

Potentiometric Flow Injection Determination of Glycerol in Distilled Spirits

ANA PAULA G. GERVASIO, EDUARDO P. BORGES,[†] ELIAS A. G. ZAGATTO, AND
BOAVENTURA F. REIS

Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Box 96,
Piracicaba SP 13400-970, Brazil

RUI A. S. LAPA AND JOSÉ L. F. C. LIMA*

CEQUP/Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto,
Rua Aníbal Cunha 164, Porto 4050, Portugal

A single-line flow injection system including a tubular periodate-selective electrode without inner reference solution is proposed for glycerol determination in distilled spirits, based on oxidation of this polyol by periodate. Interferences due to 5.0 mg L⁻¹ Cu, 5000 mg L⁻¹ sucrose, and 3000 mg L⁻¹ fructose plus glucose were investigated. The procedure is characterized by a linear response for 20–500 mg L⁻¹ glycerol ($r > 0.9999$, $n = 7$), a relative standard deviation of results of < 0.03 , and an analytical throughput of 30 determinations per hour. Accuracy was assessed by applying the procedure to distilled spirits of sugarcane and grape already analyzed by HPLC; in addition, recoveries within 96 and 120% were obtained.

KEYWORDS: Distilled spirits; flow analysis; ion selective electrode; glycerol; periodate oxidation

INTRODUCTION

Glycerol—a constituent of animal and vegetal fats and oils—is a clear, water-soluble, simple trihydric alcohol and exists as a viscous, hygroscopic, odorless liquid with a sweet taste (1). It is easily digested and does not irritate the skin or mucous membranes. Glycerol is also a secondary product in wine fermentation, contributing to the sensory properties. Adulteration by addition of industrial-grade glycerol to the wine may happen and can be detected by determining some typical substances formed during the production process (2, 3). In other alcoholic beverages, such as distilled spirits, glycerol is added also to improve taste (4).

The quality of alcoholic beverages produced from sugarcane or grape is then related to the glycerol content. As this value is subjected to strict supervision prescribed by law, the determination of glycerol should be routinely performed in a wide variety of beverage matrices. In this sense, the Office International de la Vigne et du Vin (OIVV) recommends a spectrophotometric procedure exploiting the reading of the colored product obtained after reaction of formaldehyde with phloroglucinol, the former being produced after glycerol oxidation by periodate (5). Because this method is time-consuming, gas chromatography (6) and high-performance liquid chromatog-

raphy (7, 8) have also been suggested. These separation procedures are less suitable for yielding swift readings, especially considering the complexity of the related matrices. Alternatively, enzymatic reactions can be exploited (9). The above-mentioned methodologies are usually expensive and require a long time for sample and standard preparation.

An alternative procedure combining the fast analytical frequency inherent to flow injection analysis, the low-cost associated with potentiometric detection, and the exploitation of glycerol oxidation by periodate is herein presented. With potentiometric detection, problems associated with the color of the samples are not relevant and measurements within a wide concentration range, typically within 4 orders of magnitude, can be performed. Therefore, sample lots with high variability in analyte concentration can be assayed without extensive dilutions.

The tubular periodate-selective electrode has been previously used for glycerol determination in different matrices (10, 11), and advantages such as the high sampling frequency and lack of a requirement of a derivatization step have been emphasized. However, the determination can be strongly affected by foreign chemical species usually present in distilled spirits, such as oxidized compounds other than glycerol, and by the high ethanol content that may affect the poly(vinyl chloride) (PVC) membrane of the electrode. In this way, correction of these effects is needed for a reliable determination. As an application, the analysis of distilled spirits with high alcoholic contents (cachaça and bagaceira—popular Brazilian and Portuguese spirits with ~40% v/v ethanol) was selected.

* Corresponding author (fax +351 22 200 4427; e-mail limajlfc@ff.up.pt).

[†] Ph.D. student at Departamento de Química, Universidade Federal de São Carlos, Brazil.

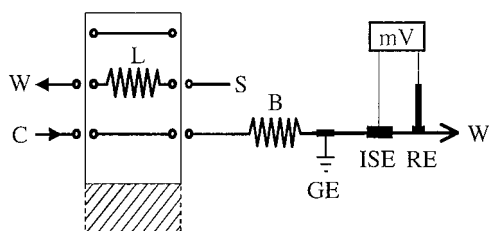


Figure 1. Flow diagram: L, 30-cm sampling loop (150 μL); C, carrier solution (1.0×10^{-3} mol L^{-1} $\text{NaIO}_4 + 5.0 \times 10^{-1}$ mol L^{-1} $\text{Na}_2\text{SO}_4 + 1.0 \times 10^{-1}$ mol L^{-1} sodium acetate/acetic acid, pH 4.7) at 2.0 mL min^{-1} ; B, 300-cm reaction coil; GE, ground; RE, reference electrode; ISE, periodate-selective tubular electrode; mV, detector; W, waste; dashed area, alternative position of the injector; full arrows, sites where pumping is applied.

MATERIALS AND METHODS

Apparatus. The potentiometric system comprised a model 2002 Crison instrument (0.1-mV resolution), a periodate-selective electrode, and a model 900200 Orion reference (Ag/AgCl) electrode. The indicator electrode without inner reference solution was prepared in a tubular geometry as described by Luca and co-workers (11). Grounding was done according to the method of Alegret et al. (12). When not in use, the indicator electrode was maintained in 1.0×10^{-1} mol L^{-1} IO_4^- .

A model IPC-4 Ismatec peristaltic pump was used with Tygon pumping tubes. The computer-controlled injector, as well as the 0.8-mm i.d. polyethylene coiled reactors, transmission lines, and accessories were described in earlier work (13).

For system control and data acquisition, a 486 microcomputer with a PCL-711S Advantech analog/digital interface card was used. Software was developed in Quick Basic 4.5, and data were stored as ASCII files for further treatment.

For chromatographic analyses, a model DX 300 Dionex high-pressure liquid chromatograph with an integrated oven compartment, a Carpac PA-1 strong cation-exchange resin column (250 \times 4 mm i.d.), and an amperometric detector (gold cathode) were used. Sample injection was done by means of a 20- μL loop inserted in a Valco rotary valve.

Solutions. The solutions were prepared with analytical grade chemicals and deionized water (specific conductivity < 0.1 $\mu\text{S cm}^{-1}$).

The glycerol stock standard solution was prepared by diluting 40 g of glycerol in 1.000 L of water and further iodometrically standardizing (14). Working standards (20–500 mg L^{-1} glycerol) were prepared to contain also 500 mg L^{-1} sucrose, 300 mg L^{-1} fructose, 300 mg L^{-1} glucose, and 40% (v/v) ethanol to match the matrix of the distilled spirit samples (cachaça and bagaceira).

The 1.00×10^{-1} mol L^{-1} periodate solution was prepared by dissolving 5.347 g of NaIO_4 in 250 mL of water. The sample carrier stream was a 2.00×10^{-3} mol L^{-1} $\text{NaIO}_4 + 5.0 \times 10^{-1}$ mol L^{-1} $\text{Na}_2\text{SO}_4 + 1.0 \times 10^{-1}$ mol L^{-1} sodium acetate/acetic acid (pH adjusted to 4.7) solution.

Samples were run without any treatment. For those with > 500 mg L^{-1} glycerol, a prior manual dilution (10 mL sample to 100 mL 40% v/v ethanol) was done.

Procedure. Working conditions of the tubular periodate-selective electrode were verified by injecting in triplicate different (1.00×10^{-4} – 1.00×10^{-1} mol L^{-1} IO_4^-) periodate solutions into the single-line system in Figure 1. In these experiments, a 1.00×10^{-4} mol L^{-1} $\text{IO}_4^- + 1.0 \times 10^{-1}$ mol L^{-1} $\text{Na}_2\text{SO}_4 + 1.0 \times 10^{-1}$ mol L^{-1} sodium acetate/acetic acid buffer solution was used as sample carrier stream. To avoid the establishment of undesirable concentration gradients along the monitored zone, the injected solutions were also 1.0×10^{-1} mol L^{-1} $\text{Na}_2\text{SO}_4 + 1.0 \times 10^{-1}$ mol L^{-1} sodium acetate/acetic acid. The solutions were successively injected by means of a 30-cm (150- μL) sampling loop, transported through the analytical path and monitored under conditions of constant ionic strength and pH. A relatively high (4.0 mL min^{-1}) carrier stream flow rate and a short (30-cm) reaction coil were selected to minimize dispersion and to permit a prompt evaluation

of any eventual limitation in measurement due to detector response time.

For glycerol determination in distilled spirits, the system in Figure 1 was designed to provide large sample dispersion (15): this feature was required in view of the high ethanol content expected in the samples. After loop-based injection, glycerol was oxidized during transportation of the dispersing sample zone through the analytical path, and details of the involved chemical reactions are given elsewhere (16). The remaining periodate was monitored during passage of the processed sample through the detector, which yielded a transient variation in potential. This variation was recorded as a peak with height reflecting the glycerol content in the sample. About 50 s after peak maximum recording, the injector was switched back to the position in Figure 1 for another cycle.

Effects of potential interfering species were evaluated by using 400 mg L^{-1} glycerol solutions containing also 5000 mg L^{-1} sucrose, 3000 mg L^{-1} fructose, 3000 mg L^{-1} glucose, or 5.0 mg L^{-1} Cu. The influence of ethanol addition was investigated by using 400 mg L^{-1} glycerol hydroalcoholic solutions with 10, 20, 38, 40, 42, or 45% (v/v) ethanol (as determined by densimetry).

Thereafter, the main analytical characteristics were evaluated, and the system was applied to large-scale analyses.

RESULTS AND DISCUSSION

In the experiments involving injections of periodate standard solutions, limitations related to baseline noise or drift were not observed, as the monitored periodate ion was always present. With a constant periodate level, the electrode membrane was preserved because dissolution of periodate immobilized into the PVC membrane was impaired in view of the presence of the analyte in the carrier stream. Precise measurements were attained, and the standard deviation of peak height was estimated as < 0.5 mV. The general recorded peak shape was maintained regardless of periodate monitored concentration, indicating that response time was not a limiting factor in the measurement. This aspect confirmed that 1.0×10^{-4} mol L^{-1} IO_4^- in the carrier stream was suitable for evaluating the electrode response. These figures are in closer adherence with the previously reported general working characteristics of the periodate tubular electrode (17). The calibration curve was linear within 1.0×10^{-3} and 1.0×10^{-1} mol L^{-1} IO_4^- in the injected solution, and the linear regression coefficient was estimated as 0.9991 ($n = 4$).

To define the experimental conditions for glycerol determination, several parameters of the flow system were investigated and dimensioned by using the univariate optimization approach. The first parameters considered were the sample injected volume and the reaction coil length. The former was varied within 50 and 150 μL (10–30 cm), and the latter was kept as 100, 200, or 300 cm. Increasing the injection volume up to 150 μL increased the glycerol analytical signal. Beyond this value, further increase in sample volume determined only a slight increase in peak height associated, however, with a pronounced drop in sampling rate, and—for higher values—with the appearance of double peaks (18). Moreover, increasing the reactor length promoted only a small decrease in the recorded signal. This performance was related to the combined influence of two opposite factors: the available time for reaction development and the dispersion of the sample plug. Furthermore, for selection of the best conditions, special attention was focused on the possible deterioration of the electrode membrane for alcohol concentrations $> 5\%$ (v/v). This concentration means $\sim 40\%$ (v/v) in the solution injected into the proposed system. This deterioration was confirmed by injecting 150 μL of distilled spirit samples with alcohol concentrations varying from 38 to 45% (v/v), using the shorter coil length. Erratic signals were

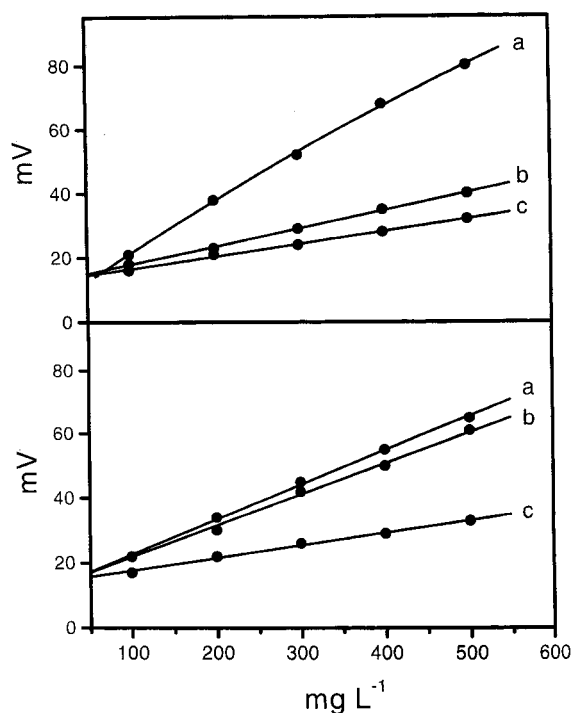


Figure 2. Influence of carrier stream flow rate and periodate ion concentration: (top) flow rates of 2.0, 5.0, and 7.0 mL min⁻¹ (a, b, and c, respectively) and periodate concentration of 2.0×10^{-3} mol L⁻¹; (bottom) periodate concentrations of 1.0×10^{-3} , 5.0×10^{-3} , and 10×10^{-3} mol L⁻¹ (a, b, and c, respectively) and a flow rate of 2.0 mL min⁻¹. Figure refers to the flow system in Figure 1.

recorded and a fast deterioration of the membrane observed. Using tubular electrodes without inner reference solutions (19) minimized the effect. Therefore, the system was redimensioned to provide high sample dispersion, so that the highest ethanol content of the processed sample reaching the flow cell was <5% (v/v). With a 150- μ L sampling volume and a 300-cm reaction coil, the injected sample aliquot was efficiently mixed with the carrier stream, sensitivity matched the 20–500 mg L⁻¹ glycerol range, and the linear regression coefficient was estimated as 0.9997 ($n = 7$).

The carrier stream flow rate and the periodate concentration play a relevant role in the proposed procedure, as they are closely related to the reaction development. The flow rate had little influence on sample dispersion but defined the mean available time for the reaction between IO₄⁻ and glycerol. Although chemical equilibrium was not required, signals recorded for 2.0, 5.0, and 7.0 mL min⁻¹ flow rates indicated a better sensitivity for lower flow rates (Figure 2a). This situation was particularly dictated by the reaction kinetics more than as a result of the response time of the potentiometric detector.

As the analytical signals were based on periodate consumption by glycerol oxidation, the IO₄⁻ concentration was of utmost relevance (Figure 2b). Moreover, the effect of alcohol concentration in the carrier stream on the reaction kinetics and on the potentiometric response was minimized but not quantified. Therefore, a preliminary study to investigate the influence of periodate concentration in the carrier stream (1.0×10^{-3} – 1.0×10^{-2} mol L⁻¹) was carried out for both aqueous and 40% (v/v) hydroalcoholic solutions. This latter value was selected in view of the expected mean ethanol content in the samples that should be introduced into the system without any prior preparation. As expected, a different behavior was observed, and the best sensitivity was attained for aqueous solutions,

reflecting the effect of organic solvents in plastic membrane electrodes.

Nevertheless, the sample matrix is ethanolic, and a step for removal of the alcohol would be cumbersome. Alternatively, standard solutions simulating the sample matrix could be used, but preparation was difficult in view of the variations in alcohol concentration within the samples. Therefore, the influence of the alcoholic content in the glycerol determination was initially evaluated by injecting solutions (400 mg L⁻¹ glycerol) with different ethanol concentrations, ranging from 10 to 45% (v/v), which corresponds to 1.2–6.0% v/v in the sample solution in contact with the electrode. As the alcohol concentration rose, slight lessening of the analytical signal ($\sim 1\%$ per ethanol degree) was observed until $\sim 35\%$ (v/v) and remained unaffected by variations in ethanol content within 38 and 45% (v/v). Therefore, the standard solutions were prepared in 40% (v/v) ethanol. In view of the large involved dispersion, addition of ethanol to the sample carrier stream was not performed. Therefore, flow rate and composition of the carrier stream were chosen as 2.0 mL min⁻¹ and 2.0×10^{-3} mol L⁻¹ NaIO₄ + 5.0×10^{-1} mol L⁻¹ Na₂SO₄ + 1.0×10^{-1} mol L⁻¹ sodium acetate/acetic acid, respectively. In this situation, the analytical curve was linear for 20–500 mg L⁻¹ glycerol.

Chromatographic analysis currently carried out in the authors' laboratory pointed out that distilled spirits generally present about 5000, 3000, and 3000 mg L⁻¹ sucrose, fructose, and glucose, respectively. These sugars are potential interfering species, as fructose and glucose are present in high concentrations in the sample and are oxidized by periodate, whereas sucrose may hydrolyze to form fructose and glucose. The influence of these species on the glycerol determination was studied by running glycerol solutions (400 mg L⁻¹) containing up to 5000 mg L⁻¹ sucrose plus 3000 mg L⁻¹ fructose or glucose. The analytical signals in the presence of these species were increased with the reducing sugar concentrations. Matrix matching between reference and sample solutions could be exploited to circumvent this effect, but this strategy was not needed here. In fact, varieties of commercially available samples with glycerol contents of <500 mg L⁻¹ usually present also lower sugar concentrations. On the other hand, samples with >500 mg L⁻¹ glycerol underwent prior manual dilution that reduced also the mean sugar contents to be about 500, 300, and 300 mg L⁻¹ sucrose, fructose, and glucose, respectively. In this situation, no interference from sugars or ethanol was observed. It should be stressed that other potential interfering polyols such as butanediol are not added to distilled spirits. With regard to copper, no interference was observed on glycerol determination by the proposed method up to 5.0 mg L⁻¹ Cu.

Under the above-defined conditions, several distilled spirit samples were analyzed, and results are shown in Table 1. The results are in agreement with those obtained by HPLC, with a maximum relative error of 7% being verified. Recovery was estimated as 96–120%. These figures are acceptable for monitoring purposes and quality assurance of the final product, in view of the high variability of the glycerol contents within the different sample lots.

The detection limit was estimated (20) as 10 mg L⁻¹ glycerol. The proposed procedure is characterized by a linear potential/concentration response for 20–500 mg L⁻¹ glycerol ($r > 0.9999$, $n = 7$). Linearity of the analytical curve is a consequence of the combined effects of electrode Nernstian response and first-order kinetics of the glycerol oxidation (21). Other favorable features are a relative standard deviation of results of <0.03, a

Table 1. Glycerol Determination in Commercial Distilled Spirits from Brazil (Cachaça, BR) and Portugal (Bagaceira, PT) As Determined by the Proposed System and by HPLC^a

sample	proposed method	HPLC	% recovery
01 BR	80.0 ± 0.9		101
02 BR	36.3 ± 0.5		96
03 BR	1260 ± 15	1210	
04 PT	88.0 ± 0.7	88.3	
05 PT	44.0 ± 0.5	43.8	
06 PT	81.5 ± 0.5		120
07 BR	850 ± 12		118
08 BR	1086 ± 10		
09 BR	765 ± 13	709	
10 BR	1462 ± 20		97
11 BR	727 ± 8		115
12 BR	2150 ± 25		99
13 BR	357 ± 5	369	

^a Data, in mg L⁻¹ glycerol, based on three replications. Uncertainties associated with HPLC data are ~3%. Recoveries were found by adding 50 µL of 4000 mg L⁻¹ glycerol to 10 mL of sample.

throughput of 30 determinations per hour, and a reagent consumption of only 350 µg of IO₄⁻ per determination.

Furthermore, the stability of electrode response was checked during ~130 h of operation by using the alcoholic standard and sample solutions, and only slight variations in the angular coefficient of the analytical curve (usually <5%) were observed.

CONCLUSIONS

The proposed system is very robust and easily operated. When applied to quality control analysis, a manually operated injector can be used to allow higher system portability. Temperature has little influence on the results, so that the system can be operated under room temperature providing that suitable air-conditioning facilities ($T = 25 \pm 2$ °C) are available.

Sample pretreatment for elimination of sugar and alcohol is not required. Moreover, the proposed procedure is fast and does not need highly hazardous reagents.

ACKNOWLEDGMENT

We are grateful to G. Sales, B. Pereira, and G. C. Luca for electrode construction and for critical comments and to L. C. Basso for chromatographic determinations.

LITERATURE CITED

- Monick, J. A. Trihydric and Polyhydric Alcohols. In *Alcohols: Their Chemistry, Properties and Manufacture*; Monick, J. A., Ed.; Reinhold Book: New York, 1968; pp 365–445.
- Nykänen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.* **1986**, *37*, 84–96.
- Lampe, U.; Kreisel, A.; Burkhard, A.; Bebiolka, H.; Brzezina, T.; Dunkel, K. Method for detection of added industrial glycerol in wine. *Dtsch. Lebensm. Rundsch.* **1997**, *93*, 103–110.
- Prodromidis, M. I.; Stalikas, C. D.; Tzouwara-Karayanni, S. M.; Karayannis, M. I. Determination of glycerol in alcoholic beverages using packed bed reactors with immobilized glycerol dehydrogenase and amperometric FIA system. *Talanta* **1996**, *43*, 27–33.
- OIVV. *Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts*; Office International de la Vigne et du Vin: Paris, France, 1990; pp 142–151.
- Ackermans, M. T.; Ruiters, A. F. C.; Endert, E. Determination of glycerol concentrations and glycerol isotopic enrichments in human plasma by gas chromatography mass spectrometry. *Anal. Biochem.* **1998**, *258*, 80–86.
- Lopez, E. F.; Gomez, E. F. Simultaneous determination of the major organic acids, sugars, glycerol, and ethanol by HPLC in grape musts and white wines. *J. Chromatogr. Sci.* **1996**, *34*, 254–257.
- Munoz, G. S.; Romero, E. G.; Coello, M. S. P.; Alvarez, P. J. M. Determination of glycerol in wines by high-performance liquid-chromatography—comparison with enzymatic method. *J. Chromatogr.* **1992**, *623*, 43–47.
- Kiranas, E. R.; Karayannis, M. I.; Tzouwara-Karayanni, S. M. An enzymatic method for the determination of ATP and glycerol with an automated FIA system. *Anal. Lett.* **1997**, *30*, 537–552.
- Gomes Neto, J. A.; Nogueira, A. R. A.; Bergamin Filho, H.; Zagatto, E. A. G.; Lima, J. L. F. C.; Montenegro, M. C. B. S. M. Multi-site detection in flow analysis. Part 3. Periodate tubular electrode with low inner volume as a relocatable detector. *Anal. Chim. Acta* **1994**, *285*, 293–299.
- Luca, G. C.; Reis, B. F.; Zagatto, E. A. G.; Montenegro, M. C. B. S. M.; Araújo, A. N.; Lima, J. L. F. C. Development of a potentiometric procedure for determination of glycerol and 2,3-butanediol in wine by sequential injection analysis. *Anal. Chim. Acta* **1998**, *366*, 193–199.
- Alegret, S.; Alonso, J.; Bartroli, J.; Machado, A. A. S. C.; Lima, J. L. F. C.; Paulis, J. M. Construction of equipment for potentiometric determinations in flow injection analysis. *Quim. Anal.* **1987**, *6* (3), 278–292.
- Martelli, P. B.; Reis, B. F.; Korn, M.; Rufini, I. A. The use of ion-exchange resin for reagent immobilization and concentration in flow systems. Determination of nickel in steel alloys and iron speciation in waters. *J. Braz. Chem. Soc.* **1997**, *8*, 479–485.
- American Oil Chemists' Society. *Official Methods and Recommended Practices of AOCS, 4th ed., Section D, Sampling and Analysis of Soaps and Soap Products*; AOCS: Champaign, IL, 1991; Tentative Method Da 23-56.
- Ruzicka, J.; Hansen, E. H. *Flow Injection Analysis*, 2nd ed.; Wiley-Interscience: New York, 1998; 499 pp.
- Jackson, E. L. Periodate Oxidation. In *Organic Reactions*; Adams, R., Bachmann, W. E., Fieser, L. F., Johnson, J. R., Snyder, H. R., Eds.; Wiley: New York, 1947; pp 341–375.
- Montenegro, M. C. B. S. M.; Lima, J. L. F. C.; Mattos, I. L.; Oliveira Neto, G.; Gomes Neto, J. A.; Zagatto, E. A. G. Development of a tubular periodate electrode for flow-injection determination of glycerol. *Talanta* **1993**, *40*, 1563–1568.
- Tyson, J. F. Analytical information from doublet peaks in flow-injection analysis. I. Basic equation and application to flow-injection titration. *Analyst* **1987**, *112*, 523–526.
- Lima, J. L. F. C.; Machado, A. A. S. C. Procedure for the construction of all-solid-state PVC membrane electrodes. *Analyst* **1986**, *111*, 799–802.
- International Union of Pure and Applied Chemistry, Analytical Chemistry Division, Commission on spectrochemical and other optical procedures for analysis. Nomenclature, symbol, units and their usage in spectrochemical analysis—III. Analytical flame spectroscopy and associated non-flame procedures. *Spectrochim. Acta* **1978**, *33B*, 247–269.
- Gomes Neto, J. A.; Zagatto, E. A. G. Linear relationship between potential and concentration in flow potentiometry. *Lab. Rob. Autom.* **2000**, *12*, 41–45.

Received for review November 28, 2000. Revised manuscript received August 9, 2001. Accepted October 9, 2001. This work was supported by a CAPES/ICCTI binational collaborative project.