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SHORT COMMUNICATION

# Spectrophotometric determination of nitrite based on its catalytic effect on the reaction of nuclear fast red and potassium bromate

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*Abstract*: A highly selective and sensitive catalytic spectrophotometric method was developed for the determination of nitrite in water samples. The method is based on its catalytic effect on the nuclear fast red–potassium bromate redox reaction in acidic medium. The reaction was followed spectrophotometrically by measuring the change in the absorbance at 518 nm of nuclear fast red 5 min after initiation of the reaction. In this study, the experimental parameters were optimized and the effects of other cations and anions on the determination of nitrite were examined. The calibration graph was linear in the range 2.0–45 µg mL$^{-1}$ of nitrite. The relative standard deviations for the determination of 15 and 30 µg mL$^{-1}$ of nitrite were 3.1 and 1.75 %, respectively ($n$ = 8). The detection limit calculated from three times the standard deviation of the blank $3S_b$ was 0.7 µg mL$^{-1}$. The method was successfully applied to the determination of nitrite in spiked tap, natural and wastewater samples.

*Keywords*: nitrite; catalytic reaction; spectrophotometric; nuclear fast red.

## INTRODUCTION

The determination of nitrite is of increasing interest in a variety of fields, such as food, water and environmental samples. Nitrite and nitrate ions participate in several important environmental transformations involving nitrogen. The toxicity of nitrite is primarily due to the fact that it can react with secondary or tertiary amines present in the human body to form nitrosamines, which are known to be carcinogens and mutagenic.[1–3] It is well known that the nitrite ion is an important intermediate in the biological nitrogen cycle and is present in soils and surface water.[4] Nitrite salts are versatile chemical agents that have found numerous applications, such as in dye manufacture, the food industry and corrosion inhibition of industrial process water.[5]

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As nitrite shows potential toxicity by forming carcinogenic nitrosamines, the determination of nitrite is important in environmental protection and public health. Therefore, many methods for nitrite determination have been developed in recent years, such as kinetic methods,[6,7] chromatography,[8,9] potentiometry,[10,11] amperometry,[12] polarography,[13] capillary electrophoresis,[14] spectrophotometry[15] and flow injection analysis (FIA) systems.[16] However, these methods have the disadvantage of the employment of large volumes of toxic reagents, low sample frequency, application of complicated flow injection systems, poor reproducibility, expensive and time-consuming separation procedures and, for certain methods, the requirement of high temperatures.[17,18]

Kinetic methods[19,20] can be implemented on very simple, inexpensive equipment and provide for low level determinations comparable to those typically offered by much more expensive techniques, such as inductively coupled plasma, atomic emission spectrometry (ICP-AES) or electro thermal atomic absorption spectrometry (ETAAS). In this paper, a simple, sensitive, and highly selective catalytic method for the determination of nitrite based on its catalytic effect on the redox reaction of nuclear fast red–potassium bromate is described.

## EXPERIMENTAL

### *Reagents and solutions*

All employed reagents were of analytical-reagent grade and double distilled water (DDW) was used throughout. Nuclear fast red (Aldrich), potassium bromate and sulfuric acid (Merck) were used without further purification. A nitrite stock solution 100 mg $L^{-1}$ was freshly prepared before each measurement by dissolving 0.1500 g sodium nitrite (Merck) in 1000 mL water. This solution was transferred into a brown bottle and stored under diffused sunlight. A bromate solution (0.25 mol $L^{-1}$) was prepared by dissolving 4.1752 g of $KBrO_3$ in water in a 100 mL volumetric flask. A nuclear fast red solution ($5\times10^{-4}$ mol $L^{-1}$) was prepared by dissolving 0.0178 g of nuclear fast red in water and diluting with water in a 100 mL volumetric flask. A sulfuric acid solution (2 mol $L^{-1}$) was prepared by diluting an appropriate volume of concentrated acid. Stock solutions interfering ions, 1000 μg $mL^{-1}$, were prepared by dissolving appropriate amounts of their suitable salts in water.

### *Apparatus*

A UV-visible spectrophotometer (Shimadzu) was used for recording and measuring the absorbance at 518 nm. A Colora C-1668 thermostat, in which the temperature could be fixed to within ±0.10 °C, was used for maintaining the temperature.

### *Procedure*

To a series of 10 mL volumetric flasks, 2.0 mL of $5.0\times10^{-4}$ mol $L^{-1}$ nuclear fast red solution, 1 mL of 2.0 mol $L^{-1}$ sulfuric acid and 1.0 mL of different concentrations of nitrite were added in sequence. Then 2.0 mL of 0.2 mol $L^{-1}$ bromate solution was added and the solution was diluted to the mark with DDW and the solution was mixed well. The zero time was taken as the moment at which the last drop of bromate solution was added. After 5 min, a portion of the solution was transferred into a 1.0 cm quartz cell and the absorbance was measured against DDW at 518 nm ($A_S$). The blank reaction was performed according to the same procedure without addition of nitrite and the change in absorbance was labeled as $A_0$. A standard curve showing the difference in absorbance between $A_s$ and $A_0$ ($\Delta A$) *vs*. the nitrite concentration was obtained. For each sample and blank, five replicate determinations were made and the mean signals were used. The catalytic and non-catalytic reactions (blank reaction) were simultaneously measured.

## RESULTS AND DISCUSSION

### *Absorption spectra*

Nuclear fast red, NFR, is a dye with an absorption spectrum in acidic medium that shows an absorbance maximum at 518 nm. In acidic medium, nuclear fast red is oxidized by bromate to produce a colorless compound. However, in the absence of nitrite, the reaction between nuclear fast red and bromate is slow and in the presence of trace amounts of nitrite, the rate of reaction is increased. This oxidation process was observed by the decrease in absorbance of the characteristic band for nuclear fast red. The catalytic effect of nitrite on the reaction was monitored spectrophotometrically by measuring the change in absorbance of nuclear fast red at 518 nm with time. The possible mechanism of the nuclear fast red reaction in its simplest form may be represented by the following reactions:

$$BrO_3^- + H^+ + NFR(Red) \rightarrow Br^- + NFR(Ox) \quad (1)$$

$$BrO_3^- + H^+ + \text{Nitrite} \rightarrow Br^- + NO_3^- + H_2O \quad (2)$$

$$BrO_3^- + H^+ + Br^- \rightarrow Br_2 + H_2O \quad (3)$$

$$Br_2 + H^+ + NFR(Red) \rightarrow Br^- + NFR(Ox) \quad (4)$$

where Red is the reduced form and Ox is the oxidized form of nuclear fast red. Reaction (1) is slow in the absence of nitrite, $BrO_3^-$ oxidizes nuclear fast red to produce a small reduction in the absorbance of dye. When nitrite is added to this system, reactions (2) and (3) are very fast and the $Br_2$ generated *in situ* in this system is a nascent oxidant.[21] To determine the optimum experimental conditions, several parameters having the biggest influence on the rate of reaction, *i.e.*, time of reaction, temperature, sulfuric acid concentration, bromate concentration and concentration of nuclear fast red, were studied.

### *Effect of reaction time*

As shown in Fig. 1, when the reaction time was between 0 and 5 min, $\Delta A$ ($A_s$ – – $A_0$) was a linear function of the reaction time. After 5 min, $\Delta A$ changed very slowly with elapsed time; $\Delta A$ was first stable and then showed a decreasing tendency. The change in absorbance, $\Delta A$, reached its maximum in 5 min. Therefore, a fixed time of 5 min was applied for all the experiments because the highest $\Delta A$ was obtained after this time (see Fig. 1).

### *Effect of reaction variables*

In order to find the optimal conditions for the catalytic determination of nitrite, the effects of acids and acidity, the concentration of the reagents, interfering ions and temperature were examined, in both the catalyzed and uncatalyzed reactions.

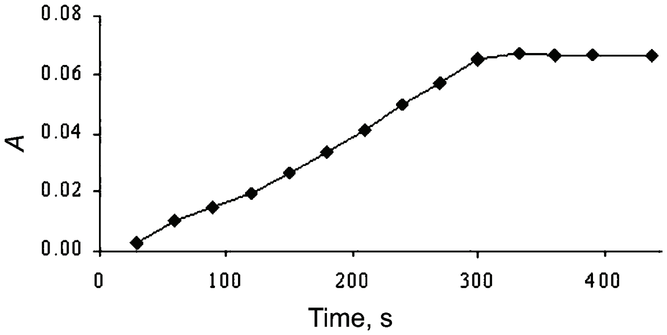

Fig. 1. Effect of time on Δ*A*.

*Effect of acidity*. Since the reaction proceeds in acidic media, the effect of the type and concentration of acid on the reaction rate was studied. The effects of various types of acids, *i.e.*, sulfuric, hydrochloric, phosphoric and nitric acid, of similar concentration were studied and sulfuric acid was selected. The effect of acidity was studied by varying the volume of the employed 2.0 mol $L^{-1}$ sulfuric acid solution between 1.0–5.0 mL. The results showed that with increasing acidity, the sensitivity of the determination of nitrite decreased. Therefore, 1.0 mL of sulfuric acid was chosen for further studies.

*Optimization of the concentrations of the reagents.* The effect of the nuclear fast red concentration on the reaction rate is shown in Fig. 2. When the nuclear fast red concentration was increased in the range $5.0\times10^{-6}$ to $2.0\times10^{-4}$ mol $L^{-1}$, the difference in the absorbance (Δ*A*) increased. A concentration of $1.0\times10^{-4}$ mol $L^{-1}$ of nuclear fast red in the final solution was selected. At higher concentrations of nuclear fast red, the absorbance of the solution became too high (see Fig. 2).

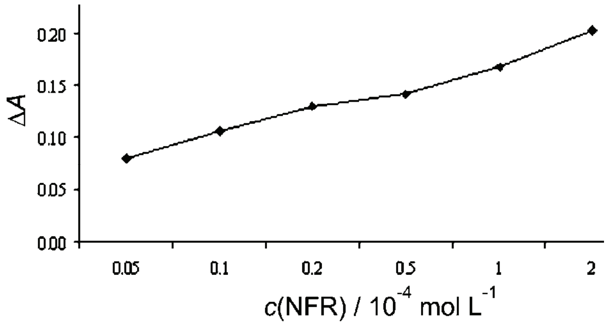

Fig. 2. Effect of the nuclear fast red concentration on Δ*A*. Conditions: 10 μg $mL^{-1}$ nitrite; 0.20 mol $L^{-1}$ sulfuric acid; 0.020 mol $L^{-1}$ potassium bromate; temperature: 25 °C; reaction time: 5 min.

The influence of bromate concentration on the reaction rate was also studied by adding different concentration of the potassium bromate and applying the recommended procedure. A concentration of 0.040 mol $L^{-1}$ of potassium bromate in final solution gave the highest $\Delta A$ and was selected for further work (see Fig. 3).

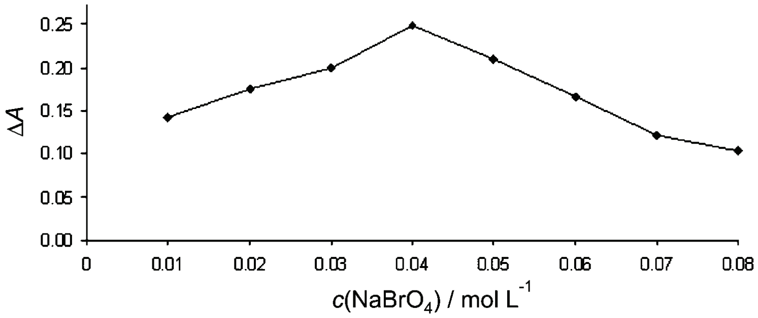

Fig. 3. Effect of the potassium bromate concentration on $\Delta A$. Conditions: 10 μg $mL^{-1}$ nitrite; 0.20 mol $L^{-1}$ sulfuric acid; $1.0\times10^{-4}$ mol $L^{-1}$ nuclear fast red, temperature: 25 °C; reaction time: 5 min

*Effect of temperature*. The effect of temperature on the sensitivity was examined in the range of 20–50 °C. At high temperatures, the absorbance difference ($\Delta A$) decreased. At temperatures from 20 to 25 °C, the highest absorbance difference ($\Delta A$) was obtained. In this study, the laboratory temperature (25±0.1 °C) was used for further experiments.

*Effect of interfering ions*. To study the selectivity of the proposed method, the interference of several ions that could occur in real water samples besides nitrite was studied by adding various concentrations of the different ions to a solution containing 20 μg $mL^{-1}$ of nitrite. The results are summarized in Table I. The tolerance limit was defined as the concentration of added ion causing a relative error less than 3 %. The results indicate that most of common anions and cations did not interfere in the determination of nitrite by the proposed method.

TABLE I. Interference of various ions in the determination of 20 μg $mL^{-1}$ of nitrite

| Ions | Tolerance ratio |
|---|---|
| $K^+$, $Ca^{2+}$, $Na^+$, $Cl^-$, $Cd^{2+}$, $Mg^{2+}$, $NH_4^+$, $IO_3^-$, $NO_3^-$, $PO_4^{3-}$, $Cr^{3+}$, $Mn^{2+}$ | 1000 |
| $Cu^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Li^+$, $Sn^{2+}$, $CH_3COO^-$, $Pb^{2+}$, $F^-$ | 500 |

## *Calibration graph and precision*

A calibration graph was obtained by plotting $\Delta A$ *vs*. the nitrite concentration using the developed method under the optimal conditions. From the results of the experiments, 5 min was chosen as the optimal time because it provided the best correlation coefficient and sensitivity. The calibration graph was linear in the range of 2.0–45 μg $mL^{-1}$ of nitrite ions. The equation of the line was $y = 0.0046 + + 0.014c$, where $y$ is $\Delta A$, and $c$ is the concentration of nitrite in μg $mL^{-1}$, with a correlation coefficient $r = 0.997$. The correlation coefficient indicates a good linear correlation between Δ$A$ and the concentration of nitrite. Each point in the calibration graph was the average of five replicates. The experimental limit of detection ($3S_b/m$, three times the blank standard deviation divided by the slope of the equation) was 0.70 μg mL$^{-1}$ and *LOQ* of the proposed method was 2.5 μg mL$^{-1}$. The relative standard deviation for 5 replicate determinations of 15 and 35 μg mL$^{-1}$ of nitrite were 3.1 and 1.75 %, respectively.

### *Analytical applications*

The proposed method was applied for the determination of nitrites in some natural waters. The samples were collected from the city of Semnan, Iran, and its surroundings. The river water sample was sampled at the Heshmat River (North of Iran)and the tap water sample was obtained from local pipes. All samples were filtered through a 0.45 μm filter (glass fiber) to remove the suspended solids and then stored in clean polyethylene bottles. When water samples spiked with nitrite standards of different concentrations were treated by the proposed method, the recoveries were all in the range of 97–99 %, with a *RSD* of 1.0–4.0 %. The characteristics of the tap water samples are given in Table II. The determination results and recoveries are listed in Table III. They show good accuracy in comparison to the standard method.[22]

TABLE II. Some parameters of the tap water sample

| | |
|---|---|
| pH | 7.6 |
| Turbidity (NTU) | 0.3 |
| Conductivity, S cm$^{-1}$ | 670 |
| Total dissolved solid, mg/L | 374 |
| Alkalinity, mg/L | 140 |

TABLE III. Determination of nitrite in various samples

| Sample | Nitrite added μg mL$^{-1}$ | Proposed method[a] μg mL$^{-1}$ | Standard method[24] μg mL$^{-1}$ | Recovery % |
|---|---|---|---|---|
| Tap water | – | Trace (< 2) | Trace (< 2) | – |
| | 12.0 | 11.8 | 11.9 | 98.3 |
| Waste water[b] | – | 2.54 | 2.63 | – |
| | 10.0 | 12.31 | 12.46 | 97.5 |
| | 20.0 | 22.43 | 22.50 | 99.5 |
| River water | – | Trace (< 2) | Trace (< 2) | – |
| | 10.0 | 9.86 | 9.90 | 98.6 |
| | 20.0 | 19.8 | 19.9 | 99.0 |

[a]Mean of four determinations; [b]Semnan University wastewater

## CONCLUSIONS

Nitrite, formed during the biodegradation of nitrate and ammoniacal nitrogen or nitrogenous organic matter, is an important indicator of fecal pollution of natural water. The determination of nitrite is of general importance because of its harmful impact on human health. In comparison with the high cost techniques for nitrite determination, the present paper describes a low-cost, very simple, selective and highly sensitive method for the determination up to 2.0–45 μg $mL^{–1}$ of nitrite with a detection limit of 0.70 μg $mL^{–1}$. In addition, the new proposed method involves neither solvent extraction[23] nor requires any separation or preconcentration steps, nor critical control of pH[24] or temperature[25] nor employs sophisticated instruments[26] and can be directly applied for the determination of nitrite in tap, natural and industrial waste water samples. In practice, the system showed high tolerance to interference from matrix elements. The results presented in this paper clearly demonstrate that the proposed method is sensitive, simple and selective and that it can also be conveniently applied for the analysis of different water samples.

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## ИЗВОД

## СПЕКТРОФОТОМЕТРИЈСКО ОДРЕЂИВАЊЕ НИТРИТА ЗАСНОВАНО НА ЊЕГОВОМ КАТАЛИТИЧКОМ ЕФЕКТУ НА РЕАКЦИЈУ ИЗМЕЂУ НУКЛЕАРНО БРЗО ЦРВЕНОГ И НАТРИЈУМ-БРОМАТА

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Развијена је високоселективна и осетљива каталитичка спектрофотометријска метода за одређивање нитрита у узорцима воде. Метода је заснована на каталитичком ефекту нитрита на нуклеарно брзо црвено–натријум-бромат редокс реакцији у киселој средини. Реакција је праћена спектрофотометријски, мерењем промене абсорбанције нуклеарно брзо црвеног на 418 nm, 5 min након почетка реакције. Испитивањем су оптимизовани експериментални параметри и утврђен ефекат других катјона и анјона на одређивање нитрита. Калиграциони график је линеаран у области концентрације нитрита 2,0–45 μg $mL^{-1}$. Релативно стандардно одступање за одређивање 15 и 30 μg $mL^{-1}$ нитрата износило је 3,1 и 1,75 %, редом ($n$ = 8). Граница детекције израчуната из троструког стандардног одступања слепе пробе, $3S_b$, износила је 0,70 μg $mL^{-1}$. Метода је успешно примењена за одређивање нитрита у узорцима бунарске, природне и отпадне воде.

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