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Flow injection kinetic spectrophotometric method for the determination of trace amounts of nitrite

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ABSTRACT

In this work, a new, simple and sensitive flow injection catalytic kinetic spectrophotometric determination of nitrite is reported based on catalytic effect of nitrite on the redox reaction between sulfonazo III and potassium bromate in acidic media. The reaction was monitored by measuring the decrease in the absorbance of sulfunazo III at 570 nm. Various chemical (such as the effect of acidity, reagents concentrations) and instrumental parameters (flow rate, reaction coil length, injection volume and temperature) were studied and were optimized. Under the optimum conditions calibration graph was linear in the nitrite concentration ranges of 8.00×10^{-3}–3.00×10^{-1} μg/ml (with slope of 2.40) and 3.50×10^{-1}–1.80 μg/ml (with slope of 0.42). The detection limit was 6.00×10^{-3} μg/ml of nitrite, the relative standard deviation ($n = 10$) was 1.25% and 0.88% for 5.00×10^{-2} and 2.00×10^{-1} μg/ml of nitrite respectively. About 60 samples in 1 h can be analyzed. The interfering effects of various chemical species were studied. The method was successfully applied in the determination of nitrite in food and environmental samples.

1. Introduction

Nitrite ion is commonly monitored for environmental protection purposes in water, agriculture and food control. Nitrite ion formation is an important step in the nitrogen cycle. Leafy vegetables which are not only an excellent source of vitamins, minerals and biologically active compounds [1,2], but they are also one of the main sources of nitrite in our bodies. Commonly nitrates are abundant in food primarily because plants take up nitrogen from the soil in this ionic form. The nitrates in foods then can be reduced to nitrite because of some bacteria's action [3]. In another way, nitrite is widely used as preservatives in meat products due to their ability to inhibit the growth of spores of *Clostridium botulinum*. It is added (particularly to ground meat products) in the meat curing process to speed curing and the formation of the required colors and flavors. Nitrite is formed during the biodegradation of domestic or industrial nitrogenous wastes as well as some fertilizers. Air-borne nitrogen oxides are converted into nitrite ion, which is also a component of acid rains. Nitrite produces nitrosamines in human body through its reaction with amines or amides, which is carcinogenic compound. Nitrite can also interfere with the oxygen transport system in the body and may result in the condition known as methemoglobinemia, in which the ability of hemoglobin to exchange oxygen is seriously reduced [4,5]. Infants under 3 months are thought to be more susceptible than adults [3]. Due to these toxic effects, it is important to develop new analytical methods for determination of nitrite in food products. Many analytical methods have been proposed to trace nitrite analysis. Most of the methods are based on the formation of strongly colored azo dyes. An amine is diazotized by means of nitrite under special conditions and the intermediate is let to react with a selected aromatic compound to couple. Several aromatic amines have been used for diazotization such as 4-aminobenzoic acid [6], 4-amino-1-naphtalenesulfonic acid [7], 2-nitroaniline [8], 3-nitroaniline [9], 4-nitroaniline [10–15], *p*-rosaniline [16], 4-aminophenylmercaptoacetic acid [17,18], 4-aminobenzotrifluoride [19], sulfamate group compounds such as sulfanilamide [20] and safranin [21]. Azo dye formation is dependent on pH, diazotization temperature and coupling time. Toxicity of certain amines is also an important point for chemists. Coupling time is relatively long (in the case of sulfanilic acid/NEDA which is a reference method, color development is completed in 40 min). In contrast to these azo dye formation methods, kinetic catalytic spectrophotometric methods are very fast, simple and sensitive. Also, the concept of flow injection analysis has been applied successfully to the determination of nitrite, using various techniques such as kinetic catalytic spectrophotometric methods [22,23].

In this paper, a new flow injection spectrophotometric method is proposed for the determination of nitrite based on catalytic effect of nitrite on the redox reaction between sulfonazo III and potassium

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bromate in acidic media. The reaction was monitored by measuring the decrease in the absorbance of sulfunazo III at 570 nm. The proposed method was used to determine of nitrite ion in real samples with satisfactory results.

2. Experimental

2.1. Apparatus and reagents

A diagram of the flow system employed is shown in Fig. 1. The decrease in absorbance was measured with an Ultrospec Model Pro-3100 spectrophotometer equipped with a flow through cell (20 μl inner volumes). A 4-channel peristaltic pump (Gilson, Minipuls 3) with three silicon rubber tubes (1.02 mm i.d.) was used. PTFE mixing joints and PTFE tubing (0.5 mm i.d.) were used for the connections and the reaction coil. The controlled water bath (home made) was used for controlling the temperature. Sample solutions were injected using a six position rotary Rheoyne valve with a sample loop of 294 μl.

All the solution was prepared using reagent grade chemicals. Triply distilled water was used throughout.

Nitrite standard solution (1000 μg/ml) was prepared by dissolving 0.15 g of dried (for 4 h at 105–110 °C) sodium nitrite (Merck) in three-ply distilled water and diluting to 100 ml in volumetric flask. A pellet of sodium hydroxide was added to prevent liberation of nitrous acid and 1 ml of chloroform to inhibit bacterial growth. Working standard solution was freshly prepared by diluting the stock solution with water. Sodium bromate solution (0.50 M) was prepared by dissolving 3.77 g of $NaBrO_3$ (Merck) in water and diluting to 50 ml in a volumetric flask. Sodium nitrate solution (1.0 M) was prepared by dissolving 8.45 g of $NaNO_3$ (Merck) in water and diluting to 100 ml in a volumetric flask. Sulfonazo III solution (1.00×10^{-3} M) was prepared by dissolving 0.194 g of the dye (Fluka) in 100 ml of distilled water, after dissolving completely, the result solution diluted with water in a 250-ml volumetric flask. Sulfuric acid stock solution (1.0 M) was prepared by diluting of 13.9 ml of concentrate solution (96%) in a 250-ml volumetric flask with doubly distilled water.

2.2. Recommended procedure

As shown in Fig. 1 each solution containing carrier (H_2O, R_1), bromate solution (R_2), and a mixture of the dye plus sulfuric acid and sodium nitrate (R_3) previously thermostated at an appropriate temperature (25 °C) is pumped at 1.4 ml/min via a peristaltic pump. The standard solution containing 8.00×10^{-3}–0.30 μg/ml and 3.50×10^{-1}–1.80 μg/ml NO_2^- was injected into a carrier stream via sample injection valve. The sample solution was directly treated with a mixture of bromate, sulfuric acid and the dye, and then passed to the sample flow cell of the spectrophotometer via reaction coil, where the decrease in absorbance at 570 nm was measured as nitrite concentration. The concentration of nitrite was evaluated from the peak height measurements by using a calibration curve prepared from the results obtained on standards.

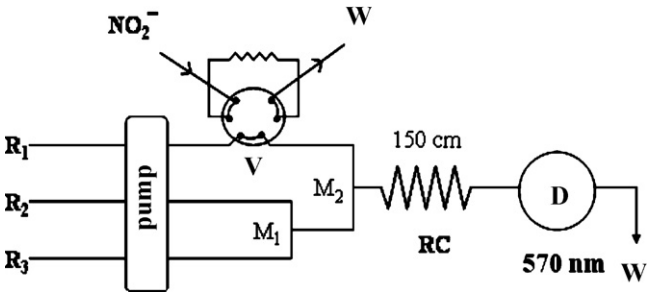

Fig. 1. Schematic representation of the FI manifold employed for the determination of nitrite; R_1, carrier; R_2, bromate solution; R_3, a mixture solution of the sulfonazo III, the sulfuric acid and the sodium nitrate; V, injection valve; M_1 and M_2, mixing zone of the reagents; RC, reaction coil; D, detector; W, waste.

2.3. Real sample analysis

For the beef sausage, 5.0 g of sample was mixed and homogenized in a mortar. The thoroughly mixed sample was taken in a 500 ml beaker and digested carefully by heating the solution content in water batch for 2 h. The mixture was filtered using filter paper (Whatman No. 1). The result solution was collected in a 250 ml volumetric flask and diluted with water to the mark. The nitrite contents were measured by recommended procedure following the method recommended by the AOAC [24]. Any of the sample solutions of sausage and tap water passed through a column (10 cm × 1.0 cm) containing cation exchange resin before analysis. Tap water sample was used without any pretreatment.

3. Results and discussion

Sulfonazo III is a dye can be oxide in acidic media with strong oxidizing agent such as bromate in slow rate. The oxidation reaction of sulfonazo III undergoes fast in the presence of trace amounts of nitrite, and the absorbance of the dye decreases at 570 nm, rapidly. Therefore, 570 nm was used as detection wavelength. In addition, nitrate does not affect directly on the oxidation reaction, even in the presence of 1000-fold and more relation to nitrite. Preliminary tests were carried out with the aid of different flow assemblies to select optimal manifold configuration. The assembly in Fig. 1 was selected as the best configuration. In order to optimize the flow injection system, the influence of the reagents concentration and temperature as well as manifold variables on the sensitivity was studied.

Considering experimental results, the reaction could run faster in sulfuric acid media than other inorganic acids in the same concentration. Therefore, we selected sulfuric acid for the study. The influence of reagents concentration on the sensitivity was checked using 0.05 μg/ml nitrite, pump flow rate of 1.4 ml/min, sample loop volume of 196 μl and reaction coil length of 150 cm at room temperature.

Fig. 2 shows the influence of sulfuric acid concentration in the range of 0.50–1.00 M, with 0.05 μg/ml nitrite at 25 °C (conditions were shown in the legend of the figures). The results show that increasing the acid concentration leads to increasing the sensi-

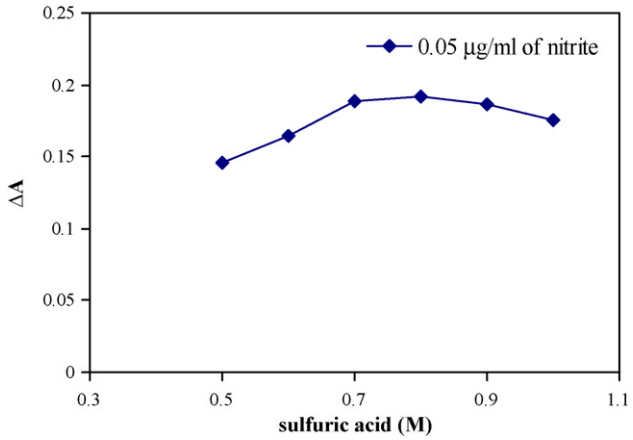

Fig. 2. Influence of sulfuric acid concentration on the sensitivity. *Conditions*: BrO_3^-, 0.30 M; sulfonazo III, 1.00×10^{-4} M; NO_2^-, 0.05 μg/ml; flow rate, 1.4 ml/min; reaction coil length, 150 cm, injection volume, 196 μl; and *t*, 25 °C.

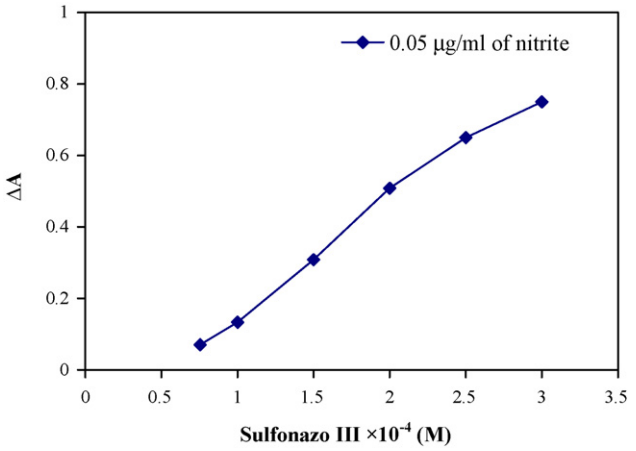

Fig. 3. Effect of sulfonazo III concentration on the peak height. *Conditions*: BrO_3^-, 0.30 M; H_2SO_4, 0.70 M; NO_2^-, 0.05 μg/ml; flow rate, 1.4 ml/min; reaction coil length, 150 cm; injection volume, 196 μl; and *t*, 25 °C.

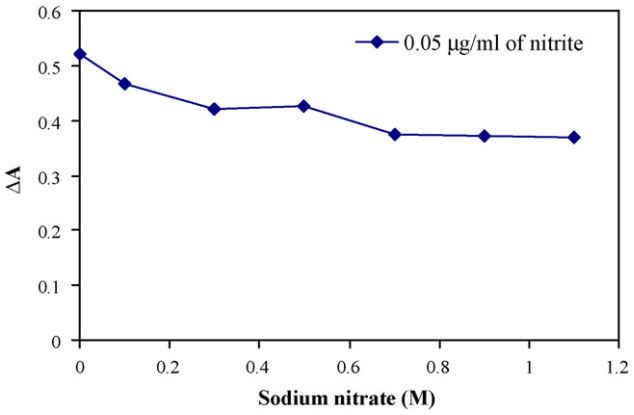

Fig. 5. Influence of electrolyte concentration on the sensitivity. *Conditions*: H_2SO_4, 0.70 M; sulfonazo III, 2.50×10^{-4} M; BrO_3^-, 0.30 M; NO_2^-, 0.05 μg/ml; flow rate, 1.4 ml/min; reaction coil length, 150 cm; injection volume, 196 μl; and *t*, 25 °C.

tivity up to 0.70 M whereas, higher concentration does not affect the sensitivity. Therefore, 0.70 M sulfuric acid concentration was selected.

The influence of the sulfonazo III concentration on the peak height was studied for the range of 7.50×10^{-5} to 3.00×10^{-4} M, with 0.70 M sulfuric acid and 0.05 μg/ml of nitrite at 25 °C (Fig. 3). The results show that by increasing the sulfonazo III concentration up to 2.50×10^{-4} M leads to increasing the sensitivity. At higher concentration of the sulfonazo III the noise of the system become larger. Therefore, 2.50×10^{-4} M (for better S/N ratio) was used for further study.

Fig. 4 shows the influence of bromate concentration on the sensitivity for the range of 8.00×10^{-2} to 5.00×10^{-1} M, with 2.50×10^{-4} M of sulfonazo III, 0.70 M H_2SO_4 at 25 °C. According to the results, the peak height goes up as bromate concentration increases up to 0.30 M, and then decreases. Therefore, 0.30 M bromate concentration was selected.

The effect of the ionic strength on the peak height was studied by addition of an electrolyte (sodium nitrate) in the range of 0.00–1.10 M, with 0.70 M sulfuric acid, 0.30 M bromate, 2.50×10^{-4} M of sulfonazo III and 0.05 μg/ml of nitrite at 25 °C (Fig. 5). Based on the results, increasing the electrolyte concentration up to 0.50 M cause decrease in the sensitivity and then reach a plateau. Therefore 0.50 M was used for further study.

The influence of instrumental variables on the sensitivity (pump flow rate, length of the reaction coil and sample loop volume) was studied with the optimized reagents concentration and 0.05 μg/ml of NO_2^- at room temperature. The peak height depends on the residence time of the sample zone in the system; e.g. on the total flow rate and the length of the reaction coil. The effect of the flow rate was checked over the range 0.92–1.70 ml/min. The results show that the peak height increases as flow rate rises up to 1.40 ml/min, and then decrease (Fig. 6). This is due to the fact that at higher flow rate the residence time of mixture of the reagents is reduced and thus the consumption of the dye decreased, causing a drop in the peak height. From the result, pump flow rate of 1.40 ml/min was chosen for study.

The influence of the length of the reaction coil on the sensitivity was investigated with flow rate of 1.40 ml/min. Considering the results, increasing the length of the reaction coil from 100 to 250 cm, initially it caused increasing the analytical signal (up to 150 cm) and then it decreased (for >150 cm) (Fig. 7). By increasing the length of the reaction coil, the rate of the uncatalyzed reaction goes more ahead and the net signal (differences of catalyzed and uncatalyzed reaction) reduces. Therefore, a 150 cm was chosen as the optimum length of the reaction coil.

The sample volume injected into the carrier line has a significant effect on the peak height. The signal rises with increasing sample volume up to 294 μl and then remains nearly constant for larger volumes up to 588 μl. In addition, using sample volume larger than

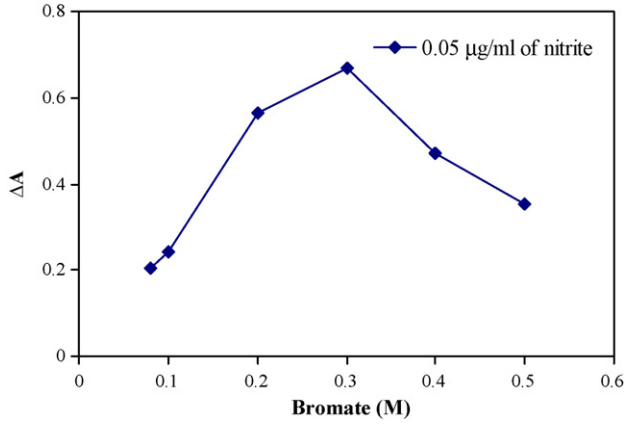

Fig. 4. Influence of bromate concentration on the sensitivity. *Conditions*: H_2SO_4, 0.70 M; sulfonazo III, 2.50×10^{-4} M; NO_2^-, 0.05 μg/ml; flow rate, 1.4 ml/min; reaction coil length, 150 cm; injection volume, 196 μl; and *t*, 25 °C.

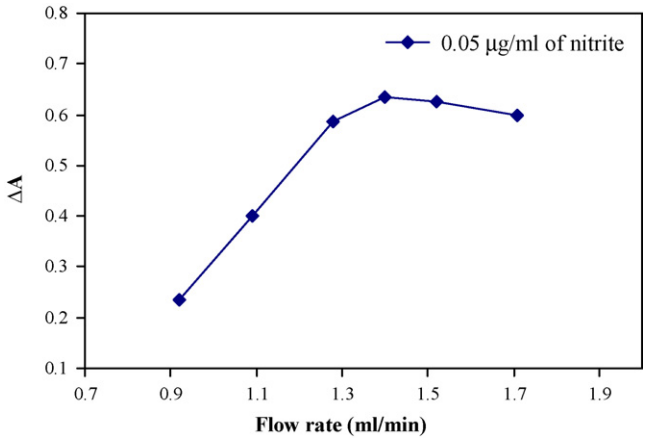

Fig. 6. Effect of pump flow rate on the peak height. *Conditions*: H_2SO_4, 0.70 M; sulfonazo III, 2.50×10^{-4} M; BrO_3^-, 0.30 M; NO_2^-, 0.05 μg/ml; reaction coil length, 150 cm; injection volume, 196 μl; and *t*, 25 °C.

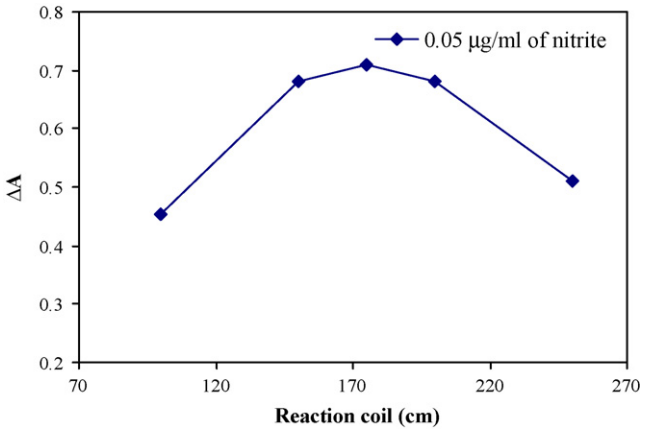

Fig. 7. Effect of length of the reaction coil on the peak height. *Conditions*: H_2SO_4, 0.70 M; sulfonazo III, 2.50×10^{-4} M; BrO_3^-, 0.30 M; NO_2^-, 0.05 μg/ml; flow rate, 1.4 ml/min; injection volume, 196 μl; and *t*, 25 °C.

294 μl leading to peak broadening and tailing. Therefore, a sample volume of 294 μl was selected for further experiments because of sharper peaks.

4. Analytical parameters

Under the optimized conditions and at 25 °C the peak height obtained with nitrite was proportional to the concentration in the range of 8.00×10^{-3}–1.80 μg/ml for nitrite ion. There are two LDR in this range of concentration (Fig. 8). The first LDR range is 8.00×10^{-3}–3.00×10^{-1} μg/ml (with slope of 2.40) and the second one is 3.50×10^{-1}–1.80 μg/ml (with slope of 0.42). The detection limit for the determination of nitrite was 6.00×10^{-3} μg/ml (S/N = 3). The relative standard deviation ($n = 10$) was 1.25% and 0.88% for 0.05 and 0.20 μg/ml of nitrite, respectively. The rate of analysis (number of sample injected in 1 h) in the optimum conditions was 60 samples per hour.

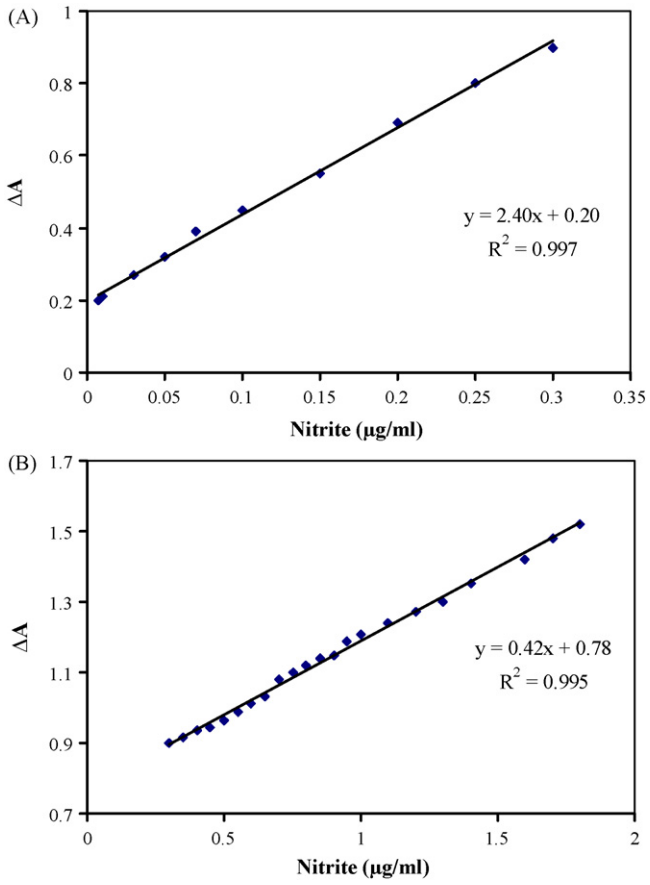

Fig. 8. Calibration curves for the determination of nitrite. (A) 8.00×10^{-3}–3.00×10^{-1} μg/ml. *Conditions*: H_2SO_4, 0.70 M; sulfonazo III, 2.50×10^{-4} M; BrO_3^-, 0.30 M; sodium nitrate, 0.50 M; flow rate, 1.4 ml/min; reaction coil length, 150 cm; injection volume, 294 μl; and *t*, 25 °C. (B) 3.50×10^{-1}–1.80 μg/ml of nitrite. *Conditions*: H_2SO_4, 0.70 M; sulfonazo III, 2.50×10^{-4} M; BrO_3^-, 0.30 M; sodium nitrate, 0.50 M; flow rate, 1.4 ml/min; reaction coil length, 150 cm; injection volume, 294 μl; and *t*, 25 °C.

5. Interference study

Under the optimized conditions, the influence of several cations and anions on the determination of 0.05 μg/ml nitrite was studied. The tolerance limit was defined as the interference ions cause less than ±3% relative error for the nitrite determination. The results are summarized in Table 1.

Table 1
The effect of foreign ions on the determination of nitrite.

Species	Tolerance limit ($W_{Ion}/W_{nitrite}$)
Al^{3+}, Ba^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, $S_2O_8^{2-}$, HCO_3^-, CO_3^{2-}, SO_4^{2-}, SCN^-, HPO_4^{2-}, PO_4^{3-}, IO_3^-	>1000
Cd^{2+}, CH_3COO^-, F^-, $C_2O_4^{2-}$	800
Sr^{2+}	500
NH_4^+	400
CN^-	300
Ag^+, $Cr_2O_7^{2-}$, Cu^{2+}	70
I^-, SO_3^{2-}	5
$S_2O_3^-$, Br^-	1

6. Applications

The present method was successfully applied to the determination of nitrite in the tap water and sausage samples. In view of the unknown composition of the samples, equivalent portions of each sample were analyzed for nitrite contents. In order to validate the

Table 2
Data for the determination of nitrite in tap water (Zanjan city).

No.	Nitrite				
	Added (μg/mL)	Found (μg/mL) ($n = 3$)		Recovery (%)	
		Proposed FIA	Standard method	Proposed FIA	Standard method
1	0.02	$2.01(\pm0.10) \times 10^{-2}$	$1.96(\pm0.10) \times 10^{-2}$	100.5	98.0
2	0.04	$4.20(\pm0.10) \times 10^{-2}$	$3.92(\pm0.08) \times 10^{-2}$	105.0	98.0
3	0.06	$6.16(\pm0.07) \times 10^{-2}$	$5.88(\pm0.05) \times 10^{-2}$	102.6	98.0
4	0.08	$7.92(\pm0.07) \times 10^{-2}$	$7.95(\pm0.07) \times 10^{-2}$	99.0	99.3
5	0.10	$9.88(\pm0.20) \times 10^{-2}$	$10.10(\pm0.06) \times 10^{-2}$	98.8	101.3

Table 3
Data for the determination of nitrite in a sausage.

No.	Nitrite				
	Added (μg/mL)	Found[a] (μg/mL) ($n = 3$)		Recovery (%)	
		Proposed FIA	Standard method	Proposed FIA	Standard method
1	0.00	$2.00(\pm0.50) \times 10^{-2}$	ND[b]	–	–
2	0.01	$3.04(\pm0.50) \times 10^{-2}$	$1.02(\pm0.10) \times 10^{-2}$	104.0	102.0
3	0.02	$4.11(\pm0.40) \times 10^{-2}$	$2.04(\pm0.09) \times 10^{-2}$	105.5	102.0
4	0.03	$5.07(\pm0.30) \times 10^{-2}$	$3.16(\pm0.08) \times 10^{-2}$	102.3	105.3
5	0.04	$5.97(\pm0.10) \times 10^{-2}$	$3.98(\pm0.10) \times 10^{-2}$	99.2	99.5
6	0.05	$6.96(\pm0.40) \times 10^{-2}$	$5.00(\pm0.08) \times 10^{-2}$	99.2	100.0

[a] After 5-fold dilution.
[b] Not detected.

accuracy of the proposed method, the data were compared with those obtained by the Griess standard method [25]. The results are shown in Tables 2 and 3. Recoveries in the spiked samples plus the results from the proposed and the standard method are in good agreement.

7. Conclusions

The new method described is significant with respect to the development of a simple manifold for the determination of traces nitrite in the real samples. Its simplicity and reproducibility are coupled with the high speed and safety analysis of the FIA technique.

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