidney weight increase [6]. Barbituric acid is also a precursor to derivates that have been shown to have antibacterial activity [7,8] and for tumor inhibitory agents [9]. Therefore, determination of trace amounts of barbituric acid is very important both in studies of biological and industrial processes. Different methods such as chromatography [10–12], mass spectrometry [13], capillary electrophoresis [14], infra-red spectrophotometry [15], spectrophotometric methods [16–21] and coulometric method [22] also have been reported for determination of barbituric acid. Some of these

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methods are time consuming and suffer from lack of selectivity or good sensitivity and/or have short linear dynamic range or have higher limit of detection and/or used reagents not commercially available. Recently, Medien and Zahran [23] used 1,4-naphthoquinone as a spectrophotometric reagent for barbituric acid determination, but the method is not sensitive and has high limit of determination (2.7–61.5 μg ml^{-1}) and has many interfering substances for barbiturate determination. R. Bartzatt [24] used sodium nitrite as a suitable reagent for colorimetric analysis of barbiturate, with linear range of 18.7–225 μg ml^{-1}. D. Nematollahi et al. [22] used controlled potential coulometric technique for barbituric acid analysis in the range of 1–200 μmol. The method is not very sensitive and has several interferences. Therefore, the need for a fast, low cost and sensitive method is obvious, especially for routine quality control analysis. According to our knowledge, there is not any kinetic-spectrophotometric procedure for determination of barbituric acid at trace level.

Kinetic spectrophotometric method [25] is one of the most attractive approaches for ultra trace determination of some species. Its advantage is that only a spectrophotometer is required as the main instrument. In this present work, a sensitive, facile, and relatively selective method was developed for the determination of barbituric (1×10^{-6}–6.0×10^{-4} M) acid based on its inhibiting effect on the reaction of bromate with hydrochloric acid. The decolorization of methyl orange by the reaction products was used to monitor the reaction spectrophotometrically at 510 nm.

2. Experimental

2.1. Reagents and chemicals

Doubly distilled water and analytical-reagent grade chemicals were used. Barbituric acid stock solution 0.010 M was prepared by dissolving barbituric acid (Fluka) in water.

A 0.010 M sodium bromate solution was prepared by dissolving 1.510 g $NaBrO_3$ (Merck) in water and diluting to 100 ml in a volumetric flask.

A solution of methyl orange (100 mg l^{-1}) was prepared by dissolving 0.0250 g methyl orange (Merck) in water and diluting to 250 ml with water. Hydrochloric acid was prepared by appropriate dilution of concentrated hydrochloric acid (Merck).

2.2. Apparatus

A Spectronic 20 (Genesys) spectrophotometer with two matched 1-cm quartz cells was used to get absorbance graph at various wavelengths. A Spectronic 20 (Genesys, Model 4001/4) spectrophotometer with a 1-cm glass cell was used to get absorption–time graphs at a fixed wavelength. A thermostat water bath (Memmert, Model KG 8540, Schwabachw, Germany) was used to keep the temperature of solutions at 35 ± 0.1 °C.

2.3. Recommended procedure

The inhibited reaction was followed spectrophotometrically by monitoring the change in absorbance of the mixed reagents solution at 510 nm. An aliquot of sample solution containing 1×10^{-5}–6.0×10^{-3} mol barbituric acid was transferred into a 10-ml volumetric flask, and then 1.4 ml of 2.33 M hydrochloric acid was added, followed by 1.0 ml 100 mg l^{-1} methyl orange solution. The solution was diluted to ca. 8 ml with water. Then 1.0 ml 1.44×10^{-3} M bromate was added to the solution and the result solution was diluted to the mark with water. The solution mixed and a portion of the solution was transferred to the spectrophotometric cell. The change in absorbance with time was measured for a 10–240 s from initiation of the addition of last drop of bromate solution. All the solutions were kept at the 35 °C.

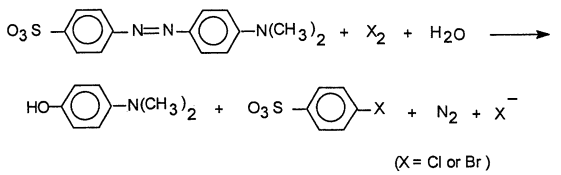
3. Results and discussion

Bromate can be reduced by hydrochloric acid as the following:

$$10Cl^- + 2BrO_3^- + 12H^+ \rightleftharpoons 5Cl_2 + Br_2 + 6H_2O$$

The produced bromine and chlorine react with methyl orange and this reaction cause decoloriza-

tion of methyl orange [26] as the following reaction:



This reaction can be monitored spectrophotometrically by measuring the decrease in absorbance of the reaction mixture at 510 nm at 35 °C. Barbituric acid can react with product of the reaction (bromine and chlorine); therefore, the induction period increases with increasing barbituric acid concentration (Fig. 1). This inhibitory effect on the reaction system depends on the concentration of barbituric acid. The induction period can be measured mathematically from the regression equations of the linear part of the absorption–time graph. The regression equation for the first linear part of the graph is:

$$A = a_1 + b_1 t$$

And for the second linear part is:

$$A = a_2 + b_2 t$$

By equating these equations the induction period can be calculated as:

$$T_{ip} = a_1 - a_2/b_2 - b_1$$

Therefore, the calibration graph can be prepared by plotting of t_{ip} versus barbituric acid concentration.

3.1. Influence of variables

The effect of reagents concentration and temperature on the reaction system was studied to get the best sensitivity and find optimum conditions. The influence of hydrochloric acid concentration on the sensitivity was studied over the range of 0.20–0.45 M with and without barbituric acid at 30 °C. Fig. 2 shows the change in absorbance with time as a function of hydrochloric acid. In addition, Fig. 3 shows that by increasing HCl concentration up to 0.35 M, the difference in absorbance change for uninhibited reaction and inhibited reaction increases, whereas greater amounts of the acid concentration decrease this difference. Therefore, 0.35 M HCl was selected for study.

Fig. 4 shows the effect of bromate concentration on the induction period time. The results show that by increasing bromate concentration, the induction period time decreased and the slope of

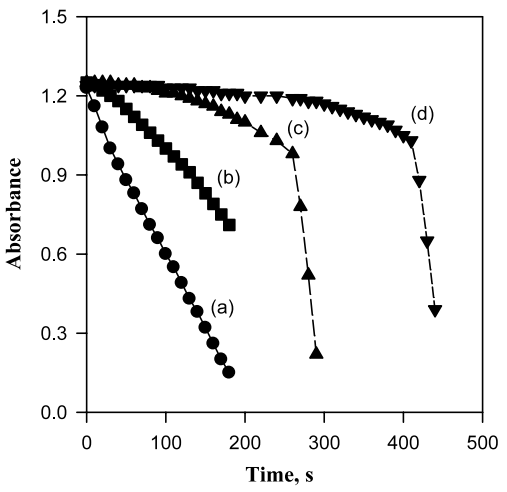


Fig. 1. Absorbance change of methyl orange–bromate–HCl system, (a) 0.00; (b) 2.6×10^{-6} M; (c) 7.2×10^{-6} M; and (d) 1.2×10^{-5} M barbituric acid. Conditions: 9.0 mg l^{-1} methyl orange, 1.0×10^{-4} M bromate, and 0.35 M HCl.

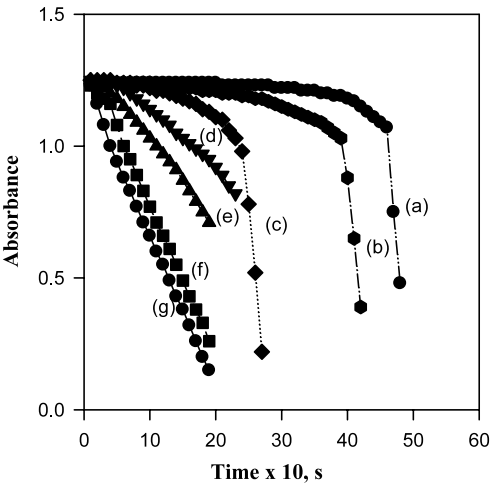


Fig. 2. Variation of absorbance with time as a function of hydrochloric acid; (a) 0.20 M; (b) 0.25 M; (c) 0.30 M; (d) 0.35 M; (e) 0.40 M HCl; (f) 0.45 M HCl; and (g) 0.50 M HCl. Conditions: 1.6×10^{-6} M barbituric acid, 1.44×10^{-4} M bromate, and 10.0 mg l^{-1} methyl orange.

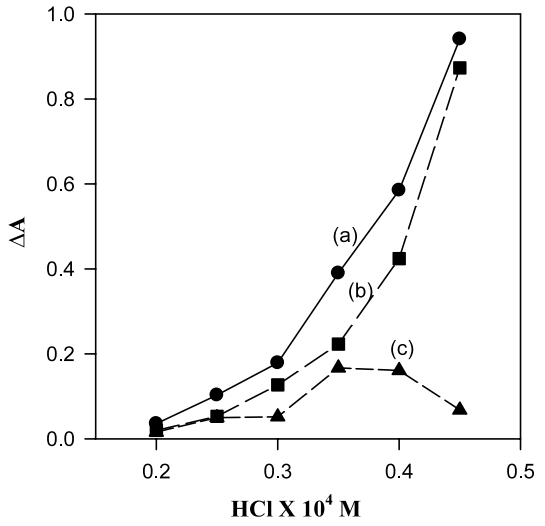


Fig. 3. Absorbance change for inhibited reaction (■), uninhibited reaction (●), and for their difference (▲) as a function of HCl concentration. Conditions: 1.6×10^{-6} M barbituric acid, 1.44×10^{-4} M bromate, and 10.0 mg l^{-1} methyl orange.

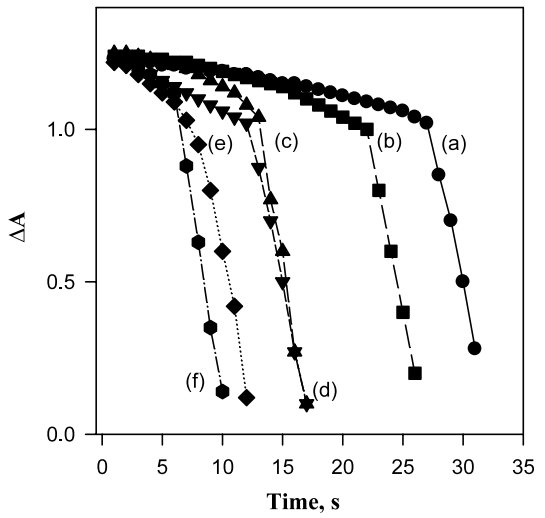


Fig. 4. Effect of bromate concentration on the induction period time; (a) 7.2×10^{-5} M, (b) 1.15×10^{-4} M, (c) 1.44×10^{-4} M, (d) 1.72×10^{-4} M, and (e) 2.16×10^{-4} M bromate. Conditions: 1.6×10^{-6} M barbituric acid, 0.35 M HCl, and 10.0 mg l^{-1} methyl orange.

the absorbance change increases for uninhibited reaction (after initiation of the reaction). In order to find the optimum concentration of bromate, the change in absorbance–time was plotted for the system with and without addition of barbituric acid (Fig. 5), for the first 10–240 s from initiation of the reaction. The results show that the best sensitivity can be achieved in the presence of 1.0×10^{-4} M bromate concentration. Therefore, this concentration was selected for the study.

The influence of methyl orange concentration on the sensitivity was studied in the range of 2–20 mg l^{-1} methyl orange and in the presence of 0.35 M HCl and 1.0×10^{-4} M bromate concentration at 30 °C with and without addition of barbituric acid. The results showed that by increasing methyl orange concentration up to 9.0 mg l^{-1}, the sensitivity increases, whereas greater amount of the dye decreased the sensitivity. Therefore, a 9.0 mg ml^{-1} methyl orange was selected for the study.

The sensitivity of the system dose not depends on the ionic strength up to 1.0 M ionic strength (using 3.0 M K_2SO_4).

The influence of temperature on the sensitivity was studied over the temperature range of 5–40 °C in the presence of optimum reagent concentration. The results showed by increasing temperature up to 30 °C the sensitivity increased, and after that the

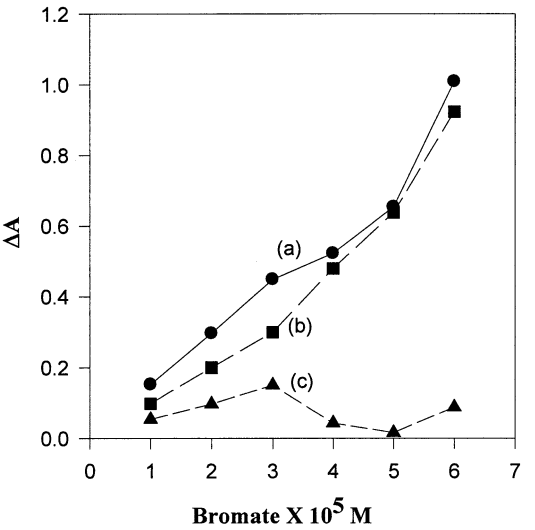


Fig. 5. Absorbance change for inhibited reaction (■), uninhibited reaction (●), and for their difference (▲) as a function of bromate concentration. Conditions: 1.6×10^{-6} M barbituric acid, 0.35 M HCl, and 10.0 mg l^{-1} methyl orange at 30 °C.

sensitivity decreased. Therefore, 30 °C was selected for the study.

3.2. Calibration graph, precision and limit of detection

Solutions of known barbituric acid concentrations are utilized for standard curve. Under the optimum conditions a linear correlation was found between the induction period and barbituric acid concentration. The linear dynamic range and depends on the bromate concentration the results are presented at Table 1. The linearity of the calibration curve is demonstrated over a 200-fold range of the concentration. The limit of detection ($3s_b/m$, three of the standard deviation blank divided to slope of the calibration curve) was 7.9×10^{-7} M, barbituric acid. The relation standard deviation for seven-replication determination of 1×10^{-6}, 5.6×10^{-6} and 1.1×10^{-5} M barbituric acid was 2.5, 1.8 and 1.5%, respectively.

4. Effect of interfering substances

The effect of various substances on the determination of 1.6×10^{-6} M barbituric acid was studied and the results are shown in Table 2. The tolerance was defined as the concentration of added substance causing a relative error less than 3%. Many substances did not interfere, even when present in 500-fold excess over barbituric acid. The results show good selectivity of the method for barbituric analysis. To evaluate the performance of the method for analysis of real sample, the determination of barbituric acid in top water as a synthetic sample was investigated. The results are shown in Table 3 with satisfactory results.

Table 2
Tolerance limit for diverse ions on the determination of 1.6×10^{-6} M barbituric acid

Substance	Tolerance limit ratio ($mole_{Substance}/mole_{Barbituric\ acid}$)
$C_2O_4^{2-}$, ClO_3^-, K^+, Na^+, NO_3^-, SO_3^{2-}, SO_4^{2-}, MoO_4^{2-}, PO_4^{3-}, Cd(II), Mn(II), Cs^+, Na^+, K^+, $B_4O_7^{2-}$, acetone, urea, ethanol, sucrose, glucose, fructose, galactose	1000[a]
Pb(II), Cu(II), Mn(II), Al(III), ClO_3^-, HCO_3^-, EDTA, acetate	500
Citrate, benzoic acid, maleic acid, tartaric acid, Co(II), Ni(II)	250
Fe(III), Zn(II), CN^-, salicyl aldehyde, phenobarbital	100

[a] Maximum concentration of substances tested.

Table 3
Determination of barbituric acid in synthetic samples

Sample number	Barbituric acid (μM)		Recovery%
	Added	Found ($n = 5$)	
I	1.50	1.46+0.06	97.3
II	2.00	1.96+0.05	98.0
III	2.50	2.48+0.04	99.0
IV	3.00	3.04+0.02	101.5
V	3.50	3.40+0.02	97.0
VI	4.00	4.12+0.03	103.0

5. Conclusion

With the proposed kinetic method it is possible to determine barbituric acid at ultra trace levels. Because the detection limit is dependent on the concentration of bromate, it is expected that the limit could be lowered further if lower concentration of bromate are selected. The method is very

Table 1
Linear regression parameters of calibration data for different concentration of bromate

BrO_3^- (M)	Slope (s/M)	Intercept (s)	r ($n = 13$)	Detection limit (M)	Calibration range (M)
1.0×10^{-4}	217.4×10^5	65.99	0.9991	7.9×10^{-7}	1.6×10^{-6}–1.8×10^{-4}
5.0×10^{-4}	15.2×10^5	90.33	0.9998	5.5×10^{-6}	8.0×10^{-5}–6.0×10^{-4}

simple, rapid, sensitive and relatively selective for determination of barbituric acid. By dependence of the rate of reaction with temperature (log(k) vs. 1/T), the activation energy of the reaction was equal to 28 ± 1.2 kJ mol^{-1}. The procedure is suitable for the analysis of various pharmaceutical samples, with satisfactory results.

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