



A renewable copper electrode as an amperometric flow detector for nitrate determination in mineral water and soft drink samples

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ABSTRACT

A novel approach was developed for nitrate analysis in a FIA configuration with amperometric detection ($E = -0.48$ V). Sensitive and reproducible current measurements were achieved by using a copper electrode activated with a controlled potential protocol. The response of the FIA amperometric method was linear over the range from 0.1 to 2.5 mmol L⁻¹ nitrate with a detection limit of 4.2 μmol L⁻¹ (S/N = 3). The repeatability of measurements was determined as 4.7% ($n = 9$) at the best conditions (flow rate: 3.0 mL min⁻¹, sample volume: 150 μL and nitrate concentration: 0.5 mmol L⁻¹) with a sampling rate of 60 samples h⁻¹. The method was employed for the determination of nitrate in mineral water and soft drink samples and the results were in agreement with those obtained by using a recommended procedure. Studies towards a selective monitoring of nitrite were also performed in samples containing nitrate by carrying out measurements at a less negative potential (-0.20 V).

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1. Introduction

Nitrate can be found naturally in soil, water, vegetables and foods. In soil, the presence of nitrate is essential for the conversion of nitrogen unused by plants and its concentration is controlled by some processes like reduction by bacteria (denitrification), uptake by plants and decay of organic matter [1–3]. Anthropogenic sources of nitrate in soil are correlated to the intensive cultivation of lands and application of nitrogen fertilizers [1–3]. As a result of the high solubility of nitrate in water, this chemical species is easily leached down into groundwater, leading to a contamination of water supplies.

In the last years, concern has been expressed about the increasing levels of nitrate ion in drinking water, due to the toxicological problems associated with the formation of *N*-nitrous compounds [1–4]. Accordingly, these compounds are classified as carcinogenic hazard to humans and animals. Moreover, high levels of nitrate in water could result in disequilibrium of the freshwater ecosystem, due to a fast eutrophication process. The intake of large amounts of nitrate for infants could result in the “blue-baby syndrome”, as a consequence of nitrate reduction in the organism to nitrite and further reaction of this metabolite with haemoglobin. Hence, the absorption and transfer of oxygen to cells is compro-

mised. For this reason, nitrate levels (as N-NO₃⁻) in potable water should not exceed 11 mg L⁻¹ (or 0.8 mmol L⁻¹) and 10 mg L⁻¹ (or 0.7 mmol L⁻¹), according to recommendations of the World Health Organization (WHO) [5] and the US Environmental Protection Agency (EPA) [6], respectively. Taking into account these aspects, it is clear that the development of fast and sensitive methods for quantification of nitrate in natural water samples is a pertinent topic in Analytical Chemistry.

Several procedures have been reported in the literature to quantify nitrate in different matrices and they were reviewed by Compton et al. [7]. Different techniques have been used such as UV/vis, chemiluminescence, fluorimetry, infrared, Raman and molecular cavity emission, chromatography, capillary electrophoresis and electrochemistry. In the case of electrochemical methods, copper [8–15], nickel [16], copper–nickel alloys [17], copper–cadmium alloys [18], copper–palladium alloys [1], platinum [19,20], lead [21], silver [22], rhodium [23], boron-doped diamond [24], gold [25] and polypyrrole nanowires [26] have been employed as substrate materials. The use of bare unmodified electrodes for the direct determination of nitrate is difficult owing to the slow kinetics of the charge transfer step. Hence, methods based on the direct reduction of nitrate are characterised by low sensitivity and irreproducibility and associated with surface passivation effects [27]. Nonetheless, procedures to increase the sensitivity in direct electroanalytical determinations of nitrate are generally associated with activation of the electrodic surface, especially when copper cathodes [13] are used. The indirect determination of nitrate

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has been proposed after a derivatisation procedure [28], as also reported in a method for nitrite analysis [29].

The use of a copper surface as amperometric detector in a flow-through configuration has been already explored by our group in the determination of sulphite in alkaline conditions [30]. As fresh copper surfaces are more effective for cathodic reactions, we have also proposed the development of a renewable process for the activation of copper electrodes without the necessity to introduce copper ions into the sample [13]. However, as the majority of other published works involving the electroanalytical determination of nitrate, measurements were obtained in a batch mode with low analytical frequency. In the present work, we describe our efforts to associate the renewable copper surface with a mechanised process (flow injection analysis). The analytical applicability of this high-throughput proposed method was examined by measuring the nitrate content in mineral water and soft drink samples.

2. Experimental

2.1. Chemicals, materials and samples

All solid reagents were of analytical grade and were used without further purification. Sulphuric acid, sodium sulphate, potassium nitrite and potassium nitrate were obtained from Merck (Darmstadt, Germany). The supporting electrolyte for flow injection experiments was a 0.1 mol L^{-1} Na_2SO_4 solution, pH 2.0 (adjusted with H_2SO_4). In almost all cases the solutions were prepared by dissolving the reagents in deionised water processed through a water purification system (Nanopure Infinity, Barnstead). Samples of mineral water and soft drink were acquired from a local market. Carbon dioxide was removed from soft drink samples by thorough bubbling with argon. Nitrite solutions were standardised as reported in the literature [31].

2.2. Electrodes and instrumentation

An Autolab PGSTAT 30 (Eco Chemie) bipotentiostat with data acquisition software made available by the manufacturer (GPES 4.8 version) was used for electrochemical measurements. Experiments were done in a conventional electrochemical cell, a Ag/AgCl (saturated KCl) electrode and a stainless-steel tube being used as reference and counter electrodes, respectively. A copper sheet (Pertech® of Brazil) fabricated by copper electroplating in a substrate composed of paper and phenolic resin was used as working electrode (area = 56 mm^2). This copper sheet is used for fabrication of printed circuit boards. The flow injection apparatus consisted of an aquarium air pump (to propel the solution by pressurization), a homemade rotatory injection valve and an acrylic cell mounted in a thin layer configuration [32,33].

2.3. Activation of the electrode surface in FIA experiments

The working electrode surface is activated by two potential steps prior current measurements, Fig. 1. To drive copper dissolution, the working electrode is polarised at +0.50 V (step a). Then, the redeposition is carried out at -0.25 V (step b). Both steps are performed in absence of flow, otherwise, copper ions would escape from the diffusion layer after the anodic dissolution. These two steps (copper dissolution and redeposition) are required to produce a fresh copper layer with an active role concerning the cathodic reduction of nitrate, as previously demonstrated [13]. The optimisation of both time parameters involved in the pre-treatment potential program (steps a and b) was investigated as an attempt of enhancing the sensitivity of nitrate determinations. After the activation process, the electrode was polarised at the potential required for nitrate reduction. Three samples were injected after each activation step.

2.4. Spectrophotometric analysis

An evaluation of the accuracy of the proposed method for quantitative determinations of nitrate in mineral water samples was performed by UV-spectrophotometry without derivatisation at 220 nm in a Hitachi U-3000 spectrophotometer using a 1.00 cm optical pathlength quartz cuvette. As soft drink samples are more complex and contain large amounts of organic compounds (glucose), comparative measurements were obtained with the UV-spectrophotometry Griess assay. The reduction of nitrate to nitrite was accomplished by using a copperised cadmium reactor column prior to spectrophotometric measurements with the Griess method at 543 nm.

3. Results and discussion

Previous reports on the literature [8,12,13] have demonstrated that the reduction of nitrate can be promoted on a fresh copper surface, in accordance with Eq. (1):



Taking into account the necessity of a fresh copper layer, a protocol involving the dissolution of the outer copper layer for further redeposition of copper ions is required. A schematic representation of the pre-treatment procedure used in the FIA system is shown in Fig. 1. At the activated detector surface, FIA experiments were carried out to investigate the best applied potential for nitrate reduction. From the results obtained in hydrodynamic voltammetric experiments (not shown), the working potential value -0.48 V was selected as the most appropriate to ensure complete analyte reduction at the copper sensor.

The optimisation of both parameters (dissolution and redeposition time) was also investigated. Data in Fig. 2, curve A, shows that no faradaic signal is obtained at a non pre-treated surface (i.e. bare copper electrode), a different situation being observed as the amount of copper ions redeposited during the cathodic process increases (for a constant dissolution time). In a similar way, the signal for nitrate reduction increases with the increase in the dissolution time (for a constant redeposition time) (Fig. 2, curve B). Optimal conditions were achieved by using 30 s, respectively, for the dissolution and redeposition steps, and this procedure was employed for all analytical determinations.

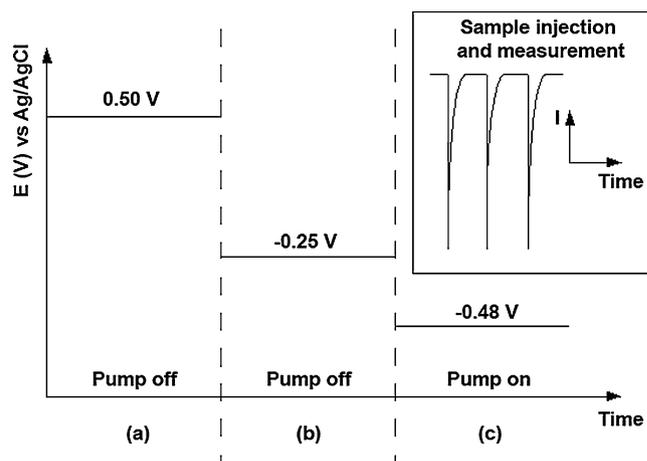


Fig. 1. Schematic representation of the copper surface activation protocol in the FIA system: copper dissolution (a); copper redeposition (b) and nitrate electroreduction (c). The inset shows the amperometric signal recorder after 3 injections of a nitrate solution in the FIA apparatus.

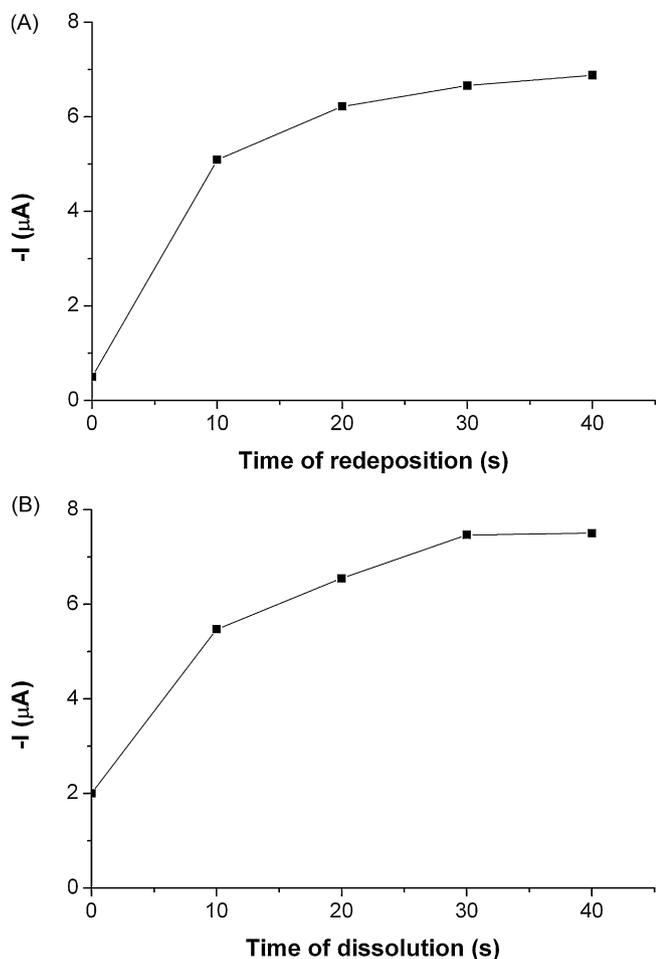


Fig. 2. Dependence of cathodic peak current values on the redeposition (A) and dissolution (B) time for injections of a $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4 + 1.0 \text{ mmol L}^{-1} \text{ NO}_3^-$ solution (pH 2.0). The dissolution time was 30 s for experiments represented by curve (A) and the redeposition time was 30 s for experiments represented by curve (B). Carrier solution: $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4$ (pH 2.0), $E = -0.48 \text{ V}$, number of measurements: 3, flow rate: 3.0 mL min^{-1} , sample volume: $150 \mu\text{L}$.

To investigate the optimal conditions for the flow injection system performance, parameters such as sample volume and flow rate were varied using a 1.0 mmol L^{-1} nitrate solution. The effect of flow rate (studied from 0.5 to 4.5 mL min^{-1}) on the analytical signal was evaluated (Fig. 3A) and the current increased with flow rate up to 3.0 mL min^{-1} . This indicates that a kinetic limitation is prevalent at more efficient mass-transport conditions. Taking into account these aspects, a flow rate of 3.0 mL min^{-1} was selected as the most favourable. The effect of sample volume was evaluated using injection loops from 25 up to $200 \mu\text{L}$, Fig. 3B. The amperometric signal increased almost linearly up to $150 \mu\text{L}$, and then a slight decrease was noticed. Hence, a $150 \mu\text{L}$ sample volume was selected as the most appropriate, based on the best compromise between reproducibility and sampling rate.

In order to confirm the importance of surface activation on the repeatability of the measurements, two independent studies were performed. Firstly, nitrate solutions were injected in the FIA system after one activation procedure (30 s of dissolution and 30 s of redeposition, Fig. 4, curve B) at the beginning of experiment. A continuous current decrease was noticed, indicating the sensor activity loss during the experiment. On the other hand, by using the protocol described in Section 2 a completely different result was obtained (Fig. 4, curve A). In this case, 3 samples were injected after each activation step and the standard deviation (SD) for 9 injections was found to be 4.7% (60 samples per hour). No significant improve-

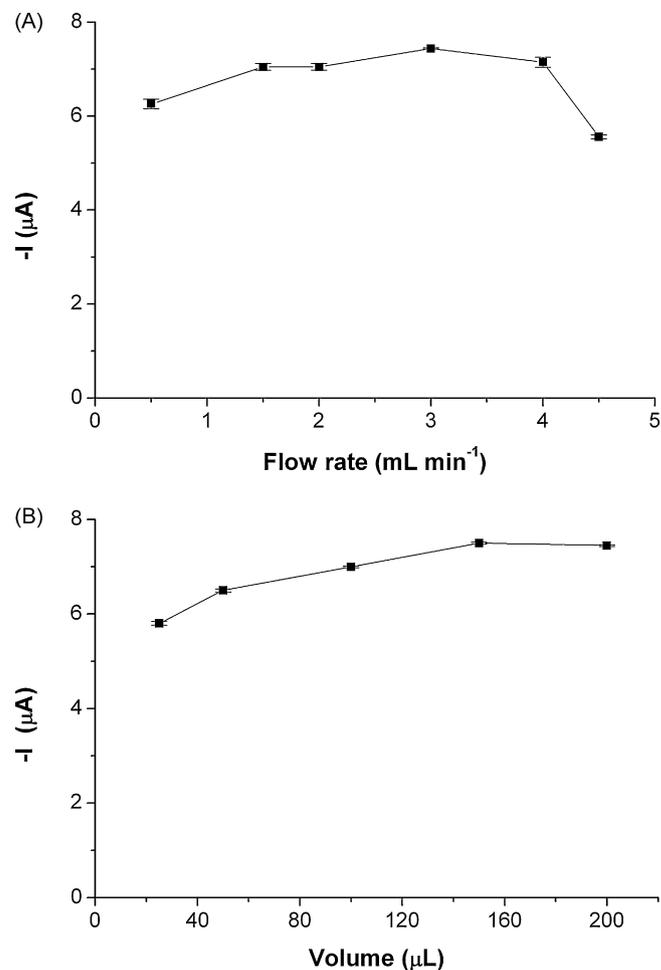


Fig. 3. Average current peak values ($n=3$) recorded for a 1.0 mmol L^{-1} nitrate + 0.1 mmol L^{-1} de Na_2SO_4 solution (pH 2.0) at various flow rates (A) (sample volume $150 \mu\text{L}$) and various sample volumes (B) (flow rate 2.0 mL min^{-1}). Carrier solution: $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4$ (pH 2.0), $E = -0.48 \text{ V}$.

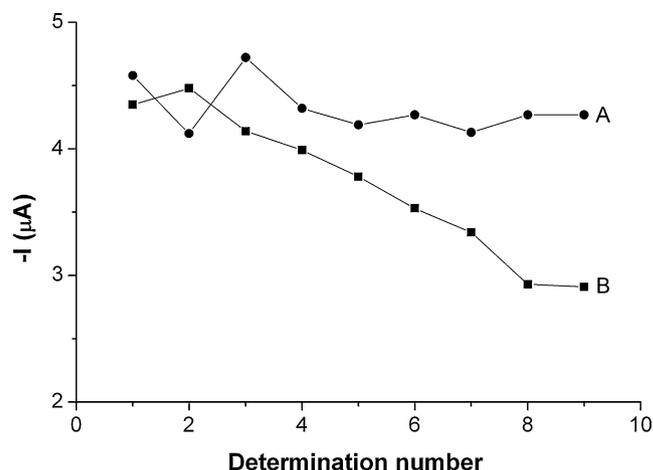


Fig. 4. Current peak values as a function of time for injections of a $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4 + 0.5 \text{ mmol L}^{-1} \text{ NO}_3^-$ solution (pH 2.0). Data were obtained by using the proposed protocol (pre-treatment of the copper electrode after each set of 3 injections) (A) and by pre-treating the electrode only in the beginning of the experiment (B). Carrier solution: $0.1 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4$ (pH 2.0), $E = -0.48 \text{ V}$, flow rate: 3.0 mL min^{-1} , sample volume: $150 \mu\text{L}$.

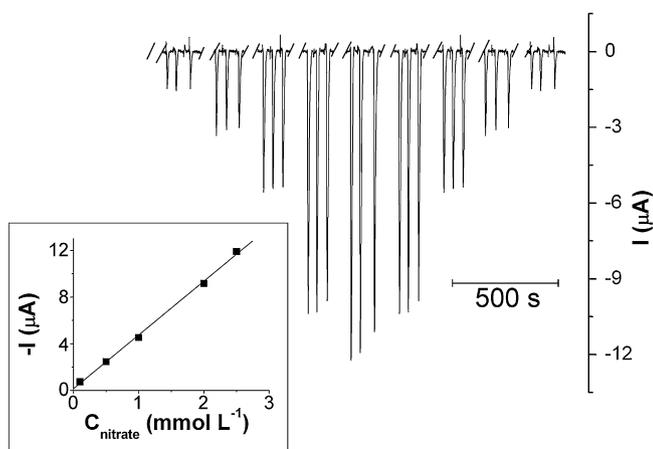


Fig. 5. Peaks recorded for injections of nitrate standard solutions at the optimised FIA conditions (0.1–2.5 mmol L⁻¹). The breaks (//) in the figure represent the pre-treatment gap (60 s). The inset shows the calibration curve. Carrier solution: 0.1 mol L⁻¹ Na₂SO₄ (pH 2.0), $E = -0.48$ V, flow rate: 3.0 mL min⁻¹, sample volume: 150 μL.

Table 1

Analytical parameters for nitrate determination.

Analytical parameters	Batch mode [13]	Proposed method
Analytical frequency (h ⁻¹)	25	60
Detection limit (μmol L ⁻¹)	11.6	4.2
Quantification limit (μmol L ⁻¹)	38.7	14.0
Linear range (mmol L ⁻¹)	0.1–2.5	0.1–2.5
Sample volume (mL)	10	0.15

ment in the precision was noticed by injecting each nitrate sample after the activation step. Hence, in order to increase the analytical frequency without lose in precision, the second protocol (one activation step prior to 3 sample injections) was elected as the most convenient for all subsequent FIA experiments.

The relationship between the amperometric peak current and the concentration of nitrate was investigated in the range 0.1–2.5 mmol L⁻¹ (Fig. 5). Using the optimised conditions previously reported, a linear response ($(-I/\mu\text{A}) = 0.13 + 4.6 (C_{\text{nitrate}}/\text{mmol L}^{-1})$, $R^2 = 0.9991$) was obtained. No memory effect was observed during the experiment, demonstrating that the adopted procedure is convenient. The detection (3 SD) and quantification (10 SD) limits were estimated as 4.2 and 14 μmol L⁻¹, respectively. In order to compare the results obtained by using the batch method [13] with those proposed in this work, figures of merit of both methods are summarised in Table 1. It is clearly seen that the limit of detection, sample volume and analytical frequency of the FIA proposed method are better, indicating its potentiality as an alternative method for routine analysis of nitrate.

The applicability of the renewable copper electrode as detector in a FIA configuration for the determination of nitrate in real samples was also performed. Table 2 shows the nitrate content in 3 mineral water and 3 soft drink samples. The results were in good

Table 2

Nitrate content (as N-NO₃⁻) in mineral water and soft drink samples.

Sample	Standard method [31] (mg L ⁻¹)	Proposed method (mg L ⁻¹)
Aquana ¹	11.03 ± 0.04	11.3 ± 0.1
Schincariol ¹	10.49 ± 0.07	10.6 ± 0.1
Lindoya ¹	5.16 ± 0.02	5.7 ± 0.2
Soda Antártica ²	6.30 ± 0.01	6.1 ± 0.1
Tônica Antártica ²	17.60 ± 0.01	18.0 ± 0.2
Sprite ²	15.27 ± 0.09	14.7 ± 0.6

Number of replicates = 3, 1 = mineral water, 2 = soft drink.

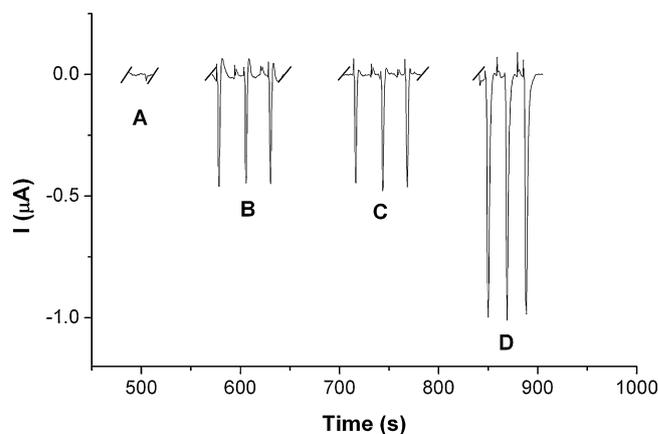


Fig. 6. Peaks recorded at the optimised FIA conditions for injections of 1.0 mmol L⁻¹ nitrate (A), 0.05 mmol L⁻¹ nitrite (B), a mixture of 1.0 mmol L⁻¹ nitrate + 0.05 mmol L⁻¹ nitrite (C) and a mixture of 1.0 mmol L⁻¹ nitrate + 0.10 mmol L⁻¹ nitrite. Carrier solution: 0.1 mol L⁻¹ Na₂SO₄ (pH 2.0), $E = -0.20$ V, flow rate: 3.0 mL min⁻¹, sample volume: 150 μL.

agreement with those obtained by using standard methods [31] (for a 95% confidence level according to the Student's *t*-test). As an additional accuracy test of the proposed method, a set of recovery experiments in which nitrate was added directly to water samples was carried out. Recovery results were satisfactory and ranged from 90 to 97%. Accordingly, we can conclude that the proposed method is reliable for nitrate measurements in the investigated samples.

Previous experiments with the activated copper electrode [13] have shown the good resolution between nitrate and nitrite reduction current peaks in differential pulse voltammograms, respectively -0.48 and -0.20 V. In order to investigate the possibility of analysing nitrite in the presence of nitrate using the proposed FIA method, a standard solution of nitrite was injected under the optimised conditions for nitrate determination. However, experiments were carried out at -0.20 V, instead of -0.48 V. A transient signal for nitrite is observed (Fig. 6, injection B), no response being obtained for nitrate at the same experimental conditions (injection A). By injecting solutions containing both nitrate and nitrite, the current response (injection C) is similar to the one observed after injection of nitrite (injection B). The FIA response for nitrite is proportional to the analyte concentration, as can be observed by comparing peaks C and D. Data shown in this figure also demonstrate that the detector sensitivity for nitrite is good, as a suitable discrimination between signal and noise is observed for 0.05 mmol L⁻¹ nitrite solutions (around 0.7 ppm N-nitrite). Accordingly, an interesting option for the simultaneous determination of both analytes in mixtures is envisaged by performing experiments using dual-detection. We are currently undertaking experiments on this subject, and determining low levels of nitrite in water samples with the proposed copper sensor is an expected challenge.

4. Conclusions

A renewable copper electrode was successfully used as an amperometric detector in a flow injection apparatus for nitrate determination. The system provides high throughput with good reproducibility and sensitivity, hence the analytical parameters are significantly improved in comparison with determinations performed using the batch method. Moreover, taking into account the principles of Green Chemistry, the proposed method is favoured as no carcinogenic compounds are required to convert the analyte. Finally, the possibility to differentiate nitrate and nitrite may consti-

tute a valuable tool in examining samples containing both analytes in a rapid, simple, inexpensive and safe way.

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