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Kinetic Spectrophotometric Method for Trace Amounts Determination of Bromide in Pharmaceutical Samples Using Janus Green-Bromate System

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Abstract

A new simple and rapid kinetic spectrophotometric method has been developed to trace amounts determination of bromide. This method is based on the catalytic effect of bromide on the reaction between Janus Green and bromate in sulfuric acid media. The reaction was followed spectrophotometrically by measuring the absorbance at 618 nm. The fixed-time method was used for the first 210 s. The influence of reagents concentration, temperature and time on the sensitivity was studied. Under optimum experimental conditions, bromide can be determined in the range of 10.0-1800.0 µg/L. The relative standard deviations (n = 10) were 0.22 and 0.19% for 100.0 and 1000.0 µg/L of bromide, respectively. The detection limit of the proposed method was 4.1 µg/L. The influence of potential interfering of some ions and biological species on the selectivity was studied. The proposed method was successfully applied for the determination of bromide in pharmaceutical samples. The new developed method was found to have fairly good selectivity, sensitivity, simplicity and rapidity.

Keywords: Bromide, Janus Green, Kinetic spectrophotometery, Pharmaceutical samples

1. Introduction

Bromide is one of the constituents of ground water, surface water and sea water. Some oxidizing agents in such waters may oxidize bromide to liberate bromine. Bromide can combine with many types of organic pollutants present in waters to form toxic compounds of bromo-derivatives, which can cause serious harm to human health and environment. Moreover, bromide in source water for potable water is the one of precursor to the formation of bromate, which is harmful disinfection byproduct in drinking water. Concentration range of bromide in natural waters and biological fluids is 10^{-8} to 10^{-4} M [1].

Some active ingredients in drug formulations are in the form of bromide salts of weak organic bases. The development of suitable methods for their analysis has been a subject of active interest. Some of the active ingredients that are nowadays frequently used, and which have been subject of this study are: hyoscine butyl bromide ($C_{21}H_{30}BrNO_4$) and clidinium bromide $C_{22}H_{26}BrNO_3$. Therefore, sensitive and selective methods are required for their reliable quantification.

Bromide has been determined using a wide variety of analytical techniques, such as inductively coupled plasma-mass spectrometry [2, 3], ion chromatography [4-6], gas

chromatography-mass spectrometry [7, 8] and liquid chromatography-mass spectrometry [9, 10]. Although these methods are highly sensitive, their instruments are expensive Moreover, they suffer from long times due to the necessity of the sample preparation steps.

Catalytic spectrophotometric methods having excellent sensitivity and sufficient accuracy without expensive or special equipments are the most suitable methods for the determination of trace elements in food, water and biological samples [11, 12]. Several kinds of kinetic-spectrophotometric methods have been reported based on the catalytic effect of bromide [13-27].

In our investigation of bromide-catalyzed oxidation of Janus Green (JG), a kinetic spectrophotometric method for the determination of trace amounts of bromide is proposed. The reaction was followed spectrophotometrically by monitoring the decrease in absorbance at 618 nm with a fixed time of 30-210 s. It was found that in acidic bromate solution bromide catalyzed oxidation of JG. Thus, we developed a simple, rapid, sensitive and selective method for the kinetic determination of bromide.

2. Experimental

2. 1. Reagents and chemicals

All chemicals used were of analytical reagent grade and were used without further purification. Doubly distilled water was used throughout in the experiments. 100 mL standard stock bromide solution of 100.0 mg/L was prepared by dissolving 0.0145 g of potassium bromide (Merck) in water. Working solutions were obtained by appropriate dilution with water. Potassium bromate stock solution of 0.25 M was prepared by dissolving 10.4380 g of $KBrO_3$ (Merck) in 250 mL volumetric flask. JG solution (1.0×10^{-3} M) was prepared by dissolving 0.1278 g of JG in appropriate amount of water and diluted to 250 mL in a volumetric flask. Sulfuric acid solution 2.0 M was prepared by dissolving of an appropriate amount of concentrated sulfuric acid in water and diluting to 500 mL.

2. 2. Sample preparation

Analysis of pharmaceutical samples was performed by grinding of some tablets. Then 0.05 g of powder dissolved in solvent (1:1 of water and ehanol) and passed through a filter paper (Whatman No. 1) in a 100 mL volumetric flask. Suitable aliquots of sample solution were analysed according to the procedure for bromide determination.

2. 3. Apparatus

A Shimadzu spectrophotometer 160A with 1-cm glass cell was used for absorbance measurement at a fixed wavelength. A thermostat (Heidolph-Germany) with temperature measurement precision ± 0.1 ^{o}C was used for controlling of temperature.

2. 4. Recommended procedure

To a series of 10mL volumetric flasks, 1.0mL of 2.0 M sulfuric acid, 0.3mL of 1.0×10^{-3} M JG solution , 1.0 mL of 10.0 mg/L (10.0 µg) bromide and 1.0 mL of 0.25 M bromate solution were added. The stopwatch was started immediately after adding last drop of bromate (oxidant). The solution was diluted to the mark with double distilled water and mixed well.

An aliquot of the reaction mixture was immediately transferred to a glass cell and the absorbance was recorded as a function of time. The catalyzed (Fig. 1) as well as uncatalyzed (inset of Fig. 1) reaction was followed spectrophotometrically by monitoring the decrease in absorbance at 618 nm after 30 s of initiation up to 210 s to use as fixed time measurement of initial rate. The absorbance changes of the catalyzed and uncatalyzed reactions were labeled ΔA_c and ΔA_u, respectively. The calibration graph was constructed by plotting analytical signal ($\Delta A=\Delta A_c - \Delta A_u$) versus the bromide concentration.

The spectrum was shown that the absorption of JG in 618 nm was reduced with adding bromide in different times.

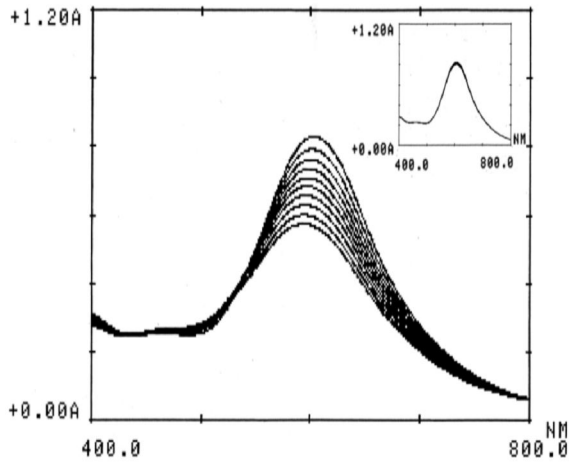


Fig. 1. Catalytic effect of bromide on the rate of reaction. Sample; H_2SO_4, 1.0 mL (2.0 M); Janus Green, 0.3 mL (1.0×10^{-3} M); Br^-, 10.0 μg; BrO_3^-, 1.0 mL (0.25 M); temperature, 25°C; time, 3.5 min. Inset shows the blank spectrum; H_2SO_4, 1.0 mL (2.0 M); Janus Green, 0.3 mL (1.0×10^{-3} M); BrO_3^-, 1.0 mL (0.25 M); temperature, 25°C; time, 3.5 min.

3. Results and Discussion

3. 1. Response behavior

JG (3-diethylamino-7-(4-dimethylamino phenyl azo)-5-phenylphenazinium chloride) is an azo dye (scheme.1) that can be oxidized by strong oxidizing agents such as bromate in acidic media at slow reaction (1). Reagent oxidation take place in N=N bond to produce a colorless oxidized form [28]. Bromide ion can increase rate of reaction at ultra-trace level. Mechanism of the JG reaction may be contributed to the following reactions:

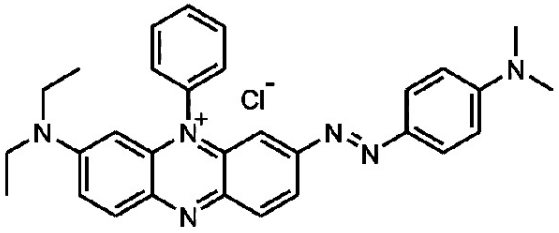
$$JG_{(Red)} + BrO_3^- + H^+ \rightarrow JG_{(Ox)} + Br^- \quad (1)$$

$$BrO_3^- + H^+ + Br^- \rightarrow Br_2 + H_2O \quad (2)$$

$$JG_{(Red)} + Br_2 \rightarrow JG\,Br^+ + Br^- \quad (3)$$

$$JG\,Br^+ + Br_2 + H^+ \rightarrow JG_{(Ox)} + Br^- \quad (4)$$

where Red is reduced form and Ox is the oxidized form of JG. In the presence of bromide, Br_2 generation (2) is very fast. Br_2 generated in situ attacked to the amine groups of JG and produced intermediate product (3). Then excess values of Br_2 oxidize JG Br^+ structure by breaking N=N bond [29, 30].



Scheme 1. Molecular structure of Janus Green.

3. 2. Optimization of reagents concentration

In order to establish experimental conditions under which the catalytic effect of bromide and therefore the sensitivity in its determination to be at maximum, the dependence of rate on temperature, time and reagents concentration were studied. The change in absorbance after fixed time as measure of initial rate, were used to plot the graph for each variables. Optimum conditions were taken from the graphs for the subsequent study of the variables. The reagent concentration optimization was carried out on the catalyzed and uncatalyzed reactions for a constant time of 210 s in the presence of 10.0 μg of bromide.

The effect of sulfuric acid on the uncatalyzed and catalyzed reactions was studied in the concentration range 0.04 to 0.16M. As shown in Fig 2, the reaction rate increases with increasing concentration of sulfuric acid up to 0.08 M. At higher concentrations, the reaction rate was decreased. This decrease at higher acidic conditions may be attributed to protonation of JG, which might stop oxidation or make oxidation quite difficult to occur. Thus, 0.08 M of sulfuric acid was used for further study.

The experimental results on the study of JG concentration effect in the range 1.0×10^{-5} to 4.0×10^{-5} M indicate that the absorbance differences increases almost linearly with the concentration of JG at 3.0×10^{-5} M (Fig. 3). Therefore, 3.0×10^{-5} M of JG was selected as the optimum concentration.

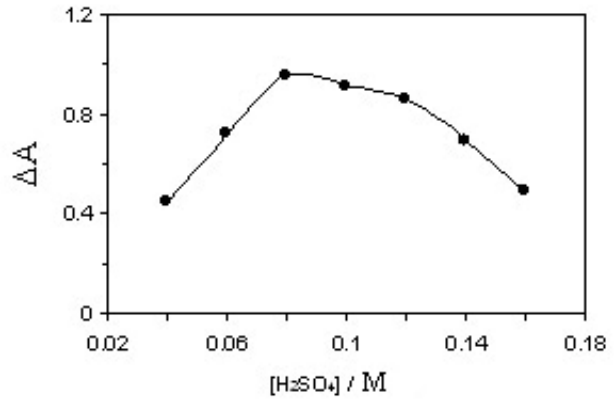


Fig. 2. Effect of sulfuric acid concentration on the rate of reaction: Janus Green, 0.3 mL (1.0×10^{-3} M); Br^-, 10.0 µg; BrO_3^-, 1.0 mL (0.25 M); temperature, 25°C; time, 3.5 min.

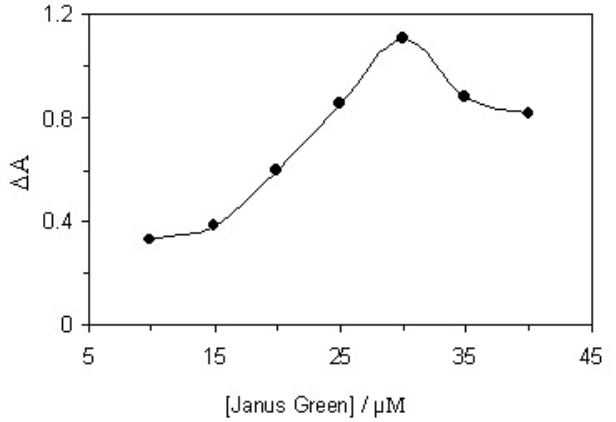


Fig. 3. Effect of Janus Green concentration on the reaction rate: H_2SO_4, 0.4 mL (2.0 M); Br^-, 10.0 µg; BrO_3^-, 1.0 mL (0.25 M); temperature, 25°C; time, 3.5 min.

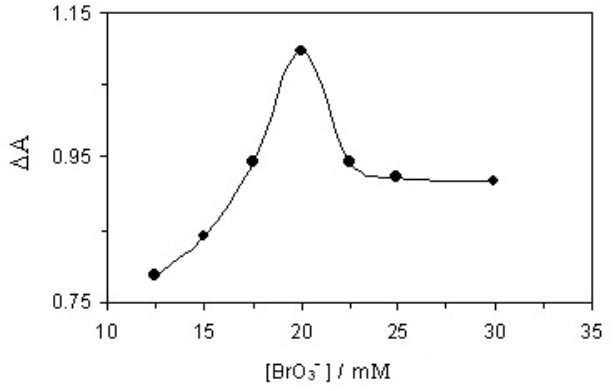


Fig. 4. Effect of bromate concentration on the reaction rate: H_2SO_4, 0.4 mL (2.0 M); Janus Green, 0.3 mL (1.0×10^{-3} M); Br^-, 10.0 µg; temperature, 25°C; time, 3.5 min.

The dependence of bromate concentration and rate of catalyzed oxidation of JG was studied in the range of 0.0125 to 0.03 M of bromate. As shown in Fig. 4, under optimum concentrations of H_2SO_4 and JG, the reaction rate increased up to 0.02 M of bromate. Therefore, 0.02 M of bromate was selected for further study.

The effect of ionic strength was studied by 3.0 M of KNO_3 solution under optimum reagents concentration in the range of 0.09 to 0.3 M. At 0.12 M of KNO_3 concentration, the change in absorbance was maximum. Therefore, this concentration was selected as optimized value.

Temperature effect on the rate of reaction was studied in the range of 15 to 45 °C under optimum reagents concentration. Increasing the temperature up to 35 °C caused the change in absorbance increased. Thus, 35 °C was selected as optimum.

90.0 s as optimized time was found by measuring the absorbance and changing in it during 30 to 210 s. The reaction rate increases up to 90.0 s. At still higher time, the rate is almost constant.

3. 3. Analytical parameters

Calibration graph was obtained by applying the fixed time method under the optimum conditions (0.08 M of sulfuric acid, 3.0×10^{-5} M of JG, 0.02 M of bromate, 0.12 M of KNO_3, 35 °C and 90.0 s). A plot of the absorbance changes versus bromide concentration in the reaction mixture is linear in the range of 10.0–1800.0 µg/L of bromide. The linear regression equation relating initial rate, is given in Eq. (5).

$$\Delta A = 0.0002\,[Br^-] + 0.9095 \quad (R^2 = 0.9994) \qquad (5)$$

The detection limit ($3S_b/m$) was 4.1 µg/L of bromide. The relative standard deviations (n = 10) were 0.22 and 0.19% for 100.0 and 1000.0 µg/L bromide, respectively.

3. 4. Interference studies

For investigation of analytical applicability of the proposed method, the influence of biological compounds and inorganic ions were tested by using the standard solution of bromide (500.0 µg/L). The results are given in

Table 1. As can be seen, most of cations and anions do not interfere up to 1000 fold. Interfering effect of nitrite was removed by adding 3.0 mL of 5% sulfamic acid solution to each sample. Addition of 3.0 mL of 5% NaF for removing of Fe^{3+} that presence in water samples was done.

3. 5. Application

To examine the applicability and validity of the proposed method, pharmaceutical products were analysed as in the procedure. The results that shown in Table 2 having good repeatability and accuracy in relation to the standard method [31] and statistical tests confirm this declare.

4. Conclusions

This paper reports a sensitive and selective spectrophotometric method for trace determination of bromide using JG as a new reagent. The method possesses distinct advantages over existing methods [2-10] that used to expensive and complex instruments at least in simplicity and ease of operation. Moreover, no extraction step is required and hence, the use of organic solvents, which are generally toxic pollutants, is avoided. The proposed method can serve as an alternative method for bromide determination. Some analytical parameters of the developed method over the several latest reported methods [23-27] are summarized in Table 3.

Table 1. Tolerance limit of biological compounds and diverse ions on the determination of 500.0 μg/L of bromide ion.

Foreign species	Tolerance limit ($W_{substance}$ / $W_{bromide}$)
Glucose, saccarose, fructose, EDTA, methanol, ethanol, sulfamic acid	1000
Benzaldehyde, acetaldehyde	400
Formaldehyde	50
Li^+, Na^+, K^+, Mg^{2+}, Al^{3+}, NH_4^+, Co^{2+}, Cd^{2+}	1000
Ca^{2+}, Ba^{2+}	850
[a]Fe^{3+}	800
As^{3+}, Cr^{3+}	100
Sn^{2+}, Zn^{2+}	50
NO_3^-, SO_4^{2-}, CO_3^{2-}, CH_3COO^-, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, F^-, $S_4O_6^{2-}$	1000
[b]NO_2^-	700
SCN^-	40
Cl^-, I^-	20
$S_2O_3^{2-}$	10
VO_3^-	5
ClO_3^-, ClO_4^-	0.4

[a] After masking with 3.0 mL of 5% NaF.
[b] Removed by adding 3.0 mL of 5% sulfamic acid.

Table 2. Determination of bromide in pharmaceutical products

Sample	Composition of tab. (w/tab.)	Certified value of bromide (mg)	Bromide found[a]		Recovery (%)	t test[b]	F test[c]
			Pro. Met.	Std. Met.[31]			
Hyoscine -N- butyl bromide[d]	$C_{21}H_{30}BrNO_4$ (10 mg)	1.81	1.76 ± 0.07	1.79 ± 0.05	97.2	1.59	0.96
Clidinium bromide [e]	$C_{22}H_{26}BrNO_3$ (5 mg)	0.83	0.85 ± 0.05	0.80 ± 0.06	98.8	1.24	1.44

[a] Mean ± standard deviation (n= 5).
[b] Tabulated t-value for 4 degrees of freedom at P (0.95) is 2.776.
[c] Tabulated F-value for (4, 4) degrees of freedom at P (0.95) is 6.39.
[d] Tehran chemie, Iran.
[e] Sobhan pharm. Co. Iran.

Table 3. Comparison of some analytical parameters of the present work with other methods

Reaction system	Dynamic range (μg/L)	Detection limit (μg/L)	Precision (RSD %)	Application	Ref.
*DPC-Cr(VI)-IO_3^-	500.0-8000.0	250.0	1.57 (n=10)[a]	Sea water	[23]
**m-CSP-IO_4^-	160.0-20000.0	150.0	2.1 (n=10)[b]	River water & tap water	[24]
Methylene blue-H_2O_2	0.0-3200.0	100.0	0.74 (n=5)[c]	Sea water	[25]
KBr-$KBrO_3$	1.28-232.80	400.0	–	Sea water	[26]
Methylene blue-H_2O_2	80.0-960.0	35.0	1.4 (n=10)[d]	Sea water	[27]
Janus Green-BrO_3^-	10.0-1800.0	4.1	0.19 (n = 10)[e]	Pharmaceutical samples	

[a] For determination of 1000.0 ppb of bromide.
[b] For determination of 1000.0 ppb of bromide.
[c] For determination of 2400.0 ppb of bromide.
[d] For determination of 480.0 ppb of bromide.
[e] For determination of 100.0 ppb of bromide.
***** DCP: Diphenylcarbazide
****** m-CSP: m-cresolsulfonephthalein

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