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Simple Flow Injection Analysis System for the Spectrophotometric Determination of Trace Amounts of Nitrite in Water Samples

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Abstract

A simple and sensitive spectrophotometric flow injection analysis system has been developed for the determination of trace amounts of nitrite. The method is based on the reaction of nitrite with sulfanilamide in acidic medium to form a diazonium salt which then couples with thenoyltrifluoroacetone in alkaline medium to give an azo dye. Beer's law is obeyed at 470 nm over a nitrite concentration range of 1-1200 μg L^{-1}, and a detection limit for nitrite is 0.3 μg L^{-1} is achieved with a relative standard deviation of 1.4% at 50 μg L^{-1} (n=8). An injection rate of about 40 h^{-1} is possible using this method, which was successfully applied to the determination of nitrite in rain water, tap water, pond water, and river water samples.

Keywords Flow injection analysis, Nitrite, Spectrophotometry, Thenoyltrifluoroacetone, Sulfanilamide

1. Introduction

Nitrite is an active intermediate in the microbiological decomposition of nitrogen-containing compounds. Nitrite-forming bacteria convert ammonia to nitrite under aerobic conditions. Bacterial reduction of nitrates to nitrites can also occur under anaerobic conditions [1-2]. Therefore, all sources of nitrogen such as organic nitrogen compounds, ammonia, and nitrogenous fertilizer residues should be considered as potential sources of nitrite in natural waters. Nitrite salts are also used as a color fixative and as a preservative in the food industry and a corrosion inhibitor in industrial process water.

Nitrite reacts with secondary and tertiary amines and amides in the human body to form N-nitrosamines which are known carcinogens and mutagens [3-5]. This ion also oxidizes iron in hemoglobin of the red blood cells to produce methemoglobin, which inhibits oxygen transport to the tissues [6]. Therefore, determination of nitrite in environmental samples is of great importance because of its harmful effects on human health, especially infants and pregnant women [7].

Several analytical methods have been proposed for the determination of trace nitrite in different samples, including spectrophotometry [8-10], fluorimetry [11-13], chemiluminescence [14-15], kinetic [16-18], electroanalytical [19-21], chromatography [22-25], and capillary electrophoresis [26-27]. Most of these methods are not suitable for routine determination of nitrite in water and environmental samples because they are either time consuming, or require sophisticated and expensive equipments, or exhibit poor precision and accuracy.

Numerous flow injection analysis methods with a variety of detection systems have also been reported for the determination of nitrite [28-34]. These methods offer some advantages, especially for routine analysis because of their high reproducibility and reliability, high sample throughput, low analytical costs, and economical sample and reagent consumption [35].

In the present work, a simple and sensitive flow injection analysis system equipped with a multi-reflection cell and a photometric detector is applied to the determination of nitrite using a diazotization-coupling reaction. Sulfanilamide is diazotized by nitrite in acidic medium, and coupled with thenoyltrifluoroacetone (TTA) to produce a highly colored azo dye in alkaline medium (Fig. 1(a) and (b)). The method is sufficiently sensitive to determine nitrite in a range of water samples without involving a preconcentration step.

2. Experimental

2.1. Reagents

All the chemicals used were of analytical reagent grade, and ultrapure de-ionized water (Millipore Milli-Q water system, USA, 18.2 MΩ cm resistivity) was used for all dilutions.

A stock solution of 1000 μg mL^{-1} nitrite was prepared by dissolving 0.15 g of pre-dried (4 h at 110 °C) sodium nitrite (E. Merck, Darmstadt, Germany) in water. A pellet of sodium hydroxide was added to the solution to prevent the decomposition of nitrite and a few drops of chloroform were also added to inhibit bacterial growth. The solution was diluted to the mark with water in a 100 mL calibrated flask and was kept in a refrigerator at 4 °C. The working solutions were prepared daily by serial diluting the stock solution with water.

A 2.5 g L^{-1} acidic solution of sulfanilamide prepared by dissolving 0.625 g of the reagent (E. Merck, Darmstadt, Germany) in 50 mL of 3 mol L^{-1} hydrocholoric acid and diluting to 250 mL with water. Thenoyltrifluoroacetone solution (3 g L^{-1} in 10% acetone) was prepared by dissolving 0.75 g of the reagent (Sigma-Aldrich, Steinheim, Germany) in 25 mL acetone and diluting to 250 mL with water. A 0.9 mol L^{-1} sodium hydroxide (E. Merck, Darmstadt, Germany) solution containing 0.05 mol L^{-1} EDTA was prepared by dissolving 9 g sodium hydroxide and 4.653 g of EDTA disodium salt (E. Merck, Darmstadt, Germany) in water and made up to 250 mL in a 250 mL standard flask.

2.2. Apparatus

The flow injection system used for the determination of nitrite is shown schematically in Fig. 2. The manifold was furnished with two peristaltic pumps P_1 (Ismatic, model IS7610, Switzerland) and P_2 (Alitea AB, Model C2-OEM, USA) and an electrically actuated rotary injection valve (Rheodyne, model 5020, USA). Tygon® pump tubing was used, and PTFE tubing (0.5 mm i.d.) was used for the injection loop and all manifold lines.

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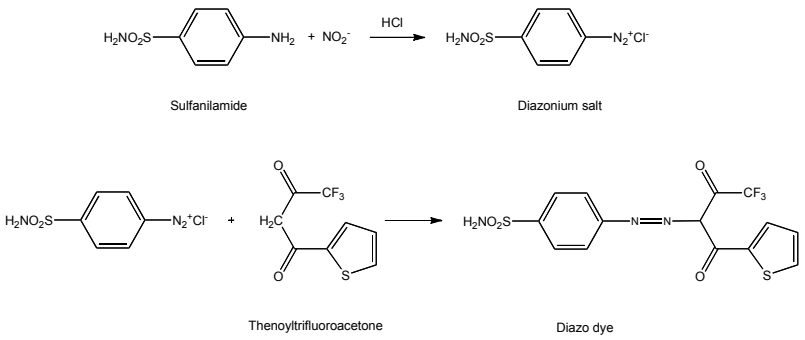
(a)



(b)

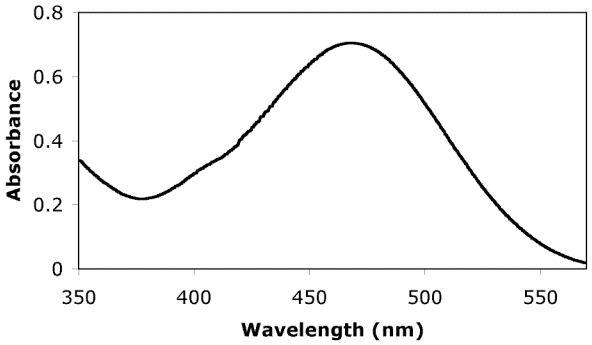


Fig 1. (a) Reaction scheme for the determination of nitrite with thenoyltrifluoroacetone (TTA). (b) UV-Visible absorption spectrum of the azo dye formed by coupling of TTA with diazotized nitrite (1.2 mg L^{-1} nitrite).

Reaction coils were knotted tightly to improve radial dispersion. The absorbance of the azo dye formed was measured in a multi-reflection cell with an effective optical pathlength of approximately 20 mm [36] in conjunction with an LED (Nichia, Ultrabright blue, λ_{max} = 470 nm) and an in-house designed photometer containing a photodiode (Infineon SFH250V) coupled with a log amplifier (Burr-Rown, LOG102A, USA). A linear relationship between Absorbance and output voltage, V, (mV) was observed such that Abs = (7.289 x 10^{-5})V - 0.0176. Flow injection system control and data acquisition were achieved using a visual programming language (Lab VIEW 8.5, National Instruments, Austin, TX, USA).

2.3. Procedure

Carrier (ultrapure water) and reagent solutions in the reservoirs R_1 (sulfanilamide solution), R_2 (thenoyltrifluoroacetone solution), and R_3 (sodium hydroxide solution) were pumped using the peristaltic pump P_2. Sample or standard solution was injected into the carrier using a 200 µL loop. Following diazotization of nitrite with sulfanilamide, the diazonium product was coupled with thenoyltrifluoroacetone in alkaline medium to form a stable azo dye that was then detected in the multi-reflection cell. The absorbance-time response was monitored and the peak response (in mV) was used for quantitative analysis.

2.4. Water samples

Water samples were collected without adding any preservative in the previously cleaned polyethylene bottles and analyzed within 6 h. after filtration through a 0.22 µm pore-size membrane filter (Millipore, Bedford, MA, USA).

3. Results and discussion

Most spectrophotometric methods for determination of nitrite are based on the formation of an azo dye [9]. In these methods, an aromatic amine or sulfamate is diazotized, and the intermediate is then coupled with a suitable aromatic compound to produce a colored azo dye. In this work, thenoyltrifluoroacetone (TTA), a β-diketone which is one of the most strongly chelating extractants for some metal ions, is used as a coupling agent for spectrophotometric flow injection analysis of trace nitrite. Sulfanilamide is diazotized in acidic medium, and coupled with TTA to give a stable color dye in alkaline medium according to the mechanism presented in Fig. 1.

Different flow assemblies were tested to select the best manifold configuration. The selected manifold configuration is shown in Fig. 2. In order to establish the best conditions for the determination of nitrite, the influence of reagent concentration, sample volume, and length of reaction coils on the sensitivity was studied, and optimized a univariate approach. Experiments were performed at room temperature.

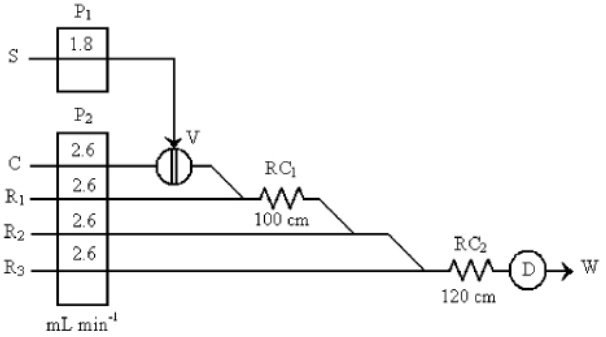


Fig 2. Schematic diagram of the flow injection system used for the determination of nitrite. S, sample; C, carrier; R_1, 2.5 g L^{-1} solution of sulfanilamide containing 0.6 M hydrocholoric acid; R_2, 3 g L^{-1} thenoyltrifluoroacetone solution containing 10% acetone; R_3, 0.9 M sodium hydroxide solution containing 0.05 M EDTA; P_1 and P_2 peristaltic pumps; V, injection valve; RC_1 and RC_2, reaction coils; D detector; W, waste.

The reaction of nitrite with sulfanilamide to form diazonium ion occurs under acidic conditions. The effect of hydrochloric acid concentration in the sulfanilamide solution was investigated over the range of 0.1-1.2 mol L^{-1}, and the largest response was obtained in the range of 0.4-1 mol L^{-1} hydrochloric acid. A concentration of 0.6 mol L^{-1} was chosen for further experiments.

The effect of sulfanilamide concentration on the sensitivity was investigated from 0.5 to 10 g L^{-1}. The analytical signal increased with sulfanilamide concentration up to 2.5 g L^{-1} before reaching a plateau. A concentration 2.5 g L^{-1} of sulfanilamide was selected as the optimum condition.

The influence of coupling agent concentration on the sensitivity was studied over the range of 0.5 to 6 g L^{-1}. The results demonstrated (Fig. 3) that the analytical signal increased with TTA concentration up to 3 g L^{-1} and then leveled off at higher concentrations. Therefore, a concentration of 3 g L^{-1} was chosen for subsequent applications.

Diazonium ion couples with TTA in alkaline media. However preliminary experiments showed that TTA was not stable for more than a few hours in sodium hydroxide solution. Therefore, TTA (R_2) and sodium hydroxide (R_3) solutions were prepared separately and mixed on-line with the diazotized sample (Fig. 2). The effect of sodium hydroxide concentration was tested over the range of 0.5-1.5 mol L^{-1}. The results showed that a 0.9 mol L^{-1} sodium hydroxide solution was sufficient for maximum color development, and thereafter no further change in the analytical signal was observed. Hence a concentration of concentration of 0.9 mol L^{-1} sodium hydroxide was used as optimum. EDTA (0.05 M) was also added to the sodium hydroxide solution as an appropriate masking agent for some metal ions, such as Fe^{3+}, Pb^{2+}, Mn^{2+} and Cu^{2+}, that may interfere when present in large quantities in real samples. These ions can form hydroxide or metal-TTA complexes in alkaline solutions.

The effect of sample loop volume on the sensitivity was examined by varying the volume from 50 to 300 µL. An injection volume of 200 µL gave the highest signal, and hence this injection volume was chosen for all subsequent.

Lastly, the effect of reaction coil length (RC_1 and RC_2) was examined over the range 50-200 cm, and coil lengths of RC_1 = 100 cm and RC_2 = 120 cm were selected as a compromise between the sampling rate and the height of the peak.

3.1. Analytical figures of merit

Under the optimum conditions, the relationship between absorbance and concentration of nitrite was linear in the concentration range of 1-1200 µg L^{-1}. The calibration equation was: H = 13.534 C + 1.629 (where H is peak height in mV and C is the concentration of nitrite in µg L^{-1}) with a correlation coefficient of 0.9999 (n = 13).

3.2. Effect of foreign ions

In order to evaluate the possible analytical applications of the proposed method, the effects of potentially interfering ions were studied by analysing a 100 µg L^{-1} nitrite standard in the presence of a variety of foreign ions.

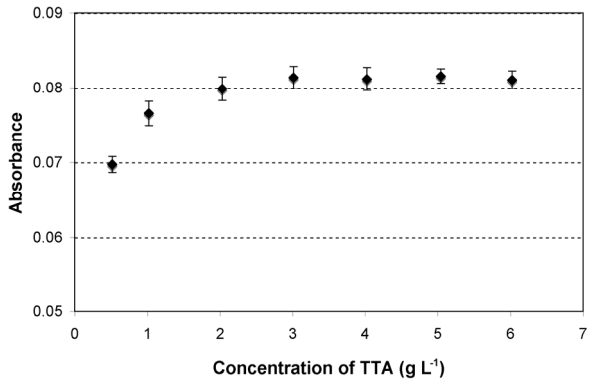


Fig. 3. Effect of TTA concentration on the peak height of 100 µg L-1 nitrite

The tolerance limits shown in Table 1 are the maximum concentration of foreign ions that cause no more than a 5% error in the determination of nitrite. Anions have no serious interference, whereas some metal ions, such as calcium, zinc and magnesium that form hydroxide or metal-TTA complexes in alkaline medium may adversely affect the determination of nitrite. Hence, these ions were masked with 0.05 mol L^{-1} EDTA throughout the experiments.

Table 1 Tolerance limits of foreign ions on the determination of 100 μg L^{-1} nitrite

Foreign ion	Tolerance limit (mg L^{-1})
Ca^{2+}, K^{+}, Li^{+}, Cl^{-}, F^{-}, Br^{-}, PO_4^{3-}, CO_3^{2-}, NO_3^{-} CH_3COO^{-}, $C_2O_4^{2-}$, SO_4^{2-}, citrate, tartrate	1000
Zn^{2+}, Mg^{2+}	500
Pb^{2+}, Cu^{2+}	100
Mn^{2+}	30
Fe^{3+}, Cr^{3+}	20

3.3. Applications

The proposed flow injection analysis method was applied to the determination of nitrite in rain water, tap water, pond water, and river water samples. To investigate the reliability of the method, samples were spiked with known amounts of nitrite solutions, and analyzed according to the proposed method. The water samples were also analyzed using a reference FIA method using the reaction of sulfanilamide with N-(1-naphthyl)ethylenediamine (NED) [37]. Table 2 shows that the recoveries of the spiked samples are quantitative (97.5-100.5%), and that there is no significant difference (paired t-test, 95% confidence level, $t_{experimental}$ = 0.25, $t_{critical}$ = 3.18, n = 4) between the analytical results obtained by the proposed method and the data obtained by reference method.

Table 2 Determination of nitrite in water samples.

Sample	Added (μg L^{-1})	Found* (μg L^{-1})	Recovery (%)	Standard method* (μg L^{-1})
Rain water	-	24.6 ± 0.3	-	24.3 ± 0.4
	20	44.3 ± 0.7	98.5	
Tap water	-	6.3 ± 0.2	-	6.1 ± 0.1
	20	26.4 ± 0.6	100.5	
River water	-	3.1 ± 0.2	-	3.2 ± 0.1
	20	22.7 ± 0.4	98	
Pond water	-	32.8 ± 0.6	-	33. 4 ± 0.5
	20	52.3 ± 0.9	97.5	

*Mean and standard deviation of three determinations.

Table 3 Comparison of the proposed method with some previously reported spectrophotometric methods based on diazotization-coupling reaction for determination of nitrite.

Diazotization reagent	Coupling reagent	Mode	DL μg L^{-1}	Linear range μg L^{-1}	Sample	Reference
p-Nitroaniline	Naphth-1-ol	Batch	-	20-140	River water	[38]
p-aminobenzoic acid	8-hydroxyquinoline	Batch	100	100-1500	-	[39]
AAP	Resorcinol	Batch	5	0-333	Water, soil	[40]
p-Nitroaniline	Guaiacol	Batch	-	30-200	Polluted water	[41]
o-Nitroaniline	ANSA	Batch	-	1-80	Polluted water	[42]
Sulfanilamide	NED	FIA	100	0-10000	River water	[43]
Sulfadiazine	α-Naphthol	Batch	40	80-960	-	[44]
3-Nitroaniline	NED	FIA	1	10-2200	Water, soil, beer, food	[45]
Sulfanilamide	NED	SIA	10	50-2500	Waste water	[46]
p-Nitroaniline	Acetyl acetone	Batch	-	50-1400	Water, soil	[47]
p-Nitroaniline	Ethyl acetoacetate	Batch	-	50-6000	Water, soil	[10]
Sulfanilamide	Ethyl acetoacetate	Batch	-	200-3000		
Sulfanilamide	NED	SIA	48 400	0-3000 0-20000	Waste water	[48]
MMCBAT	N,N-dimethylaniline	Batch	12	50-2000	Tap and lake water	[49]
Sulfanilamide	NED	FIA	43	66-1600	Soil	[50]
Sulfanilamide	NED	FIA	8	100-1000	Fresh water	[51]
Sulfanilamide	NED	FIA	72	164-5255	Soil	[52]
Sulfanilamide	TTA	FIA	0.3	1-1200	Water	*This work*

DL: detection limit; FIA: flow injection analysis; SIA: sequential injection analysis; AAP: *p*- aminoacetophenone; ANSA: 1-aminonaphthalene-2-sulphonic acid; NED: N-(1-naphthyl)-ethylenediamine hydrochloride ; MMCBAT: 4-(1-methyl-1-mesitylcylobutane-3-yl)-2-aminothiazole; TTA: thenoyltrifluoroacetone.

4. Conclusion

A simple spectrophotometric flow injection analysis method based on the diazotization-coupling reaction has been proposed for the determination of nitrite. The coupling reagent (thenoyltrifluoroacetone) used in this work provides a very sensitive method for the analyte at room temperature. The reagent is commercially available and its solution is stable at least for one month. The proposed method is convenient, economic, and enables a high sample throughput of 40 injections h^{-1} to be achieved. The method has a wide linear range and a better detection limit with many previously reported spectrophotometric flow and batch methods based on diazotization-coupling reactions (Table 3). The method has been successfully applied to the determination of trace amounts of nitrite in rain water, tap water, pond water, and river water samples.

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