FLOW-INJECTION SPECTROPHOTOMETRIC DETERMINATION OF NITRITE AND NITRATE IN WATER AND SOIL SAMPLES

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ABSTRACT

A simple and rapid flow-injection spectrophotometric method was developed for determination of nitrite and nitrate with 3-amino-5-methyl isoxazole to form a diazonium ion, which is coupled with resorcinol in an alkaline medium to form azo compound, with a maximum absorption at 354 nm. Beer's law is obeyed over the range of 0.1-8.0 µg/ml of nitrite with a detection limit 0.08 µg/ml .Nitrate is determined by it's reduction to nitrite using amalgamated cadmium column over the range of 0.2-9.0 µg/ml with a detection limit of 0.1 µg/ml. The sample volume and sample rate were 100 µl and 60 samples/h for nitrite and 150 µ l and 55 samples/h for nitrate respectively. The interfering effects of different cations and anions were studied. EDTA solution is used to avoid the interference effect of some cations. The method has acceptable precision and accuracy and it is used for determination of nitrite and nitrate in water and soil samples.

INTRODUCTION

The determination of nitrate ion is an important factor in the analysis of food and natural waters. Nitrite and nitrate are intimately involved in the overall nitrogen cycle in soil and higher plants [Ali and Kazemzadeh (1999)]. Nitrite exists in nature as a result of the incomplete oxidation of ammonia or reduction of nitrate. The use of nitrate and/or ammonium salts as fertilizers in agriculture has increased the nitrite concentration in surface and subsurface water. The presence of

nitrite in natural water gives an indication of pollution and eutrophication [Ryoichi et al., (1999)]. The recommended lower limits for nitrite in drinking water are 0.06 mg/liter [U.S Public Health Association] and 0.1 mg/liter (European Community) [Gen et al., (1998)]. An upper limit of 45 mg/liter for nitrate has been proposed on drinking water lead to methemoglobinalmia in infants [Ali and Kazemzadeh (1999)]. The most widely used methods for determination of nitrite are based on the diazodization of an aromatic amine and subsequent coupling to form an azo dye [Zhang et al., (1998)]. The flow injection technique, which is rapid, reproducible and suitable for on-line analysis, has been employed for the determination of nitrite spectrophotometrically [Keiro and Shoji, (1999); Oshima and Motomizu (2002); Legnerova et al., (2002); Fang et al., (2002) and Ali et al., (2004)].

Most of the flow-injection determination methods of nitrate have been based on the reduction of nitrate to nitrite, which has been determined spectrophotometrically of an azo dye formed by diazotization /coupling reaction [Anatol and Ewa (1995); Gen et al., (1998); Xiao et al., (1998) and Keiro and Shoji, (1999)].

The main purpose of this study was to develop a simple flow-injection procedure for determination of nitrite and nitrate based on the reaction of nitrite with 3-amino-5-methyl isoxazol to form a diazonium ion, which is coupled with resorcinol to form the azo dye. Nitrate is determined by it's reduction to nitrite using amalgamated cadmium column.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade.
Stock standard nitrite and nitrate solutions (100 µg / ml) were prepared from sodium nitrite and sodium nitrate respectively. Working solutions were freshly prepared by diluting the stock solutions. 3-Amino-5-methyl isoxazole solutions (0.2% and 0.08%) were prepared using 0.1M and 0.4M HCl solutions respectively. Resorcinol solutions (0.5%) and potassium hydroxide solutions　(2.0M and 3.0M) were also prepared.

Apparatus

Two flow- injection systems were used for determination of nitrite Fig. 1 and nitrate Fig. 2. The formed azo dye was detected and measured at 354 nm using LKB Bichrom Ultrospec 4050 uv/visible

spectrophometer with a flow cell of 10-mm path length made of quartz (Pye Unicam UV 681435). Two peristaltic pumps (Watson-Marlow 501Z, single channel) and (Watson-Marlow 501Z, Multi channel) and an injection valve with 6-way loop-valve (Omnifit) were used. The peaks were recorded on Philips PM 8251A one line recorder.
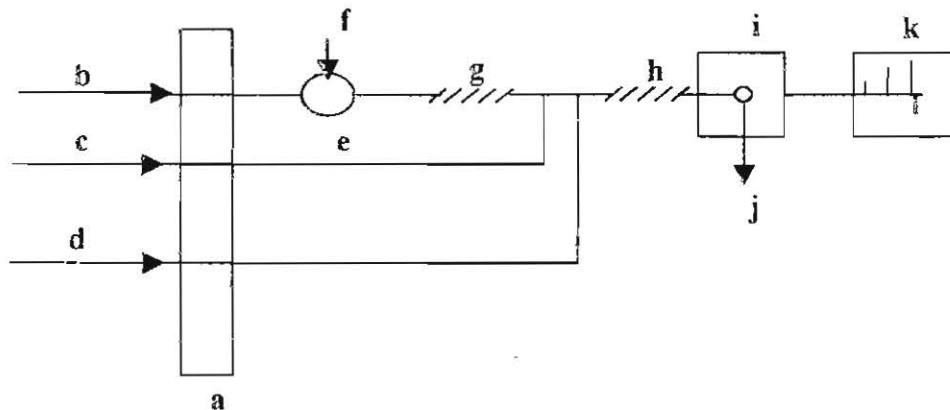

Fig. 1: Schematic diagram of the flow-injection system for determination of nitrite. a, peristaltic pump; b, 3-amino-5-methyl isoxazole solution; c, resorcinol solution; d, potassium hydroxide solution; e, injection valve; f, sample injected; g and h reaction coils; i, detector (uv/visible spectrophotometer); o, flow cell; j, waste;k, recorder; l, peaks.
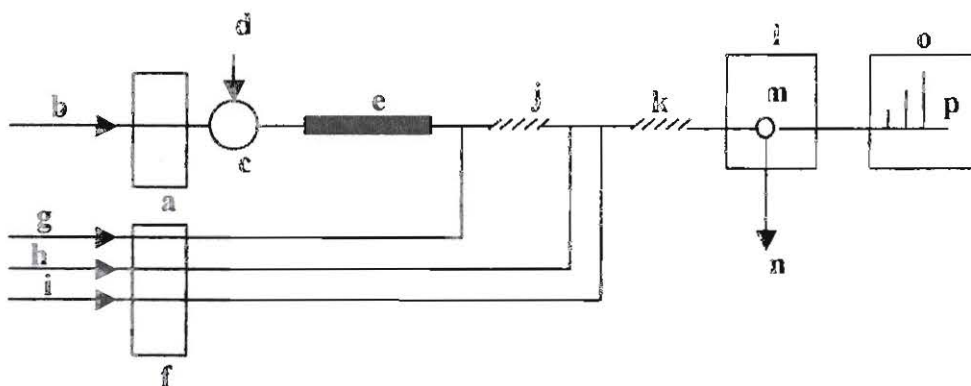

Fig. 2: Schematic diagram of the flow-injection system for determination of nitrate. a and f, peristaltic pumps; b, aqueous carrier stream; c, injection valve; d, sample injected (nitrate); e, reductor column; g, 3-amino-5-methyl isoxazole solution; h, resorcinol solution; i, potassium

hydroxide solution; j and k, reaction coils; l, detector (uv/visible spectrophotometer); m, flow cell; n, waste; o, recorder; p, peaks

RESULTS AND DISCUSSION

Preliminary tests were studied with the aim of the optimization of the flow injection analysis parameters. The optimization includes physical and chemical variables.

Physical optimization
The effects of the flow rate, sample volume and the reaction coil lengths were studied to obtain a maximum peak height.

Effect of the flow rate
Different flow rates (0.5, 1.0, 1.5, 2.0, 2.5, 3 or 3.5) ml/min., were studied. Fig. 3 shows that a flow rate of 1.5 ml/min has a maximum peak height.

Effect of the sample volume
Various sample volumes (25, 50, 85, 100, 150, 200 or 250 µl) of 2.0 µg/ml nitrite ion solution were injected through the injection valve. Fig. 4 shows that the sample volume of 100 µl has the maximum peak height.

Effect of reaction coils
Two reaction coils with various lengths (10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 cm) were used. It was found that the reaction coil lengths of 40-cm and 50-cm lengths have an optimum condition for the formation of the diazonium ion and the azo dye, respectively. Figs. 5 and 6 show the results. The peak height decreased with increasing the flow rate, considering the sensitivity and the reproducibility, the flow rate adopted on 1.5 ml/min. The analytical signal (peak height) was increased with increasing the sample volume up to 100 µl. When more than 100 µl was used, no significant changes were observed, because it seems to be reached the steady state. With increasing the coil length, the peak height was decreased due to the dispersion of the sample zone with increasing the distance traveled of the azo dye formed. Therefore, two reaction coils of (40-cm and 50-cm lengths) were used.
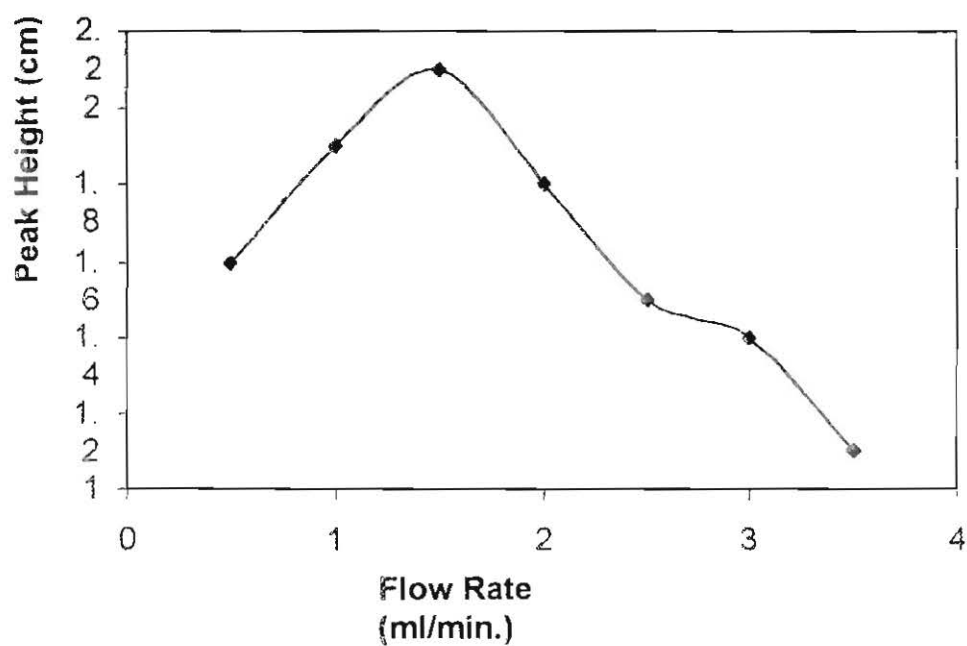
Fig. 3: Effect of the flow rate on the peak height obtained using flow-injection spectrophotometric determination of nitrite.
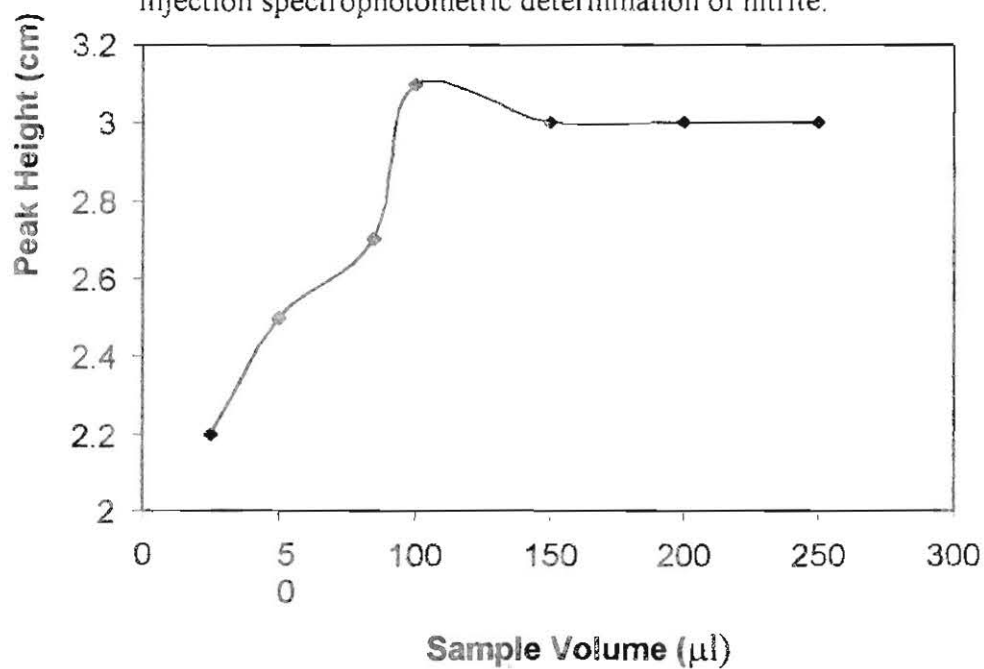

Fig. 4: Effect of the sample volume on the peak height obtained using flow-injection spectrophotometric determination of nitrite.
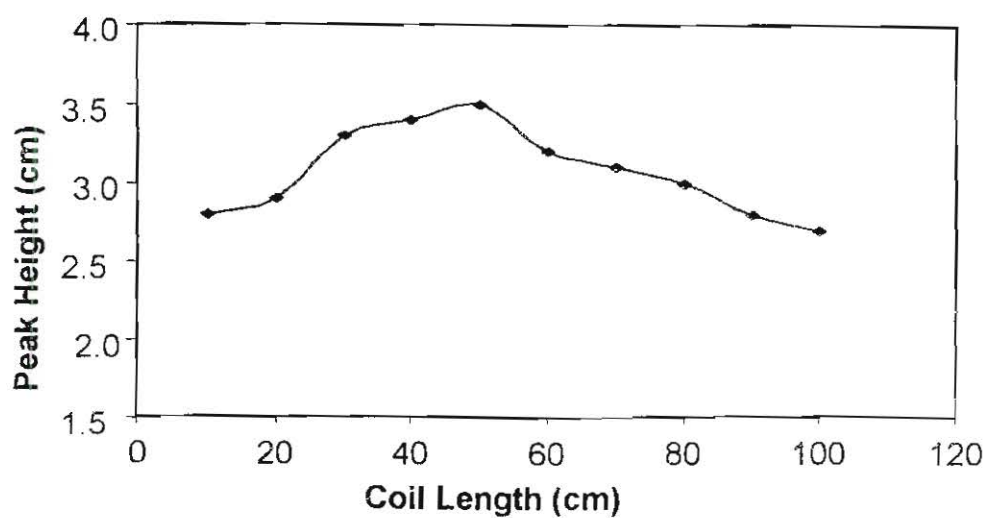
Fig. 5: Effect of the first reaction coil length for the formation of the diazonium ion on the peak height obtained using flow-injection spectrophotometric determination of nitrite.
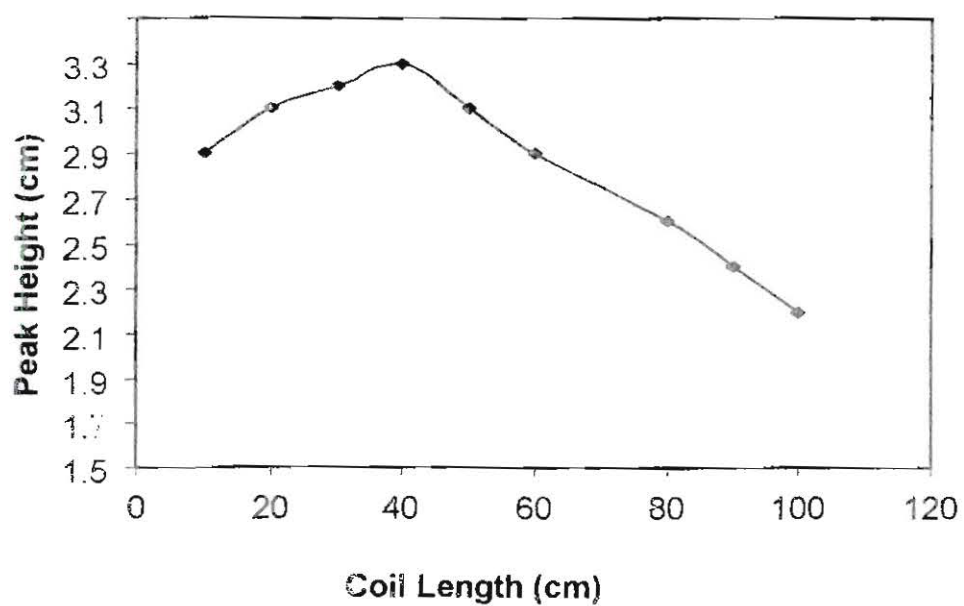

Fig. 6: Effect of the second reaction coil length on the peak height obtained using flow-injection spectrophotometric determination of nitrite.

Chemical optimization

After the physical parameters were optimized, the chemical parameters were also optimized using the following experiments. Various carrier streams were studied as 3-amino-5-methyl isoxazole, water or hydrochloric acid, the peak height was changed markedly with the carrier streams. The maximum peak height was obtained with water. Other chemical parameters of the reaction of nitrite with 3-Amino-5-methyl isoxazole coupling with resorcinol were studied and optimized as shown in the recommended procedures for determination of nitrite and nitrate.

In order to optimize the proposed flow-injection system for determination of nitrate, the influence of the hydrodynamic parameters on the magnitude of the peak height, the shape of the peaks and reproducibility of the results were studied. Two peristaltic pumps were used in the system, the first pump to propel the aqueous carrier stream, and the second pump to propel the reagents, with a flow rate of 0.8 and 1.0 ml/min. respectively. The injected sample volume was 150μl. Reaction coils of 20-cm and 30-cm length were used with the system.

The optimum reduction of nitrate to nitrite was established by testing various reduction column (amalgamated zinc, amalgamated cadimum, copperized cadimum, copperized zinc and cadimum coated zinc) lengths and internal diameter, obviously, increasing the column length can enhance the reduction efficiency and then increase the signal, but on the other hand, the dispersion also increased and reduced the intensity of the FIA signal (peak height). A column length of 25-cm (2.0-mm i.d.) was selected and the maximum reduction percentage was 93.8% which determined by comparison of the plateau achieved with that corresponding to the same concentration of nitrate ion solution.

Recommended procedures for determination of nitrite and nitrate

As shown in the schematic diagram of flow injection analysis system used for determination of nitrite, a multichannel peristaltic pump was used to propel (0.08%) 3-amino-5-methyl isoxazole, (0.5%) resorcinol and 3.0M potassium hydroxide solutions with a flow rate of 1.5 ml/min. A sample volume of 100 μl was used to inject 0.1-8.0 μg/ml of nitrite ion solution into the 3-amino-5-methyl isoxazole solution stream. Two reaction coils were used in the system. The first reaction coil was 40-cm length for the reaction of the nitrite ion with 3-amino-5-methyl isoxazole solution to form the diazonium ion, while the second reaction coil was 50-cm length used for coupling the diazonium ion with

resorcinol in the presence of potassium hydroxide solution to form the azo dye. The peak heights were recorded at 354 nm, with a sampling rate of 60 samples/ h.

As shown in the schematic diagram of the flow-injection analysis system used for determination of nitrate, two peristaltic pumps were used. The first pump was single channel to propel the aqueous carrier stream (distilled water) with a flow rate of 0.8 ml/min., while the second pump was multichannel to propel 0.08% 3-amino-5-methyl isoxazole (dissolved in 0.4M hydrochloric acid), 0.5% resorcinol and 2.0M potassium hydroxide, solutions with a flow rate of 1.0 ml/min., using tubes of 0.7 mm i.d. A sample volume of 150 µl was used to inject 0.2-0.9 µg/ml of nitrate ion into the aqueous carrier stream. A glass tube of 25-cm length and 2.0-mm i.d. tube of amalgamated cadmium reductor was used to reduce the nitrate to nitrite. Two reaction coils were used in the system. First reaction coil was 20-cm length used for the reaction the nitrite with 3-amino-5-amino-methyl isoxazole solution to form the diazonium ion, while the second reaction coil was 30-cm length used for coupling the diazonium ion with resorcinol in the presence of potassium hydroxide solution to form the azo dye. The peak heights were recorded at 354 nm, with a sampling rate of 50 samples /h.

Calibration curves

Using the optimum conditions, the calibration curve (absorbance as peak height in cm) against the concentration was linear over the concentration range of 0.1-8.0 µg /ml nitrite ion, with a detection limit of 0.08 µg /ml and regression coefficient 0.9996. Beer's law was obeyed over the concentration range of 0.2-9.0 µg /ml nitrate ion, with a detection limit of 0.1 µg /ml and regression coefficient 0.9993.

Precision and accuracy

The precision and accuracy of the determination of nitrite and nitrate ions using flow-injection spectrophotometric system were studied. The values of the relative standard deviation percentage (RSD %) and relative errors percentage (Error %) for triplicate injection of various concentrations are shown in Table 1.

Table (1): Precision and accuracy of the flow-injection
spectrophotometric determination of nitrite and nitrate.

Concentration of nitrite ion (µg/ml)	RSD %	Error %	Concentration of nitrate ion (µg/ml)	RSD %	Error %
0.1	4.6277	-4.7619	0.2	4.2253	-1.4084
4	0.6073	-0.3147	4.5	1.265	0.766
8	0.3228	-0.3322	9	0.833	0.762

Interferences

The interferences of several ions which can occur in real water and soil samples beside nitrite and nitrate, was studied by using a solution containing nitrite and nitrate separately and adding various concentrations of the foreign ions up to the amounts where the relative error reached a value of about $< \pm$ 5%. The errors were calculated with comparing the peak heights with that obtained when 50 µg of nitrite and 75µg nitrate (in the absence of foreign ions) was injected separately. The results are shown in Tables 2 and 3.

The effect of the cationic Interferences, (Cr^{3+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Mg^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}) were avoided using solution of EDTA (10%). Table (4.5) shows that 4.5 ml of EDTA was used to avoid the effect of the interference cations.

Applications of the method

The proposed flow-injection system was applied for the determination of nitrite and nitrate ions in tap water and soil samples, collected from different places of Arbil City .The aqueous extracted of the soil was prepared by placing 50 g of the soil in a 500-ml conical flask, and adding 0.5 g of $CaSO_4$ and 250 ml of distilled water. The conical flask was stoppered and shaked for 10 minutes on a mechanical shaker, allowing the suspension to settle for a few minutes, decanting, and filtering the supernatant liquid through a filter paper. If the first portion of the filtrate is not clear, the filtrated was returned to the filter and collected in a clean receiver flask **[Black et al., (1976)]**. It was found that the water and aqueous extracted of the soil samples were free of nitrite ion in the concentration range 0.1 – 8.0 µg /ml. Known amounts of nitrite ion were added to the samples and acceptable recoveries have been

obtained. Nitrate ion was found to be present in the tap water samples in the range 1.76 – 2.36 µg /ml and in the aqueous extracted of the soil samples in the range of 0.9 – 2.5 µg /ml. The results are shown in Tables 4 and 5.

Table (2): Effect of the interference anions on the determination of 50 µg nitrite ion and a 75 µg nitrate ion in a final volume of 25 ml using flow-injection spectrophotometric method.

Interfering anions	Acceptable amount to be added to nitrite (µg)	Error %	Acceptable amount to be added to nitrate (µg)	Error %
Chlorate	7375	-3.98	7250	-1.5
Iodate	7250	+2.87	7000	+3.33
Bromide	7000	+2.16	6625	+1.66
Thiosulphate	6250	+1.99	6125	+2.83
Nitrate	4500	+4.86	4125	-3.50
Phosphate	4375	-2.65	4125	-0.83
Sulphite	3625	-1.54	3750	+3.33
Bicarbonate	3500	+1.10	3500	+2.16
Periodate	3375	+4.86	3375	+3.83
Acetate	3250	-4.64	3125	-3.83
Fluoride	2500	+2.21	2250	+1.33
Oxalate	2000	+2.43	1625	-2.83
Cyanide	1750	-1.10	1500	+4.66
Carbonate	1500	+2.87	1375	+3.66
Persulphite	625	+3.53	375	+3.00
Thiocyanate	500	-3.31	375	-4.00
Sulphate	200	+2.21	150	+0.16
Metabisulphite	150	-2.65	125	+3.16
Iodide	125	+4.42	112.5	-2.50
Bromate	125	-3.98	100	-4.16

Table (3): Effect of the interference cations on the determination of 50 µg nitrite ion and a 75 µg nitrate ion in a final volume of 25 ml using flow-injection spectrophotometric method.

Interfering cations	Acceptable amount to be added to nitrite (µg)	Acceptable amount to be added to nitrate (µg)	Error %	Error %
Barium (II)	11875	12125	-2.27	-3.51
Mercury (II)	11250	11500	+0.81	+1.09
Calcium (II)	9375	9500	+3.73	+1.75
Aluminum (III)	6250	6750	+0.81	+4.61
Zinc (II)	3375	3500	+2.92	+3.29
Cadmium (II)	3125	3375	+1.62	+3.73
Chromium (III)	3125	3250	+5.36	+2.41
Magnesium (II)	2750	3125	+2.60	+3.95
Cobalt (II)	2375	2750	-3.25	-2.19
Ammonium	1325	1500	-3.66	-3.53
Iron (II)	200	250	+2.11	+3.07
Iron (III)	200	250	+3.57	+3.51
Copper (II)	125	200	+4.55	+1.31
Nickel (II)	75	125	-4.87	-2.63
Manganese (II)	50	125	+2.11	+1.75

Table (4): Determination of nitrate in tap water samples using flow-injection spectrophotometric method.

Region of the sample taken	Nitrate (μg)
Kurdistan's street	50
Bazar	53
Pzeshkan's street	46
Shekh Choli	44
Idrees Barzani's street	51
Kala	59
Shekh Allh	58
Minara	50

Table 5: Determination of nitrate in soil samples using flow-injection spectrophotometric method.

Region of the sample taken	Nitrate(μg)
Kirkuk's way	25
	30
	37.5
	33.75
Massef's way	24.5
	22.5
	23.75
	25
Kusnazan	50
	48.75
	46.25
	47.5
Mousl's way	57.5
	62.5
	52.5
	65

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تعيين النيترات والنترات فى عينات المياه والتربة
بواسطة الحقن الإنسيابى الطيفى

تم تطوير طريقة للحقن الإنسيابى الطيفى السريع لتعيين النترات والنيترات باستخدام ٣ – أمينو – ٥ – ميثيل أيزوكسازول لتكوين أيون الديازونيوم الذى يكون مع الريزورسينول فــى الوسط القلوى مركبات الأزو والتى يكون طيف إمتصاصها عند ٣٥٤ نانومير بحساسية عالــية فى المحال ١ و – ٨ ميكروجرام / لتر للنيترات. تم تعيين النترات بعد إختزالها الى النيترات بإستخدام مملغم عمود الكادينوم.

هـــذا وقــد تم دراسة تأثير داخل العديد من الأينونات والكاتيونات وإستخدام محلول الاديــتا EDTA للــتخلص مــن تداخل بعض الكاتيونات. وقد ثبت أن هذه الطريقة دقيقة وصحيحة الى حد كبير لتعيين النيترات والنترات فى عينات المياه والتربة.