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Online publication date: 01 December 2009

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ABSTRACT Sequential injection analysis (SIA) is proposed for managing microvolumes of sample and arsenic species solutions for speciation analysis by capillary electrophoresis focusing on the reduction of hazardous waste residues. An electronically controlled hydrodynamic injector was projected to introduce microvolumes of solutions prepared by SIA into the CE capillary with precision better than 2%. The determination of arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, and arsenobetaine was performed from $50\,\mu L$ volumes of lyophilized urine and extract of shrimp with the system hyphenated to inductively coupled plasma mass spectrometry (CE–ICP–SFMS).

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INTRODUCTION

Arsenic speciation analysis has been deeply investigated mainly due to the high toxicity of inorganic arsenic and its methylated forms. The most used methodologies for speciation analysis are commonly based on the species separation by liquid chromatography (HPLC) and capillary electrophoresis (CE). Both techniques have been employed for As speciation in combination with highly sensitive detection techniques such as Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) or Mass Spectrometry (ICP–MS).[1] However, in some cases the production of volatiles hydrides of the separated As species was implemented to improved the efficiency of analyte transport to the plasma.[2] A system combining capillary electrophoresis with hydride generation was successfully hyphenated to ICP OES for this purpose.[3]

In comparison with the traditional chromatographic techniques, CE presents advantages for managing low sample volumes (nL), high peak resolution, high sample throughput, and the low quantity of waste solutions. These characteristics play an important role considering the instability of the arsenite and arsenate species in aqueous medium[4] requiring daily preparation of the multi species standards and, as a consequence, producing a high quantity of hazardous waste solutions rich in As species.

Non-chromatographic speciation of arsenic compounds in fish samples has been performed by using hydride generation (HG) and atomic fluorescence spectrometry (AFS).[5] However, in these approaches the volumes of hazardous As waste increases. To generate the volatile hydrides from the different As species separated procedures are performed. Also, for total arsenic determination the sample needs to be digested.

The on-line preparation of multiple standards by flow injection systems (FIA) or sequential injection analyzers (SIA) just before the analysis have been reported[6] and several systems coupling flow analysis and capillary electrophoresis FIA–CE or SIA–CE have been proposed with hydrodynamic sample injection of small volumes into the CE capillary.[7–11] The main advantages of SIA reside on its flexibility for fully programming the pre-mix of sample and standard volumes with the possibility of managing micro volumes.

The miniaturization of SIA for managing µL of solutions (µSI) in a system with a lab-on-valve (LOV) device to enhance versatility of the on-line sample pretreatment and total automation in combination with CE was reported earlier.[11] A fully automated on-line µSIA–CE interface employing a syringe of 100 µL was described to prepare different mixed solutions of adenosine and adenosine monophosphate for calibration purposes.[9] The sequential hydrodynamic injection of samples, standards, and the capillary conditioning solutions were accomplished. However, during sample preparation an external chamber was used to improve the analyte to reagent mixing by decoupling the SIA–CE. The on-line coupling of both techniques required the automation of the whole analytical process including the CE capillary cleaning and reconditioning. A sample injection of 40 nL into the CE capillary presented

precision of the UV detected peaks area characterized by 5% RSD. Despite the small sample volume injected into the CE capillary the sample volumes introduced by the SIA system to fill the interface was not provided.[9] In a recent paper[10] a SIA–CE arrangement was early described for full automation of sample and standards injection, as well as the capillary reconditioning solutions. The required volume for each sample injection was 100 µL.

In this research the µSIA capability to handle small volumes of As species to prepare standards for calibration or addition to the sample and their hydrodynamic injection into CE aimed to decrease hazardous waste. The SIA handling of As species was performed independently of the CE cycle. This configuration is proposed looking for reducing volumes of As stock solutions during sample preparation and increase sample throughput by superposing SIA and CE actions by independent processes, such as capillary reconditioning. The system was employed for As speciation analysis in a shrimp and urine sample by CE–ICP–MS.

EXPERIMENTAL

Reagents

Stock standard solutions of 100 mg/L for arsenite, arsenate, monomethyl arsinic acid, dimethyl arsonate acid, and arsenobetaine were prepared from sodium meta arsenite ($AsNaO_2$) and disodium arsenate ($AsNa_2HO_4 \cdot 7H_2O$) (Merck, Darmstadt, Germany), monosodium acid methane arsonate (MMA), and dimethyl arsinic acid (DMA) (Supelco, Sigma-Aldrich, St. Louis, MO, USA). Arsenobetaine ($(CH_3)_3As^+-CH_2COO^-$ the BCR-626 CRM from Bureau of Reference of European Commission Community was used. All solutions were prepared using purified water at $18.2\,M\Omega \cdot cm$ in a Milli-Q system (Millipore, Bedford, MA, USA). Monosodium Phosphate, Tetradecyltrimethylammonium Bromide (TTAB), and Nitric Acid, all from Merck, were employed to prepare the electrolytic buffer.

Samples

Commercially available salted entire dried shrimps were washed carefully just to desalt. Thereafter they were lyophilized and cryogenically grinned. The aqueous extract of 500.00 mg shrimp powder was

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prepared using 20.0 mL of water, ultrasound bath during 1 hr and filtered. Lyophilized urine was weighed (250.00 mg) and extracted with 15.0 mL of water in an ultrasonic bath during 1 hr and filtered. The filtration was performed in the manual vacuum system (Nalgene, Rochester, NY, USA) using (0.45 µm) cellulose nitrate filters (Sartorius AG, Goettingen).

Apparatus and Interface

Sequential Injection Analyzer

The SIA Lab® sequential injection system (FIAlab Instruments Inc., Bellevue, WA, USA) equipped with an eight port valve was used to mix micro-volumes of multi-species standards. The SIA system with a 500 µL glass syringe pump and a polyether ketone (PEEK) holding coil (30 cm long, 0.2 mm i.d.) was employed. Capillary PEEK tubing of 65 µm i.d. and 10 cm long were used for connecting the SIA ports to the solutions vials.

Sample Introduction into CE

The CE sample injector system with a 200 µL vial (Eppendorf AG, Hamburg, Germany) was installed in a platform similar to that described earlier.[12] For CE sample introduction, an injector was constructed with a gas controller unit (DGU) (Shimadzu Corporation, Kyoto, Japan) and an electronic timer composed by a 555 IC controlled two three-way solenoid valves (NResearch, Stow, MA, USA). The sample injection characteristics were determined by using in-line UVdetection (LabAlliance, mod.500,

State College, PA, USA) and data acquisition by a chromatographic station (Data Apex Ltd., Prague, Czech Rep.).

Interface CE–ICP–SFMS

The homemade capillary electrophoresis system was nested using fused-silica capillaries with 75 µm i.d. × 45 cm length (Polymicro Technology, Phoenix, AZ, USA), Pt electrodes 350 µm (Loccus, Biotech, São Paulo, Brazil) connected to a high voltage power supply model CZE-1000R (Spellman, Hauppauge, NY, USA). The CE was interfaced with the ICP nebulizer using a PEEK T shape (Upchurch Scientific, Oak Harbor, WA, USA). The electrophoresis capillary crossed the T shape interface and by the perpendicular tube a sheath flow of 0.1 mol/L nitric acid connected to the Pt electrode was introduced. The sheath flow was pumped using a peristaltic pump (Ismatec, mp-8, Zurich, Switzerland). At the exit of the T piece a 3 cm polyethylene tubing 0.5 mm i.d. was connected to the high efficient nebulizer (HEN Meinhard, Santa Ana, CA, USA) installed in a cyclonic spray chamber connected to the torch. A sector field ICP/MS instrument (Element 1, Finnigan MAT, Bremen, Germany), operating at low-resolution mode was used.

Experimental Procedures

SIA Procedure for Standards Preparation

First, the sequential injection analyzer shown in Fig. 1 was programmed to prepare daily 500 µL of a set of arsenic species secondary standard solutions
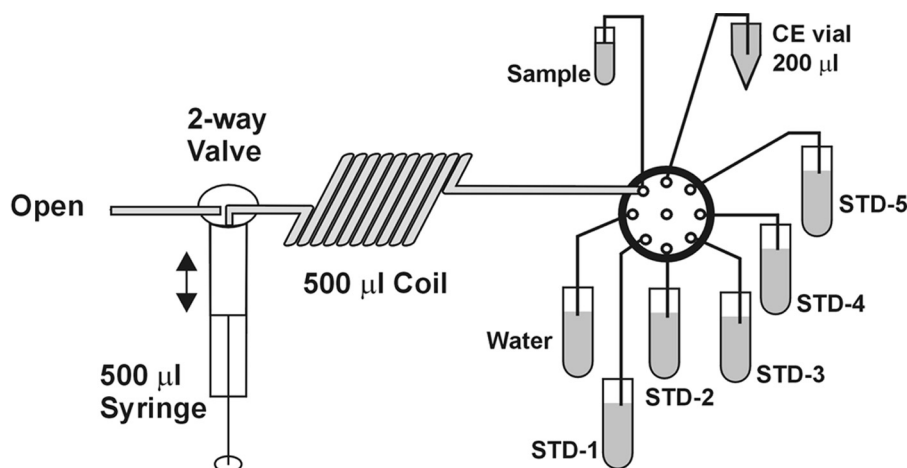

FIGURE 1 Scheme of the SIA system employed. The syringe pump of 500 µL, the As standard solutions, the holding coil and the small vials 200 µgL.

containing 200 µg/L each. The diluted solutions were dropped from the SIA holding coil into the 200 µL Eppendorf® vial by reversing the syringe pump flow direction. The vials were manually able-bodied in the CE injector device.

Volumes of the secondary standard solutions were properly mixed to perform a multi-species solution employed to adjust the electrophoresis parameters, such as capillary dimensions and applied voltage. Also, those secondary standard solutions were properly employed to identify the analyte peak sequence and to perform standard additions to the samples for quantification purposes.

Sample Introduction into CE

An electronically hydrodynamic injection device was projected for CE by nesting the components shown at the left side of the scheme depicted in Fig. 2. The injector port constructed in acrylic presents entrances for the CE capillary, the platinum electrode, and for the pressurized gas. The capillary and the Pt wire were submerged into the solution contained in the flask. The platinum electrode in the sample vial was connected to the negative pole of the high-voltage power supply.

The precision of the injected volume of solutions into the CE capillary was evaluated introducing 100 mmol/L cysteine with detection at 214 nm as described earlier.[12] The experiments were carried out by adjusting argon pressures (5, 10, and 20 kPa) for pressurizing the vial during time delays (5, 10, and 20 s), controlled by the integrated electronic circuit 555. Those parameters were controlled by
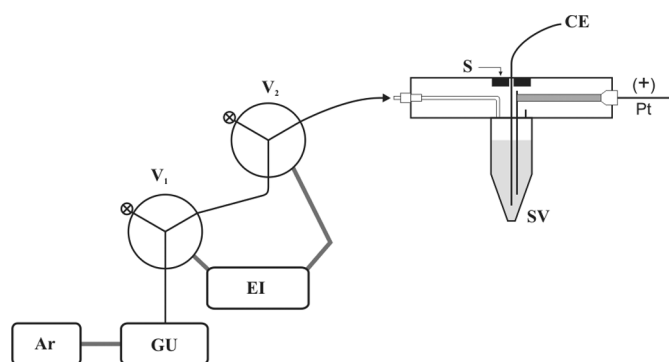

FIGURE 2 Details of the hydrodynamic sample injector constructed in acrylic. CE fused-silica capillary, S septum, V1 and V2 are solenoid valves, EI electronic circuit to control delay of pressurizing the sample vial SV using the gas controller unit GU connected to the argon cilinder Ar.

handling the gas flow-meter and the electronically temporal control of solenoid valves adjusted by a variable resistor (20 kΩ). Two solenoid valves were used in sequence to prevent gas leakage to the flask when valves are off and the electrophoresis separation is occurring. The solenoid valves supported up to 200 kPa. The influence of the flask's headspace on the injected sample volume was evaluated by filling the sample vial with 50 and 60 µL of the cysteine standard solution. The repeatability of 12 sample injections was carried out by applying 5 kPa for 10 s and the area under the peaks was compared using chi-square test (χ^2).

Capillary Reconditioning and As Species Separation Performance

Initial conditioning of capillaries was performed off-line by injecting in sequence solutions of 1 mol/L HCl, water, 0.1 mol/L NaOH, and buffer during 10 min each using the sample introduction device at 15 kPa. A simplest reconditioning procedure was carried out after 5 runs by flushing the conditioner solutions during 30 s at 25 kPa followed by 1 min at 25 kPa of the buffer solution.

The separation performance of arsenite, arsenate, MMA, and DMA was evaluated by CE-UV by injecting 10 mg/L of the species during 5 s applying 10 kPa. The repeatability of migration time and peak areas for each As specie was evaluated from 5 runs with a conditioning step of NaOH in between. The CE separation was performed using the parameters in Table 1.

CE–ICP–SFMS Interface and Parameters

Optimization of ICP–MS parameters was performed by direct nebulization of a solution of

TABLE 1 Working Conditions for CE and ICP–SFMS

Monosodium Phosphate (mmol/L)	20
Tetradecyltrimethylammonium Bromide (mmol/L)	1.5
CE Applied voltage (kV)	−15
Make-up electrolyte HNO_3 (mmol/L)	0.5
CE–ICP make-up flow rate (µL/min)	120
Capillary length (cm)	45
Plasma RF power (kW)	1.3
Plasma argon flow (L/min)	12
Nebulizer argon flow (µL/min)	100
Peak recorder single ion mode m/z	75

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100 µg/L of As(III) employing the injection device at a constant pressure of 25 kpa in order to flush this solution throught the CE capillary. Besides, the make-up solution was pumping at a constant flow rate of 10 µL/min, aiming to pretend sample dilution at the interface. The signal ratio at m/z 75 was monitored while ICP–MS parameters were modified.

The ICP–SFMS operation parameters are presented in Table 1. The CE interface was connected to the high efficient nebulizer (HEN) installed in a cyclonic spray chamber. The nebulizer gas pressure was adjusted to 800 kPa for nominal aspiration of 100 µL/min. In the CE–ICP interface the flow-rate of the sheath solution was 120 µL/min to avoid back pressure into the CE capillary avoiding undesired hydrodynamic effects during the electrophoresis separation.

RESULTS AND DISCUSSION

The electronically controlled injection device projected for CE introduces into the capillary volumes adjusted by pressurization time delays of sample vials. The effect of both parameters is presented in Table 2. The injected volume increased linearly with the injection time, which is in agreement with Altria.[13] Variations of time delays resulted in more accurate injected volumes than changes in pressures, as shown in Table 2. These results confirm the precise time control by the electronic circuit. Since the sample injected volume is desirable to be less than 10% of the total volume of the CE capillary only 5 combinations in Table 2 presented the adequate volume and would be employed. Higher volumes must be avoided as they can affect the current stability.[13]

Bearing in mind that small volumes are employed in capillary electrophoresis, the hypothesis was that a variation in the sampling flask headspace would affect the injected volume. However, the possibility of liquid volume variations up to 20% inside the flask did not affect the injected volume, demonstrating the robustness of the proposed device. Volume variations after triplicate sample injections were insignificant (<1 µL). A minimum volume of arsenic standard solution is desired for lowering the residues after analysis. Nevertheless, the volume must be enough to accomodate the Pt electrode and the capillary separately or not in contact. Nevertheless, the Pt electrode and the CE capillary must be merged together in the solution but not in contact.

The sample volume for on-line SIA–CE reported elsewhere[10] was 100 µL. This volume filled the whole pumping tube and the interface, despite just 50 µL were effectively used during the hydrodynamic injection.

The preparation of solutions by SIA performed off-line to the CE allows the increase of the sample throughtput once both systems can perform actions independently. SIA mixed solutions were prepared in less than 5 min simultaneously to the CE capillary reconditioning. Besides, it presents lower risk of contamination by solutions used for reconditioning.

The vial contains just 50 µl of buffer solution, which needs to be frequently replaced, once the electrolyte becomes biased, which could affect the separation efficiency. In our experiments the buffer solutions were replaced after 3 runs, which is less than the continuously flowing volume of electrolyte typically from FIA–CE or SIA–CE configurations.

Repeatability of sample injection and CE separation parameters for arsenite, arsenate, MMA, and DMA detected at 185 nm are presented in Table 3. Results are characterized by a relative standard deviation less than 1.6% on migration time and 2.6% on peak area for all the standard solutions species. The repeatability values are a consequence of the proposed hydrodynamic sample injection into the CE capillary allowing re-using the vial containing the unaffected solution, which is related to the residues wasted.

TABLE 2 Injected Volumes in nL by Applying Different Pressures and Time Delays, $N = 3$

Pressure/kPa	5	10	20	R^2
Time delays/s				
5	46 ± 1	99 ± 1	230 ± 6	0.9972
10	142 ± 2	297 ± 8	662 ± 16	0.9985
20	327 ± 7	674 ± 17	1429 ± 19	0.9996
R^2	0.9999	0.9998	0.9997	

TABLE 3 Arsenic Species Migration Times and Peak Areas Detected at 185 nm

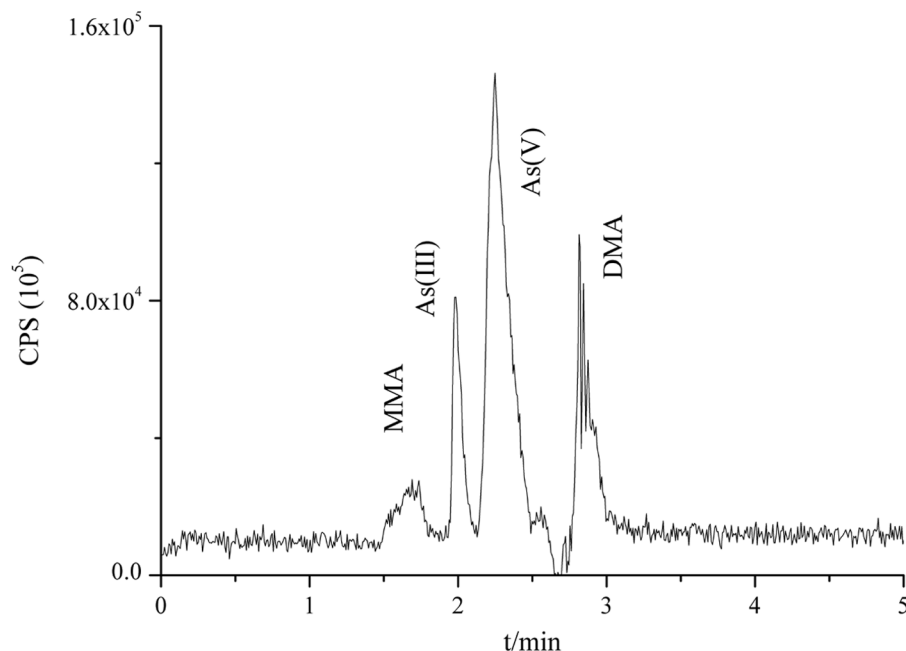| As specie	Migration time* (s)	Peak area* (mV·s)
MMA	1.533 ± 0.021	546.54 ± 12.68
As(III)	1.707 ± 0.015	153.07 ± 3.95
As(V)	2.053 ± 0.032	261.29 ± 6.53
DMA	2.190 ± 0.031	367.27 ± 8.08

$N = 5$.

FIGURE 3 Electropherograms of urine containing arsenite (As (III)), arsenate (As (V)), monomethyl arsonic acid (MMA), and dimethyl arsinic acid (DMA). With addition of 200 mg/L at final concentration of MMA (a) and As(V).

The advantages of As speciation analysis by CE–ICP–SFMS are the possibility of determination in natural samples extracts due to the high detection power and the very low residues produced. The daily maximum volume of hazardous residual solutions for analysis of 10 samples was 1.5 mL, representing about 185 µL of the initial multi-species solution, approximately 240 µL from the five standards solutions used for the calibration curve and residual contaminated water used to wash the SIA system.

The electropherogram presented in Fig. 3 corresponds to As speciation analysis from a spiked urine sample. The urine sample was spiked with 200 µg/L of MMA and arsenate. The four arsenic species presented good resolution and the run took less than 5 min. Furthermore, the aqueous extract of shrimp was analyzed and the main specie founded was arsenobetaine. The electropherogram in Fig. 4 presents also the peak of 200 µg/L of arsenate added to the shrimp extract as a flow-marker.

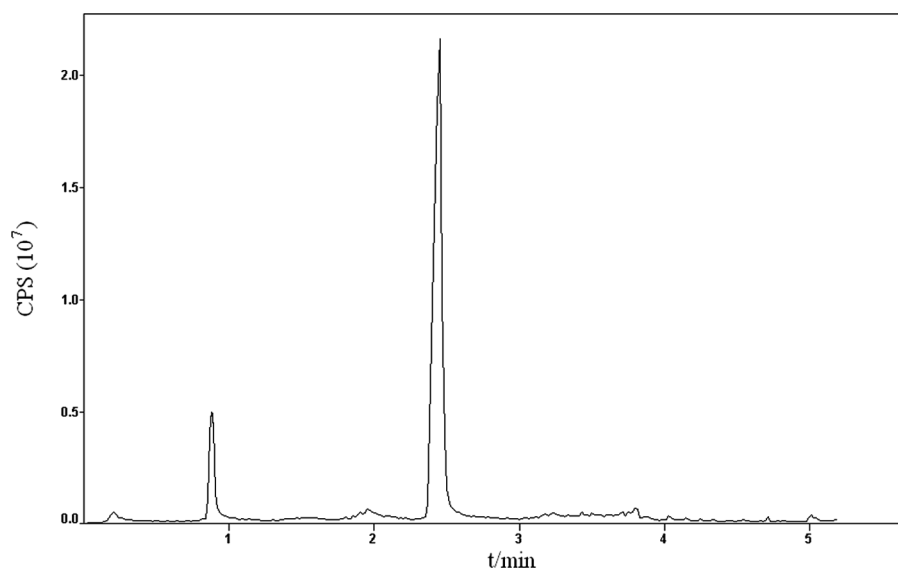

FIGURE 4 CE–ICP–SFMS electropherogram of a shrimp extract detected at m/z 75. From left to right, peaks corresponding to arsenobetaine and the spiked arsenate are shown.

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CONCLUSIONS

A SIA procedure for managing micro-volumes of As species and a miniaturized hydrodynamic injection device for CE reduced significantly the amount of hazardous waste generated during the analytical procedure.

The employment of SIA for *in-situ* preparation of micro-volume standards and to perform the sample addition presented satisfactory results. A considerable reduction (ca. 87%) of the hazardous As residues produced during standard preparation was attained. Another improvement to reduce the residues was the development of a micro-volume hydrodynamic injector for capillary electrophoresis. This device allowed the precise and reproducible hydrodynamic injection of nL of solutions into the capillary. Further, the eletropherogram presented high resolved and low dispersed peaks separated in less than 5 min.

The utilization of a high efficient micro-nebulizer coupled to a cyclonic spray chamber was characterized by efficient transportation of the separated species into the plasma reducing waste of As solutions. The eletropherogram presented high resolved and low dispersed peaks.

ACKNOWLEDGMENTS

REFERENCES

1. Terlecka, E. Arsenic speciation analysis in water samples: A review of the hyphenated techniques. *Environmental Monitoring and Assessment* **2005**, *107*(1–3), 259–284.

2. Magnuson, M. L.; Creed, J. T.; Brockhoff, C. A. Speciation of arsenic compounds in drinking water by capillary electrophoresis with hydrodynamically modified electroosmotic flow detected through hydride generation inductively coupled plasma mass spectrometry with a membrane gas-liquid separator. *J. Anal. At. Spectrom.* **1997**, *12*(7), 689–695.

3. Suárez, C. A.; Giné, M. F. A reactor/phase separator coupling capillary electrophoresis to hydride generation and inductively coupled optical emission spectrometry (CE-HG-ICP OES) for arsenic speciation. *J. Anal. At. Spectrom.* **2005**, *20*(12), 1395–1397.

4. Hall, G. E. M.; Pelchat, J. C.; Gauthier, G. Stability of inorganic arsenic(III) and arsenic(V) in water samples. *J. Anal. At. Spectrom.* **1999**, *14*(2), 205–213.

5. Cava-Montesinos, P.; Nilles, K.; Cervera, M. L.; Guardia, M. D. L. Non-chromatographic speciation of toxic arsenic in fish. *Talanta* **2004**, *66*(4), 895–901.

6. Hindson, B. J.; Brown, S. B.; Marshall, G. D.; McBride, M. T.; Makarewicz, A. J.; Gutierrez, D. M.; Wolcott, D. K.; Metz, T. R.; Madabhushi, R. S.; Dzenitis, J. M.; Colston, B. W. Development of an automated sample preparation module for environmental monitoring of biowarfare agents. *Anal. Chem.* **2004**, *76*(13), 3492–3497.

7. Kuban, P.; Pirmohammadi, R.; Karlberg, B. Flow injection analysis capillary electrophoresis system with hydrodynamic injection. *Anal. Chim. Acta* **1999**, *378*(1–3), 55–62.

8. Fang, Q.; Wang, F. R.; Wang, S. L.; Liu, S. S.; Xu, S. K.; Fang, Z. L. Sequential injection sample introduction microfluidic-chip based capillary electrophoresis system. *Anal. Chim. Acta* **1999**, *390*(1–3), 27–37.

9. Kulka, S.; Quintas, G.; Lendl, B. Automated sample preparation and analysis using a sequential-injection-capillary electrophoresis (SI-CE) interface. *Analyst* **2006**, *131*(6), 739–744.

10. Horstkotte, B.; Elsholz, O.; Cerdá Martín, V. Development of a capillary electrophoresis system coupled to sequential injection analysis and evaluation by the analysis of nitrophenols. *Intern. J. Environ. Anal. Chem.* **2007**, *87*(12), 797–811.

11. Wu, C. H.; Scampavia, L.; Ruzicka, J. Microsequential injection, anion separations using ''Lab-on-Valve'' coupled with capillary electrophoresis. *Analyst* **2002**, *127*(7), 898–905.

12. Lavorante, A. F.; Gervasio, A. P. G.; Moraes, M. C. B.; Giné, M. F.; Miranda, C. E. S.; Carrilho, E. An alternative sample introduction device for a home-made capillary electrophoresis system. *Anal. Letters* **2004**, *37*(12), 2501–2513.

13. Altria, K. D. *Capillary Electrophoresis Guidebook: Principles, Operations and Applications*; Humana Press: Totowa, New Jersey, 1996; 349 p.