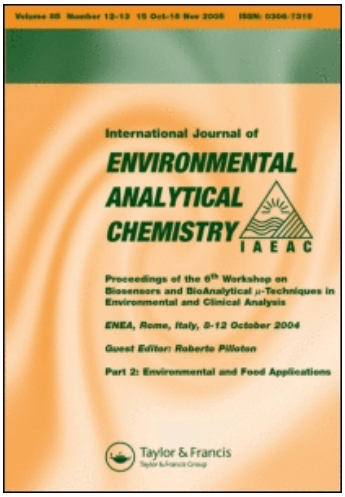

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SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF NITRITE AND NITRATE IN WATER SAMPLES BY FLOW-INJECTION ANALYSIS

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An automatic direct spectrophotometric method for the simultaneous determination of nitrite and nitrate by flow-injection analysis has been developed. The method is based on the reaction of nitrite and nitrate with the spectrophotometric reagent N-phenylanthranilic acid in sulfuric acid medium (pH=0.4~0.6) to form the same color products while the absorbance is measured at 410 nm. This method has the advantage of direct determination of nitrite and nitrate and without reducing nitrate to nitrite as other reported methods. Various analytical parameters, such as flow rate, sample size, effect of acidity(pH), reagent concentration and interfering species were studied. The detection limit is 2.5 ng ml^{-1} for nitrite and 12 ng ml^{-1} for nitrate. Up to 35 samples per hour can be analyzed with a relative precision of ca. 0.1–2%.

Keywords: Flow-injection; nitrite; nitrate; spectrophotometry; N-phenylanthranilic acid

INTRODUCTION

Nitrite and nitrate are currently monitored in natural waters and agricultural products because of the important role of the former in producing nitrosamine, a potent carcinogenic and mutagenic material [1,2]. Therefore, simultaneous determination of nitrite and nitrate is of importance in the field of environmental chemistry. Many spectrophotometric[3–5], kinetic[6–8], fluorimetric[9,10] and chromatoghraphic[11,12] methods have been described for the simultaneous

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determination of nitrite and nitrate. Some of the methods yield good sensitivity and selectivity, but require very expensive reagents or close control of pH, whereas others are time consuming.

In recent years there has been increasing interest in the development of automatic methods for the routine determination of nitrite and nitrate. These methods can be divided into two groups: flow injection (FI) methods[13–16] and sequential injection (SI) methods[17–18]. In most of those methods the nitrate is reduced to nitrite with a copper-coated cadmium column, the nitrite forms diazonium salt with sulphanilamide that is subsequently reacted with N-(1-naphthyl)ethylenediamine dihydrochloride(NED) to yield a pinkish azo dye, the absorbance of which is measured at 540 nm. Bermudez et al[19] reported that the reduction efficiency of a copper-coated cadmium column decreased after 40–50min and always results in production of poisonous waste water containing cadmium. Besides, the NED reagent is toxic[20] and it is harmful for the manipulator. So, it can not be utilized largely in the light of environmental protection.

It has been reported that the spectrophotometric reagent N-phenylanthranilic acid (PA) is very sensitive to both nitrite and nitrate ($\varepsilon' {NO_2}^{-1} = 1.70\times10^5$ $L\cdot mol\cdot cm^{-1}$; ε' ${NO_3}^{-1} = 0.87\times10^5$ $L\cdot mol^{-1}\cdot cm^{-1}$)[21], but was not previously used for the simultaneous determination of nitrite and nitrate in a FI system. N-phenylanthranilic acid reacts almost instantaneously with nitrite and nitrate and yields the same yellow product, the absorbance of which are both 410 nm (Figure 1). The aim of this study was to develop a simple flow-injection procedure based on this reaction for the simultaneous determination of nitrite and nitrate in water samples. In the proposed configuration of the manifold, the sample are divided into two parts, one portion passes directly through the column, yielding a composite response for nitrite and nitrate; in the other portion, nitrite react with sulfanilic acid (SA) reagent, which act as the masking reagent for nitrite to form diazonium salt. Then, yielding a response for nitrate alone, and the sample's nitrite content is calculated by the difference of the absorbance.

This procedure for nitrite and nitrate determination involved the use of noncarcinogenic reagents and without the reductor column. Therefore, the shortcomings mentioned above can be simply overcome. Finally, the method is comparable in sensitivity and precision to the standard method using sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride (NED)[22].

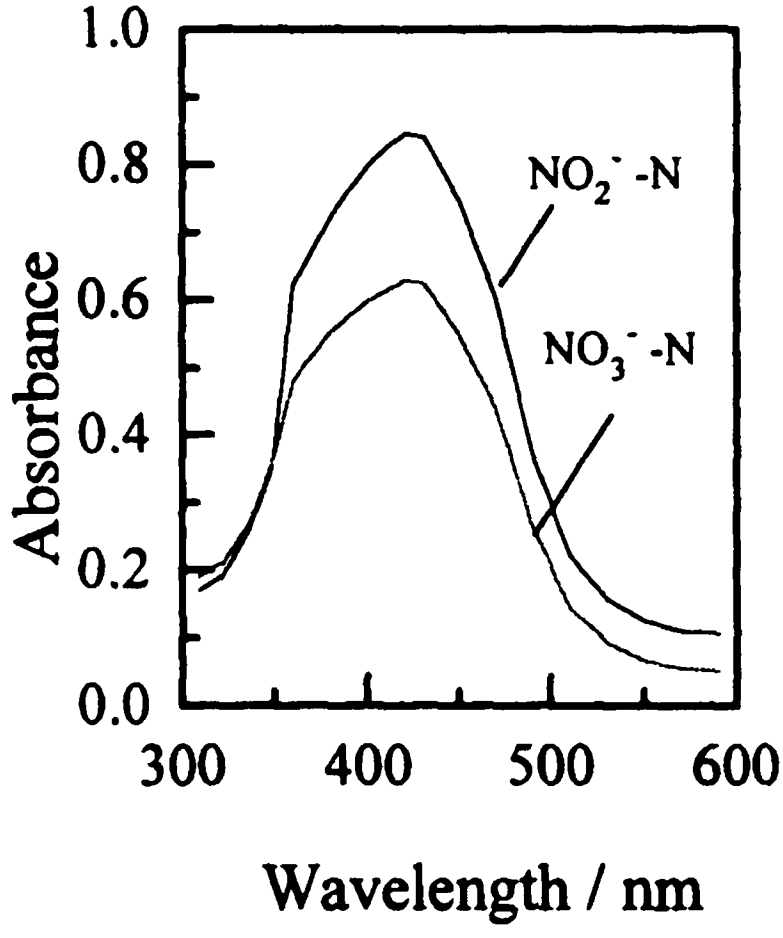

FIGURE 1 Absorption spectra of N-phenylanthranilic acid in the presence of 0.6 μg ml^{-1} of nitrite and 0.8 μg ml^{-1} nitrate, pH= 0.5

EXPERIMENTAL

Apparatus

The manifold for the simultaneous determination of nitrite and nitrate was made of poly(tetrafluoroethylene) (PTFE) tube (0.5 mm. I.D.) and the schematic diagram is shown in Figure 2. It consisted of a LZ-2000 Flow-injection apparatus

(Shengyang, China) having a four-way pneumatically actuated injection valve and an eight-channel peristaltic pump. The detector used was an U-3400 Spectrophotometer (Hitachi. Japan) which was performed to measure absorbance at 410 nm. A digital pH-3C meter (Shanghai, China) was used for the pH adjustments. Data processing and collection were performed with an IBM-compatible computer.

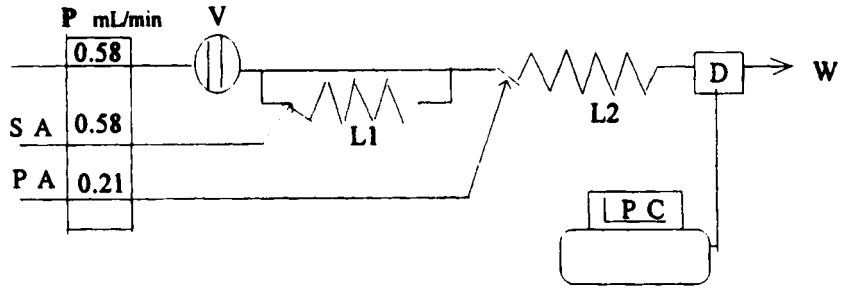

FIGURE 2 Schematic diagram of FI manifold employed for the simultaneous determination of nitrite and nitrate: P, pump; V, valve; L(1, 2), reaction coil; D, detector; W, waste; PC, personal computer; SA, sulfanilic acid; PA, N-phenylanthranilic acid

Reagents

All chemicals used were of analytical-reagent grade and doubly distilled water and spectroscopic chloroform were used throughout.

A stock nitrite solution (1mg ml^{-1}) was prepared by dissolving sodium nitrite (dried for 4h at 105–110°C) in doubly-distilled water. A pellet of sodium hydroxide was added to prevent liberation of nitrous acid and lml of spectroscopic grade chloroform to inhibit bacterial growth. The stock solution was kept in a refrigerator for preservation. Working standard nitrite solutions were freshly prepared by diluting the stock solution with 0.4 M NH_4Cl.

A stock nitrate solution (1mg ml^{-1}) was prepared by dissolving sodium nitrate dried (for 1h at 105–110 °C) in doubly-distilled water. The solution was treated with a few drops of chloroform and kept in a refrigerator for preservation. Working standard nitrate solutions were freshly prepared by appropriate dilution with 0.4 M NH_4Cl.

A stock solution of ammonium chloride solution (0.4 M) was prepared by dissolving anhydrous ammonium chloride (dried at 105 °C) in doubly-distilled water.

100 mg (0.1% w/v) of N-phenylanthranilic acid (PA) was dissolved in a mixture (100 ml) of concentrated sulfuric acid and doubly-distilled water in a 2:1 ratio. The solution was kept in a refrigerator for preservation.

80 mg of sulfanilic acid (SA) was dissolved in 100 ml of doubly-distilled water, and the sulfanilic acid (SA) solution (0.08% w/v) was kept in a refrigerator.

Solutions of a large number of inorganic ions were prepared from their analytical-reagent grade water-soluble salts, stock solutions and environmental water samples were kept in poly(propylene) bottles containing 1ml of spectroscopic grade chloroform.

Procedure

The flow diagram of simultaneous determination of nitrite and nitrate is shown in Figure 2. The sample was loaded into sample loops and introduced by carrier stream under the computer-controlled valve. The sample was divided into two channels, only in channel 2, the nitrite was masked by reacting with sulfanilic acid (SA) solution in reaction coil 1. Then, both samples were combined with the N-phenylanthranilic acid (PA) solution in reaction coil 2. The absorbances of the color products were detected by photometric detector using 410 nm filters. An IBM-compatible computer was used for data collection and analysis. The concentrations of nitrite and nitrate were evaluated from the peak heights by using calibration curves prepared from standards.

RESULTS AND DISCUSSION

Optimization of the flow-injection system

Preliminary tests were carried out with the aid of different flow assemblies to select the optimal configuration. The assembly (Figure 2) was selected as the one producing the best compromise between peak height and the shape of the peak.

In order to optimize the proposed flow-injection manifold, the influence of the hydrodynamic and chemical parameters were studied. Table I shows the results of the optimization of working conditions for 0.6 $\mu g\ ml^{-1}$ of nitrite and 0.8 $\mu g\ ml^{-1}$ of nitrate.

The optimum lengths of the reaction coils 1 (L_1) and 2 (L_2) were chosen as 35 cm and 60 cm, respectively, because with the lengths, the reactions were completed and the reproducibility was good. The sample size is sensitive to the system (Figure 4) and a sample size of 100 µl was selected. On the other hand an overall flow rate of 0.58 ml min^{-1} and a reagent flow rate of 0.21 ml min^{-1} were selected being a compromise between the sampling rate and the height of the peak. Of the various acids (phosphoric, sulfuric and hydrochloric) studied, sulfu-

ric acid was found to be the best acid as the reaction medium for the system. Different concentrations of sulfuric acid were tested in the range shown in Table I. The absorbance was therefore, maximum and constant when the pH of solution was kejtat 0.5–0.6 (Figure 3). Therefore, for all subsequent measurements, 0.16 M sulfuric acid (pH=0.5) was used.

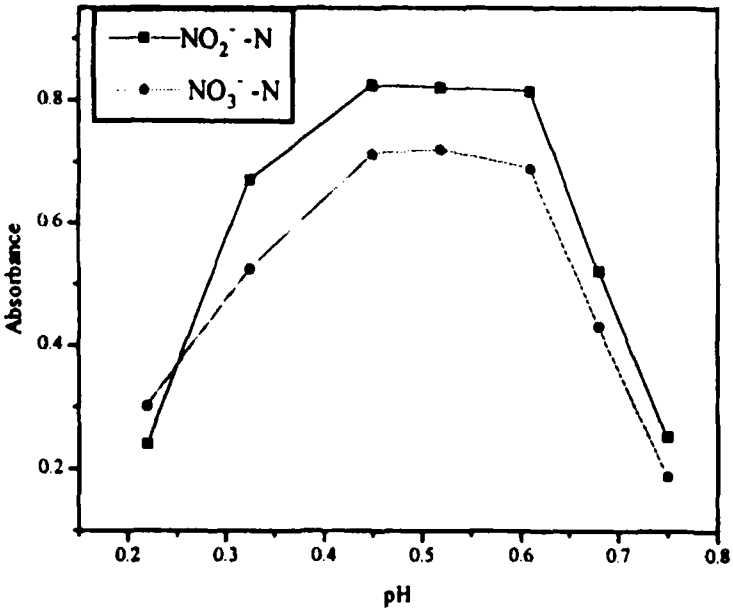

FIGURE 3 Effect of pH value on the sensitivity, 0.6 μg ml^{-1}; nitrate, 0.8 μg ml^{-1}

TABLE I Selected chemical and FIA parameters obtained with the optimization manifold

Parameter	*Studied range*	*Selected value*
Size of sample loop (μl)	40–120	100
Length of reaction coil L_1 (cm)	15–60	35
Length of reaction coil L_2 (cm)	30–110	60
Overall flow rate (ml min^{-1})	0.2–1.2	0.58
Reagent flow rate (ml min^{-1})	0.06–0.30	0.21
Concentration of reagent (M)		
H_2SO_4	0.05–0.30	0.16
Sulfanilic acid	0.0001–0.0005	0.0004
N-phenylanthranilic acid	0.002–0.006	0.004

Evaluation of the method

The reproducibility and sensitivity of the proposed procedure were determined by repeated injections of a sample (n=5) containing 0.6 μg ml^{-1}nitrite and 0.8 μg ml^{-1} nitrate. The relative standard deviations were 0.1–2% for 0.01–2.5 μg ml^{-1} nitrite and 0.1–4.5 μg ml-1 nitrate, indicating that this method is highly precise and reproducible. The detection limit, defined as three times the baseline noise (S/N=3), was 2.5 ng ml^{-1} for nitrite and 12 ng ml^{-1} for nitrate. The sample throughput was 35 measurements per hour. The effect of temperature on the peak height was studied between 10–50 °C, and a temperature range of 10–15 °C was found the most suitable, because a further increase temperature did not result in any significant change in sensitivity. Important features of the proposed method for simultaneous determination of nitrite and nitrate are summarized in Table II.

TABLE II Analytical features of the proposed method

Parameter	*Nitrite*	*Nitrate*
Acidity (pH)	0.4–0.6	0.4–0.6
Linear range (μg ml^{-1})	0.01–2.5	0.1–5.5
Detection limit (ng ml^{-1})	2.5	12
Reproducibility (% RSD)	0.1–2	0.1–2
Sample throughput (sample h^{-1})	35	35
temperature (°C)	10–15	10–15

The interference of several ions which occur in environmental water samples besides nitrite and nitrate was studied by using a mixture of nitrite (0.6 μg ml^{-1}) and nitrate (0.8 μg ml^{-1}) and adding various concentrations of interfering ions up to the amounts where the relative error reached a value of about 5%. The errors were calculated by injecting an aqueous solution of nitrite and nitrate containing no interfering ions as a reference. Unlike the common interference from Cu(II), Cr(III), and Fe(II) in most azo dye methods[13–16], in the proposed method these ions are tolerated up to concentrations of 100 μg ml^{-1}; 200 μg ml^{-1}; and 100 μg ml^{-1}, respectively. All other ions tested could be tolerated at reasonably high concentrations, as indicated in Table III.

Applications

The proposed method was applied to the simultaneous determination of nitrite and nitrate in a number of environmental water samples, the samples were col-

lected from the city of Lanzhou and its surroundings, prior filtration of the samples was necessary due to the interference caused by the turbidity of the particles in the samples. Determination results are given in Table IV. The results of the environmental samples analyses by the flow-injection (FI) method were found to be in excellent agreement with those obtained by the official standard method using sulphanilamide-NED[22].

TABLE III Effect of interfering ions on the determination of 0.6 μg ml^{-1}nitrite and 0.8 μg ml^{-1} nitrate

Interfering ion	*Concentration*[a]	
	NO_2^-	NO_3^-
Sodium	1000	1000
Potassium	1000	1000
Nickel (II)	200	200
Chromium (III)	200	200
Cobalt (II)	400	400
Iron (II)	100	100
Manganese(II)	100	100
Copper (II)	100	100
Acetate	150	150
Ammonium	1000	1000
Chloride	1500	1500
Carbonate	500	500
Phosphate	100	100
Nitrate	1000	---
EDTA	1000	1000
Iodide	50	50
Iodate	100	100

a. A 5% error criterion is adopted

TABLE IV Determination of nitrite and nitrate in environmental samples (μg ml^{-1})

Sample	*Concentration of* NO_3^-			*Concentration of* NO_2^-		
	Method			*Method*		
	FIA	*AOAC*	*Error(%)*	*FIA*	*AOAC*	*Error (%)*
Yellow River water						
Sample 1	2.52	2.51	0.39	0.02	0.02	0.00
Sample 2	2.47	2.47	0.00	0.01	0.01	0.00

Sample	Concentration of NO_3^-			Concentration of NO_2^-		
	Method			Method		
	FIA	AOAC	Error(%)	FIA	AOAC	Error (%)
Tap water						
Sample 1	1.54	1.53	0.65	0.01	0.01	0.00
Sample 2	1.46	1.44	1.38	--	--	--
Lake water	1.73	1.72	0.58	0.047	0.05	1.2
Spring water	3.25	3.24	0.30	0.098	0.10	0.8

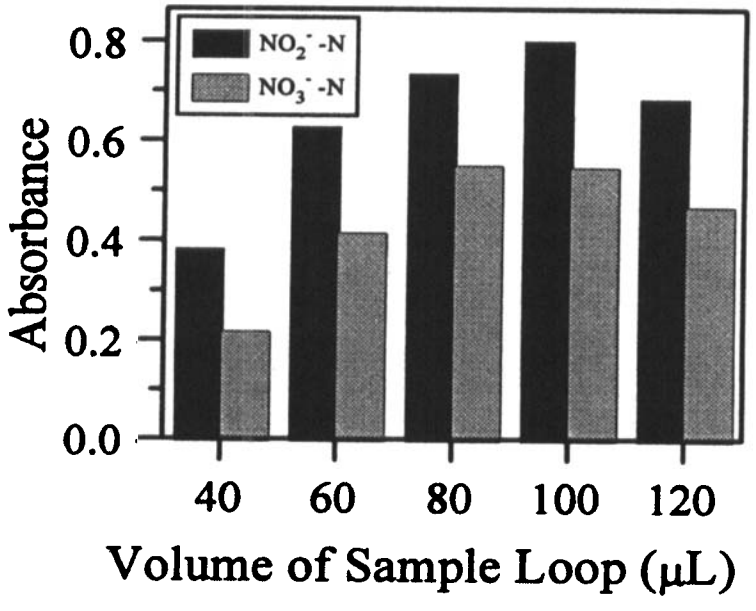

FIGURE 4 Effect of sample size on the sensitivity: nitrite, 0.6 μg ml^{-1}; nitrate, 0.8 μg ml^{-1} pH= 0.5

CONCLUSION

The results reported above show that the new proposed method could be an alternative to the rapid on-line simultaneous determination of nitrite in the procedures

reported earlier[3–16]. Although with lower sensitivity, the system is simpler and without producting the poisonous waste. Therefore, this method could be applied in the monitoring of trace amounts of nitrite and nitrate in environmental water samples.

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