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Sub-ppm determination of uric acid using a sensitive kinetic-catalytic reaction system

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Abstract: A sensitive and selective kinetic-spectrophotometric method was developed for the determination of trace amounts of uric acid. The method is based on the inhibitory effect of uric acid on the oxidation of Janus Green by bromate in the presence of sulfuric acid. The reaction was followed spectrophotometrically by measuring the absorbance at 518 nm using the fixed-time method. Under optimum experimental conditions (Janus Green, 30.0 μmol L^{-1}; sulfuric acid, 70.0 mmol L^{-1}; bromate, 3.75 mmol L^{-1}; 25 °C and 4.0 min), the calibration curve was linear over the range 0.3 - 46.1 μg mL^{-1} of uric acid and the detection limit was obtained 0.08 μg mL^{-1}. The relative standard deviations (n=6) of 1.0 and 10.0 mg L^{-1} of uric acid were 1.14 and 1.08%, respectively. The influence of different foreign species that co-exist with uric acid was investigated in different matrices. The developed method was successfully applied for the determination of uric acid in biological samples.

Key words: Uric acid, Janus Green, Kinetic spectrophotometry, Biological sample

1. Introduction

Uric acid, the final oxidation product of purine metabolism, is a heterocyclic compound firstly that synthesized by Hoffmann [1]. Gout, a type of arthritis, can be resulted to high blood concentrations of uric acid.

The chemical is associated with other medical conditions including diabetes and the formation of ammonium acid urate kidney stones. In humans, about 70% of daily uric acid disposal occurs via the kidneys, and in 5-25% of humans, impaired renal (kidney) excretion leads to hyperuricemia. In humans, over half of the antioxidant capacity of blood plasma comes from uric acid and excreted in urine. Therefore, uric acid concentrations in blood plasma and urine can be used for following the diseases and developed a sensitive method for its quantitative determination can be attractive for clinical researches [2, 3].

Various analytical techniques have been developed for the determination of uric acid such as electrochemical methods using different electrode materials and modifiers [4-8], high performance liquid chromatography [9], high performance liquid chromatography-mass spectrometry [10], capillary electrophoresis [11], ion chromatography [12] and flow injection [13]. Low repeatability is the most important limitation of the electrochemical methods. Also, hard operation and high cost are the main drawback of the chromatographic methods. Therefore, it is necessary to develop a simple, low cost and accurate method.

Kinetic-spectrophotometric method is an attractive alternative method for the determination of different species in various matrices such as morphine [14], iodide [15] and vanadate [16], because they have advantages such as high sensitivity, sufficient accuracy, simple procedures and the necessity of less expensive apparatus. To the best of our knowledge, a few reports have been found for kinetic spectrophotometric determination of uric acid [17, 18]. The methods have shortages in limit of detection and linear range.

In this study, we propose a simple and sensitive kinetic spectrophotometric method for the determination of uric acid based on its inhibitory effect of it on the oxidation of Janus Green (JG) with bromate in presence of sulfuric acid. The absorbance change of JG at 618 nm is proportional to the uric acid concentration. The reaction was followed spectrophotometrically by monitoring the decrease in absorbance at 618 nm. This method has been successfully applied to the determination of uric acid in biological samples with different matrices.

2. Experimental

2.1. Material and Methods

Analytical-grade chemicals and double distilled water were used. At first, 50 mL of a uric acid solution of 200.0 μg mL^{-1} was prepared by dissolving 0.0100 g of uric acid (Merck) in water and diluting to the mark in a volumetric flask daily. A solution of Janus Green (4.0×10^{-4} mol L^{-1}) was prepared by dissolving 0.2044 g of it in water and then diluting to 1000 mL in a volumetric flask. A sulfuric acid solution (1.0 mol L^{-1}) was prepared by diluting appropriate amount of sulfuric acid (a = 98%, d = 1.84 kg L^{-1}) to 100 mL; a 0.025 mol L^{-1} of potassium bromate stock solution was subsequently prepared by dissolving 4.1725 g of $KBrO_3$ (Merck) in water and diluting to 1000 mL in a volumetric flask.

2.2. Apparatus

A Jenway double beam UV-Vis spectrophotometer (6800, UK) with 1-cm glass cell was used to measure the absorbance. A thermostated water bath (Heidolph, Germany) was used to keep the temperature of all solutions at the working temperature (25.0 ± 0.1°C). A stop-watch was used to record the reaction time.

2.3. General Procedure

After initial kinetic spectrophotometric studies of the reaction, the reagent concentrations (except the uric acid) were judiciously chosen for the analytical procedure. To a series of 10 mL calibrated flasks, 0.8 mL of a 4.0×10^{-4} mol L^{-1} JG solution, 0.9 mL of 1.0 mol L^{-1} sulfuric acid solution, and 0.1 mL of 200.0 µg mL^{-1} of uric acid solution were added. Then, 1.2 mL of 2.5×10^{-2} mol L^{-1} bromate solution was added and diluted to the mark with double distilled water. The time measurement started just after adding the last drop of the bromate solution. After thorough mixing, a portion of this solution was transferred to a glass cell, and the absorbance was measured against water at 618 nm and 25 °C for fixed times of 0.5 and 5 min. The measurements were repeated in the absence of uric acid to obtain the value for the uninhibitored

reaction as the absorbance of blank (The visible spectrum of blank was shown in Figure 1). The absorbance changes of the inhibitored and uninhibitored reactions were labeled ΔA_s and ΔA_b, respectively. The difference in the absorbance ($\Delta A = \Delta A_b - \Delta A_s$) was considered as the response.

2.4. Biological sample preparation

Human urine and serum were used as biological samples for the determination of uric acid. At first, each sample was spiked with different amounts of uric acid (in two linear segments of calibration curve) and C_{18} cartridge (Supelco Inc., 10 mL) was used for purification and pre-concentration of uric acid [20]. Then, the extracted uric acid was determined by the proposed method.

3. Results and discussion

JG (8-(4-Dimethylaminophenyl)diazenyl-N,N-diethyl-10-phenylphenazin-10-ium-2-amine chloride, see Scheme 1 for molecular structure) is a basic dye that can be oxidized by oxidizing agents, such as bromate in acidic media at a slow reaction to produce a colorless oxidized form. It was used as an indicator for the quantitative determination of different species such as opiates [14, 19].

The reaction mechanism for JG-bromate system may be represented as follow:

$$JG_{(Red)} + BrO_3^- + 6H^+ \rightarrow JG_{(ox)} + Br^- + 3H_2O \quad (1)$$

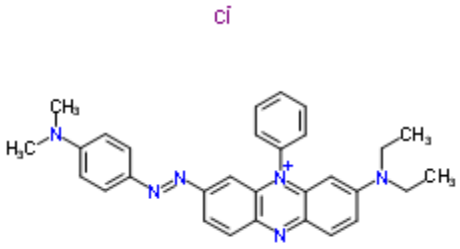
$$4\ BrO_3^- + 8Br^- + 12H^+ \rightarrow 6\ Br_2 + 6\ H_2O \quad (2)$$

$$JG_{(Red)} + Br_2 + H^+ \rightarrow JG_{(ox)} + 2\ Br^- \quad (3)$$

Since uric acid reacts rapidly with bromine, uric acid shows an inhibitory effect.

$$\text{Uric acid}_{(Red)} + Br_2 + H^+ \rightarrow \text{Uric acid}_{(Ox)} + Br^- \quad (4)$$

Where Red and Ox are the reduced and oxidized form of the reactant. Therefore, the level of inhibitation depends on the amount of uric acid in the reaction system and this behavior lead to developing an analytical method for the determination of uric acid.



Scheme 1: Molecular structure of Janus Green B

3.1. Optimization of Reaction Variables

In order to establish the experimental conditions under which the inhibitory effect of uric acid, the dependence of the reaction rate to reagents concentration, temperature and time were investigated.

The change in absorbance after fixed time as a measure of initial rate was used to optimize each variable. The reagent concentration optimization was carried out on the inhibitored and uninhibitored reactions for a constant time in the presence of 0.1 mL of 200.0 µg mL^{-1} of uric acid.

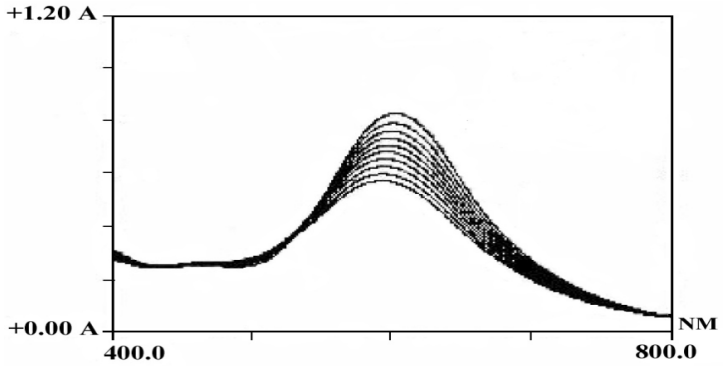


Figure 1: The visible spectrum of the reaction system. (Conditions: 2.56×10^{-5} mol L^{-1} JG, 90.0 mmol L^{-1} sulfuric acid, 3.0 mmol L^{-1} bromate , 25 °C and 5.0 min).

3.2. Effect of Janus Green Concentration

The experimental results on the study of JG concentration effect in the range 20.0 to 32.0 μmol L^{-1} indicates that difference in absorbance increases with the concentration of JG up to 30.0 μmol L^{-1} (Figure 2). Therefore, 30.0 μmol L^{-1} of JG was selected as optimum value. This may be due to aggregation of JG at higher concentrations.

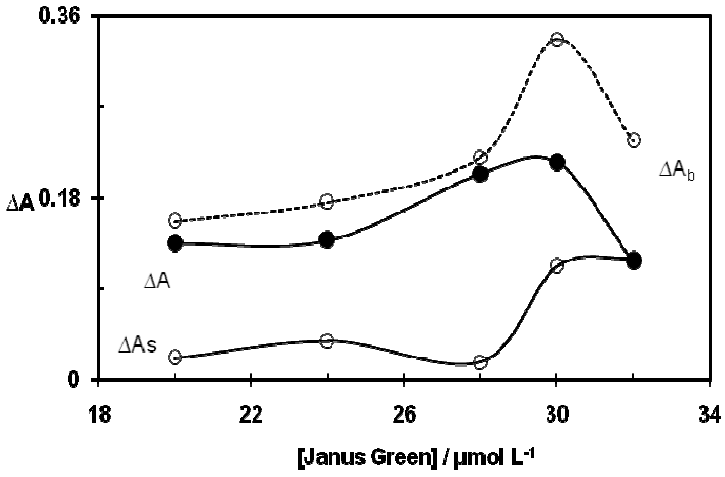


Figure 2: Effect of Janus Green concentration on the rate of uninhibitored (ΔA_b), inhibitored (ΔA_s) reactions and response (ΔA). (Conditions: sulfuric acid, 90.0 mmol L^{-1}; uric acid, 2.0 μg mL^{-1}; bromate, 3.0 mmol L^{-1}; 25 °C and 5.0 min).

3.3. Effect of Sulfuric acid Concentration

The effect of sulfuric acid concentration on the uninhibitored and inhibitored reactions was studied in the concentration range 55.0 to 120.0 mmol L^{-1}. As shown in Figure 3, the reaction rate increases with increasing concentration of sulfuric acid up to 70.0 mmol L^{-1} and decreases at higher concentrations that may be attributed to the protonation of JG which might stop oxidation or make oxidation quite difficult to occur. Thus, 70.0 mmol L^{-1} of sulfuric acid was used for further study.

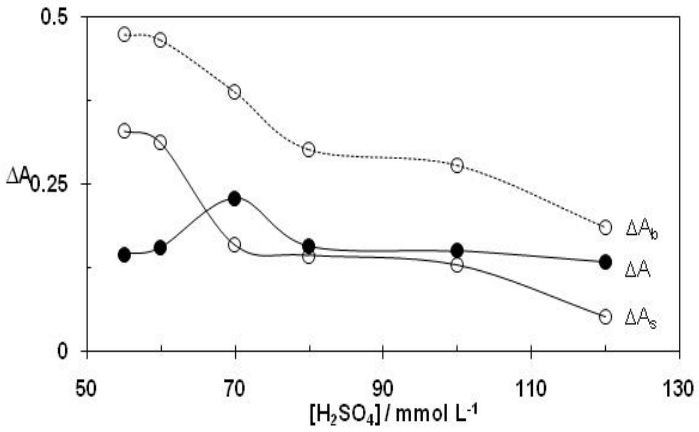


Figure 3: Effect of sulfuric acid concentration on the rate of uninhibitored (ΔA_b), inhibitored (ΔA_s) reactions and response (ΔA). (Conditions: Janus Green, 30.0 μmol L^{-1}; uric acid, 2.0 μg mL^{-1}; bromate, 3.0 mmol L^{-1}; 25 °C and 5.0 min).

3.4. Effect of Bromate Concentration

The dependence of oxidation reaction rate to bromate concentration was studied over the range of 2.5 to 5.5 mmol L^{-1}. As shown in Figure 4, under optimum concentrations of H_2SO_4 and JG, the reaction rate increased up to 3.75 mmol L^{-1} of bromate. Therefore, the optimum value of 3.75 mmol L^{-1} of bromate was selected for the following procedure. Increasing the reaction rate on inhibitored reaction along with the uninhibitored reaction resulted to decrease in ΔA at higher concentrations.

3.5. Effect of Temperature

Under optimum reagents concentration, temperature effect on the rate of reaction was studied in the range of 15 - 35 °C. As shown in Figure 5, the change in absorbance was increased up to 25 °C. Thus, 25 °C was selected as optimum.

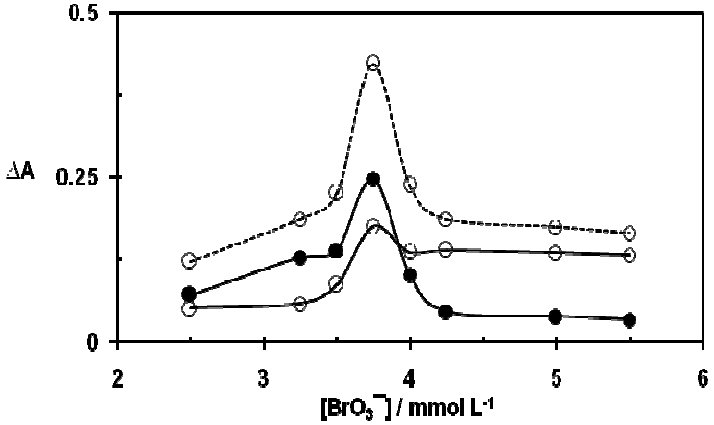


Figure 4: Effect of bromate concentration on the rate of uninhibitored (ΔA_b), inhibitored (ΔA_s) reactions and response (ΔA). (Conditions: Janus Green, 30.0 μmol L^{-1}; sulfuric acid; 70.0 mmol L^{-1}; 25 °C and 5.0 min).

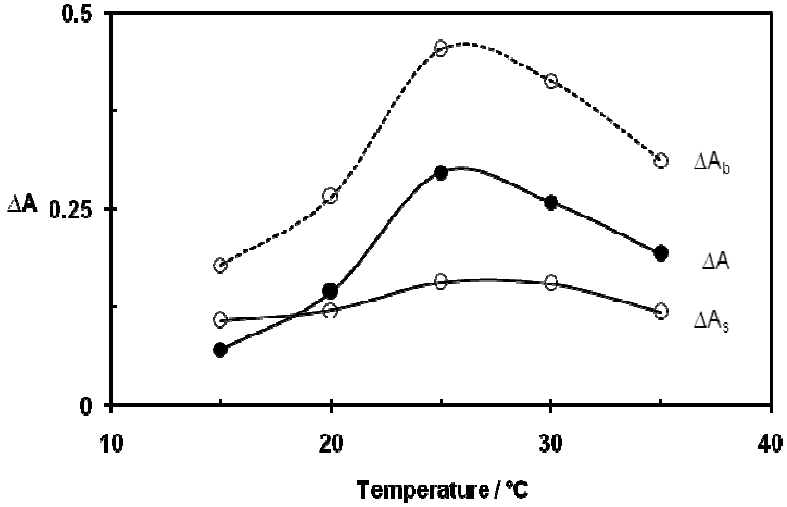


Figure 5: Effect of temperature on the rate of uninhibitored (ΔA_b), inhibitored (ΔA_s) reactions and response (ΔA). (Conditions: Janus Green, 30.0 μmol L^{-1}; sulfuric acid, 70.0 mmol L^{-1}; bromate, 3.75 mmol L^{-1} and 5.0 min).

3.6. Effect of Reaction Time

The optimum time was found by measuring the change in the absorbance during 30 - 390 s. The reaction rate was increased up to 240 s, and for longer times was almost constant. Therefore, 240 s was selected as optimum for further study.

3.7. Analytical Parameters

Under optimum experimental conditions, calibration curve was obtained over the range of 0.3 - 46.1 μg mL^{-1} of uric acid using fixed time procedure including two linear ranges (Figure 6).

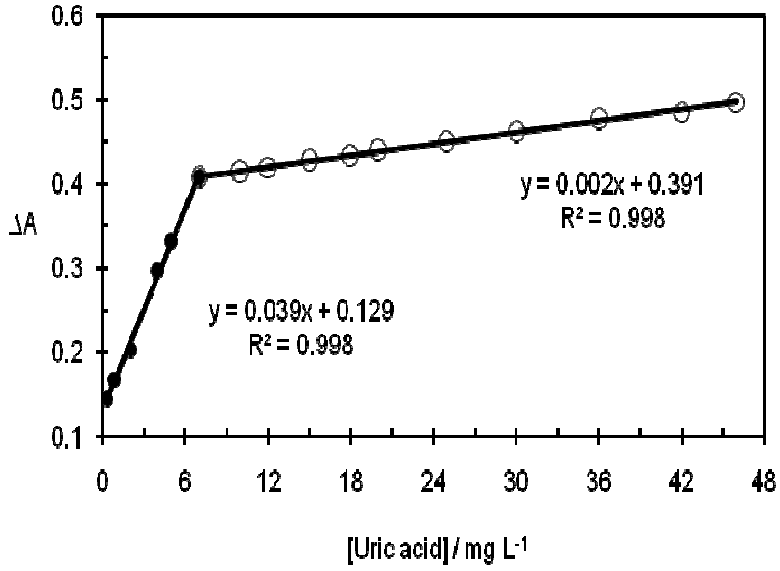


Figure 6: Calibration curve for the determination of uric acid (Conditions: Janus Green, 30.0 μmol L^{-1}; sulfuric acid, 70.0 mmol L^{-1}; bromate, 3.75 mmol L^{-1}; 25 °C and 4.0 min).

Table 1. Interfering effect of foreign species on the determination of 2.0 μg mL^{-1} of uric acid.

Foreign species	Tolerance limit ($W_{Species}/W_{Uric\ acid}$)
Li^{+}, Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}	1000
NO_3^{-}, SO_4^{-2}, $CH_3CO_2^{-}$, $PO4^{-3}$, $H_2PO_4^{-}$, F^{-}	1000
Saccarose, sulfamic acid	1000
Methanol	985
Ethanol	960
Glucose, fructose	950
Fe^{+3} [a]	700
NO_2^{-} [b]	540
Urea	140
I^{-}, Cl^{-}, Br^{-}	15

[a] After adding 3.0 mL of 5% NaF.

[b] After adding 3.0 mL of 4% sulfamic acid.

Table 2. Determination of uric acid in human urine and serum sample using the developed procedure.

Sample	Proposed method/ μg mL^{-1}		RSD (%)	Recovery (%)
	Added	Found[a]		
Human urine				
1	–	<D.L	–	–
	1.0	1.01 ± 0.02	1.98	101.0
	5.0	5.03 ± 0.06	1.19	100.6
	10.0	9.99 ± 0.10	1.00	99.9
2	–	<D.L	–	–
	1.0	0.99 ± 0.01	1.01	99.0
	5.0	4.98 ± 0.05	1.00	99.6
	10.0	10.03 ± 0.10		100.3
Human serum	–	<D.L	–	–
1	1.0	0.99 ± 0.01	1.01	99.0
	5.0	5.02 ± 0.06	1.19	100.4
	10.0	9.97 ± 0.11	1.11	99.7
2	–	<D.L	–	–
	1.0	1.01 ± 0.02	1.98	101.0
	5.0	5.01 ± 0.05	1.00	100.2
	10.0	9.97 ± 0.11	1.10	99.7

[a] Mean ± standard deviation

An analysis of the data gave the following regression equation: ΔA = 0.0399 [uric acid] + 0.3918 (R^2= 0.9984) in concentration range 0.3 – 7.0 μg mL^{-1} (first linear segment) and ΔA = 0.0023 [uric acid] + 0.3918 (R^2= 0.9983) in concentration range 7.0 – 46.0 μg mL^{-1} (second linear segment), where ΔA is the difference in absorbance between the blank and the sample, [uric acid] is the uric acid concentration in μg mL^{-1} and R^2 is the correlation coefficient. The detection limit ($3S_b/m$) was 0.08 μg mL^{-1} of uric acid. The relative standard deviations (n = 6) were 1.14, 1.08% for 1.0 and 10.0 μg mL^{-1} of uric acid, respectively.

3.8. Interference studies

The interfering effect of foreign species in the determination of 2.0 μg mL^{-1} of uric acid was studied. The tolerance limit was defined as the concentration of the added species causing an error more than ± 5%. The obtained results are given in Table 1. Interfering effect of nitrite was removed by adding 3.0 mL of 4% sulfamic acid solution to each sample. Also, the interfering effect of Fe^{+3} by adding 3.0 mL of 5% NaF was removed.

3.9. Real Sample Analysis

The applicability of the proposed method has been confirmed by the determination of uric acid in human urine and serum sample as biological sample. After sample preparation (as discussed previously), the extracted uric acid was analyzed using recommended procedure. The results were given in Table 2. The recoveries vary in the range 99.0-101.0% for urine and serum samples respectively. Therefore, the developed method is free from interfering effect of matrix effect and suitable for analysis of uric acid in different samples.

Table 3 Comparison of some of analytical parameters for the determination of uric acid.

Reaction system	LDR (μg mL^{-1})	DL (μg mL^{-1})	Ref.
$[Fe(phen)_3]^{3+}$ [a]	0.72-13.16	0.27	17
Cu(I)- BCA[b]	1.68-16.81	0.50	21
Janus Green-bromate	0.3-46.1	0.08	This work

[a] tris(1,10-phenanthroline)-iron(III) complex
[b] 2,2′-biquinoline 4,4′-dicarboxylic acid

4. Conclusion

The results presented clearly demonstrate the inhibitory effect of uric acid on the oxidation of JG by bromate in acidic media. The reaction system was applied to develop a simple method for the determination of uric acid at sub-ppm. As given in Table 3, the proposed method surpasses to other spectrophotometric procedures in linear dynamic range and detection limit.

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