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A green analytical procedure for flow-injection determination of nitrate in natural waters

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Abstract

Nitrate determination in waters is generally carried out with cadmium filings and carcinogenic reagents or by reaction with phenolic compounds in highly concentrated sulfuric acid medium. In this work, it was developed a green analytical procedure for nitrate determination in natural waters based on direct spectrophotometric measurements in ultraviolet, using a flow-injection system with an anion-exchange column for separation of nitrate from interfering species. The proposed method employs only one reagent ($HClO_4$) in a minimum amount (equivalent to 18 μL concentrated acid per determination), and allowed nitrate determination within 0.50–25.0 mg L^{-1}, without interference of up to 200.0 mg L^{-1} humic acid; 1.0 mg L^{-1} NO_2^-; 200.0 mg L^{-1} PO_4^{3-}; 75.0 mg L^{-1} Cl^-; 50.0 mg L^{-1} SO_4^{2-} and 15.0 mg L^{-1} Fe^{3+}. The detection limit (99.7% confidence level) and the coefficient of variation ($n = 20$) were estimated as 0.1 mg L^{-1} and 0.7%, respectively. The results obtained for natural water samples were in agreement with those achieved by the reference method based on nitrate reduction with copperized cadmium at the 95% confidence level.

Keywords: Green chemistry; Flow-injection analysis; Spectrophotometry; Nitrate; Waters

1. Introduction

Green chemistry (also named as clean chemistry or environmentally benign chemistry) has as objective to develop chemical processes that minimize or eliminate the use of toxic substances and the generation of toxic wastes in the prevention of environmental pollution and human hazards [1]. Researches in this field have focused mainly on the development of new synthetic rotes and the replacement or minimization of the use of organic solvents. However, there is an evident demand to the development of greener, yet reliable, analytical procedures, because several current analytical methods employ highly toxic reagents, resulting themselves in a potentially negative environmental impact. For developing a new analytical procedure, the amount and toxicity of the wastes generated are as important as any other analytical feature. In this sense, flow-based systems are an alternative because the reagent consumption and production of wastes are inherently lower. Several strategies adopted to develop greener analytical procedures by employing flow analysis were previously described [2].

Nitrate determination in waters, often required in environmental studies, is usually performed with cadmium filings for nitrate reduction and carcinogenic reagents for the diazo-coupling reaction with nitrite [3,4]. Cleaner procedures have replaced cadmium by photochemical [5–7] or enzymatic reduction [8] of nitrate by nitrate reductase. However, toxic reagents are employed for determination of the formed nitrite by spectrophotometry [5,8] or chemiluminescence [6,7]. Other current procedures involve nitration or oxidation of phenolic compounds in highly concentrated sulfuric acid medium [4,9], also generating toxic wastes.

In order to prevent the use of toxic reagents in the nitrate measurement, one of the alternatives is the direct spectrophotometric measurement in the ultraviolet, because ni-

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trate absorbs electromagnetic radiation near 200 nm [10]. However, the procedure lacks selectivity because other substances such as Cl^-, NO_2^-, Fe^{3+}, and organic matter also absorb ultraviolet radiation in the same wavelength range [11]. Strategies exploited to increase selectivity include compensation for absorption of interfering species by dual wavelength spectrophotometry [9,12], or mathematical data processing [13,14], retention of organic substances in activated charcoal [15], or nitrate separation by chromatography or capillary electrophoresis [16].

In this work, it was developed a green procedure for nitrate determination in natural waters based on direct spectrophotometric measurements in ultraviolet. A flow-injection system with an anion-exchange resin was employed for in-line separation of nitrate from interfering species, allowing selective nitrate determination without employing toxic reagents.

2. Experimental

2.1. Apparatus

The flow system was constructed with a sliding-bar injector equipped with an electronic timer (Micronal, Brazil), 0.7-mm i.d. polyethylene tubes and Perspex joint points. An Ismatec IPC-8 peristaltic pump equipped with Tygon tubes was used for fluid propelling. A UV–vis spectrophotometer (Hitachi, 3000) with a 10-mm optical path (80 μL inner volume) quartz flow cell (Hellma) was employed for signal measurements. A mini-column was lab-made by using three Perspex blocks attached by screws. The central block was drilled to form one hollow cylinder (3-mm diameter, 15-mm long) that was filled with the ion-exchange resin.

2.2. Reagents and solutions

All solutions were prepared with analytical grade chemicals and distilled-deionized water. The reference solutions were prepared in the range 0.50–25.0 mg L^{-1} NO_3^- by dilution of a 1000 mg L^{-1} stock solution, prepared using sodium nitrate previously dried at 110°C for 2 h. A 0.05 mol L^{-1} $HClO_4$ solution was employed as eluent. The mini-column was filled with ca.180 mg of an anion-exchange resin (Bio-Rad, AG 1-X8, 100-200 mesh, chloride form) and employed for nitrate retention. Before preparing the mini-column, the resin was washed with 0.05 mol L^{-1} $HClO_4$ to replace the counter ions by perchlorate. The procedure was repeated until the absorbance of the washing solution at 201 nm was negligible. The effect of foreign ions was evaluated with solutions prepared from NaCl, Na_2SO_4, NaH_2PO_4, $Fe(ClO_4)_3$, and humic acid (Aldrich, 50% carbon).

River and lake water samples were collected in polyethylene vessels and mineral water samples were purchased from a local market. Samples were filtered through 0.45-μm cellulose acetate membranes (Millipore) before analysis.

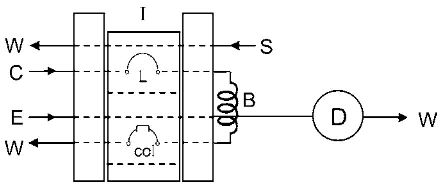

Fig. 1. Flow diagram of the system for nitrate determination. I: sliding-bar injector, S: sample, C: carrier stream (H_2O, 4.3 mL min^{-1}), E: eluent ($HClO_4$ 0.05 mol L^{-1}, 1.7 mL min^{-1}), L: 100 cm sample loop (500 μL), col: anion-exchange column (3 mm i.d., 15-mm long, filled with AG1-X8), D: spectrophotometer (201 nm), B: 300-cm long coil and W: waste.

2.3. Flow diagram and procedure

The diagram of the flow-injection system in the injection position is showed in Fig. 1. Sample is inserted into the water carrier stream (C) and transported through the 300-cm long coil (B) towards the anion-exchange column (col), where nitrate is retained. In the alternative position of the injector, sample (S) is aspirated to fill the sample loop (L), while nitrate is eluted from the column by the 0.05 mol L^{-1} $HClO_4$ solution (E), being the sample zone transported towards the flow-cell (D). The timer of the sliding-bar injector (I) is adjusted to 60 s and 150 s in the injection and elution positions, respectively. Absorbance measurements were carried out at 201 nm. All measurements were carried out in triplicate.

The flow-injection procedure based on nitrate reduction by copperized-cadmium filings followed by the formation of an azo dye with nitrite was adopted as reference for the analysis of nitrate in the water samples [17].

3. Results and discussion

3.1. Chemical variables

Nitrate absorbs radiation within 190 and 220 nm, and the absorption maximum depends on the acidity [18]. The molar absorptivity was estimated as 9.3×10^3 L mol^{-1} cm^{-1} at the absorption maximum for aqueous solutions (201 nm) in batch conditions.

Nitrate determination based on direct spectrophotometric measurements in ultraviolet is usually hindered by the presence of other absorbing species, mainly organic substances. Separation of nitrate from the concomitants is thus required for selective determination. In this sense, several alternatives were evaluated including the retention of organic substances with activated charcoal, XAD-2 resin or C_{18}-bonded silica. Preliminary tests with natural water samples showed that both, organic substances and nitrate, were retained by activated charcoal, while quantitative separation was not achieved in the short residence time (<10 s) for flowing samples with the other materials. In addition, organic substances retained in XAD-2 and C_{18}-bonded silica were difficult to remove from the solid phase, limiting the use for successive measurements.

Table 1
Effect of different eluent solutions in the nitrate separation from the sample matrix. Absorbance values measured with or without separation in the flow system

Eluent solution[a]	Natural water sample[b]	Reference solution 5 mg L^{-1} NO_3^-
Perchloric acid	0.247 ± 0.002	0.221 ± 0.002
Sodium perchlorate	0.377 ± 0.001	0.323 ± 0.003
Sodium phosphate	0.529 ± 0.001	0.393 ± 0.016
Without separation	1.290 ± 0.010	0.505 ± 0.002

[a] 0.60 mol L^{-1} for each eluent solution tested.

[b] Containing 5.04 ± 0.19 mg L^{-1} NO_3^- according to the reference method [17].

Best results were obtained by employing an anion-exchange resin for the reversible retention of nitrate. Several solutions (concentration of 0.6 mol L^{-1}) were evaluated to elute nitrate from the separation column, including those containing anions with high (sulfate) or low (acetate, phosphate, hydroxide and chloride) relative selectivity to the resin [19]. Perchloric acid and sodium perchlorate solutions were also evaluated. Sodium hydroxide and sodium acetate solutions presented strong absorption in the 200–220 nm range. Chloride and sulfate solutions showed strong absorption at 201 nm but could be employed for measurements in wavelengths higher than 210 nm. The use of these solutions was avoided in view of the lessening in sensitivity of at least 20% by carrying out the measurements out of the nitrate absorption maximum. The other solutions were evaluated as eluent and the results are shown in Table 1. Best sensitivity was achieved with phosphate that presents low relative selectivity to the anion-exchange resin (5.0 against 65 for nitrate) [19]. However, best separation from the interfering species was attained with perchloric acid and this solution was selected as eluent.

3.2. Flow system

By considering the main interferences for nitrate determination in waters, the flow system (Fig. 1) was designed to separate nitrate from cationic and neutral species. In the injection step, anions are retained in the ion-exchange column and other species passed directly through the anionic column, being removed by the carrier stream. In the alternative position of the sliding bar injector, the column is inserted into the eluent stream and the analyte is transported towards the flow cell for measurement. It was observed with dye solutions that an intense carryover between sample and previous eluent zones occurred without employing the coil B. Thus, nitrate retention was hindered by the presence of relatively higher concentrations of perchlorate ions in the sample zone. Carryover was minimized by inserting a 300-cm long coil among the sample loop and the column and as showed in Fig. 2, sensitivity and precision were improved in this condition. On the other hand, results in Fig. 2 show that the eluent concentration (initially 0.6 mol L^{-1}) could be reduced to 0.05 mol L^{-1}, improving sensitivity, minimizing reagent consumption, and decreasing the acid concentration of the residues. The effects of eluent and carrier flow-rates were evaluated and the results are shown in Fig. 3. Higher signals were obtained for lower flow-rates indicating that the kinetics of nitrate retention and elution are slow. Flow rates of 1.7 mL min^{-1} and 4.3 mL min^{-1} were selected for the eluent and carrier solutions, respectively, as a compromise between sensitivity and sampling rate. It was further verified that the precision was deteriorated by memory effects caused by the incomplete removal of nitrate in the elution step. This drawback was circumvented by increasing the resting time of the injector in the elution position to 150 s.

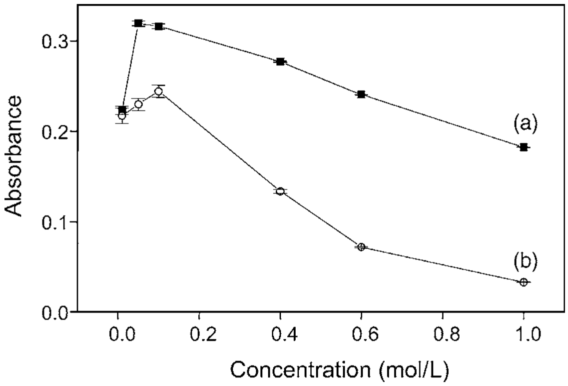

Fig. 2. Effect of the eluent concentration with (a) and without (b) the reactor coil B.

3.3. Analytical features and application

The proposed procedure was efficient to separate nitrate from the main interfering species for the analysis of natural waters. Solutions containing humic acid were employed to simulate natural organic matter, and results obtained with (a) and without (b) separation are shown in Fig. 4. While severe interferences were observed in the presence of 10 mg L^{-1} humic acid without separation, concentrations as high as 200 mg L^{-1} humic acid did not interfere in the nitrate determination when applying the proposed procedure. Similar effect was observed in the presence of Fe^{3+} that inter-

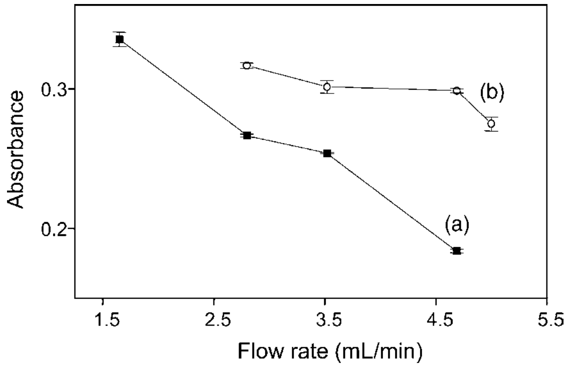

Fig. 3. Effect of the flow-rates of the eluent (a) and carrier (b) solutions on the sensitivity.

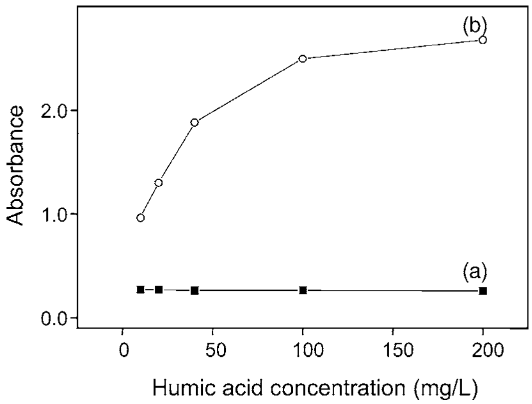

Fig. 4. Effect of the humic acid concentrations on nitrate signal with (a) and without (b) separation.

Table 2
Mean values and uncertainties (n = 3) for the analysis of nitrate in river (S_1–S_3), lake (S_4, S_6–S_9) and mineral water (S_5) samples

Sample	Nitrate concentration (mg L^{-1})	
	Proposed method	Reference method
S1	1.24 ± 0.01	1.07 ± 0.01
S2	2.32 ± 0.01	2.06 ± 0.01
S3	1.56 ± 0.01	1.52 ± 0.01
S4	4.55 ± 0.01	4.98 ± 0.01
S5	5.37 ± 0.01	5.51 ± 0.01
S6	4.41 ± 0.01	4.46 ± 0.04
S7	7.27 ± 0.03	7.87 ± 0.04
S8	6.32 ± 0.01	6.62 ± 0.12
S9	3.83 ± 0.05	4.23 ± 0.03

feres in the direct determination in concentrations lower than 5.0 mg L^{-1}. By admitting signal variations lower than 5%, up to 1.0 mg L^{-1} NO_2^-; 200.0 mg L^{-1} PO_4^{3-}; 75.0 mg L^{-1} Cl^-; 50.0 mg L^{-1} SO_4^{2-} and 15.0 mg L^{-1} Fe^{3+} also did not interfere in the determination of 5.00 mg L^{-1} NO_3^- by the proposed procedure. These concentrations thresholds for manifestation of interferences allow the application of the procedure for the determination of nitrate in freshwaters [20–22]. Even nitrite that absorbs radiation in the same wavelength range is tolerated in concentration higher than typically found in natural waters.

As a consequence of the efficiency of retention and elution of nitrate, it was evaluated that for a 500-μL sample volume, the sensitivity was 40% lower than that attained without separation. However, if necessary, sensitivity can be improved by increasing the sample volume. Absorbance is directly proportional to the sample volume at least up to 1500 μL: A = 0.061 + 0.00049 V (μL), r = 0.999.

For a 500 μL sample volume, linear response was attained within 0.50–25.0 mg L^{-1}, described by the equation A = −0.049 + 0.078 C (mg L^{-1}), r = 0.999. The detection limit was estimated as 0.10 mg L^{-1} at the 99.7% confidence level and the coefficient of variation was 0.7% (n = 20). The sampling rate was estimated as 17 determinations per hour, and 300 μmol $HClO_4$ (equivalent to 18 μL concentrated $HClO_4$) is consumed per determination. Transient signals for samples and reference solutions are shown in Fig. 5. Results obtained for natural waters samples (Table 2) were in agreement with the reference method [17] at the 95% confidence level. It should be mentioned that errors from 14% to 60 % were observed when these same samples were analyzed by UV-spectrophotometry without separation.

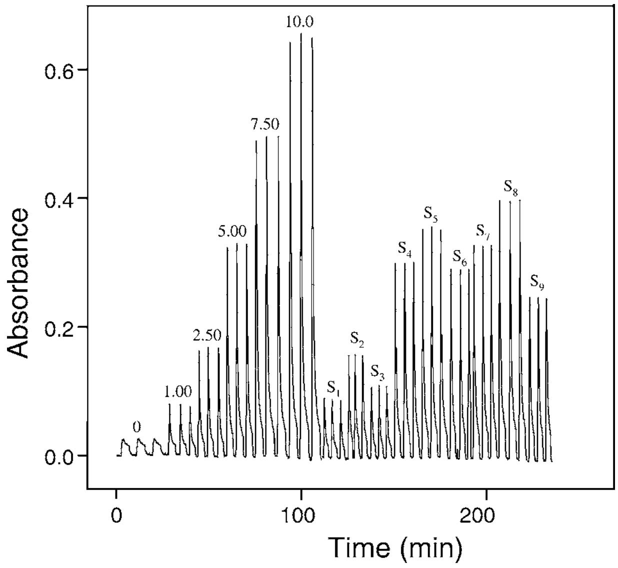

Fig. 5. Transient signals for nitrate reference solutions and natural water samples (S_1–S_9) measured in triplicate. Numbers indicate nitrate concentrations in mg L^{-1}.

4. Conclusions

The in-line separation with an anion-exchange column was effective to avoid the interference of common organic and inorganic species on nitrate determination by direct measurements in ultraviolet. The same mini-column containing ca.180 mg ion-exchange resin was employed for at least 500 measurements. The proposed procedure is a clean alternative for nitrate determination in natural waters, because neither toxic nor carcinogenic reagents are employed and the only chemical product (perchloric acid) is employed in a minimum amount.

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