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Capillary electrophoresis applied to the determination of some seleno compounds

Nicole Gilon, Martine Potin-Gautier*

Laboratoire de Chimie Analytique, CURS Faculté des Sciences, Université de Pau et des Pays de l'Adour, 64000 Pau, France

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Abstract

Selenium is well known for its both toxic and beneficial effects on living organisms. The toxicity is closely correlated with the form in which selenium is present. The need for new methods for the speciation of this element led to the study of analytical parameters applicable to the determination of selenite, selenate, selenocystine and selenomethionine by capillary electrophoresis. The separation is achieved in a chromate buffer with trimethyltetradecylammonium hydroxide as electroosmotic flow modifier. Detection limits are ca 10 $\mu g\ l^{-1}$ for inorganic species and 300 $\mu g\ l^{-1}$ for selenoamino acids. Interferences by other inorganic ions were studied and applicable to the determination of selenium in a thermal water led to results in good agreement with those given by differential-pulse cathodic stripping voltammetry.

Keywords: Selenoamino acids; Selenium, inorganic compounds; Inorganic anions

1. Introduction

Since its discovery in 1817, selenium has been considered as a toxic agent. Its important role for living organisms was only discovered in 1957 by Schwartz and Foltz [1]. Selenium levels present in the environment are very low, depending on the area. The element exists as both organic and inorganic compounds in several oxidation states. The major inorganic forms are selenium(VI) (selenate), selenium(IV) (selenite), selenium(0) (elemental selenium) and selenium(−II) (selenide) and the main organic species are amino acids (selenocysteine, selenomethionine and their derivatives [2]), methylselenides [3] and trimethylselenonium ion [4].

The gap between toxic and essential levels of total selenium is narrow, the harmfulness depending on the species. Dissolved selenium compounds are present in waters; concerning surface water, total selenium levels are in the range 10–350 ng l^{-1} with a mean value of 200 ng l^{-1}, the major form being Se(VI) [5]. The mean concentration of total selenium in seawater is 0.1 $\mu g\ l^{-1}$ depending on depth and geographic location, and selenium is found as Se(VI), Se(IV) and Se(−II) [6].

Numerous techniques have been employed to determine selenium and either measure the total element, e.g., atomic absorption spectrometry and neutron activation analysis [7], or are sensi-

* Corresponding author.

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tive to a single selenium compound, such as fluorimetry [7], electrochemical techniques [8] and hydride generation [9]. These methods, although having low detection limits (in the ng l^{-1} range), require redox reactions to form Se(IV), so that the other species are calculated from a combination of mineralized, direct and reduced sample analysis, increasing the possibility of element loss. Hence, methods able to separate and quantify simultaneously different forms of the element present in a sample, in a single analysis, are becoming very important.

Capillary electrophoresis provides the opportunity for rapid and simple analysis together with high efficiency. This method has only recently been applied to the determination of inorganic ions and led to low detection limits, usually in the μg l^{-1} range with a small sample volume of only a few nanolitres [10]. The use of various electrolytes allows the determination of different organic compounds such as amino acids [11], carbohydrates [12] and organic acids [13], and also inorganic species such as iodide, iodate, bromide and chloride [14,15]. Analyses have recently been performed on ionized arsenic species [16] and selenium compounds [17]. Selenite, selenate, selenocystine and selenomethionine were separated in a phosphate buffer followed by direct UV detection at 220 nm with detection limits in the mg l^{-1} range.

In this work, the determination of the above four compounds was attempted in a chromate electrolyte, allowing indirect UV detection, which is believed to improve sensitivity. The aim was the determination of the selenium present in natural water with a first application to the evaluation of the selenium content of a thermal water from La Roche Posay (France).

2. Experimental

2.1. Reagents

Stock solutions of Se(VI) and Se(IV) were prepared from analytical-reagent grade sodium selenate and sodium selenite, respectively, from Merck. DL-Selenocystine and DL-selenomethionine were purchased from Sigma. These products were used without further purification. Stock solutions (1000 mg l^{-1}) in deionized water (Millipore, 18 mΩ) were stored in the dark at 4°C. Hydrochloric acid (3%) (Merck, Suprapur) is required to dissolve selenocystine. Working standards were prepared daily.

The electrolyte consisted of a mixture of sodium chromate ($Na_2CrO_4 \cdot 4H_2O$) (Merck) and an electroosmotic flow (EOF) modifier, sodium trimethyltetradecylammonium bromide (TTAB, $C_{17}H_{38}NBr$) (Aldrich), prepared daily, filtered and degassed. The hydroxy form of the modifier was obtained after ion exchange on an anion-exchange resin (Amberlite GC 400, modified with NaOH).

2.2. Instrumentation

A Quanta 4000 capillary electropherograph (Waters Chromatography Division of Millipore), with a negative power supply and a twenty-position carousel, was used. The 75 μm I.D. fused-silica capillary was 52 cm to detection point with total length 60 cm. Detection was achieved using a 254-nm UV lamp equipped with an appropriate filter and window. The separations were preceded and followed by purging for 2 min with the carrier electrolyte.

Two injection modes are available with this equipment: hydrostatic injection (HYD), where the sample vessel is elevated of 10 cm before separation, and an electromigrative mode (ELM), where a high voltage is applied between the sample and buffer. In all experiments samples were injected directly after filtration (Millipore filter, 0.45 μm) or diluted in deionized water. The data were acquired with a Maxima 820 chromatography workstation (Waters) using a 20-Hz data acquisition rate.

3. Results and discussion

The separation of ionic compounds is achieved in a capillary tube filled with an appropriate carrier electrolyte. Application of a high voltage between two electrodes, located at each end of

the column, allows the charged species to migrate. The velocity results in two phenomena, electromigration and electroosmosis, which are affected by several factors such as pH of the buffer and the use of a surfactant [18]. The study of these parameters was carried out using hydrostatic injection with 4 mg l^{-1} solutions of Se(VI) and Se(IV) and 10 mg l^{-1} solutions of selenocystine and selenomethionine.

3.1. pH of the buffer

In analysis by capillary electrophoresis, pH is usually a major factor. The velocity (V_i) of the analytes is expressed as a function of electromigration and electroosmosis phenomena [19], both of which are dependent on the pH of the electrolyte. The effective charge (Q_{eff}) of the analytes strongly affects their mobility. To consider the influence of the ionic environment, some workers [14,16] use the effective charge of the species expressed per unit concentration. Q_{eff} for the four seleno compounds is calculated using the following equation, as expressed for the example of Se(IV):

$$Q_{eff} = \frac{-(2[SeO_3^{2-}] + [HSeO_3^-])}{[H_2SeO_3] + [HSeO_3^-] + [SeO_3^{2-}]} \quad (1)$$

Fig. 1 shows the evolution of Q_{eff} as a function of the pH of the buffer. All compounds are negatively charged above pH 8, giving rise to possible separation and short migration times. However, a carbonate peak is always present on the electropherograms, resulting in dissolution of carbon dioxide from the air. The manufacturer's proposed conditions, i.e., sodium chromate 5 mmol l^{-1} and TTAB 0.5 mmol l^{-1} (pH 7.8), gave an unsatisfactory separation of the selenite and carbonate peaks. An increased pH led to a better separation of the two peaks (Fig. 2).

From these results, a basic buffer was chosen for its good separation of Se(IV) and carbonate peaks, and for its ability to separate the four species in a reasonable analysis time. Under these conditions a neutral species (water) exhibits a constant migration time of 16.6 min over the studied pH range, which indicates the small contribution of the EOF to the migration time of charged compounds (0.15 cm^2 V^{-1} s^{-1}). Nevertheless, a high buffer pH leads to increased background noise and is also suspected of degrading the capillary walls, so the pH of the electrolyte was fixed at 10.5 as a compromise.

3.2. pH of the sample

Concerning hydrostatic injection, the influence of the sample pH on the analysis was ascertained by comparison of the responses of acidified solutions. Synthetic solutions containing 4 mg l^{-1} of Se(VI) and Se(IV) were adjusted to pH 3 to 8 and injected. The peak-height relative standard deviations were 5.2% and 4.8% for Se(VI) and Se(IV), respectively, and 4.2% and 6.1% for corrected area (ratio of peak area to migration time). From these results, it appears that with a

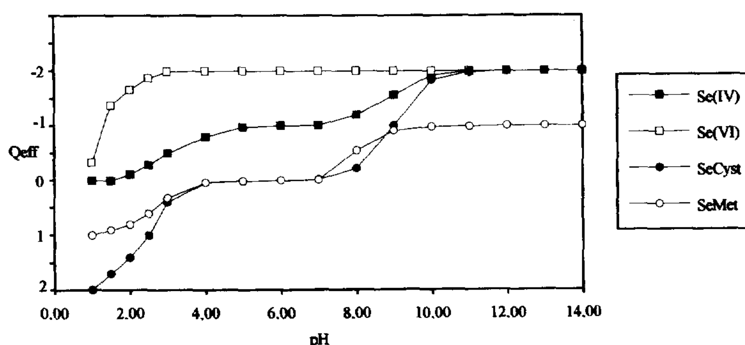

Fig. 1. Calculated effective charges of Se(VI), Se(IV), selenocystine (SeCyst) and selenomethionine (SeMet).

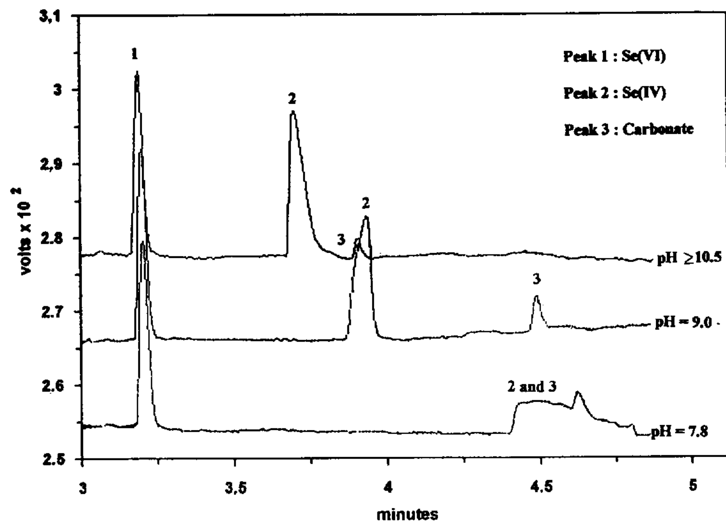

Fig. 2. Electropherograms of Se(IV), Se(VI) and carbonate with electrolytes of pH 7.8, 9 and 10.5, respectively. Hydrostatic injection (30 s, 10 cm); separation achieved in 6 min at −20 kV.

pH change of 5 units neither the corrected area nor the peak height was significantly altered.

3.3. Use of a surfactant

The working pH used in the determination of seleno compounds (10.5) should result in high electroosmosis. This factor is usually controlled via the pH [20], the application of a radial potential [21] or the addition of a cationic surfactant to the electrolyte, the last solution being mainly used with cetyltrimethylammonium bromide (CTAB), trimethyltetradecylammonium bromide (TTAB) or decyltrimethylammonium bromide (DeTAB) [20].

A sufficient concentration of cationic surfactant is used to modify the capillary wall. It is adsorbed as a double layer at the surface, creating a globally positive wall [19]. The electroosmotic flow travels in the same direction as the migration of anions.

However, to reach optimum pH values, it was necessary to add sodium hydroxide to the buffer, and the rise in ionic strength of the solution caused a high background noise. The use of the hydroxy form of the surfactant enabled the pH to be increased without any modification of the ionic strength. Fig. 3 shows that, as for TTAB [19], a TTAOH working concentration of 0.5 mmol l^{-1} led to a mobility steady state.

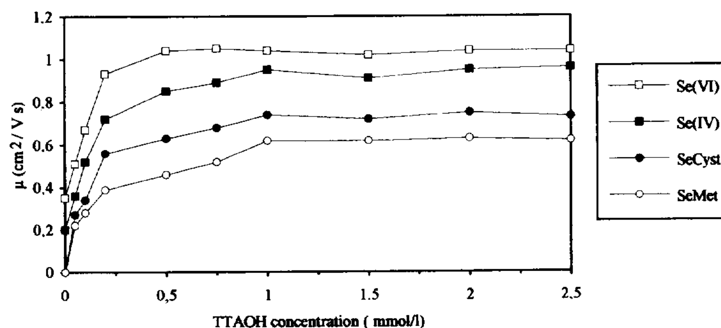

Fig. 3. Mobilities (μ) as a function of surfactant (TTAOH) concentration.

Table 1
Calculated detection limits and reproducibilities of injection for Se(VI), Se(IV), selenocystine (SeCyst) and selenomethionine (SeMet)

Parameter	Injection mode	Se(VI)	Se(IV)	SeCyst	SeMet
Migration time (min)		3.2	3.7	4.9	6.5
Calculated detection limit (μg l^{-1})	HYD	372	334	268	279
	ELM	16.4	5.7	360	353
Reproducibility (R.S.D.) (%)	HYD (C_{Se} = 1 mg l^{-1})	1.0	1.7	4.0	3.9
	ELM (C_{Se} = 60 μg l^{-1})	1.8	3.0		

Injection conditions: hydrostatic (HYD), 30 s, 10 cm; electromigration (ELM), 20 s, −10 kV. Separation conditions: 15 min, −20 kV. Detection: UV at 254 nm.

3.4. Analytical criteria

Table 1 gives the mean reproducibilities for the two injection modes. Calculations were performed on six consecutive injections of selenocystine, selenomethionine, selenite and selenate (1 mg l^{-1}) for the hydrostatic mode and Se(VI) and SE(IV) (60 μg l^{-1}) for electromigration.

The calibration graphs are perfectly linear in the ranges 5–600 μg l^{-1} using electromigrative injection and 0.1–200 mg l^{-1} for selenite and selenate and 0.2–200 mg l^{-1} for selenocystine and selenomethionine using hydrostatic injection.

Detection limits were evaluated using the IUPAC equation $C_1 = m(x_b + 3\sigma_b)$, where C_1 is the calculated detection limit, m is the slope of the calibrations graphs and x_b and σ_b are the mean value and the standard deviation, respectively, calculated from 20 blank measurements.

The low sensitivity obtained for amino acids injected using the electromigrative mode is caused by the preferential injection of more mobile species at this pH (hydroxy anions) or by the important difference in mobility between these analytes and the electrolyte.

3.5. Ionic interferences

Natural waters contain selenium at trace levels combined with an important ionic matrix. UV detection suffers a dramatic lack of specificity and, prior to analysis of natural samples, it was important to evaluate the interferences caused by major anions such as chloride, sulfate or nitrate whose peaks are very close to that of selenate one (Fig. 4).

Increasing concentrations of various major anions (chloride, sulfate, nitrate and carbonate) were injected with 1 mg l^{-1} of both selenite and selenate. Fig. 5 shows the evolution of the resolution factors. Concerning hydrostatic injection, the resolution factors remain over the quantification level ($R_s > 1.5$) for all anions except the Se(VI)–nitrate separation. Selenate determination is possible only with low concentrations of nitrate (ratio 1:5). The resolution is improved using electrokinetic injection; the peak shape is strongly altered but analysis remains possible by means of the internal standard method (Fig. 5a).

The resolution between Se(IV) and carbonate/hydrogen carbonate peaks, present at high levels in numerous waters, is insufficient only above 200 mg l^{-1}. The range of anion concentration allowing the determination of low levels of inorganic selenium is relatively large apart from nitrate (Fig. 5b).

3.6. Application

Selenium-rich waters contain only a very small amount of the element. In France the La Roche Posay region is known for its seleniferous springs. At the beginning of the century, 0.2 μg l^{-1} could be detected; in 1965, Morette and Divin (see Ref. [22]) found levels from 37 to 74 μg l^{-1},

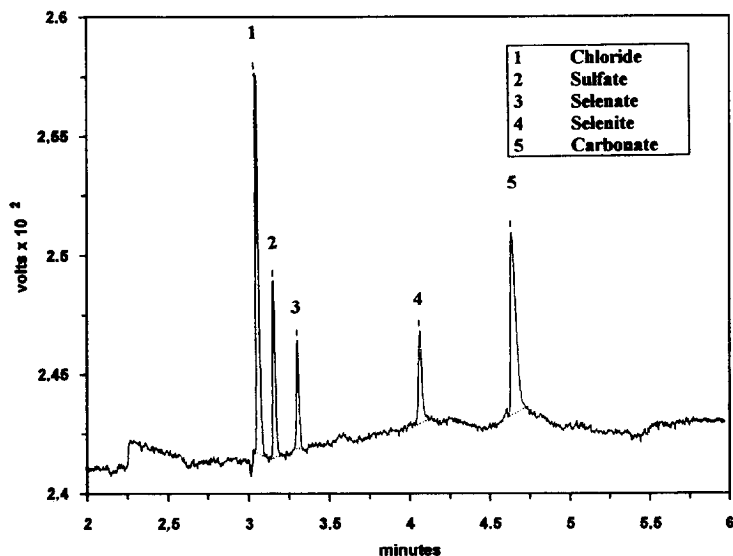

Fig. 4. Hydrostatic injection (30 s, 10 cm) of 1 mg l^{-1} of chloride, sulfate, selenate and selenite ions. Separation achieved in 6 min at −20 kV.

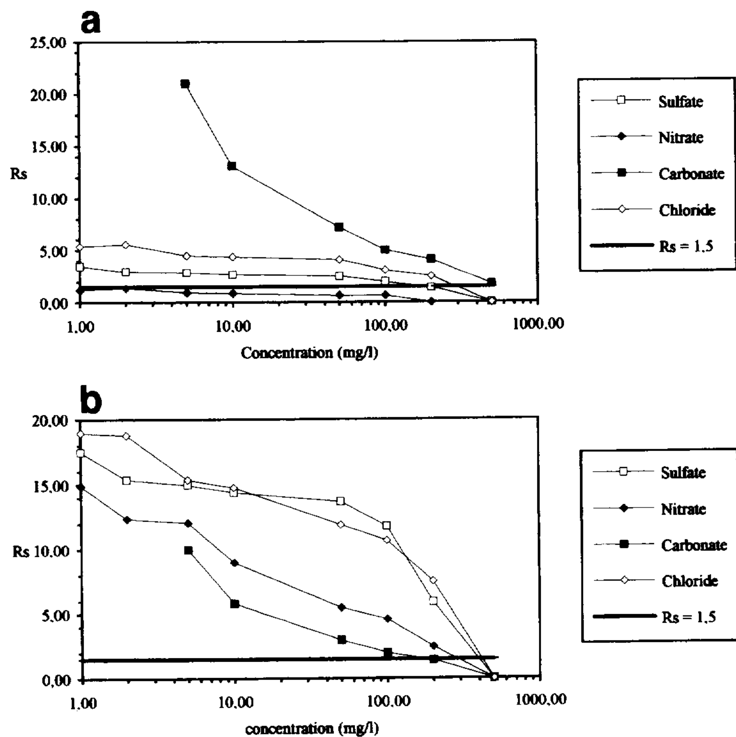

Fig. 5. Resolution factors between (a) Se(VI) and other ions and (b) Se(IV) and other ions.

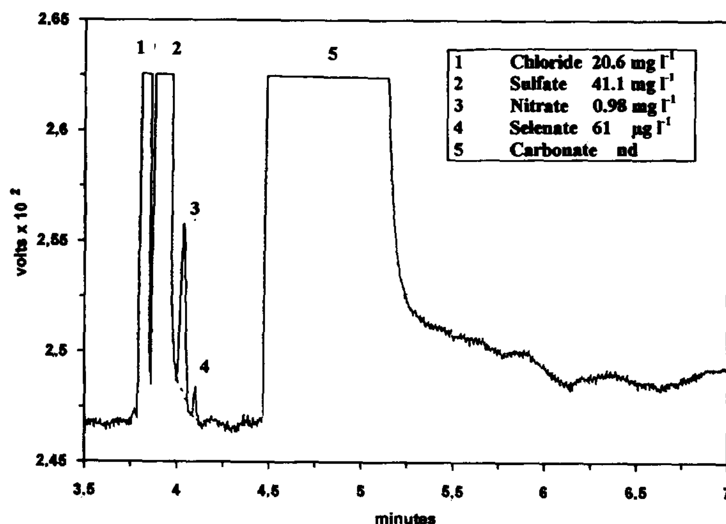

Fig. 6. Electromigrative injection of La Roche Posay thermal water. Separation achieved in 7 min at −20 kV.

depending on the spring concerned. An analysis by Saumade and Lorens [22] of the Melusine source gave 235 μg l^{-1}, whereas St. Aigny water only contained 5 μg l^{-1}. In 1991, an intercomparison exercise determined an average concentration of 53 μg l^{-1} in La Roche Posay thermal water [23].

Capillary electrophoresis (CE) analysis of a commercial sample was compared with a differential-pulse cathodic stripping voltametric (DPCSV) determination. The sample was available as a spray from a pharmacy, and in addition to selenium it contained large amounts of other anions (Fig. 6). The filtered water was placed in a sampling vessel without dilution and introduced through electromigrative injection. Se(VI) was determined the only detectable source of selenium, as shown on the electropherogram.

Selenium concentrations found using DPCSV and CE were 59 ± 3 and 61 ± 6 μg l^{-1}, respectively, which were in good agreement with the total selenium level stated on the label (60 μg l^{-1}).

4. Conclusion

Capillary electrophoresis analysis applied to the determination of ionic species or small selenated organic molecules seems to give interesting results considering the range of detection limits obtained, i.e., 10 μg l^{-1} for inorganics and 300 μg l^{-1} for amino acids. This technique requires small analyte and mobile phase volumes and led to good reliability of measurements. Hence it seems to be suitable for fresh water analysis, provided that the interferences from non-selenated compounds are evaluated and taken into account. Nevertheless, the lack of selectivity and sensitivity of the UV detector is a limit to its application to biological materials and other complex matrices.

The use of more specific and sensitive detection methods such as fluorescence [24], mass spectrometry [25], inductively coupled plasma mass spectrometry or even electrothermal atomic absorption spectrometry, and also the study of extraction procedures closely adapted to capillary electrophoretic analysis, appears to be necessary step and would make this technique more attractive for environmental trace analysis.

References

[1] K. Schwartz and C.M. Foltz, J. Am. Chem. Soc., 79 (1957) 3292–3293.
[2] T.C. Stadtman, Annu. Rev. Biochem., 59 (1990) 111–127.

[3] L. Cleveland, E.E. Little, D.R. Buckler and R.H. Wiedmeyer, Aq. Tox., 27 (1993) 265–280.
[4] J.S. Blais, A. Huygues-Despointes, G.M. Momplaisir and D.W. Marshall, J. Anal. At. Spectrom., 6 (1991) 225–232.
[5] G.A. Cutter, Science, 217 (1982) 829–831.
[6] G.A. Cutter and K.W. Bruland, Limnol. Oceanogr., 29 (1984) 1179–1192.
[7] X. Dauchy, M. Potin-Gautier, A. Astruc and M. Astruc, Fresenius' J. Anal. Chem., 348 (1994) 1–14.
[8] F. Séby, M. Potin-Gautier and A. Castetbon, J. Fr. hydrol., 24, No. 1 (1993) 81–90.
[9] G. Lespes, F. Seby, P.M. Sarradin and M. Potin, J. Anal. At. Spectrom., 9 (1994) 1433–1439.
[10] G. Bondoux, Analusis, 19 (9) (1991) 30–32.
[11] J.V. Sweedler, R. Fuller, S. Tracht, A. Timperman, V. Toma and K. Khatib, J. Microcol. Sep., 5 (1993) 403–413.
[12] W. Lu and R.M. Cassidy, Anal. Chem., 68 (1993) 2878–2881.
[13] B. Levi, K. Talmadge and M. Zhu, Am. Lab., (1993) 29–32.
[14] M.B. Amram, M.D. Lakkis, M.J.F. Leroy, J.P. Lopez-Sanchez and G. Rauret, Fresenius' J. Anal. Chem., 345 (1993) 420–423.
[15] G. Bondoux, P. Jandik and W.R. Jones, J. Chromatogr., 602 (1992) 79–88.
[16] P. Morin, M.B. Amram, R. Heimburger and M. Leroy, Fresenius'. J. Anal. Chem., 345 (1991) 342–357.
[17] M. Albert, C. Demesnay and J.L. Rocca, Analusis, 21 (1993) 403–407.
[18] P. Jandik and G. Bonn, Capillary Electrophoresis of Small Molecules and Ions, VCH, Weinheim, 1993, pp. 5–67.
[19] R. Kuhn and S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principle and Practice, Springer, Berlin, 1993.
[20] P. Gareil, Analusis, 18 (1990) 447–468.
[21] M.A. Hayes, I. Kheterpal and A.G. Ewing, Anal. Chem., 65 (1993) 27–31.
[22] P. Saumade and C. Lorens, J. Fr. Hydrol., 12 (34) (1981) 81–90.
[23] M. Potin-Gautier, M. Astruc and P. Vermeulin, Analusis, 19 (1991) 57–62.
[24] M. Albin, P.D. Grossman and S.E. Moring, Anal. Chem., 65 (1993) 389A–496A.
[25] J.H. Wahl, D.R. Goodett, H.R. Udseth and R.D. Smith, Anal. Chem., 64 (1992) 3194–3196.