A Simple Flow Injection Spectrophotometric Determination of Nitrite Based on Its Reaction with Thiourea

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A simple flow injection spectrophotometric method for the determination of nitrite is described. Nitrite injected into the flow system reacts with thiourea in acidic medium and the generated thiocyanate ion reacts with Fe(III) in the reagent solution to produce a highly colored product. The influences of chemical and physical parameters including reagent concentrations, sample volume injected, flow rates of the carrier and reagent solutions, reaction coil length and reaction temperature, were studied and optimum values of these parameters were established. Under the optimum conditions, the calibration curve for nitrite was linear over the concentration range 0.36 – 90 $\mu\text{g ml}^{-1}$ without preconcentration and over the range 3.8 – 500 ng ml^{-1} with a simple online preconcentration step using an anion exchange column. The corresponding detection limits were 0.36 $\mu\text{g ml}^{-1}$ and 3.8 ng ml^{-1} , respectively. Up to 25 samples can be analyzed per hour, with an average relative standard deviation of $\leq 1.2\%$. Interferences by various foreign ions were studied and the method was applied to the determination of nitrite in water and spiked water samples.

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Introduction

Nitrite is an anion of major importance, particularly in environmental and biological areas. It is widely found in soil, natural water and food, and can be generated by chemical transformation or biodegradation of nitrate. Since most nitrogenous materials in natural waters tend to be converted to nitrate, all sources of combined nitrogen, particularly organic nitrogen, ammonia and fertilizer residues, should be considered as potential sources for nitrite. The necessity for nitrite, and/or nitrate, monitoring has been recognized by most health authorities worldwide, with legislation often levied on permissible levels in drinking water; at present the maximum contaminant levels for nitrate and nitrite in drinking water are 10 and 1 $\mu\text{g ml}^{-1}$, respectively.¹ Excessive concentrations of nitrite in drinking water could be hazardous to health, especially for infants and pregnant women. The primary health hazard for drinking water with nitrate nitrogen occurs when nitrate is transformed to nitrite in the digestive system. Nitrite oxidizes iron in hemoglobin of the red blood cells to form methemoglobin, which lacks the oxygen carrying ability of hemoglobin. This creates the condition known as methemoglobinemia.² Nitrite could also have a direct impact on health because of its reaction with amines or amides in the human body to produce different types of nitrosamines, which are known to be very powerful carcinogens to mammals.³ Therefore, elucidation of the concentrations of nitrite, and of nitrate, is desirable from the standpoint of environmental chemistry, food chemistry, waste water treatment and drinking water quality assessment.

Various methods have been used for the determination of nitrite, including chromatography,^{4–7} gas chromatography-mass

spectrometry,⁸ capillary electrophoresis,^{9–11} colorimetric methods,^{12–23} catalytic-based methods,^{24–26} and several other methods such as electrochemical ones,^{27–29} fluorometric ones^{30,31} and chemiluminescence.^{32–34} Flow injection analysis (FIA) in combination with one of the above detection methods have been widely used for the determination of nitrite.^{14–17,35,36} Due to its inherent advantages, such as high sample throughput, improved precision and relatively simple equipment, FIA is widely used for water analysis in a rapid and efficient way.

In this work, we present a novel flow injection method for the determination of nitrite based on its reaction with thiourea in acidic media to produce thiocyanate ion, which is detected spectrophotometrically with iron(III). The reaction of nitrite ion with thiourea has been previously used for the detection of this ion,³⁷ but to the best of our knowledge, this reaction has not been applied to its determination. The proposed method is simple, and reagents are very cheap, non-toxic and not harmful to the environment. The method is suitable for routine determination of nitrite at $\mu\text{g ml}^{-1}$ levels directly or at ng ml^{-1} levels after preconcentration.

Experimental

Apparatus

A schematic diagram of the flow injection system is shown in Fig. 1. The system included a multichannel peristaltic pump (Ismatec, Model ISM 404B), a sample injector valve equipped with 100 – 400 μl loops, mixing tees, a thermostated water bath, a liquid-gas separator (Fig. 2A), a spectrophotometer detector (Shimadzu, Model UV-120-02) and an X-Y recorder (Philips, Model PM 8272). A home-made z-shaped glass flow cell (Fig. 2B) with a 3 cm path length and 270 μl capacity was used for absorbance measurements. For the preconcentration step, the injection loop was replaced with an anion-exchange column (a glass tube of 2 mm i.d. $\times$ 60 mm), which was packed with 150

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Fig. 1 Schematic diagram of the FIA system employed for the determination of nitrite without (A) and with (B) a pre-concentration step. P₁ and P₂, pumps; C, sulfuric acid carrier solution; R₂, thiourea solution; R₃, Fe(III) solution; S, sample injection valve; T, thermostated water bath; G, liquid-gas separator; D, detector; W, waste; AEC, anion-exchanger column.

mg of the anion-exchanger (Amberlite IRA-420, Merck) as shown in Fig. 1B. The sample solution is directed to waste after passing through the ion-exchanger column.

Reagents

All chemicals were of analytical reagent-grade and were used without further purification. All solutions were prepared with distilled, deionized water. The stock standard solution of nitrite (1000 mg l⁻¹) was prepared by carefully weighing sodium nitrite, which had been previously oven-dried at 110°C for several hours. Working solutions were freshly prepared by successive dilution of the stock solution with water.

Carrier solution C, and reagents R₂ and R₃ (Fig. 1) were 0.01 mol dm⁻³ sulfuric acid, 0.05 mol dm⁻³ thiourea and 0.05 mol dm⁻³ Fe(III) solutions, respectively. The thiourea was freshly prepared by dissolving appropriate amount of the reagent (Merck). The Fe(III) solution was prepared from iron(III) nitrate (Merck) in 0.01 mol dm⁻³ sulfuric acid.

Procedure

The reagents R₂ and R₃ and the carrier solution C were pumped at 0.1 ml min⁻¹ for each channel (a total flow rate of 18 ml h⁻¹) via the peristaltic pump. A 300 µl standard or a real sample was injected into the carrier stream at point S. The solution was mixed with thiourea and Fe(III), respectively, at the mixing tees. Each mixture was passed through a reaction coil (tygon tubing, 1 mm i.d. × 4.5 m) which was maintained at 55°C in a thermostated water bath and then through a liquid-gas separator. The absorbance of the resulting highly absorbing iron(III)-thiocyanate complex was measured spectrophotometrically at 460 nm by passing the solution through the flow cell.

For the pre-concentration step, an appropriate volume of the sample solution containing 3.8 – 500 ng ml⁻¹ NO₂⁻ was passed through the anion-exchange column (Fig. 1B) at a flow rate of 2.2 ml min⁻¹. The accumulated nitrite in the column is then eluted by the carrier stream, 0.01 mol dm⁻³ sulfuric acid carrier solution, into the analytical path.

Fig. 2 (A) Schematic diagram of the liquid-gas separator. (B) Side-view of the flow cell comprised of a, O-ring; b, optical glass window; c, the curved region of the cell wall for efficient removing gas bubbles. The cell volume is 270 µl.

Results and Discussion

Nitrite reacts selectively with thiourea in acidic media to form thiocyanate (SCN⁻) according to the following irreversible reaction:

The reaction is monitored spectrophotometrically by measuring the absorbance of the complex formed between thiocyanate ion and iron(III) using the flow injection system.

In order to optimize the proposed flow injection method, we examined the influences of manifold and chemical parameters on the sensitivity and reproducibility of the results.

Optimization of reagent concentration

Experimental results showed that, among different inorganic acids, the reaction of nitrite and thiourea could run efficiently in sulfuric acid medium. The influence of sulfuric acid concentration was studied in the range 5×10^{-3} – 1 mol dm⁻³. Maximum sensitivity was observed at 0.01 mol dm⁻³ sulfuric acid carrier solution in C. In order to examine the effect of concentration of thiourea on the peak height of nitrite, we studied various concentrations of this reagent in the range 0.01 – 0.5 mol dm⁻³. Maximum peak height was observed at a thiourea concentration of 0.05 mol dm⁻³ in R₂.

For measuring the effect of concentration of Fe(III) on the sensitivity of determination, we tested solutions in the range 5×10^{-3} – 0.1 mol dm⁻³. The peak height was increased with increasing the Fe(III) concentration up to 0.05 mol dm⁻³, above which it remained virtually constant. Therefore, 0.05 mol dm⁻³ Fe(III) was selected as the optimum concentration of this reagent.

Optimization of the manifold variables

The sample volume injected into the carrier line via the sample loop had a significant effect on the sensitivity of the measurement. The peak height increased with increasing sample volume up to 300 µl. This was used as the optimum injection volume in subsequent measurements.

Fig. 3 Calibration curve of measured peak height vs. nitrite concentration.

The sensitivity of the determination strongly depended on the reaction time. Longer reaction time could produce larger peak heights, and hence higher sensitivity could be obtained. Longer residence time of the sample zone in the flow system could be achieved with lower flow rate of the reagents and longer reaction coil. However, the sample throughput is slower at lower flow rates and peak broadening occurs with very long reaction coils. Several reagent flow rates and various lengths of the reaction coil were examined according to the procedure. A reaction coil of 4.5 m in length and 1 mm in internal diameter, and a total flow rate of 18 ml h⁻¹ (6 ml h⁻¹ for each of the channels) were chosen as a compromise among the sensitivity, sampling rate and peak width.

The dispersion value (D), defined by C°/C , where C° is the analyte concentration of the injected sample and C is the peak concentration at the detector, was obtained at the optimum operating conditions using Methylene Blue at 661 nm according to the recommended procedure.³⁸ The dispersion value thus obtained was 5.2; therefore, the system can be considered a medium dispersion FIA method.

Effect of temperature

The influence of temperature on the sensitivity of the reaction of nitrite with thiourea was studied by placing the reaction coil in a water bath thermostated at various temperatures between 25 – 75°C. The experimental results showed that the sensitivity was increased by increasing the reaction coil temperature. However, temperatures $\geq 60^\circ\text{C}$ gave poor reproducibility. A temperature of 55°C was found to be suitable for the reaction as a compromise between sensitivity and reproducibility of the measurements.

Optimal physical and chemical parameters are presented in Table 1.

Analytical parameters

Using the flow injection setup shown in Fig. 1 and under the above mentioned conditions, we found that the peak heights were proportional to the nitrite concentrations. The calibration graph, which is shown in Fig. 3, was linear over the range 0.36 – 90 µg ml⁻¹ nitrite and is described by the regression equation $Y = 0.0693 + 0.0208C$ ($r = 0.9995$), where Y is the peak height and C is the nitrite concentration in µg ml⁻¹.

The reproducibility of the method was determined by repeated injection ($n = 6$) of several samples containing 1, 20 and 60 µg ml⁻¹ nitrite. The relative standard deviations thus obtained were 1.2, 0.37 and 0.45%, respectively. The limit of detection, defined as three times the baseline noise, was 0.36 µg ml⁻¹

Table 1 Optimal chemical and physical parameters of the method

Parameter	Studied range	Selected value
Concentration of solutions (mol dm ⁻³)		
H ₂ SO ₄ carrier solution	0.005 – 1	0.01
Thiourea reagent	0.01 – 0.5	0.05
Iron(III) nitrate reagent	0.005 – 0.1	0.05
Manifold variables		
Flow rate (ml h ⁻¹)	18 – 180	18 (6 ml for each channel)
Reaction coil length (m)	1 – 4.5	4.5
Reaction coil temperature (°C)	22 – 75	55
Sample loop volume (µl)	100 – 400	300

Table 2 Analytical features of the proposed method

Parameter	Value/range
Linear range	0.36 – 90 µg ml ⁻¹ , 3.8 – 500 ng ml ⁻¹ after preconcentration
Detection limit	0.36 µg ml ⁻¹ , 3.8 ng ml ⁻¹ after preconcentration
Relative standard deviation (% , $n = 6$)	
At 1 µg ml ⁻¹	1.2
At 20 µg ml ⁻¹	0.37
At 60 µg ml ⁻¹	0.45
Sample throughput	25 sample h ⁻¹

NO₂⁻.

It takes about 2.5 min from the injection of the sample for the peak to reach the baseline; so the sample throughput of the system was 25 samples per hour. Important features of the proposed method for the determination of nitrite are summarized in Table 2.

Preconcentration

A preconcentration system was used for determination of trace amounts of nitrite, as described in the experimental section. The preconcentration process was carried out by passing the sample solution through an ion-exchange column. The effect of sample flow rate through the column was studied. Highest efficiency was recorded when the sample flow rate was 2.2 ml min⁻¹. Elution was effectively carried out with carrier solution C, which was 0.01 mol dm⁻³ sulfuric acid solution with the same flow rate of 6 ml h⁻¹, as described in the section above. The solution eluted from the column was introduced into the analytical path.

The calibration graph, which was obtained after preconcentration of nitrite from 40 ml solution and plotted as peak height vs. nitrite concentration, was linear in the range 3.8 – 500 ng ml⁻¹. The corresponding equation was $Y = 0.0473 + 4.52 \times 10^{-4}C$ with a correlation coefficient of 0.9993. The limit of detection was 8×10^{-8} mol dm⁻³ (3.8 ng ml⁻¹).

Interference by foreign species

In order to assess the possible analytical applications of the above described method, we studied the effect of concomitant species on the determination of nitrite in real samples by analyzing synthetic sample solutions containing 5 µg ml⁻¹ nitrite and various excess amounts of cationic and anionic species that may be present in various samples. The tolerable level of each

Table 3 Tolerance to different species in the determination of 5 µg ml⁻¹ nitrite

Interfering species	Tolerance limit/µg ml ⁻¹
Cl ⁻	9000
NH ₄ ⁺ , Zn ²⁺	1500
Ni ²⁺	1200
Ca ²⁺ , Mg ²⁺ , ClO ₄ ⁻	1000
Co ²⁺	350
Br ⁻	250
HCO ₃ ⁻	200
CH ₃ COO ⁻ , SO ₃ ²⁻	150
Cr ³⁺ , I ⁻	100
C ₂ O ₄ ²⁻	70
F ⁻	30
CN ⁻	7

Table 4 Determination of nitrite in water samples

Sample	Concentration of nitrite/µg ml ⁻¹		Recovery, %
	Added	Found ^a	
River water	—	ND ^b	—
	1.5	1.4 ± 0.1	93
	2.5	2.4 ± 0.1	96
	4.0	4.0 ± 0.2	100
Well water	—	ND	—
	0.80	0.76 ± 0.1	95
	2.0	2.0 ± 0.1	102
	3.5	3.7 ± 0.2	105
Tap water	—	ND	—
	1.0	0.96 ± 0.1	96
	1.6	1.6 ± 0.1	100
	3.0	3.1 ± 0.2	103

a. Mean ± standard deviation.

b. Not detected.

Table 5 Determination of nitrite in water samples after preconcentration^a

Sample	Concentration of nitrite/ng ml ⁻¹		Recovery, %
	Added	Found	
River water	—	21	—
	50	67	93
Tap water	—	ND	—
	50	51	102
Well water	—	ND	—
	50	47	94

a. Sample volume used for preconcentration, 40 ml.

species was taken as the concentration which caused an error of not more than 5%. The error was calculated by comparing the peak height with that obtained by injecting an aqueous solution of nitrite containing no foreign ions as a reference. The results are summarized in Table 3. As can be seen, the proposed method can tolerate the foreign species tested in satisfactory amounts and, therefore, is adequately selective for the determination of nitrite in a wide range of water samples. In other words, no significant interference arises from the foreign species for the determination of nitrite in natural waters, because the foreign species generally are not above the tolerance concentration levels given in Table 3.

Determination of nitrite in water samples

The proposed method was applied to the determination of nitrite in several water samples. The samples were collected from three different sources in Isfahan and filtered as necessary to remove the interference caused by turbidity of the samples. Determination results and recoveries are listed in Tables 4 and 5. The recoveries of the nitrite added to the real samples are good enough for practical applications. As it is seen, only the river water sample had a measurable nitrite concentration, which could be determined only after preconcentration (Table 5).

Conclusions

The results reported here show that the flow injection system based on the reaction of nitrite with thiourea can be used as an alternative for the rapid, simple and precise determination of nitrite by a simple method. The system is economical because it does not require uncommon or expensive reagents. It could be used for the determination of nitrite in various water samples from µg ml⁻¹ concentrations down to ng ml⁻¹ (after preconcentration using an online column) concentrations.

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