

SPECTROPHOTOMETRIC REACTION RATE METHOD FOR THE DETERMINATION OF NITRITE BY CATALYTIC ACTION ON NILE BLUE A BROMATE REACTION

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Abstract

A kinetic method developed for the determination of ultra-trace amounts of nitrite based on its catalytic effect on the reaction between potassium bromate and Nile blue A in strongly acidic media is reported. The reaction was monitored spectrophotometrically by measuring the decrease in absorbance of Nile blue A at 645 nm by the fixed-time method. The decrease in absorbance during the first 0.5-3.0 min of the reaction is related to the concentration of nitrite in the range of 0.002-0.500 $\mu\text{g}\cdot\text{ml}^{-1}$. The detection limit of 0.001 $\mu\text{g}\cdot\text{ml}^{-1}$ was achieved for the nitrite ion. The relative standard deviation for 0.0100 $\mu\text{g}\cdot\text{ml}^{-1}$ nitrite is 1.0%. Most common anions and cations did not interfere in the measurement. The procedure was successfully applied for the determination of nitrite in natural water and salt.

Introduction

There is an increasing need for a highly sensitive and selective method for the determination of trace amounts of nitrite in different natural and artificial samples because of its environmental, geochemical and ecological importance. Recent developments in the determination of trace amounts of nitrite have been reviewed [1-3]. Among these methods, spectrophotometry based on the diazotation of an aromatic amine and subsequent coupling to and from an azo dye is the most widely used. These methods are generally sensitive but often have some drawbacks such as serious interferences, toxicity of the reagents used and a relatively long coupling time [4-6]. Other methods such as spectrofluorometry [7], liquid chromatography [8,9] and polarography [10-13] are also used. However, they are time-consuming procedures and use complicated instrumentation.

The catalytic kinetic method [14,15] is one of the most attractive for the determination of ultra-trace amounts of

nitrite. Nevertheless, only a few methods for nitrite determination based on catalytic effects have been previously described [14-20].

To the best of our knowledge, no work has been reported on the oxidation properties for analytical use of Nile blue A for the determination of anions and cations. This paper describes the development of a new method for the determination of nitrite based on its catalytic effect on the oxidation reaction of Nile blue A by bromate, which is very rapid, simple, highly selective and sensitive and has proved effective in determining nitrite in water and salts.

Experimental Section

Reagents

All chemicals were of analytical-reagent grade. Doubly distilled water was used throughout.

Nitrite solution (1.00 mg/ml) was prepared by dissolving 0.3750 g sodium nitrite (Merck), dried at 110°C for 4 h, in 250 ml water containing a few drops of chloroform as preservative in a 250 ml volumetric flask. The solution was protected from light, and kept in a refrigerator. Working

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standard solutions were prepared daily.

Nile blue A (NBA) was purchased from Aldrich and used without purification. Its solution (2.40×10^{-4} M) was prepared by dissolving 0.0200 g of NBA in water and diluting to 250 ml in a 250-ml volumetric flask with water. A stock solution of potassium bromate (0.100 M) was prepared by dissolving 16.70 g KBrO_3 (Merck) in water in a 1000-ml volumetric flask.

Apparatus

A UV-240 spectrophotometer (Shimadzu) was used for measurements of the absorption spectra. A model 35 spectrophotometer (Perkin-Elmer) with 1.0 cm glass cuvettes was used to measure the absorbance at 645 nm. A thermostat (Gallenkamp Griffin, B JL-240-V) was used to keep the reaction temperature at 25°C. A stop-watch was used for recording the reaction time.

Recommended Procedure

The catalyzed reaction was monitored spectrophotometrically by measuring the change in absorbance at 645 nm during the first 0.5-3.0 min of the reaction. All the

solutions were kept in a thermostated water bath at 25°C for 30 min, before initiation of the reaction.

To a solution containing 0.02-5.00 μg of nitrite in a 10-ml volumetric flask, 1.0 ml of 1.20 M H_2SO_4 and 1.0 ml of 2.0×10^{-4} M NBA solution were added and the solution was diluted to ca. 8 ml with water. Then 1.0 ml of 0.010 M potassium bromate solution was added and the solution was diluted to the mark with water. The stop-watch was turned on when the last drop of bromate solution had fallen. Immediately, an appropriate quantity of the reacting solution was transferred to the chamber at the appropriate temperature and the change in absorbance at 645 nm was recorded against water for the 0.5-3.0 min from initiation of the reaction. A calibration graph was constructed as the change of absorbance vs. nitrite concentration at a fixed-time of 3.0 min.

Results and Discussion

In the absence of nitrite, there is very slow reaction between NBA and bromate in acidic media. However, this solution underwent rapid oxidation when nitrite was added at part per billion levels (Fig. 1). This oxidation process

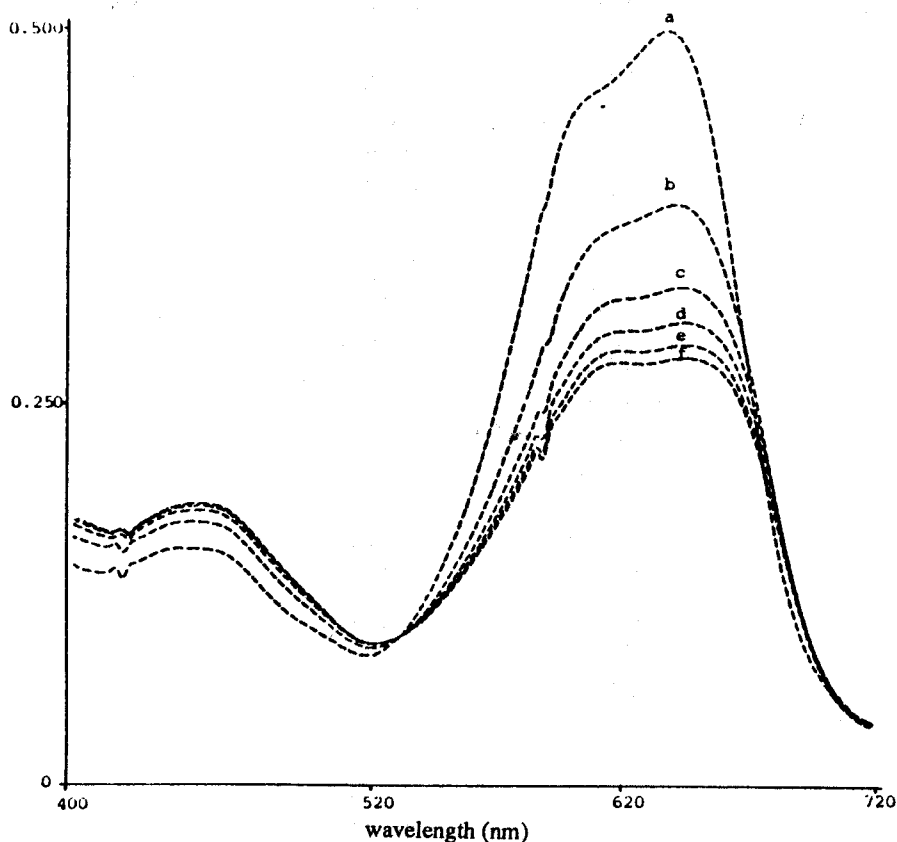


Figure 1. Variation of NBA- BrO_3 -nitrite system with time. Conditions: H_2SO_4 , 0.12 M; NBA, 2.0×10^{-5} M; Temperature, 25°C; NO_2^- , 0.090 $\mu\text{g}\cdot\text{ml}^{-1}$ and BrO_3^- , 0.0010 M. after a. 30 sec; b. 100 sec; c. 170 sec; d. 240 sec; e. 310 sec; and f. 380 sec from initiation of the reaction

was observed by means of the decrease in absorbance of the characteristic band of NAB (645 nm, maximum wavelength at the corresponding pH).

Effect of Variables on the Reaction Rate

Initially, the influence of acid type and acid concentration on the catalyzed reaction rate was studied in order to determine the optimum type and working acid concentration. It was found that oxidation of NBA can occur in the presence of bromate in acidic media (pH<3) at very slow reaction, but the reaction proceeds very fast in the presence of trace amounts of nitrite.

Among sulfuric, hydrochloric and phosphoric acids, sulfuric acid was the best. Chloride interfered with the reaction (at >250 $\mu\text{g}.\text{ml}^{-1}$) and phosphoric acid is weaker. The effect of H_2SO_4 concentration was studied over a range of 0.01-0.14 M H_2SO_4 , in the presence of 2.4×10^{-5} M NBA, $0.050 \mu\text{g}.\text{ml}^{-1}$ nitrite and 0.0010 M bromate at a temperature of 25°C (Fig. 2). It was observed that the rate of catalyzed reaction increased with increasing H_2SO_4 concentration, whereas the rate of uncatalyzed reaction is nearly constant. Thus, 0.120 M H_2SO_4 was selected for the study.

The effect of bromate concentration on the rate of catalyzed and uncatalyzed reaction was studied in the concentration range of 2.7×10^{-4} - 1.8×10^{-3} M in the presence of 0.120 M H_2SO_4 , $0.050 \mu\text{g}.\text{ml}^{-1}$ nitrite, 2.4×10^{-5} M NBA and at a temperature of 25°C. The results show that by increasing bromate concentration, both the catalyzed and uncatalyzed reaction rate was increased (Fig. 3). From the results, 0.0010 M BrO_3^- was the

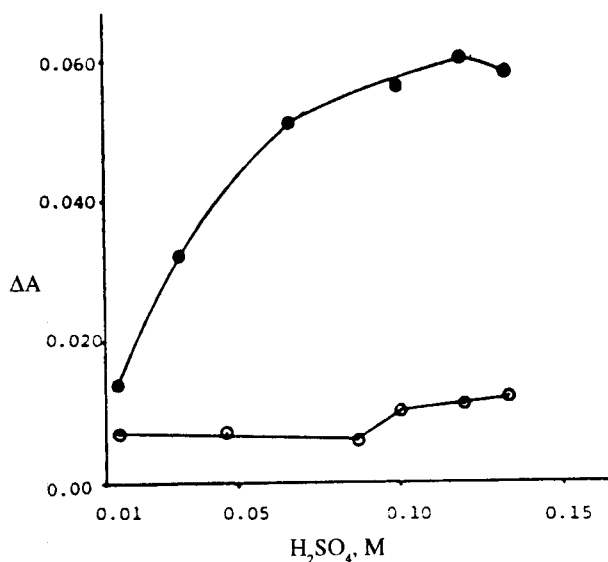


Figure 2. Effect of H_2SO_4 concentration on the rate of catalyzed (•) and uncatalyzed (o) reaction, (condition: NBA, 2.4×10^{-5} M; BrO_3^- , 0.0010 M and nitrite, $0.050 \mu\text{g}.\text{ml}^{-1}$ at 25°C)

optimum bromate concentration.

The effect of NBA concentration on the rate of catalyzed and uncatalyzed reaction was studied in the concentration range of 4.8×10^{-6} - 4.8×10^{-5} M, in the presence of 0.120 M H_2SO_4 , 0.0010 M BrO_3^- , $0.050 \mu\text{g}.\text{ml}^{-1}$ nitrite and at a temperature of 25°C (Fig. 4). The results show that the rate of catalyzed reaction increased with increasing NBA concentration up to 2.0×10^{-5} M, whereas the uncatalyzed reaction rate decreased in this region. From the results, 2.0×10^{-5} M NBA was selected for the study.

The effect of reaction temperature was studied for the catalyzed and uncatalyzed reaction in the range of 3-60°C under optimum reagents concentration with $0.050 \mu\text{g}.\text{ml}^{-1}$ of nitrite (Fig. 5). The results showed that by increasing the temperature up to 40°C the change in absorbance during the 0.5-3.0 min from initiation of reaction for catalyzed reaction increased, whereas at higher temperatures, the change in absorbance was decreased. This is due to the fact that the reaction rate at a temperature of above 40°C becomes very fast and in the first 0.5 min from initiation of the reaction, the reaction was nearly completed, and the change in absorbance between 0.5-3.0 min was decreased. From the results, 25°C was selected for simplicity.

The rate of catalyzed reaction increased very slightly by increasing ionic strength from 0.15-1.0 M (by using 3.0 M sodium nitrate solution).

Calibration Graph

Calibration graph was obtained by applying the fixed

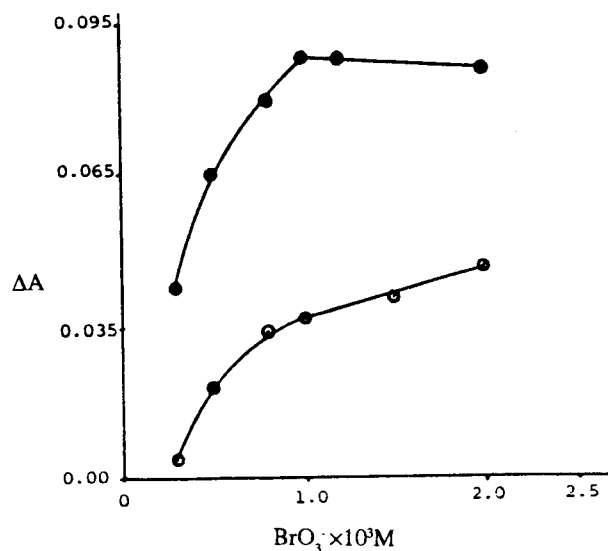


Figure 3. Effect of bromate concentration on the rate of catalyzed (•) and uncatalyzed (o) reaction, (condition: H_2SO_4 , 0.120 M; NBA, 2.4×10^{-5} M; nitrite, $0.05 \mu\text{g}.\text{ml}^{-1}$ at 25°C)

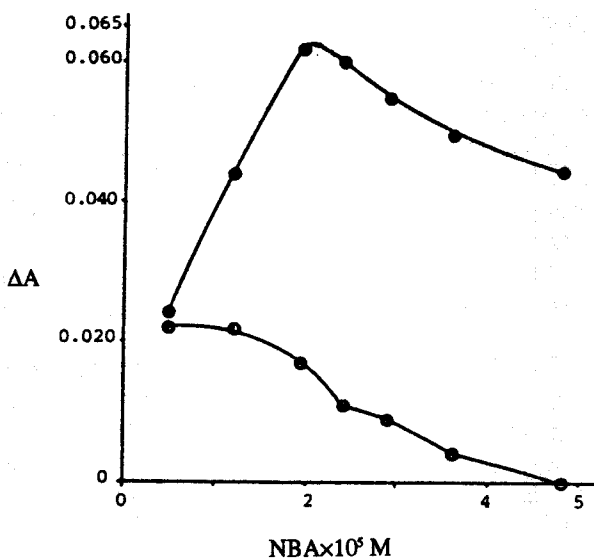


Figure 4. Effect of NBA concentration on the rate of catalyzed (●) and uncatalyzed (○) reaction; (condition: H₂SO₄, 0.120 M; BrO₃⁻, 0.0010 M; nitrite, 0.050 μg.ml⁻¹ at 25°C)

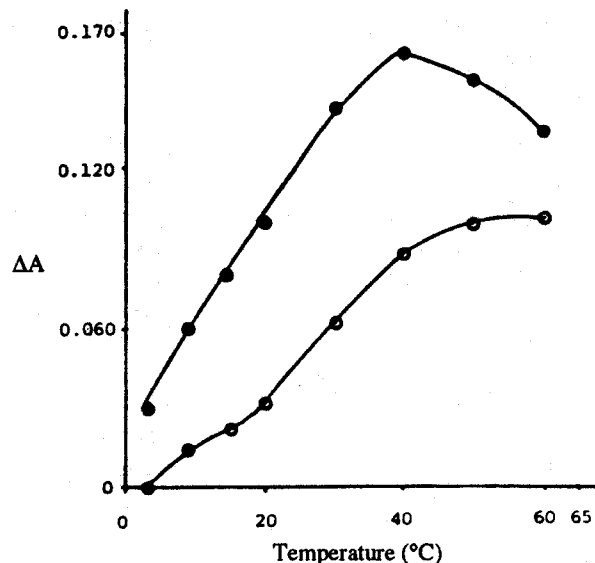


Figure 5. Effect of temperature on the rate of catalyzed (●) and uncatalyzed (○) reaction, (at the optimum reagents concentration with 0.050 μg. ml⁻¹ nitrite)

time method under the optimum conditions. The effect of measuring time on the correlation coefficient and sensitivity for the calibration graphs was studied. From these results, 3.0 min was chosen as the working measuring time. The decrease in absorbance is directly proportional to the nitrite concentration range 0.002-0.500 μg.ml⁻¹, with the regression equation of $\Delta A = 1.97 \times 10^{-2} + 0.923C$ ($n=6$ and $r=0.9998$) where ΔA is the absorbance change for the sample minus for the blank and C is the μg. ml⁻¹ of nitrite.

The experimental limit of detection is 1.0 ng.ml⁻¹ whereas the theoretical limit of detection [21] (defined as the average blank signal plus three times its standard deviation) is 0.2 ng. ml⁻¹

The relative standard deviations for eight replicate determinations of 0.100 and 0.500 μg. ml⁻¹ of nitrite are 1.0% and 0.8%, respectively.

Effect of Common Ions

More than 40 ions were tested for their possible interference with determination of 0.050 μg. ml⁻¹ of nitrite. The results showed that at least 100000-fold alkali metals, alkaline earth metals and PO₄³⁻, SO₄²⁻, B₄O₇²⁻, ClO₃⁻, CN⁻, F⁻, BO₃⁻, CO₃²⁻, C₂O₄²⁻, Ni(II), Cu(II), Zn(II), Co(II), Mn(II), Th(IV), U(VI), Mo(VI), acetate, tartarate, citrate and EDTA, 5000-fold Cl⁻, Cd(II), La(III), Zr(IV), Hf(IV), 500-fold Ce(III), In(III), did not interfere, and up to 200-fold, Fe(III), Hg(II), Br and CrO₄²⁻, and 100-fold Ag(I) and V(III) had little interference. However, more than 20-fold VO₃⁻ and Ce(IV) interfered. It is important to note that up to 10⁶-fold of nitrate and ammonium had little effect,

making possible the determination of trace amounts of nitrite in nitrate and ammonium salts. The tolerance limit of Hg(II) and Fe(III) can be increased up to 600-fold in the presence of 0.5% EDTA solution.

Determination of Nitrite in Real Samples

In order to check the applicability of the proposed method for the determination of nitrite, a series of natural surface water samples from wells, ponds, and rivers around Isfahan, as well as two nitrate salts (Fluka) were analyzed by the proposed method without any additional pretreatment.

For the determination of nitrite in the water samples, initially, 1 ml of saturated aluminum hydroxide was added to precipitate the suspended particles. Then the solution was filtered (using Whatman No. 1). The filtrate was used for the determination of nitrite. Two sets of experiments were carried out. In the first set, the recovery of nitrites was checked by adding various amounts of standard nitrite solution to the real sample solutions (standard addition method). In the second set of experiments, the samples were diluted to a constant volume with distilled water, then analyzed by the recommended procedure and by the standard method [22].

The results are given in Table 1 and show that the proposed method is comparable to the standard method. For the determination of nitrite by the standard addition method, various amounts of standard nitrite solution were transferred to seven 10-ml volumetric flasks containing constant volumes of the sample (5.0 ml of water or 3.0 ml

Table 1. Determination of nitrite in water and salts

Sample	Nitrite found ($\mu\text{g. ml}^{-1}$)		
	Proposed method (n=5)	Standard method [22] (n=5)	RSD% ^b (n=5)
water 1	0.025	0.023	1.2
water 2	0.037	0.038	0.9
water 1 ^a	0.024	0.023	1.1
water 2 ^a	0.038	0.038	0.9
water 3	0.046	0.043	1.3
water 3 ^a	0.044	0.043	1.0
NaNO ₃ ^a	0.026	0.025	0.8
KNO ₃ ^a	0.022	0.021	0.9

a, By the standard addition method; and b, for the proposed method

of 1.0 M salt solution). The total nitrite concentration was determined by the recommended procedure. The results are given in Table 1.

Conclusion

The present investigation shows that the Nile blue A-bromate-acid system could be successfully used for the quantitative determination of ultra trace amounts of nitrite in real samples. The rapidity, simplicity and relative selectivity attained using this technique encourage the application of the proposed method in fast routine analysis of nitrite in waste water without any preconcentration.

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