Atomic Spectroscopy

May/June 2005 Volume 26, No. 3

In This Issue:

ASPND7 26(3) 89–130 (2005)
ISSN 0195-5373

Issues also available electronically.

(see inside front cover)

Atomic Spectroscopy
Vol. 26(3) May/June 2005

Printed in the United States and published six times a year by:

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
http://www.perkinelmer.com

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Speciation of Chromium With Cloud Point Extraction Separation and Determination by ICP-OES

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ABSTRACT

A sensitive and selective method has been developed for the speciation of chromium in natural water, based on cloud point extraction (CPE) and inductively coupled plasma optical emission spectrometry (ICP-OES). Cr(III) reacts with 1-phenyl-3-methyl-4-benzoylpyrazol-5-one (PMBP), yielding a hydrophobic complex which is entrapped in the surfactant-rich phase, whereas Cr(VI) remains in the aqueous phase. Thus, separation of Cr(III) and Cr(VI) can be realized. Total chromium was determined after reduction of Cr(VI) to Cr(III) with ascorbic acid, the reducing reagent. The detection limit for Cr(III) was 0.81 µg L^{-1}, with an enrichment factor of 20 and a relative standard deviation (RSD) of 3.2% (n=11, C=100 µg L^{-1}). The proposed method was applied to the speciation of chromium in natural water samples with satisfactory results.

INTRODUCTION

In recent years, there has been an increasing demand for information about speciation because the toxicity of some elements depends on their chemical form (1). Dissolved chromium is usually found in natural waters in two different oxidation states, such as Cr(III) and Cr(VI), which have contrasting physiological effects. Cr(III) is considered an essential trace element for the maintenance of an effective glucose, lipid, and protein metabolism in mammals (2). On the other hand, Cr(VI) can be toxic for biological systems (3,4). It is water-soluble and extremely irritating and toxic to human body tissue owing to its oxidizing potential and permeability of biological membranes (5,6). Therefore, it is extremely importance to accurately define the quantity of both valence forms in environmental samples. A great number of speciation studies of chromium have been carried out in solid and liquid samples (7,8).

The Cr content in natural waters is normally at the low µg L^{-1} levels. Few analytical techniques are capable of the direct differentiation of the chemical forms of Cr in water. The direct determination of chromium by inductively coupled plasma mass spectrometry (ICP-MS) is possible, but these instruments are expensive and not commonly available in an analytical laboratory. As a result, preliminary species separation and preconcentration is required before their detection by sensitive analytical techniques (9). For the speciation of chromium, the separation methods reported in the literature are usually based on coprecipitation (10), solvent extraction (11), solid phase extraction (12,13), and ion chromatography (14).

Separation and preconcentration based on cloud point extraction (CPE) is becoming an important and practical application of surfactants in analytical chemistry (15,16). The technique is based on the property of most non-ionic surfactants in aqueous solutions to form micelles which become turbid when heated to a temperature known as the cloud point temperature. Above the cloud point temperature, the micelle solution separates into a surfactant-rich phase of small volume and a diluted aqueous phase, where the surfactant concentration is close to the critical micelle concentration (CMC). The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, low cost, highly efficient, fast, and of lower toxicity to the environment than those extractions that use organic solvents. Any species that interacts with the micelle system, either directly (generally hydrophobic organic compounds) or after a prerequisite derivatization reaction (e.g., metal ions after reaction with a suitable hydrophobic ligand), may be extracted from the initial solution and preconcentrated.

Cloud point methodology has been used to separate and preconcentrate organic compounds as a step prior to their analysis in hydrodynamic analytical systems such as liquid chromatography (17) and capillary electrophoresis (18). The phase separation phenomenon has also been used for the extraction and preconcentration of metal ions after the formation of sparingly water-soluble complexes (19,20). Recently, CPE as a preconcentration step in conjunction with detection by FIA spectrofluorimetry, FAAS, ETAAS, and HPLC for the speciation analysis of Cr, Cu, and Fe species has also been reported (21–28).

The aim of the present work was to apply CPE as a separation and preconcentration step for inductively coupled plasma optical emission spectrometry (ICP-OES) speciation of chromium. In the developed system, 1-phenyl-3-methyl-4-benzoylpyrazol-5-one (PMBP) was used as the chelating agent and Triton® X-100 as the extractant. The experimental para-

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meters affecting the CPE separation/preconcentration and the subsequent ICP-OES determination of the chromium species were investigated in detail. The proposed method has been applied to the determination and speciation of chromium in tap and lake water samples with satisfactory results.

EXPERIMENTAL

Instrumentation

A PerkinElmer Optima™ 2000 DV ICP spectrometer (PerkinElmer Analytical and Life Sciences, Shelton, CT, USA), equipped with the standard torch assembly and conventional cross-flow nebulizer, was used for the determination of chromium. The instrumental operating conditions and the analytical wavelength used are listed in Table I. The pH values were measured with a Mettler Toledo 320-S pH meter [Mettler Toledo Instruments (Shanghai) Co. Ltd., Shanghai, P.R. China], supplied with a combined electrode. A thermostated bath maintained at the desired temperatures was used for the cloud point experiments. An 80-2 centrifuge (Changzhou Guohua Electric Appliance Co. Ltd., P.R. China) was used to accelerate the phase separation.

Standard Solutions and Reagents

Stock solutions (1.000 g L^{-1}) of Cr(III) were prepared by dissolving $CrCl_3 \cdot 6H_2O$ (The First Reagent Factory, Shanghai, P.R. China) in 0.1 mol L^{-1} hydrochloric acid. Stock solutions (1.000 g L^{-1}) of Cr(VI) were prepared by dissolving $K_2Cr_2O_7$ (The First Reagent Factory, Shanghai, P.R. China) in 0.1 mol L^{-1} nitric acid. The non-ionic surfactant Triton X-100 was obtained from Amresco and was used without further purification. A 1.0×10^{-2} mol L^{-1} solution of PMBP was prepared by dissolving appropriate amounts of this reagent in absolute ethanol from the commercially available product. A buffer solution of 0.1 mol L^{-1} NaAc-HAc was used to control the pH of the solutions. A 10% aqueous ascorbic acid solution was prepared fresh daily. All other reagents were of analytical reagent grade or better. Doubly distilled water was used throughout the study. The pipettes and vessels used were kept in 10% nitric acid for at least 24 h and subsequently washed four times with doubly distilled water.

Cloud Point Extraction Procedure

For CPE, 10-mL aliquots of a solution containing the analytes, Triton X-100, and PMBP, buffered at a suitable pH, were kept in the thermostatic bath and maintained at 80°C for 25 min. Since the surfactant density is 1.07 g mL^{-1}, the surfactant-rich phase can settle through the aqueous phase. Phase separation was accelerated by centrifuging the solution at 3000 rpm for 5 min. After cooling in an ice-bath, the surfactant-rich phase became viscous and was retained at the bottom of the tube. The aqueous phases can readily be discarded simply by inverting the tube. In order to decrease the viscosity and facilitate sample handing prior to the ICP-OES assay, 0.5 mL 0.1 mol L^{-1} HNO_3 was added to the surfactant-rich phase. The final solution was introduced into the nebulizer of the spectrometer by conventional aspiration.

Sample Analysis

Tap water was collected in our laboratory. Lake water was collected from East Lake, Wuhan, P.R. China. All water samples were filtered through a 0.45-mm membrane filter (Tianjin Jinteng Instrument Factory, Tianjin, P.R. China) and stored at 4°C. The water sample must not be acidified before storage because this would change the chemical species (29).

The water sample was divided into two parts:

- Cr(III) determination: To a 10-mL water sample, 1.0 mL of a solution (containing 36 g L^{-1} Triton X-100 and 1.0×10^{-2} mol L^{-1} PMBP) and 1.0 mL of 0.1 mol L^{-1} NaAc-HAc buffer solution (pH 5.0) were added for CPE separation. After phase separation, 0.5 mL 0.1 mol L^{-1} HNO_3 was added to the surfactant-rich phase. The final solution was analyzed by ICP-OES.
- Total chromium determination: To a 10-mL water sample, 0.1 mL 10% aqueous ascorbic acid was added. Then it was assayed as described above.

TABLE I
ICP-OES Instrumental Operating Conditions and Analytical Wavelength

Incident power	1100 W
Plasma gas (Ar) flow rate	15 L min^{-1}
Auxiliary gas (Ar) flow rate	0.5 L min^{-1}
Nebulizer gas (Ar) flow rate	0.8 L min^{-1}
Observation height	15 mm
Integration time	3 s
Solution pump rate	0.8 L min^{-1}
Wavelength	Cr 283.5 nm

RESULTS AND DISCUSSION

Effect of pH on CPE of Cr(III) and Cr(VI)

The separation of metal ions using the CPE method involves the prior formation of a complex with sufficient hydrophobicity in order to be extracted into the small volume of the surfactant-rich phase, thus obtaining the desired preconcentration. The formation of a metal complex and its chemical stability are the two important factors influencing CPE. The pH plays a unique role on the metal complex formation and subsequent extraction, and it proved to be a critical parameter for the determination of the chromium species.

Figure 1 shows the effect of pH on the extraction of Cr(III) and Cr(VI). It can be seen that extraction was quantitative for Cr(III) in the pH range of 3.0~7.0, and Cr(VI) could not be extracted in the study pH range. In order to separate Cr(III) and Cr(VI), a pH of 5.0 was selected for subsequent work.

Effect of PMBP Concentration

A 10-mL solution containing 1.0 µg of Cr(III) in 3.0 g L^{-1} Triton X-100, at a medium buffer of pH 5.0 containing various amounts of PMBP, was subjected to the cloud point preconcentration process. The extraction yield for Cr(III) as a function of the concentration of the chelating agent is shown in Figure 2. The yield increases up to a PMBP concentration of 5×10^{-4} mol L^{-1} and reaches near quantitative extraction efficiency. A concentration of 8×10^{-4} mol L^{-1} was chosen to account for other extractable species that might potentially interfere with the assaying of Cr(III).

Effect of Triton X-100 Concentration

A successful CPE would be that which maximizes the extraction efficiency through minimizing the phase volume ratio, thus maximizing its concentrating factor. The variation in the extraction efficiency of Cr(III) within the Triton X-100 range of 0.1~6.0 g L^{-1} was examined and the results are shown in Figure 3. Quantitative extraction was observed when the Triton X-100 concentration was above 3.0 g L^{-1}. A concentration of 3.0 g L^{-1} was chosen as the optimum surfactant concentration in order to achieve the highest possible extraction efficiency.

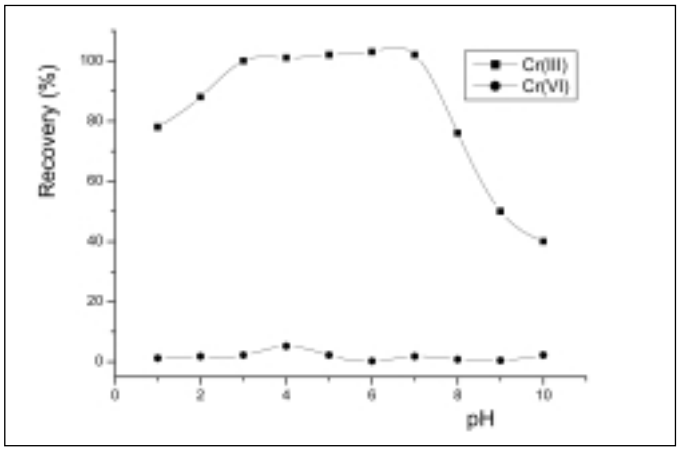

Fig. 1. Effect of pH on the extraction recoveries of Cr(III) and Cr(VI)100 ng mL^{-1} Cr(III) and Cr(VI); 8×10^{-4} mol L^{-1} PMBP; 3.0 g L^{-1} Triton X-100.

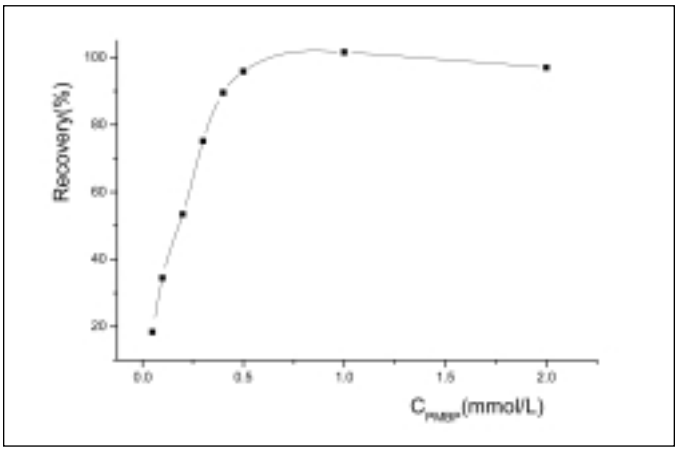

Fig. 2. Effect of PMBP concentration on the extraction recovery of Cr(III) 100 ng mL^{-1} Cr(III); 3.0 g L^{-1} Triton X-100; pH 5.0.

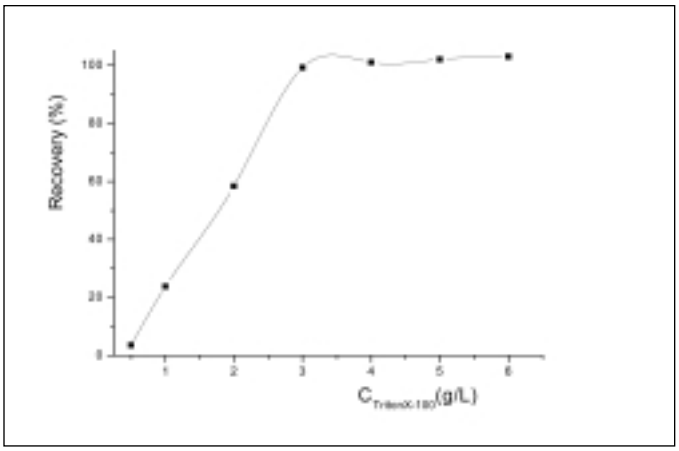

Fig. 3. Effect of Triton X-100 concentration on the extraction recovery of Cr(III)100 ng mL^{-1} Cr(III); 8×10^{-4} mol L^{-1} PMBP; pH 5.0.

Effects of Equilibration Temperature and Time

It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of phases. The dependence of extraction efficiency upon equilibration temperature and time was studied ranging from 60–120°C and 5~30 min, respectively. The results showed that an equilibration temperature of 80°C and an equilibration time of 25 min were adequate to achieve quantitative extraction.

Effect of Viscosity on ICP-OES Determination

In order to decrease the viscosity of the surfactant-rich phase and to facilitate its handling and introduction into the nebulizer of the spectrometer, some solutions, such as 0.1 moL L^{-1} HNO_3, methanol, and ethanol alone or containing 0.1 moL L^{-1} HNO_3, have been used as the diluent after separation of the two phases. Due to the difficulties of introducing organic solvents into the plasma, 0.1 moL L^{-1} HNO_3 was chosen as the diluent for this study. For added volumes of 0.1 moL L^{-1} HNO_3 larger than 0.5 mL, the final solution has no influence on the plasma by conventional aspiration. Thus, 0.5 mL 0.1 moL L^{-1} HNO_3 was added to the surfactant-rich phase after CPE.

Interferences

In view of the high selectivity provided by ICP-OES, the only interferences studied were those related to the preconcentration step. Cations that may react with PMBP and are extracted to the micelle phase were studied. The tolerance limits of the coexisting ions, defined as the largest amount resulting in the recovery of Cr(III) less than 90%, are given in Table II. It can be seen that the major cations in the water samples have no obvious influence on CPE of Cr(III) under the selected conditions.

Detection Limits and Precision

According to IUPAC definition, the detection limit (3σ) of this method for Cr(III) with an enrichment factor of 20 is 0.81 µg L^{-1}; and the relative standard deviation (RSD) is 3.2% (n=11, C=100 µg L^{-1}).

Sample Analysis

The accuracy of the proposed method was examined by determining total chromium in environmental water reference materials (ERMs, GSBZ 5009-88, P.R. China). The analytical value (637±40 µg L^{-1}) was in good agreement with the certified value (640±36 µg L^{-1}).

The proposed method was applied to the speciation of Cr(III) and Cr(VI) in tap and lake water samples collected in Wuhan, P.R. China. For calibration purposes, the working standard solutions were subjected to the same preconcentration procedure as used for the analyte solutions. In addition, the recovery experiments of different amounts of Cr(III) and Cr(VI) were carried out. The results in Table III show that the recoveries were reasonable for trace analysis, ranging from 96–104%.

CONCLUSION

The feasibility of chromium speciation in water has been demonstrated based on cloud point extraction of Cr(III) with PMBP in the presence of the surfactant Triton X-100 and sequential determination by ICP-OES. The developed method is definitely simple, reproducible, and highly sensitive, because of the distinct and advantageous features of CPE (*in situ* and single-step extraction). The method was successfully applied to the speciation of chromium in tap and lake water samples, and the precision and accuracy of the method are satisfactory. The method may also be used for the speciation of chromium in matrices other than water.

Received August 27, 2004.

TABLE II
Effect of Coexisting Ions on the Determination of Cr(III) (100 ng mL^{-1})

Coexisting Ions	Tolerance Limit of Ions
K^+, Na^+, Ca^{2+}	1000 mg L^{-1}
Mg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}	100 mg L^{-1}
Mn^{2+}, Zn^{2+}	10 mg L^{-1}

TABLE III
Determination of Cr(III) and Cr(VI) in Natural Water Samples

Samples	Added (µg L^{-1})		Found[a] (µg L^{-1})			Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)[b]	Total	Cr(III)	Cr(VI)
Tap water	0	0	4.53	1.85	6.38	–	–
	5.0	5.0	9.56	6.63	16.19	101	96
	10.0	10.0	14.20	12.05	26.25	97	102
Lake water	0	0	3.17	5.52	8.69	–	–
	5.0	5.0	8.06	10.47	18.53	98	99
	10.0	10.0	13.57	15.32	28.89	104	98

[a] Mean of five determinations. [b] Calculated value.

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Direct Generation of Stibine From Slurries and Its Determination by ETAAS Using Multivariate Optimization

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ABSTRACT

A simple and fast analytical method combining slurry preparation, hydride generation, and trapping on iridium-treated graphite tubes is presented to determine Sb in soil samples by electrothermal atomic absorption spectrometry (HG-ETAAS). Chemometric optimization of the slurry preparation and generation of stibine was carried out (Plackett-Burman designs and Simplex optimizations). Slurries were prepared in 9 mol L^{-1} HCl, and stibine was generated using 0.4 mol L^{-1} HCl and 2.5% (m/v) $NaBH_4$ (150 mL min^{-1} Ar flow). Further, a heated quartz tube atomization system was used (HG-AAS).

The slurry HG-ETAAS method was validated with nine certified reference materials: three soils, two sediments, two coal fly ashes, and two coals. The lowest LOD obtained was 0.08 µg g^{-1} and good overall precision was achieved (RSD <7%). The coal samples required a previous ashing step (450°C, up to constant weight). The analyte extraction to the liquid phase was also studied.

INTRODUCTION

Up to the present, hydride generation coupled to atomic absorption spectrometry (HG-AAS) has been the most convenient technique used to determine hydride-forming elements in aqueous samples and digested solid samples (1). The chemical vapor generated from slurried samples has been an attractive methodology since the early 1980's because (a) the combination of using slurries and hydride generation offered a simple and fast sample pretreatment step (better than conventional methods), (b) selectivity and sensitivity were good enough to determine low concentrations of analytes in complex matrices, (c) there is low risk of sample contamination, and (d) there is low analytical cost (2). It was Haswell et al. (3) who first determined arsenic in solid environmental samples as slurries by HG-AAS. This approach has also been applied to the determination of lead (4–7) and antimony (8).

Hydride generation and graphite furnace, employing tubes coated with permanent modifiers, lead to in situ trapping of the vapors generated directly from the samples. In this case, the graphite tube acts either as a pre-concentration or as an atomization medium. This methodology was reviewed by Matusiewicz and Sturgeon (9) who emphasized its advantages, namely achieving higher sensitivity and less prone to interferences. They reported that many works focused on the use of permanent modifiers as trapping agents, with the Pt-group of elements (Ir, Zr, Nb, and W) as the most employed. Degradation of the coating of the atomizer (and its control) is of great concern because it is possible to enlarge the useful analytical lifetime of the tube by recoating the atomizer. Additionally, other operational conditions must be considered, including the automatic control of the capillary transfer line.

Only recently has an extension of this methodology been developed which allows the analysis of solid samples in the form of slurries. Flores et al. (10) determined Hg in coal slurries (1 mol L^{-1} HNO_3) by HG-ETAAS (Au as the permanent modifier), where particle size and leaching time are critical. The combination of batch hydride generation and in situ preconcentration in a commercial device demonstrated its advantages to quantify As, Sb, Se, Sn, and Hg in beer and wort (11). Ir-treated graphite tubes were used with satisfactory results to determine As, Bi, Ge, and Se (12,13) in environmental and biological samples as well as As in sediments and coal slurries (14).

Over the last several years, some authors coupled slurry-hydride generation and atomic emission (15,16) or used atomic fluorescence spectrometry (17) for multielemental analysis. Hydride generation from solid samples also revealed itself as a suitable procedure for metal speciation (2). The sequential and on-line extraction of Sb(III) and Sb(V) from blood and liver tissues was reported for HG-AAS determination (18). A method to determine As(III) and total inorganic As in inorganic (marine sediment, soil, rock salt) and biological samples without sample digestion required a continuous flow of hydride into the graphite tube (19).

To the best of our knowledge, the slurry-HG-ETAAS methodology has not yet been applied to the determination of Sb in environmental matrices. Such a procedure would be useful to analyze environmental matrices and monitor anthropogenic input of heavy met-

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als in the environment because, as already reported (20), Sb is a good tracer of pollution in soils and sediments. Therefore, a new analytical method combining slurry preparation, hydride generation (batch system), and trapping of the vapors on an Ir-coated graphite furnace tube was implemented. Robustness was sought out by using chemometric tools: 8-trial Plackett-Burman fractional factorial experimental designs to extract the significant variables for slurry preparation and Simplex optimization. Two atomization modes were studied as well: Ir-coated graphite tubes (HG-ETAAS) and heated quartz tubes (HG-AAS). In contrast to previous works (12), a true optimization algorithm was used (Simplex) and all of the main analytical stages were reviewed to (a) assess the influential variables and (b) to optimize them by taking their intrinsic interaction into account.

EXPERIMENTAL

Instrumentation

A PerkinElmer AAnalyst™ 800 atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) was employed for sample analysis, equipped with Zeeman background correction, AS-800 autosampler, Sb electrodeless discharge lamp (415 mA, 217.6 nm and 0.7 nm spectral bandwidth), and pyrolytically coated graphite tubes with pre-inserted platforms, treated with Ir. A PerkinElmer MHS-10 hydride generator was coupled to the autosampler quartz capillary using a PTFE transference line.

A vibrating ball mill, Pulverisette 7 Fritsch Planetary Micro Mill, (Fritsch Industries, Idar-Oberstein, Germany), equipped with zircon cups and zirconia balls, was used to reduce the particle size. A J2-MC Beckman centrifuge (Fullerton, CA, USA) was employed when necessary.

Reagents and Standard Solutions

All reagents were of analytical grade. High-purity water (Milli-Q™ Water System, Millipore, Madrid, Spain) was employed throughout. The acids (HNO_3 and HCl) were Baker Instra-analyzed grade (J.T. Baker, Phillipsburg, PA, USA).

The Sb(III) stock solution (1000 g L^{-1}) was from Panreac (Barcelona, Spain) and working Sb standards were prepared daily by diluting appropriate aliquots of the stock solution.

Sodium tetrahydroborate (Merck, Darmstadt, Germany), dissolved in 2% (m/v) sodium hydroxide (Panreac), was used as the reducing agent, prepared daily and filtered before use. A 5.5-g L^{-1} iridium chloride solution was prepared from solid reagent (99.9%, Aldrich, Milwaukee, WI, USA) and injected onto the graphite tubes to provide a trapping surface.

All glassware, plastic ware, pipette tips, and storage bottles were soaked in 10% (v/v) HNO_3 for 24 h and then rinsed with high-purity water at least three times prior to use.

Nine certified reference materials (CRMs) were used to assess accuracy (trueness): two NCR CRM soils (GBW 07401 and GBW 07409; National Research Council of Certified Reference Materials, P.R. China), one NCR CNRC marine sediment (BCSS-1, National Research Council of Canada), two BCR CRM geological materials (calcareous loam soil, CRM 141, and estuarine sediment, CRM 277, EU Community Bureau of Reference), four NIST coal-related materials (coal fly ash 1633a and 1633b, coals 1632c and 1435, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Analytical Procedure

The slurries were prepared by suspending 0.5 g of a sample in 9 mol L^{-1} HCl, adding 1 mL of ethanol (to wet the particles), and homogenizing magnetically. The final volume was 10 mL or 20 mL depending on the Sb concentration in the sample.

Stibine was generated using a batch system in order to avoid eventual blockage in the manifold and the connectors (frequently when continuous systems are used). A slurry aliquot (0.1–0.2 mL) was placed in the reaction vessel and acidified to 0.4 mol L^{-1} with HCl. Next, a 2.5% (m/v) $NaBH_4$ solution was added (0.38 mL min^{-1}) and an Ar flow rate of 150 mL min^{-1} applied to improve contact between slurry and reductant. For HG-ETAAS, Ar sweeps the stibine through the transfer line and to the autosampler quartz capillary, and then introduces it into the graphite tube (1000ºC, 30 s). The hydride is retained on the Ir-coated graphite tube and then atomized at 2300ºC for 5 s. Table I summarizes the experimental conditions. For HG-AAS analysis, the hydride vapor was directly introduced into the heated quartz cell.

TABLE I
Experimental Conditions for the Direct Generation of Stibine From Slurries and Its Determination by HG-ETAAS

Slurry Aliquot	0.1–0.2 mL
Reaction Volume	5 mL
$NaBH_4$	2.5 % (m/v)
HCl	0.40 mol L^{-1}
Ar Flow	150 mL min^{-1}
Atomizer	Ir-coated graphite tube
Wavelength/Slit	217.6 (0.7) nm
Lamp Current	415 mA

RESULTS AND DISCUSSION

HG-AAS

All of the variables influencing the slurry preparation and stibine generation, as well as the instrumental conditions, were carefully examined in order to measure Sb by HG-AAS. An 8-trial Plackett-Burman design revealed their influence on the slurry preparation (preliminary studies not shown here) as follows: (a) volume of HNO_3 and HCl to prepare the liquid media, (b) particle size (grinding, as a binary variable yes/no), and (c) use of the ultrasonic bath (to homogenize the slurries and increase analyte extraction). Only the HCl volume was a statistically significant factor. Accordingly, a univariate scheme was adequate for optimization and 3-mL of HCl (c) was found to be the optimum volume.

In order to evaluate the analytical figures of merit, both an aqueous and standard additions calibration were performed. The latter mode showed an undesirable double peak; the explanation could be that the Sb added by the spikes leads to a very easy-to-release stibine, which is atomized first and thus yields a frontal narrow peak. However, stibine generated from the Sb in association with the solid particles will be released a little later and will lead, presumably, to the broadest profile. This is due to the different kinetic mechanisms involved in stibine generation and depends on whether the analyte is in the solid or liquid phase (21). No samples were quantified by this procedure.

HG-ETAAS

The unsatisfactory results obtained using the methodology described above, even for the calibration standards, made it necessary to look for an alternative for the determination of Sb. Although vapor trapping in pre-coated graphite tubes has been reported as a suitable way for trace metal determination in slurries (10,13), only one reference was found for measuring Sb in solid environmental samples (11). In the present work, stibine was generated directly from soil/sediment slurries, trapped in an Ir pre-coated atomizer, and Sb was determined by electrothermal atomization. Two main stages needed to be studied, such as slurry preparation and hydride generation.

Slurry Preparation

A saturated Plackett-Burman experimental design was employed to study the slurry preparation in the same way as HG-AAS (Table II). From the experimental *t*-values, only the amount of HNO_3 (c) and HCl (c) used was statistically significant (t_{tab} = 1.96, 95% confidence). The negative sign of HNO_3 (c) can be due to the oxidative character of Sb, which might form the insoluble Sb(V) silicate complexes in the presence of the oxidizing acids (yielding low extraction recoveries) (22). Accordingly, HNO_3 (c) was not added. The most remarkable drawback of the Plackett-Burman designs is that confounding effects have to be evaluated, i.e., to elucidate whether the effects can be attributed only to a particular variable or to the variable itself plus some other two-variable combinations (23). In our study, no confounding effects were observed.

TABLE II
Experimental Conditions for Each Trial of the Experimental Design and Statistical Effect Calculated for Each Variable During Slurry Preparation, HG-ETAAS Analysis

Variable								
Trial	HNO_3 (mL)	HCL (mL)	'Grinding'	Ultrasonic Bath (min)	'Dummy'	'Dummy'	'Dummy'	Absorbance
1	2.5	7.5	Yes	0	-	-	-	0.298
2	0	7.5	Yes	30	-	-	-	0.402
3	0	0	Yes	30	-	-	-	0.246
4	2.5	0	No	30	-	-	-	0.178
5	0	7.5	No	0	-	-	-	0.307
6	2.5	0	Yes	0	-	-	-	0.187
7	2.5	7.5	No	30	-	-	-	0.278
8	0	0	No	0	-	-	-	0.291
Statistical Effect								
Variable	HNO_3 (mL)	HCl (mL)	'Grinding'	Ultrasonic Bath (min)	'Dummy'	'Dummy'	'Dummy'	
$t_{experimental}$	–2.6	3.3	0.6	0.2	–1.1	–0.3	–1.3	

Following, a univariate approach was taken to optimize the volume of HCl (c). The acid increased the analyte response, although the highest volumes (>7.5 mL) caused an irregular trend. Hence, 7.5 mL of HCl (c) was set as the optimum value (9 mol L^{-1}). In addition, 4 mL of ethanol was added to improve slurry withdrawal [final concentration 20% (v/v)], but this induced a violent reaction resulting in sample sputtering. After assessing different amounts of ethanol (0.5 to 3 mL), it was observed that 1 mL was good enough to ensure good pipetting, precise results, and low blank values. However, a slight reduction in the atomic signal was observed.

Two additional factors have to be taken into account since accuracy and precision greatly depend on (a) volume of the slurry withdrawn to generate stibine and (b) sample-mass/ liquid phase-volume ratio which is a very important factor in slurry analysis (24). This ratio, in turn, depends on the expected analyte content of the sample.

Two slurry volumes (10 and 20 mL), several sample masses (from 0.05 g to 1.00 g), and different volumes of slurry aliquots withdrawn to generate stibine (10 to 200 µL) were investigated. Poor precision figures (RSD >15%) were obtained for both slurry volumes when low sample mass and low aliquot volumes were employed (even though they yielded slightly higher responses). Therefore, high levels of sample mass (0.25–0.50 g for 10 mL; 0.5–1.0 g for 20 mL) and 100 to 200 µL slurry aliquots had to be employed.

Optimization of the Generation of Stibine

Once the slurry is prepared, hydride generation can be considered. Currently, a classical trial and error approach is employed even though it cannot assure that a real optimum is found when all variables are considered simultaneously (mainly, if they interact). Therefore, a multivariate optimization procedure was employed. We carried out a Simplex optimization which is an iterative experimental process whereby the algorithm proposes new experimental trials and evaluates the responses. The Simplex starts from an initial design of n+1 experiences (n = number of variables) and follows a sequential process where the worst conditions are eliminated based on the response variable. This results in the Simplex evolving towards more favorable conditions. Additionally, experiences retained during the n+1 steps must be re-evaluated, which prevents the Simplex to be stuck around a false favorable response. Finally, experiments lying outside the practical limits of the variables are given very unfavorable responses, forcing the Simplex to leave those impractical values; more details are given elsewhere (25). For this study, a modified Nelder and Mead Simplex was employed.

The concentrations of HCl and $NaBH_4$, as well as the argon flow rate, were optimized simultaneously to generate stibine from a GBW 07401 certified soil (0.5 g/20 mL). An irregular Simplex was developed using the Matlab® program (Mathworks Inc., Newton, MA, USA), where the maximum absorbance of the atomic peak is the dependent variable. Two Simplex were developed starting from different experimental conditions to guarantee that an absolute optimum is reached. Since the multidimensional space generated by the Simplex is hard to visualize, the response surface obtained after a principal component analysis of the experimental conditions is shown in Figures 1A and 2A. The 'principal component scores vs. absorbance' plot resembles the 'true' response surface quite well, because the PC1 and PC2 subspace explains up to 99% (Simplex 1) and 98% (Simplex 2) of the variance. It is quite easy to follow the Simplex movement in the PC1-PC2 subspace (Figures 1 and 2); this approach was also proposed by Koch et al.(26). Both Simplex lead to similar optimum conditions: 0.4 mol L^{-1} HCl, 150 mL min^{-1} Ar flow and 2.5% $NaBH_4$ (Simplex 1) and 0.5 mol L^{-1} HCl, 180 mL min^{-1} Ar flow and 3% $NaBH_4$ (Simplex 2). For this study, the first optimum was selected to preserve tube lifetime and for economic reasons.

Furnace Temperature Program

Electrothermal atomization of Sb from stibine needs different furnace temperature programs than for conventional Sb atomization. The drying and pyrolysis steps are substituted with a hydride-trapping step, followed by Sb atomization itself. Then, a cleaning step is recommended to avoid memory effects (although this is unlikely because the sample matrix is not introduced into the graphite tube). For trapping, several coatings were proposed in the literature although Pd is generally recommended. Nevertheless, Ir was chosen due to its lower volatility and good properties (27,28). Three replicates (85 µL each) of a 5.5-g L^{-1} Ir solution were used for tube coating following the furnace program presented in Table IV.

A GBW 07401 soil slurry was prepared to evaluate the best furnace temperature for trapping the hydride. Stibine was generated and a trapping curve built (temperatures ranged from 200–1000ºC, atomization temperature were fixed at 2000ºC). Temperatures lower than 1000ºC did not retained the analyte, while 1000ºC was enough to quantitatively trap the hydride. The minimum collection time required to obtain maximum absorbance was determined by testing the dif-

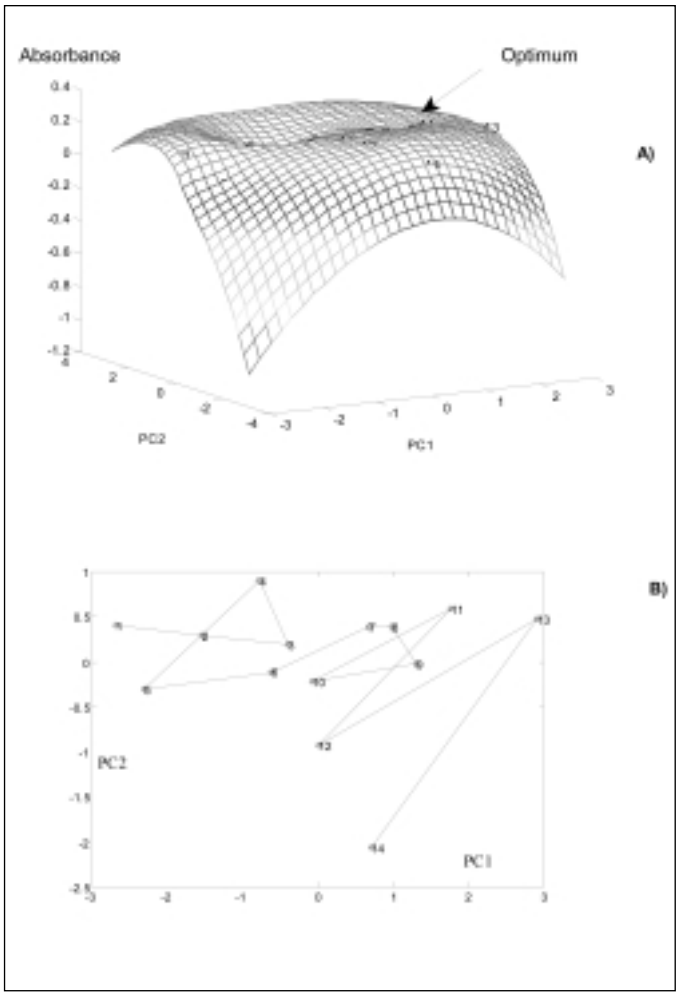

Fig. 1. Response surface obtained for Simplex 1. Absorbances vs. PC1 and PC2 scores of the experimental conditions.
Optimum values: HCl = 0.4 mol L^{-1}, $NaBH_4$ = 2.5% (m/v), Ar flow = 150 mL min^{-1}.

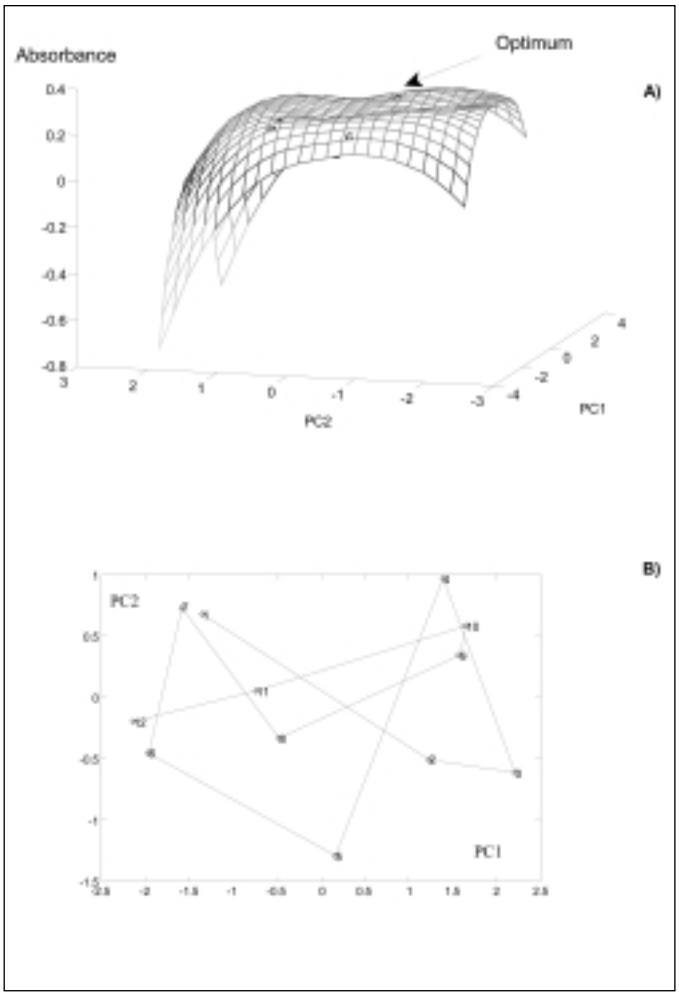

Fig. 2. Response surface obtained for Simplex 2. Absorbances vs. PC1 and PC2 scores of the experimental conditions.
Optimum values: HC] = 0.5 mol L^{-1}, $NaBH_4$ = 3% (m/v), Ar flow = 180 mL min^{-1}.

ferent lengths of time required for vapor generation plus stibine sweeping; and 30 s was selected for this study. Finally, the atomization temperature was evaluated from 2000–2500ºC. Based on the profile of the atomic peak, 2300ºC was chosen, and the final program is presented in Table III.

The lifetime of the Ir-coated tubes was monitored with control charts and 200 firing cycles were found to be the average useful lifetime. After recoating, the graphite tube can be used for an additional 100 cycles.

TABLE III
Experimental Conditions Used to Coat Ggraphite Tube With Ir and Furnace Program to Determine Sb in Soil Slurries by HG-ETAAS (including trapping)

Ir Coating Program	Temp. (ºC)	T_{ramp} (s)	T_{hold} (s)	Ar Flow (mL min^{-1})
Dry 1	150	30	40	250
Dry 2	200	20	30	250
Atomization	2000	0	5	0
Furnace Program				
Trapping 1	1000	1	30	250
Trapping 2	1000	1	2	250
Atomization	2300	0	5	0
Cleaning	2300	1	3	250

Figures of Merit

The linear response extended up to 2 ng mL^{-1} of Sb. The standard additions calibration method was selected because its slope differed statistically from that of the aqueous calibrations (0.1782 mL ng^{-1} and 0.1142 mL ng^{-1}, respectively, 95% confidence level).

The instrumental limits of detection (LOD) and limits of quantitation (LOQ) were 0.16 and 0.52 ng mL^{-1}, respectively. The method LOD and method LOQ depend on sample mass, volume of slurry, and volume of the aliquot of slurry used to generate the stibine. The lowest LOD was 0.08 µg g^{-1} (LOQ of 0.25 µg g^{-1}), which agrees with that reported for the analysis of wort slurries by HG-ETAAS (0.07 µg g^{-1}) (11). As expected, both the method LOD and the method LOQ are higher than the instrumental LOD/LOQ because of the dilutions the samples suffer during the analytical procedure (slurry preparation and stibine generation), although they seem to be adequate to determine Sb in environmental samples. The characteristic mass (± standard deviation) was 11 ± 2 ng for aqueous standards.

The instrumental precision was studied using two aqueous standards (0.5 and 1.0 ng mL^{-1} Sb) and two soil samples with quite different Sb concentrations. The RSDs were satisfactory, <1.6% and <3.3 % for standards and samples, respectively. Furthermore, the overall precision of the method was quite good, the RSD values <7%, which is within the range usually reported for this methodology (2).

Accuracy (trueness) of the method was studied in two ways. It is important to note that since the use of an oxidizing medium was avoided to prepare the slurry and a strong reductant was used to generate the hydride, it follows that stibine is generated only from Sb(III). First, the instrumental accuracy was tested using recovery assays. Different amounts of an aqueous Sb standard were added to several slurries of the GBW 07401 soil sample and the final slurries were analyzed six times. All recoveries were good, from 103–109%. The overall accuracy of the method was assessed by analyzing several CRMs: three soils, two sediments, two coal fly ashes, and two coals. The latter three matrices were selected to ascertain that the method might be extended to such complex matrices. Good agreement was found between the experimental and reference (guide) values for all CRMs (Table IV); the experimental confidence intervals overlapped with the certified values (Student's *t*-test, 95% confidence level). Accordingly, it can be concluded that the combination of slurry preparation, hydride generation, and HG-ETAAS is a suitable method for the direct determination of Sb in soils, sediments, and coal fly ash. Unfortunately, low recoveries were achieved for the coal samples (30%).

Four alternatives were studied to improve the results for coals. First, a surfactant [sodium pyrophosphate, 0.05% (m/v)] was added to the slurries to improve manual pipetting. Second, an extraction step using an ultrasonic bath was performed (30 min) before generation of the stibine. This would increase the extraction of the analyte to the liquid phase and, accordingly, the efficiency during the generation of the stibine. Nevertheless, the recoveries were lower than 35%. Next, the composition of the liquid media was changed. The use of HNO_3 and some mixtures with other reagents such as H_2O_2 or H_2SO_4 were tested to obtain an oxidant medium and, hence, to eliminate the organic matrix present in carbon. Unfortunately, recoveries did not improve, maybe because the organic matrix hindered the

TABLE IV
Sb Concentrations of CRMs Analyzed by HG-ETAAS
(± confidence interval; n=6; 95% confidence level)

	Sb Concentration (± IC, μg g^{-1})		
Material	Certified Value	Obtained Value	Recovery (%)
Soil GBW 07401	0.87 ± 0.03	0.80 ± 0.10	92
Soil GBW 07409	0.21 ± 0.03	0.20 ± 0.04	95
Calcareous Loam Soil BCR 141	(0.7 ± 0.3)[a]	0.5 ± 0.2	(71)
Marine Sediment BCSS-1	0.59 ± 0.06	0.5 ± 0.2	88
Estuarine Sediment BCR 277	(3.9 ± 1)[a]	4.2 ± 1.2	(108)
Coal Fly Ash 1633a	6.8 ± 0.4	6.0 ± 2.3	88
Coal Fly Ash 1633b	(6)[a]	5.1 ± 0.9	(85)
Coal 1632c (Bituminous)	0.461 ± 0.029	0.14 ± 0.15	30
Coal 1632c (Bituminous)[b]	0.461 ± 0.029	0.54 ± 0.03	117
Coal 1635 (Subbituminous)[b]	(0.14)[a]	0.16 ± 0.02	(118)

( )[a] Not certified.
[b] Previous ashing step (450°C up to constant weight).

generation of stibine as reported previously (29). Finally, it was decided to ash the samples to remove the organic matrix before carrying out any other analytical steps (450ºC up to constant weight). This option yielded satisfactory results (Table IV).

Extraction of Antimony into the Liquid Phase of the Slurry

The percentage of analyte extracted into the liquid phase of the slurry was determined to evaluate whether stibine is generated from the solubilized analyte or from the solid particles (only the supernatant was analyzed after centrifuging the slurry at 9000 rpm). The figures were 68 and 91% for soils GBW 07401 and GBW 07409, respectively. Since their total recoveries were 92 and 95%, respectively (Table IV), it has to be assumed that stibine is generated from both the liquid and the solid phases.

This hypothesis seems to be confirmed by the fact that although a low extraction was obtained for sediment BCSS-1 (~30%), the overall recovery was good (88%). Even though the extraction for coal fly ash was much higher (~85%), the total recovery was similar (88%), probably because stibine is not easily developed from such refractory particles. Coal samples showed close to 100% extraction when they were ashed previously.

CONCLUSION

In this study it was demonstrated that the direct determination of Sb in slurries through its previous conversion to stibine, which becomes trapped on an Ir-coated graphite tube, constitutes a reliable analytical procedure for the analysis of solid samples. The procedure avoids typical problems associated with the sample matrix when the direct analysis of slurries is carried out.

Chemometric tools (experimental design and Simplex optimization) allowed the successful optimization of both the generation of stibine and its quantitation. The HCl concentration was critical for the slurry preparation.

The HG-AAS technique was not valid for the direct analysis of slurries, whereas the HG-ETAAS technique yielded accurate and precise results (LOD 0.08 μg g^{-1}, precision RSD <7%). The direct determination of Sb in several complex matrices (soils, sediments, coal fly ash, and coals) was suitable as evidenced by the analysis of certified reference materials. For coals, a previous sample ashing is mandatory.

ACKNOWLEDGMENTS

This work was partially supported by the Vicerrectorado de Investigación of the University of A Coruña.

M.J.C.P. and M.F.S. acknowledge the Galician government (Xunta de Galicia) and the Spanish Ministry of Education, respectively, for their Ph.D. grants.

Received January 18, 2005.

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Application of Biological Substrates to the Speciation Analysis of Cr(III) and Cr(VI)

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ABSTRACT

Baker's yeast (Saccharomyces cerevisiae) was used as substrate for the selective retention of Cr(III) in the presence of Cr(VI) from aqueous solutions. The experiments were performed in batch as a suitable and simple method to obtain information of chromium uptake. The effect of chemical and physical variables affecting the degree of uptake of chromium was tested in order to select the optimum analytical conditions for the selective Cr retention by *Saccharomyces cerevisiae*. The parameters studied were reaction media and pH, amount of biomass, temperature, and contact time. The influence of some concomitant ions up to 50 mg L^{-1} was also tested.

During all the steps of the optimization process, Cr(VI) remained in solution, while Cr(III) was accumulated by the yeast cells. Quantitative determinations of the Cr species in the biomass and supernatant solution were carried out by means of flame atomic absorption spectrometry (FAAS). A preconcentration factor of 15 was achieved for Cr(III) when 75 mL of water was processed. The detection limits for Cr(III) and Cr(VI) were 0.2 and 3.0 ng mL^{-1}, respectively.

Compared with other methods, this approach allows the determination of Cr(III) and Cr(VI) in different phases. A morphological study and microscopical characterization of the yeast cell wall were carried out by Scanning Electron Microscopy (SEM) complemented with energy dispersive X-ray analysis (EDAX). The speciation analysis of inorganic Cr in different kinds of spiked natural waters was performed following the proposed method. The recoveries in each phase and in all cases were between 89 and 108%.

INTRODUCTION

The persistence, fate, bioavailability, and toxicological effects of trace elements in the environment are directly related to the physicochemical forms in which they occur. Chromium represents one of the most widely studied elements due to the different toxicological and biological behavior of its species in living organisms. Trivalent Cr compounds are much less toxic than those of hexavalent chromium (1,2). Owing to the high oxidation potential of Cr(VI) and the ease to penetrate biological membranes, it is approximately 100 times more toxic than Cr(III) (3). Hexavalent chromium is also a potent carcinogenic agent, and acute and chronic toxicity problems associated with Cr(VI) exposure include ulceration of the skin, inflammation of the larynx, as well as damage to the kidneys and lungs (4).

A variety of separation and detection techniques have been proposed for the determination of Cr species in water samples. More recently, advances have been made with more sophisticated techniques. Hyphenated techniques based on the combination of a powerful separation technique with a sensitive element-specific detector are among the most promising approaches to determine selectively Cr species in a variety of matrices. Reported methods normally require relatively complex separation schemes, such as reversed phase ion-pair high performance liquid chromatography (HPLC) (5) or capillary electrophoresis (CE) (6), coupled column HPLC-inductively coupled plasma mass spectrometry (ICP-MS) (7), and isotope dilution (ID-MS) techniques (8) that are not always available in all laboratories. It is therefore essential to investigate other alternatives for the reliable and low-cost determination of species at trace levels. One alternative to inorganic sorbents, such as bentonite (9), charcoal (10), and alumina (11), is to use biological substrates for metal speciation since microorganisms are capable of binding dissolved metals. Cell walls have a common composition of proteins and carbohydrates with which the metallic species can react.

Biological substrates have been used with preconcentration and, to a lesser extent, for speciation purposes. Cámara and her group (12–14) employed baker´s yeast biomass to determine selectively CH_3Hg^+ and Hg^{2+}, Sb(III) and Sb(V), Se(IV) and Se(VI) based on their

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different toxicities. Smichowski et al. (15) demonstrated the effectiveness of yeast cells for the speciation analysis of inorganic As.

In the specific case of chromium, different biological substrates were evaluated for speciation purposes. Neidhart et al. (16) used human red erythrocytes under physiological conditions for the selective determination of chromate in the presence of Cr(III). The removal of trivalent and hexavalent Cr by seaweed biosorbent was reported by Kratochvil and coworkers (17). The use of intact and dehydrated cells of *Candida utilis* (18) was useful for the selective sorption of Cr(VI) in the presence of other metals. Other authors used immobilized yeast in off-line column procedures. Bağ and coworkers (19) employed sepiolite and Pérez-Corona et al. (20) used alginate as support.

The aim of this work was to examine the potential of yeast cells as a selective substrate for speciation analysis of Cr using a simple and inexpensive batch procedure that allowed the characterization of the metal-binding process. The effect of chemical and physical parameters affecting the degree of bioaccumulation of Cr(III) and Cr(VI) in yeast cells was examined.

EXPERIMENTAL

Instrumentation

A PerkinElmer Model AAnalyst™ 300 flame atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA), equipped with a deuterium background corrector, was used. A PerkinElmer Cr hollow cathode lamp was employed as the light source for Cr determination. All signals were monitored at 357.9 nm with a spectral slit width of 0.7 nm. The instrumental parameters used for the Cr determinations are summarized in Table I.

Microscopy characterization of the yeast cells was carried out by scanning microscopy with a Philips 515 microscope (Philips Export B.V., Eindhoven, The Netherlands), equipped with an EDAX PV9100 probe (EDAX International Inc., Prairie, View, IL, USA). An Altronix pH meter (Buenos Aires, Argentina), equipped with a glass electrode and an AgCl/Ag reference electrode, was used for measuring the pH of the solutions. For separation of the aqueous phase in contact with the yeast cells, a laboratory centrifuge was used.

Reagents

All reagents were of analytical reagent grade unless otherwise stated. Deionized water from Nanopure (Barnstead, Dubuque, IA, USA) was used throughout. Standard solutions of Cr(III) and Cr(VI) were prepared by stepwise dilution of a 1000-mg L^{-1} standard solution (Merck, Darmstadt, Germany) just before use. Diluted working solutions were prepared daily by serial dilutions of this stock solution.

All solutions containing the acids studied were prepared at the required concentrations by dissolving appropriate amounts of each compound in deionized water or by dilution. A range of different pH values was prepared by adjusting with HCl or NaOH.

The cation and anion solutions [(As(III), Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg(II), Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sb(III), V(V), Zn^{2+}] used in interference study were prepared by appropriate dilution of the stock solutions.

Biosorbent Material

The organisms used in this study as biosorbent material were from commercial dry baker's yeast. The yeast cells were microbiologically identified by the CNEA Radiobiology Laboratory as a pure culture of *Saccharomyces cerevisiae*. Identification of the biomass was performed by their morphological characteristics and other properties. These included the ability for fermentation of sucrose, maltose, and galactose and the inability to assimilate potassium nitrate, to degrade urea, and to grow in creatinine.

The chemical composition and morphological modification on the yeast surfaces was analyzed in individual particles and in the bulk using Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Analysis (EDAX). The yeast samples, before and after Cr accumulation, were mounted on aluminum stubs, coated with gold before the analysis.

Experimental Procedure

All experiments were performed in batch (by triplicate) as a suitable and simple method to obtain information of Cr uptake by *Saccharomyces cerevisiae*. A deionized water blank was also processed and analyzed. Baker's yeast (0.75 g) was placed in centrifuge tubes and 0.25 mL of a 2.0-mg mL^{-1} solution of Cr(III), 0.25 mL

TABLE I
Operating Conditions for Cr Determination by FAAS

Instrument	PerkinElmer, Model AAnalyst 300 AAS
Flame type	Air-C_2H_2 (reducing, fuel-rich)
Burner height	12 mm
Wavelength	357.9 mm
Slit width	0.7 nm
Lamp current	15 mA
Measurement mode	Time average, Read time: 2.0 s, Read delay: 2.0 s

of a 2.0-mg mL^{-1} solution of Cr(VI) and 11.5 mL of acetic acid solution, adjusted to pH 4.0, were added to the tubes. The samples were manually agitated for 5 min and then centrifuged 10 min at 5000 rpm; both phases were separated by filtration. Cr(VI) was determined in the liquid phase by FAAS. The solid phase was re-dissolved in 10 mL of a 1.0-mol L^{-1} HCl solution.

To evaluate the effect of different variables on the Cr uptake, the separation process was carried out on each oxidation state separately. Cr(III) showed high affinity for the yeast cells and was almost completely retained by the biomass, while Cr(VI) remained in the supernatant. After optimization, mixtures of Cr(III) and Cr(VI) in water were prepared to check the ability of the yeast cells to selectively retain Cr(III) species.

RESULTS AND DISCUSSION

Morphological Study of Yeast Cells

The morphological study of the commercial yeast revealed the uniform and small size of the particles. The average particle size of the Saccharomyces cerevisiae was 2.5–3.0 mm, and when dried, aggregates of larger sizes are formed. The cell wall is the initial site of action for toxic element species. A diversity of active binding sites is contained in the cell wall. An important mechanism for the accumulation of metal ions is their adsorption on the surface of the substrate through interactions with chemical functional groups such as the carboxylate, hydroxylsulphate, phosphate, and the amino groups (21). As a result of metal binding, membrane damage may occur. Figure 1-a shows a close micrograph of the biomass before Cr uptake, where spherical particles can be observed. After Cr uptake, no morphological changes were observed (Figure 1-b), and the particles remained intact. This study evidenced that no effects of a chemical process on the cell walls were detected under the experimental procedure adopted.

Chemical and Physical Parameters Affecting Cr Uptake

The effect of chemical and physical parameters on the Cr uptake by yeast cells was evaluated. Chromium was quantified in the solid and in the liquid phase. The nature of the medium and the pH, temperature, contact time, amount of analyte, amount of biomass, and sample volume were the variables considered.

Effect of the Medium and pH on Cr Uptake

The retention of Cr(III) and Cr(VI) was evaluated in five media: acetic, phosphoric, citric, oxalic, and tartaric acids. The acid solutions (0.1 mol L^{-1}) were prepared with the pH adjusted by dropwise addition of NaOH or HCl solutions in order to cover the range from 2–10.

In all cases, Cr(III) was sequestered by the yeast cells and Cr(VI) remained in the supernatant. The results shown in Figure 2 illustrate that the uptake mechanism depends on the medium studied and to a lesser extent on the pH. It is evident from Figure 2 (a, b, c, d, e) that the acetic and oxalic acids enable the best separation of both species. For further experiments, acetic acid at pH 4 was selected as the more convenient acid for Cr(III) and Cr(VI) separation because the separation of the species is maximum. When this acid was tested, both species were completely separated at pH 4 because Cr(VI) exhibited the lower recovery, while Cr(III) was quantitatively sequestered by the biomass. This effect could be attributed to the different binding sites (functional groups) involved in Cr(III) accumulation which requires different conditions.

Hexavalent Cr retention exhibits low dependence on pH but is more sensitive to pH changes than Cr(III). It is related to the mechanism of metal uptake on the surface of the yeast cells and reflects the nature of the physico-chemical interaction of both species. Despite the expected complexity of the biosorption process, the slight dependence of Cr(III) binding on pH with the different acids evaluated suggests that yeast cells may interact with the analyte through

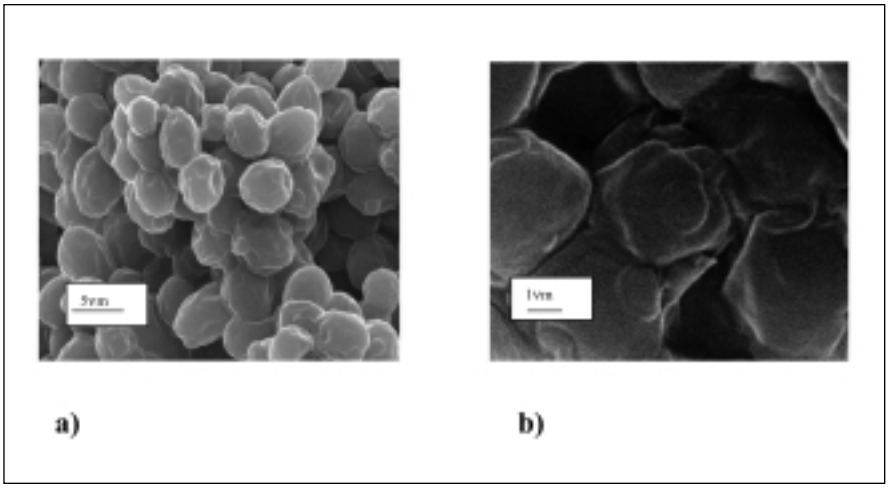

Fig. 1. Scanning electron micrograph of yeast cells: (a) before Cr uptake; (b) after Cr uptake.

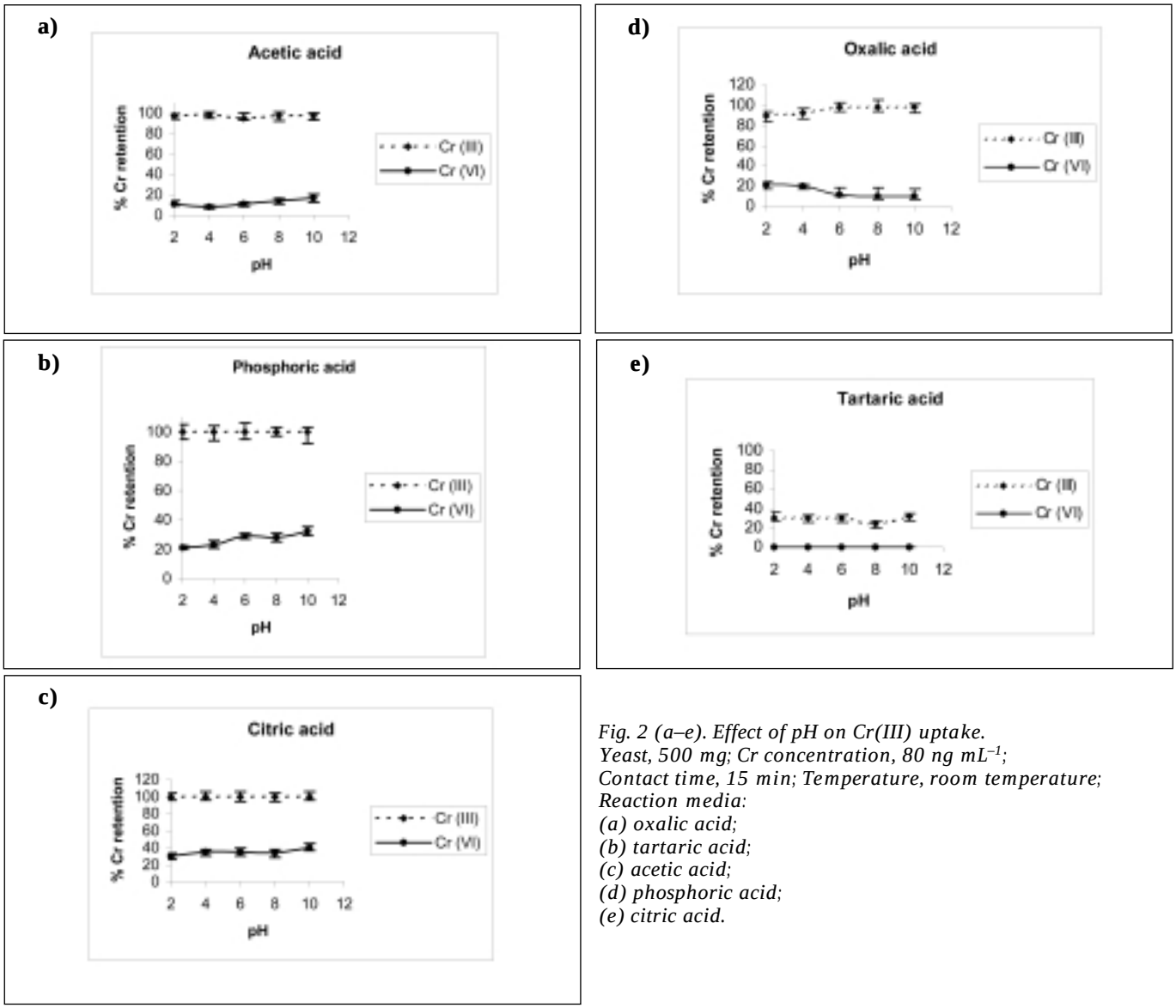

Fig. 2 (a–e). Effect of pH on Cr(III) uptake. Yeast, 500 mg; Cr concentration, 80 ng mL^{-1}; Contact time, 15 min; Temperature, room temperature; Reaction media:
(a) oxalic acid;
(b) tartaric acid;
(c) acetic acid;
(d) phosphoric acid;
(e) citric acid.

covalent binding. We observed similar results for As when *Saccharomyces cerevisiae* was used for the same purpose (15). Even when the major mechanisms responsible for metal biosorption may include ionic interaction and complex formation between metal ions and biomass (22), other studies regarding the nature of bonding between metallic ions and algae cell walls explained the greater adsorption of Cu, in comparison with other elements, as an indication of covalent bindings (23).

Effect of Temperature on Cr Uptake

The influence of temperature on Cr sequestration by yeast cells was tested from 0 to 80ºC. Figure 3 shows that Cr(III) was almost quantitatively sequestrated by the yeast cells in all of the temperature ranges evaluated. For Cr(VI), a maximum biosorption was obtained at a temperature of 40ºC. This increase in Cr(VI) retention at an increased temperature may be explained by a higher affinity of the sites for the metal or to an increase in binding sites on the surface of the biomass. Our results are in good agreement with those reported by Goyal and coworkers (24). Room temperature was adopted for further experiments since a good separation of Cr(III) and Cr(VI) species was obtained.

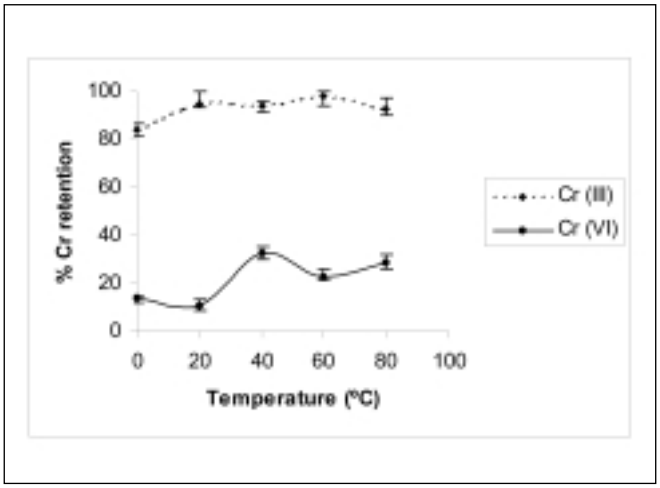

Fig. 3. Effect of temperature on Cr uptake. Yeast, 500 mg; Acetic acid, 0.1 mol L^{-1}; pH, 4; Cr concentration, 80 ng mL^{-1}; Contact time, 15 min.

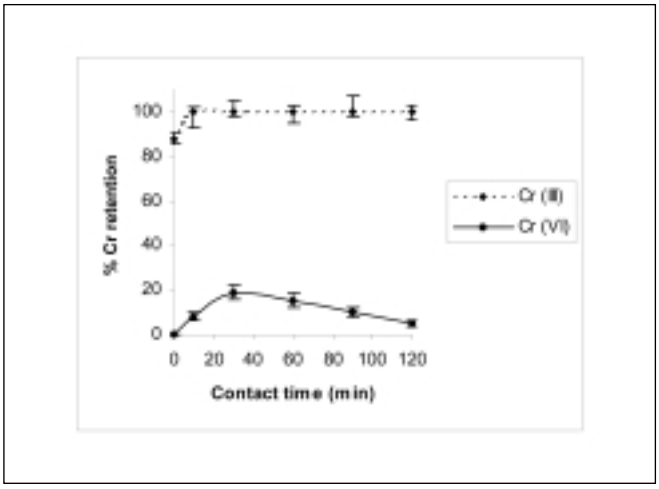

Fig. 4. Effect of contact time on Cr uptake. Yeast, 500 mg; Acetic acid, 0.1 mol L^{-1}; pH, 4; Cr concentration, 80 ng mL^{-1}; Temperature, room temperature.

Effect of Contact Time on Cr Uptake

The retention of metals and metalloids by different microorganisms in batch systems occurs in two steps: (a) A passive uptake takes place in the first stage which is very rapid; (b) the second step is active and slow and the metal is accumulated by the biomass. Under the conditions previously optimized, a series of experiments was carried out at different contact times. Figure 4 demonstrates that the sorption rate of Cr(III) appeared to go through two stages. In the first stage, retention was fast and about 88% of the total Cr(III) uptake by the biomass took place within 10 min. In the second stage, between 10 and 120 min, the biosorption was quantitative and unaffected by contact time. We can consider that in a batch system, Cr(III) was completely retained by the biomass in the first step. A contact time of 10 min was chosen for further work.

On the other hand, Cr(VI) exhibited a little retention with a maximum (retention: 20%) at 20 min. Neidhart et al. (16) reported that yeast cells are able to accumulate chromates, but the carrier mechanism for penetration of the cell membrane is too slow. These findings should explain why it is possible to separate both species under the conditions of our work.

Capacity of Yeast Cells for Cr Retention

The biomass amount is another variable that may affect biosorption. The effect of biomass on Cr(III) and Cr(VI) uptake was evaluated by using the same concentration of the analyte (80 ng mL^{-1}) and varying the biomass between 50 and 750 mg. Figure 5 illustrates that 750 mg is the minimum amount of biomass necessary to remove 98% of the Cr(III) from solution, while

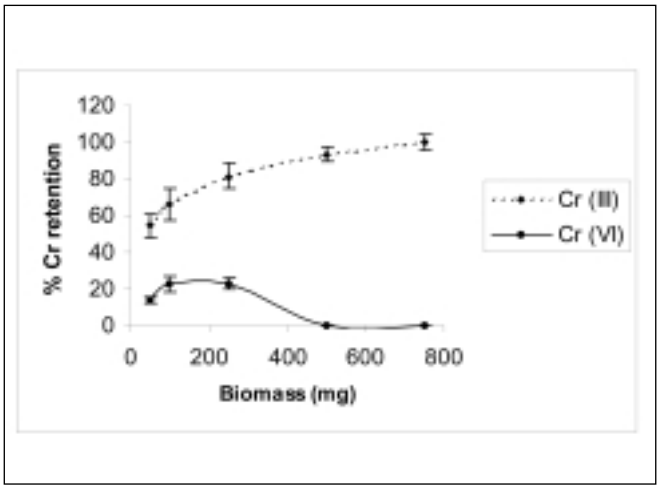

Fig. 5. Effect of amount of biomass on Cr uptake. Acetic acid, 0.1 mol L^{-1}; pH, 4; Cr concentration, 80 ng mL^{-1}; Contact time, 10 min; Temperature, room temperature.

no retention of Cr(VI) was observed. Beyond this value, the retention of the analyte is constant. These are the optimal conditions to achieve the speciation analysis of the inorganic Cr species.

Specific uptake has been demonstrated to depend on the analyte species and on the nature of the microorganisms involved (25). Itoh et al. (26) suggested that electrostatic interactions between cells might be a significant factor in biomass concentration dependency.

In order to estimate the achievable preconcentration factor, it is necessary to evaluate the maximum volume of a sample that can be contacted with the biomass. Increased sample volumes (5–100 mL) of solutions con-

taining 5.0 mg of Cr(III) were placed in contact with 750 mg of yeast. After following the procedure described, the solid phase was dissolved in 5 mL of 1.0 mol L^{-1} HCl for Cr(III) determination. This experiment showed that recovery of Cr(III) was independent of the sample volume up to 75 mL. The recovery was evaluated by comparing the signal obtained from a suspension [containing 5.0 mg of Cr(III) in 5 mL] with that obtained after preconcentration of more diluted solutions containing the same amount of analyte. A preconcentration factor of 15, calculated as the ratio of initial to final volume, was achieved. It is important to note that the recovery obtained is dependent on the complexity of the matrix. We observed lower preconcentration factors when tap water was processed.

Study of Potentially Interfering Elements

The interference of different concomitants is, in general, attributed to the strength of the interaction between the metal and the cell components. The effect of several ions on Cr(III) retention was evaluated to assess the selectivity of the method. All tests were carried out under optimum operating conditions and following the general procedure. The test samples analyzed contained 80 ng mL^{-1} of Cr(III) and 5 µg mL^{-1} of the potential interferent (50 µg mL^{-1} for Ca and Mg), and the results are the average of three replicate measurements.

Figure 6 depicts the percentage of Cr(III) retention in the presence of the potential interferent in the acid selected (acetic acid) and in oxalic acid. This study confirms that acetic acid is the best alternative not only for the purpose of speciation but also from the point of view of selectivity. Since no significant inhibitory effects were observed, it is plausible to conclude that there is no competition for binding sites between the species evaluated and the analyte in the medium selected.

Analytical Performance

The detection limits were calculated following the IUPAC rules on the basis of the 3σ criterion for 10 replicate measurements of the blank signal. For Cr(VI), it was calculated in the liquid phase. The detection limit for Cr(III) was calculated in the solid phase after dissolution of the biomass in 10 mL of 1.0 mol L^{-1} HCl. The limit of detection of Cr(III) was then corrected for the preconcentration factor. The detection limits in each phase were 3.0 ng mL^{-1} for Cr(VI) and 0.2 ng mL^{-1} for Cr(III).

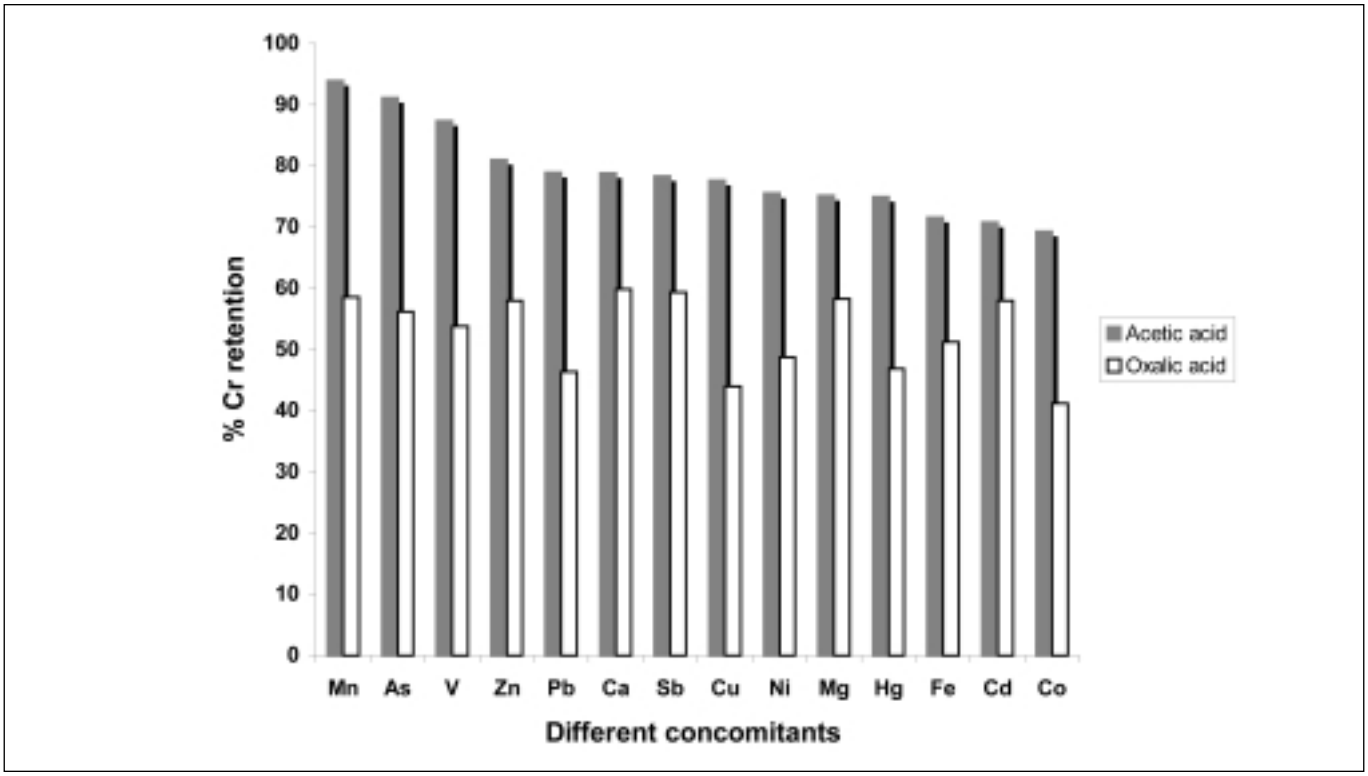

Fig. 6. Effect of foreign ions on Cr(III) retention. Yeast, 750 mg; Acetic acid, 0.1 mol L^{-1}; pH, 4; Cr concentration, 80 ng mL^{-1}; Contact time, 10 min; Temperature, room temperature.

TABLE II
Quality Parameters for Cr(III) and Cr(VI) Speciation Analysis

	Detection Limit (3σ) (ng mL^{-1})	Precision (%)[b] for 100 ng mL^{-1} Cr
Cr(III)[a]	0.2[a]	2.2
Cr(VI)	3.0	2.0

[a] Preconcentration factor: 15, [b] (n=10).

TABLE III
Recovery of Mixtures With Different Concentration Ratios of Cr(III) and Cr(VI)
[Mean value ± standard deviation (n=3), concentrations are expressed in ng mL^{-1}]

	Cr Added		Cr Found		Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
Tap	50	50	48.9±2.5	53.3±2.8	97	107
Well	10	100	8.9±0.5	98.1±3.9	89	98
River	25	25	26.3±1.5	27.0±1.7	105	108

The relative standard deviation (RSD) for 10 successive measurements of a sample containing a final concentration of 80 ng mL^{-1} of both, Cr(III) and Cr(VI), were 2.2% and 2.0%, respectively. Table II summarizes the analytical performance of the method.

Application to the Selective Determination of Cr(III) and Cr(VI) in Natural Waters

To demonstrate the capability of *Saccharomyces cerevisiae* to selectively retain Cr(III), different kinds of waters containing both species were analyzed. The samples were filtered through glass microfiber filters (1.2 mm), acetic acid was added to a final concentration of 0.1 mol L^{-1}, and the pH was adjusted to 4. An analytical curve plotted with mixed standard solutions containing 0–4.0 µg mL^{-1} of Cr(III) and Cr(VI) was used to quantify Cr(III) and Cr(VI) in the samples.

The results of the analysis and the spike recovery data for Cr(III) and Cr(VI) in spiked water samples are presented in Table III. Recoveries between 89 and 108% were obtained, which confirms the reliability of the proposed method for Cr speciation analysis.

CONCLUSION

This study demonstrated the utility of a biological substrate for preconcentration and speciation of chromium. Cr(III) was quantitatively retained and preconcentrated in the yeast cells, while Cr(VI) remained in the supernatant. This fact made possible the determination of each Cr species in separate phases in contrast to other studies (19) where Cr(VI) was calculated by substraction. Acetic acid demonstrated to be an appropriate reaction medium because in the experimental conditions the fixed Cr(III) uptake by *Saccharomyces cerevisiae* was quantitative, selective, and unaffected by the pH. No strict control of the pH is necessary which is another advantage of the method. A preconcentration factor of 15 was reached for Cr(III). The yeast cells present no chemical hazards, and are inexpensive and easy to obtain. The method proposed is simple, sensitive, highly selective, and suitable for routine analysis.

ACKNOWLEDGMENTS

Financial support was provided by the Agencia Nacional de Promoción Científica y Tecnológica through Project PICT-2000 N° 13-08772. One of the authors (PS) wishes to acknowledge the International Atomic Energy Agency (Research contract RC: 11549/R1) for partial financial support.

Received February 10, 2005.

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Flow Injection Determination of Cd in Meat Samples Using a Continuous Lixiviation/Preconcentration System Coupled to a Flame AAS

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ABSTRACT

A simple flow injection method is proposed, which combines continuous acid extraction, preconcentration, and flame atomic absorption spectrometry for the determination of cadmium in meat samples. The dynamic acid extraction step was carried out by using a continuous ultrasound-assisted extraction system. The acid extract was preconcentrated on-line on a minicolumn packed with a chelating resin (Chelite P, with aminomethylphosphoric acid groups), and elution was carried out with hydrochloric acid. Once eluted, cadmium was continuously monitored by flame atomic absorption spectrometry. These steps were optimized applying an experimental design.

The method allowed a total sampling frequency of 16 samples per hour. Good precision of the procedure was obtained [2.9%, expressed as relative standard deviation (RSD)], including a high enrichment factor (20.5), and detection and quantification limits of 0.014 and 0.067 µg/g for 60 mg of sample, respectively. The accuracy of the proposed method was verified using CRM 183 Pig Kidney standard reference material; the results were in good agreement with the certified values. The analytical procedure was applied to the determination of trace amounts of cadmium in real meat samples.

INTRODUCTION

Contamination of the environment by metals is a problem that has become of regional and even global concern. High metal concentrations occur within sites contaminated by atmospheric emissions from point source mining and smelting operations, and from combustion of fossil fuels.

The determination of inorganic elements in foods and biological materials has become of considerable interest because of the importance or toxicity of many of these elements to human health. Cadmium is an important environmental toxicant, having adverse health effects following long-term chronic exposure. This metal can be easily absorbed by plants and accumulates in the tissues of herbivores, mostly in the liver and kidney. Since meat and meat products form an important part of the human diet, cadmium can find its way into the human food chain (1). Therefore, the monitoring of endogenous and exogenous toxic trace elements, such as cadmium, in foods is an important aspect of food analysis.

Inductively coupled plasma (ICP) (2), inductively coupled plasma mass spectrometry (ICP-MS) (3-4), and differential pulse anodic stripping voltammetry (DPASV) (5) have been used for the determination of cadmium in meat samples. Atomic absorption spectrometry [graphite furnace (GFAAS) or flame atomic absorption spectrometry (FAAS)] has shown to meet most of the requirements for a reliable and fast determination of this element in meat. GFAAS has greater sensitivity than flame atomic absorption spectrometry and preconcentration steps are usually not required, but the method is subject to more chemical interferences and some form of matrix modification is required. Flame AAS is simple and inexpensive and therefore widely used for the determination of metallic elements. Flame AAS, using air-acetylene, provides accurate and sensitive results for cadmium determination. Nevertheless, the concentration of metallic ions in different types of matrices (such as cadmium in meat) is often below the detection limits attainable even with the most sensitive detectors. Therefore, in order to determine trace levels of trace metals using FAAS, it is necessary to enrich the sample. Evans et al. (2) used a chelating reagent solution [1.0% (m/v) solution of APDC-DDDC] to form a metal chelate with cadmium; this chelate was then extracted with chloroform. Falandysz (6) preconcentrated cadmium by forming a complex with APDC that was extracted with methyl isobutyl ketone. Gomes Neto et al. (7) proposed a minicolumn packed with a strongly basic anion exchanger (AG-X8 resin) for the spectrometric determination of cadmium in foodstuffs, plants, and similar products based on the formation of a cadmium iodide-Malachite Green (MG) associate.

The analysis of solid foodstuffs usually requires a rather complicated and time-consuming sample preparation. Loss of test elements and sample contamination with these elements may occur during this step, which deteriorates the reliability of the analysis. Moreover, sample preparation substantially adds to the duration and cost of analysis. Wet digestion is the preferred sample preparation proce-

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dure for the determination of cadmium in foods. The Analytical Methods Subcommittee recommends use of a sulphuric acid-hydrogen peroxide wet digestion with the usual precautions (8). A number of methods for cadmium determination in meat samples based on wet digestion have also been proposed. However, the principal disadvantages of this method are a very time-consuming process [i.e., overnight at room temperature and 1 hour at 120ºC (9), 6.5 h at different temperatures (10)] and the use of concentrated acids [nitric acid and hydrogen peroxide (11), a nitric, perchloric and sulphuric acid mixture (10)]. Microwave-assisted digestion was carried out by Crete et al. (11), Cubadda et al. (4), Zhou et al. (3), and Jorhem et al. (12). Despite the use of microwave digestion, which is fast and effective, the digestion operation requires concentrated acids, which generate carcinogenic nitrous vapors as a consequence of the organic matrix destruction, so safety control in the laboratory has to be taken into account. If dry ashing is used, the temperature must be kept below 500ºC to prevent volatilization losses. This procedure is also very time-consuming [2 h at 100ºC, 1 h at 150ºC, 1 h at 300ºC, and 15 h at 450ºC (13)].

The use of ultrasonic energy is of great help in the pre-treatment of solid samples because it facilitates and accelerates operations such as the extraction of organic and inorganic compounds, slurry dispersion, homogenization, and other analytical stages. It also eliminates total sample matrix introduction into the nebulizer of the spectrometer, which occurs with solid sampling, slurry sampling, and the digestion procedures (14–15).

Since its introduction, flow injection analysis (FIA) has established itself as a very useful and versatile technique (16-17). Some of the advantages are flexibility, reliability, reproducibility, ease of automation, high sample throughput, small sample volume, and low reagent consumption and interferences.

In this paper, we describe a simple and continuous ultrasound-assisted extraction system (CUES) connected to an on-line flow injection manifold for acid leaching of cadmium, on-line concentration of this trace metal from the acid extract using a chelating resin (Chelite P, with aminomethyl-phosphoric acid groups) , and its determination by flow injection-flame atomic absorption spectrometry (FI-FAAS). This procedure combines the benefits of the ultrasound-assisted extraction methods, the high sensitivity of the preconcentration techniques, and the advantages of the FI system. The method was applied to the analysis of meat samples for the determination of cadmium.

EXPERIMENTAL

Instrumentation

A PerkinElmer Model 5000 atomic absorption spectrometer was used (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA), equipped with a cadmium hollow cathode lamp. The instrument was set at 228.8 nm. The spectrometer output was connected to a PerkinElmer Model 50 Servograph Recorder with a range of 5 mV. The FI system was equipped with two Gilson® Minipuls™ 3 peristaltic pumps (Gilson, France) fitted with Viton® tubes, an ultrasonic bath (Selecta, Barcelona, Spain), six Rheodyne® injection or switching valves (USA), Models 5041 and 5301, a glass minicolumn (100 mm x 3 mm i.d., bed volume 700 µL) (Omnifit, UK). The ends of the minicolumn were plugged with filter paper (Whatman 541) and the Viton minicolumn (120 mm x 1 mm i.d.) was packed with 50 mg chelating resin for the preconcentration step. The ends of the minicolumn were plugged with glass wool.

Reagents

Ultrapure water of 18.2 MΩ.cm resistivity, obtained from a Milli-Q™ water purification system (Millipore, Bedford, MA, USA), was used for preparation of the reagents and standards. Hydrochloric acid, nitric acid (Scharlau Chemie, Barcelona, Spain), and 1000 µg/mL cadmium standard solution (Merck, Darmstadt, Germany) were of reagent grade. The chelating resin used was Chelite P, with aminomethylphosphoric acid groups (Serva Electrophoresis GmbH, Heidelberg, Germany). The sample particle size was 0.3–0.8 mm and ammonium acetate (Merck) of 16 mol/L in aqueous solution. The certified reference material was CRM 183 Pig Kidney (Community Bureau of Reference, Brussels, Belgium).

Sample Preparation and Procedure

The meat, liver, and kidney samples were purchased at a local supermarket. Visible fat, connective tissue, and major blood vessels were excised. The samples were cut into small pieces, dried at 50ºC, triturated and pulverized in a porcelain mortar and, after sieving, fractions with a particle size less than 30 µm were taken for analysis.

The continuous cadmium determination system is shown in Figure1. Meat samples of 50–60 mg were weighed directly into the glass minicolumn. Then, the minicolumn was connected to the CUES. First, the CUES circuit (2 mL) was loaded with the acid leaching solution (3 M nitric acid). Once the CUES circuit was closed, the leaching solution circulated through the minicolumn under ultrasonic energy action at a flow rate of 3.5 mL/min for 2 min. The direction of the flow was changed each 30 s in order to avoid sample accumulation

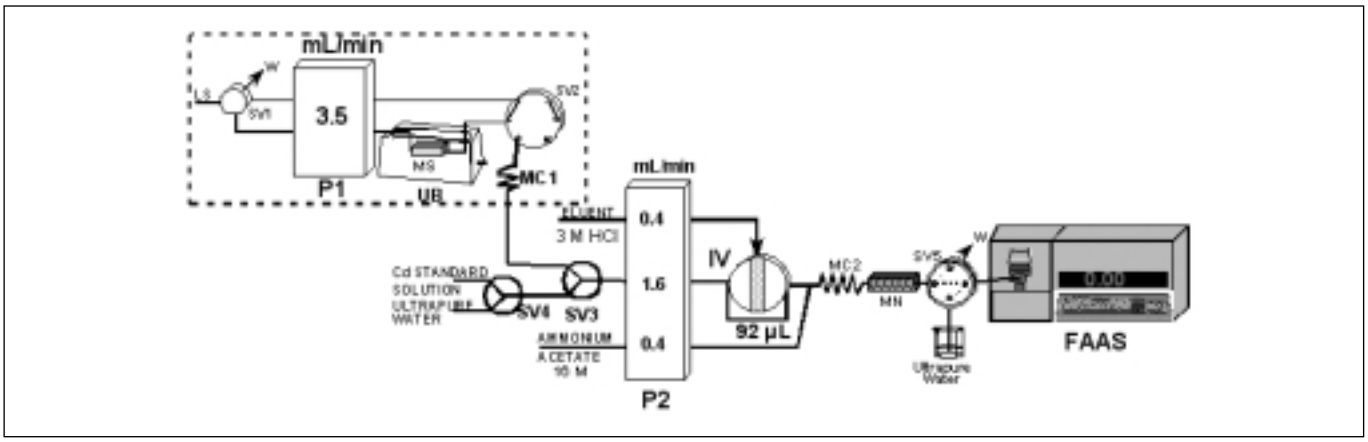

Fig. 1. Flow injection manifold for the whole procedure (CUES and preconcentration device) for cadmium determination in meat samples. P1 and P2, peristaltic pumps; LS, leaching solution; W, waste; UB, ultrasonic bath; MS, minicolumn containing the sample; IV, injection valve; SV1-SV5, switching valves; MC1 and MC2, mixing coils; MN, minicolumn containing the chelating resin (Chelite P) and FAAS, flame atomic absorption spectrometer.

at the end of the minicolumn. Then, the switching valve (SV2) was switched to its opposite position and the acid extract was homogenized in the mixing coil. After this, the acid extract channel converged with a buffer solution stream and 16 M ammonium acetate was added in order to obtain a pH value of >3. The mixture was homogenized in a second mixing coil and then passed through the preconcentration minicolumn at a flow rate of 2 mL/min. The cadmium was retained quantitatively by formation of a metal chelate, eluted by injection of 92 µL of 3 M hydrochloric acid into a water carrier stream, and swept to the detector where cadmium was continuously monitored.

Standard solutions containing between 0.000 and 0.036 µg/mL of Cd in the same acid medium as the leaching solution were introduced into the flow system.

RESULTS AND DISCUSSION

In the proposed approach, the order used for optimizing the steps involved was as follows: (a) the variables affecting the cadmium preconcentration step and (b) the leaching step.

TABLE I
Factor Levels Studied in the Plackett-Burman Design (2^6 3/16) for the Cd Preconcentration-Elution Process

Variable	Key	Low	High	Optimum
Sample pH	A	1	7	3-8
Sample flow rate (mL/min)	B	0.5	4.0	2.0
Eluent concentration (HCl, M)	C	0.1	3.0	3.0
Eluent volume (µL)	D	70.4	190	92
Elution flow rate (mL/min)	E	3.0	5.0	3.0
Minicolumn diameter (mm)	F	1.0	2.0	1.0

Optimization of the Cd Preconcentration Step

The number of variables that affect the cadmium preconcentration step is very large: HCl concentration (eluent solution), elution flow rate, sample pH to obtain a quantitative retention on the chelating resin, flow rate of the sample, eluent volume, and minicolumn diameter. Plackett-Burman designs are principally used when the number of variables that influence the analytical system is very large, because such designs permit the use of a full factorial design and the number of factor combinations can be obtained in multiples of four (18).

In this paper, a Plackett-Burman 2^6 (3/16) factorial type III resolution design with one center point was selected, allowing 6 degrees of freedom and involving 13 non-randomized runs. This factorial design was applied to a standard solution containing 0.04 µg/mL of cadmium. One mL of this solution was passed through the preconcentration minicolumn. The lower and upper levels for each studied variable are listed in Table I. These values were chosen from available data reported in previous experiments.

From the results of these analytical data, it can be concluded that the cadmium preconcentration-elution process appeared to be affected positively by one statistically significant factor, namely the sample pH. This means that the cadmium preconcentration-elution

efficiency is directly proportional to the pH and best responses (cadmium recovery, %) are obtained at the highest tested level of this variable.

All other parameters were not statistically influential. The diameter of the minicolumn, eluent concentration, and eluent volume were affected by a positive estimated effect, while the sample flow rate and eluent flow rate were affected by a negative estimated effect.

In order to improve the analytical characteristics of the method, other experiments outside the framework of the design were carried out. Table II shows the results of these experiments. The minicolumn diameter is affected by the lower positive estimated effect. In order to avoid a system connection fit, a minicolumn with a diameter of 1 mm was chosen as optimum.

The eluent volume is the variable with an upper non-significant estimated effect and was affected by a positive sign. Eluent volumes of 70.4, 92, and 190 µL were studied to obtain a greater concentration factor. An eluent volume of 70.4 µL was not enough to obtain a quantitative recovery (65%), but 92 µL provided the complete elution of cadmium from the minicolumn; and 92 µL was selected as the optimum eluent volume.

Eluent and sample flow rates were affected by a negative estimated effect. In order to achieve the greatest sampling frequency, the upper values of the flow rates were studied. The eluent flow rate was studied between 3–4 mL/min. A flow rate of 4 mL/min provided a 44.3% recovery, while the lower value studied for this variable (3 mL/min) was chosen as the optimum value because a quantitative elution was obtained. On the other hand, a sample flow rate between 2–3 mL/min was also studied. It was found that a flow rate of 3 mL/min provides a 56% recovery, while a flow rate of 2 mL/min gave a quantitative cadmium recovery of >95%; and this value was selected as the optimum for cadmium retention.

TABLE II
Experiments Outside the Framework of the Design to Fine-tune the Preconcentration-Elution Process

A	B	C	D	E	F	Cd Recovery (%)
7	0.5	3.0	190	3	1	99.7
7	0.5	3.0	70.4	3	1	65.0
7	0.5	3.0	92	3	1	103.4
7	0.5	3.0	92	4	1	44.3
7	2.0	3.0	92	3	1	96.1
7	3.0	3.0	92	3	1	56
7	2.0	2.0	92	3	1	74.9

TABLE III
Study of pH Range for Quantitative Retention of Cd

A	B	C	D	E	F	Cd Recovery (%)
8	2.0	3.0	92	3	1	106.1
9	2.0	3.0	92	3	1	46.2
3	2.0	3.0	92	3	1	98.6
2	2.0	3.0	92	3	1	72

In order to reduce the hydrochloric acid concentration (eluent), one experiment was performed using a lower concentration (2 M). The recovery obtained was not quantitative, so 3 M hydrochloric acid concentration was selected.

Finally, a few experiments were carried out with the aim of establishing the pH range for quantitative retention of cadmium. The results of these experiments are listed in Table III. Cadmium was retained quantitatively in the pH range of 3–8. This is a very important factor because the sample in the acid extract has a very low pH value (3 M nitric acid medium). An ammonium acetate buffer solution was proposed in order to achieve the optimum pH for cadmium retention. Since quantitative extraction of Cd in meat samples needs an acid medium and the pH optimum for Cd retention on the chelating resin ranges from 3–8, an ammonium acetate buffer solution was proposed. In order to carry out this pH increase on-line, the concentration, volume, and flow rate of the ammonium acetate buffer solution were studied. A 0.61-g amount of ammonium acetate was required to increase the pH from 3–5 for 2–5 mL of acid extract.

The buffer solution concentration was studied between 2.65–16 M. These concentrations implied a buffer solution volume ranging from 0.5–3.0 mL and a buffer solution flow rate from 0.4–1.0 mL/min. The principal aim was to achieve a greater sampling frequency, and the sum of both flow rates (sample flow rate and buffer solution flow rate) could not

exceed a total flow rate of 2 mL/min (maximum flow rate to obtain a quantitative cadmium retention). A 16 M concentration, 0.5 mL buffer solution, and 0.4 mL/min flow rate were selected as a compromise for the ammonium acetate channel. The optimum conditions for the cadmium preconcentration-elution are listed in Table I.

Optimization of Continuous Ultrasonic Acid Extraction of Cd

In this case, the same experimental design was used: a Plackett-Burman 2^6 (3/16) factorial type III resolution design. This factorial design was applied to 60 mg of rabbit liver with a cadmium concentration of 0.165 µg/g. The sample concentration was obtained using an off-line acid digestion procedure. Six experimental variables were optimized: nitric acid and hydrochloric acid concentrations (leaching solution), sonication time, leaching temperature, flow rate of the CUES, and leaching volume. To optimize the CUES, cadmium was measured on-line by FAAS with a flow injection system that involves the preconcentration step previously described. Table IV summarizes the lower and upper levels for each studied variable.

The analytical data show that cadmium extraction appeared to be affected positively by two statistically significant factors: nitric acid concentration and sonication time. The flow rate of the CUES, hydrochloric acid concentration, and leaching volume were affected by a negative estimated effect, which suggests that they inhibit the extraction at the highest levels tested. Thus, a 3.5 mL/min flow rate, 0 M hydrochloric acid concentration, and 2 mL volume were selected as the optimum values.

The sonication temperature was affected by a positive estimated effect but was not a statistically significant factor. In order to simplify the determination, a room temperature of 20ºC was selected as the optimum temperature for cadmium extraction. In this case, only three experiments outside of the framework of this study were carried out and the results are listed in Table V. The aim of this study was to increase the sample frequency and to reduce the nitric acid concentration. The sonication time was studied between 2.0–1.5 min. Since 2 min was enough to extract cadmium quantitatively, but 1.5 min provided an 82.3% recovery, 2 min was selected as the optimum sonication time.

Finally, the possible reduction of nitric acid concentration was studied. A 1.5 M nitric acid concentration was tested but was found not to be enough to obtain a quantitative cadmium extraction. Best results were obtained with 3 M HNO_3 and this value was chosen as the leaching solution. The optimum conditions for continuous ultrasonic acid extraction are listed in Table IV.

TABLE IV
Factor Levels Studied in the Plackett-Burman Design (2^6 3/16) for the Continuous Ultrasonic Acid Extraction of Cd From Meat Samples

Variable	Key	Low	High	Optimum
HNO_3 concentration (M)	G	0	3	3
HCl concentration (M)	H	0	3	0
Sonication time (min)	I	0.5	5.0	2
Leaching volume (mL)	J	2.0	5.0	2
Leaching temperature (ºC)	K	20	70	20
Flow rate of the CUES (mL/min)	L	3.5	6.0	3.5

TABLE V
Experiments Outside the Framework of the Design to Fine-tune the Continuous Ultrasonic Acid Extraction Process

G	H	I	J	K	L	Cd Recovery (%)
3	0	2	2	20	3.5	99.7
3	0	1.5	2	20	3.5	82.3
1.5	0	2	2	20	3.5	70.8

Two sample variables that can affect the acid extraction process were studied, namely, meat particle size and amount of sample. Using the optimum CUES and preconcentration conditions, particle sizes between 30–100 µm were tested. The results obtained indicate that this variable does not affect the extraction process. Since optimum results were obtained when a sample amount of 60 mg was used to optimize the CUES system, we supposed that smaller quantities would also be suitable. We also studied the maximum amount of sample that is quantitatively leached in order to increase the sensitivity of the method and selected 80 mg. The results demonstrated that sample amounts greater than 60 mg produce high pressure in the CUES and cause loss of sample. Therefore, the maximum sample amount that can be used is 60 mg.

Features of the Method

The calibration graph was run (n=7) under optimum chemical and flow conditions for the global process, the equation was absorbance = 7.8 x10^{-4} + 3.12 X (r = 0.999), where X is the Cd concentration expressed as µg/mL. The linear ranged from 0.067–3.243 µg/g.

The precision of the preconcentration step was verified using 1 mL Cd standard solution containing 0.024 µg/mL Cd (n = 11). The precision for the global process obtained for the real samples was checked on a rabbit liver sample containing 0.165 µg/g Cd (n = 11). The results obtained and expressed as the relative standard deviation were 1.9 and 2.9%, respectively. The limit of detection (LOD), based on three times the standard deviation (n = 30) of the blank, was found to be 0.014 µg/g. The quantification limit (LOQ) based on 10 times the standard deviation (n = 30) of the blank was found to be 0.067 µg/g. The validation of the method was performed using a certified reference material CRM183 Pig Kidney, with a cadmium content of 2.71 ± 0.15 µg/g. The cadmium content obtained for the certified reference material by the proposed method (mean ± SD, n = 3) was 2.76 ± 0.05 µg/g, which agrees with the certified value.

The concentration factor of the method based on the rate between the direct calibration graph and FIA calibration graph was found to be 20.5. The resin capacity based on the maximum amount of cadmium that was able to retain the chelating resin was 7.07 µmol Cd/g resin. The sample throughput using the proposed method was about 16 samples/h.

Analysis of the Samples

The method was applied to the determination of cadmium in various meat samples. The concentration of cadmium in these samples ranged from 0.096–0.417 µg/g. The results obtained with the proposed method were compared with those achieved by a conventional off-line sample digestion method with concentrated nitric acid, a preconcentration step previously optimized by using the chelating resin Chelite P, and determination by FAAS. To compare the results obtained by both methods, the paired *t*-test was applied (19). As shown in Table VI, both methods do not give significantly different values, thus the agreement between the two methods is satisfactory.

TABLE VI
Determination of Cd in Meat Samples and Paired t-Test

Sample	$[Cd]_A$ (µg/g)	$[Cd]_B$ (µg/g)	Recovery (%)
Chicken	0.134	0.135	99.3
Turkey	0.157	0.157	100
Pig	0.133	0.130	102.3
Calf	0.101	0.096	105.2
Mutton	0.107	0.106	100.9
Rabbit liver	0.165	0.168	98.2
Mutton kidney	0.408	0.417	97.8

A: Off-line acid digestion, preconcentration with a chelating resin (Chelite P) and FAAS Cd determination.
B: Cd concentration obtained by the present method.
Experimental value of t = 0.33; Critical value of (t_{n-1}=6, P=0.05) = 2.45

CONCLUSION

The continuous ultrasound-assisted extraction system (CUES) combined with a preconcentration step and coupled with a FI manifold has demonstrated to be a rapid, precise, and accurate sample pretreatment procedure for the acid leaching of cadmium, preconcentration of this trace metal, and its determination by FAAS in solid samples. The main goals obtained with the method proposed are a reduction of sample contamination as well as analyte loss. This is because less manipulation of the sample is required, reduced sample and reagent amounts are needed, and a reduction in the sample preparation time is achieved which significantly increases sample throughput.

This procedure combines the benefits of ultrasound-assisted extraction with the high sensitivity provided by the preconcentration step and the inherent advantages of the FI systems.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). Ministerio de Ciencia y Tecnología (Project No. CAL01–043). S.C. acknowledges a grant from the Xunta de Galicia.

Received July 14, 2004.

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Medical Application of Fast Furnace Program Used in the ETA-AAS Determination of Cu and Zn in Blood Plasma of Children With Down Syndrome

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ABSTRACT

The medical application of a fast furnace program is presented using ETA-AAS-based methods for the determination of copper and zinc in blood plasma of children with Down syndrome. The analytical parameters were evaluated to establish the reliability and reproducibility of the ETA-AAS method for clinical purposes. The accuracy was verified by analyzing two certified materials, comparing the target and experimental values for each metal, and obtaining mean relative errors of 0.63% for Cu and 1.50% for Zn. Additionally, the recovery studies were carried out, with recoveries of 100.6% and 100.2% for Cu and Zn, respectively.

The average precision (expressed as RSD) was 0.97% for Cu and 1.21% for Zn. The detection limit (3 σ) was 0.1 µg/L for both analytes. The characteristic mass was 5.3 pg for Cu and 0.9 pg for Zn using a 20-µL sample injection volume. The metals concentrations were established for 70 blood plasma samples from children with Down syndrome and from controls (35 samples each), with concentration ranges from 366–1616 µg/L Cu and 1183–2660 µg/L Zn for the experimental group, and 500–1500 µg/L Cu and 683–3817 µg/L Zn for the control group.

The ETA-AAS-based methods described for Cu and Zn represent an analytical and clinical tool for the evaluation of this kind of genetic anomaly, and permit the accurate, precise, and interference-free determination of copper and zinc in clinical samples.

INTRODUCTION

Copper and zinc are essential elements for the human organism and are indispensable components of a great number of enzymes and proteins of the body's systems. These metals are obtained through the daily intake of foods (1) and an inadequate consumption affects the body's immune system (2).

Copper is found in various tissues and organs of the human body, where the liver, brain, and bones contain the highest Cu concentrations. High levels of plasmatic Cu are responsible for gastrointestinal malfunctions, anorexia, infantile malnutrition, nausea, frequent vomiting, dysfunction of the central nervous system, and the well-known copper fever (3). Low levels of Cu can cause certain types of anemia since ceruloplasmine (an enzyme associated with Cu) diminishes the metal's capacity to promote hematopoiesis (3) and affects mental development (4).

Zinc has catalytic and regulatory functions in many biological systems (4), and is absorbed from the duodenum and the jejunum like a protein complex in the mucous tissues. Zinc is a component of the proteins that participate in the formation of collagen and in the production of insulin, is required for the duplication and development of cells, responsible for sexual maturation (4,5), and involved in the repair and transcription of deoxyribonucleic acid (6). A deficiency in Zn manifests itself in poor growth development, hypogonadysm, lack of appetite, and alteration in the scarring of wounds. A lack of Zn intake is also related to illnesses and vesicular lesions of the skin and causes breathing infections (6,7).

Down syndrome is a very frequent chromosomal anomaly, occurring in about one in every 700 births (3). It is widespread around the world, and its congenital dysfunctions are characterized by different degrees of mental retardation and multiple physical defects (7). In children with Down syndrome, the immune system is depressed (2,6,7), and causes breathing infections, where high levels of the thyrotropin regulatory hormone of the thyroid (TRH) are observed (6,7).

Since Cu and Zn are present at trace levels in different types of clinical samples, it is important to use an analytical instrumental technique reliable and modern to determine these elements with adequate accuracy and precision. Numerous analytical methods for Cu and Zn determination have been developed due to the clinical relevance of these metals (4,8–12). The techniques most widely used for the determination of Cu and Zn are inductively coupled plasma mass spectrometry (ICP-MS) (8), anodic stripping voltametry (ASV) (9), flame atomic absorption spectrometry (FAAS) (4), and electrothermal atomization atomic absorption spectrometry (ETA-AAS) (10–12).

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The ETA-AAS technique is most widely used for the determination of Cu and Zn in biological, environmental, clinical, and food samples. It allows the trace determination of these two metals and provides low detection limits (i.e., in µg/L). This technique favors the determination of these metals in blood plasma at trace levels due to its high sensitivity, excellent selectivity, and minimum requirements in the manipulation and treatment of the samples, thus decreasing contamination problems (12). ETA-AAS also requires small sample volumes, which is a very important aspect for this type of clinical analysis. In addition, the graphite furnace is able to reach high and controlled temperatures, allowing the efficient elimination of the concomitant without significant loss of the analytes under study. Another advantage of this technique is the use of parameters that maximize instrumental performance such as the ability to use an analytical isoformer, the use of air or another gas (as internal alternate gas) during the pyrolysis step for the removal of carbon residues, the addition of surfactants, and the use of normal or pyrolytically coated graphite tubes with or without L'vov platform. The main difficulties with this technique are volatilization problems of the analyte and spectral interferences. However, these types of interferences are corrected by using the background corrector, which allows the correction of high background absorbance values generated by the matrix. The volatilization problems are frequently corrected by the addition of an analytical isoformer (13). Despite the above-mentioned analytical advantages, sequential multielemental determinations using ETA-AAS are very time-consuming. Therefore, it is necessary to develop analytical methods that use fast temperature programs of ≤45 s in comparison to the conventional temperature programs of ≥90 s required for the complete dry-pyrolyze-atomize cycles in the graphite furnace.

The aim of this work is to present two fast furnace programs for the ETA-AAS determination of copper and zinc in blood plasma of children with Down syndrome and of a control group (healthy infants). These analytical methods are employed like reliable and reproducible tools for the clinical evaluation of this kind of genetic anomaly.

EXPERIMENTAL

Instrumentation

For the analytical determinations of Cu and Zn, a PerkinElmer Model 2380 atomic absorption spectrometer was used, equipped with a deuterium arc continuum background correction system and coupled to a PerkinElmer Model HGA®-500 graphite furnace (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). A PerkinElmer Model AS-40 autosampler was also employed. Wall atomization on the pyrolytically coated graphite tubes was used for all experiments. Hollow cathode lamps were individually used as the primary radiation sources for Cu and Zn. Table I shows the instrumental operating parameters and temperature programs used for the determination of Cu and Zn in the blood plasma samples.

Reagents and Standard Solutions

All chemical reagents used for this study were of analytical grade. The concentrated solutions of copper and zinc (~1000 mg/L) were prepared from copper metal powder (Fisher Scientific, Pittsburgh, PA, USA) and using a commercial concentrated solution of Zn (Riedel of Haën, Hannover, Germany).

The standard solutions for the calibration curves were performed daily by direct dilution of the concentrated solution of each analyte in 0.01 M nitric acid (Merck, Darmstadt, Germany). For both metals, the concentrations of the aqueous standard solutions for the working curves were 5, 10, 15, 20, 25, and 30 µg/L. These standards were prepared from intermediate solutions of 100 mg/L Cu and 100 mg/L Zn.

TABLE I
Operational Parameters and Fast Temperature Programs Used in the Graphite Furnace for the Determination of Cu and Zn by ETA-AAS

Wavelength	324.8[a]/213.9[b] nm	Inert gas	Argon
Slit width	0.7 nm	Injection temp.	20°C
Lamp current	10.0 mA[c]	Graphite tube	Pyrolytically coated
Sample volume	20 µL	Atomization type	Wall

Temperature Program

Step	Dry 1	Dry 2	Pyrolysis	Atomization	Clean-out
Temp. (ºC)	80	200	900[a]/1000[b]	1800[a]/2200[b]	2700
Ramp (s)	1	10	5	0	1
Hold (s)	5	1	5	5	2
Read				0	
Rec.				–5	
Internal flow rate					
Ar (mL/min)	300	300	300	0	300

[a] For Cu.
[b] For Zn.
[c] For both metals using hollow monocathode lamps for each analyte.

Triton® X-100 0.01% (v/v) (Riedel of Haën) was used to dilute the blood plasma. All of the solutions were prepared with water grade I ASTM (14) in polypropylene calibrated flasks (Nalgene Labware, Nalge, Rochester, NY, USA). The concentrated nitric acid (Merck) contained concentrations of Cu and Zn which are not detectable by ETA-AAS.

Evaluation of the accuracy of the methods was carried out in the analysis of certified reference material HPS 590321 Trace Metals in Drinking Water (from High-Purity Standards, HPS, Charleston, SC, USA) and a standard reference material NIST 1566a Oyster Tissue (from the National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA).

Population Under Study

This study was carried out for a group of 70 children: 35 children with Down syndrome and 35 healthy children (the control group), ranging in age from 6 months to 6 years. Informed consent was obtained from the parents of both groups studied. The clinical protocol was approved by an *ad hoc* committee. These children belonged to poor families located in the IV and V socio-economic stratum according to the modified Graffar's method for Venezuela (15).

Collection of Blood Plasma Samples

The blood plasma samples were collected in the Unit of Genetics, University Hospital of Maracaibo City, Venezuela. Approximately 5 mL of whole blood was collected from each individual and placed into polypropylene tubes, which contained sodium heparin as the anticoagulant (i.e., 50 µL for each mL of blood sample). The blood samples were centrifuged at 15,000 rpm for 10 minutes. Supernatants were used as the plasma samples. The obtained plasma was placed in another clean polypropylene tube, and stored at 4ºC to avoid decomposition and bacterial growth. Prior to the spectrometric determination of Cu and Zn, the plasma samples were diluted 100-fold with Triton X-100 0.01% (v/v); the certified materials were diluted 10-fold with 0.01 M nitric acid. Each test portion was prepared in triplicate and analyzed five times in order to obtain the mean, standard deviation, and relative standard deviation. Statistical analyses were carried out by conventional methods using commercial statistical programs (i.e., Origin 6.0, Excel® 2000, etc.). Differences were considered statistically significant when p was <0.05.

RESULTS AND DISCUSSION

The methodologies used for the ETA-AAS determination of Cu and Zn in blood plasma of children with Down syndrome were based on the physicochemical characteristics of these elements [i.e., melting (mp) and boiling (bp) points of the atomic precursor of the element considered]. These elements are among the intermediate metals and are neither volatile nor refractory, which suggests that atomization of both occurs via an oxide (13). Thus, Cu and Zn have metal oxides with a relatively high mp and bp [Cu: mp of 1235°C and bp of 1800°C; and Zn: mp of 1975°C and bp of >2000°C (16)], which makes them very stable at the pyrolysis temperatures considered. Based on these physicochemical characteristics, it can be concluded that (a) the use of STPF conditions for the determination of Cu and Zn by ETA-AAS is unnecessary, and (b) moderate pyrolysis temperatures (~1000°C) can be applied in the graphite furnace for the efficient removal of the concomitants without significant volatilization loss of Cu and Zn.

Consequently, it is possible to develop fast temperature programs (FTP) for the determination of Cu and Zn by ETA-AAS without using STPF conditions. Thus, the interference effects of the background on the analytical signals (spectral interferences), were minimized using a FTP for the graphite furnace.

The fast temperature programs were optimized for the ETA-AAS determination of Cu and Zn using moderate temperatures and short ramp and hold times in the pyrolysis step.

Selection of Graphite Furnace Programs

The temperature programs for the graphite furnace were optimized using aqueous standard solutions of Cu and Zn (~5.00 µg/L of each, equivalent to 100 pg of Cu or Zn); a 10-fold diluted Trace Metals in Drinking Water sample, which has Cu and Zn masses of 41 and 1394 pg, respectively; and a 100-fold diluted real sample of blood plasma (164 pg of Cu and 192 pg of Zn). The temperatures of the pyrolysis and atomization steps were optimized by increasing the temperature and taking the integrated absorbance from the diluted real blood plasma samples with a known mass of Cu and Zn [as previously reported for ETA-AAS methodologies (17)], and observing the maximum values for the integrated absorbance (Figure 1). From the analysis of the pyrolysis and atomization curves, two interesting inferences were observed: (a) the optimum pyrolysis temperatures that guarantied the efficient removal of the concomitant of inorganic and organic origin in the evaluated matrix were 900°C and 1000°C for Cu and Zn, respectively; and (b) the optimum atomization temperatures for Cu and Zn were 1800°C and 2200°C, respectively (Figure 1). These temperatures were necessary to ensure good elimination of the matrix and also to achieve high sensitivity, reliability, and reproducibility. Moreover, the results demonstrated that with

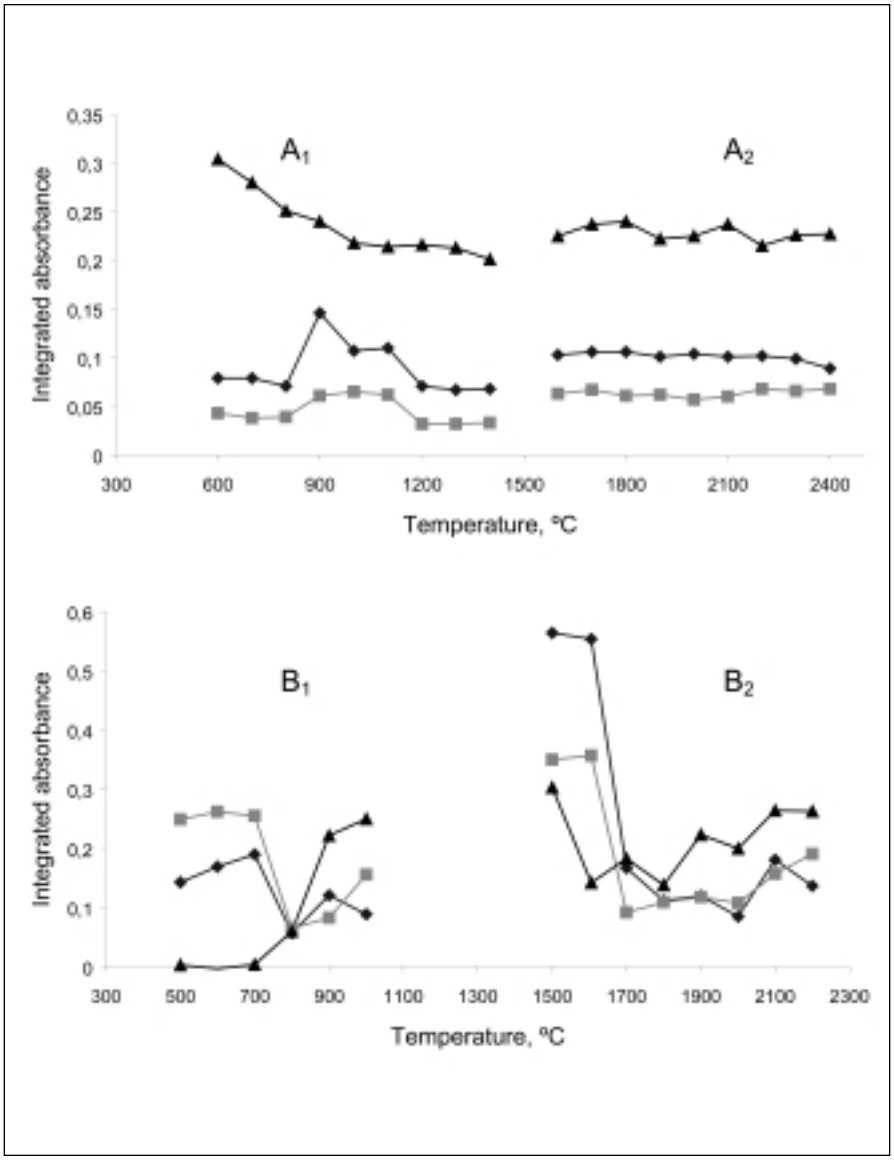

Fig. 1. Pyrolysis (A_1 and B_1) and atomization (A_2 and B_2) temperature optimization curves for Cu (A) and Zn (B) determinations by ETA-AAS, where: -◆- aqueous standard (100 pg of Cu or Zn), -■- diluted Trace Metals in Drinking Water HPS CRM 590321 (41 pg of Cu and 1394 pg of Zn), and -▲- real blood plasma sample (164 pg of Cu and 192 pg of Zn).

these temperatures, the determination of these metals can be carried out without loss due to volatilization, and thus obtain excellent accuracy and precision for the real samples and the certified materials.

There is controversy regarding the pyrolysis and atomization temperatures used in the graphite furnace for the determination of Cu and Zn in different types of matrices. Some authors report high pyrolysis temperatures ranging from 1200–1300°C for Zn (10,11,18); and for copper, low temperatures ranging from 700–1000°C (11) or between 300 and 800°C (19). Other authors report atomization temperatures ranging from 2100–2300°C for Cu and 1900–2300°C for Zn (10,11,18). These authors used short ramp and hold times (~10–25 s) in the dry step, ~40 s in the pyrolysis step, and a few seconds (~3–5 s) for the atomization step. These differences in the pyrolysis and atomization temperatures are related principally to the type of sample matrix used (i.e., organic or inorganic).

Evaluation of Analytical Parameters

The accuracy of the spectrometric methods proposed for the determination of Cu and Zn was verified analyzing two certified materials: HPS 590321 Trace Metals in Drinking Water and NIST 1566a Oyster Tissue. These materials were previously mineralized by using a reported method (17) to provide aqueous solutions whose final Cu and Zn concentrations were within the range of the metal concentrations expected in the clinical samples evaluated. The results obtained in this study are presented in Table II, which shows a similarity between the target and the experimental values. No significant statistical differences ($p < 0.05$) were observed. This parameter was also evaluated by performing recovery studies of each analyte added to the

TABLE II
Accuracy Studies for the ETA-AAS Determination of Cu and Zn in Certified Materials

Certified Materials	Concentration of Cu and Zn[a] (mean ± SD, µg/L) Target	Experimental	Relative Error (%)
CRM 590321[b]	20.00 ± 0.10	20.01 ± 0.60	0.05
	(70.00 ± 0.50)	(72.01 ± 1.70)	(2.85)
SRM 1566a[c]	66.30 ± 4.30	67.10 ± 3.60	1.20
	(830.30 ± 57.00)	(831.50 ± 59.00)	(0.14)

[a] Values in parenthesis are for zinc.
[b] Trace Metals in Drinking Water from the High-Purity Standards (HPS, USA).
[c] Oyster Tissue from the National Institute of Standards and Technology (NIST, USA).

TABLE III
Within- and Between-run Precision Studies for the Determination of Cu and Zn by ETA-AAS[a]

Sample	Mean[b] (µg/L)	Within-run[c] SD (µg/L)	RSD (%)	Between-runs[d] SD (µg/L)	RSD (%)
Plasma					
1	1.60	0.01	0.60	0.02	1.20
	(1.40)	(0.01)	(0.70)	(0.02)	(0.05)
2	11.20	0.05	0.44	0.16	1.40
	(1.02)	(0.03)	(3.00)	(0.01)	(0.98)
3	12.68	0.08	0.59	0.17	0.01
	(1.62)	(0.04)	(2.50)	(0.02)	(0.94)
4	32.26	0.39	1.22	0.01	0.01
	(2.03)	(0.02)	(0.75)	(0.18)	(1.90)
Drinking water	2.00	0.01	0.50	0.01	0.05
	(7.10)	(0.02)	(0.28)	(0.14)	(1.97)

[a] Values in parenthesis are for zinc.
[b] Concentrations of Cu and Zn in the diluted test portion of real sample.
[c] Aliquot samples prepared by triplicate, five runs each.
[d] Triplicate samples per analysis; five runs each and analyzed over a five-day period.

blood plasma samples, resulting in average recoveries of 100.6% and 100.2% for Cu and Zn, respectively, and verifies appropriate accuracy for the described methods.

The within- and between-run precisions of the developed methods were evaluated for four real blood plasma samples diluted 100-fold (Table III). Three aliquots of each real sample were analyzed (five runs each) using the instrumental and operating conditions previously listed. The obtained results show average RSDs of 0.60% (Cu) and 1.08% (Zn) for the within-run and 1.34% (Cu) and 1.33% (Zn) for the between-run precisions. These results can be considered adequate for these types of analyses.

A spectral interference study was performed by carrying out a background study in which the performance of the continuum source background corrector was evaluated to ensure its efficacy in the appropriate compensation of the spectral interferences. This procedure was described in detail elsewhere (4,13). Briefly, comparisons between background-corrected integrated absorbances and those obtained by subtracting the background signals from the uncorrected absorbances were established. Mean relative errors of 1.87% and 1.16% were found for Cu and Zn, respectively. These facts indicate that the deuterium arc was effective in compensating for the spectral interferences.

Non-spectral interference studies were carried out by comparing the slopes of the working curves with those obtained by the method of standard additions. For ETA-AAS determinations of Cu in blood plasma, the equations obtained for the standard addition and calibration curves were:
$A = 0.0151c + 0.0216$, $r = 0.9998$ ($p < 0.001$); and
$A = 0.0146c - 0.0013$, $r = 0.9999$

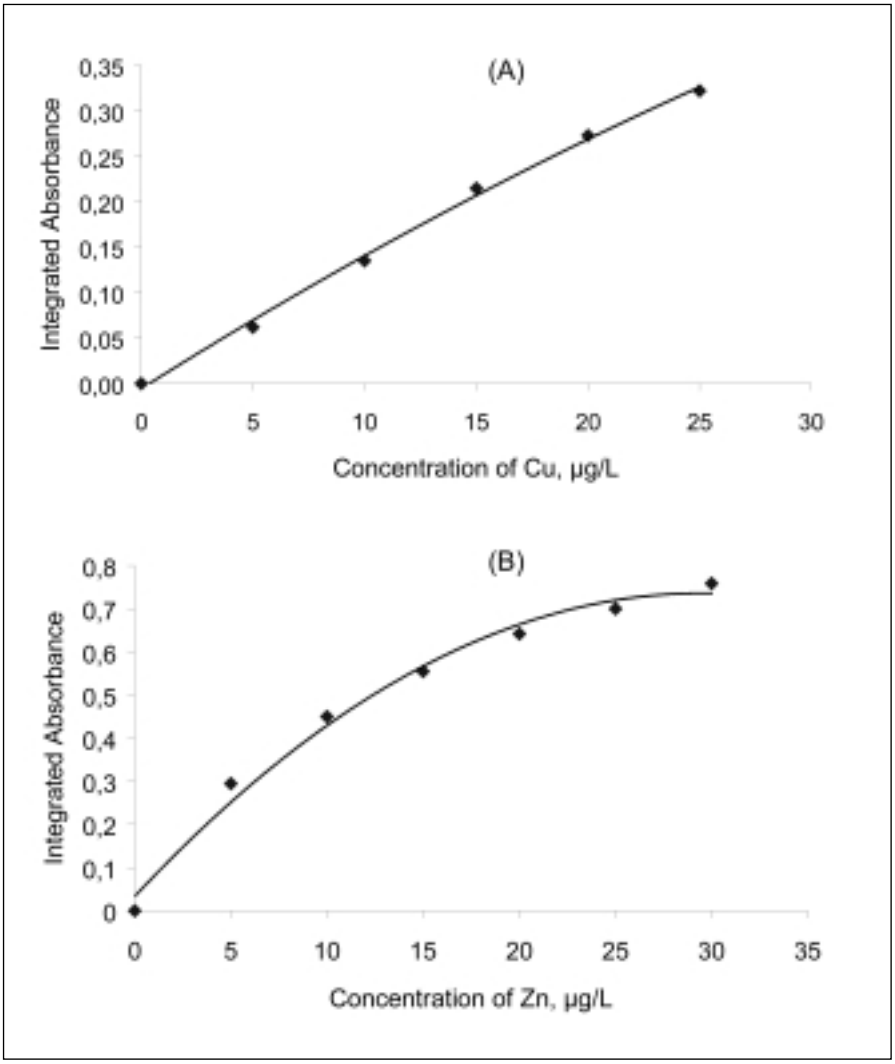

Fig. 2. Calibration curves for the ETA-AAS determination of Cu (A) and Zn (B) in blood plasma of children with Down syndrome.

($p < 0.001$), respectively (where A = integrated absorbance, c = concentration, p = statistical error, and r = correlation coefficient). The mean relative error between slopes was 3.3%. These results implied the absence of non-spectral interferences in the ETA-AAS analyses for Cu and permitted the use of either the calibration curves or the standard additions methods for metals quantification. However, blood plasma Cu levels were evaluated against aqueous copper standard calibration curves.

The linearity of the calibration curve for Cu and Zn is very small and only appears up to 15 µg/L Cu and 10 µg/L Zn, which indicates that its dynamic interval is quite limited. As a consequence, quantification of these metals in the blood plasma samples was carried out for extrapolation of the calibration curve, which has the form of a parabola arc (Figure 2). Similar concentrations of Cu and Zn were used to elaborate the calibration curves (~ 5, 10, 15, 20, 25, and 30 µg/L). These curves allowed the analytical determination of Cu and Zn in the blood plasma samples.

The quantification of Zn in the blood plasma samples was performed by extrapolation of the working curve in the form of a parabola arc because the spectrometric dynamic range of Zn is very small (i.e., up to 10 µg/L). Figure 3 shows the parallelism between the slopes of the calibration and standard addition curves obtained for Zn, indicating that non-spectral interferences did not exist in the FTP-ETA-AAS determinations of this metal. These results reinforced the analytical use of the calibration curves for quantification purposes of Cu and Zn in blood plasma of children with Down syndrome by ETA-AAS.

The characteristic masses were 5.3 and 0.9 pg/0.0044 s^{-1} for Cu and Zn, respectively, using a 20-µL

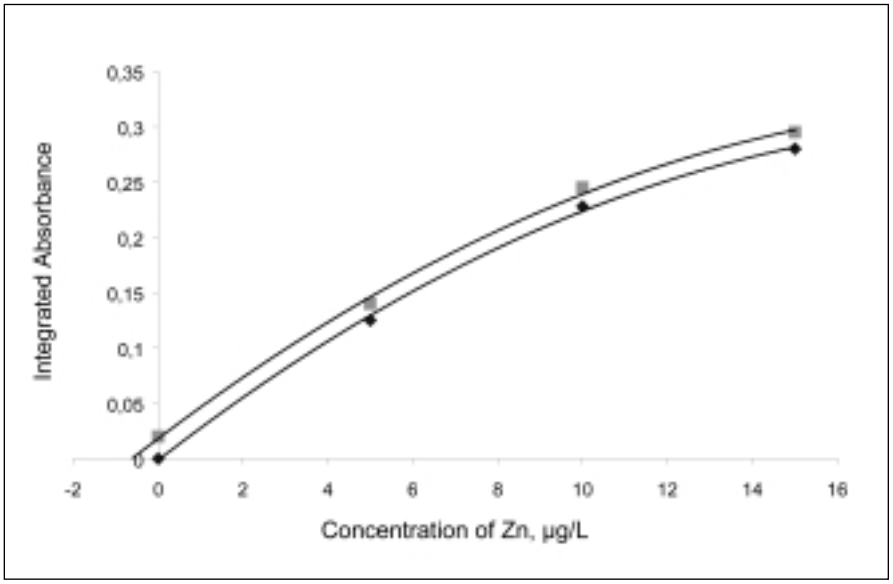

Fig. 3. Study of non-spectral interferences for the determination of Zn in blood plasma samples of children with Down syndrome by ETA-AAS, showing the adequated parallelism between calibration (◆) and standard addition (■) curves.

injection volume was used. Experimental characteristic masses for Cu were lower than the reported value (~6.4 pg) of the instrument used (20). The characteristic mass for Zn was higher than the reported value (~0.1 pg) by the spectrophotometer used (20).

The limits of detection (defined and calculated as three times the standard deviation of the blanks, expressed in µg/L as a concentration unit) in the blood plasma samples analyzed were 0.1 µg/L for both copper and zinc. In the case of Cu, this value was lower than the reported value (~0.3 µg/L) by the instrument employed (20). In solid samples, Vale et al. (10) reported a detection limit of 0.014 µg/g Cu. For Zn, the detection limit was similar to the reported value by the instrument. Several authors reported detection limits ranging from 0.2 to 4.0 µg/L (7,11,18).

The methods developed were used in our laboratory and allowed the analysis of 70 samples within an 8-hour working day. In addition to reducing the total analysis time, the number of samples analyzed was increased, excellent reproducibility and reliability was achieved, showing that the methods are suitable for use in clinical laboratories of hospitals. From an analytical point of view, it can be stated that there is no difference between samples coming from children with or without Down syndrome, implying that the matrices of the evaluated blood plasma samples are similar in nature.

Levels of Cu and Zn in Down Syndrome

The concentrations of copper and zinc in blood plasma samples of children with Down syndrome and the controls are listed in Table IV. For Cu, no statistically significant differences ($p > 0.01$) between Down and the control infantile populations were observed. As shown in Table IV, the Cu mean values (± SD, µg/L) were 805 ± 261 and 767 ± 288, for Down and the controls, respectively. For Zn, a significant statistical difference ($p < 0.01$) was found between Down children and the controls, with respective mean values of 1839 ± 261 µg/L and 1374 ± 867 µg/L. These results could be due to similar food intake of the infantile population under study and the aleatory sampling carried out.

In accordance with the established Cu and Zn norm for healthy individuals, the blood plasma Cu mean concentrations listed in Table IV show a deficit of the metal in both infantile populations [~900–1300 µg/L Cu (4)]. The low Cu levels found could become harmful for the children studied since levels below those considered normal can cause illnesses such as anemia, high cholesterol, and coronary dysfunction (21). The copper levels for Turkish Down children as reported by Cenquiz et al. (1) (~750 ± 170 µg/L Cu) are not statistically different from those found in this work, indicating that the nutritional state of children living in poor socio-economic areas is very similar to those in other parts of the world.

Table IV also shows that the Zn concentrations found in the blood plasma samples indicate a significant increase of this metal in comparison to the established Zn norm for good health [~900–1100 µg/L Zn (4)]. According to studies carried out by Artacho et al. (6) and Madaric et al. (22) no clinical pathologies are associated with high levels of zinc in blood plasma. It can therefore be concluded that the high Zn level of the studied populations does not pose any health risks. Statistical differences ($p < 0.01$) were found between the blood plasma Zn concentrations of Down infants and the controls (Table IV). As is true for Cu, the findings for Zn are related to the low socio-economic stratum of the children studied (15). Cengiz et al. (2) reported blood plasma Zn concentrations of 660 ± 180 µg/L for Turkish children with Down syndrome, which are below the norm as previously indicated. The blood

TABLE IV
ETA-AAS Determination of Copper and Zinc in Blood Plasma of Children With Down Syndrome and a Control Group From the City of Maracaibo, Venezuela

Sample	Metal	Mean ± SD[a]	C.V.(%)	Experimental Range[a]
Down	Cu	805 ± 261	32.37	366 – 1616
	Zn	1839 ± 361	19.6	1183 – 2660
Control	Cu	767 ± 288	37.57	500 – 1500
	Zn	1374 ± 867	63.1	683 – 3817

[a] In µg/L.

plasma Zn mean values reported by Cengiz et al. were very different from those found in this investigation (~1839 ± 361 µg/L Zn). This elevated Zn mean concentration can be ascribed to the nutritional conditions of the children selected for this work.

CONCLUSION

A medical application of the fast furnace program for the ETA-AAS determination of copper and zinc in blood plasma of children with Down syndrome and healthy controls was successfully carried out. This method provides analytical and clinical tools for the evaluation of this kind of genetic anomaly.

These fast electrothermal atomization atomic absorption spectrometry methodologies allowed for the accurate, precise, and interference-free determination of copper and zinc in clinical samples without the use of two basic paradigms of the STPF conditions: isoformation and platform atomization.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to La Universidad del Zulia (LUZ, Maracaibo, Venezuela). The authors also acknowledge the previous instrumental endowment obtained through of the finished researches and financed by Fondo Nacional de Ciencia y Tecnología (FONACIT, Caracas, Venezuela) and Consejo de Desarrollo Científico y Humanístico (CONDES, LUZ). This research was partially supported by the researchers from Laboratorio de Instrumentación Analítica (LIA-LUZ).

Received June 7, 2004.
Revision received March 17, 2005.

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Determination of Tellurium by ETAAS With Preconcentration by Coprecipitation With Lanthanum Hydroxide

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ABSTRACT

A procedure for the determination of traces of total tellurium is described which combines electrothermal atomic absorption spectrometry (ETAAS) with preconcentration of the analyte by coprecipitation. The samples, each spiked with lanthanum nitrate (20 mg L^{-1}), are introduced into the Amberlite XAD-4 resin, and mixed with ammonium buffer (pH 9.1). Tellurium is preconcentrated by coprecipitation with the generated lanthanum hydroxide precipitate. The precipitate is quantitatively collected in the resin, and subsequently eluted with 5% (v/v) nitric acid. The determination is developed with ETAAS.

Considering a sample consumption of 25 mL, an enrichment factor of 10 was obtained. The detection limit (3σ) was 0.04 µg L^{-1} and the precision (relative standard deviation) was 3.5% (n=10) at the 10-µg L^{-1} level. The calibration curve, using the preconcentration system for tellurium, was linear with a correlation coefficient of 0.9993. Satisfactory results were obtained for the determination of tellurium in the standard reference material NIST 1643d Trace Elements in Water and in a tap water sample.

INTRODUCTION

Tellurium is a rare trace element and used as an additive to improve alloys. Powdered tellurium is employed as a secondary vulcanizing agent in various kinds of rubbers (natural rubber and styrene-butadiene rubbers) since it reduces curing time and endows the rubbers with increased resistance to heat and abrasion. Due to its photoelectric properties, tellurium and its compounds are also employed in the semiconductor and electronic industries (1). Because of the extremely low levels of tellurium in various matrices, a sensitive method is required for its determination.

In the past years, several methods have been developed for the determination of Te at low concentrations, among them anodic stripping voltammetry (2–4), inductively coupled plasma optical emission spectrometry (ICP-OES) (5,6), inductively coupled plasma mass spectrometry (ICP-MS) (7,8), and electrothermal atomic absorption spectrometry (ETAAS) (8–11).

Electrothermal atomic absorption spectrometry appears to be one of the most attractive approaches for trace element determination (12–14). However, the direct determination of ultratrace amounts of elements by ETAAS is usually difficult owing to an insufficient instrument detection power. Consequently, preconcentration procedures, such as ion exchange, adsorption, solvent extraction and coprecipitation, are often needed before ETAAS determination.

Separation and preconcentration techniques using sorption extraction (2–4), solvent extraction (10), ion exchange (9), and coprecipitation (6) have been employed for the preconcentration of tellurium in batch and flow injection modes.

The use of a knotted reactor (KR) as collector for precipitates has been found feasible in flow injection (FI) on-line precipitation-preconcentration systems. Besides, using KR, stainless-steel filters and packed-bed filters have also been used to retain precipitated compounds (15–17). Although XAD adsorption resins have been employed in on-line preconcentration systems for retaining soluble complexes (18–20), in a previous work we have reported the use of XAD-7 resin as a filter packing to retain Pb-diethyldithiocarbamate precipitate (21).

In the present work, a method for the preconcentration of tellurium and its determination by ETAAS is proposed. Tellurium was preconcentrated by coprecipitation with lanthanum hydroxide using a packed-bed filter with Amberlite XAD-4 resin packing. The methodology was applied for the analysis of tellurium in the standard reference material NIST 1643d Trace Elements in Water and in tap water.

EXPERIMENTAL

Instrumentation

The measurements were performed with a Shimadzu Model AA-6800 atomic absorption spectrometer (Tokyo, Japan), equipped with a deuterium background corrector, a Model 6500 electrothermal atomizer, and an ASC-6100

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autosampler. Wall atomization with standard high-density graphite tubes (Shimadzu-Tokyo-Japan) was used. Some experiments were developed comparing the performance of high density graphite tube, pyrolytic graphite tube, and graphite tube with L'vov platform. Best results were obtained with the high density graphite tube. The use of a matrix modifier was not necessary because with the furnace program used there were no analyte losses. The introduction of oxygen was not necessary due to the simplicity of the matrix under study. A tellurium hollow cathode lamp (Hamamatsu Photonics K.K., Japan) was employed as the radiation source.

The instrument settings and furnace program are detailed in Table I. A Minipuls™ 3 peristaltic pump (Gilson, Villiers-Le-Bell, France) was used. A home-made microbore glass column (50 mm length; 3 mm internal diameter) fitted with porous 25 µm glass frits was used as the resin holder. Pump tubes, Tygon® type (Ismatec, Cole-Parmer Instrument Company, Niles, IL, USA), were employed to propel the sample, reagent, and eluent.

TABLE I
Instrumental Operating Parameters and Furnace Temperature Program for Te Determination

Parameters				
Wavelength	214.3 nm			
Spectral Bandpass	0.2 nm			
Lamp Current	14 mA			
Background Correction	Deuterium Lamp			
Furnace Program				
Stage	Temp. (ºC)	Time (s)		Argon Gas Flow (L min^{-1})
		Ramp	Hold	
Drying	120	30	-	0.10
	250	10	-	1.0
Pyrolisis	600	-	20	1.0
	600	-	5	0.0
Atomization	2500	-	3	0.0 (Read)
Cleaning	2600	-	2	1.0

Reagents

Amberlite XAD-4 resin (Rohm & Haas, Philadelphia, PA, USA) was used. The particle size was between 20 and 50 mesh with a surface area of 450 m^2 g^{-1}. Before use, the surface of the resin was activated by immersion in a solution of 4 mol L^{-1} methanol/hydro chloric acid (1:1). Subsequently, the metal impurities were removed by further washing with 2 mol L^{-1} HCl solution.

Tellurium standard solution was prepared by appropriate dilutions of a 1000 mg L^{-1} stock solution (Merck, Darmstadt, Germany) immediately before use.

Lanthanum nitrate solution (0.5% m/v) was made by dissolving 0.66 g of lanthanum nitrate hexahydrate in 100 mL of ultrapure water.

The buffer solutions used were in all instances 0.2 mol L^{-1} ammonium chloride, adjusted to the appropriate pH (9.0–9.2) by addition of 0.2 mol L^{-1} ammonia; the optimum pH was found to be 9.1.

Ultrapure water (18 MΩ cm^{-1}) was obtained from an EASY pure RF (Barnstedt, Dubuque, IA, USA).

All solvents and reagents were of analytical reagent grade or better, and the presence of tellurium was not detected in the working range.

Sample Pretreatment

Water samples were filtered through a 0.45-µm pore size membrane filter. An appropriate amount of 0.5% lanthanum nitrate solution was added (so that each sample solution contained a final concentration of 20 mg L^{-1} lanthanum nitrate) and then acidified to pH 3.0 with 0.1 mol L^{-1} hydrochloric acid.

Operational Procedure

A 25-mL sample and buffer solution at 5.0 mL min^{-1} and 1.0 mL min^{-1} loading flow rates, respectively, was loaded by means of peristaltic pump into the column. The sample and buffer streams merged at a point about 1 cm upstream of the column. The precipitate, which is formed instantaneously after the merging point, was collected on the XAD-4 resin. The effluent emerging from the resin was discarded. The coprecipitated analyte was eluted from the resin with nitric acid up to a final volume of 2.5 mL.

After the preconcentration step, 50 µL of the analyte solution was automatically introduced into the graphite tube by means of the furnace autosampler. Then, the autosampler arm was moved back to the wash position and the atomization program was started. The absorbance measurements (peak height) were proportional to the tellurium concentration in the sample, and were used for all measurements. With respect to the characteristic mass for tellurium, the manufacturer does not report any value for this analyte. The characteristic mass obtained in our laboratory was 20 pg.

Atomic Spectroscopy
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RESULTS AND DISCUSSION

Effect of pH and Lanthanum Concentration

Preliminary experiments showed that the pH and the lanthanum concentration in the sample were critical for the precipitation reaction itself and the subsequent recovery of tellurium. Measured on standards all containing 10 µg L^{-1} of tellurium, the absorbance remained almost constant in the pH ranges of 9.0–9.2. The results obtained are shown in Figure 1. Moreover, the lanthanum nitrate concentration was within the range: 15–30 mg L^{-1}. In the present work, 20 mg L^{-1} lanthanum nitrate and a pH of 9.1 were selected for subsequent studies. These results are in agreement with those obtained by Tao et al. (15) who used knotted reactors for the determination of Se .

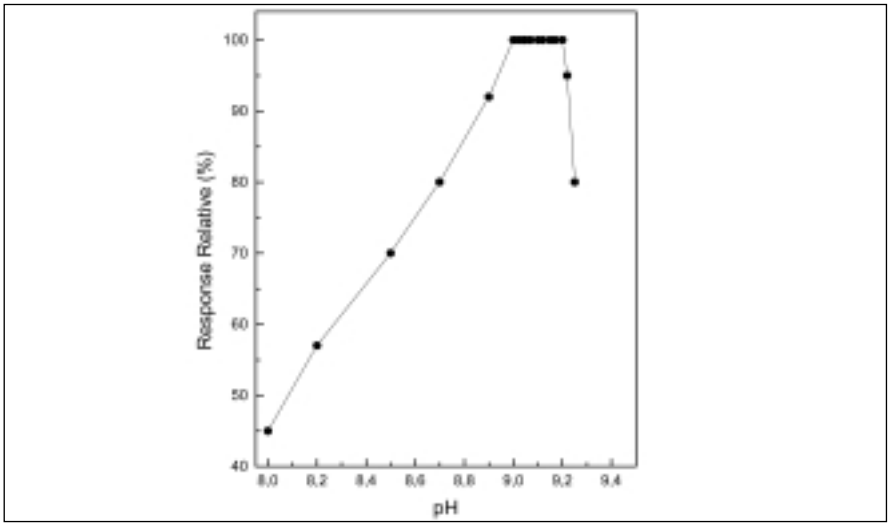

Fig. 1. Effect of pH of loading solutions. Sample loading volume 25 mL, loading flow rate 5 mL min^{-1}, elution flow rate 1.5 mL min^{-1}, Te concentration 10 µg L^{-1}, lanthanum nitrate concentration 20 mg L^{-1}.

Selection of Resin

A resin Amberlite XAD-4 packing filter allowed the tellurium present in the samples to be efficiently preconcentrated. This might have been due not only to retention by filtration of the precipitate, but also possibly due to adsorption effects of the precipitate on the resin surface. The resin size constitutes an important parameter, since it must allow appropriate precipitate retention with low hydrodynamic impedance. The particle size used in this work (20–50 mesh) permitted us to obtain optimum retention (95%) at sample flow rates of 5 mL min^{-1}.

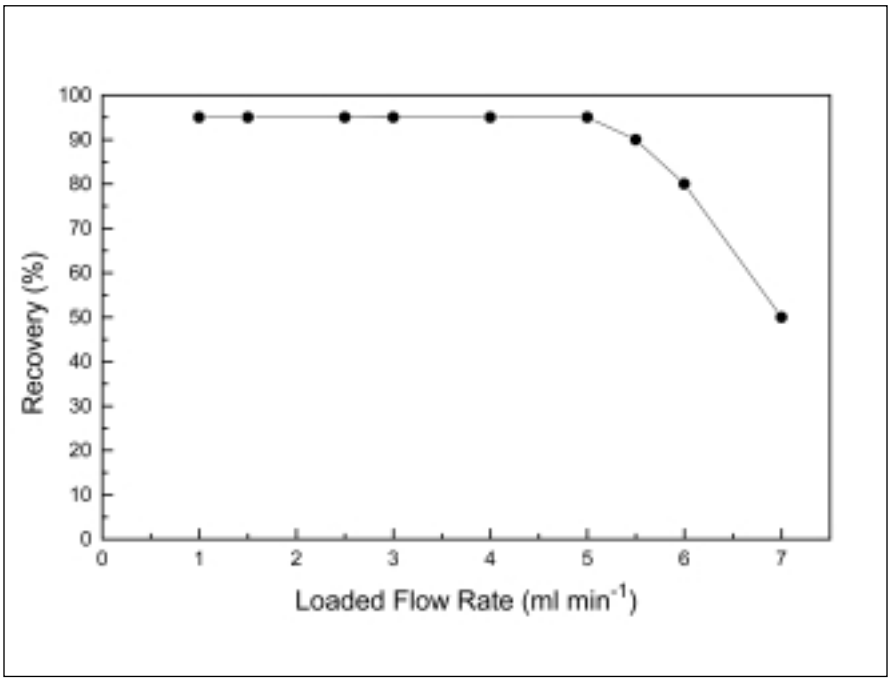

Fig. 2. Analysis of sample loading rate. Sample loading volume 25 mL, elution flow rate 1.5 mL min^{-1}, Te concentration 10 µg L^{-1}, lanthanum nitrate concentration 20 mg L^{-1}.

Sample Loading Rate

The sample flow rate through the column is one of the steps that controls the preconcentration time. In this study it was verified that with flow rates up to 5.0 mL min^{-1} there is no effect on analyte recovery, which under optimum conditions is 95%. Figure 2 shows that at higher flow rates the recovery decreases.

Effect of Eluent

A satisfactory eluent should effectively dissolve the precipitate with a discrete volume in order to obtain a better enrichment factor. Nitric acid turned out to be a good eluent for the tellurium coprecipitate with lanthanum hydroxide. It was found that 5% (v/v) nitric acid was the minimum concentration necessary to obtain best response. The optimum flow rate of the eluent used was 1.5 mL min^{-1}.

Interference Studies

The proposed coprecipitation system can tolerate the presence of ions at the concentration levels that may be found in natural water samples. Thus, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Fe^{3+} could be tolerated up to at least 2500 µg L^{-1}. Commonly encountered matrix components, such as alkali and alkaline earth elements, are not retained on the column.

Analytical Performance

The time required for the preconcentration of 25 mL of sample (5.0 min, flow rate of 5 mL min^{-1}), elution (1.7 min, flow rate of 1.5 mL min^{-1}), and conditioning (0.2 min) was about 6.9 min. Additionally, the time required for the ETAAS determination was about 1.0 min, resulting in a sample throughput of seven samples per hour.

A 10-fold total enrichment factor for a sample volume of 25 mL was obtained with respect to the tellurium determination by ETAAS without preconcentration.

The relative standard deviation (RSD) for 10 replicates containing 10 µg L^{-1} of Te was 3.5%. The calibration curve was linear, with a correlation coefficient of 0.9993. The detection limit (DL), calculated as the amount of Te required to yield a net peak equal to three times the standard deviation of the background signal (3s), was 0.04 µg L^{-1}.

TABLE II
Recovery Study

Aliquots	Base Value (µg L^{-1})	Te Added (µg L^{-1})	Te Found (µg L^{-1})	Recovery (%)[a]
1–6	–	0.0	0.0	–
7	0.0	1.0	0.95	95.0
8	0.0	2.0	1.98	99.0
9	0.0	3.0	3.06	102.0
10	0.0	5.0	5.0	100.0

[a] 100 x [(Found-Base)/Added].

In comparison to our method, the procedure of multiple injections would be too time-consuming. Besides, it would require 10 injections of 50 µL each to obtain a signal comparable with that of the preconcentation procedure. On the other hand, we believe that a drying stage would be indispensable in order to inject 500 µL due to the limited capacity of the graphite tube (approximately 100 µL).

Recovery and Validation Studies

Since tellurium was not detected in the tap water samples, we spiked the samples with a known quantity of tellurium and applied the preconcentration procedure.

In order to evaluate the tellurium recovery of this method, 250 mL of tap water sample was collected in our laboratory and divided into 10 portions of 25 mL each. The proposed method was applied to six portions and the average quantity of tellurium obtained was taken as the base value. Then, increasing quantities of tellurium were added to the other sample aliquots and tellurium was determined by the same method. The results obtained were between 95–102 % (Table II). Even though the method of standard addition is not as useful as the method of validation; it is considered as a method of validation (22).

Additionally, the proposed method was applied to a standard reference material, NIST SRM 1643d Trace Elements in Water, with a reference tellurium content of 1.0 µg L^{-1}. The density of the SRM 1643d sample at 22ºC was 1.016 g mL^{-1}. Using our method, the tellurium concentration found in this SRM was 1.1 ± 0.1 µg L^{-1}.

CONCLUSION

Although the proposed methodology was not completely automated, the on-line preconcentration system increases the speed of the preconcentration and analysis process.

The preconcentration procedure with lanthanum hydroxide using a packed-bed filter with Amberlite XAD-4 resin packing and coupled to ETAAS allowed better detection limits, approximately 10-fold, in comparison to tellurium determination without preconcentration. The recovery studies performed indicate that the method shows good reproducibility and accuracy.

ACKNOWLEDGMENTS

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID); and Universidad Nacional de San Luis (Argentina).

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Received August 13, 2004.
Revision received March 14, 2005.

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