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Speciation Analysis of Arsenic, Chromium and Selenium in Aquatic Media

Proceedings of a final research coordination meeting held in Vienna, 26–29 April 2004

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SPECIATION ANALYSIS OF
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FOREFOREWORD WORD

The initiation of the Coordinated Research Project (CRP) on Development and Validation of Speciation Analysis using Nuclear Techniques resulted from the recognition that knowledge of total element concentration does not provide adequate information to understand the effects of trace and heavy metals observed in the environment and in living systems. Their toxicity, bioavailability, physiological and metabolic processes, mobility and distribution are greatly dependent on the specific chemical form of the element. Speciation analysis has yet to be developed to its full potential for biochemical, clinical and environmental investigations and still more work is needed in the near future.

Seven participants from seven countries participated in this CRP covering a range of analytical techniques including GC, HPLC, AAS, and ICP-MS. The first Research Coordination Meeting (RCM) of the Coordinated Research Project on the Development and Validation of Speciation Analysis using Nuclear Techniques was held at the Reactor Centre of the Jozef Stefan Institute, Podgorica (near Ljubljana), Slovenia, 20–23 June 2001. The second RCM was held at the Technical University of Vienna, 18–22 November 2002, where, in addition to the participants, two external researchers could present their views and experience in the field. The last RCM was held in Vienna, 26–29 April 2004, and this publication is a summary of the results achieved and presented at this last RCM.

The participants have developed several new procedures for the reliable analysis of As, Cr, and Se species, mainly in aquatic media. A new instrument was designed and several recommendations for speciation analysis were issued. It is hoped that this publication will make a contribution to enhancing the awareness of the importance of speciation analysis and add to the reliability of speciation results in Member State laboratories.

The IAEA officers responsible for this publication were M. Campbell, Agency's Laboratories in Seibersdorf, and M. Rossbach, Division of Physical and Chemical Sciences.

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CONTENTS

SUMMARY 1

ARSENIC SPECIATION ANALYSIS

Arsenic speciation using HPLC-HGAFS or selective coprecipitation combined with FI-HGAFS or INAA 21
Z. Šlejkovec, V. Stibilj, I. Falnoga, M. Tušek-Žnidarič, D. Mazej, R.O Jaćimović, J. Ščančar, J. T. Van Elteren, M. Manuel Farinha, U. D. Woroniecka, H. Th. Wolterbeek

Selective hydride generation of inorganic arsenic species and subsequent determination by flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS) 28
P. Smichowski, L. Valiente

Determination of AS(III) and AS(V) in waters by electrothermal atomic absorption spectrometry (ETAAS) after ion-exchange chromatography 33
P. Smichowski, A. Ledesma, J. Marrero, L. Valiente

Validation of procedure for speciation of inorganic AS and metabolites in aqueous media by HPLC-HGAAS 40
Ch. Wei, Zh. Zhu, R. Zhao, H. Liu, X. Zhang

Certification of water soluble AS(III) and AS(V) contents in a traditional chinese medicine as a candidate certified reference material 50
Z. Zhu, Ch. Wei, X. Zhang

Potentiality of axial view, simultaneous ICP OES for the direct determination of AS and SE species after HPLC separation 59
P. Smichowski, R.T. Gettar, R.N. Garavaglia, S. Farías, D.A. Batistoni

CHROMIUM SPECIATION ANALYSIS

Chromium speciation in sewage sludge using liquid chromatography 69
J. Ščančar, R. Milačič

SELENIUM SPECIATION ANALYSIS

Home-made capillary electrophoresis coupled to a flow system for speciation of SE^{IV} and SE^{VI} in water samples 81
C.E.S. Miranda, E. Carrilho, A.P.G. Gervasio, A.F. Lavorante, M.F. Giné

ANNEX: VALIDATION OF SPECIATION ANALYSIS USING RADIOISOTOPES 91

LIST OF PARTICIPANTS 113

SUMMARY

INTRODUCTION

It is recognized that total element concentration determination does not provide adequate information to understand the effects observed in the environment and in living systems. The toxicity, bioavailability, physiological and metabolic processes, the mobility and fate are greatly dependant on the specific chemical form of the element. Elemental speciation is yet to be developed to its full potential for biochemical, clinical and environmental investigations and this CRP tried to emphasize on validation of methods and to alert laboratories embarking in the field of speciation analysis to observe strict quality assurance procedures before meaningful results can be obtained. More research is needed to establish recommended procedures for speciation analysis in environmental and biological systems.

The speciation of different arsenic species in biological samples is essential to evaluate the toxic effect and medical function. A number of laboratories have been involved in As speciation analysis using various analytical techniques. The reliability of speciation data depends on the accuracy of the speciation procedure. A common way to verify analytical procedures is to check them with certified reference materials (CRMs). Accordingly, a demand for CRMs for As speciation analysis is rapidly growing. A few CRMs would have been available from Community Bureau of Reference (BCR, EU), National Institute for Environmental Studies (NIES, Enviromental Agency of Japan) and other organizations based on public information. Table I lists some CRMs with different level of arsenic species. The matrix that has been studied includes seafood, seaweeds, urine, animal tissues and artificially synthesized materials. There is still no reference material for medical validation. In this paper, a full description on the preparation, homogeneity and certification of a new candidate CRM for As speciation in traditional Chinese medicine is reported. The data provided in the present paper indicated that the certified material could be recommended as a reference material for As(III)/(V) speciation in medicine matrix.

The toxicity of chromium depends primarily on its chemical form. Trivalent chromium compounds are much less toxic than those of hexavalent chromium. Due to its high toxicity hexavalent chromium is under environmental scrutiny. Concern about the presence of hexavalent chromium in the environment resulted in the development of numerous analytical techniques for the determination of Cr(VI) in different sample matrices. Among them spectrophotometry has been widely used for the determination of Cr(VI). However, the technique is liable to various interference effects, e.g. the presence of coloured species, turbidity and colloidal particles. Therefore, its use should be critically evaluated for the particular sample matrix analysed. Selective extraction of Cr(VI)-HCl complex at 277 K into methyl isobutyl ketone (MIBK) and determination of the Cr(VI) in the organic phase by flame AAS was found to be a sensitive technique for the determination of hexavalent Cr in the presence of large amounts of Cr(III). However, the technique cannot be applied if the sample matrix forms a gel with MIBK. Alkaline extraction (sodium carbonate/sodium hydroxide) was also frequently used for determination of Cr(VI) in environmental samples. Applying this technique, soluble Cr(III) could be oxidised during extraction resulting in positive errors for Cr(VI). Ultrasonic extraction in alkaline solutions was reported to provide good extraction efficiency of Cr(VI) from workplace samples and allowed the retention of Cr(VI) on an anion-exchange resin. For speciation of Cr in natural waters, selective pH-dependent preconcentration of Cr(VI) and Cr(III) was performed on high surface area adsorbents of Al_2O_3 and TiO_2, followed by ETAAS determination of the separated Cr species. Thermal lens spectrophotometric detection and on-line thermal lens spectrophotometric detection after

separation by ion chromatography were found to be selective sensitive techniques for determination of hexavalent and trivalent Cr in various environmental samples at low ng cm^{-3} concentration levels. In our group anion exchange fast protein liquid chromatography (FPLC) with AAS detection has been developed for the simultaneous determination of Cr(III) complexes and Cr(VI). The procedure was applied to determination of Cr species in the sap of cabbage plants exposed to various concentrations of chromate and Cr(III)-EDTA. As an alternative to FPLC columns containing ion-exchange resins, ion-exchange separation supports based on convective interaction media (CIM) were developed recently. The matrix supports of poly(glycidylmethacrylate-co-ethyleneglycoldimethacrylate) substituted with strong or weak cation and anion exchangers offer very fast separation of biomolecules and are also used for fast separation of organic acids. In our group CIM discs were successfully applied in the speciation of zinc in environmental samples.

Selenium was considered a toxic element from its discovery at the beginning of the 19th century until middle of the 20th century when its importance for health of living organisms was finally revealed. This element is essential in lower concentrations, around 100 ng mL^{-1}, however it becomes toxic at higher concentrations. The main natural source of selenium is metal-sulphur minerals but it can be also founded in the environment due to combustion of fossil fuels and to the widespread use in agriculture, electronics and glass industry. Therefore, selenium represents a serious contamination problem not only in urban areas but also in country areas. Selenium inorganic forms, selenite and selenate, are the most commonly found species in polluted water and soils. Since the gap between essentiality and toxicity of selenium is narrow, its harmfulness depends on the species. Selenium exists as both organic and inorganic forms in several oxidation states. Thus, many efforts have been made to develop speciation methods in order to determine and to identify each form.

Nuclear techniques, particularly the use of radioisotopes, can help in methodology development, optimization and stability tests of chemical species. These are often very labile and are prone to changes/destruction/reformation during chemical separation procedures. It was suspected that the analytical result of many speciation exercises reflects more on the separation scheme than on the original species distribution in the matrix from where the species were extracted. Validation of speciation analytical procedures is an important prerequisite to estimate the influence of artefacts on the overall analytical result. Due to the toxicological importance, it was decided to concentrate work on the speciation of As^{+3}/As^{+5}, Cr^{+3}/Cr^{+6}, and Se^{+4}/Se^{+6} in water samples.

The IAEA has initiated a Coordinated Research Project (CRP) entitled Development and Validation of Speciation Analysis using Nuclear Techniques between 2001 and 2004 to explore possibilities to validate speciation methods for As, Se and Cr in liquid matrix using nuclear and non-nuclear techniques. Participants from ARG, AUS, BEL, BRA, CPR, GHA, IND, and SLO developed their techniques and tried to give recommendations on cost-effective speciation methods, reliable and easy to adopt by beginners and more advanced analytical laboratories. Soon it became obvious that validation of speciation methods is difficult to achieve, as virtually no reliable reference materials in this field exist. In one case it was shown that an existing certified reference material was unsuitable for the purpose as the distribution of species in the matrix was changed during sample preparation. This awkward situation could not be overcome as the production and characterization of reference materials (RM) was far bejond the scope of this CRP. However, to some extend the reports compiled in this publication can provide valuable assistance in setting up speciation procedures for certain elements and type of matrices. It is assumed that the reports given by the participants will help to overcome technical problems in speciation laboratories in developing countries with little or no background in this important field of analytical chemistry.

This report provides the summary of the work done in the frame of the CRP as presented by the participants in the final research coordinated meeting, followed by a section on specific chemistry aspects of the element, toxicological aspects and average concentrations found in unpolluted specimen. Some individual laboratory reports, with a general structure according to the elements investigated, As, Cr, and Se, on speciation techniques applied to particular materials will follow. An annex on the use of radioisotopes for method validation authored by one of the CRP participants has been added with permission of John Wiley & Sons, Ltd [in print version only].

SUMMARY OF THE ACHIEVEMENTS OF THE CRP

Argentina

Gas or liquid chromatographic separation of As and Se species, followed by ICP optical or mass spectrometries is well documented in the literature. In spite of this, relatively few recent papers are devoted to explore the direct HPLC-ICP OES coupling by employing modern sequential or simultaneous instruments. Topics such as the dispersion effects caused by the interfacing of HPLC with cross-flow or thermospray nebulizers have been addressed in an earlier work by Laborda and coworkers. Hyphenation of ion exchange chromatography with an optimized sequential Czerny-Turner optical monochromator has been discussed by Chausseau et al. These authors tested five different nebulizers in combination with three spray chambers, allowing them to reach detection limits for arsenicals in the 7 to 18 $\mu g\ L^{-1}$ ranges. An axial-view, simultaneous, echelle type ICP spectrometer with CCD detection was employed by Ebdon et al. for silicon speciation analysis. A particular procedure for manipulation of the instrument software and data acquisition was briefly described to permit the recording of chromatographic peaks and to generate a conventional chromatogram. B´Hymer and coworkers [4] compared four nebulizer-spray chamber interfaces for HPLC-ICP MS. Also the simultaneous multielemental speciation analysis of As, Se, Sb and Te has been reported by Guerin et al. These authors employed ion exchange chromatography combined with ICP MS detection.

Three reports on the effect of different reaction media on the selective generation of arsine from As(III) and As(V) using sodium tetrahydroborate(III) as a reductant; development of a simple approach for the separation and determination of inorganic arsenic species using solid phase extraction; and electrothermal atomic absorption spectrometry and the performance of a simultaneous inductively coupled plasma optical emission spectrometer with axial plasma observation and CCD detection for the direct on-line determination of As and Se species previously separated by ion exchange-high performance liquid chromatography, were presented.

Brazil

The work was focused on selenium speciation analysis. Solid phase extraction (SPE) is a simple, convenient and inexpensive method that has been successfully applied to chemical speciation studies. González Soto et al. and Pacey and Ford used ion-exchange methods for separating and determine four arsenic species.

In the present approach, As(III) and As(V) were separated using a micro-column filled with an ion-exchange resin (Dowex 1-X8). The difference in dissociation constants between arsenous acid ($pKa = 9.2$, $pKa_2 = 12.1$ and $pKa_3 = 13.4$) and arsenic acid ($pKa_1 = 2.2$, $pKa_2 =$

6.8 and $pKa_3 = 11.5$) allows the separation of both species on the basis of ion-exchange. Their separation is pH dependent. At neutral pH, As(III) is not dissociated and is present as a neutral species, $As(OH)_3$, and it is not expected to be retained on an anion-exchange resin. On the other hand, As(V) is dissociated to $H_2AsO_4^-$ and will be retained in the micro-column. Using a convenient eluent, both species can be separated.

The method is rapid (36 samples/h) and requires no sample pretreatment because deposition of Cr(VI) is independent of sample acidity. This is particularly important to preserve the species avoiding their interconversion.

The present study demonstrated that ion-exchange chromatography in conjunction with GF-AAS is fully able to determine selectively As(III) and As(V) in different categories of waters. The method is simple, selective and suitable for routine analysis. Low detection limits were achieved, especially for As(V) that is the most abundant species in oxygenated waters. In addition, the detection limits are more than adequate in view of the maximum concentration fixed for arsenic in drinking waters by different international regulations. At no time was there any evidence that the system allowed any interconversion of species.

China

The speciation of different arsenic species in biological samples is essential to evaluate the toxic effect and medical function. A number of laboratories have been involved in As speciation analysis using various analytical techniques. The reliability of speciation data depends on the accuracy of the speciation procedure. A common way to verify analytical procedures is to check them with certified reference materials (CRMs). Accordingly, a demand for the CRM for As speciation analysis is rapidly growing. A few CRMs would have been available from Community Bureau of Reference (BCR, EU), National Institute for Environmental Studies (NIES, Enviromental Agency of Japan) and other organizations based on public information. The matrix that has been studied includes seafood, seaweeds, urine, animal tissues and artificially synthesized materials. There is still no reference material for medical validation. In the report presented a full description on the preparation, homogeneity and certification of a new candidate CRM for As speciation in traditional Chinese medicine is described. The data provided in the present work indicated that the certified material could be recommended as a reference material for As(III)/(V) speciation in medicine matrix.

In addition, a hyphenated method based on high performance liquid chromatography–hydride generation atomic absorption spectrometry (HPLC–HGAAS) for the speciation of As(III), As(V), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) was developed, validated and repoted.

Ghana

Arsenic, a known toxic element occurs naturally in igneous sedimentary rocks and ores mainly in the form of sulphides, arsenides and sulpharsenides. Arsenopyrites ores containing high levels of gold abound in Ghana and processing of these ores releases significant amounts of arsenic (mainly as As_2O_3) into the local environment. Goldmining is a very important industry in Ghana (since the beginning of the last century) and as such the threat of arsenic pollution of the environment is a major problem. In 1990 one of the major players in this field, the Ashanti Goldfield Company commissioned an Arsenic Recovery Plant to remove arsenic trioxide from the waste gas. Around the same time residents in and around the town of

Obuasi, the center of goldmining activity, were advised not to consume water from streams and rivers in the area. For their benefit many boreholes were sunk.

Most studies to assess arsenic pollution in the Ghanaian environment have focused on total arsenic concentrations. In general high levels of arsenic were found in all cases. As(III)/As(V) in sediments and gold tailings have been speciated by a simple distillation method. Separation of the species was based on the fact that only arsenic(III)chloride was formed when the arsenic species were treated with about 10M HCl. After distilling off the arsenic(III)chloride at 105^{o} C, it was detected by INAA. Validation of the method was achieved by analysing high purity As_2O_3 and As_2O_5.

In was reported, how progress was made in the speciation of AsIII/AsV in bore hole and tap waters obtained from Obuasi and its environs. Validation of the method will be by analysing high purity As_2O_3 and As_2O_5 solutions and measurement by Instrumental Neutron Activation Analysis (INAA).

India

The different toxicity and bio-availability of Cr(III) and Cr(VI) are a public health concern and therefore require strict control. Trivalent chromium is found to be essential for man where it is involved in glucose, lipid and protein metabolism, whereas Cr(VI) is known to be carcinogenic. Hence monitoring of the separate species in e.g. drinking water, occupational exposure or environmental samples is necessary. In order to assess the reliability of the methods developed for the speciation of chromium, we need to analyze appropriate reference materials certified for Cr(III) and Cr(VI). At present the BCR CRM 544 is the only available reference material in aqueous medium (after reconstitution), certified for both Cr(III) and Cr(VI) at μg/L levels. A HPLC-ICPMS procedure was developed for the determination of Cr(VI) and Cr(III) species in lyophilised BCR CRM 544 solution.

Good separation for the Cr(VI) and Cr(III) species among themselves as well as from expected molecular ion interference was obtained using the HPLC-ICPMS procedure, for Cr(VI) and Cr(III) in-house standard solutions, prepared under the same conditions as described for the BCR CRM 544. But when the actual BCR CRM 544 reference material was analyzed, after the reconstitution in the recommended buffer, the recovery of Cr(III) was very poor (7.4%) compared to Cr(VI) (95.8%). The total Cr content estimated using the different procedures agree closely with the certified value. These observations show that the Cr(III) in the reconstituted BCR 544 sample might have been present in a different (non-ionic?) form and not suitable for ion-chromatographic separation. The 88% recovery observed for Cr(III) using the activated alumina procedure indicates that the Cr(III) (whatever the form may be) is retained on the alumina under the alkaline conditions and eluted later using 1M nitric acid. Further experiments are on to improve the recovery of Cr(III) and Cr(VI) using the alumina procedure.

Reconstitution of the BCR CRM 544 in a suitable buffer in the pH range of 2-3 might preserve the species for further analysis using HPLC-ICPMS. This buffer preferably should not contain species that would cause molecular ion interference at mass 51 and 53. The preparation of a Cr(VI)-Cr(III) standard solution and its stability aspects in a medium free of carbonate and Cl should be investigated, so as to make the standard suitable for IC/HPLC-ICPMS studies.

Slovenia

The toxicity of chromium depends primarily on its chemical form. Trivalent chromium compounds are much less toxic than those of hexavalent chromium. Due to its high toxicity hexavalent chromium is under environmental scrutiny. Concern about the presence of hexavalent chromium in the environment resulted in the development of numerous analytical techniques for the determination of Cr(VI) in different sample matrices. Among them spectrophotometry[3-8] has been widely used for the determination of Cr(VI). However, the technique is liable to various interference effects, e.g. the presence of coloured species, turbidity and colloidal particles. Therefore, its use should be critically evaluated for the particular sample matrix analysed. Selective extraction of Cr(VI)-HCl complex at 277 K into methyl isobutyl ketone (MIBK) and determination of the Cr(VI) in the organic phase by flame AAS was found to be a sensitive technique for the determination of hexavalent Cr in the presence of large amounts of Cr(III). However, the technique cannot be applied if the sample matrix forms a gel with MIBK. Alkaline extraction (sodium carbonate/sodium hydroxide) was also frequently used for determination of Cr(VI) in environmental samples. Applying this technique, soluble Cr(III) could be oxidised during extraction resulting in positive errors for Cr(VI). Ultrasonic extraction in alkaline solutions was reported to provide good extraction efficiency of Cr(VI) from workplace samples and allowed the retention of Cr(VI) on an anion-exchange resin. For speciation of Cr in natural waters, selective pH-dependent preconcentration of Cr(VI) and Cr(III) was performed on high surface area adsorbents of Al_2O_3 and TiO_2, followed by ETAAS determination of the separated Cr species.Thermal lens spectrophotometric detection and on-line thermal lens spectrophotometric detection after separation by ion chromatography[15] were found to be selective sensitive techniques for determination of hexavalent and trivalent Cr in various environmental samples at low ng cm^{-3} concentration levels. In our group anion exchange fast protein liquid chromatography (FPLC) with AAS detection has been developed for the simultaneous determination of Cr(III) complexes and Cr(VI). The procedure was applied to determination of Cr species in the sap of cabbage plants exposed to various concentrations of chromate and Cr(III)-EDTA. As an alternative to FPLC columns containing ion-exchange resins, ion-exchange separation supports based on convective interaction media (CIM) were developed recently. The matrix supports of poly(glycidylmethacrylate-co-ethyleneglycoldimethacrylate) substituted with strong or weak cation and anion exchangers offer very fast separation of biomolecules and are also used for fast separation of organic acids. In our group CIM discs were successfully applied in the speciation of zinc in environmental samples.

Applicability of an anion-exchange FPLC - ETAAS procedure for the determination of trace amounts of Cr(VI) in environmental samples e.g. natural waters, chromium enriched sewage sludge was investigated. Oxidation-reduction processes of Cr in highly alkaline samples of lime-treated sewage sludge were studied.

In addition, selective coprecipitation (SC) of As(III) and total As combined with FI-HGAFS or INAA was used for arsenic speciation in five mineral water samples from Slovenia. Extraction of arsenic compounds followed by HPLC-HGAFS was used for arsenic speciation in food samples (hen eggs) and in lichen samples from biomonitoring survey in Portugal.

CONCLUSIONS

Major achievements of the CRP group in the field of speciation analysis can be summarized as follows:

— A cost effective hydride generation module for AAS determination of As species was developed and is ready for being distributed to other laboratories.

— Several low-tech speciation methods for As, Cr, and Se were developed that can be easily adopted by laboratories without access of ICP-MS and other expensive equipment.

— Several attempts to prepare intercomparison materials for quality control in speciation work for As have been made (freeze dried urine, Chinese traditional medicine) but these materials could not yet be evaluated as of lack of time.

— Particularly in Cr speciation an obvious lack of suitable RMs for quality control purposes was identified.

— For Se speciation the large number of possible organically bound Se species should be considered in future work. Inorganic Se species seem to be of minor importance.

As a general conclusion the participants stressed the growing need to improve speciation analysis capabilities in Member States and to continue the support for enhancing awareness and to improve capabilities in Member States laboratories for speciation analysis.

The necessity of producing certified reference materials for elemental species as an important tool for method development and validation is emphasized. Still, it is recognized that radiotracer techniques are superior for method validation, despite their limited general availability and high costs.

BACKGROUND INFORMATION ANALYSIS

ARSENIC

Arsenic is an element that has been extensively studied because it raises much concern from both environmental and human health aspects. It is a ubiquitous element in the environment, originating from natural sources (geological formations, geothermal and volcanic activity), as well as human activities. Major anthropogenic sources of arsenic include wood preservatives (chromate copper arsenate), chemicals used in agriculture (monosodium methanearsonate, as a pesticide and disodium methanearsonate, as a herbicide), industrial processes and products (electrophotography, catalysts, pyrotechnics, antifouling paints, pharmaceutical substances), mining and smelting. Due to its natural and anthropogenic occurrence, the entire population is exposed to arsenic through food, water and air.

Nowadays it is well known that the toxicity of arsenic depends strongly on its speciation, because different species exert diverse toxicological and biological effects on animals and humans.

Water

The total ingested arsenic from food and water is estimated to be 900 μg per day. The World Health Organization (WHO) established the provisional tolerable weekly intake of inorganic arsenic via food and water to be 15 μg kg-1 body weight (equivalent to 150 μg per day for a 70 kg person). Arsenic in dietary products is mainly present as organo-arsenic

compounds which are (much) less toxic than the inorganic ones [1]. Drinking water, including natural mineral water, may contain high inorganic arsenic concentrations (no organo-arsenic is reported), depending on geographical area. Some Asian, Mexican and South American populations are exposed to arsenic in drinking water generally at or above several hundred micrograms per liter. An increased risk of skin, bladder and lung cancer is reported [2]. As an example, in Bangladesh 97% of the population currently consumes drinking water supplied from groundwater that contains As at ppm levels (1-2500 ug/L). In Table 1 some orientation values on arsenic toxicity are given.

TABLE I. TOXICITY OF SOME ARSENIC COMPOUNDS FOR HUMANS [3]

Compound	Dose	Effect
AsH_3	25 ppm, 30 min	lowest conc. in air which can cause death (LCL_0)
As_2O_3	1.5 mg kg^{-1} body weight	dose which causes death at 50% of subjects (LD_{50})
As_2O_5	5 mg kg^{-1} body weight	lowest dose which can cause death (LDL_0)
MMAA	50 mg kg^{-1} body weight	lowest dose which can cause death (LDL_0)
DMAA	500 mg kg^{-1} body weight	lowest dose which can cause death (LDL_0)

Recently the European Union lowered the maximum contaminant level (MCL) for total arsenic in drinking water from 50 to 10 μg l^{-1}. Risk assessment shows that, at this new standard, the mean risk of the exposed population developing bladder and lung cancer is 1.2-1.47×10^{-4} and 1.21-1.46×10^{-4}, respectively [2].

Although the above mentioned guidelines do not distinguish between the toxic effects of the different arsenic species, it is important to note that As(III) is more toxic than As(V) [4]. In this context, it is necessary to develop sensitive and reliable methods to identify and quantify inorganic arsenic species in water.

For this purpose, different approaches based on a separation step and a specific detector for detection were evaluated. Instrumental methods based on HPLC separation on an ion exchange column combined with HG and plasma based techniques [5], AAS or AFS [6, 7] detection have been extensively used in research studies. Although the organoarsenic species monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) have been determined as well, in practice these compounds are normally not found in drinking water, including natural mineral water. Detection limits of As species are generally on the sub-μg l^{-1} level.

First methods used for speciation analysis of As involved laborious wet chemical separation of the species based on selective preconcentration procedures (extraction, coprecipitation, flotation, etc.) followed by off-line detection of the preconcentrated arsenic species. Some of them are still used due to their simplicity, low cost, possibility of field sampling and no requirement for sophisticated instrumentation. Irgolic [8] reviewed methods for the determination of total arsenic and arsenic compounds in drinking water, including the mentioned selective preconcentration procedures. Although the detection limits in these procedures may be lowered by increasing the sample volume for preconcentration, practical detection limits are of the same order of magnitude as for the instrumental methods.

Biological samples

In living organisms arsenic can be found in various compounds ranging from inorganic arsenite (As(III)) and arsenate (As(V)) to methylated compounds like monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), trimethylarsine oxide (TMAO), arsenobetaine (AsB), tetramethylarsonium ion (TETRA), arsenocholine (AsC), arsenosugars and arsenolipids [9]. Analytical methods for arsenic speciation in biological tissues normally include a previous extraction step, and in some cases an additional extraction step is necessary to remove lipids [10]. The aqueous extracts undergo separation of the species, mainly by HPLC or capillary electrophoresis and subsequent detection using an element specific technique such as FAAS, AFS, ETAAS, ICP-OES, ICP-MS, electrospray MS. In many cases hydride generation techniques (HG) are used to reach the levels at which this species is found in biological matrices. If HG is used, non-hydride forming arsenic compounds (AsB, TMAO, TETRA, AsC, arsenosugars) need to be decomposed to inorganic forms to allow arsine formation. Usually this is achieved by subjecting the column effluent to in-line UV [11] or MW [12] digestion or, less often, by thermo-oxidation [13].

Arsenic species distribution in marine ecosystems is a permanent subject of study. Inorganic arsenic, present at very low concentrations in seawater, is taken up by various organisms and methylated via various mechanisms [9, 14]. In algae inorganic arsenic and arsenosugars prevail, and in marine animals the most abundant and stable arsenic compound is AsB. On the other hand, not much is known about arsenic species in terrestrial and freshwater systems. Byrne et al. [15] reported that AsB is the main arsenic compound in some mushrooms (Agaricus haemorrhoidarius, Agaricus placomyces and Sarcodon imbricatum). Arsenic speciation in terrestrial plants was recently reviewed by Dembitsky and Rezanka [16].

SELENIUM

Selenium is an essential mineral constituent of general body proteins. About 18 mammal proteins containing seleno amino acids have been identified. The most common are seleno methionine and seleno cysteine. The body produces the antioxidant Se compound glutathion peroxidase (p-GSH-Px), which plays an important role as a protective agent against lipid peroxidation and prevents cell damage. It is also essential for the normal functioning of the immune system and thyroid gland. Lower incidences of different cancer anomalies have been attributed to high Se intake levels. In human serum, the fractions p-GSH-Px and seleno proteins contain mainly Se-cystein in their active sites. In Se, albumin is unspecifically incorporated as selenomethionine [17, 18]. Studies in human nutrition demonstrated that the absorption of Se-methionine from foods is >95%, while that of selenate is >90% and selenite is 60%. The recovery of those species in faeces and urine was 82-95% of the inorganic Se and 26% for Se-methionine [18]. This behaviour was associated with other factors, such as other dietary components, the selenium status of the body, age, etc.

In the environment, selenium thresholds in waters, sediments, diets from essentiality to toxicity for fish and birds are very narrow. Also, the threshold concentrations should not be considered safe concentrations once Se presents bioaccumulative effects. There is a controversy among Se thresholds in the environment [19]. The concentrations of dissolved inorganic Se species in lakes, rivers and oceanic waters [20] are affected by temporal variations depending on the algal activities producing Se-organic compounds. Mortality of fish in lakes and rivers was attributed to high Se concentrations and birds' eggs were analyzed to define the Se threshold in the food chain [19]. Plants could be classified as non-accumulators (presenting less than 100 μg/g), accumulators (Se from 100 to 1000 μg/g) and

hyper-accumulators (more than 1000 μg/g). Several Se species had been found in accumulator plants, such as *Brassica juncea* (Indian mustard) [21], *Allium cepa* L. (onion) [22], *Allium fistulosum* (green onion) [23] and Brazilian nuts [24,25]. Depending on the source of Se, *Brassica juncea* incorporated the selenium into different organic species [21]. The species found in *Brassica juncea* were Se methionine and Se-methyl-selenomethionine, while in sprouts of several plants Sugihara and co-workers [26] found Se methyl-selenocysteine, Se-methionine and γ-glutamyl-Se-methylselenocysteine. The anticancer Se effect of some vegetables is attributed to the metabolic conversions producing methylated forms of seleno amino acids.

Products from animals fed with Se supplements and industrial meals enriched with Se are other common sources of Se for humans. Yeast (*Saccharomyces cerevisiae)* enriched with inorganic Se produces mainly Se-methionine [27-29], which is sold as food supplements in the market. The recommended daily dietary allowance is about 50 μg of Se. The U.S. Environment Protection Agency (EPA) has determined that selenium sulfide is carcinogenic, but it is not usually present in foods and in normal environmental conditions. The amount of selenium allowed in a public water system is 50 μg/ml. Chronic exposure to high levels of oral selenium results in selenosis, with typical symptoms like loss of hair and nail brittleness. It also has some deleterious effects on the neurological system and people excessively contaminated have a strong garlic odour.

Selenium deficiency results in the Keshan disease, with typical symptoms of an enlarged heart and poor heart function. This name derives from a province of China where soils are poor in selenium and dietary intake was found to be less than 19 μg. The typical biomarker tissues for Se are blood and urine.

The bioavailability and the protective action of the individual species is a research challenge. In addition to metabolic pathways of selenium, its role in mitigating arsenic and mercury poisoning also are being studied. Decontamination of the selenium-rich agricultural lands through bio-remediation also is an active field of research, which might critically depend on the selenium species that can be bio-accumulated by certain plant species.

Speciation analysis of Se

The identification of seleno compounds in urine samples presents two main challenges. One is concerned with the species preservation during the sample treatment and the other with the need for various separation systems due to the sample complexity [30]. According to Uden et al. [31], Se speciation is vital to reveal its significance in clinical chemistry, biology, toxicology and nutrition.

Selenium interactions in the environment are largely regulated by interactions with plants, soil, bacteria and redox conditions. The aqueous reduction of selenate is slow, but under action of microbial organisms, the catalytic reduction of selenate and selenite to elemental Se is accelerated [32]. Organic selenium species (selenocysteine, selenomethionine and trimethylselenium ions) in aqueous solutions at pH 4.5, at temperatures ranging from 4 to 20˚C (in dark conditions), remained stable for 1 year, when stored in Pyrex or Teflon containers [33]. For inorganic selenium species the stability is strongly affected by the pH, presence of chloride, light, temperature and type of container. The loss of selenite solutions (10 to 50 ng/ml) in polyethylene containers at pH 2 was observed after 1 month and was completed after 1 year. Meanwhile at pH 6 the maximum storage without risk was 2 months.

The presence of chloride enhanced the stability of solutions of selenite and selenate. Storing solutions at –20˚C was considered free from risk of Se losses during 1 year [34].

Besides stability problems, there are several drawbacks to performing selenium speciation. Quantification of some species is hindered, since only a few species are commercially available. Another reason is the difficulty involved in producing a reference material. A freshwater solution spiked with inorganic selenate and selenite proposed as a reference material [35] failed, once the original ratio was affected by instability. A candidate laboratory reference material for Se speciation based on Brazil nuts was prepared [36]. After the nuts were deshelled, they were peeled and rasped, and Soxhlet was extracted for lipid removal with cyclohexane. After eliminating the solvent, the powder was homogenized and sieved to <125 μm, packed, γ-irradiated and submitted to stability tests under temperatures –18˚C, +4˚C and 24˚C. The results of stability tests found no significant difference among selenomethionine content indicating stability after 3 months. The production of a reference material by enriching yeast with Se was discussed. The main drawback is the reproducible production under controlled fermentation of a selected yeast strain.

Sample preparation for Se speciation

The solubilization of Se compounds in solid samples could be partial or total. The water-soluble Se species were described as being around 10 to 15%. Separation of proteins could be accomplished by size exclusion chromatography (SEC). In the case of nuts, the lipid fraction could be removed using Soxhlet extraction with cyclohexane [36]. A mixture of proteolytic enzymes dissolved the Se species which were bounded to proteins. These extracts were centrifuged and the supernatant was filtered (0.2 μm) and preserved in the freezer at –20˚C in order to avoid species transformations [37]. Methanesulfonic acid was used for protein hydrolysis in yeast and nuts samples to release Se-methionine. However, the results were higher than those obtained by proteolytic enzymes [40].

Techniques used for Se speciation

Several analytical procedures had been proposed for selenium speciation. In general, the most successful are those combining separation techniques with a powerful detection technique. Separation of the organic and inorganic selenium species was performed by ion chromatography (IC) [38, 39], high performance liquid chromatography (HPLC) [22-26, 33, 41-52], liquid chromatography (LC) [30, 43] and capillary electrophoresis (CE) [45-50]. Some applications described the on-column UV detection followed by the post-column derivation using hydride generation combined with atomic fluorescence spectrometry (AFS) [43, 44]. Inductively coupled plasma optical emission spectrometers (ICP-OES) [44] or mass spectrometers (ICP-MS) [22-29], and microwave induced plasmas with mass spectrometers MIP-MS [41] are powerful detectors. The on-line detection of the Se content in separated species produces transient signals. The derivation procedure, after column generating volatile hydrides, increases the transport efficiency to the plasma and the limits of detection.

The combined system using ion chromatography (IC) and ICP-OES [39] applied to bacteria cell extracts allowed the separation of Se methionine and the inorganic Se(IV) and Se(VI), but was unable to separate Se-cystine from Se-cysteine. Also, detection limits in mg/L were 0.4 for Se-Met, 0.5 and 0.7 for Se(IV) and Se(VI) respectively. The IC separation of inorganic Se(IV), Se(VI) and SeCN in petroleum refinery waste water was successfully attained. The species were pre-reduced with HBr in a heating loop before generating the hydrides. Detection limits close to 30 ng/L were attained for all the species by atomic

fluorescence (AFS). The separation using HPLC techniques (ion-pairing and anion exchange) is the most frequently cited for Se speciation in yeast and nuts.

Isotope dilution ICP-MS for Se speciation

The quantification procedure based on isotope dilution (ID) presents several options when applied to speciation analysis. ID considers spiking the sample with the enriched isotope, and after attaining the isotopic equilibrium the isotope ratio of the product is measured. The concentrations are calculated performing a mass balance between the sample and spike mass and isotope abundances and using the experimentally obtained isotope ratio measurement. The accurate quantification depends on the accuracy of sample preparation, the accurate mass of the spike and the precision of the isotope ratio measurements.

The post column isotope dilution consists of spiking the separated species with Se, which allows the quantification of the Se content in each separated species. Spikes can be added on-line. Applications were described for Se species from serum samples [51] and cod muscle [52].

The synthesis of the isotopically enriched Se species of selenite, selenate and trimethyl selenium [52] made it possible to perform speciated-isotope dilution. In this case the quantification is based on the original species and not on the inorganic Se content only. The potential and limits of speciated isotope dilution by using ICP-MS are explained well elsewhere [53]. The main advantage is that the species is quantified and also the interconversion of species is easily detected by the isotope ratios. The addition of the isotopically enriched specie to aqueous samples during the sample collection is also a tool for monitoring species transformations and in situ quantification [55, 56]. The previous synthesis of the enriched species is necessary. Then some authors added the spike to the onion or garlic sample while it was growing, or in yeast before fermentation, and in this way induced the biosynthesis of labeled species.

CHROMIUM

Chromium and its compounds have a very widespread use in a range of industries and applications, including metallurgical applications (mainly as alloys) and as components in arc welding, leather tanning (where it is used to denature proteins in the skins). Consequently, there are many potential exposure routes to man and the potential detrimental health effects of chromium species are of concern. The relevant oxidation states of chromium are Cr(0), Cr(III) and Cr(VI). The different oxidation states of Cr exhibit different levels of toxicity. Cr (VI) also has the potential to be carcinogenic.

Occupational exposure to Cr species is of concern since they have been linked to respiratory, renal and dermatological disorders. One of the earliest known symptoms of occupational exposure, reported at the end of the 19^{th} century, was observed in workers processing chromate for pigments. It was detectable as perforations of the nasal septum, which developed into sino-nasal cancer. Exposure to Cr (VI) in aerosols produced during the production of ferrochromium and stainless steel and in welding fumes are of particular concern since the species can easily enter the body via the respiratory tract.

The systematic monitoring of Cr(VI) has been imposed by the European Commission, the USA, etc.

In occupational health, the exposure limit Occupational Exposure Limit (OEL) in the air of the workplace of the soluble and insoluble compounds in water is: 0.5 mg/m^3 Cr, 0.5 mg/m^3 Cr(III) and 0.05 mg/m^3 Cr(VI). These OELs clearly reflect the difference in toxicity of the Cr-species.

The concentration of the various chromium species released into the environment needs to be monitored. There are many potential sources of pollution, which may be of concern. For example, effluent from tannery wastes may be directed into the sewage processing system, where they are amended with clean soil, as part of the waste treatment process, and ultimately applied to agricultural land, making it possible for Cr compounds to enter the food chain. In some countries, such wastes are discharged directly into the aquatic system with the consequence of the localised destruction of fauna and flora. In the developed world, a great effort is being made to reduce the chromium content in the waste stream by reclaiming the element, so that it can be recycled. This is driven by environmental and financial concerns.

Cr mobility in soils: Cr(VI) species are more mobile than the Cr(III) species in soils, and furthermore also behave differently. While the sorption of Cr(VI) decreases with increasing pH, for Cr(III) the opposite is the case, although this may depend on the pH range considered! In the environment, the major species of hexavalent Cr are $HCrO_4$- and CrO_4^{2-}. Dichromate species are only present at very high concentrations and/or at low pH. As Chromium (VI) is a strong oxidizing agent, it reacts with a wide range of reducing compounds in the environment to form Cr(III). The oxidation of Cr(III) to Cr(VI) is theoretically possible. However, as it requires a very strong oxidant, it is unlikely to occur under environmental conditions. It is thus expected that the majority of the chromium released into the environment is converted into Cr(III), although this will not necessarily occur very quickly in all cases.

Due to the different solubilities of Cr compounds, various LD_{50} values have been reported. They are summarised in the following table:

TABLE II: LD50 VALUES FOR CR COMPOUNDS AND DIFFERENT UPTAKE ROUTES

Compound	LC_{50} for inhalation	Oral LD_{50}	Dermal LD_{50}
$K_2Cr_2O_7$, $Na_2Cr_2O_7$, $(NH_4)_2Cr_2O_7$	99 (35) 200 (70) 200 (83) mg compound/m3 (mg Cr/m3)	74 (26) 59 (23) 55 (23) mg compound/kg, (mg Cr/kg)	960 (380) 1150 (410) 1860 (770) mg compound/kg, (mg Cr/kg)
$NaCrO_4$	104 (35) mg compound/m^3, (mg Cr/m^3)	87 mg/kg, corresponding to 28 mg Cr/kg	1330 (430) mg compound/kg, (to mg Cr/kg)
CrO_3	217 (113) mg compound/m^3, (mg Cr/m^3)	52-113 mg compund/kg, corresponding to 27-59 mg Cr/kg	57 (30) mg compound/kg, (mg Cr/kg) *check !* *very strange !*
$CrCl_3$		1870 mg/kg body weight or diet (?) *(to be checked !)*	

(Source: SIAM 14, 26-28 March 2002: SIDS INITIAL ASSESSMENT PROFILE, http://www.jetoc.or.jp/HP_SIDS/pdffiles/Chromates.pdf)

Chromium compounds found at trace levels in most foodstuffs are probably endogenous components, rather than a result of contamination. Indeed, no evidence has ever been reported of deleterious effects arising from external exposure to Cr (III) from foodstuffs.

Some research has suggested that chromium may be an essential trace element and that it could play a role in regulating insulin.

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ARSENIC SPECIATION ANALYSIS

ARSENIC SPECIATION USING HPLC-HGAFS OR SELECTIVE COPRECIPITATION COMBINED WITH FI-HGAFS OR INAA

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Abstract Selective coprecipitation (SC) of As(III) and total As combined with FI-HGAFS or INAA was used for arsenic speciation in five mineral water samples from Slovenia. Extraction of arsenic compounds followed by HPLC-HGAFS was used for arsenic speciation in food samples (hen eggs) and in lichen samples from biomonitoring survey in Portugal.

INTRODUCTION

Any detailed and realistic study of environmental, metabolic, toxicological or other properties of arsenic requires differentiation between its individual compounds, so-called speciation. High performance liquid chromatography (HPLC) interfaced with element-specific detection is the main technique for high-resolution determination [1]. High-sensitivity detectors in use are inductively coupled plasma/mass spectrometry (ICP/MS) [2], quartz furnace atomic absorption spectrometry (QFAAS) [3] and atomic fluorescence spectrometry (AFS) [4,5]. The last two detectors require on-line decomposition (UV or MW) and volatilisation (hydride generation (HG)) of the compounds separated.

The objectives of this work were to determine arsenic species and their content in different matrices namely, water, foodstuff and environmental samples. It includes i) the separation of inorganic arsenic in some bottled Slovene mineral waters using HPLC-HG-AFS and selective coprecipitation combined with FI-HG-AFS (6), ii) to study the effect of arsenic trioxide on metallothionein and its conversion to different arsenic metabolites in hen liver (7), iii) speciation analysis of As in lichen samples (8), iv) the study of the stability of arsenic compounds in water and urine samples and v) underestimation of the total arsenic concentration by hydride generation techniques as a consequence of the incomplete mineralization of arsenobetaine in acid digestion procedures (9). The short overview of analytical techniques used in all this studies is given in this document.

METHODS AND MATERIALS

Reagents and standards

All chemicals were at least of analytical grade. Millipore. Milli-Q Plus water (18.2 MΩ cm) was used for all solution preparations. As(III), As(V) and DMAA were purchased from Merck (Darmstadt, Germany), AsB from BCR (CRM 626, arsenobetaine solution) and MMAA, TMAO, AsC and TETRA-iodide were prepared in Karl-Franzens University Graz, Austria. Stock solutions of the arsenic compounds containing about 1000 mg L^{-1} arsenic were prepared in water and kept at 4°C. The exact concentration of arsenic in the stock solutions was determined by instrumental neutron activation analysis (INAA). Working solutions with arsenic concentrations of 5-50 ng mL^{-1} were prepared fresh daily. Dibenzyldithiocarbamic acid, sodium

salt, 97% was prepared in methanol (1% m/v final solution) andkept at 4°C and prepared fresh weekly. A 1.5% m/v solution of $NaBH_4$ was prepared in 0.1% m/v sodium hydroxide fresh daily.

Total arsenic determination

Total arsenic in various solid or liquid samples was determined by radiochemical neutron activation analysis (RNAA), employing mineralization of the neutron irradiated sample with a mixture of HNO_3 and H_2SO_4 followed by addition of H_2O_2 to complete the destruction, boiling, addition of KI, extraction of arsenic tri-iodide into toluene and measurement of the ^{76}As activity at 559 keV (10). Alternatively, instrumental neutron activation (INAA) was applied for total arsenic determination on filters after preconcentration of traces of arsenic from water samples.

HPLC-HGAFS for trace determination of As(III) and As(V) in water

A portion of water was acidified to a pH of 2±0.2 with 4 mol L^{-1} HCl. A system set-up as shown in Figure 1 was used for inorganic arsenic speciation. As(III) and As(V) were separated using an HPLC pump fitted with a Hamilton PRP-X100 anion exchange column (250 × 4.1 mm) and a guard column of the same material. As a mobile phase 50 mmol L^{-1} KH_2PO_4 solution (pH adjusted to 6.1). The separated As species underwent hydride generation by reaction with HCl and $NaBH_4$ in a PEEK mixing cross yielding hydrogen and arsine; both reagents were added by a peristaltic pump. The gas-liquid mixture was delivered to an "A" type gas-liquid separator (PS Analytical, Orpington, UK), and the arsine generated was swept from the separator with a flow of argon. AsH_3 was on-line dried in a "Perma-Pure" mini-dryer and measured with an AFS detector. The experimental details are similar to that for biological samples and can be found in Table 1.

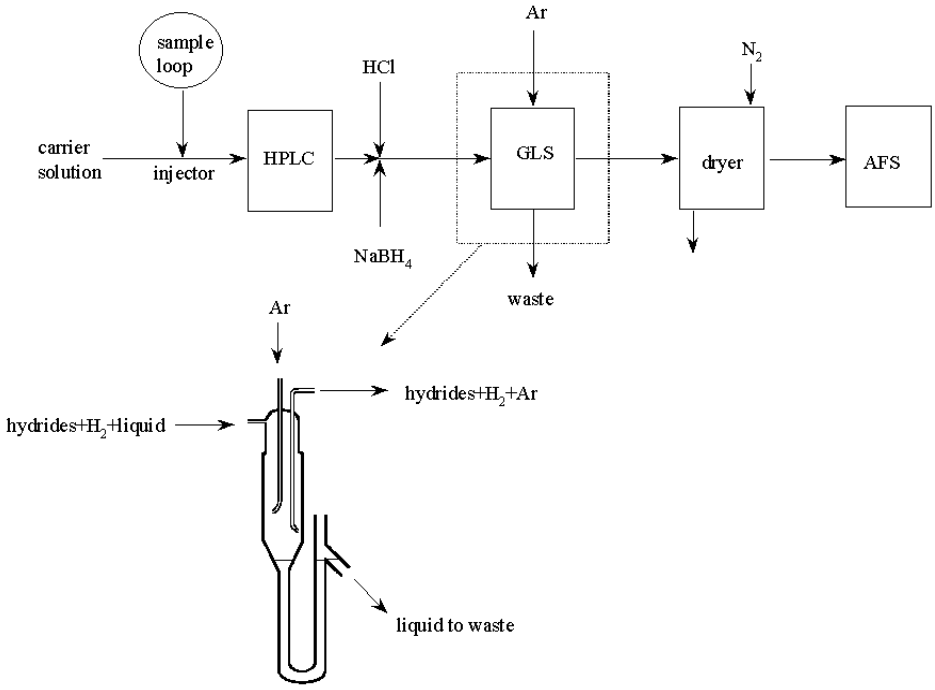

FIG. 1. Schematic diagram of the HPLC-HGAFS system with details of the gas-liquid separator (GLS).

Selective coprecipitation combined with FI-HGAFS for ultratrace determination of As(III) and Ast in water

Immediately after sampling water was subjected to a selective coprecipitation procedure for separation of As(III) and As_t: two 100-mL portions were weighed and one portion underwent the procedure for As(III) determination and the other portion underwent a procedure for As_t determination. For As(III) determination, sample was acidified with 4 mol L^{-1} HCl to a pH of 2±0.2 and 1 ml 1% m/v DBDTC (in methanol) was added. The turbid solution was gently swirled for ca. 1 min before passing through a 0.45-μm Millipore Durapore® HVLP membrane filter under reduced pressure with a vacuum filtration system (1-l unit, Schott, Germany). For total As determination the water sample was acidified to a pH of 2±0.2 as well, followed by reduction of As(V) to the trivalent state by addition of 1 ml of 20% m/v KI and 1 ml of 25% m/v potassiumthiosulphate pentahydrate. Reduction was complete in 15-20 min after which the total inorganic arsenic could be coprecipitated as As(III). The filters with coprecipitated arsenic were folded and stored in 10-mL vials with screw cap at room temperature prior to further treatment. Total arsenic amount on filters can be either determined by INAA or after releasing from filters. Arsenic can be released by addition of 4 mL of 0.01 mol L^{-1} NaOH and 1 mL of 30% H_2O_2 to the vials containing the filters, followed by heating of the vials in a water bath at 85ºC for 30 min. Keeping the leached filters for several weeks in their vials at room temperature no change in As concentration was observed. A FI set-up, *i.e.*, the same instrumental configuration as above with exclusion of the chromatographic column, was used for determination of As(III) and total As. Measurements were carried out against a calibration curve with As(V) in the presence of 8 mmol L^{-1} NaOH and 6% H_2O_2.

Preparation of biological samples

Biological samples (animal tissue, plants, food, etc.) have to be homogenized and weighed into ampoules for total arsenic determination and into extraction vials for arsenic speciation. Care should be taken to avoid changes in the speciation pattern, i.e. not allowing enzymatic or microbial activity in the samples. Samples should to be kept freeze dried or frozen at -18˚C till analysis. Urine samples only need to be diluted and filtered prior to the analysis.

Preparation of the extracts

To 3-4 g of fresh sample or about 300 mg of a dry sample, 30 mL of a mixture of methanol and water (9+1) was added. Samples were shaken overnight at room temperature. After shaking, the samples were centrifuged at 3000 rpm (15 min), decanted and the pellets washed with another 10 mL of methanol/water mixture using short shaking (30 min) followed by centrifugation. The two extracts were pooled and evaporated to dryness using a rotary evaporator. To the dry residue, 5 mL of water was added, the solution filtered through a 0.45 µm membrane filter (Millipore Millex HV hydrophilic PVDF) and kept at 4˚C till speciation analysis.

TABLE I. EXPERIMENTAL CONDITIONS FOR ARSENIC SPECIATION WITH THE HPLC-(UV)- HG-AFS SYSTEM.

HPLC	
Anion exchange:	
Column	Hamilton PRP-X100, 250×4.1 mm
Mobile phase	KH_2PO_4 solution, 15 mmol L^{-1}, pH 6.1 (NH_4OH), 1 mL min^{-1}
Cation exchange:	
Column	Alltech Adsorbosphere SCX 5U, 250×4.6 mm
Mobile phase	pyridine, 2.5 mmol L^{-1}, pH 2.65 (HCl), 1 mL min^{-1}
On-line UV-reactor	
Ultraviolet lamp	8 W (Camag), 254 nm
Digestion coil	FEP Teflon tubing (3.1 m, 0.5 mm i.d.)
$K_2S_2O_8$	3% (m/v) in 3% (m/v) NaOH, 1.35 mL min^{-1}
Hydride generation	
HCl	4.4 mol L^{-1}, 3.0 mL min^{-1}
$NaBH_4$	1.5% (m/v) in 0.1% (m/v) NaOH, 3 mL min^{-1}
AFS	
Detector	Excalibur (PS Analytical, Kent, UK)
Lamp	Arsenic, 189.04, 193.76 and 197.26 nm (Photron Pty. Ltd., Superlamp 803S)
Primary current	27.5 mA
Boost current	35 mA

Speciation analysis using HPLC-(UV)-HGAFS

A previously developed HPLC-(UV)-HGAFS system was used for determination of As compounds in extracts. Anion and cation exchange columns with (cation exchange) or without (anion exchange) on-line UV decomposition were used to separate As species prior to HG and AFS. Experimental details are given in Table 1 and the chromatograms of standards are shown in Figures 2 and 3. As can be seen from the chromatograms, baseline separation of eight arsenic compounds can be achieved using a combination of anion and cation exchange chromatography.

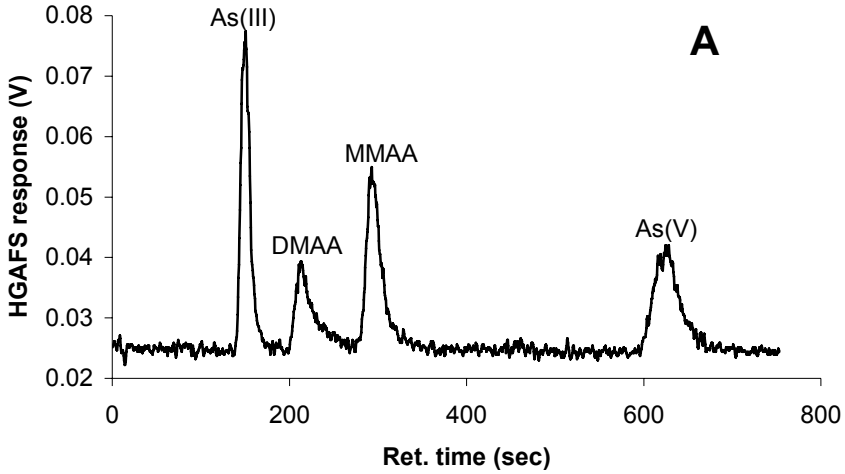

FIG. 2. Anion exchange separation of eight As compounds (10 ng g^{-1} each), without UV decomposition. Cationic compounds (TMAO, AsB, AsC and TETRA) coelute with As(III) but are not visible since they do not form hydrides.

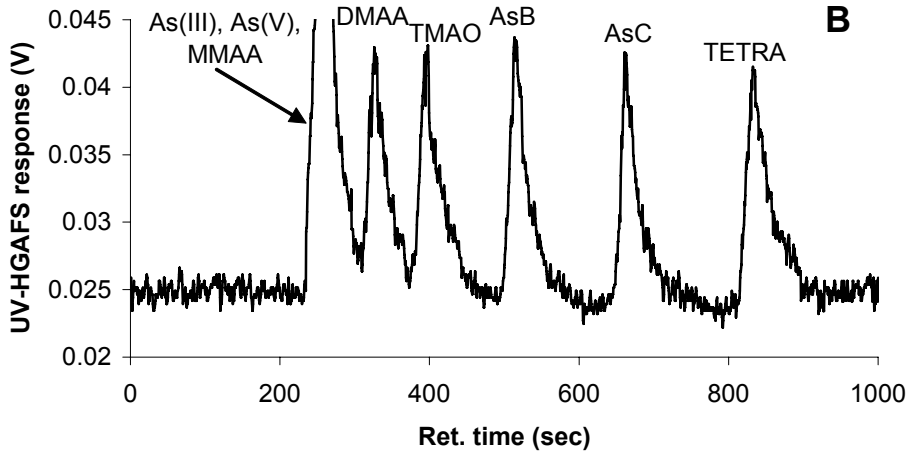

FIG. 3. Cation exchange separation of eight arsenic compounds (10 ng g^{-1} each), with on- line UV decomposition. As(V) and MMAA elute in or close to the void volume together with As(III).

RESULTS AND DISCUSSION

Both, selective coprecipitation (SC) and HPLC-HGAFS methods were used for inorganic arsenic speciation in five different bottled Slovene mineral waters (6) with HPLC-HGAFS going down to 1 μg l^{-1} levels of As(III) and As(V) and SC/FI-HGAFS going down to 0.05 μg l^{-1} levels of As(III) and As_t. For both methods no As(III) was detectable whereas SC/FI-HGAFS was able to detect As_t, thus As(V), in all mineral waters whereas HPLC-HGAFS was able to detect As(V) in the Rogaška mineral waters only (Table 2). Direct comparison of the data for these Rogaška mineral waters shows that both methods are not significantly different at the 5% level (6).

Since the arsenic (As(V)) concentration in Rogaška, Donat Mg was high - above the current maximum limit for arsenic in natural mineral waters of 50 μg l^{-1} - additional bottles of Rogaška, Donat Mg mineral water with different production dates were analyzed with HPLC-HGAFS to find out whether this high concentration was exceptional or not. The results show that the arsenic (As(V)) concentration as a function of production date varied close to the maximum limit; the average arsenic (As(V)) concentration in the bottles with production dates from April to August 2000 was 47.8±11.2 μg l^{-1} (ξ±sd; n=6). The producer is aware of the potential problem and carries out an arsenic monitoring program.

TABLE II. COMPARISON OF TWO INDEPENDENT SPECIATION TECHNIQUES FOR INORGANIC ARSENIC SPECIATION IN MINERAL WATERS SPECIFIED IN TABLE 1. AVERAGE CONCENTRATIONS (ξ±SD; N=3) ARE GIVEN

Type	HPLC-HGAFS		SC/FI-HGAFS	
	[As(III)] (μg l^{-1})	[As(V)] (μg l^{-1})	[As(III)] (μg l^{-1})	[As_t] (μg l^{-1})
Radenska, vrelec Radin	<1*	<1*	<0.05**	0.17±0.04
Radenska, vrelec Miral	<1*	<1*	<0.05**	0.24±0.01
Radenska, Kraljevi Vrelec	<1*	<1*	<0.05**	0.05±0.01
Rogaška, Tempel	<1*	5.5±0.6	<0.05**	5.5±0.5
Rogaška, Donat Mg	<1*	60.6±2.0	<0.05**	63.9±3.8

*, DL for HPLC-HGAFS
**, DL for SC/FI-HGAFS

In the second part we studied the As species in eggs from hens fed with a diet containing 7.5 and 30 μg As_2O_3 /g. The only As species found in water methanol extracts from egg yolk and white samples was dimethylarsinic acid (DMAA). In all cases, 85-100% As was present in he form of DMAA in the extract. The presence of As (III), As(V), or MAA was not detected under the described optimal conditions of extraction and separation. We did not find cationic arsenic species. It can be concluded that the liver is not the only site of inorganic As (III) methylation in the organism.

In a second part of the project a total of 29 lichen samples (*Parmelia Sulcata* Taylor) was selected from wider biomonitoring survey in Portugal for detailed arsenic speciation. Samples relating to specific source factors were chosen for speciation in order to establish whether the type of source factor may be responsible for arsenic speciation. The extractability in water ranged from 1.9 to 32 % of the total arsenic (8). In all of the samples arsenate was detected, in many of them as the main arsenic compound; the second most widely found compound was arsenite followed by an unidentified cationic compound. Some of the samples also contained an unidentified anionic compound. So far it seems there is no relationship between the source factor and arsenic speciation but this is being further investigated.

CONCLUSIONS

A short overview of analytical techniques for arsenic speciation in water, foodstuffs and in environmental samples, which were analysed as a part of this project, is given. For water samples, HPLC-HGAFS with high resolution but limited sensitivity and a coprecipitation technique with excellent sensitivity, offer an opportunity to get an insight in arsenic speciation with high resolution and high sensitivity. For food and biological samples extraction of arsenic species followed by high resolution technique such as in this case HPLC-UV-HGAFS, is a method of choice.

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SELECTIVE HYDRIDE GENERATION OF INORGANIC ARSENIC SPECIES AND SUBSEQUENT DETERMINATION BY FLOW INJECTION-HYDRIDE GENERATION-ATOMIC ABSORPTION SPECTROMETRY (FI-HG-AAS)

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Abstract The effect of different reaction media on the selective generation of arsine from As(III) and As(V) using sodium tetrahydroborate(III) as a reductant was evaluated for the subsequent determination of As(III) and As(V) in natural waters by flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS). The effect of concomitant elements on arsine generation was also studied.

INTRODUCTION

Arsenic is an element that has been extensively studied because it raises much concern from both the environmental and human health aspects. Nowadays it is well known that the toxicity of arsenic depends strongly on its speciation because different species of arsenic exert diverse toxicological and biological effects in animal and human systems. Arsenite is ten times more toxic than arsenate and 70 times more toxic than the methylated species [1]. From this point of view it is important to develop analytical methodologies that can determine the concentrations of individual species of As in biological and environmental matrices in order to evaluate the risk associated with exposure to As compounds.

Numerous and diverse analytical methods of separation and detection have been proposed for speciation analysis of arsenic [2-5]. Each approach possesses both advantages and disadvantages that must be considered with respect to the scope of the study and also the laboratory facilities available. The selective reduction of As species is a reliable, rapid and low cost technique that is well suited to apply in routine analysis. Braman and coworkers [6] reported that the reduction of As compounds with sodium tetrahydroborate(III) was pH dependent and was related to pK_a of the individual species. Many research groups have used the pK_a differences of As(III) and As(V) in order to separate both species. Pahlavanpour and Thompson [7] investigated the possibility of applying a selective reduction and continuous hydride generation to also determine organic species of arsenic. However, there appeared to be discrepancies between the responses of some organic species in the media proposed by Hinners [8], Arbab-Zavar and Howard [4] and Pahlavanpour and Thompson [7]. Anderson et al [9] studied the use of pH, reaction media, chelating agents and redox agents for the determination of As(III), As(V), MMAA and DMAA using continuous hydride generation with sodium tetrahydroborate(III) as reductant and atomic absorption spectrometry (AAS) or inductively coupled plasma optical emission spectrometry (ICP OES) for detection.

In this context, it was deemed of interest to study the performance of different reaction media for the selective reduction of the more toxic arsenic species namely As(III) and As(V) and their subsequent determination by flow injection (FI)-AAS.

METHODS AND MATERIALS

Reagents

All reagents were of analytical reagent grade unless otherwise mentioned. Deionized water from Barnstead E-pure was used throughout. Commercially available 1000 mg L^{-1} As(III) and As(V) standard solution was used. Dilute working solutions were prepared daily by serial dilutions of this stock solution. A 3% (m/v) sodium tetrahydroborate solution was prepared by dissolving $NaBH_4$ powder in de-ionized water, stabilizing in 1% (m/v) NaOH and filtering through Whatman N 42 filter paper to eliminate turbidity. The solution was stored in a polyethylene flask at 4 ^{0}C. Diluted working solutions were prepared before use. All the solutions containing the acids studied were prepared at the required concentrations by dissolving appropriate amounts of each compound in de-ionized water or by dilution. The cation solutions used in the study of interferences were prepared from analytical-reagent chemicals.

Instrumentation

An automated HG-AAS system based on a PerkinElmer 4000 atomic absorption spectrometer equipped with a PerkinElmer MHS-20 mercury/hydride device was used for the hydride generation studies. Electrodeless discharge lamps (EDL, PerkinElmer) were the sources of radiation used for As determination

RESULTS AND DISCUSSION

3.1. Effect of different reaction media on arsenic response

The influence of hydrochloric, citric, mercaptoacetic and acetic acids on arsenic response from As(III) and As(V) are depicted in Figures 1-4. Arsenic hydride species were generated in all cases from a 500 μL sample containing 5 and 15 ng mL^{-1} of As(III) and As(V) respectively. The hydride generation conditions were as follows: $NaBH_4$: 0.2%; NaOH: 0.05%; KI and ascorbic acid: 0.5% each (for total As).

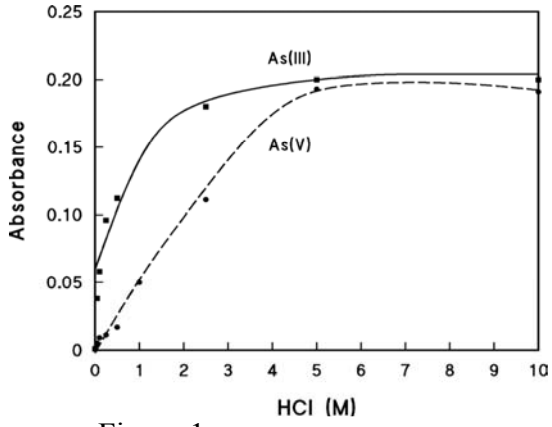

Figure 1

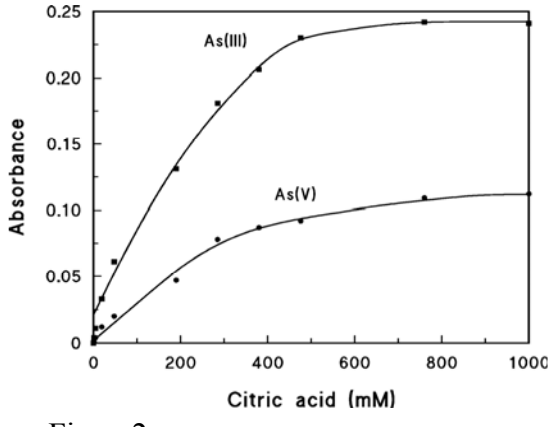

Figure2

FIG. 1. and FIG. 2. Effect of hydrochloric and citric acids on As(III) and As(V) response.

With HCl (Fig. 1) the response of As(III) and As(V) increased rapidly with higher concentrations of acid. Both species present similar and constant response at HCl concentrations higher than 5 M. It is important to remark that these results were obtained when a reaction coil of 1 m (between the mixing point and the gas-liquid separator) was used.

Citric acid was employed (Fig. 2) in order to assess its effect on As(III) and As(V) signal. The signal from As(V) was lower than that of As(III) and in these conditions it was not possible to determine selectively As(III) and As(V). The response produced by using mercaptoacetic acid (Fig. 3) evidences that both species reach a maximum (about 40 mM) and then the signal of As(III) and As(V) drop drastically.

The behavior obtained may be attributed to the presence of the sulphur containing ligands of the acid by producing a rapid hydride generation either through the formation of a more volatile arsine or by altering the reaction mechanism.

Figure 3 Figure 4

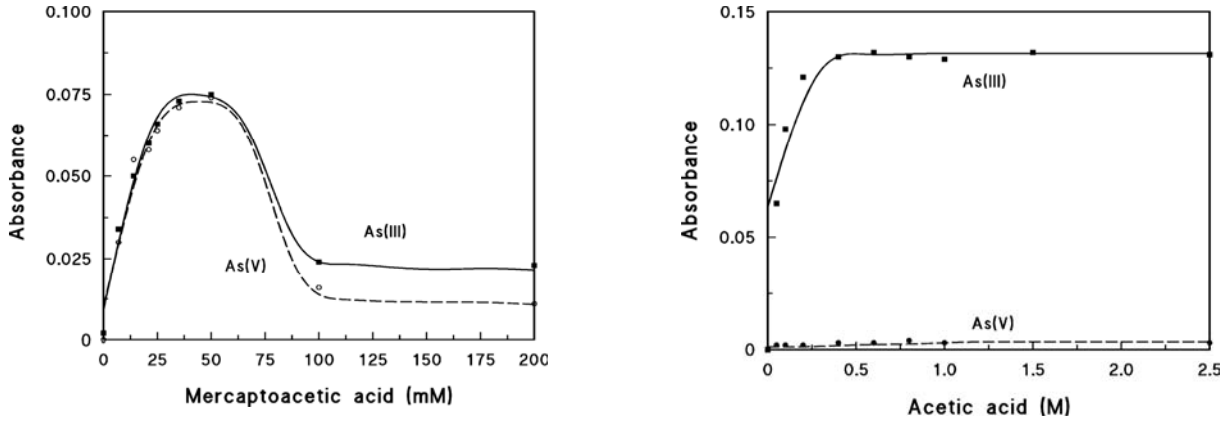

FIG. 3. and FIG. 4. Effect of mercaptoacetic and acetic acids on As(III) and As(V) response.

When acetic acid was tested (Fig. 4) it is clear evident that arsine was not generated from As(V). The response from As(III) increased rapidly with acid concentration and reached a plateau at acetic acid concentrations greater than 0.5 M.

From the analysis of the Figures 1-4 it is possible to conclude that total arsenic can be determined in 5 M HCl and 40 mM mercaptoacetic acid. Taking into account that a higher response is obtained in HCl, it was selected as the best alternative for the determination of total arsenic. On the other hand, 0.5 M acetic acid was selected for As(III) determination.

3.2. Interference study

In order to evaluate the selectivity of the method, the effect of transition metals namely, Cr, Cu, Co, Fe, Mn, Ni and Zn and other hydride forming elements (Bi, Pb, Sb, Se and Sn) on arsenic species (in hydrochloric and acetic acid) was tested. Solutions containing 5 and 15 ng mL^{-1} of As(III) and As(V) were spiked with 10 μg mL^{-1} of the potential interfering ions. This study showed that the method is highly selective. Only Ni and Zn produced a significant reduction of arsenic signal. Ni reduced 72% and 61% As(III) and As(V) signals respectively.

In the case of Zn the percentage of reduction was: 13 and 25 for As(III) and As(V) respectively.

3.3. Quality parameters

The acids selected for the analysis of inorganic arsenic speciation were also compared in terms of their analytical performance as a preliminary step to the selective determination of As in natural waters. Table I summarizes the quality parameters.

TABLE I. ANALYTICAL PERFORMANCE OF THE AS(III) AND AS(V) DETERMINATION BY FI-HG-AAS

Parameter	**As(III) (acetic acid)**	**Total As (hydrochloric acid)**
Working range	0.3 – 15 ng mL^{-1}	0.7 – 20 ng mL^{-1}
Sensitivity	0.0246 u. a./ppb	0.0233 u. a./ppb
Detection limit*	0.1 ng mL^{-1}	0.1 ng mL^{-1}
Precision*	4.0 % (for 5 ng mL^{-1})	1.9 % (for 15 ng mL^{-1})
Linearity (as R^2)	0.99576	0.9981

(n=10)

3.4. Analysis of real samples

Since no reference materials are available for As species in waters and to demonstrate the reliability of the method, a recovery test was carried out by adding known amounts of As(III) and As(V) to different categories of waters. Results are shown in Table II.

TABLE II. DETERMINATION OF AS(III) AND AS(V) IN DIFFERENT KIND OF WATERS (CONCENTRATIONS IN NG ML^{-1})

Sample	Acetic acid 0.5 M As(III)		Hydrochloric acid 5 M As(III) + As(V)	
	added	found	added	found
Tap water	2.0	2.2±0.1	1.0+1.0	1.8±0.1
Waste water 1	2.0	2.0±0.1	1.0+1.0	1.8±0.1
Waste water 2	5.0	4.9±0.1	5.0+5.0	10.9±0.3

A series of groundwater collected in Venado Tuerto (Santa Fe province, Argentina) from wells where a contamination phenomenon with As had been detected were analyzed. Results are collected in Table III.

TABLE III. DETERMINATION OF TOTAL AS, AS(III) AND AS(V) IN REAL WATER SAMPLES; MEAN VALUE±STANDARD DEVIATION (N=3). RESULTS IN NG ML^{-1}

Sample	As(III)	As(V)	Total As
VT-1	4.4±0.3	103±3	112±4
VT-2	14.9±0.8	155±5	167±5
VT-3	8.8±0.6	46.2±0.8	53.1±1.8
VT-4	33.3±1.3	308±9	316±10
VT-5	8.5±0.5	29.3±0.8	40.0±4.3

CONCLUSIONS

This study demonstrated that both the reaction media and the pH affect the selective hydride generation of As(III) and As(V). The maximum separation between both species was obtained with 0.5 M acetic acid. Total As was determined in 5M HCl and As(V) was calculated as the difference. The method is simple, sensitive, highly selective and avoids the use of relatively complex hyphenated couplings that are not always available for routine analysis.

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DETERMINATION OF AS(III) AND AS(V) IN WATERS BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY (ETAAS) AFTER ION-EXCHANGE CHROMATOGRAPHY

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Abstract A simple approach was developed for the separation and determination of inorganic arsenic species using solid phase extraction and electrothermal atomic absorption spectrometry (ETAAS). A Dowex 1-X8 anion exchange mini-column was used to separate As(III) and As(V). The chemical (pH, type and concentration of eluent) and physical (flow rate of sample and eluent) parameters affecting the separation were studied. Under optimized conditions, As(V) showed a strong affinity for the mini-column, while As(IIII) was collected in the effluent. As(V) was recovered by elution with 0.8 mol L^{-1} hydrochloric acid. The influence of other competing ions on the separation of As(III) and As(V) was also evaluated. The detection limit achieved for As(III) was 4 ng mL^{-1} and for As(V) 4 ng L^{-1}. The relative standard deviation (%RSD) ranged from 0.7 to 1.3% for replicated tap, lake, and well water samples at the 20 ng mL^{-1} level. A preconcentration factor of 100 was achieved for As(V) when 300 mL of water was processed. Arsenic recoveries (full procedure) ranged from 92 to 106%.

INTRODUCTION

Solid-phase extraction (SPE) using mini- or micro-columns offers advantages such as simplicity of operation, low cost, the possibility to achieve high preconcentration factors, the ability to combine with different detection techniques, and relative freedom from matrix interferences. The possibility of "field sampling" is another important advantage, which combines preconcentration of water samples in the field and subsequent transport of the columns for elution and analysis in the laboratory.

Numerous SPE methods for separation and experimental approaches have been proposed in recent years which separate and measure inorganic and organic species of As. Yalçin and Le [1] employed solid-phase extraction cartridges as low-pressure chromatographic columns for the separation and subsequent determination of inorganic arsenic species. Detection limits of 0.2 and 0.4 ng mL^{-1} were achieved for As(III) and As(V), respectively. Grabinski [2] used a single column containing both cation and anion-exchange resins to separate four arsenic species. The overall analytical detection limit was 10 ng mL^{-1} for each individual arsenic species. According to another study, As(III), As(V), MMA, and DMA were determined by graphite furnace atomic absorption spectrometry (GFAAS) after separation of the species by ion-exchange chromatography [3]. In the separation scheme proposed, As(III) was calculated by establishing the difference.

The aim of this study was to develop a simple off-line method based on the use of a mini-column filled with an anion-exchange resin to separate As(III) and As(V) at trace levels. After separation, the As species were quantified by GFAAS. The method's simplicity and low cost make it suitable for routine inorganic arsenic speciation analysis.

MATERIALS AND METHODS

Instrumentation

A PerkinElmer Model 5100 ZL atomic absorption spectrometer (PerkinElmer), equipped with a PerkinElmer Model THGA graphite furnace, a PerkinElmer Model AS-71 autosampler, and longitudinal Zeeman-effect background corrector, was used for the atomic absorption measurements. Electrodeless discharge lamps were used as the sources of radiation for As determination. Arsenic was measured at its most sensitive line at 193.759 nm. Pyrolytically coated graphite tubes with pyrolytic graphite L´vov platforms were employed. High-purity Ar (flow rate 300 mL min^{-1}) was used to purge air from the graphite tubes, except during the atomization step where stopped flow conditions were used. The analytical measurements were based on peak area. Autosampler volumes of 20 μL of sample followed by 5 μL of chemical modifier were employed for all studies. Each analysis was repeated at least three times to obtain the average value and its relative standard deviation (%RSD). The program was optimized using water samples spiked with As. The main ETAAS operating conditions and matrix modifier used are summarized in Table I.

Reagents

All chemicals were of analytical reagent grade unless otherwise stated. Deionized water was used throughout. All solutions were stored in high-density polypropylene bottles. Commercially available 1000 mg L^{-1} As(III) and As(V) standard solutions were prepared daily by serial dilutions of the stock solutions.

A 0.3% (m/v) magnesium nitrate solution was prepared by dissolving an appropriate amount of $Mg(NO_3)_2.6\ H_2O$ in the deionized water. The mixed Pd and $Mg(NO_3)_2$ matrix modifier solution was prepared by adding 5 mL of 0.3% $Mg(NO_3)_2$ solution and 2.5 mL of 10 g L^{-1} Pd solution into a volumetric flask of 25 mL and completing to the mark with deionized water. The final concentration of the matrix modifier solution was: 0.06% $Mg(NO_3)_2$ and 0.1% Pd.

Column Packing and Conditioning

The resin Dowex 1-X8 (100-200 mesh; Cl^- form; analytical grade) was loosely packed into a glass column (7 cm x 3 mm i.d.). Glass wool plugs were placed at both ends of the column so that the net length of the resin zone was about 5 cm. The method consists of the separation of As(III) from As(V) on the acetate form of the Dowex 1-X8 ion exchange resin. Before running the sample, the resin was converted into the acetate form by passing 3 mL of 1.0 mol L^{-1} sodium hydroxide, followed by 5 mL of 4.0 mol L^{-1} acetic acid at a flow rate of 1.0 mL min^{-1}. Then, the mini-column was washed with 10 mL of deionized water. Column degradation was not observed after several weeks of usage.

Table I depicts the optimized conditions selected for the determination of As by ETAAS.

TABLE I. GRAPHITE FURNACE PARAMETERS FOR DETERMINING ARSENIC

Parameter	Drying	Pyrolysis	Atomization	Conditioning
Temperature (°C)	120	800	2400	2700
Ramp time (s)	1	10	0	1
Hold time (s)	30	20	5	2
Ar flow rate (mL/min)	300	300	0 (read)	300

RESULTS AND DISCUSSION

Optimization of the separation conditions

To evaluate the effect of different variables affecting As(III) and As(V) separation, the separation process was carried out on each oxidation state separately.

Influence of pH on As(III) retention

Five ml of sample containing 100 ng of As(III) were passed through the column. The samples pH was varied between 3 and 8, and no retention of the uncharged As(III) was observed. As(III) was collected quantitatively in the effluent.

Elution of As(V) from the column

When five mL of sample containing 100 ng of As(V) were passed through the column the negatively charged As(V) was strongly bound to the anion exchange resin. Pentavalent arsenic was stripped with hydrochloric acid. In this screening experiment 5 ml of acid were used for the elution. Figure 1 shows the influence of acid concentration on As(V) recovery.

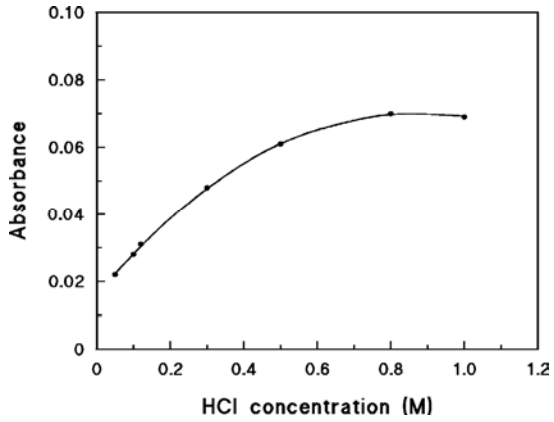

FIG. 1. Influence of hydrochloric acid on As(V) recovery.

Influence of sample and eluent flow rates

Different sample flow rates (0.2-1.0 mL/min) were tested to determine the efficiency of As(V) retention; using in all cases a 20 ng/mL standard arsenic solution. A flow rate of 1.0 mL/min was chosen for further work (Figure 2). Overpressure was observed when higher flow rates were tested.

A parallel study showed that the recovery of As(V) did not varied significantly when the elution flow rate (0.8 M HCl eluent) was varied from 0.2 to 1.0 mL/min A elution flow rate of 1.0 mL/min was chosen for further work.

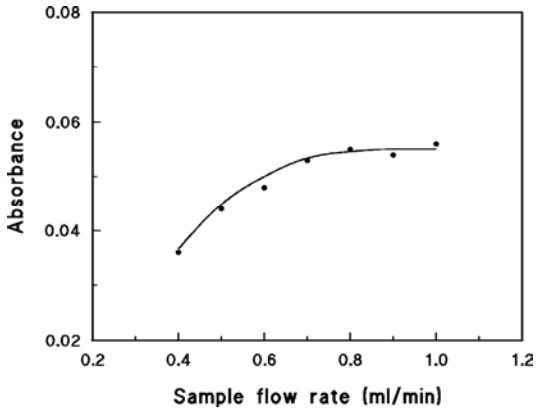

FIG. 2. Influence of sample flow rate on As(V) retention.

Elution volume

The next step in assessing the efficiency of As(V) preconcentration was to determine the minimum volume required to elute the analyte. After running 5 ml of 20 ng/mL arsenic solution, increasing volumes (0.1-5 mL) of 0.8 M HCl were used to elute the analyte retained. The eluate was successively collected and measured by GFAAS. Figure 3 shows that 3 mL is the minimum volume of acid required to quantitatively strip the analyte from the column.

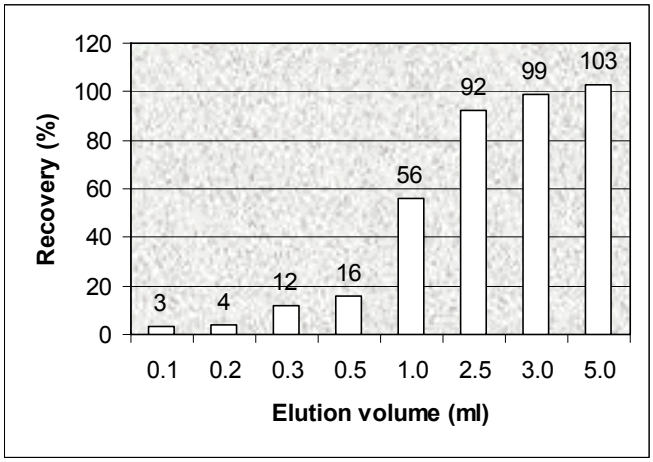

FIG. 3. Elution volume.

Influence of sample volume on As(V) retention

A total constant amount of arsenic (20 ng) in different volumes (5 to 300 mL) was passed through the column. Figure 4 shows that in deionized water the amount of As(V) retained in the column remains constant up to a volume of 300 mL. For higher volumes a significant reduction in the absorbance signal was observed.

The maximum volume that can be run through the column without any decrease in the recovery of As(V) depends on the complexity of the matrix. The recovery achieved was lower when tap and sea water spiked with 20 ng of As(V) were passed through the column.

Evaluation of As(V) retention capacity of microcolumn

The enrichment factor was calculated as the ratio between the maximum volume of deionized water spiked with As(V) that was possible to pass through the column with respect to the minimum volume of 0.8 M HCl required to elute the analyte. According with the results obtained, As(V) can be preconcentrated by a factor of 100. The column properties remained constant during about 100 cycles of retention/elution of As(V). Slight column degradation was observed after 100 cycles and the column was repacked with new resin.

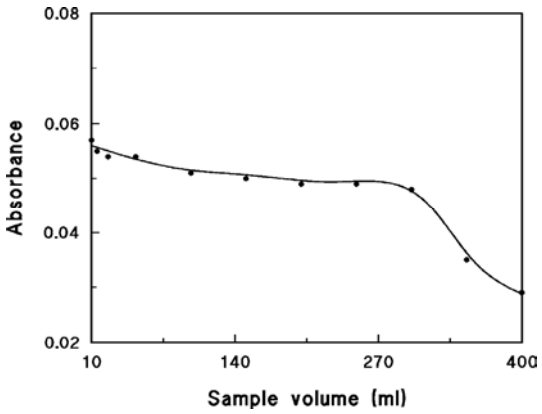

FIG. 4. Influence of sample volume on As(V) retention.

Interference study

The possible interferences produced by different cations namely, Ca(II), Cd(II), Co(II), Cu(II), Fe(III), Hg(II), Mn(II), Na(I), Ni(II), Pb(II), Sb(III), Se(IV) and Zn(II) were studied. To perform this study, 10 mL of sample containing 20 ng mL^{-1} of a mixture of As(III) and As(V) and the interfering ion tested, was passed through the column. As(III) was collected in the eluent and As(V) was stripped from the column with 3 mL of 0.8 M HCl. Variations over ± 5% in the analytical signal of As were taken as an interference. Under optimized conditions, no variation in As(III) or As(V) recovery was observed in the presence of up to 1000 ng mL^{-1} of the ions evaluated except for Cu(II), Hg(II) and Pb(II) and Sb(III). A slight depression in As(III) was observed in the presence of more than 750 ng mL^{-1} of these ions. The possible interference from typical anions (Cl^-, NO_2^-, SO_4^{2-}) found in waters was also investigated. Concentrations higher than 100 ng mL^{-1} did not allow quantitative retention of arsenic on the column, which is the major drawback of the method when seawater samples are analyzed.

Analytical performance

The detection limits calculated on the basis of the 3 σ criterion were: 4 ng mL^{-1} for As(III) and 4 ng L^{-1} for As(V) (preconcentration factor: 100). The relative standard deviation (RSD) ranged from 0.7 to 1.3 % for tap, lake and well water replicated samples at the 20 ng mL^{-1} level.

Unfortunately certified reference materials (CRM) of arsenic species are not available and for this reason a recovery test was performed (Table II). Although it cannot replace accuracy tests, some information is gained about the good performance of the overall procedure. Different combinations of spiked water samples and elution volumes were tested. Recovery data ranged between 92 and 106 %.

TABLE II. RECOVERY OF MIXTURES WITH DIFFERENT CONCENTRATION RATIOS OF AS(III)/AS(V)

	Present in mixture		**Found**		**Recovery (%)**	
	As(III)	**As(V)**	**As(III)**	**As(V)**	**As(III)**	**As(V)**
Tap	5	5	4.9±0.2	4.7±0.2	98	94
Well 1	10	50	9.2±0.4	46±1.8	92	92
Lake 1	0	0.5	-	0.53±0.03	-	106
Lake 2	50	100	47.1±2.1	105±4	94	105

concentrations are expressed in ng mL^{-1}

Application to natural water samples

Table III reports the levels of arsenic in natural waters. Only As(V) was detected in this kind of waters. Results were compared with those obtained by high performance liquid chromatography (HPLC)-hydride generation (HG)-atomic fluorescence spectrometry (AFS). Groundwater samples were collected in Venado Tuerto (Santa Fe province, Argentina) from wells where a contamination phenomenon with arsenic had been detected in some areas. Geological studies demonstrated that this contamination was from natural causes.

TABLE III. ANALYSIS OF WELL WATER SAMPLES

Sample	As(V)	As(V)
	This work	HPLC-HG-AFS
VT-1	20.2 ± 1.1	19.8 ± 0.9
VT-2	62.2 ± 3.4	63.3 ± 3.3
VT-3	197 ± 9	194 ± 8

concentrations are expressed in ng mL^{-1}

CONCLUSIONS

The present study demonstrated that ion-exchange chromatography in conjunction with GF-AAS is fully able to determine selectively As(III) and As(V) in different categories of waters. The method is simple, selective and suitable for routine analysis. Low detection limits were achieved, especially for As(V) that is the most abundant species in oxygenated waters. In addition, the detection limits are more than adequate in view of the maximum concentration fixed for arsenic in drinking waters by different international regulations. At no time was there any evidence that the system allowed any interconversion of species.

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VALIDATION OF PROCEDURE FOR SPECIATION OF INORGANIC AS AND METABOLITES IN AQUEOUS MEDIA BY HPLC-HGAAS

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Abstract A hyphenated method based on high performance liquid chromatography–hydride generation atomic absorption spectrometry (HPLC–HGAAS) for the speciation of As(III), As(V), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) is developed and validated. A Hamilton PRP-X100 anion-exchange column is used for carrying out the arsenic species separation. 12 mM phosphate buffer (pH 6.0) as the mobile phase is used for As(III), As(V), MMA and DMA separation. Then different arsenic species quantified by hydride generation atomic absorption spectrometry. The validated method has the following performance characteristics: The limit of detection values of arsenite, arsenate, DMA, MMA were 8.9 μg L-1, 14.4 μg13.8 μg L-1, 11.6 μg L-1 respectively. Repeatability was better than 6.6% for As concentrations at 150 μg L-1 Linear range was 40-400 μg L-1. An inter-laboratory candidate reference material (prepared by National Research Centre for Certified Reference materials, NRCCRM) was used for method validation. The investigating comparability between these two techniques was also carried out between HPLC-HGAAS and HPLC-ICP-MS. There is no significant difference between the values obtained by the present method and the values recommended. This method would be recommended as a standard operation procedure for speciation of the four arsenic species in water, biological fluids, food and natural plant extracts.

INTRODUCTION

Arsenic is a ubiquitous element. Its toxicity, environmental mobility and accumulation in living organisms usually depend on the form in which the element is present. Of the inorganic forms, arsine is highly toxic, and arsenite is accepted as being more toxic than arsenate. The methylated organic species monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are less toxic than the inorganic forms, and organoarsenicals, arsenobetaine and arsenocholine are generally considered to be non-toxic [1]. Information on the chemical forms is important for understanding the role of the element present as well as revealing its environmental cycle. This requirement stimulates the need for information on the speciation of arsenic and the development of suitable analytical methodology.

The most widely analytical methods used for element speciation are hyphenated techniques, favoured for both the efficient separation and elemental-specific detection. Some of the most suitable methods proposed in the literature to carry out arsenic speciation, are based on the coupling HPLC with on-line detection by hydride generation coupled with AAS [2-10], ICP OES [11,12], ICP-MS [13-19] or AFS [20-23]. Although HPLC coupled to ICP-MS is an effective method for speciation analysis it is not always necessary to obtain such sensitivity. In addition, ICP-MS instruments are too expensive for many laboratories, especially for laboratories in the developing countries. The combination of HG and is AAS available in many laboratories and is one of the most inexpensive and convenient ways of enhancing the sensitivity of arsenic determination. The introduction of the hydride generation as a post-column derivatization method, leads to increased sensitivity and matrix removal, because the volatile hydrides are separated from the liquid residue.

The aim of this paper is to report a validation study for the arsenic speciation by HPLC separation, hydride generation AAS detection. It was proved to be a low-cost, efficient and reliable method for arsenic speciation in biological and environmental analysis. The operation procedure recommended in the present paper would be fit to laboratories in the developing world where they have this urgent As problem.

MATERIALS AND METHODS

Apparatus

A commercial HPLC system or home-made HPLC system with a sample loop volume of 50 μL can be used; a Hamilton PRP-X100 column (250×4.1 mm id. 10 μm particles) is recommended; a low-pressure UV lamp 8 W around quartz tube (0.4m length) provides the energy needed for the decomposition of the organoarsenicals. The flow rates of carrier, oxidant and acid were fixed at 2 mL min^{-1}. In all experiments an atomic absorption spectrometry was used. The software of the AAS instrument offers the chromatography peaks time and area data automatically. Operating conditions of the AAS and the chromatographic systems are listed in Table I. For ICP-measurements, signals at m/z 75 and 77 were monitored in the graphic mode of the instrument. The operation conditions are listed as follows:

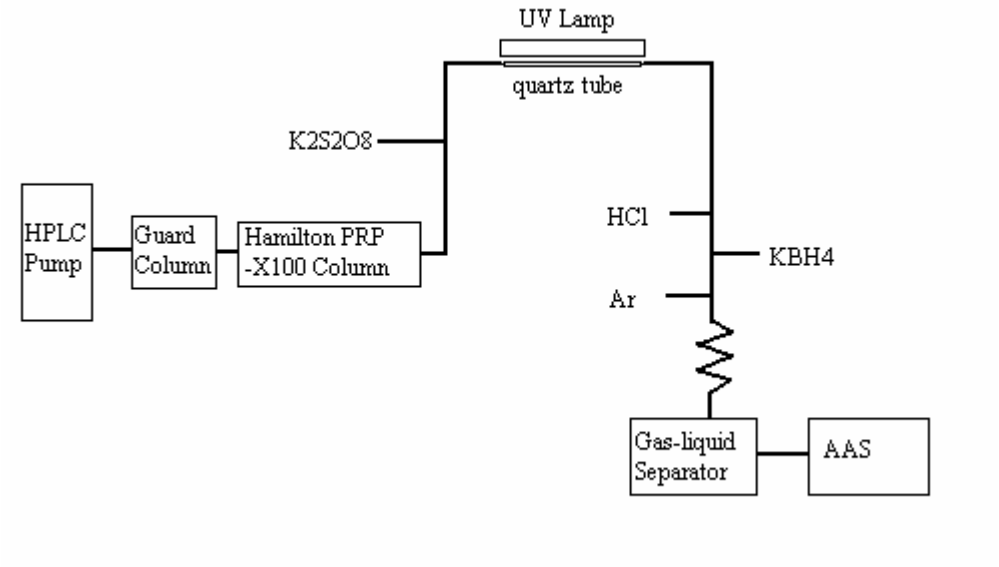

FIG. 1. HPLC–UV–HG–AAS SYSTEM FOR ARSENIC SPECIATION ANALYSIS.

TABLE I. OPERATION CONDITIONS RECOMMENDED

HPLC—	
Column	PRP-X100 Anion exchange column
Dimensions: guard column	250mm×4.1mm id, 10μm
Dimensions: analytical column	250mm×2.3mm id, 12-20μm
Mobile phase	20 mM $NH_4H_2PO_4$ adjusted to pH 6.0 with HCOOH
Flow rate	1 ml/min
Injected volume	50 μL
Hydride generation condition—	
$NaBH_4$ solution concentration	1.5% m/v, stabilized with 0.3% m/v NaOH
$NaBH_4$ solution flow rate	2.0 mL min^{-1}
HCl solution concentration	30% v/v
HCl solution flow rate	2.0 mL min^{-1}
Argon purge flow rate	40 mL min^{-1}
Atomic absorption spectrometer—	
Wavelength	193.7 nm
Bandpass	0.8 nm
Lamp power(EDL)	8 W
Quartz cell temperature	900 °C
Inductively coupled plasma-mass spectrometry	
Radio frequency power	1200W
Sampler and skimmer cones	Nickel
Spray chamber	Double-pass (Scott type)
Argon coolant flow	15 L min^{-1}
Argon nebulizer flow	0.96 L min^{-1}
Data acquisition mode	Graphics (signal intensity versus time)

Reagents

All reagents were of analytical-reagent grade or higher purity. Water was de-ionized and further purified with a Milli-Q water-purification system. Stock solutions (1000 mg L^{-1}) of As(III) and As(V) were prepared by dissolving appropriate amounts of As_2O_3 and $Na_2HAsO_4 \cdot 7H_2O$ in 50 mL 0.1 mol L^{-1} NaOH and diluting the solution to 1000 ml. Stock solutions (1000 mg Ll^{-1}) of MMA disodium salt and DMA sodium salt in water were provided by the Commission of the European Communities, Standard, Measurement and Testing Programme. Each day, aliquots were diluted with mobile phase to obtain working solution.

A candidate reference material, dry powder of traditional Chinese medicine (TCM) was also analyzed for arsenic speciation by HPLC-HGAAS. The recommended values are As(III)(394.3 ± 14.2 mg kg^{-1}), As(V) (432.6 ± 8.3 mg kg^{-1}). Inter-laboratory test is still being undertaken, organized by China National Research Centre for Certified Reference Materials. Although we had doubts about the applied technique with the corresponding results it was considered interesting to analyze this sample by HPLC-HGAAS and check if the presented indicative arsenic concentration was correct.

Speciation Arsenic Species by HPLC-UV-HG-AAS

HPLC separation: Wash the column with ultrapure water and then equilibrate it with the corresponding eluent for 30 min before connecting it on-line to HGAAS instrument. The AAS (including the As hollow cathode lamp and the heater that heat the quartz tube atomizer) was opened and warmed up for at least 60 min before analysis to ensure its stabilization.

The operating parameters used for HGAAS are given in Table I. Sample acquisition is started (manually) at the same time the sample is injected into the column. The calibration standards should be prepared fresh every day. The samples should be analyzed the same day. Analytical results are obtained by calculating the mean values of three injection of each sample.

In order to obtain the method performance characteristics the following parameters were determined:

– Resolution factor. Defined as $R = (t_1-t_2)/(T_{1/2,1}+T_{1/2,2})$, where t is the retention time, $T_{1/2}$ is the width of the peak in the middle high.

– Limit of detection. Defined as LOD = 3 s.d., where s.d. is the standard deviation of the arsenic concentration in the sample near the LOD.

– Repeatability. sr is the repeatability standard deviation of three times analysis

– Linearity range. The standard mixed solutions of the four compounds at different concentrations were injected into the HPLC system, the separation was obtained and the peak area of each compound was calculated.

Validation by HPLC-ICP-MS

The method developed was checked by an independent method without any sample digestion step. The method of choice is ICP-MS after separation with HPLC. In this method any sample digestion step is absent, the diluted sample is directly injected into the HPLC system.

Verification of the method by another technique was performed by analyzing a candidate reference material and spiked samples by HPLC- HG-AAS and HPLC-ICP-MS. HPLC-ICP-MS determinations yields As-concentrations for individual arsenic species.

RESULTS

Speciation analysis of arsenic by HPLC-UV-HG-AAS

A good separation of the four arsenic compounds can be obtained with a single column and with isocratic elution. Fig.1 shows the chromatogram obyained. The baseline separation of the four As species of As(III), DMA, MMA, As(V) can be achieved. The resolution and retention time values are summarized in Table II.

TABLE II. THE SEPARATION RESULTS OF THE FOUR ARSENIC COMPOUNDS

	As(III)	DMA	MMA	As(V)
Retention time/min	2.20±0.02	3.43±0.04	5.55±0.05	13.41±0.12
Resolution	1.620	2.211	6.252	

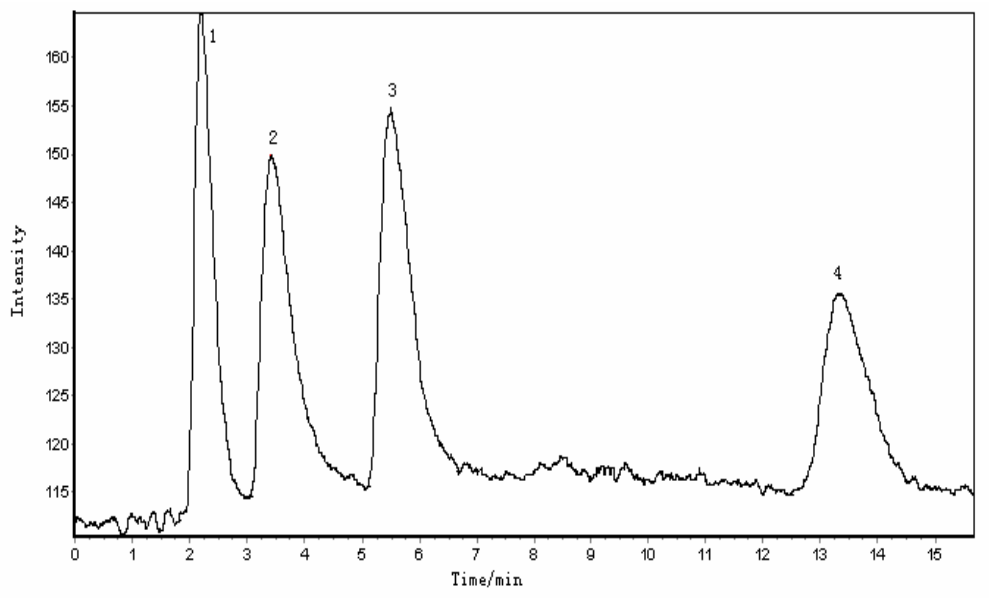

FIG. 2. Chromatogram corresponding to the separation of arsenite, arsenate, MMA, DMA by HPLC-HG-AAS. 1=arsenite(150 μg /L), 2=DMA (300 μg /L), 3=MMA (180 μg L), 4=arsenate(150μg/L).

The repeatability of the method was tested as follows: standard solution of the four compounds (150μg L^{-1})were injected three times

TABLE III. THE DATA OF REPEATABILITY EXPERIMENT USING THE HPLG-HG-AAS METHOD

No.	Peak area			
	As(III)	DMA	MMA	As(V)
1	982.3	576.6	885.3	955.3
2	987.4	532.1	900.7	963.8
3	988.3	506.85	883.	980.6
Mean ± SD	986.0±3.2	538.5±35.3	889.7±9.5	966.6±12.8
RSD (%)	0.33	6.56	1.07	1.33

The linearity of the calibration curves established using peak area for all the arsenical compounds was examined. Standard mixed solutions from 40 to 400 μg L^{-1} of the four compounds at different concentrations were injected into the HPLC system, the separation was obtained and the peak area of each compound was calculated. The correlation coefficients of each compound are shown in Table IV. The linearity range may be wider because all the correlation coefficients are much better than 0.99. Therefore, the concentration ranges for As(III), DMA , MMA , As(V) of 40-400 μg L^{-1} were achieved. However, since the determination of low As concentration is the main purpose of the work, we didn't examine the higher concentrations.

TABLE IV. LINEARITY EXPERIMENT USING THE HPLG-HG-AAS METHOD

	Peak area (n=6)			
Concentration(ppb)	As(III)	DMA	MMA	As(V)
40	392.7±4.6	291.1±9.24	275±6.8	309.3±6.3
80	698.6±4.2	500.2±16.4	605±6.5	529.9±10.5
100	888.9±3.9	566.4±17.2	748.4±8.0	695.3±11.2
150	1212.6±7.5	904.8±33.6	1016.9±14.6	1089.4±26.0
200	1542.1±6.6	1128.6±31.7	1384.4±19.4	1584.6±25.1
300	2311.2±14.7	1630.4±54.8	2075.6±18.2	2370.8±39.5
400	2882.8±23.6	2050.0±52.9	2769.9±29.6	3145.4±34.8
R^2	0.9977	0.9968	0.9990	0.9982

The limit of detection (LOD) for each species was calculated as three times the standard deviation of the background signal (n=11). The LOD for arsenite, arsenate, DMA, MMA are 8.9 μg L^{-1}, 14.4 μg L^{-1}, 13.8 μg L^{-1}, 11.6 μg L^{-1} respectively.

Validation of the HPLC-UV-HG-AAS by recovery experiments and comparison with HPLC-ICP-MS technique

Performance characteristics of the HPLC-ICP-MS method (Tables V and VI):

The calibration curve was established using standards of 1.0, 2.0, 5.0, 10.0, 20.0 and100 ng mL^{-1} of each As species. By using a sample volume of 50 μL, the LOD for As species were 0.2 μg L^{-1}, calculated as three times the signal-to-noise ratio.

TABLE V. PEAK AREA OF FOUR ARSENIC COMPOUNDS AT DIFFERENT CONCENTRATIONS

Species / Concentration	As(III)	DMA	MMA	As(V)
1ppb	1106±28	1012±37	1115±42	1041±55
2ppb	2014±26	2017±41	2062±37	2074±51
5ppb	5315±68	5212±88	5137±45	5044±104
10ppb	10948±108	10782±118	10954±136	10687±152
20ppb	20416±175	20368±224	20348±309	20853±257
100ppb	107860±1206	108147±1012	107667±740	105600±1350
r^2	0.9998	0.9999	0.9999	0.9998

(n=3)

TABLE VI. INTRA AND INTER-ACCURACY OF FOUR ARSENIC COMPOUNDS WITH DIFFERENT CONCENTRATIONS

Species / Concentration		As(III)	DMA	MMA	As(V)
1ppb	intra	2.6%	3.6%	3.7%	4.9%
	inter	2.1%	3.0%	2.4%	3.8%
10ppb	intra	1.0%	1.1%	1.2%	1.4%
	inter	1.3%	1.7%	0.9%	2.1%
100ppb	intra	1.2%	0.9%	0.7%	1.3%
	inter	0.9%	1.1%	1.5%	1.2%

(n=3)

The samples diluted to concentration in the linearity range of the calibration curves were injected into the column for arsenic speciation. By comparing the retention times of each peak of the real sample with that of standard sample, the existence of As (III) and As (V) had been determined and there's no other arsenical speciation. The concentrations of As (III) and As(V) obtained from calculating the peak areas and then using calibration curves are shown in Table VI and the chromatogram of the diluted sample is shown in Fig. 3.

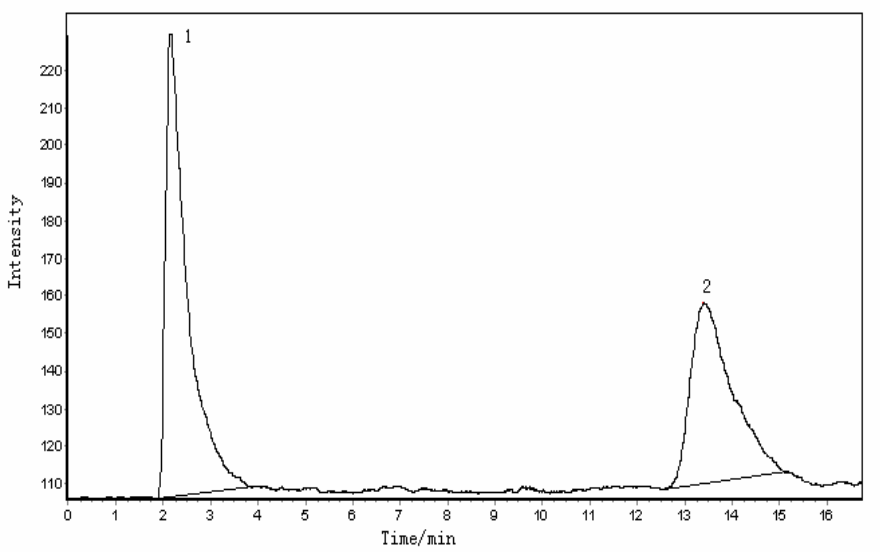

FIG. 3. Chromatogram corresponding to the separation of As species in bezoar detoxification tablet. 1=arsenite, 2=arsenate.

TABLE VII. DETERMINATION OF AS FORMS IN REFERENCE MATERIAL CANDIDATE BY HPLC-HGAAS, IN MG KG^{-1}

	As (III)	DMA	MMA	As (V)
Certified Value (mean and confidence interval)	394 ± 14	N.D.*	N.D.	432 ± 8
Found (mean ±SD) (n=5)	390 ± 8	N.D.	N.D.	442 ± 17

N.D.* : not detected.

TABLE VIII. ARSENIC SPECIES CONCENTRATIONS IN THE REFERENCE MATERIAL CANDIDATE AND SPIKED SAMPLES ANALYZED BY HPLC-HG-AAS AND HPLC-ICP-MS

Spike		HPLC-ICPMS			HPLC-HGAAS		
		Conc. (ng mL^{-1})	RSD (%)	Recovery (%)	Conc. (ng mL^{-1})	RSD (%)	Recovery (%)
No As Added	As^{III}	98.3 ± 1.2	1.2	-	97.5 ± 2.0	2.1	-
	DMA	N.D.	-	-	N.D.	-	-
	MMA	N.D.	-	-	N.D.	-	-
	As^{V}	111.9 ± 2.3	2.1	-	110.4 ± 4.2	3.8	-
+50 ng mL^{-1}	As^{III}	145.1 ± 1.7	1.2	94	143.5 ± 2.0	1.4	92
	DMA	48.3 ± 1.2	2.5	97	49.4 ± 2.3	4.7	99
	MMA	51.3 ± 0.6	1.2	103	50.7 ± 3.2	6.4	101
	As^{V}	160.9 ± 2.6	1.6	98	158.4 ± 3.6	1.0	96
+100 ng mL^{-1}	As^{III}	193.6 ± 2.0	1.0	95	189.5 ± 5.0	2.6	92
	DMA	98.4 ± 1.1	1.1	98	93.7 ± 4.8	5.1	94
	MMA	106.7 ± 1.5	1.6	106	107.8 ± 3.3	3.1	108
	As^{V}	204.4 ± 4.0	2.0	93	198.2 ± 4.9	2.5	88
+200 ng mL^{-1}	As^{III}	289.8 ± 6.0	2.1	96	287 ± 10	3.4	94
	DMA	186.4 ± 5.1	2.7	93	183.7 ± 2.1	1.1	92
	MMA	206.7 ± 3.5	1.7	103	204.8 ± 8.4	4.1	102
	As^{V}	310.4 ± 4.2	1.4	99	302 ± 12	4.0	96

(n=3)

All these individual As-concentrations were given together with the HPLC- HG-AAS As-concentrations in Table 8. The obtained results were evaluated by applying the so called Deming regression [24]. With this type of regression, two independent methods of similar dispersion can be compared. It was calculated that the two methods yield slightly different results. The HPLC- HG-AAS gives 2–5% (95%, 5 df) lower results than the HPLC-ICP-MS method. The difference in the results obtained is rather small compared to the repeatability of both methods.

Recovery experiments:. As (III), As (V), MMA and DMA were added to a candidate reference material (initial As^{III} and As^{V} concentration approx. 100 mg L^{-1}) at concentration levels of 50, 100 and 200 mg L^{-1}. As^{III} recovery at the 50 mg L^{-1} addition was 92% while added spikes at 100 and 200 mg L^{-1} yielded recoveries of 92 % and 94 %, respectively. The same sample (spiked with 50, 100 and 200 mg L^{-1} As^{III}) was tested by HPLC-ICP-MS. The recoveries were 94, 95, and 96%. The samples spiked other arsenic forms (at 50, 100, 200 mg L^{-1} level) were found with good recovery results. All spikes added to the original samples could be recovered with efficiencies of 88 – 108 %. The recovery study confirmed the suitability of the HPLC-HGAAS method for As speciation analysis.

CONCLUSION

HPLC-UV-HG-AAS and HPLC-ICP-MS are good alternatives for speciation analysis of As. By applying the proposed method arsenic can be quantified precisely and accurately in aqueous samples. The method has been validated by comparing it with HPLC-ICP-MS technique. The detection limits were 8.9 μg L^{-1}, 14.4 μg L^{-1}, 13.8 μg L^{-1}, 11.6 μg L^{-1}l for As (III), As (V), MMA and DMA, respectively. The HPLC separation, HG and AAS measurement could be completed on-line within 10 min.

This is a standard operating procedure recommended for speciation analysis of arsenic species As^{III}, As^{V}, MMA and DMA in aqueous samples of exposed subjects. The method is suitable for monitoring occupational exposure to arsenic pollution in developing world especially.

ACKNOWLEDGEMENTS

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CERTIFICATION OF WATER SOLUBLE AS(III) AND AS(V) CONTENTS IN A TRADITIONAL CHINESE MEDICINE AS A CANDIDATE CERTIFIED REFERENCE MATERIAL

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Abstract A study was undertaken to certificate a material for the chemical speciation of water-soluble arsenic species in a traditional Chinese medicine (TCM) for use in preparing a candidate certified reference material (CRM). The medicine which has been extensively used in China were ground, sieved and dried. The obtained power was homogenized and filled into brown bottles. The homogeneity and stability at -20ºC, 4ºC, 20ºC were tested. Five individual methods were applied in the certification, including ICP-MS, ICP OES, HG-AAS and HG-AFS, coupled with off-line/on-line HPLC techniques. The recommended values are: As(III), 394 ± 14 mg kg^{-1}, As(V), 432 ± 8 mg kg^{-1}. An inter-laboratory test is still being undertaken, organized by China National Research Center for Certified Reference Materials.

INTRODUCTION

Arsenic is a ubiquitous element. Its toxicity, environmental mobility and accumulation in living organisms usually depend on the form in which the element is present [1]. As(III) is known as a poison and a cancer causing agent, therefore, the World Health Organization guideline for arsenic in drinking water is 10 μg L^{-1}. The U.S. Environmental Protection Agency has recently reduced its maximum contaminant level from 50 to 10 μg L^{-1}. The Canadian interim maximum acceptable concentration is 25 μg L^{-1} and is currently under review by Health Canada [2].

Although it was well known as a toxic species, As(III) has been successfully used as a chemotherapeutic agent to treat acute promyelocytic leukemia (APL) in recent years [3,4]. Researchers are currently conducting more than 20 clinical trials in the United States to evaluate the use of As(III) for the treatment of other types of cancers [2]. Several traditional Chinese medicines containing inorganic arsenic have been used for treatment of diseases for several hundred years.

The speciation of different arsenic species in biological samples is essential to evaluate the toxic effect and medical function. A number of laboratories have been involved in As speciation analysis using various analytical techniques [5-11]. The reliability of speciation data depends on the accuracy of the speciation procedure. A common way to verify analytical procedures is to check them with certified reference materials (CRMs). Accordingly, a demand for the CRM for As speciation analysis is rapidly growing. A few CRMs would have been available from Community Bureau of Reference (BCR, EU), National Institute for Environmental Studies (NIES, Enviromental Agency of Japan) and other organizations based on public information. Table I list some CRMs with different level of arsenic species. The matrix that has been studied includes seafood, seaweeds, urine, animal tissues and artificially synthesized materials. There is still no reference material for medical validation. In this paper a full description on the preparation, homogeneity and certification of a new candidate CRM for As speciation in traditional Chinese medicine is reported. The data provided in the present paper indicated that the certified material could be recommended as a reference material for As(III)/(V) speciation in medicine matrix.

TABLE I. SOME CRMS CONTAINING DIFFERENT ARSENIC SPECIES IN LITERATURE

CRM numbers	Material	Species	Reference
NIES CRM NO .14	Brown Alga	As(V), Arsenosugar	[12]
NIES CRM NO.15	Scallop	AsB	[12]
NIES CRM NO.18	Human urine	AsB, DMAA	[13]
BCR CRM 626	Artificially Synthesized	AsB , AsC	[14]
BCR CRM 627	Tuna fish	AsB, DMAA	[15]
NRCC DORM-1	Dogfish Muscle	AsB, DMAA	[16]
NRCC DOLT 1	Dogfish liver	AsB, DMAA, TMAs+	[16]
NRC DORM-2	Dogfish muscle	AsB, TMAs+	[1]

AsB: Arsenobetaine; AsC: Arsenochloline; DMAA: Dimethylarsinic acid; TMAs+: Tetramethylarsonium ion ; NRC: Nuclear Regulatory Commission; NRCC: National Research Council of Canada.

METHOD FOR CERTIFICATION

Sample preparation

All bottles, tanks and other apparatus used in the sampling and preparation were cleaned by soaking overnight in 3 mol L^{-1} HNO_3, followed by vigorous rinsing with high purity water (MQ)[13].

The sample (about 3 kg) was obtained from a pharmaceutical company in Beijing. The material was ground**,** sieved over a sieve with apertures of 200 μm. The fraction >200 μm was discarded [15]. The collected power was dried at 50ºC under N_2 protection overnight**.**

An electric oven-drying procedure at 85 ℃ for 4 h followed by 30 min cooling in a silica gel desiccators recommended in literature [17, 18] indicated that the prepared CRM contained approximately 2% moisture. Finally it was homogenized in a V-blender made of stainless steel. The bulk homogeneity of the power was tested by XRF, choosing a number of key minor and trace elements. After reached the required bulk homogeneity, the material was further mixed for 20 h and filled into 300 pre-cleaned brown glass bottles with plastic inserts and screw caps, 10 g each. Before closing, bottles were flushed with dry nitrogen. The power was stored at －20ºC in the dark to reduce microbial activity and photodegradation. Some of them were stored at 4ºC and 20ºC individually to determine the stability of the material.

Extraction protocol

Accurately, 0.2000 g of the test material was placed in a centrifuge tube and 20 mL of deionized water was added. The mixture was extracted during 5 min with ultrasonic assistance. Then, the mixture was centrifuged at 4000 rpm for 10 min. The extract was decanted. At first, the extraction efficiency has been investigated. The arsenic was keeping extracted from the powder owing to the main arsenic compound in the medicine was slightly soluble. The procedure was repeated for 3 times to determine the soluble arsenic species. All the supernatant was transferred into 100 mL volumetric flask and diluted to 100 mL for further measurements.

Homogeneity study

The homogeneity studies were performed for water soluble As(III) and As(V) species because there were only two As-species present in the samples. The As species were determined by high performance liquid chromatography coupled to hydride generation - atomic adsorption spectrometry (HPLC–HG–AAS). The hydride was generated by using HCl (30% v/v) and sodium tetrahydroborate (III) (1.5% in 0.3% NaOH solution). Twenty bottles were set aside during the filling procedure for homogeneity study. For the within–bottle homogeneity, ten test samples were taken from each of the two bottles after one min shaking by hand. For the between–bottle homogeneity, 0.2000 g samples from each of ten randomly selected bottles were analyzed. The coefficients of variation (CV) [15] of the sets of results obtained in the homogeneity study of the candidate CRM are presented in Table II.

TABLE II. WITHIN- AND BETWEEN-BOTTLE HOMOGENEITY

	Within bottles CV%		Between bottle
	Bottle 1	Bottle 2	CV%
As(III)	1.7	1.8	3.2
As(V)	1.6	1.7	2.8

The results did not reveal any significant differences between the within- and between-bottle variances. The within-bottle CVs are very close to the CVs of the repeatability of the method and, therefore, no inhomogeneity in the material was demonstrated. Hence, it was concluded that the homogeneity of the material was suitable for a candidate CRM [15].

Stability study

The stability of the water-soluble contents of As (III) and As (V) was tested to determine the suitability of the candidate reference material. Bottles were kept at, respectively, -20ºC, 4ºC, 20ºC. The water-soluble contents of As (III) and As (V) were determined at regular intervals during the storage period. Tests had been made after 1 day, 5 days, 15 days, 30 days, 60 days and 90 days. Samples were analysed using the same procedure as for the homogeneity study.

Any change with time indicates instability, provided that a good long-term analytical reproducibility has been achieved. By comparing the contents of the different species at different temperatures, instability was detected [19]. At low temperature, changes of As(III)/As(V) ratio proceed slowly, so the samples stored at -20ºC were used as comparators for the samples stored at higher temperature. The results of the stability are given in the Table 3 as the relative variations (R_t) of the mean values ($\overline{X}_t$): $R_t = \overline{X}_t / \overline{X}_{-20°C}$, where $\overline{X}$ is the mean value of 3 independent determinations at the different temperatures. U_T represents the combined uncertainty on R_T and is established as follows: $U_T = (CV_T^2 + CV_{-20°C}^2)^{1/2} \cdot R_T$ [20].

TABLE III. STABILITY STUDY OF THE CANDIDATE CRM

The significance of R_T and U_T are given in the table.

Species	Time(dates)	T(°C)	$R_T \pm U_T$
As(III)	1	20	1.00±0.05
	5		0.99±0.03
	15		1.00±0.02
	30		1.01±0.03
	60		1.00±0.04
	90		1.00±0.02
	1	4	1.00±0.03
	5		1.00±0.03
	15		0.99±0.02
	30		1.00±0.04
	60		1.01±0.03
	90		1.00±0.01
As(V)	1	20	1.01±0.04
	5		0.99±0.03
	15		1.00±0.04
	30		0.99±0.05
	60		1.01±0.02
	90		1.01±0.03
	1	4	1.00±0.04
	5		0.99±0.05
	15		1.00±0.03
	30		0.99±0.04
	60		1.01±0.02
	90		1.00±0.01

In the case of ideal stability, the ratios R_T should be 1. In practice, however, there are some random variations due to the error of the measurement. Nevertheless, the water soluble content of As(III) and As(V) in the power did not show any significant changes during last month. In all cases the expected value of 1.0 was comprised between $R_T + U_T$ and $R_T - U_T$. [15]. The results also were given in Fig 1 and Fig 2.

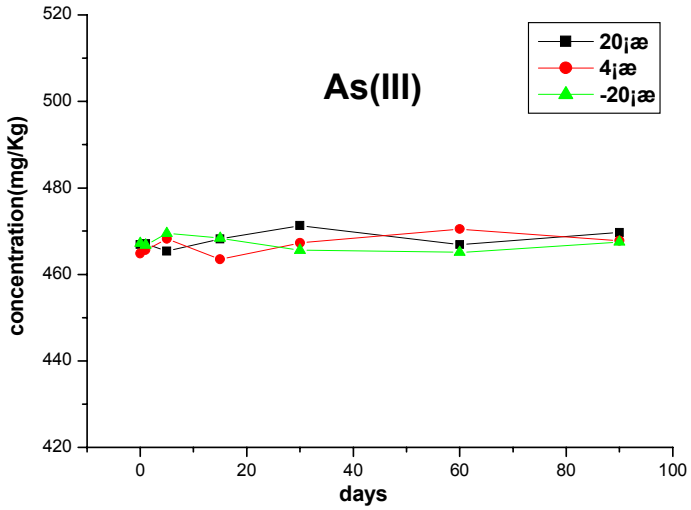

FIG. 1. Stability of water- soluble As(III) species.

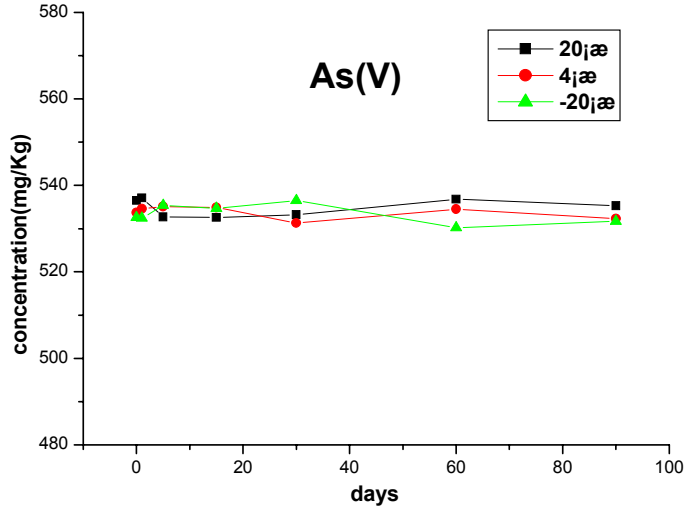

FIG. 2. Stability of the water-soluble As (V) species.

CERTIFICATION USING DIFFERENT TECHNIQUES

Certification was carried out by ICP-MS, HG-AAS, ICP-OES and HG-AFS. A minimum of five fully independent replicate determinations on at least three bottles of the material was done with each method. The mean and SD of the results gained by each method were calculated. If the SD was larger than expected for the analytical method used and the concentration of the analyte, the value was excluded from the certification procedure. The mean of the remaining means was designated as the Certified Value, and twice its SD was taken as the uncertainty range of the Certified Value [13].

A Hamilton PRP X-100 column (250mm×4mm id, 5 μm particles) was used for As(III) and As(V) separation. The following conditions were used: mobile phase, 17 mmol L^{-1} $(NH_4)_2HPO_4$ adjusted to pH 6.0 with HCOOH; flow rate, 1 mL min^{-1}.

Certification by HPLC-ICPMS

For ICP-MS measurements, signals at m/z 75 and 77 were monitored in the graphic mode of the instrument. The operation conditions are listed in Table IV.

TABLE IV. OPERATING CONDITIONS FOR ICP-MS

Radio frequency power	1200W
Sampler and skimmer cones	Nickel
Spray chamber	Double-pass (Scott type)
Argon coolant flow	15 L min^{-1}
Argon nebulizer flow	0.96 L min^{-1}
Data acquisition mode	Graphics (signal intensity versus time)

Certification by HPLC-ICP OES

The operating conditions for ICP OES measurements are listed in Table V. Wavelengths were selected based on their sensitivity and freedom from spectral interferences.

TABLE V. THE OPERATING CONDITIONS OF ICP OES INSTRUMENT

Nominal frequency (MHz)	27.12
RF power (W)	1350
Outer gas flow rate (L min^{-1})	15
Intermediate gas flow rate (L min^{-1})	1.5
Observation height (mm)	8
Working wavelengths (nm)	As (I) 189

Certification by HPLC-HGAFS

Atomic fluorescence spectrometer (AFS) was employed in the test. A high-intensity As hollow cathode lamp was used as the radiation source. A quartz tube (7-mm id.×14-cm length) was used as the atomizer[21]. The operate conditions are list in Table VI.

TABLE VI. OPERATING CONDITIONS FOR HG AFS MEASUREMENTS

Atomic Fluorescence Spectrometry	
As hollow cathode lamp	70 mA (primary current) 70 mA (boost current)
quartz furnace height	10 mm
negative high voltage of photomultiplier	-280 V
Hydride Generation System	
flow rate of 5% v/v HCl	2.5 mL min^{-1}
flow rate of 2% m/v KBH_4	2.0 mL min^{-1}
Carrier gas (argon) flow rate	320 mL min^{-1}

Certification by HPLC-HGAAS

Atomic absorption spectrometry was used. The operation conditions are listed below (Table VII).

TABLE VII. OPERATING CONDITIONS OF THE HG-AAS

HG system	
$NaBH_4$ solution concentration	1.5% m/v, stabilized with 0.3% m/v NaOH
$NaBH_4$ solution flow rate	2.0 mL min^{-1}
HCl solution concentration	30% v/v
HCl solution flow rate	2.0 ml L^{-1}
Argon purge flow rate	40 mL min^{-1}
Atomic absorption spectrometer	
Wavelength	193.7 nm
Bandpass	0.8 nm
Lamp power(EDL)	8 W
Quartz cell temperature	900 ºC

Evaluation of the methods

Results were provided by the four independent methods. The mean values were 384.6-403.1 mg kg^{-1} for As(III) (Fig. 3) and 421.9-443.9 mg kg^{-1} for As(V) (Fig. 4). The CV of the means for As(III) was < 4% for all results, and the CVs for As(V) were 2.0-4.2 %. These CVs demonstrated that the determination of As(III) and As(V) in all of the different methods was highly repeated. On the basis of these results, the recommend values are given in the table 8.

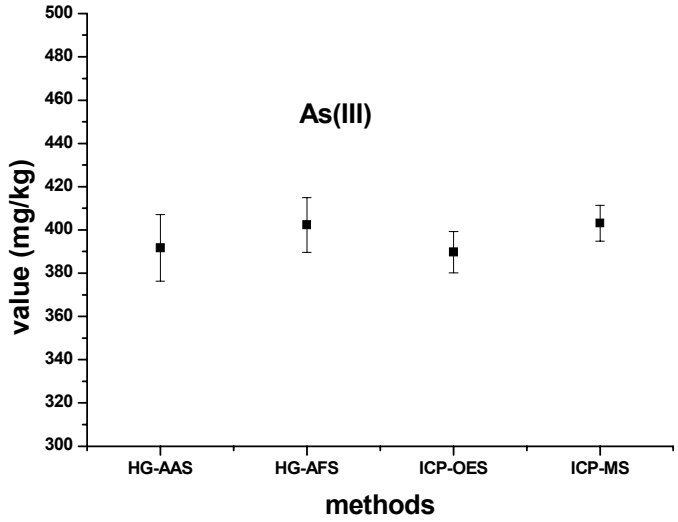

FIG. 3. Concentrations of the water-soluble contents of As(III) determined by different methods.

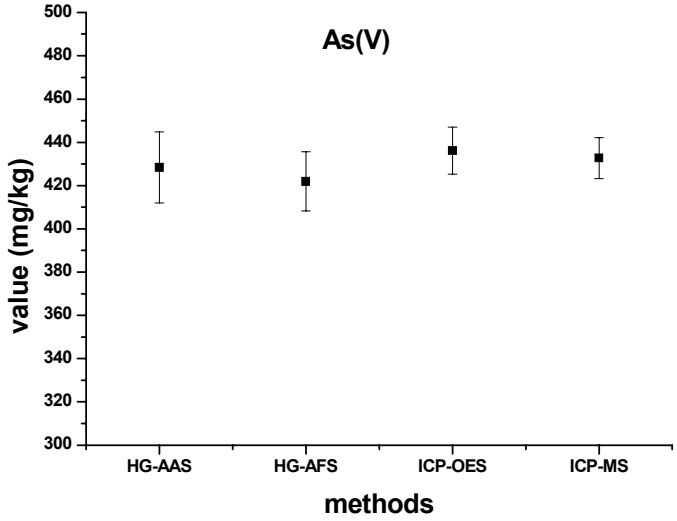

FIG. 4. Concentrations of the water-soluble contents of As(V) determined by different methods.

TABLE VIII. CERTIFIED VALUES FOR SOLUBLE AS(III),AS(V) IN THE CANDIDATE CRM

component	value(mg kg^{-1})	uncertainty(mg kg^{-1})
As(Ⅲ)	394	14
As(Ⅴ)	432	8

CONCLUSIONS

A candidate CRM for inorganic arsenic speciation has been prepared from a ground, sieved power of a traditional Chinese medicine. Its composition was homogeneous to be used as a candidate CRM. The water-soluble contents of As (III) and As (V) are stable for 90 days. Concentrations of As were determined for soluble contents of As (III) and As (V), which are typically found in traditional Chinese medicine. Therefore this candidate CRM would be particularly suitable for use in quality assurance of arsenic in pharmaceutical samples.

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POTENTIALITY OF AXIAL VIEW, SIMULTANEOUS ICP OES FOR THE DIRECT DETERMINATION OF AS AND SE SPECIES AFTER HPLC SEPARATION[1]

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Abstract The performance of a simultaneous inductively coupled plasma optical emission spectrometer with axial plasma observation and CCD detection was evaluated for the direct on-line determination of As and Se species previously separated by ion exchange-high performance liquid chromatography. The column effluent was injected into the plasma by pneumatic nebulization without prior derivatization. A simple data acquisition procedure was adapted from the commercial software of the instrument to allow multi-wavelength recording of the transient chromatographic peaks. After optimisation of the separation operating conditions, three nebulizers and three spray chambers, employed in seven combinations, were tested regarding sensitivity and signal to noise ratio. Measured element detection limits (3 σ) were around 10 ng mL^{-1} for all the species considered, making the method a viable alternative to similar procedures that employ volatile hydride generation previous to sample injection into the plasma. Analytical recoveries both for inorganic and organic species ranged between 92 and 107 %.

INTRODUCTION

Gas or liquid chromatographic separation of As and Se species, followed by ICP optical or mass spectrometries is well documented in the literature. In spite of this, relatively few recent papers are devoted to explore the direct HPLC-ICP OES coupling by employing modern sequential or simultaneous instruments. Topics such as the dispersion effects caused by the interfacing of HPLC with cross-flow or thermospray nebulizers have been addressed in an earlier work by Laborda and coworkers [1]. Hyphenation of ion exchange chromatography with an optimized sequential Czerny-Turner optical monochromator has been discussed by Chausseau et al. [2] These authors tested five different nebulizers in combination with three spray chambers, allowing them to reach detection limits for arsenicals in the 7 to 18 μg L^{-1} range. An axial-view, simultaneous, echelle type ICP spectrometer with CCD detection was employed by Ebdon et al. [3] for silicon speciation analysis. A particular procedure for manipulation of the instrument software and data acquisition was briefly described to permit the recording of chromatographic peaks and to generate a conventional chromatogram. B´Hymer and coworkers [4] compared four nebulizer-spray chamber interfaces for HPLC-ICP MS. Also the simultaneous multielemental speciation analysis of As, Se, Sb and Te has been reported by Guerin et al. [5]. These authors employed ion exchange chromatography combined with ICP MS detection.

METHODS AND MATERIALS

Instrumentation

The HPLC module consisted of an isocratic pump, a syringe injection valve, fitted with a 200 μL loop that was used to inject the sample into the mobile phase carrier stream, and an

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analytical column. Separation of As(III), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and As(V) by ion chromatography (IC) was carried out on a Hamilton PRP-X100 column (25 cm x 4.1 mm i.d.), polymeric (polystyrene-divinylbenzene), 10 μm particle size. A small guard column with the same packing material was placed directly before the column to prevent damage of the main column.

An ICP optical emission spectrometer was employed. RF power and nebuliser Ar flow rates were optimized, with the signal to noise ratio (SNR) used as response function. Arsenic and Se signals were monitored at 188.979 and 196.026 nm respectively.

Three nebulizers and three associated spray chambers were tested as IC-ICP interfaces. The following combinations were considered for comparison: A) Cross-flow / Double pass Scott; B) Concentric Meinhard / Glass Cyclonic; C) Concentric Meinhard / Double pass Scott; D) Concentric Meinhard / Plastic cyclonic; E) Concentric PolyCon / Glass cyclonic; F) Concentric PolyCon/ Double pass Scott; and G) Concentric PolyCon / Plastic cyclonic.

Reagents and standard solutions

All reagents were of analytical grade. Deionised water (18.3 MΩ) was used for the preparation of all standard and reagent solutions. Four species of As and two species of Se were employed in the chromatographic experiments. Stock solutions of 1.0 μg mL^{-1} of As(III) and As(V) were prepared by dissolution of the corresponding oxides in 0.2% (w/v) NaOH solution. Monomethylarsonic acid (MMA), sodium form and dimethylarsinic acid (DMA), sodium form, both 100 μg mL^{-1} (in As) solutions were prepared by dissolving the appropriate amounts of the reagents in water. A commercially available 1000 mg L^{-1} selenite standard solution was used to prepare Se(IV) calibrant solutions. Se(VI) stock solution was prepared by dissolving the appropriate amount of sodium selenate (Merck) in deionised water. All solutions were stored in polyethylene bottles, refrigerated at 4 ºC. Dilutions were prepared daily.

Di-sodium hydrogen phosphate dihydrate and NaH_2PO_4 were employed for preparation of mobile–phase buffer solutions. The resulting solutions were filtered through a 0.22 μm Millipore filter before injection.

Analytical procedure

Filtered samples and standards solutions were loaded into a 200 μL loop and injected into the anion-exchange column (PRP-X100, 25 cm x 4.1 mm i.d.) using phosphate buffer solution 0.020 mol L-1 at pH 6.0 (for As species), or 0.08 mol L^{-1} at pH 6.8 (for Se species), and eluted at 1.5 mL min-1. After chromatographic separation, the effluent was driven directly through the nebulizer and spray chamber into the plasma source for their quantification. The four As species were eluted in less than 10 min. The separation of inorganic species of As and Se was achieved in less than 6 min.

Data acquisition

For the ICP OES instrument used in this study, the software (Perkin-Elmer WinLab 32, version 2.2) enables the measurements of transient signals by manipulation of three readout parameters: sample integration time, read time and number of replicates. The manual mode integration was used during this study and time parameters were set at 0.3 s (integration time) and 3 s (read time). The number of data points (replicates) was significantly increased to 230.

The peak areas of the spectrum lines were calculated by integrating 3 pixels (peak centred) on the corresponding peak. All data were stored as a database in WinLab and chromatograms were constructed by plotting net emission intensity (NEI) values as a function of time using Microsoft Excel 5.0.

RESULTS AND DISCUSSION

Optimization of chromatographic parameters

Inorganic As and Se species: Fig. I depicts chromatograms of inorganic species of As and Se. A 80 mmol L^{-1} phosphate buffer was required to separate inorganic species of As and Se in less than 6 min. Effective resolutions, estimated by considering the chromatograms simultaneously obtained at the individual element wavelengths, were significantly higher than 1.5 in the range of flow rates tested. Average peak asymmetry factors were in all cases lower than 1.7, and may be ascribed to a small broadening effect introduced by the column. This is supported by the fact that band asymmetries lower than 1.3 were observed for direct injection of 200 μL volumes of sample without interposing the column.

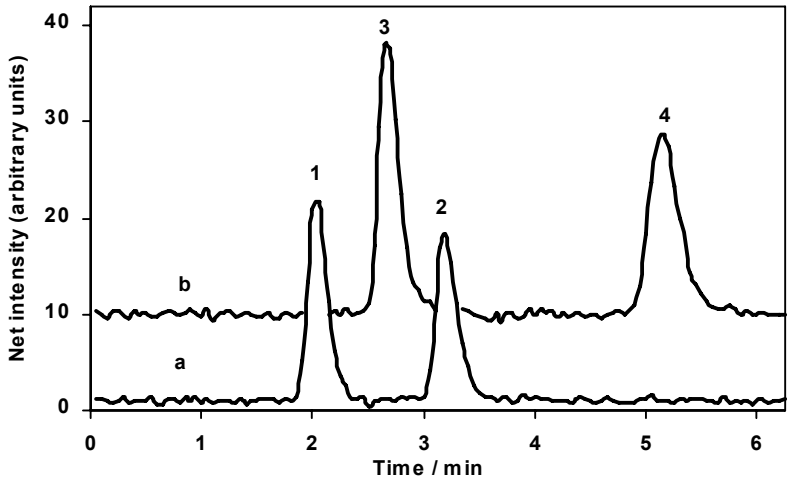

FIG. 1. Simultaneous chromatograms 0f 200 ng of a) As and b) Se. Observed peaks: 1) As(III), 2) As(V). 3) Se(IV), 4) Se(VI).

Inorganic and organic As species: The best resolution was achieved at pH about 6. At pH 5.0, As(III) and DMA co-eluted and at pHs higher than 6.0 the resolution for the organic species was insufficient. The four As species were separated with acceptable resolution in less than 10 min by employing a 20 mmol L^{-1} phosphate buffer at a flow rate of 1.5 mL min^{-1}. Chromatograms obtained for a standard solution containing 1.0 μg mL^{-1} of each species ran under optimized separation conditions are illustrated in Fig. II.

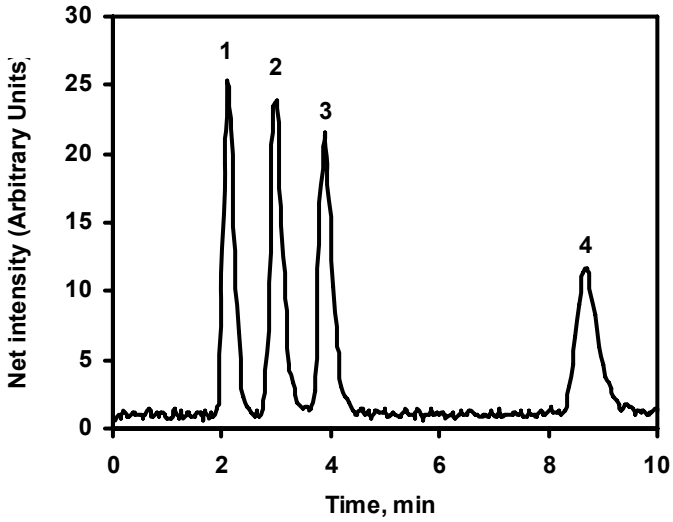

FIG. 2. Chromatogram of 200 ng of each As species obtained under optimized conditions. 1) As(III), 2) DMA, 3) MMA, 4) As(V).

Effect of nebulizer and spray chamber combinations

The following combinations were considered for comparison: A) Cross-flow / Double pass Scott; B) Concentric Meinhard / Glass Cyclonic; C) Concentric Meinhard® / Double pass Scott; D) Concentric Meinhard / Plastic cyclonic; E) Concentric PolyCon / Glass cyclonic; F) Concentric PolyCon / Double pass Scott; and G) Concentric PolyCon / Plastic cyclonic. Fig. III illustrates that the best sensitivity and signal to noise ratio (SNR) for 1.0 µg mL^{-1} As (A) and 1.0 µg mL^{-1} Se (B) was obtained with the combination Concentric Polycon/glass cyclonic.

(1) (2)

(E) PolyCon / Glass cyclonic

(F) PolyCon / Double pass Scott
(B) Meinhard / Glass cyclonic
(A) Cross flow / Double pass Scott
(C) Meinhard / Double pass Scott
(G) PolyCon / Plastic cyclonic
(D) Meinhard / Plastic cyclonic

FIG. 3. Response of As (1) and Se (2) to the variation of the nebulizer Ar flow rate and power using different combination of nebulizer and spray chambers.

Analytical Performance

The figures of merit are set forth in Table I. Calibration functions were always calculated from chromatographic peak areas, with element concentrations ranging from 50 to 1000 μg L^{-1}. Detection limits (LOD) were calculated using the 3 σ criterion. Relative standard deviations were averaged from values obtained from mixtures of the four species of As or mixtures of inorganic As and Se (As(III), As(V), Se(IV) and Se(VI)). Results are averages of 10 consecutive runs conducted over two hours using multielement standards.

Recovery of analyte species was tested from spiked well and tap water samples. Concentrations equivalent to 500 ng mL^{-1} of As and Se were added. Results have been also included in Table I.

TABLE I. FIGURES OF MERIT FOR THE DETERMINATION OF AS(III), DMA, MMA, AS(V), SE(IV) AND SE(VI)

Species	Calibration equation	Detection limit / μg L^{-1}	Recovery range / %	% RSD*
As(III)	a = 3.4, b = 12.0 (r = 0.9996)	10	98-103	5.1
DMA	a = -20.7, b = 13.8 (r = 0.9998)	7	99-103	3.1
MMA	a = -160, b = 12.9 (r = 0.9997)	8	92-102	3.3
As(V)	a = -8.8, b = 11.5 (r = 0.9999)	13	98-103	4.0
Se(IV)	a = 124, b = 12.8 (r = 0.9999)	6	104-107	3.1
Se(VI)	a = 374, b = 10.9 (r = 0.9998)	7	104-107	4.1

Fitted equation: y = a + bx, where y = peak area, x = concentration (μg L^{-1})
r = correlation coefficient
10 replicates for a concentration of 1000 μg L-1.

Analysis of real samples

The proposed methodology was assessed for the analysis of the speciation analysis of As and Se in natural water samples. Four groundwater samples were collected in Santiago del Estero province (Argentina) from wells where a contamination phenomenon with As had been detected in some areas. Samples were filtered through a 0.22 μm Millipore filter before injection. Only As(V) was detected after chromatographic separation. Determination of total As was also carried out by graphite furnace atomic absorption spectrometry (GFAAS). Good agreement between both techniques was obtained, as shown in Table II.

TABLE II. LEVELS OF AS SPECIES IN NATURAL WATERS

Sample	As[a] / μg L^{-1}	
	This work	**GFAAS**
S 1	260 ± 12	240 ± 12
S 2	691 ± 25	675 ± 38
S 3	280 ± 13	260 ± 11
S 4	ND[b]	13.0 ± 0.9

a Reported with 95 % confidence limits

CONCLUSIONS

The results obtained in this study indicate that with a simple manipulation of the software (number of sample replicates and integration time), transient measurements suitable for chromatographic-spectrometric coupling can be performed with the instrument employed.

In general terms, concentric nebulizers seem more adequate for chromatographic interfacing regarding SNR. In spite of the different volumes of the spray chambers tested, no significant increase in signal dispersion was introduced. Sensitivity was more favourable with the lower volume glass cyclonic design, possibly due to its higher nebulization efficiency. Improvements in sensitivity could be attained by a further reduction in effluent flow rates (i.e. by employing shorter columns).

The detection limits obtained when a Concentric PolyCon® nebulizer associated to a glass cyclonic spray chamber was used as sample introduction system, appear adequate to determine As and Se species in aqueous samples without derivatization. The optimized axial ICP OES system may thus be considered as a valid alternative to HG ICP OES for speciation studies.

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CHROMIUM SPECIATION ANALYSIS

CHROMIUM SPECIATION IN SEWAGE SLUDGE USING LIQUID CHROMATOGRAPHY

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Abstract Applicability of an anion-exchange FPLC - ETAAS procedure for the determination of trace amounts of Cr(VI) in environmental samples e.g. natural waters, chromium enriched sewage sludge was investigated. Oxidation-reduction processes of Cr in highly alkaline samples of lime-treated sewage sludge was investigated. The objective was also to develop the procedure for determination of Cr(VI) by the use of connvective interaction media (CIM) anion-exchange fast monolithic chromatography with ETAAS detection and to validate FPLC-ETAAS and CIM-ETAAS procedures by the use of certified reference materials.

INTRODUCTION

The toxicity of chromium depends primarily on its chemical form. Trivalent chromium compounds are much less toxic than those of hexavalent chromium[1,2]. Due to its high toxicity hexavalent chromium is under environmental scrutiny. Concern about the presence of hexavalent chromium in the environment resulted in the development of numerous analytical techniques for the determination of Cr(VI) in different sample matrices. Among them spectrophotometry[3-8] has been widely used for the determination of Cr(VI). However, the technique is liable to various interference effects, e.g. the presence of coloured species, turbidity and colloidal particles. Therefore, its use should be critically evaluated for the particular sample matrix analysed[4-8]. Selective extraction of Cr(VI)-HCl complex at 277 K into methyl isobutyl ketone (MIBK) and determination of the Cr(VI) in the organic phase by flame AAS was found to be a sensitive technique for the determination of hexavalent Cr in the presence of large amounts of Cr(III)[5,9]. However, the technique cannot be applied if the sample matrix forms a gel with MIBK. Alkaline extraction (sodium carbonate/sodium hydroxide) was also frequently used for determination of Cr(VI) in environmental samples[8,10]. Applying this technique, soluble Cr(III) could be oxidised during extraction resulting in positive errors for Cr(VI)[8]. Ultrasonic extraction in alkaline solutions was reported to provide good extraction efficiency of Cr(VI) from workplace samples and allowed the retention of Cr(VI) on an anion-exchange resin[11]. For speciation of Cr in natural waters, selective pH-dependent preconcentration of Cr(VI) and Cr(III) was performed on high surface area adsorbents of Al_2O_3[12] and TiO_2[13], followed by ETAAS determination of the separated Cr species.Thermal lens spectrophotometric detection[13,14] and on-line thermal lens spectrophotometric detection after separation by ion chromatography[15] were found to be selective sensitive techniques for determination of hexavalent and trivalent Cr in various environmental samples at low ng cm^{-3} concentration levels. In our group anion exchange fast protein liquid chromatography (FPLC) with AAS detection has been developed for the simultaneous determination of Cr(III) complexes and Cr(VI)[16]. The procedure was applied to determination of Cr species in the sap of cabbage plants exposed to various concentrations of chromate and Cr(III)-EDTA. As an alternative to FPLC columns containing ion-exchange resins, ion-exchange separation supports based on convective interaction media (CIM) were developed recently. The matrix supports of poly(glycidylmethacrylate-co-ethyleneglycoldimethacrylate) substituted with strong or weak cation and anion exchangers offer very fast separation of biomolecules[17-19] and are also used for fast separation of organic acids[20]. In our group CIM discs were successfully applied in the speciation of zinc in environmental samples[21].

The project objectives were to examine the applicability of an anion-exchange FPLC - ETAAS procedure for the determination of trace amounts of Cr(VI) in environmental samples e.g. natural waters, chromium enriched sewage sludge and to investigate oxidation-reduction processes of Cr in highly alkaline samples of lime-treated sewage sludge. The objective was also to develop the procedure for determination of Cr(VI) by the use of connvective interaction media (CIM) anion-exchange fast monolithic chromatography with ETAAS detection and to validate FPLC-ETAAS and CIM-ETAAS procedures by the use of certified reference materials.

EXPERIMENTAL

Apparatus

A strong anion-exchange FPLC column (Pharmacia, Uppsala, Sweden) of Mono Q HR 5/5 (column dimensions 5x50 mm, 10 μm beaded hydrophilic polyether resin substituted with quaternary amine groups, pH stability 2-12) was employed for separation of Cr(VI). The column was connected to a Varian (Mulgrave, Victoria, Australia) Star gradient high pressure pump, equipped with a Rheodyne (Cotati, CA, USA) Model 7161 injector (0.5 cm^3 loop).

A weak anion-exchange CIM DEAE disc (Bia Separations, Ljubljana, Slovenia) (disc dimensions 12x3 mm, poly(glycidylmethacrylate-co-ethyleneglycoldimethacrylate) matrix support substituted with diethylamine (DEAE) groups, pH stability 1-13) was used. The disc was connected to Waters (Milford, Massachusetts, USA) Model 600E gradient high-pressure pumps, equipped with a Rheodyne (Cotati, California, USA) Model 7725i injector (0.5 cm^3 loop). Separated Cr species were determined by ETAAS on a Hitachi (Tokyo, Japan) Z-8270 polarized Zeeman atomic absorption spectrometer.

Reagents

Merck (Darmstadt, Germany) suprapur acids and water doubly distilled in quartz were used for the preparation of samples and standard solutions. All other chemicals were of analytical- reagent grade.

A standard $(CrO_4)^{2-}$ stock solution (1000 ± 2 mg dm^{-3}) was purchased from Merck.

Potassiumhydrogenphthalate-NaOH buffer was used to adjust the pH from 4.0 to 6.0, HCO_3^-/H_2CO_3 buffer to adjust the pH from 6.0 to 7.0, TRIS-HCl buffer to adjust the pH from 7.0 to 9.0 and $Na_2CO_3/NaHCO_3$ buffer to adjust the pH from 9.0 to 12.0.

The certified reference material CRM 544, Cr(VI) in lyophilised solution, obtained from the Community Bureau of Reference (BCR, Geel, Belgium), was used to validate the method for determination of Cr(VI).

Sartorius (Goetingen, Germany) 0.2 μm cellulose nitrate membrane filters of 25 mm diameter were used in the filtration procedure.

Design of the experiment and sample preparation

Samples of untreated sewage sludge and sludge mixed with a sawdust (volume ratio 1+1) with pH of 8.0 and 7.8, respectively were analysed. For sludge stabilisation and disinfection 5% of quicklime was added to sludge and sawdust amended sludge samples. The

addition of quicklime raised the pH of the sludges to 12. In order to investigate the potential process of Cr oxidation at high pH, analyses of sludge and its mixtures were performed in a time span of 6 months after the quicklime treatment. To 2.00 g of moist sludge sample 8 cm^3 of water was added, the mixture shaken for 2 h, centrifuged (10000 rpm, 20 min), decanted and filtered through a 0.2 μm pore size filter. In the sludge extracts Cr(VI) by FPLC-ETAAS.

ANALYTICAL PROCEDURE

Recommended FPLC-ETAAS procedure

The stability of the chromatographic column in the pH range 2 to 12 enabled analysis of untreated sewage sludge and sludge mixed with sawdust (volume ratio 1+1) with pH of 8.0 and 7.8, respectively, as well as their mixtures with 5% of quicklime with a pH of 12.

0.5 cm^3 of sample was injected onto the column. Buffer A consisting of TRIS-HCl (0.005 mol dm^{-3}, pH 8.0) and buffer B (buffer A plus 0.5 mol dm^{-3} NaCl) were employed in linear gradient elution from 0-100% of buffer B for 15 min at a flow rate of 1.0 cm^3 min^{-1}. The column was regenerated with 2 mol dm^{-3} NaCl for 5 min and equilibrated with buffer A in the following 10 min at a flow rate of 1.0 cm^3 min^{-1}. The chromatographic run was completed in 30 min. The separated chromium species were determined "off line" by ETAAS in 0.5 cm^3 fractions. In order to reduce the salt deposit and to obtain reproducible measurements in ETAAS analysis, 5 μl of 32% HNO_3 was added to the graphite tube before each determination[22]. Eluent-matched standard solutions of Cr(VI) were prepared in eluent solution of the same molarity that eluted Cr(VI) from the column (0.4 mol dm^{-3} NaCl).

Recommended CIM fast monolithic chromatography - ETAAS procedure

0.5 cm^3 of sample was injected onto a CIM fast monolithic disc. Buffer A (0.005 mol dm^{-3}) adjusted to the pH of sample and buffer B (buffer A plus 3 mol dm^{-3} NH_4NO_3) were employed in the separation procedure. In the first 5 min buffer A was eluted through the disc (from 0 to 2 min at a flow rate of 1.0 cm^3 min^{-1}, and from 3 to 5 min at a flow rate of 2.0 cm^3 min^{-1}). Afterwards linear gradient elution from 0-100% of buffer B was employed from 5 to 10 min at a flow rate of 1.0 cm^3 min^{-1}. The disc was then regenerated with 100% of buffer B for 0.5 min at a flow rate of 5.0 cm^3 min^{-1} and at same flow rate equilibrated for the following 4.5 min with 100% of buffer A. The chromatographic run was completed in 15 min. The separated chromium species were determined "off line" by ETAAS in 0.5 cm^3 fractions. In order to reduce the salt deposit and to obtain reproducible measurements in ETAAS analysis, 5 μl of 32% v/v HNO_3 was added to the graphite tube before each determination[22]. Eluent-matched standard solutions of Cr(VI) were prepared in eluent solution of the same molarity that eluted Cr(VI) from the disc (2.1 mol dm^{-3} NH_4NO_3).

RESULTS AND DISCUSSION

Anion exchange with FPLC-ETAAS

Determination of Cr(VI) in standard CrO42- solutions by anion-exchange FPLC-ETAAS

It was experimentally proven that Cr(VI) was reproducibly and quantitatively eluted from 12.0 to 13.5 min with a peak maximum eluting from 12.5 to 13.0 min when CrO_4^{2-} standard solutions with a pH of 8.0 or 12.0 were applied. Linearity of measurement for the injected sample solution (0.5 cm^3) was obtained over a concentration range from 5.0 to 50.0 ng cm^{-3} of Cr(VI) with a correlation coefficient better than 0.998. The repeatability of

measurement and efficiency of Cr(VI) separation was tested for six consecutive separations of a standard solution of Cr(VI) (25.0 ng cm^{-3}, pH=8.0). The results are presented in Table 1.

TABLE I. REPEATABILITY OF MEASUREMENT AND EFFICIENCY OF SEPARATION FOR STANDARD SOLUTION OF CR(VI) BY FPLC-ETAAS TECHNIQUE

Replicate	Cr(VI) Added (ng cm^{-3})	Time 11.5-12.0 min (ng cm^{-3})	Time 12.0-12.5 min (ng cm^{-3})	Time 12.5-13.0 min (ng cm^{-3})	Time 13.0-13.5 min (ng cm^{-3})	Time 13.5-14.0 min (ng cm^{-3})	Cr(VI) Found (ng cm^{-3})	Recovery (%)
1.	25.0±0.2	<0.5	1.0±0.1	21.8±0.3	1.3±0.1	<0.5	24.1±0.6	96.4
2.	25.0±0.2	<0.5	2.9±0.1	21.4±0.3	1.9±0.1	<0.5	26.2±0.6	104.8
3.	25.0±0.2	<0.5	1.9±0.1	22.9±0.3	1.3±0.1	<0.5	26.1±0.6	104.4
4.	25.0±0.2	<0.5	1.0±0.1	22.0±0.3	1.6±0.1	<0.5	24.6±0.6	98.4
5.	25.0±0.2	<0.5	2.4±0.1	21.8±0.3	2.1±0.1	<0.5	26.3±0.6	105.2
6.	25.0±0.2	<0.5	1.5±0.1	23.8±0.3	1.3±0.1	<0.5	26.6±0.6	106.4

Mean = 25.7 ng cm^{-3} ± 1.0 ng cm^{-3}; RSD = 4.0%

It is evident from data of Table 1 that Cr(VI) was quantitatively and reproducibly eluted from 12.0 to 13.5 min with a peak maximum eluting from 12.5 to 13.0 min. The recoveries were calculated on the basis of the ratio between the found and added Cr(VI) concentrations and ranged from 96 to 106%. The repeatability of measurement was found to be 4.0%. The LOD for the injected sample solution (0.5 cm^3) calculated on 3 *s* basis (a value of three times the standard deviation of the blank) was 1.5 ng cm^{-3} of Cr(VI).

The behaviour of other negatively charged Cr(III) complexes e.g. Cr-EDTA and Cr-oxalate as well as Cr^{3+} on the anion-exchange FPLC column was investigated in our previous work[9]. It was demonstrated that Cr-EDTA and Cr-oxalate were eluted from 6.0 to 7.0 min and 8.0 to 9.0 min, respectively, and were therefore completely separated from Cr(VI). The elution of Cr^{3+} depended significantly on the pH of sample. At pH below 5, Cr^{3+} was quantitatively eluted with the solvent front. At pH 7.0 only 40% of Cr^{3+} was eluted with the solvent front. The other 60% of Cr that corresponded to hydroxo-Cr(III) species were strongly adsorbed on the column resin and did not disturb the following separations. At pH higher than 10 no measurable Cr(III) was detected during the chromatographic run due to the strong adsorption of hydroxo-Cr(III) species on the column resin. Therefore, anion-exchange FPLC column enabled quantitative separation of Cr(VI) in the presence of aqueous Cr(III) species and some other negatively charged Cr complexes (Cr-EDTA, Cr-oxalate).

Validation of the procedure

Validation of the procedure was performed by the analysis of certified reference material CRM 544, Cr(VI) in lyophilised solution. After opening the vial, containing lyophilised solution, the sample was reconstructed with 20 cm^3 of HCO_3^-/H_2CO_3 buffer at pH 6.4. The analysis of the total Cr and Cr(VI) were performed immediately after sample reconstruction. Total Cr was determined by ETAAS. The average of six determinations was found to be 49.0 ng cm^{-3} with the standard deviation of measurement ± 0.7 ng cm^{-3}. The result agreed well with the reported certified value for total Cr 49.4 ± 0.9 ng cm^{-3}. Cr(VI) was determined by the anion-exchange FPLC-ETAAS procedure by injecting six sample aliquots on the column. The pH of buffers A and B used in chromatographic run were adjusted to 6.4.

The average of six parallel determinations of Cr(VI) was found to be 23.5 ± 1.0 ng cm^{-3}. Good agreement between Cr(VI) determined and the reported certified value (22.8 ± 1.0 ng cm^{-3}) was obtained.

Determination of Cr(VI) in untreated and lime-treated sewage sludge extracts by FPLC-ETAAS and validation of the procedure

In order to reduce the moisture content, sawdust (volume ratio 1+1) was added to the sludge. To reduce microbial density liming was applied for sludge stabilisation and disinfection by raising the pH above 12. It was experimentally proven that addition of 5% of quicklime fulfilled this criterion. However, at so high a pH oxidation of Cr(III) may potentially occur, despite the highly reducing characteristics of the sludge matrix.

The FPLC-ETAAS technique was applied for the determination of Cr(VI) in untreated and lime-treated sewage sludge. In untreated sludge the concentration of total Cr was found to be 1064 mg kg^{-1} and 522 mg kg^{-1} in sawdust amended sludge, while the concentrations of total water soluble Cr in the corresponding sludge extracts were 7.9 mg kg^{-1} and 1.2 mg kg^{-1}, respectively. The pH of the sludge extract was 8.0 and in sawdust amended sludge the extract pH was 7.8, respectively. In these sludge extracts the concentration of Cr(VI) was found to be below 0.02 mg kg^{-1}.

In lime-treated sludge and lime-treated sawdust amended sludge the concentrations of total Cr were 792 mg kg^{-1} and 427 mg kg^{-1}, respectively. The concentrations of total water soluble Cr in sludge extracts (over a time span of 6 months after liming) ranged from 0.7 mg kg^{-1} to 1.2 mg kg^{-1}. During the course of the experiment the pH of lime-treated sludge extracts ranged from 12.0 to 9.6. In these sludge extracts measurable concentrations of Cr(VI) were found. In order to prove that the Cr species eluted from the column after 12.0 to 13.5 min corresponded to Cr(VI), spiking of untreated and lime-treated sludge extracts with 50 ng cm^{-3} of Cr(VI) was performed. Spiked samples were eluted at the same retention time. The results are presented in Table 2.

TABLE II. RECOVERIES OF CR(VI) IN SPIKED EXTRACTS OF SEWAGE SLUDGE SAMPLES. CR(VI) WAS DETERMINED BY THE FPLC-ETAAS TECHNIQUE*

Extract	Concentration of Cr(VI) (ng cm^{-3})	Cr(VI) added (ng cm^{-3})	Cr(VI) found (ng cm^{-3})	Recovery (%)
Sludge	<1.5	50.0±0.4	47.5±3	95.0
Sludge amended with sawdust	<1.5	50.0±0.4	47.0±3	94.0
Sludge + 5% of quicklime**	20.5±1	50.0±0.4	71.4±4	101
Sludge amended with sawdust + 5% of quicklime**	3.5±0.2	50.0±0.4	51.3±3	95.8

*Mean of two parallel samples ± deviation of measurement
**Three months after liming

It is evident that recoveries for Cr(VI) in spiked samples lay between 94 and 101%. The repeatability of measurement was tested for six consecutive separations of a lime-treated sewage sludge extract. The results are presented in Table 3.

TABLE III. REPEATABILITY OF MEASUREMENT FOR CR(VI) IN LIME-TREATED SEWAGE SLUDGE EXTRACT* BY THE FPLC-ETAAS TECHNIQUE.

Replicate	Time 11.5 min (ng cm^{-3})	Time 12.0 min (ng cm^{-3})	Time 12.5min (ng cm^{-3})	Time 13.0 min (ng cm^{-3})	Time 13.5 min (ng cm^{-3})	Cr(VI) (ng cm^{-3})
1.	<0.5	4.0±0.1	10.4±0.2	1.8±0.1	<0.5	16.2±0.4
2.	<0.5	4.7±0.1	11.7±0.2	2.0±0.1	<0.5	18.4±0.4
3.	<0.5	3.1±0.1	10.3±0.2	2.0±0.1	<0.5	15.4±0.4
4.	<0.5	3.9±0.1	9.6±0.2	2.2±0.1	<0.5	15.7±0.4
5.	<0.5	4.0±0.1	9.7±0.2	2.4±0.1	<0.5	16.1±0.4
6.	<0.5	4.6±0.1	9.5±0.2	2.4±0.1	<0.5	16.5±0.4

*48 h after liming

Mean: 16.4 ng cm^{-3} ± 1.1 ng cm^{-3}; RSD = 6.5%

It can be seen that the repeatability of measurement for a lime-treated sewage sludge extract was ± 6.5%. The LOD (3 *s*), expressed on a dry mass basis was found to be 20 ng g^{-1} of Cr(VI).

Variation of Cr(VI) concentrations with time elapsed after quicklime treatment

Cr(VI) was determined in samples of sludge and sludge amended with sawdust 48 h after liming and then monitored over a time span of 6 months. The results of these measurements (average of two parallel determinations) are presented in Fig. 1.

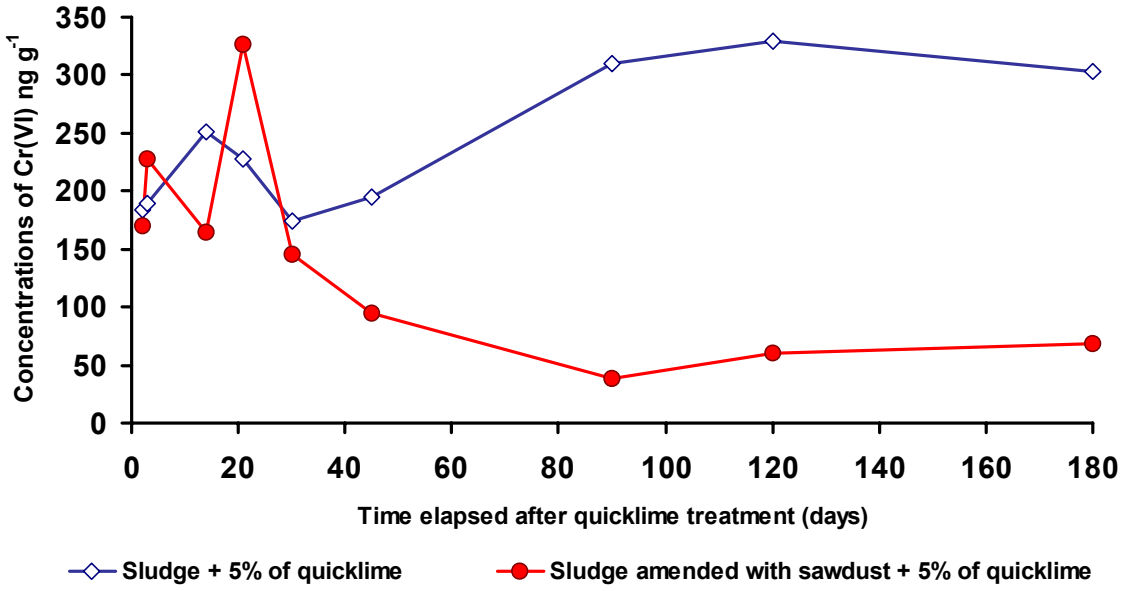

FIG. 1. Variation of Cr(VI) concentrations with time elapsed after quicklime treatment in aqueous extracts of sludge samples treated with 5% of quicklime and sludge samples amended with sawdust and treated with 5% of quicklime. Cr(VI) was determined by FPLC-ETAAS.

The data of Fig. 1 indicate that despite the highly reducing characteristics of the sewage sludge, liming caused oxidation of Cr(III) to Cr(VI) at pH 12, probably through the action of $MnOOH_{(s)}$ and dissolved oxygen. This prediction is based on the reported mechanisms and kinetics of Cr oxidation in synthetic solutions of Cr(III) by β-$MnO_{2(s)}$ in the pH range from 6.0 to 10.0[23] and the observed slow oxidation by dissolved oxygen in buffered solutions of Cr(III) in the pH range from 5.5-9.9[24]. Data from Fig 1 further indicate that the amount of Cr(VI) in lime-treated sludge slightly increased with time and after three months was almost constant (about 300 ng g^{-1} Cr(VI)). Nevertheless, the concentration of Cr(VI) was very low, representing only about 0.04 % of the total Cr. During the course of the experiment no significant changes in pH of these sludge extracts were observed. The concentration of Cr(VI) in the lime-treated sludge amended with sawdust increased in the first three weeks elapsed after lime treatment to about 300 ng g^{-1}, and appreciably decreased after one month to a concentration of about 50 ng g^{-1} Cr(VI) (Fig. 2). In the latter sludge extracts a decrease of pH from 12.0 at the start of the experiment to 9.6 after six months was also observed. The decrease in pH and reduction of Cr(VI) to Cr(III) was presumably caused by the release of organic acids from decaying sawdust.

CIM fast monolithic chromatography –ETAAS

Development of the procedure for determination of Cr(VI) in standard solutions of Cr2O72- and CrO42- by anion-exchange CIM fast monolithic chromatography — ETAAS and investigation of the behaviour of standard solutions of Cr(III) nitrate

The recently developed cation- and anion-exchange separation supports based on convective interaction media (CIM) offer very fast separation of biomolecules[17-19]. In general, CIM are produced in disc units and are also used for fast separation of organic acids[20]. In our group CIM discs were successfully applied for the speciation of zinc in soil extracts and wastewater samples[21]. In the present work the novel approach of the use of CIM fast monolithic chromatography was investigated for the speciation of chromium on anion-exchange discs. The study was performed on synthetic standard solutions containing 40 ng cm^{-3} of Cr(VI) ($Cr_2O_7^{2-}$ and CrO_4^{2-}) and 40 ng cm^{-3} of Cr(III) (aqueous solution of the nitrate salt) at pH 6.4, using strong QA and weak DEAE anion-exchange discs. It was demonstrated that a better separation of Cr(VI) from Cr^{3+} was obtained on weak DEAE anion-exchange discs. On strong QA anion-exchange discs Cr(VI) appeared immediately after the elution of Cr^{3+} and was not completely separated from this species. Therefore, an analytical procedure was developed and optimised to quantitatively separate Cr(III) from Cr(VI) on a weak DEAE anion-exchange disc at pH 4.0, 6.4, 8.0, 10.0 and 12. Various eluent solutions were investigated at pH 6.4. It was experimentally proven that gradient elution in 5 min from 0-100% of 2 mol dm^{-3} NaCl or 0-100% of 2 mol dm^{-3} NH_4Cl, did not quantitatively elute Cr(VI) from the disc. 2 mol dm^{-3} $CaCl_2$ eluent resulted in severe matrix interference effects in determination of separated chromium species by ETAAS. Good results were obtained with NH_4NO_3 eluent (gradient elution from 0-100% of 3 mol dm^{-3} NH_4NO_3 in 5 min). It was experimentally proven that Cr(VI) was reproducibly and quantitatively eluted from a CIM DEAE disc from 3.0 to 4.0 min with peak maximum eluting from 3.0 to 3.5 min when $Cr_2O_7^{2-}$ and CrO_4^{2-} standard solutions (40 ng cm^{-3} Cr(VI)) in a pH range from 4.0 to 12.0 were applied. In order not to disturb the equilibrium between chromium species in the synthetic standard solutions investigated, the eluents were buffered to the same pH as that of the sample analysed. The behaviour of Cr(III) (aqueous solution of nitrate salt, 40 ng cm^{-3} Cr) depended significantly on the pH. The percentage of Cr(III) eluting with the solvent front decreased with increasing pH due to formation of hydroxo-Cr(III) species which were partially adsorbed on the disc and partially eluted from 2.5 to 3.0 min, preventing the efficient separation of Cr(VI) from Cr(III). In order to assure effective separation of Cr(VI) from Cr(III) species,

isocratic elution of buffer A was applied in the first 5 min of the chromatographic run to efficiently elute weakly adsorbed Cr(III) species from the disc. Afterwards linear gradient elution from 0-100% buffer B was employed from 5 to 10 min as described in *2.5.* By applying the recommended chromatographic procedure quantitative and reproducible elution of Cr(VI) on CIM DEAE disc from 8.0 to 9.0 min with a maximum peak eluting from 8.0 to 8.5 min was obtained over a pH range from 4.0 to 12.0. In the same pH range Cr(III) did not disturb determinations of Cr(VI) since it was partially eluted with the solvent front and was not detected in the continuation of the chromatographic run. Therefore, the weak anion-exchange CIM DEAE disc enabled quantitative separation of Cr(VI) to be performed in the presence of aqueous Cr(III) species.

Linearity of measurement for the injected sample solution (0.5 cm^3) was obtained over a concentration range from 5.0 to 50.0 ng cm^{-3} of Cr(VI) with a correlation coefficient better than 0.998. The repeatability of measurement and efficiency of Cr(VI) separation was tested for six consecutive separations of a standard solution of Cr(VI) (40.0 ng cm^{-3}, pH=8.0). The recoveries were calculated on the basis of the ratio between the found and added Cr(VI) concentrations and ranged from 97 to 102%. The repeatability of measurement was found to be ± 2.0%. The LOD for the injected sample solution (0.5 cm^3) calculated on a 3 *s* basis (a value of three times the standard deviation of the blank) was 1.5 ng cm^{-3} of Cr(VI).

Validation of the procedure

Validation of the procedure was performed by the analysis of certified reference material CRM 544, Cr(VI) in lyophilised solution. After opening the vial, containing lyophilised solution, the sample was reconstructed with 20 cm^3 of HCO_3^-/H_2CO_3 buffer at pH 6.4. The analysis of the total Cr and Cr(VI) were performed immediately after sample reconstruction. Total Cr was determined by ETAAS. The average of six determinations was found to be 49.0 ng cm^{-3} with the standard deviation of measurement ± 0.7 ng cm^{-3}. The result agreed well with the reported certified value for total Cr 49.4 ± 0.9 ng cm^{-3}. Cr(VI) was determined by the anion-exchange FPLC-ETAAS procedure by injecting six sample aliquots on the column. The pH of buffers A and B used in chromatographic run were adjusted to 6.4. The average of six parallel determinations of Cr(VI) was found to be 23.8 ± 0.8 ng cm^{-3}. Good agreement between Cr(VI) determined and the reported certified value (22.8 ± 1.0 ng cm^{-3}) was obtained.

CONCLUSION

The applied anion-exchange FPLC-ETAAS procedure was found to be a reliable and sensitive technique for the determination of trace amounts of Cr(VI) in highly alkaline samples of lime-treated sewage sludge and suitable for investigation of oxidation-reduction processes of Cr in such a complex sample matrix.

The developed CIM fast monolithic chromatography –ETAAS procedure was found to be a reliable, sensitive and fast analytical procedure for the determination of trace amounts of Cr(VI) and will be applied in determination of Cr(VI) in natural waters.

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SELENIUM SPECIATION ANALYSIS

HOME-MADE CAPILLARY ELECTROPHORESIS COUPLED TO A FLOW SYSTEM FOR SPECIATION OF SE^{IV} AND SE^{VI} IN WATER SAMPLES

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Abstract A coupled FIA-CE system for inorganic selenium speciation with on-column direct UV detection is presented. An interface to couple capillary electrophoresis to the flow system was specially designed. The interface is an acrylic-made chamber, which accommodates a grounded tubular electrode and inlet/out-let channels. System performance was evaluated by analysing inorganic Se species in water samples. Since UV sensitivity was not enough to detect analytes in the considered sample, analytes spike was done. Recovery values from 70 to 95.3% were attained. Relative standard deviations calculated from signals of a water sample with 1,5 μg mL^{-1} of both species (n=5) were 8.32 and 6.48 % for Se(IV) and Se(VI), respectively. Other characteristics are analytical throughput of 15 h^{-1}, limits of detection of $8{,}3.10^{-4}$ and $2{,}7.10^{-3}$ mmol L^{-1} for Se (IV) and Se(VI), respectively.

Keywords: capillary electrophoresis, flow injection, interface, selenium speciation

INTRODUCTION

Selenium was considered a toxic element from its discovery at the beginning of the XIX century until middle of the XX century when its importance for health of living organisms was finally revealed [1]. This element is essential in lower concentrations, around 100 ng mL^{-1}, however it becomes toxic at higher concentrations [2,3]. The main natural source of selenium is metal-sulphur minerals but it can be also founded in the environment due to combustion of fossil fuels and to the widespread use in agriculture, electronics and glass industry [4,5]. Therefore, selenium represents a serious contamination problem not only in urban areas but also in country areas. Selenium inorganic forms, selenite and selenate, are the most commonly found species in polluted water and soils [6]. Since the gap between essentiality and toxicity of selenium is narrow, its harmfulness depends on the species [7]. Selenium exists as both organic and inorganic forms in several oxidation states. Thus, many efforts have been made to develop speciation methods in order to determine and to identify each form.

Techniques such as neutron activation and atomic absorption have been employed to determine selenium. Nevertheless, using these techniques the total element concentration is determined [8]. Other techniques such as fluorimetry [8], voltametric methods and hydride generation are sensitive just for Se (IV) [9,10]. However, there is an increasing demand for methods capable to separate and to identify simultaneously different forms of elements contained in a given sample, preferentially in a single analysis. Some hyphenated techniques that associate chromatographic procedures to GF-AAS or ICP-MS, which are very useful in speciation analysis, have been also employed to determine selenium [11]. In the last decade capillary electrophoresis (CE) has become one of most remarkable analytical separation techniques due to its simplicity, and speediness associated to high resolution efficiency. Despite the long-term applicability of CE in the analysis of organic species this technique was only employed for inorganic analysis from 1993 [12].

In 1997, two research groups simultaneously and independently proposed coupling capillary electrophoresis to a flow injection system [13,14]. Flow injection analysis (FIA) presents among its advantages features such as: reagent economy, high analytical throughput and on-line solution management including any sample pre-treatment (pre-concentration, dilution, standard addition, *etc.*) [15]. On its turn, capillary electrophoresis presents some shortcomings related to sample introduction mode and sensitivity, which were overcome by employing both on-line sample introduction and pre-concentration methods, respectively, provided by the flow systems.

Our group has recently published an article describing a FIA-CE-ICP-MS system [16] for chromium speciation in water samples. The three parts of the system were coupled by employing two interfaces specially designed, FIA-CE and CE-ICP-MS. In this article, the speciation of inorganic selenium species by employing the FIA-CE system is proposed and a further description of the FIA-CE interface is also presented. The system was evaluated analysing Se species in tap and natural spring waters by employing on-column direct UV detection.

EXPERIMENTAL

Reagents and solutions

All reagents were analytical grade and all solutions were prepared with deionized water obtained from a Milli-Q system (Millipore, Milford, MA). Stock solutions (1,26 mmol L^{-1}) of Se(IV) and Se(VI) were prepared from sodium selenite and selenate, respectively. Dibasic sodium phosphate (Mallinckrodt, Xalostoc, Mexico) was employed as background electrolyte. Cethyltrimethylammonium bromide (CTAB) (VETEC, Rio de Janeiro, Brazil) was used as electro-osmotic flow reverser. Acetonitrile (Mallinckrodt, California, USA) was added to the background electrolyte to modify the electrophoretic mobility of the analytes. Phosphoric acid (Merck, Rio de Janeiro, Brazil) was employed for pH adjustment.

Selenium working solutions were stored in the dark at 4 ^{o}C. The electrolyte consisted of a mixture of sodium dibasic phosphate ($Na_2HPO_4.H_2O$), Cethyltrimethylammonium bromide ($C_{19}H_{42}BrN$), acetonitrile (CH_3CN) and phosphoric acid (H_3PO_4), which were filtered and degassed. Reference solutions were prepared from suitable dilution of the stock solution.

Instrumentation

A peristaltic pump (Ismatec, model IPC), equipped with Tygon tubes, was employed to propel and to aspirate solutions. Polyethylene tubes (0.8 mm i.d.) were used as transmission lines.

A variable wavelength UV detector (LabAlliance, USA) was employed. for direct detection at 200 nm. Uncoated capillary columns (75 μm i.d., 375 μm o.d; Polymicro Technologies, Phoenix, USA) were used throughout the study. The effective length was 21 cm and the total length was 42 cm. The CE system was operated with no thermosetting control under constant voltage given by a high voltage power supply (Spellman, model CZE 1000R), which provided difference of potential up to 30 kV. A platinum electrode (anode), placed in a glass vial, and a tubular electrode (cathode), placed inside the interface, were used to establish electrical connections. All analyte electrofiagrams were generated and treated by a chromatographic station.

FIA and CE systems were connected using a cylindrical acrylic made chamber, whose design is depicted in Figure 1 in a three-dimensional view.

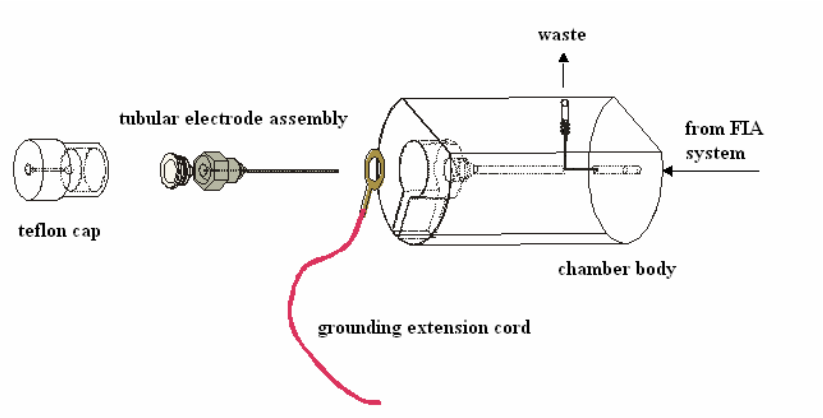

FIG. 1. Exploded perspective view of the FIA-CE chamber.

The tubular electrode (cathode) was screwed to the chamber by using a hexagonal key. A copper ring connected to the extremity of a high-tension cable was employed to close the electrical circuit. This ring was fixed between the tubular electrode and the chamber itself. A Teflon end-cap was screwed to the chamber used to isolate the metallic electrode from the system to prevent any current breakthrough. There are three channels, the first one is in where the tubular electrode (L = 52 mm) is inserted, the second one (L = 9.4 cm) is in where the capillary is inserted and the last one (L = 7.7 cm) connects the chamber to the FIA system.

Procedure

In Figure 2, a schematic diagram of the proposed system is presented. According to this figure, the anodic capillary end was introduced in a 10 mL glass reservoir, which was filled with the same buffer contained in the capillary. The capillary was introduced all the way through the UV detector, in which it was fastened with polyethylene tubing. The capillary "window" was held in position by a set of ferrules. The cathode extremity was concentrically introduced into the tubular electrode through the end-cap hole and it was placed in the halfway of the 9.4 x 0.8 mm channel.

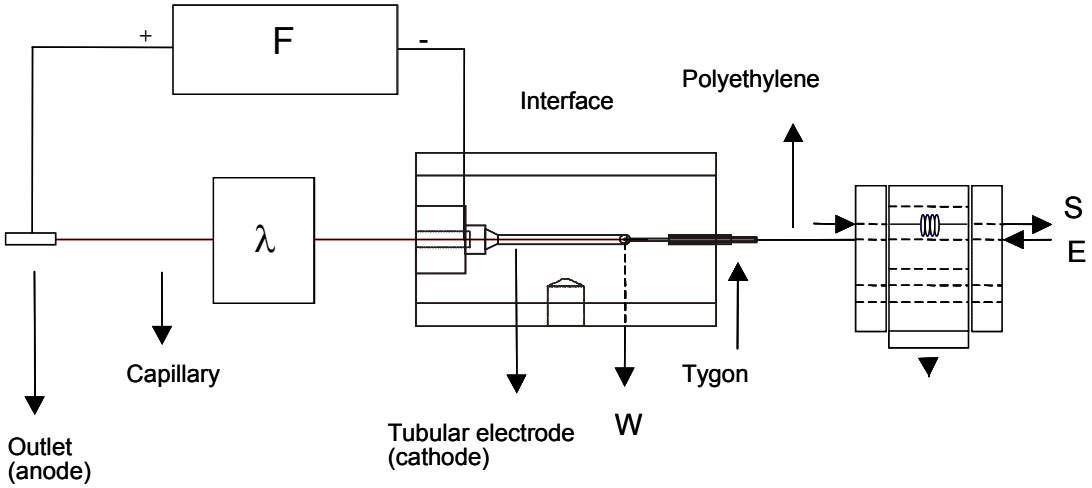

*FIG. 2. FIA-CE system. F: high-tension power supply; λ: detector; S: analyte solution; E: buffer solution; W: waste.*The platinum electrode, placed in the glass reservoir, was connected to the high voltage power supply. The tubular electrode (cathode) was grounded through the copper ring located in the extremity of the high voltage cable. The flow system was connected to the chamber by the chamber inlet (point x). The electrolyte solution continuously flows through the chamber and was discharged by the chamber outlet (point y). Concomitantly, analyte solution is aspirated to fill up the loop *L*. In the next step, the injector is commuted to the alternative position and the sample filled loop is inserted in the analytical path and the solution containing the analytes is transported by the background electrolyte solution into the chamber. When the analyte solution reaches the capillary inlet (cathode), the analyte ions are electrokinetically injected.

The bare silica capillary tube was firstly conditioned with 1.0 mol L^{-1} NaOH for 10 min, then with 0.1 mol L^{-1} NaOH for 5 min, followed by water rinse for 5 min. Afterwards, the capillary was washed with running buffer for 5 min prior analysis. After each five runs, a new conditioning was carried out. This conditioning consisted washing the capillary with buffer solution during 2 min. The running buffer was prepared with 20 mmol L^{-1} dibasic sodium phosphate, 0.5 mmol L^{-1} CTAB and 20% (v/v) acetonitrile at pH 8,5. The sample was electrokinetically injected at 10 kV. Separation procedure was performed at 10 kV and 238 V cm^{-1} at room temperature.

RESULTS AND DISCUSSION

The interface was acrylic-made not only because of the inertness of this material but also due its translucency, which allows the complete visualization of all phenomenons that might occur inside the interface. The channel dimensions inside the interface were designed to be as small as possible, specially the inlet capillary channel, to avoid excessive dispersion of the sample zone. Additionally a Tygon tube was used to wrap up the commercial tubular electrode in order to fill up the channel almost entirely. This procedure avoided the interface large internal volume, which could cause not only an undesired dispersion of the sample zone but also carryover effect during sample running. The principle of the proposed FIA-CE interface functioning is quite similar to those of the interfaces projected by Kuban *et al.*[13] and Fang *et al*.[14]. Structurally, however, the design of the present interface is closest to that proposed by Kuban *et al.* [13]. Both of them are closed systems, which is an advantage because it minimizes risks of contamination, especially when sample analytes present

concentration at trace level. Electrophoretic capillary was concentrically introduced into tubular electrode and this set was positioned against the interface inlet. Since flow profile in FIA systems is parabolic the most concentrated part of the sample could be easier introduced into the capillary allowing an analytical signal increase. The distance between sample loop and the capillary end was as short as it was physically possible in order to avoid severe sample dispersion during carrying. The interface outlet was located at the interface superior part to easily eliminate any air bubbles, which could be generated inside it. It should be pointed out this interface can carry out not only electrokinetic injection but also the hydrodynamic. To implement the last one it is only necessary to close the interface outlet so that sample solution is forced into the capillary.

Considering voltages up to 30 kV are currently used in CE, some security procedures were taken to protect the system operators from any possibility of electrical shock and also to prevent loss of current. First of all, the power supply was properly grounded using three 2.5 m copper bars, which were attached to each other and buried. The high-tension cable was connected to buffer reservoir, which was isolate from the rest of the system except by the capillary. All system components were made with no conductive material.

Since the investigated species are negatively charged, the electroosmotic flow had to be reversed in order to separate and detect Selenium species in a shorter time interval. A suitable concentration of a cationic surfactant, CTAB, was added to the electrolyte solution to modify the capillary wall. This surfactant is adsorbed as a double layer at the surface reversing the electrosomotic flow by creating a positively charged wall [17]. Acetonitrile was also added to the electrolyte solution to improve peak shape and resolution of Se species.

Selenite and selenate were analysed keeping constant sample introduction mode, which was electrokinetic, and employing direct on-column UV detection at 200 nm. Electrophoretic parameters such as pH, applied voltage and electrolyte concentration were evaluated. Flow parameters such as sample loop volume and electrolyte flow rate were also investigated.

Electrophoretic parameters

In capillary electrophoresis, pH is probably the most important parameter, which affects electroosmotic flow and electrophoretic mobility. Normally, the electrolyte is selected to provide the best separation and not necessarily the optimal electroosmotic velocity [18].

The influence of the pH was studied for the range between 7.5 and 9.5 and the pH was adjusted by adding to the buffer solution diluted phosphoric acid. Figure 3 presents the signal magnitudes and peak profiles of the selenium species as a function of pH. Good resolution for Se species was observed for all pH values. However, as it can be observed, as pH was increased resolution decreased but not enough to make an incomplete separation of the considered species. On the other hand, pH increasing led to a shorter analysis time. Additionally, higher pH values allowed sensitivity improvement especially for selenite considering its second pK value is 7.94. Considering the obtained results, a sodium dibasic phosphate electrolyte with pH 8.5 was chosen because it provided good separation selectivity in a reasonable analysis time.

The influence of phosphate sodium concentration ranging from 20 to 80 mmol L^{-1} was studied at pH 8.5. As ionic strength increases the eletroosmotic flow may increase due to current increments and consequently the temperature increase, which lowers the viscosity when there is no thermosetting device [18]. Despite the proposed system presents no

temperature control as the electrolyte concentration was increased an electroosmotic flow decrease was observed. According to VanOrman *et al.* [19], which found out that eletrosmotic flow is reduced as the electrolyte ionic strength is augmented. A 20 mmol L^{-1} sodium phosphate was selected since it allowed enhancing the analytical frequency; satisfactory signal magnitude and low baseline noise were obtained.

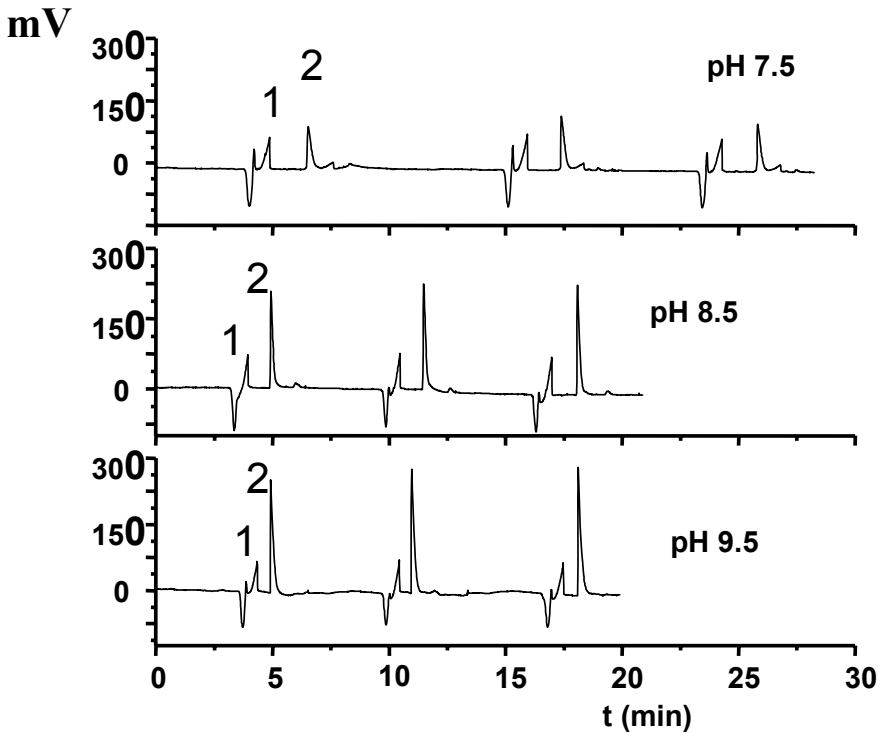

FIG. 3. Effect of pH on sensitivity, separation and migration of Se species. Peak 1 corresponds to Se(VI) and peak 2 to Se(IV). Peaks were obtained by injecting a mixed solution of 5 mg/L of each Se species. The electrical currents were 18, 20 and 22 μA for pH 7.5, 8.5 and 9.5 respectively

The simplest way to vary the electroosmotic flow is changing the voltage and consequently modifying the electric field [18]. Despite higher voltages lead to a shorter analysis time and to a higher efficiency, it was not possible to surpass 10 kV once the selenate peak presented an overlap from a vacancy peak. Although analytical throughput has been harmed, 10 kV was selected as separation voltage to avoid any possibility of peak overlapping.

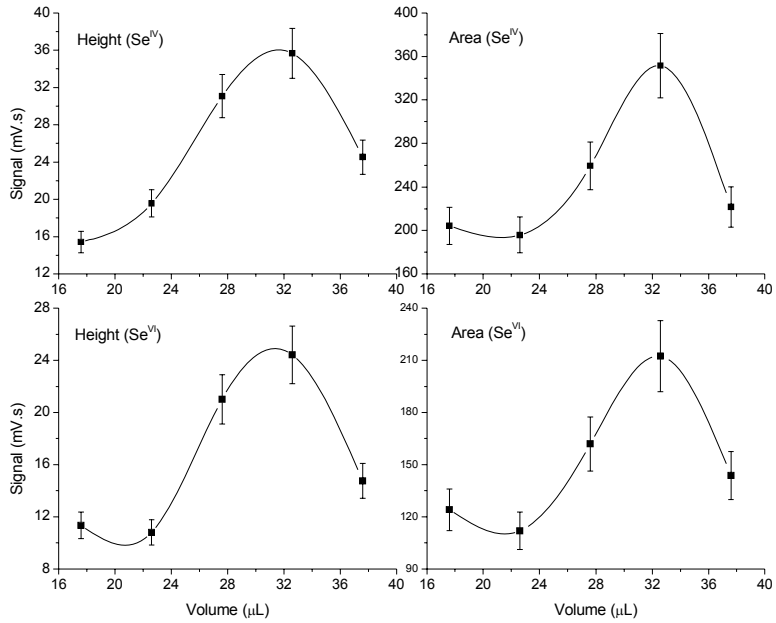

FIG. 4. Effect of the buffer flow rate. Area and height for Se^{IV} and Se^{VI}. Buffer solution: 20 mmol L^{-1} sodium dibasic phosphate, 20 % (v/v) acetonitrile, 0,5 mmol L^{-1} cethyltrimethylammonium bromide; pH = 8,5; sample loop: 17,6 μL; λ = 200 nm; electrokinetic injection, capillary column: 40 (20 cm efective), 75 μm i.d.; applied voltage = 5 kV, I = 36 μA, standard solution: 1,26.10-1 mmol L^{-1} (10 μg mL^{-1}) Se(VI) and Se(IV).

Flow parameters

One of the variances that contributes to sample zone enlargement is the variance due to the injection system, σ^2_{inj}, given by $\sigma^2_{inj} = l^2_{inj}/12$ in which l_{inj} is the length of the sample plug in the capillary [20]. The previous equation shows, as l_{inj} becomes larger the σ^2_{inj} increases, indicating the zone width get wider and peak broadening. Thus, it is possible to observe in Fig. 4, as the sample volume introduced into the interface was increased there was an analytical signal increasing up to 32.6 μL. From this point the signal magnitude started to decrease in terms of both height and area. The higher sample loop volume the spreader sample zones. Thus, sample zones remains a longer time in front of capillary extremity, which means a greater quantity of sample is injected into capillary. In 75 μm i.d. capillaries, the typical sample zone spreading is ranged from 1 to 2 mm. Inside the capillary, sample aliquots acquire the Gaussian distribution due to diffusion process [18]. In according to Huang *et al.* [21] sample zone spreading is mainly caused by peak enlargement if it is higher than the extension of the analyte diffusion. In the proposed system, the sample zone spreading depends on the sample injection delay, which depends on carrier flow rate (buffer solution). Vinther and Søeberg [22] affirmed that peak efficiency sensitively decreases when time injection is increased. It is also necessary to consider the solute in the sample should be hundred times less than concentration of the electrolyte [23]. High concentrations of the solute ions can

produce distortion in the electric field, which causes deformation of peak shapes. This could explain the peak height and area decrease when a 37.7 μL sample was injected. Considering the obtained results a 32.6 μL sample loop was chosen.

As it can be seen in Fig. 5, the higher the flow rate the less height and area in the analytical signals. In the case of extremely low flow rate an excessive sample zone occurred which reflected in peak height decrease and area increase. On the other hand, high electrolyte flow rates hinder the analytes remain time enough in front of the capillary to be introduced into it. Thus, a few ions were able to get inside the capillary. Considering this aspect, a flow rate of 165 μL min^{-1} was selected since it provided an analytical signal with a suitable magnitude. The flow rate was selected considering the pressure, which could be generated inside the chamber. High flow rates could even cause rupture of FIA connections or also cause solution leakages. However, it is necessary to consider that flow rates excessively low could reduce not only analytical throughput but also to produce severe sample zone enlargement. Therefore, it must be a compromise between these two parameters.

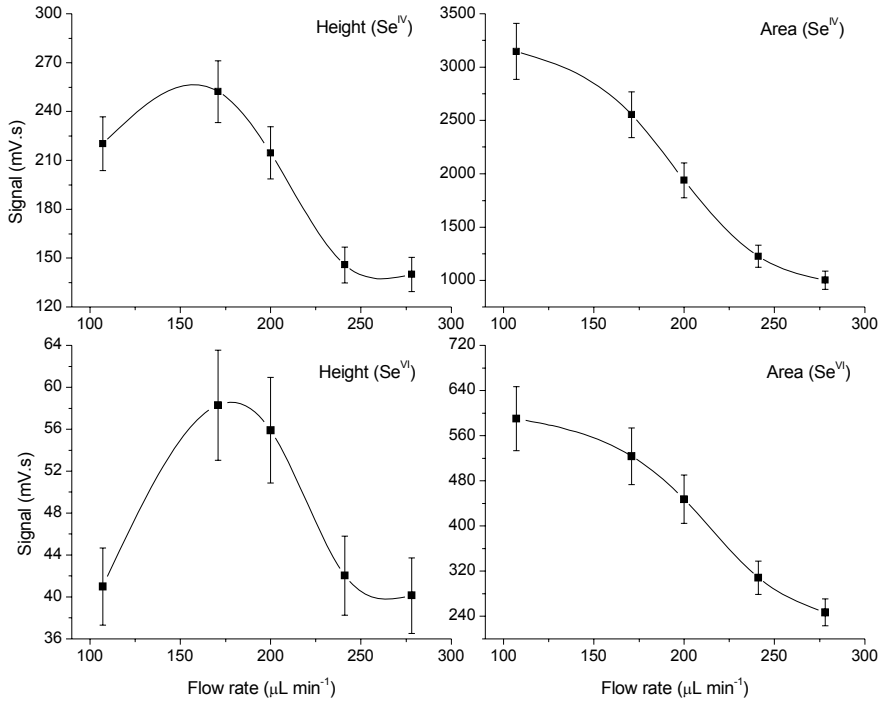

FIG. 5. Effect of the sample loop volume. Area and height for Se^{IV} and Se^{VI}.

The applicability of the proposed FIA-CE system for selenium speciation in real samples was verified by analyzing water samples. Unfortunately, selenium concentrations of both species in the considered samples were too low to be detected by UV. Thus, the mineral water samples were spiked with 1.5 μg mL^{-1} of both selenium species and tap water was spiked with 3 μg mL^{-1} of both selenium species. For each water sample three replicates were prepared. Afterwards, all water samples were analysed in duplicate. The obtained recoveries for Se(IV) and Se(VI) are presented in Table I. As it can be observed the analyte recoveries

were satisfactory except for Se(VI) from Mineral Water I. The other recoveries were ranging from 70 to 95.3%.

Relative standard deviations, calculated for a water sample with 1,5 μg mL^{-1} of both species (n=5), were 8.32 and 6.48 % for Se(IV) and Se(VI), respectively. Limits of detection, calculated in accordance to IUPAC [24] were $8{,}3.10^{-4}$ and $2{,}7.10^{-3}$ mmol L^{-1} and limit of determination [25], calculated by using k=10, were $2{,}7.10^{-3}$ and $9{,}2.10^{-3}$ mmol L^{-1} for Se (IV) and Se(VI), respectively.

TABLE I. RECOVERY VALUES FOR SE SPECIES IN WATER SAMPLES

Samples	Recovery (%) *	
	Se^{IV}	Se^{VI}
Mineral water I	70.66 ± 1.15	34.63 ± 5.78
Mineral water II	86.63 ± 1.15	81.50 ± 13.37
Tap water	77.86 ± 6.31	71.40 ± 3.15

* (n=3)

CONCLUDING REMARKS

The FIA-CE system described in this article was successfully assembled, since inherent problems related to electrophoretic systems such as current loss, grounding, and electrical isolation were suitably overcome. Other shortcomings related to the differences between electrophoretic and flow systems, which are micro and macro-scaled, respectively, in terms of sample volume and flow rate were also overcome. Careful investigation of all parameters involved in the FIA-CE coupling allowed their optimization obtaining calibration data with good linearity and satisfactory reproducibility for inorganic selenium speciation. Both forms were separated in less than 8 min by using CTAB to reverse the electroosmotic flow. The main advantage of the proposed interface is that it facilitate the sample introduction with no interruption of the applied voltage during sample loading step.

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