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A Simple Method for the Determination of Lead Isotope Ratios in Ancient Glazed Ceramics Using Inductively Coupled Plasma - Quadrupole Mass Spectrometry

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ABSTRACT

A method for the determination of lead isotope ratios in glazes by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) has been developed for distinguishing antique lead-glazed ceramics from different ages, workshops and geographical regions. Glazes were leached in 4% (v/v) acetic acid for 24 hours, and the leachates diluted with 2% (v/v) nitric acid to get final lead concentrations around 100 ng mL^{-1}. Acquisition parameters were optimized to get the highest precision attainable by ICP-QMS. Integration times of 20 seconds per isotope were selected, resulting in an analysis time per sample of less than six minutes. Relative standard deviations of the lead isotope ratios ranged from 0.2% for $^{206}Pb/^{204}Pb$ to 0.05% for $^{207}Pb/^{206}Pb$. For the lead glazes studied, it was found that the attainable precision was not a limitation because the lead-isotope-ratio variations between groups of samples were large enough, allowing classification of ceramics with respect to production workshops or glaze types, and proving that different lead raw materials were used according to the quality of the ceramics. In addition to the performance of the technique, the simplicity of the sample treatment proposed, based on the leaching of a glaze fragment with acetic acid, offers several advantages linked to cultural heritage studies, such as handling very small samples and using short analysis times necessary for dealing with a large number of samples to be analyzed.

INTRODUCTION

The abundance of lead isotopes in nature depends on the formation process of rocks and ore deposits and their evolution. ^{204}Pb is non-radiogenic, whereas ^{206}Pb, ^{207}Pb, and ^{208}Pb are radiogenic isotopes, produced by the radioactive decay of U and Th. As a consequence, since lead isotopic composition varies with the geographical location of rocks and ores, this property is exploited for the characterization and identification of lead sources in a number of fields, including geology (1,2), environmental sciences (3–6), and cultural heritage (7,8).

The determination of isotope ratios can be achieved with accuracy and precision by several mass spectrometry techniques (9–10). Although lead isotope ratio measurements have traditionally been carried out by thermal ionization mass spectrometry (TIMS), inductively coupled plasma mass spectrometry (ICP-MS) is increasingly replacing it. In ICP-MS, attainable precision with a single detector ranges from 0.1–0.5% for quadrupole mass spectrometers to 0.02–0.1% for double-focusing sector field instruments, whereas precision as low as 0.002% can be obtained with multicollector sector field ICP-MS (9). Although similar low precisions can be achieved by TIMS methods, they involve longer sample preparation and data acquisition times than those for ICP-MS.

Until the present, most studies based on the determination of lead-isotope ratios in cultural heritage materials have been carried out by TIMS (7,11,12). Accurate results with typical precisions of TIMS analysis have been reported (0.005% for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ ratios and better than 0.02% for $^{206}Pb/^{204}Pb$ ratio) (7). The sample treatments involve the dissolution of the sample, followed by the separation and purification of lead by either electrodeposition or ion-exchange chromatography, or even by both methods. In order to obtain such low precision figures, the measurement times range from 30 minutes up to 20 hours (4,8). By comparison, inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) just involves sample dissolution, whereas measurement times to achieve its limiting precision are in the range of a few minutes. Although ICP-QMS is limited by its attainable precision (around 0.1%), secondary analytical properties (cost and time) and the large range of naturally occurring lead isotope ratios can fulfil the "fitness for purpose" requirements and make ICP-QMS an appropriate technique for solving problems in archaeology and art. Lead isotope ratios have been used to differentiate provenances of samples in several applications, including metals (11,13), glass (14) and glazes (12,15), and to find possible sources of raw materials (8,16,17).

Since one of the main aims in ancient ceramic research is the material characterization for provenance or technological studies, the knowledge of some major components could help to establish identi-

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fication patterns for periods or ceramic production centres. Lead has been used as a main component for glazes in ceramic since antiquity (18). Lead is added as a fluxing and stabilizing agent to the glazing mixture in the form of lead oxides or powdered metal. It contributes to lower the surface tension, reduces the risk of glaze crazing and increases the refractive index and brilliance of the glazes (19). In the Iberian Peninsula, lead-glazed ceramics have been used since Roman times until today, although the proportion of lead in the diverse glazes has varied. Analytical studies of ancient pottery provide important information about the composition, technology, and raw materials used, allowing the characterization and the identification of the ceramics produced in different workshops and phases. However, most studies carried out on glazed ceramic are focused on the composition of ceramic bodies and glazes. Lead isotope ratios, which are a unique fingerprint of the ores, can complete the basic ceramic studies by providing workshop features, possible origin of the lead ores used in the manufacture of the glazes in different periods (12) or archaeological sites, technological characteristics, and cultural and trade contacts.

The aim of the present work is to evaluate the performance of ICP-QMS in terms of precision, bias, analysis time, and sample treatment, for the determination of lead isotope ratios applied to archaeometric studies related to the provenance of glazed ceramics, production workshops and manufacturing periods. The identification of the lead raw materials in archaeological objects could be useful to characterize and distinguish the possible production areas, determine their origin or even the trade routes.

Conventional determination of lead isotope ratios in ceramic samples involves a dissolution step by using hazardous acids. A leaching procedure with acetic acid is proposed for sample preparation, avoiding the total dissolution of the sample, as well as the use of hydrofluoric, perchloric and nitric acids for the attack of the siliceous matrix.

EXPERIMENTAL

Instrumentation

The ICP-QMS instrument was a PerkinElmer SCIEX ELAN® 6000 quadrupole ICP-MS instrument (Concord, Ontario, Canada). The sample introduction system consisted of a cross-flow nebulizer and a double-pass Scott-type spray chamber. Default instrumental parameters are listed in Table I. Total lead in sample leachates was determined by atomic absorption spectrometry using a PerkinElmer® AAnalyst™ 300 (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) flame atomic absorption spectrometer.

Standards and Reagents

A stock lead isotopic standard reference solution (1000 mg L^{-1}) was prepared by dissolving 1 g of NIST SRM-981 (National Institute of Standards and Technology, Gaitherburg, MD, USA) in nitric acid (J.T. Baker, Holland). Standard solutions were prepared daily from the stock solution in 2% (v/v) nitric acid.

Acetic acid, hydrofluoric acid and perchloric acid (Merck, Darmstadt, Germany) used were analytical grade reagents. Milli-Q™ ultrapure water (Millipore, Bedford, MA, USA) was used throughout the study.

Samples

In order to investigate the characteristics of the lead used in pottery production, 12 lead-glazed ceramics from the 11th to 16th centuries were selected (see Table II). The samples belong to two different production areas of Aragon (Spain): Zaragoza and Albarracin. Albarracin samples were manufactured in the 11th century AD (Islamic period) and the fragments correspond to three different decoration styles: honey glaze, green glaze, and green-and-black on white tin glaze. The samples produced in Zaragoza belong to different periods (11th century AD – Islamic and 16th century AD – Hispano-Moresque) and have different decorations.

As well as the ceramics, a sample of lead ore (galena) from a mine (Linda Mariquita) exploited since antiquity and located in the north-eastern Iberian Peninsula (Catalonia area) was analyzed by ICP-QMS. This sample was previously analyzed by TIMS (using a Finningan@ MAT 262 in static collection mode with four Faraday

TABLE I
ICP-MS Default Operating Conditions and Selected Data Acquisition Parameters

ICP Conditions	
Argon Gas Flow Rates	
Plasma	15 L min^{-1}
Auxiliary	1.2 L min^{-1}
Nebulizer	0.9 L min^{-1}
Pump Rate	1.2 mL min^{-1}
RF Power	1000 W

Acquisition Parameters	
Scanning Mode	Peak Hopping
Number of Sweeps	1000
Dwell Time	20 ms
Integration Time	20000 ms
Settle Time	3 ms
Number of Replicates	3
Isotopes Measured:	^{202}Hg
	^{204}Pb
	^{206}Pb
	^{207}Pb
	^{208}Pb
Analysis Time	5 min 45 s

Table II
Description of Studied Glazed Ceramic Samples and Pb Leached Following the Procedure Proposed

Sample	Site	Century A.D.	Glaze Decoration	Leached Pb (μg mL^{-1})
ZA6	Zaragoza	11th c.	Green & black on white tin glaze	306.2
ZA7	Muel-Zaragoza	16th c.	Blue on white tin glaze	6.6
ZA9	Muel-Zaragoza	16th c.	Golden lustre on white tin glaze	73.0
AL15	Albarracin	11th c.	Green & black on white tin glaze	45.5
AL16	Albarracin	11th c.	Green & black on white tin glaze	38.2
AL17	Albarracin	11th c.	Green & black on white tin glaze	33.2
AL22	Albarracin	11th c.	Honey glaze	71.4
AL26	Albarracin	11th c.	Honey glaze	16.7
AL27	Albarracin	11th c.	Honey glaze	250.6
AL30	Albarracin	11th c.	Green glaze	10.0
AL34	Albarracin	11th c.	Green glaze	13.6
AL35	Albarracin	11th c.	Green glaze	13.6

cups, from Thermo Fisher Scientific, Inc., Waltham, MA, USA) at the Geochronology and Isotope Geochemistry Research Facility, University of Basque Country (Spain).

Sample Treatment

Glazes and Ores Dissolution

For glaze dissolutions (20), about 2 mg of glaze were obtained by scraping a surface of 1.5–2.0 cm^2 with a diamond drill. They were weighed and placed into a PTFE beaker with 3 mL of concentrated HNO_3 and 3 mL of ultrapure water and heated in a sand bath for one hour. After cooling, 2 mL of $HClO_4$ and 10 mL of HF were added to the sample and the solution was kept at room temperature for 12 hours. After this period, the solution was evaporated to dryness and $HClO_4$ and HF were added several times until the entire residue was dissolved. Then, 5 mL of $HClO_4$ was added and evaporated to dryness. Finally, 2.5 mL of HNO_3 and 2.5 mL of ultrapure water were added and heated in a sandbath. The solution obtained was filtered and transferred into a 10-mL flask.

For the analysis of the lead ores, about 0.5 mg of sample was dissolved with 2 mL of concentrated HNO_3 and 4 mL of ultrapure water.

Leaching

Approximately 1 cm^2 fragments of glaze were cut with a diamond saw to avoid contamination. Each fragment was covered with 2 mL of 4% (v/v) acetic acid in a closed PTFE beaker at room temperature for 24 hours. After that period, the lead glazed fragments were removed and the leached lead was measured by atomic absorption spectroscopy. Leachates were diluted with 2% (v/v) HNO_3 to keep the concentration range of 50–100 ng mL^{-1} Pb for lead-isotope ratio determination by ICP-MS.

The same method was followed for the lixiviation of ore sample. In this case, 5–7 mg of ore was placed in a beaker with 2 mL of 4% (v/v) acetic acid. After 24 hours, the leachate was filtered and diluted to a final concentration of 100 ng mL^{-1} Pb.

Data Processing

Default data acquisition parameters are listed in Table I. Prior to ratio-ing, the measured signals were automatically corrected by the instrument software for signal losses due to the detector dead time. The detector dead time was determined experimentally according to the procedures summarized by Nelms et al. (21) and loaded into the instrument software. The signals were further corrected for the procedure blank, which was below 0.03 ng mL^{-1} of lead. Mass 202 was measured in addition to masses 204, 206, 207, and 208 to correct for the potential interference from ^{204}Hg. The equation:

$$N_{204}(\text{corrected}) = N_{204}(\text{measured}) - 0.2298\ N_{202}(\text{measured})$$

was used, although the contribution of mercury in samples, blanks, and standards was negligible.

RESULTS AND DISCUSSION

Data Acquisition Parameters

Attainable precision in ICP-MS is limited by shot noise, which depends on ion counting and is described by the Poisson distribution. Flicker noise also contributes to the overall precision, and is related to the nebulization process and the fluctuations of the plasma. In isotope ratio measurements, the contribution of the flicker noise can be reduced by using high scanning speeds along with short dwell times and a large number of sweeps (22). Shot noise is reduced by increasing the number of ions counted and, hence, by increasing the integration time (22).

The influence of data acquisition parameters (integration time, dwell time, and number of sweeps) on lead isotope ratio precision was evaluated through the relative standard deviation of 10 replicate measurements in a 50-μg L^{-1} lead standard solution. In the conditions described in Table I, five isotopes were measured in peak hopping mode, measuring one point per

spectral peak. Firstly, combinations of dwell times and number of sweeps to obtain a constant integration time of 5000 ms per isotope were studied. The same integration time was applied to all the isotopes, because no improvements were observed when integration times inversely proportional to the isotope abundance were used. Theoretical relative standard deviations (RSD_t) were calculated as the square root of $1/N_1 + 1/N_2$, where N_1 and N_2 are the number of counts measured for the two isotopes involved (see Table III). Relative standard deviations between 0.2–0.3% were obtained in any case. In order to improve the attainable RSDs through counting statistics, the number of sweeps was fixed to the maximum value of the instrument (1000), and the integration time increased by increasing the dwell time. RSDs below 0.1% were attained for isotope ratios involving the three most abundant isotopes (^{206}Pb, ^{207}Pb, ^{208}Pb) at 20 seconds integration time (Table III). This precision is in agreement with the limiting precision attainable with quadrupole mass spectrometers (9) and is very close to twice the theoretical attainable precision under the conditions studied.

Mass Discrimination Correction and Bias of the Procedure

Mass discrimination can occur in ICP-MS because of space charge effects and the preferential loss in transmission of light ions (9). Here, mass discrimination correction was performed by external correction, using the lead isotopic reference material NIST SRM-981. Comparison of the experimental lead isotope ratios (obtained from a lead standard solution prepared from the reference material) and the corresponding certified values allowed the calculation of mass-discrimination correction factors by ratioing the certified and experimental data. Internal correction by using the $^{203}Tl/^{205}Tl$ ratio of thallium spiked to the samples did not provide reliable results as was pointed out by other authors working with ICP-QMS (23).

The frequency of external correction between samples was studied by measuring 57 replicates of a 100-ng mL^{-1} lead isotopic standard for over two hours. Because each result was the average of three replicates following the proposed acquisition parameters (Table I), the 57 replicates were equivalent to 19 measurements. By using this set of data, one measurement every ten, five, three, or one measurement/s was used to compute the correction factors. The average of two consecutive standards was then applied to the samples measured between them. The difference between the certified and corrected values was lower than 0.1% when the number of samples between two consecutive standards was five or less. Figure 1 shows the effect of correcting the mass discrimination every five samples on the $^{206}Pb/^{204}Pb$ ratio.

The bias of the developed procedure was evaluated using the galena sample, for which the lead isotope ratios had been previously determined by means of multicollector TIMS. Table IV shows the results obtained by both techniques. Bias ranged from 0.06 for $^{207}Pb/^{206}Pb$ to 0.26% for $^{208}Pb/^{206}Pb$, with an average bias of 0.1%.

Leaching Procedure

Figure 2 shows the lead leached from one square centimetre of a glaze [20 % (w/w) PbO] in a 4% (v/v) acetic acid solution as a function of time. The maximum amount of lead was obtained with a minimum of 16 hours of leaching; thereafter, a leaching time of 24 hours was selected for convenience, although shorter times can be used. The final amount of lead leached from the glazes studied depended on the percentage of lead in the vitreous layer and ranged from 5 µg mL^{-1} to 300 µg mL^{-1} (see Table II). Solutions diluted to about 100 ng mL^{-1} of lead were used to measure the isotope ratios in order to get counting rates around one million counts per second but lower than two million counts per second, the minimum number of counts on the pulse state of the detector. Thus, fifty- to three thousand-fold dilutions were required to obtain this range.

In order to check a potential mass fractionation due to the leaching process, a glaze sample was dissolved following the procedure for glaze dissolution and the isotope ratios were compared with the leaching procedure. Averaged results of three samples are summarized in Table V and show excellent agreement between both methods,

TABLE III
Theoretical (RSD_t) and Measured (RSD_m) Relative Standard Deviations Obtained Under Different Data Acquisition conditions (t_i: integration time, t_d: dwell time, n_s: number of sweeps; Pb concentration: 50 ng mL^{-1} ; n=10)

			$^{206}Pb/^{204}Pb$		$^{207}Pb/^{206}Pb$		$^{208}Pb/^{206}Pb$	
t_i (ms)	t_d (ms)	n_s	RSD_t (%)	RSD_m (%)	RSD_t (%)	RSD_m (%)	RSD_t (%)	RSD_m (%)
5,000	20	250	0.236	0.322	0.080	0.172	0.067	0.255
5,000	10	500	0.241	0.254	0.082	0.174	0.069	0.190
5,000	5	1000	0.245	0.292	0.088	0.242	0.073	0.292
10,000	10	1000	0.178	0.185	0.061	0.175	0.051	0.167
20,000	20	1000	0.132	**0.175**	0.043	**0.047**	0.036	**0.075**

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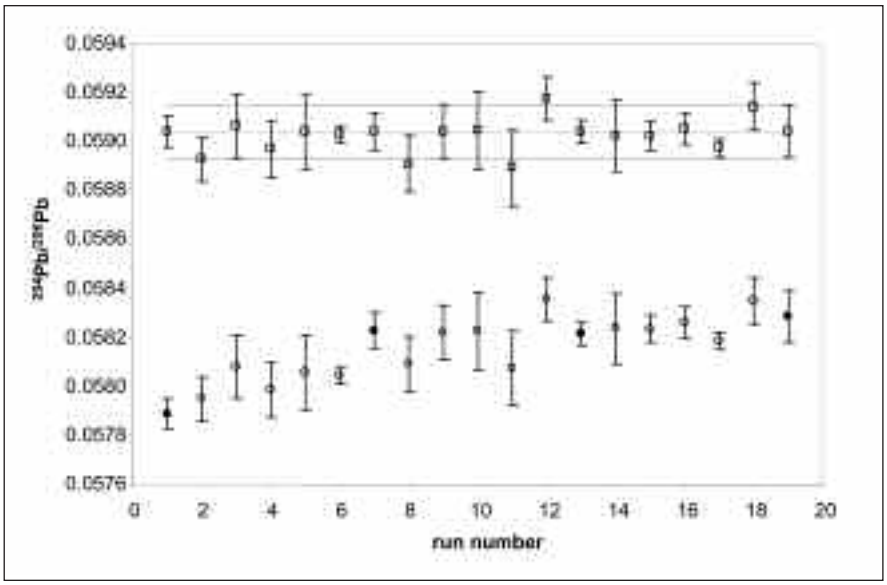

Fig. 1. Repetitive measurements of NIST SRM-981 before (circles) and after mass bias correction (squares). Filled circles are data used for correction. The solid line represents certified value and bars ±sd.

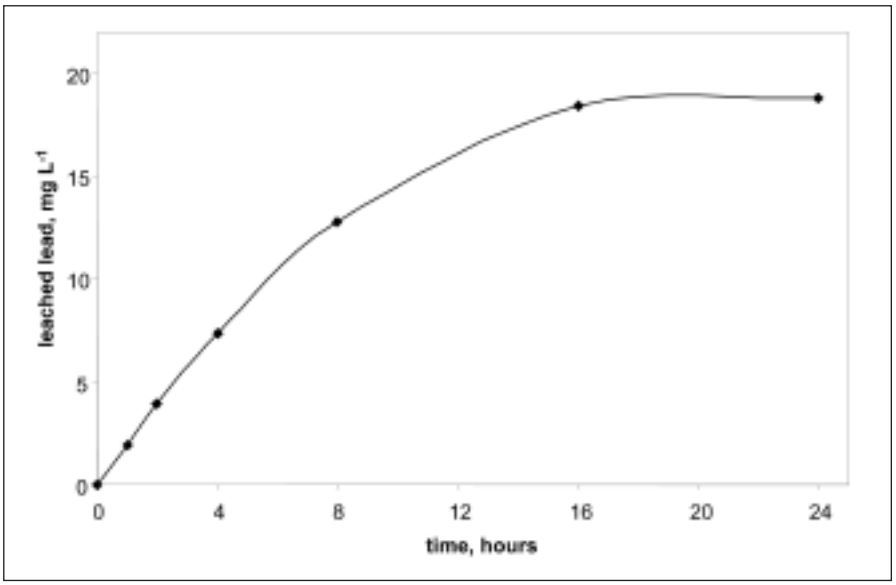

Fig. 2. Leached lead in a 4% (v/v) acetic acid solution from 1 cm^2 of glaze [20% (w/w) PbO].

TABLE IV. Lead Isotope Ratios for the Galena Sample Measured by ICP-QMS and TIMS

Technique	$^{206}Pb/^{204}Pb$ ± sd	$^{207}Pb/^{206}Pb$ ± sd	$^{208}Pb/^{206}Pb$ ± sd
ICP-QMS	18.418 ± 0.007	0.8518 ± 0.0009	2.0919 ± 0.0010
TIMS	18.431 ± 0.003	0.85116 ± 0.00004	2.0973 ± 0.0002

TABLE V. Comparison of Leaching and Dissolution Procedures for Glaze Analysis in the Determination of Pb Isotope Ratios (average of 3 samples)

Procedure	$^{206}Pb/^{204}Pb$ ± sd	$^{207}Pb/^{206}Pb$ ± sd	$^{208}Pb/^{206}Pb$ ± sd
Leaching	18.833 ± 0.005	0.8537 ± 0.0002	2.107 ± 0.002
Dissolution	18.836 ± 0.006	0.8540 ± 0.0002	2.109 ± 0.003

which implies that no significant mass fractionation occurred during the leaching process.

Analysis of Glaze Samples

Results of the determination of lead isotope ratios in the glaze samples studied are summarized in Table VI. Figure 3 shows the lead-isotope ratio composition, plotted as $^{208}Pb/^{206}Pb$ vs. $^{207}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ vs. $^{207}Pb/^{206}Pb$. These plots offer the possibility to establish similarities and differences related to the lead used in different periods and areas of pottery production. Glazed ceramic samples can be clearly separated into four different groups. One of the groups is related to the samples from Zaragoza (samples ZA6, 7, 9: white tin glazes). The samples show no differences in isotope composition, although they belong to different periods of production (Islamic-11th century AD and Hispano Moresque-16th century AD ceramics); even their decoration is different. This fact seems to indicate that the lead used in fine ceramic workshops in the Zaragoza area during the medieval and post-medieval periods could be supplied from the same mine area, and its use was independent of the type of decoration on the white tin glaze. The second group (AL30, 34, and 35 fragments) was made with green glazes from Albarracin. The third group includes green-and-black-decorated tin glazes (AL15, 16, and 17) produced in Albarracin in the Islamic period. Finally, the fourth group uses honey glazes (AL22, 26 and 27). All of these glazed ceramics, manufactured in the 11th century AD in Albarracin, have lead isotope abundances very well characterized in three groups related to the decoration applied on the pottery surface. This suggests diverse provenance of lead sources for glaze raw materials depending on the type and quality of glaze, and also different from that used in Zaragoza.

TABLE VI
Lead Isotope Ratios for the Glazed Ceramic Samples Studied (n=3)

Sample	$^{206}Pb/^{204}Pb \pm sd$	$^{207}Pb/^{206}Pb \pm sd$	$^{208}Pb/^{206}Pb \pm sd$
ZA6	18.348 ± 0.051	0.8539 ± 0.0001	2.1026 ± 0.0020
ZA7	18.395 ± 0.056	0.8547 ± 0.0007	2.1048 ± 0.0022
ZA9	18.381 ± 0.038	0.8536 ± 0.0011	2.1028 ± 0.0016
AL15	18.537 ± 0.045	0.8450 ± 0.0001	2.0932 ± 0.0019
AL16	18.555 ± 0.010	0.8448 ± 0.0002	2.0871 ± 0.0017
AL17	18.579 ± 0.032	0.8451 ± 0.0001	2.0928 ± 0.0026
AL22	18.250 ± 0.023	0.8560 ± 0.0006	2.1230 ± 0.0009
AL26	18.347 ± 0.009	0.8563 ± 0.0004	2.1168 ± 0.0005
AL27	18.287 ± 0.010	0.8556 ± 0.0011	2.1179 ± 0.0013
AL30	18.648 ± 0.008	0.8431 ± 0.0001	2.1052 ± 0.0003
AL34	18.630 ± 0.004	0.8436 ± 0.0005	2.1065 ± 0.0016
AL35	18.625 ± 0.008	0.8428 ± 0.0008	2.1105 ± 0.0017

From these lead isotope ratios of glazed ceramics produced in the Aragon area, it is possible to obtain some additional information about the pattern of manufacture in the different workshops. For instance, the diverse lead-isotope ratios from glazes produced in the Islamic period in Albarracin show a selection of different sources of lead linked to the type of glaze (white tin glaze, transparent green, or honey glazes). However, in Zaragoza the similar lead isotope abundances reflect that the provenance of lead could be linked to the use of white tin glazes and be independent of the type of decoration (green and black, blue, or golden lustre) and of the period of production.

CONCLUSION

Although the precision achievable in lead-isotope ratio determinations by ICP-QMS is poorer than obtained with TIMS or ICP-multicollector MS, this work proves that relevant data can be derived from ICP-QMS related to lead isotope ratios in vitreous materials. In the case of glaze analyses, sensitivity is not a limitation because of the relatively high levels of lead found in the samples, whereas variations in lead isotope ratios between groups are larger than the attainable precision.

In addition to the performance of the technique, the simplicity of the sample treatment proposed, based on the leaching of a glaze fragment with acetic acid, avoids digestion of the glaze and potential contamination during handling. Due to the high lead proportion in the glazes, leaching times could be shortened to leach the required lead concentration. The amount of sample required, a serious limiting factor in the art and archaeological fields, is only one square centimetre. However, this amount could even be smaller where lead is in high concentrations and the glaze should not be altered.

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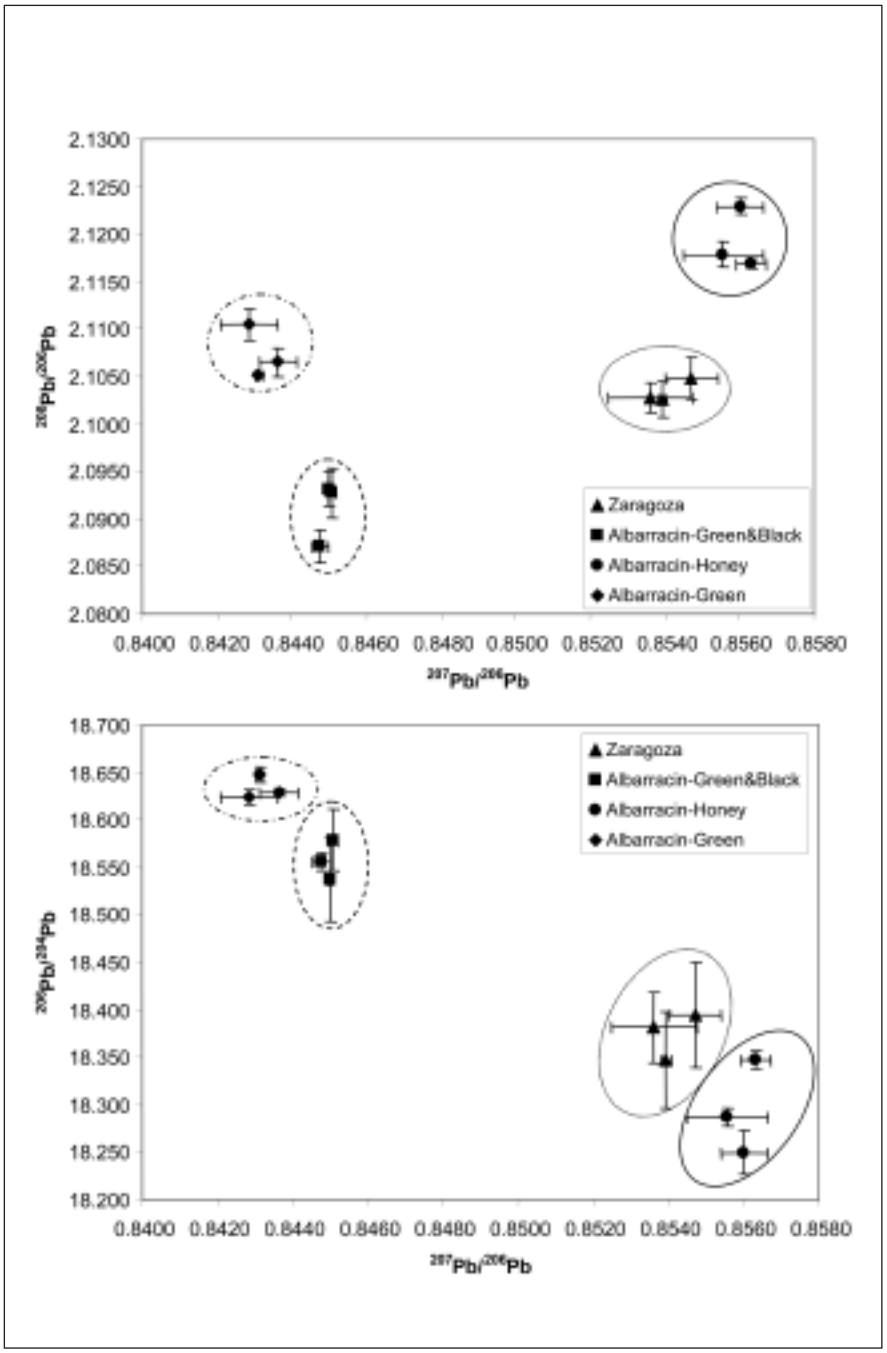

Fig. 3. Lead-isotope-ratio composition of glazed ceramics from different origins (see Table II for the description of the samples). Bars represent ±sd.

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Multielement Analysis of Soils by Wavelength-Dispersive X-ray Fluorescence Spectrometry

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ABSTRACT

This paper introduces a calibration procedure and provides the data achieved for accuracy of the results and the detection limits of major and trace elements in soils. Soil reference materials were used to calibrate and evaluate an analytical method for the determination of major (Si, Al, Fe, Mg, Ca, Na, K, Mn, P, Ti) and trace elements (As, Ba, Cd, Co, Cr, Cu, Se, Sr, Mo, Ni, Pb, Rb, S, U, Th, V, Y, Zn, Zr) by sequential wavelength dispersive X-ray fluorescence spectrometry.

The samples were prepared as pressed pellets, and analysis was performed within a total measuring time of 20 minutes per sample. A set of 22 reference materials was used for calibration of the spectrometer. Another set of 10 reference materials were analyzed for the evaluation of accuracy. The detection limits obtained for the trace elements (1–2 mg/kg) are adequate both for geochemical and environmental purposes and the accuracy of the method is within the expected interval of certified values in most cases.

INTRODUCTION

The sustainable management of soils requires that they be protected from chemical contamination. Such contamination can arise from atmospheric or waterborne deposition (1). In many Asian countries, current research and development is focused on the problems of waste management and on issues of improving nutrient recycling. As organic matter is returned to the land for agricultural use, it is essential that toxic metals be monitored to avoid their entry into the food chain (2,3).

The chemical analysis of soils can reveal anomalous high metal values and help authorities to the presence of polluted elements in the eco system. These may originate from natural sources such as mineralization of a specific rock or metals found in runoff from mines or in effluents from industries, etc. However, in many soils, the true threat to an ecosystem may be obscured by the chemical analysis of a total soil sample with respect to environmental pollution monitoring (4,5).

Concerning the analytical techniques that can be used, probably one of the most appropriate is X-ray fluorescence (XRF) spectrometry. Several reasons explain the wide acceptance of XRF, not the least being sample preparation, instrumental precision, and longevity of calibrations. Besides sample preparation, the quality of results also relies strongly on the calibration strategy, i.e, the method used to correct the loss in X-ray intensities caused by absorption and secondary excitation effects (2). Several empirical algorithms have been proposed to deal with interelemental effects and how to correct them mathematically. In most cases, the correction factors are calculated based on XRF measurements from a set of certified reference materials (CRMs). Several X-ray fluorescence spectrometry methods have been described in the literature with focus on geochemical mapping, geochemical exploration, and environmental pollution assessment studies (6,20). Moreover, the new generation of computer-assisted spectrometers has significantly lowered the detection limits, so that background elemental levels in remote areas, as well as enhanced levels near industrial activities, can effectively be analyzed in soils.

The purpose of the present study was to develop a quantitative analytical XRF method for the routine analysis of soil samples, prepared as pressed pellets. In such an application, the homogeneity and particle size of the powders can significantly influence the quality of the final results. Besides sample preparation, the final accuracy depends strongly on the calibration strategy, which was evaluated as thoroughly as possible by the analysis of 10 independent reference materials. The results are judged by fitness of accuracy as well as the detection limits of trace elements that are of environmental concern. When complex samples are concerned, a frequent calibration strategy refers to matrix-matched reference materials, ideally certified for the constituents of interest. Many of the soil reference materials available have certified values (7,8). Frequently, the samples employed for calibration are not clearly mentioned or samples from only one source are used (9). In this work, 10 international soil reference materials were screened, considering data quality and elemental concentrations, to select a group of 22 reference samples that were used to calibrate the instrument.

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EXPERIMENTAL

Instrumentation and Operational Parameters

A Philips MagiX PRO, Model PW 2440, wavelength dispersive X-ray fluorescence spectrometer, coupled with an automatic sample changer PW 2540 and provided with suitable software SUPER Q 3.0, was used for this study (Philips, Eindhoven, The Netherlands). The MagiX PRO is a sequential instrument with a single goniometer-based measuring channel covering the complete elemental measurement range from F to U in the concentration range from 1.0 ppm to % level, determined in vacuum media. The instrument is microprocessor-controlled for maximum flexibility and consists of an end-window X-ray tube with an Rh anode and a maximum voltage/current of 60 kV / 125 mA at a maximum power level of 4 KW. The analytical lines and instrumental parameters used for each element are listed in Table I.

A prerequisite in any analytical scheme is the use of the correct operating conditions. For the vast amount of geochemical work, most elements are known to fall within certain ranges, and these are considered when selecting operating conditions. In general one set of operating conditions is sufficient for each element, designed initially to avoid spectral overlaps (on peaks and backgrounds) and to optimize the count rate. Also, it is preferable to count for a longer period on a well-separated peak than to introduce a correction for overlapping peaks where the choice exists. In quantitative analysis, the intensity of any given line is proportional to concentration, but modified by a combination of absorption and enhancement effects, which are in turn a function of the composition of the sample and the primary spectrum from the X-ray tube (4,10,15). The current and voltage values were chosen based on the results of a preliminary investigation concerning the effect of voltage value on the intensity of the characteristic peak areas.

To compensate for possible instrument drift, a monitor sample was analyzed at the beginning and at the end of each day when the samples were run. For all elements, the net intensities were calculated by subtracting the background from the raw peak intensity. The net intensity (or raw intensity where backgrounds were not measured) for a given element divided by the average of the two monitor sums for the day gives the monitor-normalized intensity. The difference in count rate of the monitor sample between the start and the end of the day was typically ±0.2% relative to the mean of the two runs of the day.

In the present study, the elements were determined using pressed powder pellets (with only a few exceptions) to minimize dilution of the sample. The spectral lines used for trace element analysis in most natural samples lie at a shorter (higher energy) wavelength than those of the associated major elements, and the dominant problem to overcome is that of absorption. The simplest and probably the most effective approach to correcting for absorption makes use of the fact that the intensity of the background, and of the coherent and incoherent scattered lines of the anode element, vary systematically with the mass absorption of the sample matrix. Measurement of this scattered radiation (usually that of the Compton Kα for most anode) provides an absorption correction factor (3,9).

All samples were used as received without drying because of concerns about loss by volatization. Pressed pellets (40 mm diameter) were prepared by using collapsible aluminum cups (10). These cups were filled with boric acid and about 2 g of the finely powdered soil sample after mixing and pressed under a hydraulic press at 25-ton pressure to get a pellet (Hydraulic Press, Herzog, Germany).

Calibration

The spectrometer was calibrated after measuring intensities of 22 international reference materials (see listing in Table II). The criteria to select these samples were the required interval of concentration, the quality of the known data for each reference material, and also previous calibration tests. Source/reference for the certified values was taken from Govindaraju 1994 (21). Table III provides the interval of concentration of each analyte in the calibration.

RESULTS AND DISCUSSION

Calibration lines were obtained with the SUPER Q 3.0 analytical software issued by the instrument manufacturer and using linear regression of the net intensities versus concentration. For some elements (As, Cd, Mo, Sr, V, Y, and Zr), the matrix effects were corrected using empirical coefficients, more specifically alphas, based on count rate. Most well-characterized geological reference materials, i.e., those with reference values and associated uncertainties for almost every element present at detectable concentrations, are of common silicate matrices. When reference materials with abnormally high concentrations of trace elements are considered, there are too few available with reference values and their associated uncertainties to permit calibration of the spectrometer. As a consequence, difficulties arise when samples with anomalously high concentrations of one or more element are to be determined. Attempts were made to correct matrix effects with empirical alphas based on concentration which did

TABLE I
Instrumental Parameters Used in X-ray Fluorescence Spectrometry

Element	Line	Crystal	Detector	kV	mA	Peak (20)	Bkg (±20)	Counting Time (s)	Line Overlap/ Correction
Traces									
As	Kα	LiF 220	Duplex	40	90	48.7106	0.9962	60 + 40	Cd/alpha
Ba	Lα	PX9	Duplex	40	90	87.1508	0.9652	60 + 40	nil
Cd	Kα	LiF 220	Duplex	55	70	21.6048	–0.933	60 + 40	Cu, Zn/alpha
Co	Kα	LiF 220	Duplex	40	90	77.837	0.8774	60 + 40	nil
Cr	Kα	PX9	Duplex	40	70	69.3876	0.771	60 + 40	nil
Cu	Kα	LiF 220	Duplex	40	90	65.5022	0.8652	60 + 40	nil
Mo	Kα	LiF 220	Duplex	40	90	28.8422	0.8064	60 + 40	V/alpha
Ni	Kα	LiF 220	Duplex	40	80	71.2262	0.8594	60 + 40	nil
Pb	Lα	PX9	Duplex	40	90	33.8972	0.9486	60 + 40	nil
Rb	Kα	LiF 220	Duplex	40	80	37.9122	0.6884	60 + 40	nil
Se	Kα	LiF 220	Duplex	50	80	45.6346	0.6756	60 + 40	nil
Sr	Kα	LiF 220	Scint.	60	66	35.7714	0.9226	60 + 40	Y/alpha
Th	Lα	LiF 220	Scint.	60	66	39.2058	0.8344	60 + 40	nil
U	Lα	LiF 220	Duplex	60	66	37.2436	–0.6448	60 + 40	Rb/alpha
V	Kα	PX9	Flow	40	90	77.2036	–1.1426	60 + 40	nil
Y	Kα	LiF 220	Duplex	40	90	33.8118	0.7838	60 + 40	Zr/alpha
Zn	Kα	PX9	Duplex	40	80	41.7432	0.9124	60 + 40	nil
Zr	Kα	LiF 220	Duplex	40	80	32.0112	0.814	60 + 40	Ba/alpha
Major									
Si	Kα	PE 002-C	Flow	40	60	108.9726	2.4836	20 + 10	nil
Al	Kα	PE 002-C	Flow	40	60	144.843	2.6008	20 + 10	nil
Fe	Kα	PX9	Scint.	30	40	57.4932	0.9372	20 + 10	nil
Mn	Kα	PX9	Scint.	40	80	62.9406	0.9482	20 + 10	nil
Mg	Kα	PX1	Flow	40	60	23.0708	2.3298	20 + 10	nil
Ca	Kα	PX9	Flow	40	70	113.1196	–1.0578	20 + 10	nil
Na	Kα	PX1	Flow	40	70	27.9086	2.3194	20 + 10	nil
K	Kα	PX9	Flow	50	40	136.6522	1.99	20 + 10	nil
Ti	Kα	PX9	Flow	40	70	86.1484	0.9272	20 + 10	nil
P	Kα	Ge 111-C	Flow	40	70	141.0412	1.8622	20 + 10	nil

Duplex = Flow proportional and sealed xenon counter. Scint. = Scintillation counter.

TABLE II
International Reference Materials Studied

S.No	Reference Material	Source
1.	ASK-1 (Larvikite)	Analytisk Sporelement Komite, Oslo, Norway
2.	ASK-2 (Schist)	Analytisk Sporelement Komite, Oslo, Norway
3.	CRM-141R (Soil)	Community Bureau of Reference, Brussels, Belgium
4.	CRM-142R (Soil)	Community Bureau of Reference, Brussels, Belgium
5.	G-2 (Granite)	USGS, United States Geological Survey, Reston, CO, USA
6.	JG-2 (Granite)	GSJ, Geological Survey of Japan
7.	NIM-G (Granite)	MINTEK, Council of Mineral Technology, South Africa
8.	NIST-2709 (Soil)	NIST, National Institute of Standards and Technology, USA
9.	NIST-2710 (Soil)	NIST, National Institute of Standards and Technology, USA
10.	NIST- 2711(Soil)	NIST, National Institute of Standards and Technology, USA
11.	SO-1 (Soil)	CCRMP, Canadian Certified Reference Materials Project, Canada
12.	SO-2 (Soil)	CCRMP, Canadian Certified Reference Materials Project, Canada
13.	SO-3 (Soil)	CCRMP, Canadian Certified Reference Materials Project, Canada
14.	SO-4 (Soil)	CCRMP, Canadian Certified Reference Materials Project, Canada
15.	TILL-1 (Soil)	CCRMP, Canadian Certified ReferenceMaterials Project, Canada
16.	TILL-3 (Soil)	CCRMP, Canadian Certified Reference Materials Project, Canada
17.	LKSD-1 (Lake Sediment)	CCRMP, Canadian Certified Reference Materials Project, Canada
18.	JP-1 (Peridotite)	GSJ, Geological Survey of Japan
19.	JR-1 (Rhyolite)	GSJ, Geological Survey of Japan
20.	JR-2 (Rhyolite)	GSJ, Geological Survey of Japan
21.	SY-3 (Syenite)	CCRMP, Canadian Certified Reference Materials Project
22.	JB-2 (Basalt)	GSJ, Geological Survey of Japan

TABLE III
Concentration Interval Covered by the Canadian Reference Materials Project
Used to Calibrate the Spectrometer

Major Oxides	% (m/m)	Trace Elements	(mg/kg)	Trace Elements	(mg/kg)
SiO_2	12.2 - 77	As	0.3 – 626	Se	0 – 17
TiO_2	0 - 4	Ba	7.7 – 8000	Sr	3.3 – 4600
Al_2O_3	0.6 - 18	Cd	0 – 310	Th	1 – 1003
Fe_2O_3	0.9 - 18	Co	0.4 – 116	U	1 – 650
MgO	0.04 – 44.72	Cr	2.3 – 2970	V	2 – 313
CaO	0.45 – 44.63	Cu	0.4 – 2950	Y	1 – 718
Na_2O	0.05 – 8.37	Mo	0.2 – 60	Zn	1 – 6952
K_2O	0.01 – 5.51	Ni	0.7 – 2460	Zr	6 – 502
MnO	0.01 – 1.30	Pb	0 – 5532		
P_2O_5	0.01 – 0.95	Rb	1 - 320		

produce acceptable calibration. When based on intensities, matrix correction was achieved by trial and error and was mainly based on which elements would more strongly absorb the emitted intensities of the element of interest. Matrix corrections based on empirical coefficients are only valid for the analysis of samples with a composition within the interval of standard. For this reason, for many analytes, especially heavy metals, reference materials with unusually high concentrations were also included as standards.

The results obtained for major and minor elements in a group of reference materials are presented in Tables IV and V. Two lines of data are presented for each sample. In the first line, the results are presented with the associated uncertainty, calculated during analysis. The second line represents certified values of the reference materials. The uncertainty includes the sum of the following components: counting statistics, systematic errors in background calculation, and spectral correction and sensitivities used to calibrate the spectrometer. Counting statistics is the dominant source of uncertainty for trace elements. All data were obtained for single pellet analysis, but some pellets were analyzed two or three times, and this instrumental uncertainty is of the same order as that presented with the data.

Detection Limits

An important statistical consideration in XRF analysis is the capability of an instrument to merely detect whether an element is present in a specimen or not. In fact, one wants only to be able to claim with some defined statistical certainty that a given element is present if its concentration is greater than a certain limit. The most current detection limit used in XRF analysis is the lower limit of detection, which is assumed to be the concentration equivalent to three standard counting errors (11) of a set of measurements of the background intensity (12). The expression "lower limit of detection" (LLD) is often used in X-ray literature to represent the smallest amount of an element in a given specimen that can be detected by an instrument in a specific statistical context for a given matrix.

Our recent studies using the PW 2440 spectrometer enabled us to scan the elements of interest and to calculate the counting statistical error (CSE) and the lowest limit of detection (LLD) for elected counting times on peaks and backgrounds. When reasonable times are selected (60 peak – 40 background for trace elements and 20 peak – 10 background for major elements) the LLD and CSE are good (Figure 1). These studies have also shown that standard pellets made from 2 g of powder are critically thick enough for measuring major and trace elements.

However, the line intensity measuring conditions are the most important factor in determining the final detection limit. The detection limits for some elements (As, Cd, Cr, Cu, Pb, Zn) of environmental concern according to Canadian Council of Ministers of the Environment (CCME) guidelines (16) are between 0.5–1.0 mg/kg (13) for geochemical prospecting and the suggested values for As in geochemical mapping (14). The CCME (15) provides maximum provisory values for heavy metal toxicity in environmental and risk assessment, as for instance, for soil in agriculture, fresh water sediments, and health effect levels. These guideline detection limits are listed in Table VI.

Accuracy

Tables IV and V show the results obtained for major and trace elements, respectively, after the analysis of the following 10 international reference samples:
1. SO-1 (regosolic clay soil),
2. SO-3 (calcareous soil),
3. SO-4 (chermozemic A horizon soil), CCRMP, Canadian Certified Reference Materials Project, CANMET, Ottawa, Ontario, Canada.
4. NIST-SRM-2709 (San Joaquin soil),
5. NIST-SRM-2710 (Montana soil),
6. NIST-SRM-2711 (Montana soil), NIST, National Institute of Standards and Technology, Gaithersburg, MD, USA.
7. TILL-1,
8. TILL-3, B and C Horizon Soil, CCRMP, Canadian Certified Reference Materials Project, CANMET, Ottawa, Ontario, Canada.
9. BCR-141R (Calcareous loam soil),
10. BCR-142R (Light sandy soil), BCR, Community Bureau of Reference, Brussels, Belgium.

For each sample (see Table IV), the average of three results and the respective standard deviation are given in the first line; the second line represents the certified values of the reference material; the uncertainty in the results refers to the precision since only one pellet was prepared for each reference material.

The accuracy of the results was evaluated by comparison with the certified values of the analyzed reference materials. When the certified values are known, the results should ideally be within the confidence interval: CV± Cl. Some reference materials used in the present work contain anomalous concentrations of one or more elements and the results obtained are presented in Table IV. The major elements of SiO_2 in SRM- 2710, and Al_2O_3 and MgO in SRM-2709 have certified values only and are not within the 95% confidence interval.

A seriously limiting factor in the final accuracy of XRF analysis of common geological samples is the lack of information concerning constituents not usually determined by

TABLE IV
Analytical Results Obtained for Major Elements (in oxide form)
Values are given in (%) (m/m).

Sample	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	MnO	TiO_2	P_2O_5
SO-1	55.68±0.3	18.4±0.5	7.81±0.01	3.68±0.08	2.18±0.13	2.86±0.07	3.2±0.35	0.10±0.01	0.74±0.03	0.13±0.01
	54.98	17.59	8.58	3.83	2.46	2.70	3.18	0.11	0.87	0.15
SO-3	30.28±0.16	5.98±0.06	1.94±0.01	7.2±0.12	21.22±1.01	0.84±0.03	1.66±0.06	0.05	0.24±0.01	0.12±0.01
	33.72	5.80	2.22	8.42	20.71	1.01	1.40	0.07	0.33	0.11
SO-4	67.27±0.2	10.18±0.4	3.85±0.01	0.91±0.02	1.70±0.06	0.99±0.01	2.17±0.12	0.08	0.22±0.01	0.27±0.01
	68.74	10.22	3.37	0.89	1.55	1.33	2.07	0.08	0.58	0.2
SRM-2709	62.2±0.2	16.5±0.2	5.1±0.01	3.1±0.10	2.52±0.09	1.24±0.02	2.35±0.014	0.07±0.01	0.48±0.02	0.14±0.01
	63.45	14.17	5.00	2.50	2.64	1.56	2.45	0.06	0.57	0.14
SRM-2710	64.13±0.06	12.4±0.36	4.86±0.01	1.45±0.02	1.57±0.04	1.43±0.01	2.52±0.14	1.15±0.01	0.42±0.01	0.21±0.01
	61.97	12.16	4.83	1.41	1.75	1.54	2.54	1.30	0.47	0.24
SRM-2711	64.43±0.15	12.10±0.10	3.92±0.02	1.96±0.05	3.64±0.12	1.39±0.03	2.93±0.17	0.07±0.01	0.42±0.01	0.19±0.01
	65.11	12.34	4.13	1.74	4.03	1.54	2.95	0.08	0.51	0.19
TILL-1	60.86±0.97	13.33±0.40	6.37±0.45	2.09±0.11	2.27±0.38	2.30±0.46	2.03±0.21	0.17±0.06	0.97±0.06	0.17±0.06
	60.9	13.7	6.82	2.15	2.72	2.71	2.22	0.18	0.98	0.22
TILL-3	68.37±0.81	11.40±0.72	3.73±0.21	1.33±0.32	2.50±0.10	2.43±0.48	2.23±0.15	0.10±0.01	0.47±0.06	0.10±0.01
	69.1	12.2	3.92	1.71	2.63	2.64	2.42	0.06	0.49	0.11
BCR-141R	54.94±0.21	10.93±0.45	3.30±0.08	1.34±0.04	14.60±0.61	0.43±0.01	1.83±0.10	0.08±0.01	0.42±0.02	0.33±0.02
	**	**	**	**	**	**	**	**	**	**
BCR-142R	58.40±0.18	19.72±0.30	4.68±0.01	2.03±0.05	1.36±0.04	1.76±0.03	2.89±0.18	0.12±0.01	0.62±0.02	0.32±0.01
	**	**	**	**	**	**	**	**	**	**

First-line values (standard deviation) of each sample were obtained in this work (±1S, n=3).
Second-line values are certified values, issued by the producers of the reference materials.
** values not provided.

TABLE V
Analytical Results for Trace Elements
Values are given in mg/kg.

Sample	As	Ba	Co	Cr	Cu	Mo	Ni	Pb	Sr	V	Zn	Zr
SO-1	1.9±0.1	862.1±2.2	29.5±0.9	162.9±2.7	58.8±1.5	1.6±0.2	84.9±1.1	18.6±0.7	297.3±	130.5±3.8	134.8±0.9	82.7±0.9
	2.0	870.0	29.0	170.0	61.0	2.0	92.0	20.0	331.0	133.0	140.0	84.0
SO-3	2.2±0.3	285.5±3.6	4.7±0.5	28.3±1.1	16.3±0.7	1.5±0.2	13.1±0.7	13.1±0.1	220.7±4.4	35.7±1.3	47.5±0.9	154.8±1.6
	2.51	290.0	5.5	27.0	17.0	2.0	14.0	13.0	222.0	36.0	48.3	156.0
SO-4	6.6±0.4	700.8±2.1	12.3±1.3	64.1±2.3	19.1±0.6	0.9±0.2	24.2±0.2	12.6±0.7	161.5±1.5	88.5±0.8	92.6±0.8	265.3±1.7
	7.4	700.0	10.4	64.0	21.0	1.0	24.0	14.0	168.0	85.0	94.0	270.0
SRM-2709	16.1±0.8	948.1±16.3	17.2±0.8	106.6±2.0	35.7±0.5	2.0±0.5	82.4±2.2	17.2±0.8	208.4±0.4	92.6±4.5	109.0±0.9	158.1±2.7
	17.7	968.0	13.4	130.0	34.6	2.0	88.0	18.9	231	112	106.0	160.0
SRM-2710	606.8±7.7	681.6±6.4	13.8±0.8	40.2±0.8	2513±6.0	17.7±1.7	8.1±1.5	4307±10.2	261.2±0.1	72.74±1.4	5359±11.8	154.1±2.2
	626	707	10.0	39.0	2950	19.0	14.3	5532	240	76.6	6952	**
SRM-2711	461.6±6.4	646.8±10.8	12.7±1.2	44.3±1.6	104.1±0.6	0.8±0.1	11.8±1.1	994.1±1.6	238.4±0.4	72.6±2.0	316.4±1.4	295.8±3.1
	105	726	10.0	47.0	114.0	1.6	20.6	1162	245.3	81.6	350.4	230.0
TILL-1	17.5±0.8	697.1±6.6	17.6±0.66	64.4±0.60	46.5±0.64	2.0±0.1	24.2±0.3	21.4±0.7	289.4±1.7	98.5±0.51	97.5±0.61	500.1±1.9
	18.0	702.0	18.0	65.0	47.0	2.0	24.0	22.0	291.0	99.0	98.0	502.0
TILL-3	86.4±0.6	483.5±4.9	14.5±0.58	121.5±1.5	21.5±0.28	1.9±0.1	38.4±0.60	25.4±0.76	294.1±5.3	61.3±0.76	55.5±0.7	229.1±0.7
	87.0	489.0	15.0	123.0	22.0	2.0	39.0	26.0	300.0	62.0	56.0	230.0
BCR-141R	14.8±0.8	381.0±5.1	10.9±1.3	191.9±1.4	46.3±1.8	2.5±0.4	100.7±1.0	54.0±1.3	403.5±1.1	65.6±1.3	281.0±0.5	199.5±3.3
	**	**	10.5±0.4	195±7	46.4±1.8	**	103±3	57.2±1.2	**	**	283.0	**
BCR-142R	23.5±0.8	673.3±2.1	15.9±0.9	112.7±1.2	73.5±0.6	0.6±0.2	56.6±0.6	62.2±1.1	184.0±1.0	105.0±2.9	109.5±0.4	359.6±1.4
	**	**	12.1±0.7	**	69.7±1.3	**	64.5±2.5	40.2±1.9	**	**	**	**

First-line values (standard deviation) of each sample were obtained in this work (±1S, n=3).
Second-line values are certified values, issued by the producers of the reference materials.
** values not provided.

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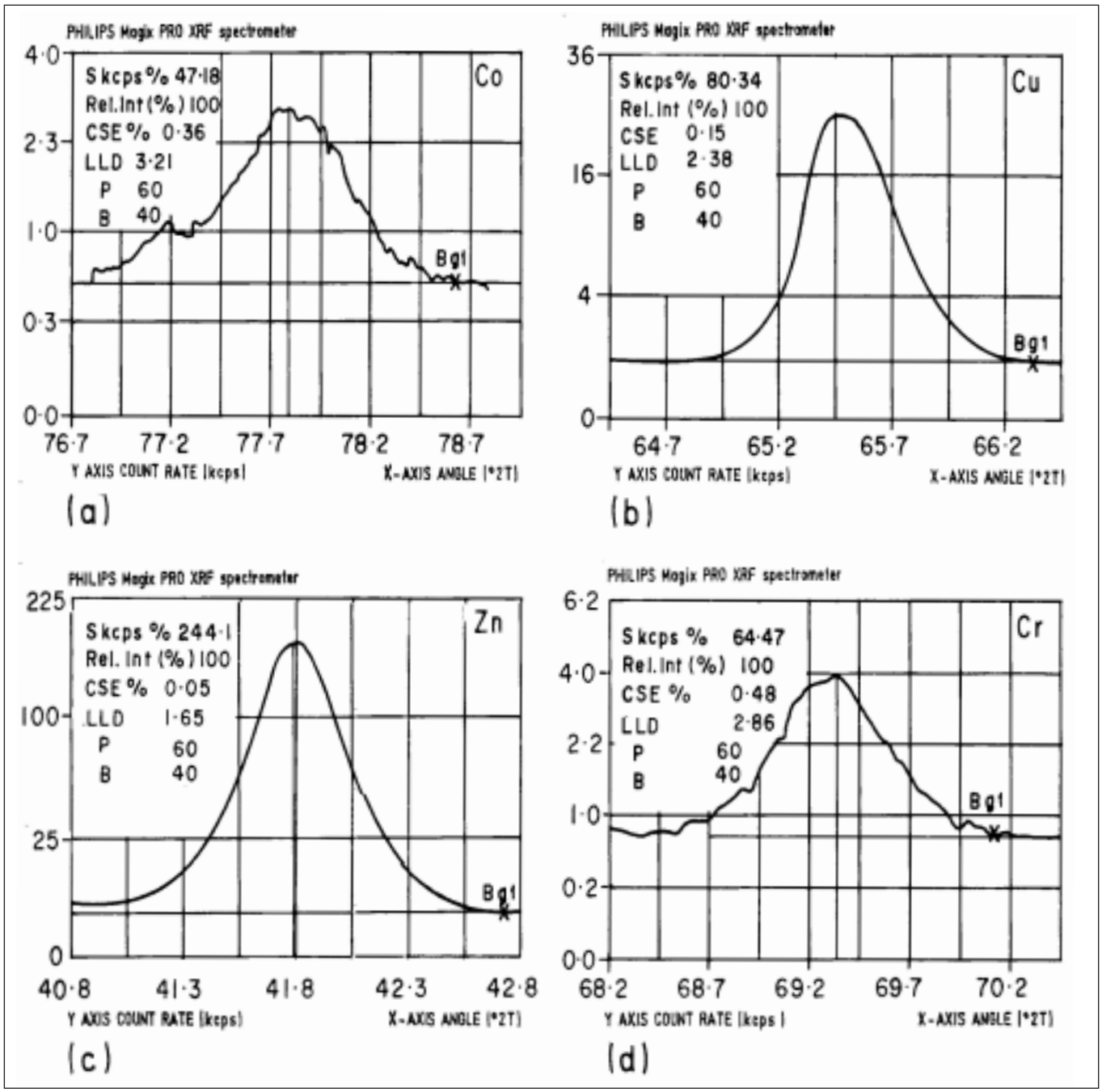

Fig. 1. A scan of elements in soil pellets with a Philips PW 2440 X-ray Spectrometer enables the determination of the lowest limit of detection and counting statistical error (CSE).
(a) Cobalt conc. = 0.05%, Peak rate = 2.671 kcps, Background rate = 0.8774 kcps;
(b) Copper conc. = 0.295%, Peak rate = 24.746 kcps, Background rate = 0.8652 kcps;
(c) Zinc conc. = 0.6952%, Peak rate = 169.41 kcps, Background rate = 0.9124 kcps;
(d) Chromium conc. = 0.0496%, Peak rate = 3.818 kcps, Background rate = 0.7710 kcps.

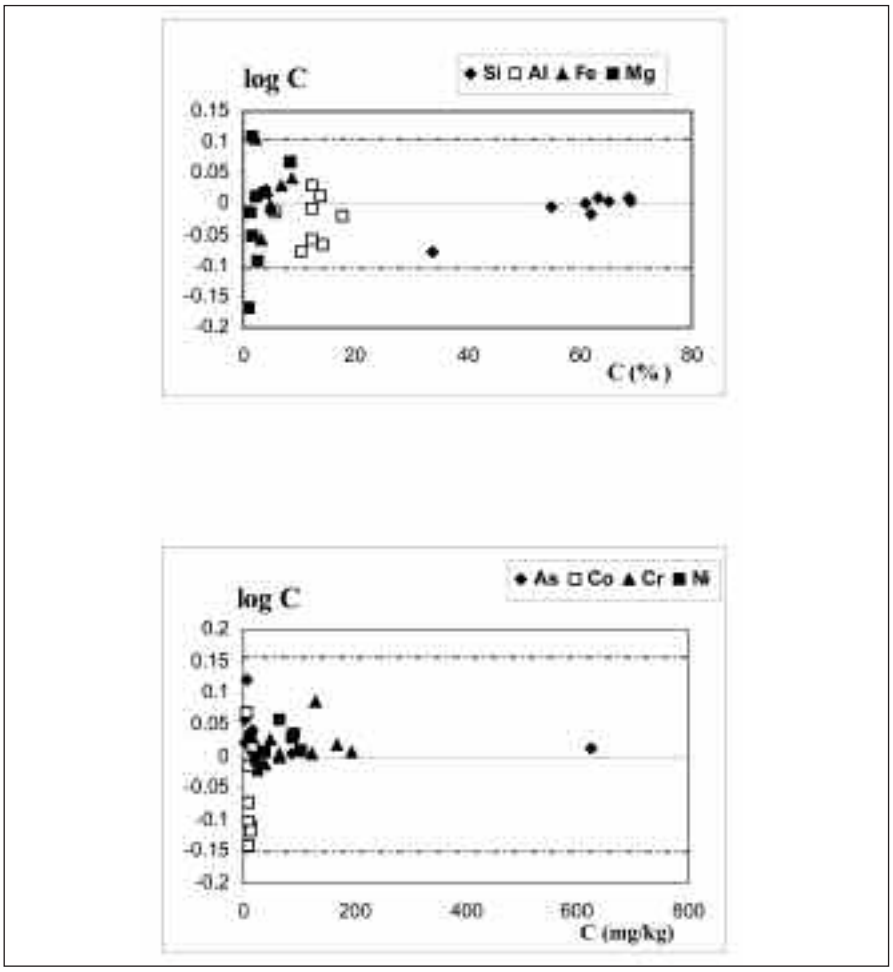

Fig. 2. Test proposed by IGCP for accuracy evaluation in geochemical mapping. Results obtained for some major and trace elements are displayed, taking log C_c – log C_f (C_c = Certified and C_f = found concentration). The plot's external lines represent the expected accuracy for first-grade standards (e.g., international reference materials), while the internal interrupted lines represent the accuracy expected for second-grade standards.

TABLE VI
Comparison of Detection Limits for Trace Metals With Several Applications Used in Soil and Sediment Analysis
(Table taken from Zambello et al., 2002, see Ref. 13)

Element	Detection Limit (DL)	Soils				Sediments	
		Required[a] DL	Agriculture[b] BRA[e]	GER[f]	CAN[g]	ISQG[c] CAN[g]	PEL[d]
As	1.0	1.0	55	50	20	5.9	17
Cd	1.6	-	3	5	3	0.6	3.5
Cr	1.0	10 - 20	250	500	750	37.3	90
Cu	1.0	1.0	1100	200	150	35.7	197
Pb	1.5	1 - 10	140	1000	375	35	91.3
Zn	1.0	10	4000	600	600	123	315

[a]Rrequired detection limit for geochemical mapping program.
[b] Maximum provisory value for soil use in agriculture.
[c] ISQG = Interim freshwater sediment quality guidelines.
[d] PEL = Probable health effect levels.
[e] BRA = Brazilian Sopciety of Oncology Pharmacy.
[g] CAN = British Columbia Cancer Agency, Canada.
[f] GER = German Cytotoxic work group.

XRF. The most important of these are water and carbon (present either as carbonate or as organic carbon). For example, according to a study performed by Jacinta et al. in 2004 (2), the reference material NIST SRM-2710, for which the data are provided on the certification by the suppliers, contains 3% m/m C. When this value for C is used in the fundamental parameter calculations, the Zn result obtained is 6900 ± 20 mg/kg; which is within the CV± Cl value of 6952 ± 91 mg/kg. When the C content was omitted, a higher result was obtained: 7190 ± 10 mg/kg. In many geoanalytical laboratories, water and carbon are not determined but just estimated in sum from loss on ignition (LOI). Comparing major and trace element results, the latter showed a tendency towards better results. This can be attributed to the fact that trace elements tend to be better characterized in soil samples and also because trace elements are less affected by mineralogical effects when analyzed in pressed pellets.

The fitness for purpose of the results was also evaluated by the quality test proposed by the International Global Geochemical Mapping Program (IGCP) (17) and as performed by Jacinta et al. in 2004 (2), which compares the differences between obtained and recommended values by the expression of $\log C_c - \log C_f \leq \pm 0.05 - 0.3$, where C_c and C_f are the certified and found concentrations, respectively. The values of the interval that should be satisfied depend both on analyte concentration and on sample. The sample can be either first or second grade, corresponding to international and in-house reference samples, respectively. Figure 2 shows the plots obtained by applying the IGCP test to the results of SiO_2, Al_2O_3, Fe_2O_3, and MgO from Table IV and for some trace elements (As, Co, Cr, Ni) from Table V. According to IGCP criteria, the reference samples analyzed in this work would be considered first-grade standards and $\log C_c - \log C_f \leq \pm 0.1$ for the four major oxides. Lines were drawn at $\log C_c - \log C_f \leq \pm 0.1$, representing limits for second-grade standard samples. The same was done for trace element results for which $\log C_c - \log C_f \leq \pm 0.2$ was used for first-grade standards, while the lines represent limits for second-grade standards, i.e., $\log C_c - \log C_f \leq \pm 0.15$. From Figure 2 it can be deduced that the method is adequate considering mapping application.

Precision

Precision of triplicate analysis is expressed as the relative standard deviation (%RSD). Although the RSD varies from sample to sample and for each element, the range of RSD based on the highest and lowest concentrations gives a good indication of the precision at high concentration levels. Figures 3 and 4 show the relative standard deviation (RSD) of the elemental concentration measured for major and trace elements. For major elements, the RSD is well below 5%, except for K showing 5.7% for SRM 2710, SRM-2711, and 5.65% and 5.48% for samples BCR 141R and BCR 142R, respectively. However, for trace elements, the RSD is also below 5% for some elements like Ba, Co, Cu, Zn, Sr, and Zr, and above 5.52% for As, 5.07% for Cr, and 5.32% for Ni. Elements with high RSDs, such as As, Cr, and Ni, may be due to suppression of the peaks, as for instance with As whose peak strongly overlaps with that of Pb and Cd.

In general the wavelength dispersive spectrometer (WDS) results have the smallest RSDs, and this is consistent with all standards and measurements of the elements sequentially. The WDS, however, has good precision and sensitivity and is less prone to inter-element interferences than other X-ray analytical techniques. Although matrix correction procedures are relatively well understood in X-ray analysis, some of the correction models produce more accurate results than others (18,19). In addition, corrections to light elements (e.g., Si, Al, and K) in the presence of heavy elements at major concentrations (e.g., As, Pb, and Cu), as in the SRM-2710 and SRM-2711 standards, will be relatively less accurate unless the calibration standard is of similar composition to the "unknown".

CONCLUSION

The analysis of soils by X-ray fluorescence spectrometry is advantageous because little effort is necessary in sample preparation. The analysis method proposed is very robust since reliable results could be obtained for a representative set of major and trace elements in several distinct types of geochemical reference materials with anomalous concentrations of many elements. Comparing the results for major and trace elements, the latter tend to be more accurate, excluding the cases where the analyte concentration approaches the detection limit. All of the results lie within the limits of the fitness for the purpose test of the international geochemical mapping (IGCP) program. The spectrometer calibration with empirical coefficients is a critical step because matrix effects are quite severe and results in a time-consuming process with a wide concentration range of the analytes. The accuracy of the method, when only major elements are considered, improves from lighter to heavier elements.

The monitoring of trace metals in the environment has been a subject of great concern over the last decade and will continue to be so since there is an ever-increasing amount of metals at toxic levels found in the environment. The

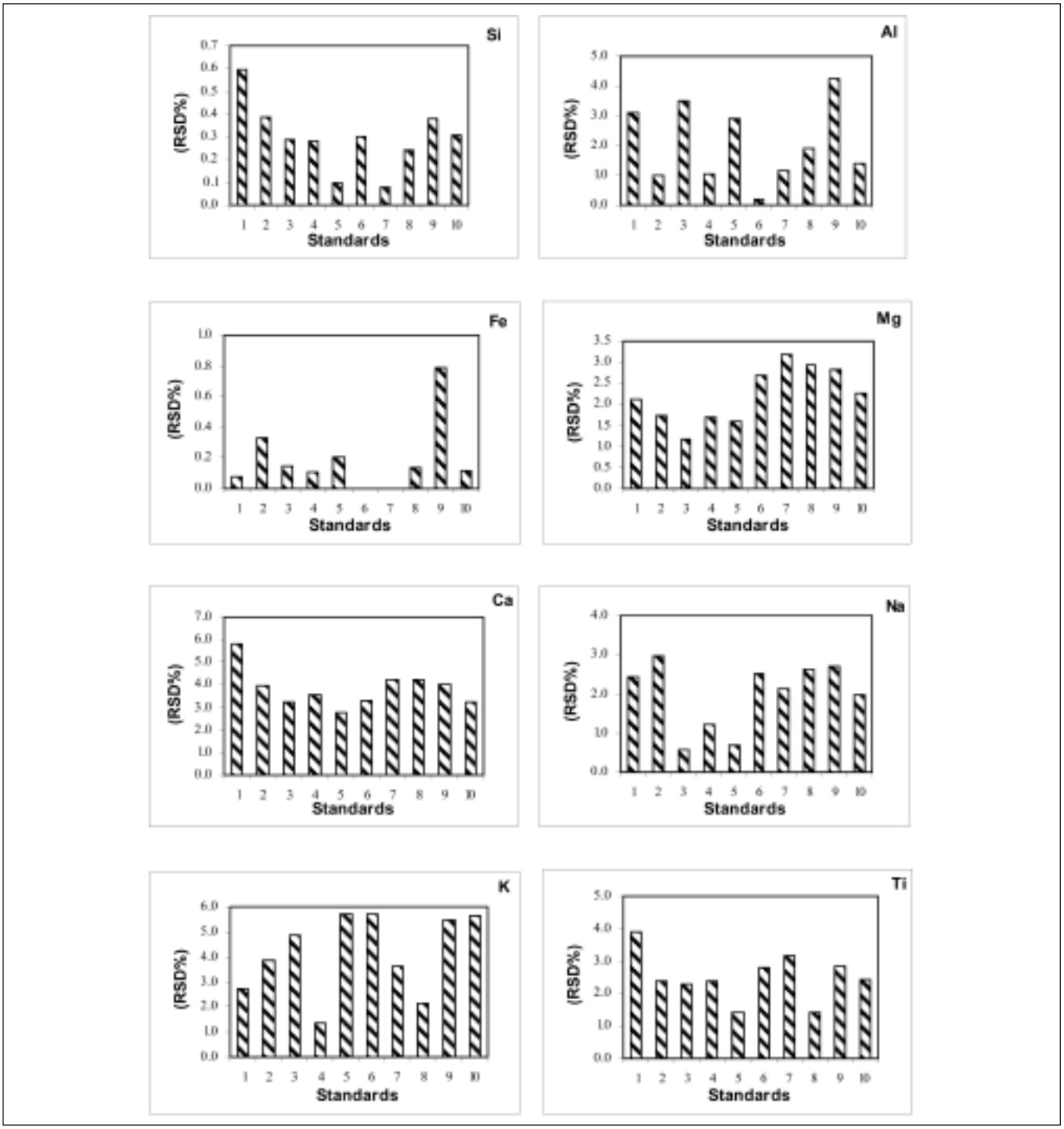

Fig. 3. The relative standard deviation (%RSD) on y-axis for major elemental concentration and numbers on x-axis indicate standards 1: SO_1, 2: SO_3, 3: SO_4, 4: SRM-2709, 5: SRM-2710, 6: SRM-2711, 7: TILL-1, 8: TILL-3, 9: BCR-141R, 10: BCR-142R.

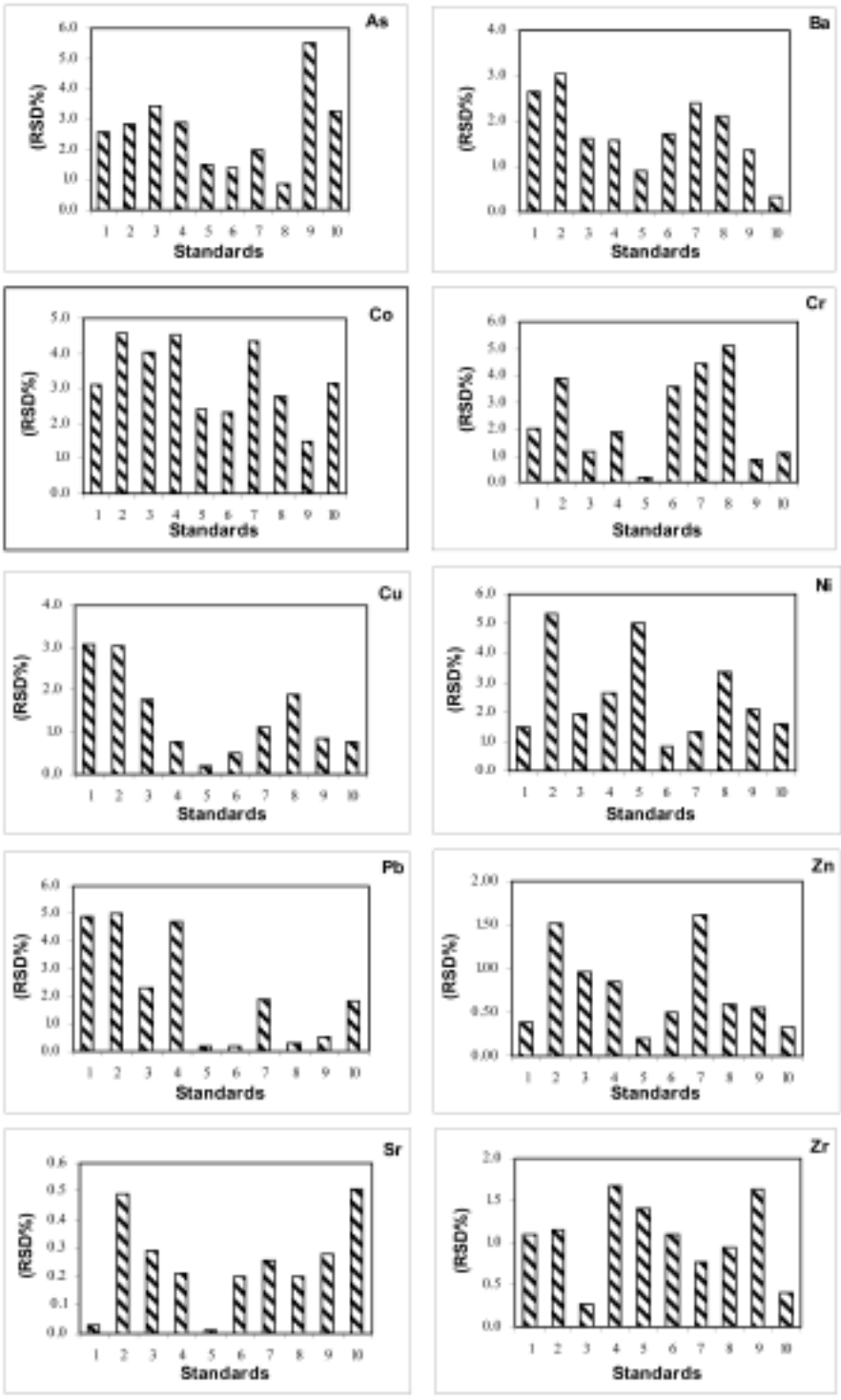

Fig. 4. The relative standard deviation (%RSD) on y-axis for trace elemental concentration and numbers on x-axis indicate standards 1: SO_1, 2: SO_3, 3: SO_4, 4: SRM-2709, 5: SRM-2710, 6: SRM-2711, 7: TILL-1, 8: TILL-3, 9: BCR-141R, 10: BCR-142R.

sources of heavy metals in the environment have been attributed primarily to anthropogenic sources, such as waste discharge and stack emissions from industrial sources. Research over the last 20 years indicates that consumption of heavy metals, for instance Pb, may be detrimental to human health at levels lower than previously thought to be safe. Thus, there exists the need for the continuous monitoring of soils in order to establish the source and to determine the amount of these toxic metals present in order to prevent a health crisis.

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Determination of Trace Pb in Water Samples by Electrothermal Atomic Absorption Spectrometry After Single-Drop Microextraction

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ABSTRACT

A simple method of single-drop microextraction (SDME) combined with electrothermal atomic absorption spectrometry is proposed for the determination of trace Pb using dithizone as the extractant. Several factors influencing the microextraction efficiency (pH, extraction time, dithizone concentration, organic drop volume, stirring rate, and sample volume) were investigated and optimized. Under the optimized conditions, a detection limit (3σ) of 0.73 ng mL^{-1} and an enrichment factor of 74 were achieved. The relative standard deviation was 7.7% (c=10 ng mL^{-1}, n=5). The developed method was applied to the determination of trace Pb in water samples with satisfactory results.

INTRODUCTION

It is now known that Pb has serious influences on the environment and human health. Its influence on the environment is through contamination of the air and water. Lead accumulates in the body, including the brain, liver, kidney, and heart and has a high potential for toxicity. Inorganic lead, in particular, can be absorbed into the bloodstream where it is distributed to soft tissues, bones, and teeth (95% in bones and teeth) (1). In addition, it also can destroy the central nervous and reproductive systems. Therefore, the ability to determine trace amounts of Pb is of great importance.

For the determination of trace Pb in a variety of materials, highly sensitive and selective analytical techniques have been used including inductively coupled plasma atomic emission spectrometry (ICP-AES) (2), atomic fluorescence spectrometry (AFS) (3), atomic absorption spectrometry (AAS) (4–6), and inductively coupled plasma mass spectrometry (ICP-MS) (7). In particular, electrothermal atomic absorption spectrometry (ETAAS) has long been considered one of the most suitable instrumental techniques for the determination of trace Pb in different samples due to its excellent analytical performance, simple operating procedures, low cost of instrumentation, high sensitivity and selectivity. However, the determination of trace Pb in natural water samples is difficult because the low concentrations present fall below the detection limits of conventional analytical techniques and because of matrix effects. Hence, preconcentration and separation techniques are still necessary.

Solid-phase extraction (8–11) and liquid-liquid extraction (LLE) (12–15) are classic pretreatment techniques that have been widely employed in analytical chemistry. However, conventional LLE consumes large amounts of expensive and potentially hazardous organic solvents, the disposal of which is problematic and very time-consuming to perform.

Recently, liquid-liquid microextraction (LPME) (16–19) has attracted increasing attention as a novel technique for sample preparation. Jeannot and Cantwell (20) were first to report a novel single-drop microextraction (SDME) technique. In their report, a small organic solvent drop that was immiscible with water was held at the end of a Teflon® rod, which was immersed in a stirred aqueous sample solution. After extracting for a prescribed period of time, the micro-drop was retracted back into the microsyinge and transferred to a gas chromatograph for further analysis. This technique is simple, inexpensive, and employs a minimal amount of toxic organic solvents. Therefore, further studies have exploited the analytical application of SDME (21,22). In the past few years, this method was primarily applied to the determination of organic compounds in water and various environmental and biological samples. Recently, there have been several reports on the study of single-drop microextraction for inorganic analytes (23,24). However, few analytical applications of the SDME method for extraction and preconcentration of metal ions have been reported.

SDME is a miniaturized sample pre-treatment technique and ETAAS is a microvolume sample analysis technique. Therefore, it will be a perfect combination if SDME is combined with ETAAS. In this work, a dithizone-carbon tetrachloride single-drop microextraction system is combined with electrothermal atomic absorption spectrometry for the determination of trace Pb in water samples.

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EXPERIMENTAL

Instrumentation

A TAS-990 atomic absorption spectrometer (Beijing Puxi Instrument Factory, Beijing, P.R. China), equipped with deuterium lamp background correction and transversely heated graphite atomizer, was used in this study. Pyrolytically coated graphite tubes (Beijing Puxi Instrument Factory, Beijing, P.R. China) were used. A Pb hollow cathode lamp was employed as the radiation source at 283.3 nm. The spectral band pass was 0.4 nm. Argon (99.99% pure) was used as a protective and purge gas. The graphite furnace temperature program for the determination of Pb is given in Table I.

The solvent microextraction apparatus was similar to the device described by Jeannot and Cantwell (25). A 10-µL microsyringe (Agilent Technologies, Santa Clara, CA, USA) was employed to introduce the extraction solution of 3.0 µL of organic phase and inject the organic phase into the ETAAS after single-drop microextraction.

Standard Solution and Reagents

A Pb stock standard solution (1.0 mg mL^{-1}) was prepared by dissolving appropriate amounts of $Pb(NO_3)_2$ (Shanghai Chemicals, Shanghai, P.R. China) in 100 mL 1% (v/v) nitric acid. The standard working solutions of the metal ions were prepared by step dilution of standard stock solutions with doubly distilled water. The 1.2 mmol L^{-1} solution of dithizone-carbon tetrachloride was prepared by dissolving appropriate amounts of dithizone (Shanghai Reagent Factory, Shanghai, P.R. China) in carbon tetrachloride. The pH was adjusted with 0.1 mol L^{-1} nitric acid and 0.1 mol L^{-1} ammonia solutions before use. All reagents used were of Specpure® grade or at least of analytical reagent grade. Doubly distilled water was used throughout.

TABLE I
Graphite Furnace Temperature Program for the Determination of Pb

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (mL min^{-1})
Solvent Evaporation	80	10	10	250
Drying	100	5	10	250
Pyrolysis	500	5	10	250
Atomization	1900	0	3	0
Furnace Cleaning	2200	1	2	250

The certified reference material used in this work was GBW(E) 080072 Water Sample (National Institute of Standards and Technology, Beijing, P.R. China).

Sample Collection and Preparation

River water (Linfen, P.R. China) was acidified to a pH value of about 2.0 with concentrated nitric acid prior to storage and use. Man-made seawater (pH=4.0) was prepared by dissolving $CaCl_2$ (0.2775 g), $MgSO_4 \cdot 7H_2O$ (3.275 g), and NaCl (6.975 g) in 250 mL doubly distilled water. The certified reference water sample GBW(E) 080072 was measured after 100-fold dilution.

Analytical Procedure

A 3-µL amount of dithizone-carbon tetrachloride solution was first withdrawn into a 10-µL microsyringe; then the needle of the microsyringe was immersed into the 5-mL sample solution containing a stirrer bar. As the organic solvent was pushed out, it formed a drop which was held at the end of the needle. Then the sample solution was stirred at 400 rpm. After extraction, the micro-drop was retracted into the microsyringe and injected into the graphite furnace for analysis. Calibration was performed using a standard solution subjected to the microextraction procedure.

RESULTS AND DISCUSSION

Optimization of the SDME Method

The selection of an appropriate extraction solvent is important for SDME. Four solvents used in conventional liquid–liquid extraction were evaluated as the extraction solvents, i.e., carbon tetrachloride, chloroform, benzene, and methyl isobutyl ketone (MIBK). The experimental results demonstrated that carbon tetrachloride provided a higher extraction efficiency than the other organic solvents. Therefore, carbon tetrachloride was selected as the extraction solvent for SDME.

Because pH value plays an important role in metal-chelate formation and subsequent extraction, the influence of sample pH values on the extraction efficiency was investigated. The pH value of sample solutions was studied in the pH range of 2.5 to 9.0; the results are shown in Figure 1. As can be seen, the Pb signal intensity increased with a pH increase from 2.5 to 4.0; however, after the pH value of 4.0, the signal intensity decreased. Thus, the optimum pH used for this procedure was 4.0.

SDME is a procedure of mass transfer kinetics equilibrium; however, it is a time-consuming process for the system to reach complete equilibrium. The effect of extraction time on the extraction efficiency of Pb was investigated and the results are shown in Figure

2. The signal intensity of Pb increased with increasing extraction times of 2 to 10 minutes. After 10 minutes, the increase of the analytical signal levelled off. To avoid the drop dissolution, the extraction time of 10 minutes was selected for all further experiments.

The effect of dithizone concentration on the extraction efficiency of Pb was investigated. The results in Figure 3 show that the signal intensity of Pb increased with an increase of dithizone concentration from 0.2 to 1.0 mmol L^{-1} and remained constant when the dithizone concentration continued to increase to 1.4 mmol L^{-1}. Because of the coexisting ions' influence on the real samples, a dithizone concentration of 1.2 mmol L^{-1} was chosen for all further experiments.

To increase the signal intensity for the determination of Pb using the SDME method, the influence of the organic micro-drop volume was investigated. The experimental results showed that the signal intensity of Pb increased with the drop volume increasing from 1 to 4 µL. However, if micro-drop volumes larger than 4 µL were used, the microdrops easily fell from the needle of the microsyringe. Therefore, 3.0-µL drop volumes were chosen for further experiments.

Based on the convective-diffusive kinetic model (20), a change of stirring rate is expected to affect the kinetic procedure of SDME. The effect of stirring rates ranging from 100 to 600 rpm was investigated. The experimental results showed that the signal intensity of Pb increased with increasing stirring rates. However, stirring rates above 400 rpm resulted in the micro-drop falling from the needle of the microsyringe. Thus, a sample stirring rate of 400 rpm was selected for this work.

The effect of sample volume on the extraction of Pb (60 ng) was investigated in the range of 5 to 15 mL. It could be concluded that the signal intensity of Pb decreased with an increase in sample volume. A sample volume of 5.0 mL was selected for this work.

Interference Studies

It has been well documented that dithizone does not chelate alkali and alkaline earth metals. But many transition metals such as Fe(II), Cu(II), Co(II), Ni(II), Mn(II), and Zn(II) react with dithizone to form stable chelate complexes within a wide pH range. The potential interferences of these ions were therefore investigated. Solutions of 10 ng mL^{-1} Pb containing the corre-

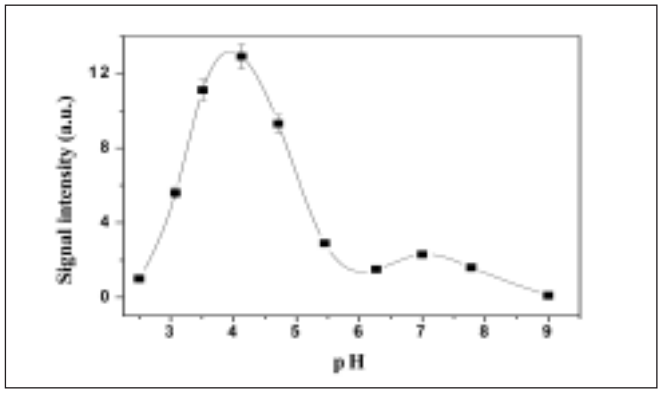

Fig. 1. Effect of pH on signal intensity of Pb. Other experimental conditions: Pb concentration level at 10 ng mL^{-1}; 400 rpm stirring rate; 10 min extraction time; 5.0 mL aqueous volume; 3.0 µL dithizone-carbon tetrachloride drop volume.

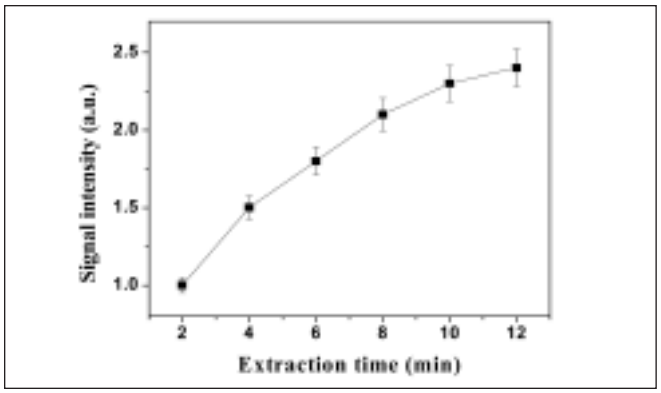

Fig. 2. Effect of extraction time on signal intensity of Pb. Other experimental conditions: Pb concentration level at 10 ng mL^{-1}; 400 rpm stirring rate; 3.0 µL dithizone-carbon tetrachloride drop volume; pH=4.0; 5.0 mL aqueous volume.

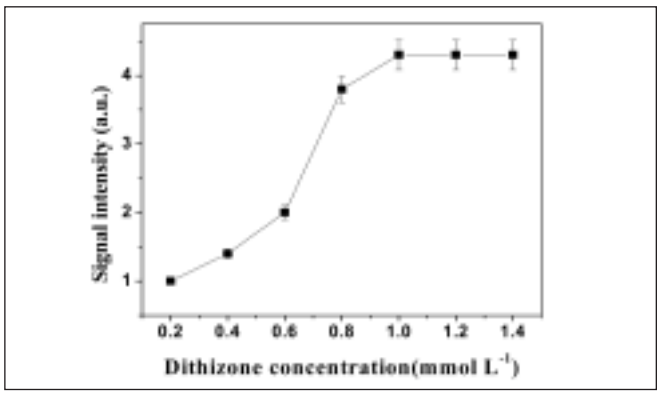

Fig. 3. Effect of dithizone concentration on signal intensity of Pb. Other experimental conditions: Pb concentration level at 10 ng mL^{-1}; 400 rpm stirring rate; 3.0 µL dithizone-carbon tetrachloride drop volume; pH=4.0; 5.0 mL aqueous volume.

sponding interfering ions were prepared and used according to the recommended procedure. The tolerable limit was taken as a relative error ≤ ±10 %. The tolerable concentration of foreign ions for 10 ng mL^{-1} Pb was found to be 3.0 mg L^{-1} for Fe(II), Mn(II), and Zn(II); 1.0 mg L^{-1} for Co(II) and Ni(II); and 0.1 mg L^{-1} for Cu(II). It was found that the developed method was not affected by the potential interferences.

Analytical Performance

The working range of the calibration curve of the method was from 0.73 to 80 ng mL^{-1} for Pb. The calibration function was A=0.0838+0.0124C with a correlation coefficient (r^2) of 0.9972, where A was the signal intensity and C was the concentration of Pb (ng mL^{-1}). The detection limit, calculated as three times the standard deviation of seven blank signals and obtained using the SDME method, was 0.73 ng mL^{-1}. The precision of the method, calculating the relative standard deviation (RSD) of five standard solutions of Pb (10 ng mL^{-1}) after extraction by dithizone-carbon tetrachloride solution, was 7.7%. The enhancement factor, defined as the concentration ratio of Pb in the micro-drop and in the initial solution, was 74.

Application to Real Samples

The proposed method was also applied to the determination of Pb in real water samples. The analytical results are given in Table II. The recoveries for the spiked samples were in the acceptable range (94.8–109 %). In addition, in order to verify the accuracy of the method, the developed method was applied to the determination of Pb in certified reference material GBW(E)080072 Water Sample. The measured and certified values were 0.96±0.15 μg mL^{-1} and 1.00±0.09 μg mL^{-1}, respectively. As can be seen, good agreement was obtained between the determined value and the certified value, and no significant differences were observed (*t*-test, P=0.05).

CONCLUSION

In this work, an effective method for the preconcentration of Pb has been achieved by single-drop microextraction (SDME) using 3 μL of dithizone-carbon tetrachloride solution and electrothermal atomic absorption spectrometric (ETAAS) determination. The method is simple, fast, effective, inexpensive, and virtually solvent-free. Under the optimized experimental conditions, the detection limit of the present SDME-ETAAS method for the determination of Pb was 0.73 ng mL^{-1}. The proposed method was applicable to the determination of trace Pb in water samples.

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Table II
Analytical Results (mean±3s, n=3) for Pb in Water Samples

Samples	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)
River Water	0	3.19±0.38	–
	2.5	5.69±0.39	100
	5.0	7.93±0.48	94.8
Artificial Seawater	2.0	2.17±0.28	109
	4.0	4.24±0.38	106

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Determination of Antimony(III) and Antimony(V) in Natural Waters at Ultratrace Levels by Flow Injection On-line Sorption Preconcentration Coupled With Hydride Generation Atomic Fluorescence Spectrometry

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ABSTRACT

A novel non-chromatographic approach is described for the determination of Sb(III) and Sb(V) in natural water samples using flow injection on-line sorption preconcentration coupled with hydride generation atomic fluorescence spectrometry (HG-AFS). With the sample pH kept at 1.0, only Sb(III) formed complexes with ammonium pyrrolidine dithiocarbamate (APDC) and was retained on the inner walls of the knotted reactor in the presence of Sb(V). Then, 1.5 mol L^{-1} HCl was used to elute the retained analyte complex and the KBH_4 solution added for HG-AFS detection. An enhancement factor of 17 was obtained at a sample frequency of 24 h^{-1} with a sample consumption of 12.0 mL. The limit of detection was 2.3 ng L^{-1} and the precision (RSD) for 11 replicate measurements of 0.1 µg L^{-1} Sb(III) was 4.5%.

INTRODUCTION

China is rich in antimony (Sb) mines and has the world's largest antimony deposits. Consequently, the pollution of antimony and other related environmental problems in China are very severe (1). As a result of rock weathering, soil runoff and anthropogenic activities, antimony is present in the aquatic environment and has been detected in natural waters at ultratrace levels (2). Inorganic species of antimony are more toxic than the organic forms. Sb(III) is ten times more toxic than Sb(V), which is the predominant species in solution under oxidative conditions, and inhalation exposure to antimonial products produces pneumonitis, fibrosis, bone marrow damage, and carcinomas (3,4). The variable toxicity of the different forms of Sb makes it imperative to develop suitable methods of speciation analysis. However, speciation of antimony still represents a real analytical challenge due to its extremely low levels in natural water samples.

On-line hyphenation of suitable element-specific detectors with various modern separation techniques are powerful tools for antimony speciation (4,5). However, the complexity and high cost of these techniques makes the use of the simple non-chromatographic approach an attractive alternative for selective determination of the two major antimony species, i.e., Sb(III) and Sb(V). It is well documented that the most popular non-chromatographic procedures are based on the selective generation of stibine from Sb(III) and Sb(V) (3,6). More recently, many methods have also been reported for this purpose, based on the suppression of hydride generation from Sb(V) using complexing agents (7–9), different analytical sensitivity obtained for Sb(III) and Sb(V) (10,11), the sequential extraction (12), and acidity control in combination with prereduction of Sb(V) (13,14), photo-oxidation of Sb(III) (15), or addition of fluoride used as a modifier (16). Besides significant improvements in sensitivity and selectivity, separation and preconcentration prior to analysis have been shown to be efficient in distinguishing element speciation. Coprecipitation (17), ion exchange (18), solvent extraction (19), and sorbent extraction (20–22) are the most commonly used separation and preconcentration methods for antimony speciation. Among them, a particular on-line preconcentration approach using a knotted reactor (KR) has been receiving considerable attention (17,22) due to its excellent properties of collecting precipitate (23) and organometallic complexes (24).

Some of the special advantages of hydride generation atomic fluorescence spectrometry (HG-AFS) are low instrument cost, high sensitivity, short analysis time and easy operation. Therefore, the method is well suited for the determination of antimony in real samples such as Chinese medicinal herbs (14), air (25), plant (26), milk (27), soils and vegetables (9,28). Even so, this analytical technique is sometimes insufficient in the direct assay of antimony in natural waters due to the very low concentration, complicated matrix, and interference of Sb(V). The aim of the present work was to develop a flow injection (FI)

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on-line preconcentration and separation system coupled with HG-AFS for ultratrace antimony determination. Ammonium pyrrolidine dithiocarbamate (APDC) was selected as the complexing agent due to its stability in acid medium and solubility in aqueous solution (22). Selective retention of the Sb(III)-APDC complex on the inner wall of the KR was used for the rapid and sensitive determination of Sb(III) and Sb(V) in natural water samples.

EXPERIMENTAL

Instrumentation

A Model AF-610A non-dispersive atomic fluorescence spectrometer (AFS) (Beijing Rayleigh Analytical Instrument Co., Beijing, P.R. China) was coupled to a Model FIA-3100 flow injection system (Vital Instrumentals Co. Ltd, Beijing, P.R. China). A detailed description of the instrumentation can be found elsewhere (29). A high-intensity antimony hollow cathode lamp (Beijing Tian-Gong Analytical Instrumental Factory, Beijing, P.R. China) was used as the radiation source at 217.6 nm. The operating parameters of the AFS instrument are given in Table I.

Reagents

All chemicals were of analytical grade (Shanghai Chemicals Co., P.R. China) unless otherwise stated. Doubly de-ionized water (DDW), obtained by sub-boiling distillation of de-ionized water in a quartz still, was used. Working standard solutions of Sb(III) were prepared by appropriate stepwise dilution of a 1000 mg L^{-1} stock standard solution [National Research Center for Standard Materials (NRCSM), Beijing, P.R. China] to the required μg L^{-1} levels just before use, and the pH of the solutions was adjusted to 1.0 with diluted HCl. The Sb(V) stock solution (1000 mg L^{-1}) was prepared by dissolving 0.2160 g of potassium hexahydroxyantimonate in 100 mL DDW. Ammonium pyrrolidine dithiocarbamate (APDC) solution was prepared by dissolving adequate amounts of APDC (Sigma-Aldrich, Canada) in DDW. A 1.0% (m/v) KBH_4 solution was prepared by dissolving KBH_4 in 0.2% (m/v) NaOH solution.

TABLE I
Operating Parameters of the AFS Instrument

Parameter	Setting
Negative High Voltage of Photomultiplier	300 V
Lamp Current	80 mA
Flow Rate of Carrier Gas (Ar)	700 mL min^{-1}
Atomizer Temperature	200 °C
Atomizer Height	7 mm
Signal Recording Mode	Peak Area

Sample Pretreatment

Two seawater samples were taken from the Yellow Sea (Lianyungang, P.R. China). River water, lake water, and groundwater were collected locally using polyethylene bottles. One mineral water sample was obtained from commercial sources. Immediately after sampling, all water samples were filtered through a 0.45-μm membrane. The filtered samples were subdivided into aliquots of 40 mL each for selective treatment. For Sb(III) determination, aliquots of the samples were acidified to pH 1.0 with hydrochloric acid (HCl). For total inorganic Sb determination, the aliquots were added to 0.2 g of L-cysteine (30), acidified with 2.0 mL of concentrated HCl to achieve a quantitative reduction of Sb(V) present in the sample and cooled to room temperature, then followed by the pH adjustment to 1.0 with diluted ammonia solution. All resultant samples were diluted to 50 mL and stored at 4 °C in low-density polyethylene bottles.

Certified reference material GBW(E) 080001 Tea Leaf, obtained from NRCSM (National Research Center for Standard Materials, Beijing, P.R. China), was used to validate the proposed method for total inorganic Sb determination. The tea powder sample was dried at 80 °C, and then around 0.5 g of the sample was accurately weighed and transferred to a PTFE microwave digestion vessel containing 3 mL concentrated HNO_3 and 2 mL 30% H_2O_2. Once sealed, the vessels were placed in the microwave chamber of a Model MK-III closed microwave digester (Shanghai SINEO Microwave Chemistry Technology Co. Ltd., Shanghai, P.R. China). The optimized digestion procedure was run through the pressure program (31) shown in Table II. After cooling, the clear digests were transferred into a 25-mL calibrated flask. The subsequent procedures were the same as indicated above for total inorganic Sb determination. Blanks were subjected to the same procedures as the samples.

TABLE II
Pressure Program of the Microwave Digestion System

Step	Pressure	Time
1	0.5 MPa	3 minutes
2	1.0 MPa	3 minutes
3	1.5 MPa	3 minutes

Procedure

As shown in Figure 1, the manifold of the FI on-line KR sorption preconcentration coupled with HG-AFS is a variation of previously reported manifolds (22,29). The procedure is briefly summarized as follows:

(a) the sample and the APDC solution were pumped to prefill the tubing before entering the KR;

(b) the sample and the APDC solution were passed through the KR where the complex of Sb(III)-APDC was produced on-line and quantitatively retained;

(c) an air segment was introduced into the KR to remove residual reagents and matrix while the KBH_4 solution and the HCl solution were propelled to be mixed in the gas-liquid separator to define the baseline signal;

(d) a 1.5-mol L^{-1} HCl solution was pumped to elute the collected analyte complex and to merge with the KBH_4 solution for detection of the analyte by HG-AFS.

Method Development

With the acidity of the standard Sb(III) solution kept at pH 1.0, the optimal values of the FI variables including flow rates, loading time, KR length, and KR washing were established in the preliminary optimization. A sample flow rate of 6.0 mL min^{-1} was selected since no significant sensitivity improvement was observed above this level. The flow of the complexing agent (APDC) was kept at 1.2 mL min^{-1} in order to minimize the total flow rate through the KR and thereby avoid leakage in the preconcentration process. The flow rate of the eluent (HCl) and KBH_4 was fixed at 10.7 mL min^{-1} and 8.0 mL min^{-1}, respectively, as recommended by the instrument manufacturer. Studies on the effect of sample loading time showed that the intensity signal of Sb(III) increased linearly up to a loading time of 120 s and then levelled off with further increases; a sample loading time of 120 s was therefore selected. As longer KR lengths imply generation of increased back pressure, a KR length of 150 cm was used as the optimum with respect to quantitative complexation and complete collection of the complex formed. Air was found to remove the residual matrix effectively without stripping off the collected analyte at a rinsing flow rate of 5.0 mL min^{-1} for 10 s.

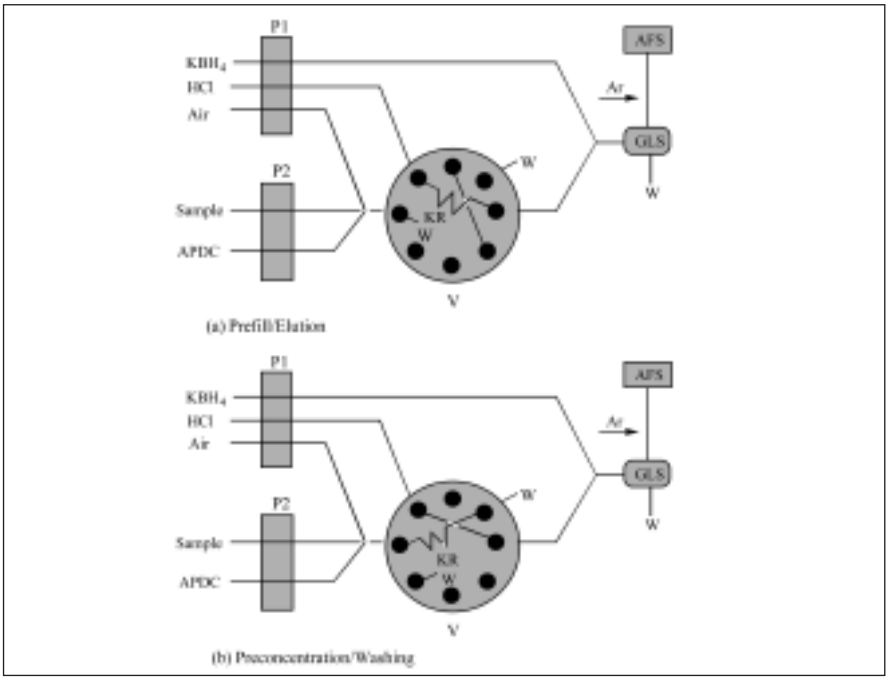

Fig. 1. FI manifold for on-line sorption preconcentration coupled with HG-AFS. P1, P2, peristaltic pump; W, waste; KR, knotted reactor (150-cm long × 0.5-mm i.d. PTFE tubing); V, injector valve; GLS, gas-liquid separator; AFS, atomic fluorescence spectrometry.

RESULTS AND DISCUSSION

Speciation Scheme

The effects of sample acidity on the preconcentration of Sb(III) and Sb(V) with APDC used as the complexing agent are shown in Figure 2. As can be seen, the Sb(III)-APDC complex could effectively be collected on the inner wall of the KR with a pH ranging from 1.0–6.0. In contrast, no fluorescence signal of Sb(V) was observed over the sample acidity range tested since Sb(V) does not form a dithiocarbamate complex with APDC (18,22). Considering that the preconcentration efficiencies on the KR using APDC as the complexing agent for potential coexisting ions such as Fe(III), Ni(II) and Cu(II) decreased sharply as the sample acidity increased (32), the sample solution was adjusted to pH 1.0 for the selective preconcentration of Sb(III). In this way, a speciation scheme was developed based on the selective preconcentration for Sb(III) determination. Total inorganic Sb concentration was obtained in combination with a pre-reduction of Sb(V), and Sb(V) was calculated by subtraction.

Effect of Concentration of Complexing Agent

As shown in Figure 3, the signal intensity of 0.1 μg L^{-1} Sb(III) was extremely low when the APDC concentration was below 0.01% (m/v), implying insufficient APDC for Sb(III) to completely produce a complex. Maximum signal was obtained within an APDC concentration range from 0.02 to 0.1% (m/v); further increases in the concentration from 0.1 to 0.2% (m/v) resulted in a decrease in the signal intensity due to the competition of

excessive complexing agent for the active sites on the KR walls (32). Thus, the APDC concentration of 0.025% (m/v) was used.

Effect of Eluent Concentration

In the flow injection on-line sorption preconcentration system coupled with HG-AFS, the eluent should not only have sufficiently strong elution capability for the collected analyte complex, but also provide the required acidic medium for hydride generation. In this work, hydrochloric acid was tested as the eluent. As shown in Figure 4, the optimal concentration of HCl was in the range of 1.0–2.0 mol L^{-1}. Therefore, 1.5 mol L^{-1} HCl, which was sufficient for the fast elution of the analyte complex, was employed throughout this work.

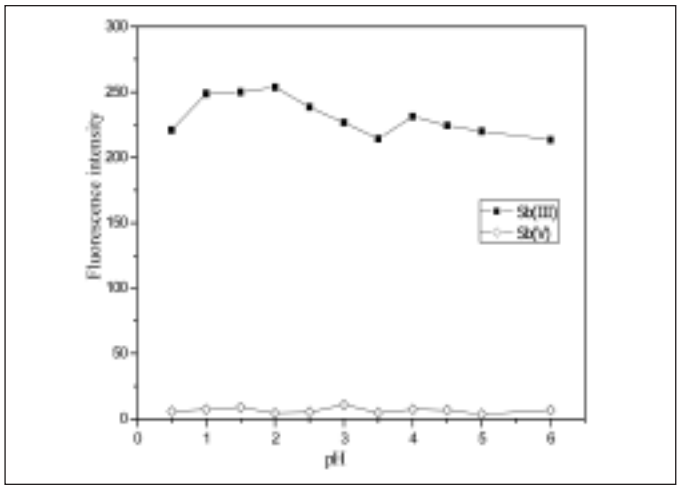

Fig. 2. Effect of sample acidity on the atomic fluorescence signal of Sb(III) and Sb(V) at 0.1 µg L^{-1}. Sample flow rate 6.0 mL min^{-1}; APDC flow rate 1.2 mL min^{-1}; Eluent flow rate 10.7 mL min^{-1}; KBH_4 flow rate 8.0 mL min^{-1}; Sample loading time 120 s; KR length 150 cm.

Effect of KBH_4 Concentration

KBH_4 was used as both a reductant and a hydrogen supplier, which was necessary to sustain the argon-hydrogen flame (33). Studies on the influence of KBH_4 concentration showed that the concentration of KBH_4 strongly affected the fluorescence and the background intensity. It was observed in this work that low concentrations of KBH_4 [<0.5% (m/v)] could not effectively reduce the analyte to hydride nor sustain the argon-hydrogen flame, while too high concentrations [>2.0% (m/v)] caused very high background signals, which yielded poorer detection limits. Therefore, a KBH_4 concentration of 1.0% (m/v) was chosen.

Interference Evaluation

Alkali and alkaline earth elements, which exist as the most common matrix constituents in the examined real samples, were separated from Sb(III) and caused no interferences due to the group-specific character of APDC and the efficient washing step (32). Potential interferents come from transition metals, heavy metals, and other hydride-forming elements, which might compete with the analyte for

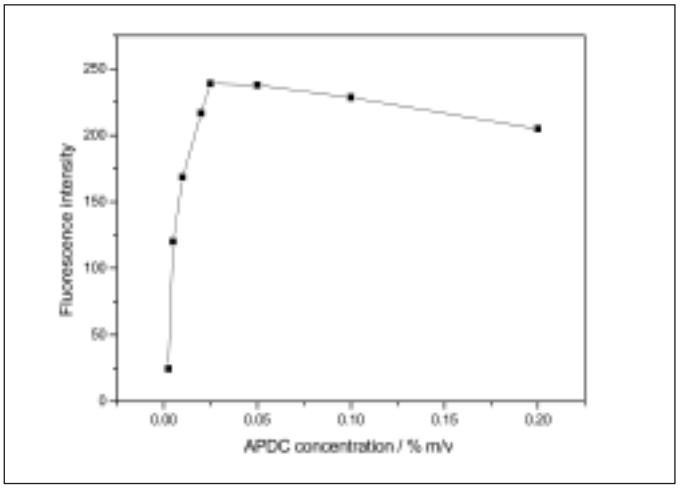

Fig. 3. Influence of APDC concentration on the atomic fluorescence signal of 0.1 µg L^{-1} Sb(III). All other conditions as in Figure 2.

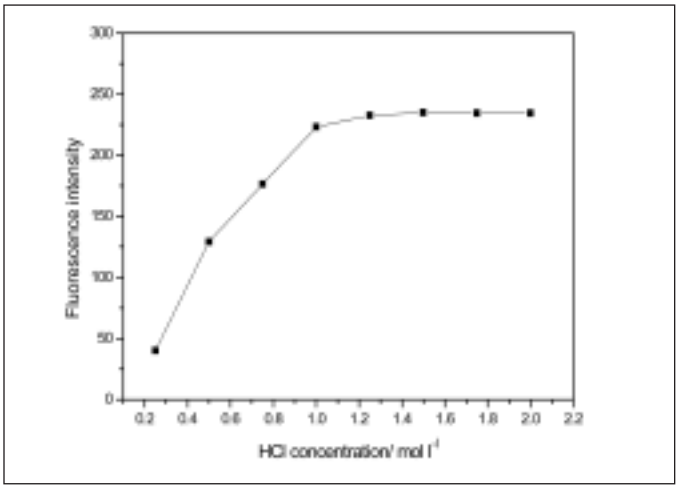

Fig. 4. Influence of HCl concentration on the atomic fluorescence signal of 0.1 µg L^{-1} Sb(III). All other conditions as in Figure 2.

the complexing agent and/or affect the analyte in the gaseous and liquid phase during hydride generation. Thus, the influence of those potential interferents on the signal of 0.1 μg L^{-1} Sb(III) was investigated. The tolerance limits (μg L^{-1}), defined as interferent concentration varying the analyte signal by 10%, are presented as follows:
Fe(III) 150 μg L^{-1};
Cu(II), Zn(II), Ni(II) 100 μg L^{-1};
Mn(II), Co(II), Cd(II) 50 μg L^{-1};
Bi(III) 30 μg L^{-1};
Hg(II), Se(IV), Pb(II) 20 μg L^{-1};
As(III) 10 μg L^{-1}.
Evaluation of the interference demonstrated that the potential interferents in natural water samples in the majority of cases had no significant effect on the determination of Sb(III).

Analytical Performance

For a 120-second preconcentration time, the sampling throughput was 24 h^{-1} and the enhancement factor was 17 (as calculated by comparison with the direct injection of 0.5 mL aqueous standard solution) with a sample consumption of 12.0 mL. The calibration line for fluorescence intensity was I = 12.59 + 2478.2C (C, in μg L^{-1}), with r = 0.9989. The detection limit was calculated on the basis of three times the standard deviation of the blank and was found to be 2.3 ng L^{-1} with the liner range of the method between 0.01 and 1.0 μg L^{-1}. The precision (RSD) of the 11 replicate measurements of 0.1 μg L^{-1} Sb(III) was 4.5%. Compared with some reported sensitive methods for antimony determination in water samples (Table III), the sensitivity of the proposed system is not only much improved in comparison to those obtained by HG-AFS (5), HG-AAS (15), differential pulse anodic stripping voltammetry (DPASV) (34) and are comparable to those of ICP-MS methods (35,36), but are also superior to those preconcentration systems with HG-ICP-OES (17), ETAAS (20) and GFAAS (21).

Real Analytical Application

Certified reference material GBW(E) 080001 Tea Leaf, with an Sb content of 0.036 ± 0.004 μg g^{-1}, was used to validate the method for total inorganic Sb determination. Using the proposed method, the Sb content determined in this sample was 0.038 ± 0.005 μg g^{-1}. The accuracy of Sb(III) and Sb(V) determination was checked by recovery measurements on spiked samples of a synthetic water solution with the composition of 25.9 g L^{-1} NaCl, 13.6 g L^{-1} $MgCl_2 \cdot H_2O$, 4.2 g L^{-1} Na_2SO_4 and 2.0 g L^{-1} $CaCl_2$. As shown in Table IV, the recoveries of the Sb(III) and Sb(V) spiked samples varied from 93.0 to 108.5%, demonstrating the validity of the developed method.

The developed method was applied to the determination of Sb(III) and Sb(V) in several types of natural water samples. The analytical results and recoveries for spiking with 0.100 μg L^{-1} Sb(III) and 0.200 μg L^{-1} Sb(V) are shown in Table V. The recoveries of the spiked samples varied from 94.4 to 105.4% for Sb(III) and from 94.1 to 106.9% for Sb(V), indicating that the low levels of Sb(III) and Sb(V) originally present were determined with a comfortable degree of confidence.

TABLE III
Comparison of Detection Limits of the Present System With Some Reported Sensitive Methods in the Determination of Sb in Water Samples

Methods/Preconcentration Procedure	Samples	Detection Limits (ng L^{-1})	Ref.
HG-AFS/KR on line sorption	Natural water	2.3	This work
HPLC-HG-AFS	Sea water	70–130	5
HG-AAS	Seawater	5–10	15
HG-ICP-OES/KR coprecipitation	Sea water	270	17
ETAAS/microcolumn sorption	Natural water and soil	300	20
GFAAS/microcolumn sorption	Tap water, snow and urine	7	21
DPASV	Seawater	11	34
ICP-MS/solid phase extraction	Water samples	1	35
ICP-MS/solvent extraction	Natural water	0.7	36

CONCLUSION

The results obtained in this work demonstrate that the coupling of hydride generation atomic fluorescence spectrometry (HG-AFS) to a flow injection (FI) on-line knotted reactor (KR) sorption preconcentration is applicable for the determination of ultratrace Sb(III) and Sb(V) in natural water samples. With the sample pH kept at 1.0, only Sb(III) formed complexes with ammonium pyrrolidine dithiocarbamate (APDC) and was retained on the inner walls of the knotted reactor in the presence of Sb(V). An enhancement factor of 17 was obtained with the detection limit of 2.3 ng L^{-1} for Sb(III). In particular, the proposed system is very simple and cost-effective because of the ease of construction and the unlimited lifetime of the KR.

TABLE IV
Analytical Results for Sb(III) and Sb(V) Determination in Spiked Samples of Synthetic Water
(Mean value ± standard deviation, n=5)

Added (μg L^{-1})		Found (μg L^{-1})		Calculated (μg L^{-1})	Recovery (%)	
Sb(III)	Sb(V)	Sb(III)	Total Sb	Sb(V)	Sb(III)	Sb(V)
0.100	0.100	0.103 ± 0.007	0.196 ± 0.014	0.093 ± 0.014	103.0	93.0
0.200	0.200	0.189 ± 0.012	0.406 ± 0.023	0.217 ± 0.023	94.5	108.5
0.400	0.400	0.385 ± 0.015	0.776 ± 0.028	0.391 ± 0.028	96.3	97.8

TABLE V
Analytical Results for Sb(III) and Sb(V) Determination in Natural Water Samples
(Mean value ± standard deviation, n=5)

Sample	Found (μg L^{-1})		Calculated (μg L^{-1})	Recovery (%)	
	Sb(III)	Total Sb	Sb(V)	Sb(III)[a]	Sb(V)[b]
Lake water	0.043 ± 0.006	0.125 ± 0.024	0.082 ± 0.024	94.4	96.6
Groundwater	0.413 ± 0.023	0.491 ± 0.030	0.078 ± 0.030	97.5	102.6
Mineral water	N.D.	0.226 ± 0.028	0.226 ± 0.028	98.0	104.4
River water	0.012 ± 0.003	0.071 ± 0.013	0.059 ± 0.013	105.4	94.1
Sea water 1	0.022 ± 0.007	0.215 ± 0.024	0.193 ± 0.024	96.7	106.9
Sea water 2	N.D.	0.155 ± 0.018	0.155 ± 0.018	103.0	95.2

[a] Recovery for spiking with 0.100 μg L^{-1} Sb(III).
[b] Recovery for spiking with 0.200 μg L^{-1} Sb(V).
N.D. =not detectable, less than detection limit.

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Factorial and Doehlert Design Used as Optimization Procedures for the Direct Determination of Vanadium in Serum Samples by Graphite Furnace Atomic Absorption Spectrometry With *in-situ* Matrix Removal

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ABSTRACT

In the present study, the determination of vanadium in human serum by graphite furnace atomic absorption spectrometry was investigated after multivariate optimization using the fragmental factorial and Doehlert design. Optimization of the method involved the study of pyrolysis and atomization temperature, and use of sample dilution and a chemical modifier.

Rhodium was the best modifier and 1:4 the best dilution of the sample. The pyrolysis and atomization temperatures were refined by using a Doehlert matrix, and 1100 and 2700 ºC were found to be optimum, respectively. With these conditions, the recoveries of the spiked serum samples were 101.7 ± 5.7%, with a relative standard deviation (RSD) lower than 20%. The obtained inter- (n=3 days) and intra-assay (n=7) studies showed average values of 4.35 and 5.53, respectively. Analysis of the serum samples obtained locally from healthy people showed an average vanadium level of 12.04 ± 1.64 μg L^{-1}. A limit of detection (LOD) of 1.36 μg L^{-1} and a tube lifetime of more than 500 cycles were obtained using the proposed methodology.

INTRODUCTION

Because of their biochemical importance, the concentration of trace and ultra-trace elements in body fluids such as serum, plasma, and urine can often be used as biological indicators of human health, disease, and nutrition, and their determination is of great interest in the biological and medical sciences (1). To accurately determine ultra-trace elements in biological fluids, various highly sensitive techniques have been employed such as neutron activation analysis (NAA) (2), graphite furnace atomic absorption spectrometry (GFAAS) (3), inductively coupled plasma atomic emission spectrometry (ICP-AES) (4), inductively coupled plasma mass spectrometry (ICP-MS) (5), and double-focusing sector field inductively coupled plasma mass spectrometry (HR-ICP-MS) (6). The NAA technique needs access to a nuclear reactor and elaborate radiochemical separations, which is very time-consuming and can take up to one month. The ICP-MS technique shows a severe polyatomic interference with $^{35}Cl^{16}O^{+}$ (arising from the sample matrix) on the major ^{51}V isotope and hampers the accurate determination of V in matrices such as urine and serum, particularly when using quadrupole-based ICP-MS. As a result, separation of the analyte due to such interferences prior to ICP-MS determination in often needed to ensure accurate results (7). GFAAS is widely employed because of its high sensitivity and low sample consumption (10–50 μL); however, it is basically a single-element technique which suffers from matrix and memory effects, especially for refractory elements. In some applications, the process of analyte-matrix separation before determination is necessary to alleviate interference from the sample matrix.

Human serum has a complicated chemical composition and contains large amounts of proteins (about 60–80 g L^{-1}) and approximately 9 g L^{-1} dissolved salts. In addition, the concentrations of Na, K, Ca, Mg, Cl, S, and P are higher than of all other elements, except for C, H, O, and N (8).

Vanadium is released in large quantities into the environment, mainly from the combustion of fossil fuels during various industrial processes which use vanadium as a catalyst, as well as an alloying agent for steel, welding and metal plating processes (9).

Numerous studies have since been made to try to establish the relationship of vanadium levels to human cerebral activity, growth, and reproduction. But one of the main difficulties of such a study is the fact that the organism seems to adjust its metabolism to the presence or absence of vanadium during food intake (10). On the other hand, it is known that vanadium increases muscular mass; it is even suggested that athletes take 30 mg

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a day (11), while other works (12) affirm that a daily ingestion of 10 mg vanadium produces patent toxicity in humans. Even lesser amounts (1 µg/g V, that is, 10 to 100 times the amount usually present in the diet) have been reported (12) to have pharmacological effects on animals and humans. The toxicity threshold may be even below 10 mg vanadium/day. This is a very serious situation considering that manufacturers advise the athletes to take 30 mg of vanadium a day. Vanadium is currently undergoing clinical trials as an oral drug for patients with noninsulin-dependent diabetes mellitus. Furthermore, vanadium occurs in elevated concentrations in the blood of patients receiving intravenous albumin solutions containing large amounts of the metal ion as an impurity. This study was conducted on five healthy volunteer subjects who received intravenously 90 mL of a commercial 20% albumin infusion solution containing 47.6 µg vanadium as an impurity (13).

Three types of pressure digestion systems used prior to the determination of vanadium by electrothermal atomic absorption spectrometry (ETAAS) were evaluated by Vogt et al. (14): the HPA (high pressure ashing) system, Model DAB III (No. 5225090, Berghof, USA) pressure digestion system and the pressurized microwave digestion (PMD) system. Complete sample digestion and no loss of graphite tube sensitivity as well as reliable vanadium values could only be achieved with HPA digests of freeze-dried serum. The mean recovery rate was 98%, and they observed no loss of tube sensitivity.

A simple, fast, and sensitive method is described by Yang et al. (7), for the determination of vanadium in biological fluids using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). The samples were diluted 20-fold in 0.3% HNO_3. Spectral interference from $^{35}Cl^{16}O^+$, present in urine or serum matrices, was completely resolved from the major vanadium isotope $^{51}V^+$ at a medium resolution of 4000.

Chery et al. (15) used Slab-gel electrophoresis for the speciation of vanadium in serum. The electrophoresis separation is an adaptation of the blue native polyacrylamide gel electrophoresis separation necessary to ensure the stability of the vanadium protein complex. Coomassie blue was used to shift the charges of the proteins and to stabilize the vanadium complex. Detection of the vanadium species was made possible by the use of the ^{48}V radiotracer and phosphor-screen technology. In another work (16), vanadium concentration in hair samples was measured by atomic absorption spectrophotometry using a Model Hitachi Z-5000 apparatus, following sample incineration and dissolution in a 1 M solution of spectrally pure HNO_3. The results were verified by using a hair reference material.

The instrumental conditions recommended by the manufacturers of graphite furnace atomic absorption spectrometers (GFAAS) are usually only appropriate for aqueous solutions. In the analysis of real matrices, especially when previous sample digestion is required, optimization of the furnace temperature program and the modifier should always be optimized and appraised through the figure of merit in the development of a new method.

Frequently, the optimization of experimental conditions in GFAAS studies (chemical modifiers, pyrolysis time, pyrolysis and atomization temperatures) requires a large number of time-consuming and costly experiments. Moreover, the interactions between optimized variables are not evaluated. Multivariate optimization seems to be more adequate when many variables are involved. The factorial design (17,18) is a good and simple statistical tool that can be used to verify the effects of variables and their interactions and requires few experiments. In this work, a factorial and Doehlert (19–21) design was employed to optimize the experimental conditions for the direct determination of vanadium in diluted serum samples by graphite furnace atomic absorption spectrometry with *in-situ* matrix removal.

EXPERIMENTAL

Instrumentation

All measurements were carried out with a PerkinElmer AAnalyst™ 400 atomic absorption spectrometer, equipped with a Model HGA®-800 graphite furnace, an AS-800 autosampler, and a deuterium lamp arc background correction operated under the conditions recommended by the manufacturer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA), unless specified otherwise.

A hollow cathode vanadium lamp from Instrumentos Científicos LTDA, São Paulo, Brazil, was operated at 20 mA, with a slit width of 0.8 nm and a wavelength of 318.4 nm.

Argon 99.996% (White Martins, Belo Horizonte, MG, Brazil) was used as the purge gas with a flow rate of 250 mL min^{-1}. Pyrolytically coated graphite tubes with integrated platforms (PerkinElmer Part Numbers B-3001264 and B-3001263) were used for all studies. The studies showed that in the determination of vanadium in serum samples, the sensitivity was better using graphite tubes with integrated platforms than with graphite tubes without platforms. Using the readings for vanadium in serum samples prepared as described above, the average signal (±deviation, n=3) was 0.086±0.002 with platform (plus 500 µg Rh) and 0.066±0.005 with wall atomization.

The amount of diluted sample and calibration solutions pipetted into the graphite tube was 20 µL. In the studies using a permanent modifier, the tubes were treated with the method previously described for either Ir, Rh, Ru, W, or Zr, i.e., by applying 50 µL of 1000 mg L^{-1} Rh and submitting the tube to a specific temperature program as published elsewhere (22-24). This procedure was repeated 10 times in order to obtain a deposit of 500 µg of permanent modifier. The optimized graphite furnace temperature program for the determination of V in serum is shown in Table I.

TABLE I
Temperature Program for the Determination of V in Serum Samples by GF-AAS with Rh as a Permanent Modifier

Step	Temperature (ºC)	Ramp (s)	Hold (s)	Ar Flow Rate (mL min^{-1})
Dry	100	10	20	250
Dry	140	20	20	250
Dry	200	10	30	250
Pyrolysis	1100	10	20	250
Atomization	2700	0	5	0 (read)
Clean	2700	1	4	250
Cool	20	1	5	250

Reagents

The following reagents were used:

• Suprapur® nitric acid, 65% (Merck, Darmstadt, Germany).

• Titrisol® vanadium standard solution (Merck).

• Iridium standard solution (No. 58195) in 1 mol L^{-1} hydrochloric acid (Fluka, Buchs, Switzerland).

• Ruthenium standard solution (No. 84033) in 1 mol L^{-1} hydrochloric acid (Fluka).

• Rhodium standard solution (No. 83722) in 1 mol L^{-1} hydrochloric acid (Fluka).

• Zirconium standard solution in 1 mol L^{-1} hydrochloric acid (Aldrich, Milwaukee, WI, USA).

• Tungsten standard solution (1.0 g L^{-1} W) prepared by dissolving 0.18 g of Na_2WO_4 (Merck) in 100 mL of water.

• Water – All solutions were prepared with deionized water with a specific resistivity of 18 mΩ cm^{-1} obtained by double-filtering distilled water through a Milli-Q™ Model RO15 purifier immediately before use (Millipore, Gig-sur-Yvette, France) .

Plastic bottles, autosampler cups, and glassware were cleaned by soaking in 20% (v/v) HNO_3 for one day, rinsing many times with Milli-Q water and drying. The autosampler washing solution containing 0.1% (v/v) Triton® X-100 (Merck) plus 0.2% (v/v) nitric acid was used to avoid analyte adsorption onto the surface of the container and clogging of the capillary sampling tip, as well as to improve the dispersion of the sample solution onto the platform.

Tricethyl methyl ammonium chloride (TMAC), 0.02% (w/v), No. 29273-7 (Aldrich (Milwaukee, WI, USA), was used as a diluent. TMAC acts as a detergent to eliminate carbonaceous residues formed inside the graphite tube and also helps in the cleaning the autosampler capillary between sampling.

Procedure

Sample Preparation

This study was conducted under voluntary participation of healthy individuals (students). Approximately 20-mL blood samples were collected from each person in the Laboratory of Molecular and Cellular Immunology in the Department of Biochemistry and Immunology at the Institute of Biological Science of Federal University of Minas Gerais (UFMG), Brazil. This study was approved by the Ethics Committee in Research of the UFMG (CONEP). Informed consent was obtained from each subject before blood collection.

The collected serum samples were diluted 1:4 with nitric acid 1% (v/v) containing 0.02% (w/v) TMAC for the factorial design studies (17,18). Quantification was performed with matrix-matched calibration standards. Each point of the calibration curves presented the same volume of serum with different concentrations of the analyte. For verification of the accuracy of the proposed methodology, the recovery of the serum samples spiked with 2.0 to 40.0 µg L^{-1} of vanadium was determined (see Table VI in Analytical Figures of Merit section). The limit of detection (LOD, µg L^{-1}) was calculated by using the equation LOD = 3 x SBL, where SBL is the standard deviation of 10 measurements of the first calibration point in the matrix-matched calibration curve [serum sample diluted 1:4 with nitric acid 1% (v/v) with 0.02% (w/v) of TMAC and 6 µg L^{-1} of vanadium]. The LOQ (µg L^{-1}) calculed was 10 x SBL.

Multivariate Method Optimization

A fragmental factorial design (factorial 2^{4-1}, 8 experiments) was used to optimize the best pyrolysis and atomization temperatures (pyrolysis between 1100 and 1700 ºC

and atomization between 2300 and 2700 ºC), dilution factor (1:1 and 1:4), and either without a modifier or with 500 µg rhodium modifier. Previous to this stage, selection of the best modifier (for use in the factorial design) was made in a serum sample diluted 1:4 and using the temperature program recommended by the manufacturer with the following permanent modifiers: rhodium, iridium, ruthenium, zirconium, tungsten (500 µg each, independently), as well as without modifier. The results of this study showed that the best sensitivity was obtained with rhodium as the modifier and without a modifier. The other parameters of the temperature program were the same as presented in Table I. Based on the results obtained in the factorial design, the modifier (rhodium) and dilution factor (1:4) were fixed and the pyrolysis and atomization temperatures refined through a Doehlert design. The levels evaluated were dilution and atomization temperatures of 1:1 to 1:4 and 2300 and 2700 ºC, respectively, were used.

Precision

Precision of the method was evaluated by the variation coefficient of intra- and inter-assay studies using solutions of diluted serum, as described; and adding concentrations of 6, 12, and 18 µg L^{-1} of vanadium. To evaluate the intra-assay precision of the method, seven replicates of each concentration were analyzed on the same day. In the inter-assay precision study, the solutions were analyzed in seven replicates on three consecutive days.

RESULTS AND DISCUSSION

Factorial and Doehlert Design

The diluent was selected based on the results obtained for Bi, Al, and Cr determinations in serum samples of previous studies (24–26). According to the factorial design (17,18), the variables that have a significant effect on the response (integrated absorbance) obtained at the 95% significance level were the dilution factor, and best results were obtained by using the 1:4 ratio for serum:diluent, 500 µg rhodium modifier; best signal was obtained with an atomization temperature of 2700 ºC. The pyrolysis temperature by itself was not a significant factor, but the interaction between pyrolysis temperature, modifier use, sample dilution factor, and atomization temperature all were significant. In the determination of vanadium, the use of rhodium as a modifier led to a higher absorbance and the atomization temperature of 2700 ºC was more effective than 2300 ºC (see Table II).

Since pyrolysis was not significant, a second study was made using the Doehlert program (19–21) to refine the dilution factor and the atomization temperature results. The surface response (Figure 1) shows that the best signal was obtained using an atomization of 2700 ºC with a sample dilution factor of 1:4. Table III presents the optimal experimental conditions obtained by the multivariate procedure for the determination of vanadium in serum samples by GFAAS. Using these conditions, the background (corrected by deuterium arc lamp) was lower than 0.05 units of absorbance. Table II also shows that for other conditions, the background is close to 0.5 which is the maximum value that can be corrected for by the instrumental corrector (deuterium arc lamp).

TABLE II
Fragmental Factorial Design Experiments

Experiment	Pyrolysis	Atomization	Dilution	Modifier	Analyte Average Signal (n=3)	Background Average Signal (n=3)
1	1100 (-)	2300 (-)	1:1(-)	No modifier (-)	0.094	0.406
2	1700 (+)	2300 (-)	1:1(-)	Rh (+)	0.130	0
3	1100 (-)	2700 (+)	1:1(-)	Rh (+)	0.336	0.407
4	1700 (+)	2700 (+)	1:1(-)	No modifier (-)	0.338	0
5	1100 (-)	2300 (-)	1:4(+)	Rh (+)	0.145	0.364
6	1700 (+)	2300 (-)	1:4(+)	No modifier (-)	0.104	0.001
7	1100 (-)	2700 (+)	1:4(+)	No modifier (-)	0.405	0.307
8	1700 (+)	2700 (+)	1:4(+)	Rh (+)	0.422	0.005

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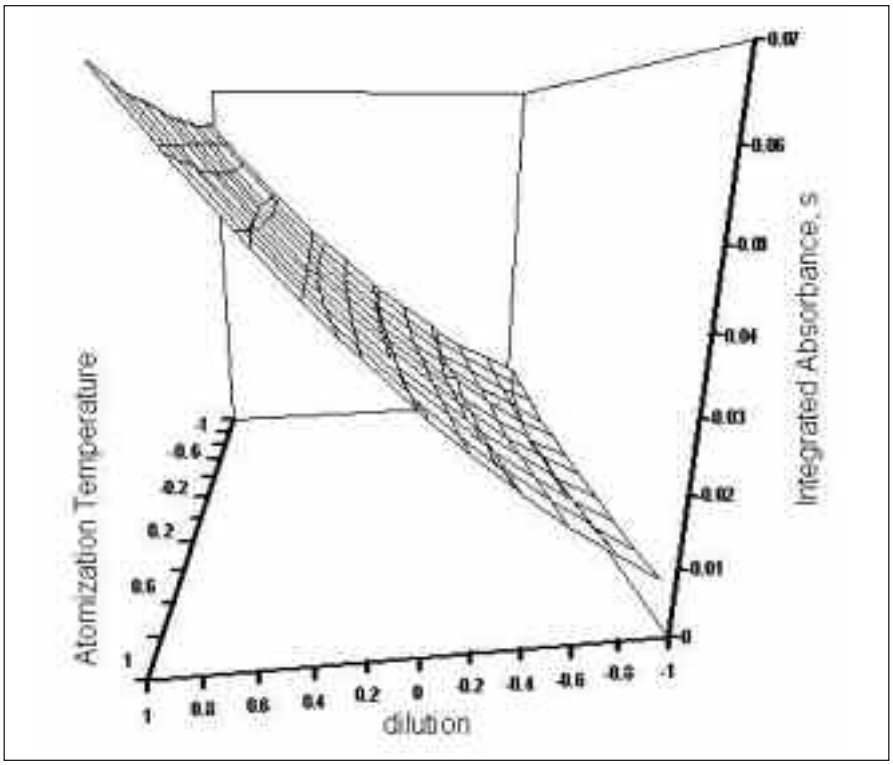

Fig. 1. Response surface obtained in the Doehlert Design.

For obtaining the best atomization temperature based on the data in Figure 1, we used the following equation:

Calculation of the best atomization temperature - V in serum:

$Abs = 0.006d^2 + 0.002T_a^2 + 0.029d + 0.002dT_a + 0.029$

$\partial z/\partial d = 0.012d + 0.002T_a + 0.029 = 0$ {1}

$\partial z/\partial T_a = 0.004T_a + 0.002d = 0$ {2}

$d = -0.004T_a/0.002 \Rightarrow d = -2T_a$ {3}

where,
Abs = integrated absorbance
d = dilution
T_a = atomization temperature

Substituting {3} in {1}:

$0.012(-2T_a) + 0.002\ T_a + 0.023=0$
$0.22\ T_a = 0.023$
$T_a = 1.045$
$d = -2T_a$
$d = -2(1.045)$
$d = -2.090$

Decodification:
$x_i = [(z_i - z_i^o)/\Delta z_i]\beta d$
$1.045 = [(T_a^o - 2500)/200] \cdot 1$
$T_a^o = 2709\ ^oC$

Analytical Figures of Merit

Table IV shows the principal figures of merit of the present study. It was observed that the calibration curve presented a satisfactory linear correlation coefficient; the average characteristic mass was in accordance with the recommended value; a comparable precision with GFAAS determination was obtained in the intra- and inter-assay studies; the limit of detection and quantification was also appropriate. In this work, the limit of detection and quantification was 1.36 and 4.53 μg L^{-1}, respectively (Table IV).

TABLE III
Optimized Experimental Conditions Obtained by Multivariate Procedure for the Determination of V in Diluted Serum Samples

Parameter	Optimized Conditions
Modifier	Rhodium
Dilution Factor	1:4
Pyrolysis Temperature	1100 ºC
Atomization Temperature	2700 ºC

Other workers reported similar limits of detection and quantification in the GFAAS determination of vanadium as shown in our study. Cassela et al. (27) report the direct determination of vanadium in highly saline water (obtained from an offshore petroleum exploration area) by employing electrothermal atomic absorption spectrometry and obtained a LOD and LOQ of 1.9 μg L^{-1} and 6.3 μg L^{-1}, respectively. Chéry et al. (15) report the use of an ICP-MS instrument with dynamic reaction cell (DRC) as the specific detector for the determination of vanadium at therapeutic levels in serum and obtained a LOD of 40 ng L^{-1}. Safari et al. (28), using a spectrophotometric method based on the catalytic effect of V(VI) on the oxidation of aniline blue bromate in environmental samples, obtained a LOD of 2 μg L^{-1}. Su and Huang (29) presented a method for the determination of vanadium in seawater samples by GFAAS using magnesium nitrate as the modifier and obtained a LOD of 0.42 μg L^{-1}.

Table V lists the precision obtained through inter- and intra-assay studies using the proposed methodology. The obtained values are in agreement with the values reported in the literature.

TABLE IV
Analytical Characteristics of Proposed Method

Parameters	Results
Regression Equation (n=21)	Y = –0.0028 (s=0.004)
	X = +0.0027 (s=0.0006)
R (n=21)	0.99618 ± 0.003
Linear Range	0 – 50 μg L^{-1}
LOD	1.36 μg L^{-1}
LOQ	4.53 μg L^{-1}
Characteristic Mass[a]	30.8 ± 2.8 pg
Recovery (%)	93.8 – 117.0%
Intra-assay Average Precision	4.35 ± 1.76
Inter-assay Average Precision	5.53 ± 1.78
Recommended Mass	30 pg

[a] Recommended mass = 30 pg.

TABLE V
Variation in the Intra- and Inter-assay Coefficients Obtained in the Vanadium Determination in Human Serum by GFAAS

Vanadium Concentration (μg L^{-1})	CV Intra-assay (%, n=7)	CV Inter-assay (%, n=3)
6	4.21 ± 0.24	7.47 ± 2.75
12	6.18 ± 0.75	5.15 ± 2.34
18	2.67 ± 0.46	3.98 ± 2.22
Average	4.35 ± 1.76	5.53 ± 1.78

TABLE VI
Vanadium Recoveries From Spiked Serum Samples With Rh as Permanent Modifier

Spike (μg L^{-1})	Determined (μg L^{-1})	Recovery (%)	RSD (n=3)
2	2.34	117.0	20.0
6	5.63	93.8	6.7
10	9.89	98.9	5.0
14	13.82	98.7	3.7
18	18.41	102.3	1.9
20	20.71	103.5	1.6
24	24.60	102.6	2.7
28	28.25	100.9	0.2
32	32.18	100.6	1.6
36	36.10	100.3	1.4
40	40.05	100.1	5.3

TABLE VII
Levels of Vanadium in Serum Samples of Healthy Persons Obtained Using the Proposed Methodology

Individual	Level of Vanadium (μg L^{-1})
1	12.75
2	12.50
3	12.39
4	12.50
5	11.43
6	16.00
7	13.46
8	11.21
9	12.14
10	10.71
11	11.32
12	11.93
13	13.21
14	12.64
15	11.79
16	12.89
17	12.04
18	8.82
19	12.89
20	8.11

Table VI shows that the recoveries obtained for 11 serum samples, spiked from 2 to 40 μg L^{-1} vanadium, were close to 100% which shows good accuracy of the proposed methodology.

In Table VII we report the level of vanadium in the serum samples of 20 healthy persons from Belo Horizonte, Brazil. It can be seen that the average level of vanadium in the serum of these individuals is 12.04 ± 1.64 μg L^{-1}.

CONCLUSION

Use of the fragmental factorial design, followed by a Doehlert design, is a simple and fast procedure to evaluate the GFAAS conditions for vanadium determination in diluted serum samples. It requires minimal use of procedures, and the matrix is removed in situ with a rhodium permanent modifier. A simple dilution of the sample before analysis helps to minimize analytical time and cost. The obtained figures of merit are very adequate for GFAAS determination. The average level of vanadium present in the serum of the healthy persons was found to be 12.04 ± 1.64 µg L–1. A limit of detection (LOD) of 1.36 µg L^{-1} and a tube lifetime of more than 500 cycles were obtained using the proposed methodology.

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On-line Speciation of Cr(III) and Cr(VI) Using Microcolumn Packed With Immobilized Used Green Tea Leaves (UGTLs) and Determination by ICP-OES in Environmental Water Samples

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ABSTRACT

A simple and sensitive method has been developed using microcolumn (20-mm length x 2.0 -mm i.d) packed with immobilized used green tea leaves (UGTLs) for the speciation of Cr(III) and Cr(VI) prior to their determination by inductively coupled plasma optical emission spectrometry (ICP-OES).

The optimal experimental conditions including pH, eluent concentration and volume, sample volume and sample flow rate were investigated and established. The adsorption capacity of immobilized used green tea leaves for Cr(III) was found to be 35 mg g^{-1}. A preconcentration time of 72 s and an elution time of 8 s, with an enrichment factor of 10 and a sampling frequency of 20 h^{-1}, were obtained. The detection limit corresponding to three times the standard deviation of the blank was found to be 87 pg mL^{-1}.The precision for seven replicate determinations at the 2-ng mL^{-1} level of Cr(III) gave a relative standard deviation (RSD) of 5%.

The method was applied to the determination of Cr(III) and Cr(VI) in environmental water samples with satisfactory results.

INTRODUCTION

Chromium species enter the environment as a result of effluent discharge from the steel, electroplating, tanning, oxidative dyeing, and chemical industries. They may also enter drinking water supply systems from the corrosion inhibitors used in water pipes and containers or by contamination of the underground water from sanitary landfill leaching (1). In aqueous systems, chromium usually exists in both the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms. Cr(III) compounds are one of the essential trace nutrients in human bodies, and play an important role in the metabolism of glucose and certain lipids, whereas Cr(VI) compounds are toxic and carcinogenic (2,3). The toxicity of Cr(VI) ions is attributed to their high oxidation potential and their relatively small size, which enables them to penetrate through biological cell membranes (4,5). In view of the above, the development of a sensitive method for the speciation method of chromium in its different oxidation states in the environment is of paramount importance. However, the concentration of chromium in natural waters is very low and the concentrations of chromium species are even lower. Hence, for speciation of chromium in natural waters, an effective separation/preconcentration procedure is usually mandatory.

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The most widely used separation/preconcentration techniques for speciation of chromium include liquid-liquid extraction (6), coprecipitation (7), and solid-phase extraction (8-10). Of all these techniques, solid-phase extraction (SPE) has proved to be effective due to the following merits:

(a) higher preconcentration factor;
(b) rapid phase separation;
(c) easy automation;
(d) time- and cost-saving; and
(e) low contamination risk over conventional procedures.

Up to this date, various detectors including inductively coupled plasma mass spectrometry (ICP-MS) (5,11,12), soft-x-ray spectrometry (13), flame atomic absorption spectrometry (FAAS) (7,4), electrothermal atomic absorption spectrometry (ETASS) (10,15,16), and inductively coupled plasma optical emission spectrometry (ICP-OES) (8,9,17) have been reported for chromium speciation. ICP-OES has gained strong recognition in environmental analysis due to the following advantages:
multi-elemental analysis capability;
large dynamic linear range;
low detection limits; and
high productivity (18).

It should be stressed that the coupling of flow injection (FI) on-line microcolumn separation and preconcentration techniques to ICP-OES have proven to be very successful (19,20). This combination not only provides an improvement in detection limits and reduces interference from the matrix, but also has significantly enhanced the analytical performance of the methods (21–23). In comparison to their off-line batch counterparts, these systems have a number of significant advantages for trace element determination including greater efficiency, lower consumption of sample and reagent, improved precision, possibility of working in a closed system with a significant reduction of airborne contamina-

tion, and increased sampling frequency. On-line column preconcentration systems coupled with ICP-OES are based on the retention of the analytes in a microcolumn packed with an adsorbent that determines the sensitivity and selectivity of the analytical method. Therefore, new adsorbents are explored and searched actively for use with SPE technique. Many adsorption materials have been reported such as nanometer-sized TiO_2 (8), activated carbon (10), surfactant coated alumina (11), and Amberlite XAD-16 resin (24).

Biological materials of plant origin have been proposed for the preconcentration and speciation of trace metals. Immobilized moss (5), Saccharomyces cerevisae immobilized on sepolite (25), milled peat (26), and Garcinia cambogia (27) were found to be suitable for chromium speciation. Hossain et al. (28) explored the dynamic characteristic of Cr(VI) adsorption on used black tea leaves (UBTLs) by batched experiment. According to their report, Cr(VI) could easily be adsorbed on UBTLs. In addition the use of UBTLs was found to be superior to activated carbon for the removal of Cr(VI) from aqueous solution. In view of the above findings, it is evident thatUGTLs may be employed for preconcentration and speciation studies.

In this study, the possibility of using UGTLs as a sorbent packed in the microcolumn for preconcentration speciation of Cr(III) and Cr(VI) has been explored. A new method of flow injection microcolumn separation/preconcentration coupled on-line to ICP-OES has been developed for the speciation of chromium in natural waters. The experimental parameters affecting the separation/preconcentration of two chromium species were investigated and the optimal conditions were established. The developed method has been applied to the speciation of chromium in natural waters with satisfactory results.

EXPERIMENTAL

Instrumentation

ICP-OES determination was performed with an Intrepid XP Radial ICP-OES (Thermo, Waltham, MA, USA) with a concentric nebulizer and a Cinnabar spray chamber. The operating conditions and wavelengths of the emission lines used are summarized in Table I. The analytical lines of the analytes were selected on the basis of their net and background intensities and their freedom from spectral interference overlaps. The pH adjustment was conducted by means of a Mettler Toledo 320-S pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, P.R. China) supplied with a combined electrode. the separation/preconcentation process was performed by means of an HL-2 peristaltic pump (Shanghai Qingpu Huxi Instrument Factory, Shaghai, P.R. China). A self-made PTFE microcolumn (20-mm length x 2.0-mm i.d), packed with immobilized used green tea leaves, was used for the speciation of Cr(III) and Cr(VI). In order to minimize dead volume, a PTFE tubing with an i.d. of 0.5 mm was used for all connections.

Standard Solutions and Reagents

Standard stock solutions containing (1g L^{-1}) of Cr(III) and Cr(VI) were prepared separately by dissolving $CrCl_3 \cdot 6H_2O$ and $K_2Cr_2O_7$ (The First Reagent Factory, Shanghai, P.R. China) in 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} HNO_3. A 10% ascorbic acid solution was prepared daily. Diluted standard solutions and model solutions were prepared daily from the stock standard solutions. High purity deionized water and high purity analytical grade reagents were used throughout for preparation of the standard and sample solutions.

Sample Preparation

Water samples were collected from the East Lake and Yangtze River of Wuhan, P.R. China. The water samples were filtered before analysis through a cellulose membrane filter of 0.45 μm pore size (Tianjin Jinteng Instrument Factory, Tianjin, P.R. China) and analyzed immediately after sampling. The water sample was divided into two parts: One part for the determination of Cr(III) and the other part for the determination of total chromium. Total chromium determination was adopted from the literature cited (8). To 49 mL of water

TABLE I
ICP-OES Operating Condition and Selected Emission Line

Parameters	
RF generator	1150 W
Frequency of RF generator	27.12 MHZ
Auxiliary gas (Ar) flow rate	0.5 L min^{-1}
Carrier gas (Ar) flow rate	0.5 L min^{-1}
Plasma gas (Ar) flow rate	14 L min^{-1}
Observation height	15 mm
Solution uptake	1.0 mL^{-1}
Integration time	30 s
Nebulizer	Concentric nebulizer
Spray chamber	Cinnabar model
Atomic emission line	Chromium: 267.716 nm

sample, 0.5 mL aqueous ascorbic acid was added and allowed to stand for 3 hours. The pH was adjusted to pH 6.0 with dilute ammonia, and the solution was then diluted to 50 mL with deionized water in a calibration flask.

Sorbent Preparation

Green tea leaves were purchased from Wuhan City Tea Leaves Company, Wuhan, P.R. China.

The used green tea leaves (UGTLs) were obtained after extracting tea liquor from green tea leaves by boiling with distilled water for 8 hours. After extraction, the leaves were dried at 105 °C for 24 hours and then sieved.

Immobilization of Used Green Tea Leaves on Sodium Silicate

It is suggested that immobilizing biomass in a granular or polyatomic matrix improves biomass performance and facilitates faster separation of metal ions from the solutions (29). Hence, when performing column experiments, it is preferable to immobilize the UGTLs to avoid reduction in flows due to clumping. A polysilicate matrix support material was used in this study to immobilize the used green tea leaves. This combines the physical properties of polysilicate and the binding properties of the used green tea leaves.

The method to be adopted for immobilization of the material within a polysilicate matrix was similar to that reported in the literature (5,25). Briefly, 75 mL of 5% H_2SO_4 was mixed with sufficient sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. Five grams of powdered UGTLs was added to the silica solution and stirred for 15 minutes. The pH was then raised slowly by the addition of 6% Na_2SiO_3 to reach pH 7.0. The ratio of silica to UGTLs was approximately 1:10 (500:5000 mg). The polymer gel was then washed with water enough times to remove all sulphates. This was further confirmed by the addition of a few drops of barium chloride ($BaCl_2$) to water so as to confirm that there is no formation of a white precipitate. The polymer gel with immobilized used green tea leaves was dried overnight at 45–50 °C and ground by mortar and pestle to powdered form.

Column Preparation

Twenty milligrams of immobilized used green tea leave adsorbent was filled into a PTFE microcolumn (20-mm length x 2.0-mm i.d) plugged with a small portion of glass wool at both ends. Before use, 1.0 mol L^{-1} HCl solution and high purity deionized water were passed through the column in order to clean and condition it.

General Procedure

Sample solutions containing the analytes Cr(III) and Cr(VI) were prepared by appropriate dilution of their stock solutions and then adjusted to the desired pH value with 0.1 mol L^{-1} HCl and $NH_3 \cdot H_2O$ before use.

The operation sequence of the FI on-line column preconcentration and determination is shown in Figure1. In the preconcentration step, (a) pump P1 was activated, so that the sample was drawn through the column. And in the elution step, (b) pump P2 was activated, so that the eluent was propelled through the column reversibly. In this instance, the continuous impact on the sorbent could be avoided. Then, the eluting solution was introduced into the ICP-OES for analysis.

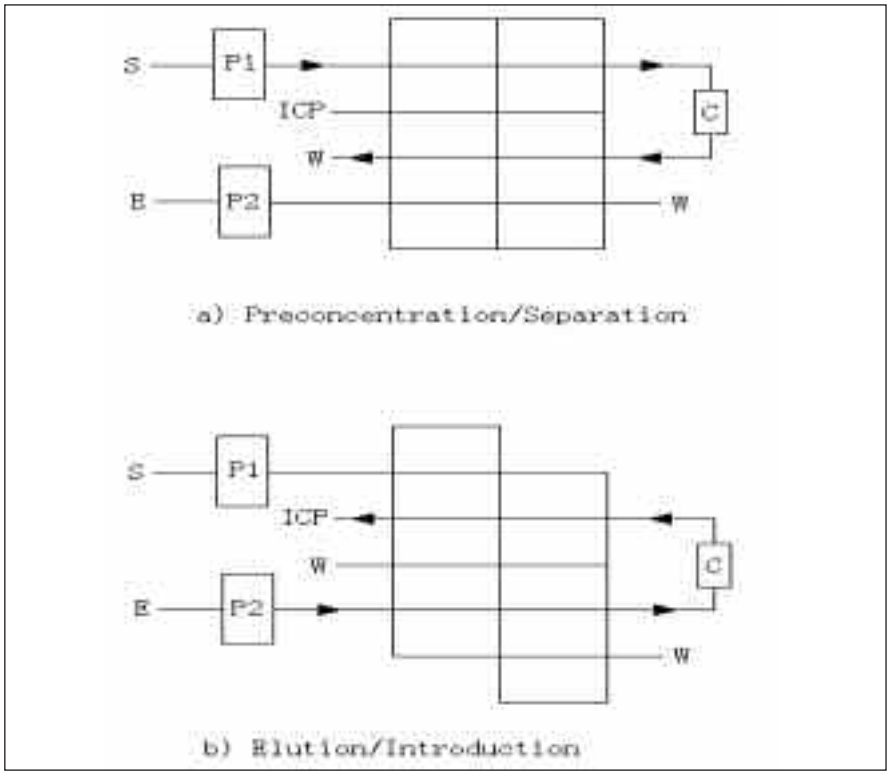

Fig. 1. Operation sequence for on-line column preconcentration and determination of Cr(III) and Cr(VI).

High purity deionized water was used as the blank solution and subjected to SPE, and the blank values were determined. The determined values for the Cr species were obtained by subtracting the blank values.

RESULTS AND DISCUSSION

Effect of pH

The effect of pH on the retention of Cr(III) and Cr (VI) on the microcolumn packed with immobilized used green tea leaves was studied. The pH of the sample solutions containing 0.2 ng mL^{-1} of the analytes were adjusted to a pH range of 1–8 by adding 1.0 mol L^{-1} $NH_3 \cdot H_2O$ or HCl and passed through the column. The retained analytes were eluted from the column and determined by ICP-OES as described above in the general procedure. As can be seen in Figure 2, quantitative adsorption (>90%) could be obtained at pH 6-8 for Cr(III), while the adsorption percentage of Cr(VI) was low (<10%). This clearly indicates the possibility of separating Cr(III) and Cr(VI).

The obtained result was quite contrary to the studies conducted by Hossain et al. (28) on the optimization of parameters for Cr(VI) adsorption on used black tea leaves in which Cr(VI) was quantitavely adsorbed at pH 1.54. In the literature (5) on chromium speciation using plant biomass it was shown that in the pH range of 3–8, the possible chromium species are Cr^{3+}, $Cr(OH)^{2+}$, and $Cr(OH)^{2+}$. The possible reason for the maximum retention of Cr^{3+} may be due to the exchange of various cationic forms of Cr(III) with ions of the carboxylic acid functional groups or various functional groups (sulphates, amino, phosphate, and thiol moieties that are associated with cellulose, proteins and lignins) found on the surface of the plant cell wall (5,30). In this work, the sample solution of pH 6 was adopted for all subsequent studies as a compromise with respect to the effective separation or speciation of Cr(III) and Cr VI).

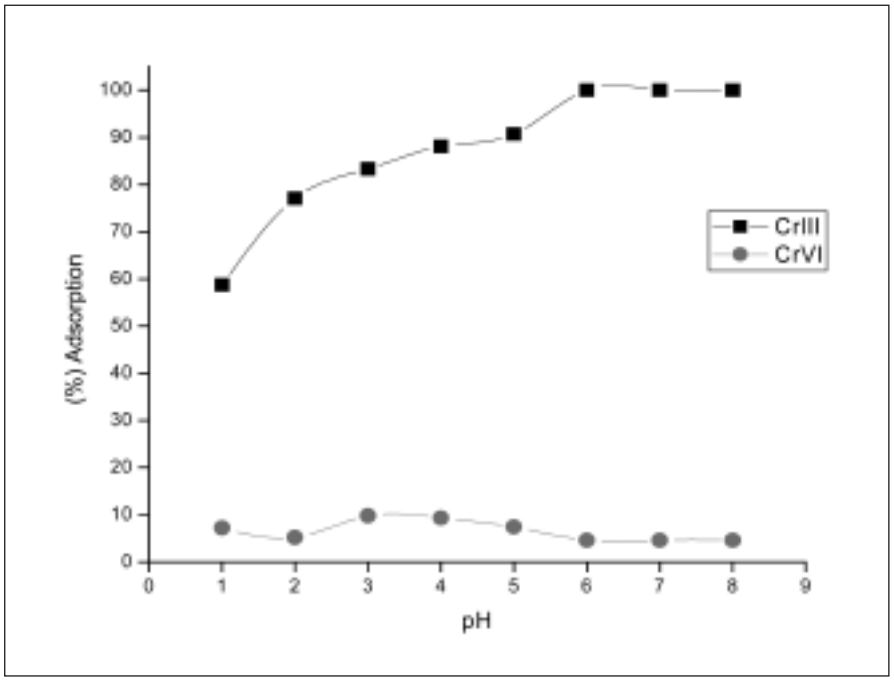

Fig. 2. Effect of pH on adsorption (%) of Cr(III) and Cr(VI): 0.2 µg mL^{-1}, Sample volume = 3 mL, Eluent volume = 3 mL, Eluent concentration =1 M HCl, Flow rate = 1 mL min^{-1}, n = 3.

Effect of Eluent Concentration

In order to determine the optimum eluent concentration required for the quantitative recovery or desorption of Cr(III) at a concentration of 0.2 µg mL^{-1} from the adsorbent, various HCl concentrations (0.5–3.0 mol L^{-1}) were studied. As can be seen in Table II, quantitative recovery (>97%) was obtained with 1 mol L^{-1} HCl. Therefore, 1 mol L^{-1} HCl was selected for further experiments.

TABLE II
Effect of Eluent Concentration on the Recovery of Cr (III)
(0.2 µg of Cr (III) in 3-mL sample)

Concentration (HCl)	Recovery[a]
0.5 mol L^{-1}	90%
1 mol L^{-1}	98%
1.5 mol L^{-1}	98%
2 mol L^{-1}	99%
2.5 mol L^{-1}	99%
3 mol L^{-1}	99%

a Mean of three determinations, analyzed by ICP-OES

Effect of Eluent Volume

The effect of eluent volume on the desorption of Cr(III) at a concentration of 0.2 µg mL^{-1} was studied. The eluent concentration was kept constant (1 mol L^{-1}), while the eluent volume was varied from 0.2–3.0 mL. It was found that with 0.3 mL HCl, quantitative recoveries (>98%) could be obtained. Therefore, 0.3 mL of eluent volume was selected for subsequent experiments.

Effect of Elution Flow Rate

The effect of elution flow rate on the recovery of the anlytes was investigated by keeping the volume of 0.3 mL containing 1 mol L^{-1} HCl.

The results indicated that the analytes could be recovered quantitatively at flow rate range of 1.0–2.5 mL min^{-1}. Hence, an elution flow rate of 2.5 mL min^{-1} was selected for this study.

Effect of Sample Flow Rate

The effect of sample solution flow rate on the retention of Cr(III) on immobilized UGTLs was investigated by passing 3 mL of sample solution through the microcolumn and studying the flow rate ranging from 0.5–2.5 mL min^{-1}. As can be seen in Figure 3, the retention of Cr(III) remained unchanged from 1.0 up to 2.5 mL min^{-1} and a quantitative recovery of >90% was obtained. Hence, the flow rate of 2.5 mL min^{-1} was selected as the optimum condition for all further experiments.

Effect of Sample Volume

In order to achieve a high preconcentration factor of very dilute analyte solution from large volumes, it is important to determine the effect of sample volume on the retention of Cr(III) on immobilized used green tea leaves.

The effect of the sample volume on the recovery of analytes was investigated by passing 1, 3, 5, 10, 20, 50, 100, and 150 mL sample solutions containing 0.2 µg of Cr(III) through the microcolumn using the optimum conditions and then the above-mentioned general procedure. As can be seen from Figure 4, the recovery of Cr(III) was approximately quantitative (>%95) up to 100 mL of sample. Above 150 mL, the recovery decreased to about 89%. In this work, for on-line purposes, a sample volume of 3 mL was employed for real sample analysis.

Effect of Coexisting Ions

The effect of some coexisting ions on the preconcentration and determination Cr(III) was studied. In this study, the concentrations of the coexisting ions were varied while the concentrations of Cr(III) were kept at 0.2 µg mL^{-1} and treated according to the recommended procedure. The tolerance limit of the coexisting ions is defined as the largest amount making the recovery of the studied elements less than 90% (12). The results of the analysis conducted are depicted in Table III. It can be seen that the presence of the coexisting ions studied has no obvious effect on the determination of the analytes under the optimum experimental conditions.

TABLE III
Tolerance Limits for Coexisting Ions

Coexisting Ions	Tolerance Limit (mg L^{-1})
Na^+, K^+, Ca^{2+}	10,000
Mg^{2+}, Al^{3+}	1000
Fe^{3+}	200
SO_4^{2-}, Cl^-	10,000
NO_3^-	1000
PO_4^{3-}	200

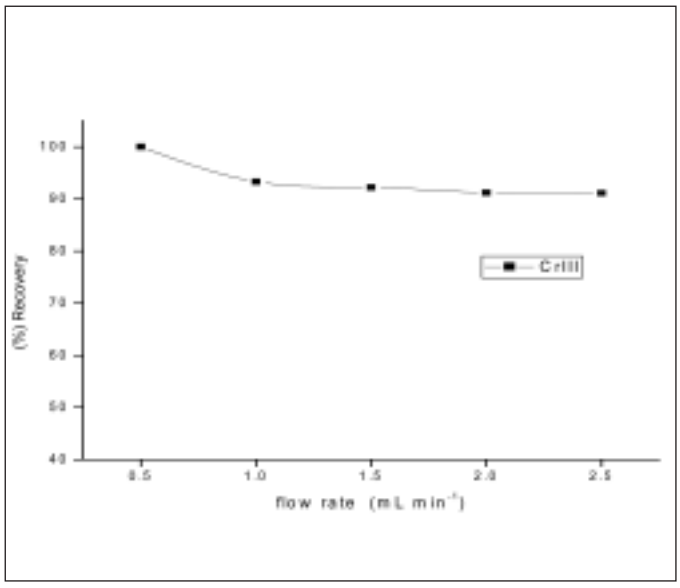

Fig. 3. Effect of sample flow rate: Cr(III), 0.2 µg mL^{-1}, Sample volume = 3 mL, Eluent concentration =1M HCl, Eluent volume = 1 mL, n = 3.

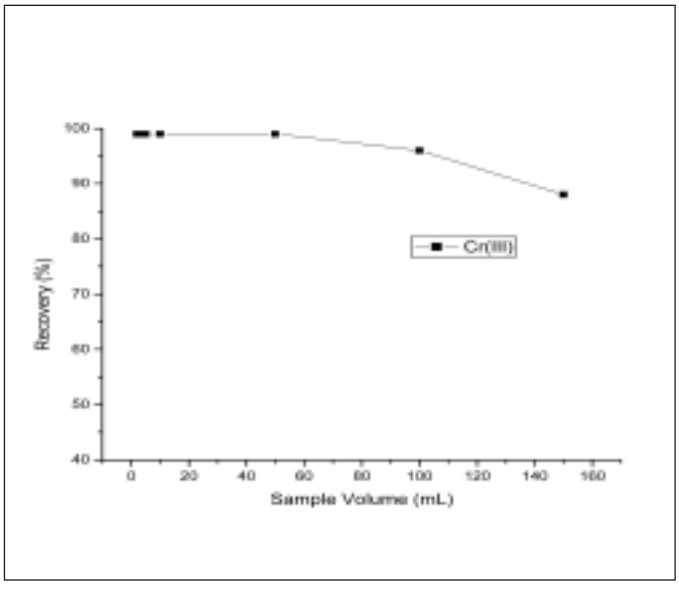

Fig. 4. Effect of sample volume on recovery (%) of Cr(III): 0.2 µg, Eluent concentration=1M HCl, Eluent volume=1.0 mL, Flow rate=1.0 mL min^{-1}, n = 3.

Column Reuse

The regenerability and stability of the column was investigated by passing the analytes through the column packed with 20 mg of immobilized used green tea leaves and then followed by passing throuhg 2 mL 1.0 mol L^{-1} HCl and 5 mL of distilled water. It was observed that the column could be reused up to 50 runs without decrease in the recoveries of the studied analytes.

Adsorption Capacity

The adsorption capacity of immobilized used green tea leaves was studied. This was with the view to evaluate the amount of immobilized used green tea leaves required to quantitatively concentrate the analytes from a given solution. The method used was adapted from that recommended by Maquieira et al. (31). It was then followed by the separation and preconcentration procedure described above. The adsorption capacity of Cr(III) was found to be 35 mg g^{-1}.

Analytical Performance

Under the optimal experimental conditions described above, the analytical data of the on-line microcolumn preconcentration and ICP-OES determination of chromium are summarized in Table IV. As can be seen, a sampling frequency of about 20 h^{-1} and an enrichment factor of 10 were obtained. According to the IUPAC definition, the detection limit (3σ) of this method for Cr(III) was 87 pg mL^{-1}, and the relative standard deviation (RSD) was 5% (n = 7, C = 2 ng mL^{-1}). A comparison of the proposed method with other literature is given in Table V.

Real Sample Analysis

The applicability of the proposed method was tested by speciation of Cr(III) and Cr(VI) in environmental water samples. In this work, a standard calibration curve was employed for real sample analysis.

The water samples were not acidified before storage because this also would change the chemical species. Table VI lists the analytical results of the sample and the recovery for the spiked samples. It can be seen that quantitative recoveries of >90% were obtained for the target analytes in East Lake and Yangtze River water samples, with the exception of Cr(VI) of the East Lake water where the recovery was about 89%.

TABLE IV
Analytical Performance Data for On-Line Speciation of Chromium Using Microcolumn Packed With Immobilized (UGTLs)

Correlation Coefficient (R^2)	0.9985
Sampling Frequency (*f*)	20 h^{-1}
Detection Limit (3σ)	87 pg mL^{-1}
Precision (2 ng mL^{-1}) (%RSD, n =7)	5
Enrichment Factor (EF)	10

Table V
Comparison of Analytical Performance Data for Cr(III) With Some Literature

Sorbent	Detection Limit	Adsorption Capacity	Detector	Literature
Immobilized UGTLs	87 pg mL^{-1}	35 mg g^{-1}	ICP-OES	This work
Nanometer TiO_2	320 pg mL^{-1}	7.6 mg g^{-1}	ICP-OES	(8)
Saccharomyces cerevisiae immobilized sepiolite	94,000 pg mL^{-1}	11.9 mg g^{-1}	FAAS	(25)
Immobilized moss	150 pg mL^{-1}	11.5 mg g^{-1}	ICP-MS	(5)

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

Sample	Added (ng mL^{-1})		Found[a] (ng mL^{-1})		Total (ng mL^{-1})	Recovery (%)	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)		Cr(III)	Cr(VI)
East Lake	0	0	0.3±0.002	0.8±0.07	1.1±0.11	–	–
Water	1	1	1.3±0.09	1.6±0.10	2.9±0.10	100	89
Yangtze River	0	0	0.5±0.01	1.0±0.03	1.5±0.05	–	–
Water	1	1	1.4±0.11	2.2±0.15	3.6±0.20	93	110

[a]Mean±S.D. (n =3)
Sample volume passed = 3 mL

CONCLUSION

A new method using a microcolumn packed with immobilized used green tea leaves for the speciation of Cr(III) and Cr(VI) has been developed. The method possesses some attractive features including good sensitivity and selectivity, is cost effective and simple, environmentally friendly and has a high adsorption capacity as compared to other existing literature (5,8,25). The proposed method has been applied to the speciation of Cr(III) and Cr(VI) in lake water and river water samples with satisfactory results.

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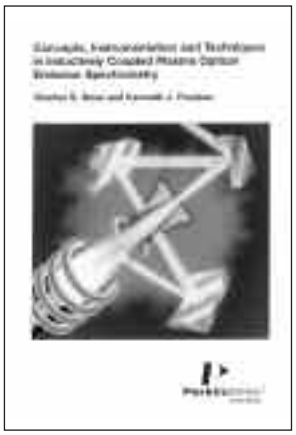
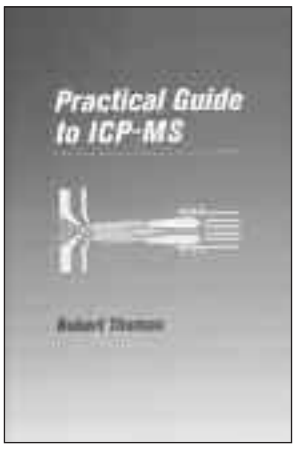
This book provides insight into the theoretical and practical aspect of graphite furnace AA making it the perfect reference resource for all laboratories wanting to use their graphite furnace more effectively.
Using an easy-to-follow style, the reader is guided from method development to calibration and validation of the instrument to the use of accessories and software in modern graphite furnace AA.



3. Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry

Authors: Charles B. Boss and Kenneth J. Fredeen
Order No. 005446B (free of charge)
Ordering information: http://www.las.perkinelmer.com or contact your local PerkinElmer representative.

This book presents the general characteristics of ICP-OES and ICP-OES instrumentation. It discusses ICP-OES methodologies including their application for the analysis of samples in the various industries such as agriculture and foods, biological and clinical, geological, environmental and water, metals, and organics.



4. Practical Guide to ICP-MS

Author: Robert Thomas, Scientific Solutions (www. scientificsolutions1.com)
Published in 2004 by Marcel Dekker
Ordering and price information: http://www.crcpress.com/shopping_cart/products/product_detail.asp?sku=DK2933&parent_id=1151&pc=

The brand new reference book presents this powerful trace-element technique as a practical solution to real-world problems. The basic principles of ion formation/transportation/detection, common interferences, peak quantitation, sample preparation, contamination issues, routine maintenance and application strengths of ICP-MS are described in a way that is easy to understand for both experienced users and novices of the technique. In addition ICP-MS is compared with AA and ICP-OES in the areas of detection capability, dynamic range, sample throughput, ease of use and cost of ownership. The book concludes with an excellent chapter on the most important testing criteria when evaluating commercial instrumentation.

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