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A Guide for the Quantitative Elemental Analysis of Glass Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT

The elemental analysis of glass samples for forensic use has been an interest in our group at Florida International University for over eight years as glass evidence can provide a way of associating a suspect to a crime. Laser ablation has been incorporated in our analytical scheme due to the many advantages that solid sampling holds over digestion of the glass followed by solution analysis using ICP-MS. The optimized conditions developed in our laboratory and by others are presented here for a specific combination of laser ablation system and ICP-MS instrument. The expected instrument responses for specific analytical conditions are presented. The method recommends using ^{29}Si as an internal standard, employing single spot ablation modes at a spot size of ~50 µm and collecting ~60 seconds of signal. Helium is used as a scavenging gas and a matrix-matched solid standard is recommended for quantification purposes. The procedure described should permit anyone with the described instrument combination or similar setup to achieve good accuracy and precision for the elemental analysis of glass using LA-ICP-MS.

INTRODUCTION

Glass evidence can provide important information regarding associations between individuals and crime events, or between individuals that are involved in actions that lead to the breaking of glass. The transfer and persistence of glass can be used to associate a person to a particular crime event.

Elemental analysis of glass has been shown to provide very good discrimination between glass fragments from different sources, and it complements the use of refractive index comparisons for discriminating fragments (1–5). It has also been shown that when glass fragments are compared by quantitative elemental analysis and the elemental profiles are found to be indistinguishable by statistical means (such as a *t*-test), that it can be stated with high confidence that the glass fragments originate from the same manufacturing source (6).

Digestion of small (~2 mg) glass fragments from forensic casework for elemental analysis by solution inductively coupled plasma mass spectrometry (ICP-MS) has been previously reported (7,8). Laser ablation (LA) ICP-MS has since been shown to be a viable, alternate method to solution ICP-MS for quantitative analysis of glass in forensic cases. Advantages of laser ablation include a dramatically lower sample preparation time and sample consumption during an analysis, thus enabling additional analyses of the same sample.

Quantitative analysis by ICP-MS allows for more robust comparisons to be made between glass samples than refractive index, the traditional method used for comparing glasses. With knowledge of the sample analysis precision and variances within a population of the type of glass investigated, more reliable conclusions may be made regarding the likelihood that two or more samples originate from the same source. Several organizations, including the European Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensics (NITECRIME) network, have worked towards developing a standardized method for the quantitative analysis of glass by LA-ICP-MS (9).

This study reports basic parameters used for the forensic analysis of glass by LA-ICP-MS through the determination of an element menu known to provide good discrimination between glass samples commonly found in forensic casework. The optimization and analysis parameters of a laser ablation system coupled to an ICP-MS are described, and the results are presented.

EXPERIMENTAL

Sample Preparation

Minimal sample preparation of glass fragments for LA analysis is required. Glass fragments were washed by first soaking in methanol (reagent grade, Fisher Scientific, USA) for 10 minutes, followed by a 30-minute soak in 10% nitric acid (v/v) (Trace Metal grade, Fisher Scientific, USA). The fragments were then rinsed with deionized water (18 MΩ-cm), dried, and placed in

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the ablation cell for analysis. The volume of wash solutions depends on the size of the fragments, but enough volume is needed to completely cover the samples.

Laser Conditions

The laser used for this work was a New Wave UP-213 laser ablation system (New Wave Research, Fremont, California, USA). The analytical parameters for both single-spot and scan analyses are listed in Table I; a schematic of the laser system is shown in Figure 1.

ICP-MS Conditions

An ELAN® DRC™II (PerkinElmer Sciex, Concord, Ontario, Canada) was used for all analyses. An RF power of 1500 W was used, and the following elements were measured with a dwell time of 10 ms: ^{7}Li, ^{25}Mg, ^{27}Al, ^{29}Si, ^{39}K, ^{42}Ca, ^{49}Ti, ^{55}Mn, ^{57}Fe, ^{85}Rb, ^{88}Sr, ^{90}Zr, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{139}La, ^{140}Ce, ^{146}Nd, ^{178}Hf, ^{208}Pb.

TABLE I
New Wave UP-213
Laser Parameters

Spot Analysis	
Spot size	55 µm
Power	100%
Fluence	27.25 J/cm^2
Repetition rate	10 Hz
He flow into the cell	0.86 L/min
Ar make-up gas after the cell	0.90 L/min
Time of ablation	60 s
Scan Analysis	
Spot size	55 µm
Power	100%
Fluence	27.25 J/cm^2
Repetition rate	10 Hz
He flow-through cell	0.86 L/min
Ar make-up gas after the cell	0.90 L/min
Scan Rate	15 µm/s
Time of Ablation	60 s

For all analyses, a transient signal of intensity versus time was obtained for each element using a 20-second background (blank) collection, followed by a 60-second ablation of the sample, and finished with a 20-second post-ablation collection of signal at background levels. Typical transient signals for ^{88}Sr by both scanning and single-spot ablations are shown in Figure 2.

Optimization of the System

The analysis of solids by LA-ICP-MS may require a preliminary optimization of instrumental parameters with aqueous standards to provide a general check of instrument performance. The instrument response must first pass quality control requirements before any measurement can be conducted with the LA system. A typical instrument response for aqueous standards is given in Table II (note: the expected counts per second will vary between instruments and should be determined by each laboratory). Once the optimization with aqueous standards has been performed, the LA system is coupled to the ICP-MS and a second optimization with a glass standard is recommended. Table III lists the typical response for the laser ablation of NIST 612 glass. Since this glass contains Ga (m/z 69), it is not suitable for the determination of the % Ba^{++}/Ba parameter, unless a correction for ^{69}Ga is made by measuring ^{71}Ga and considering the isotopic relative abundances. Instead, it is recommended to use a standard which does not contain Ga (such as FGS02, BKA, Germany) for determining the Ba^{++}/Ba ratio.

Figure 3 summarizes a basic scheme for conducting an analysis of glass samples by LA-ICP-MS. It is strongly recommended that at least 3–4 replicates (i.e., different spots) per sample are analyzed in order to be able to apply statistical analyses. The NITECRIME group has

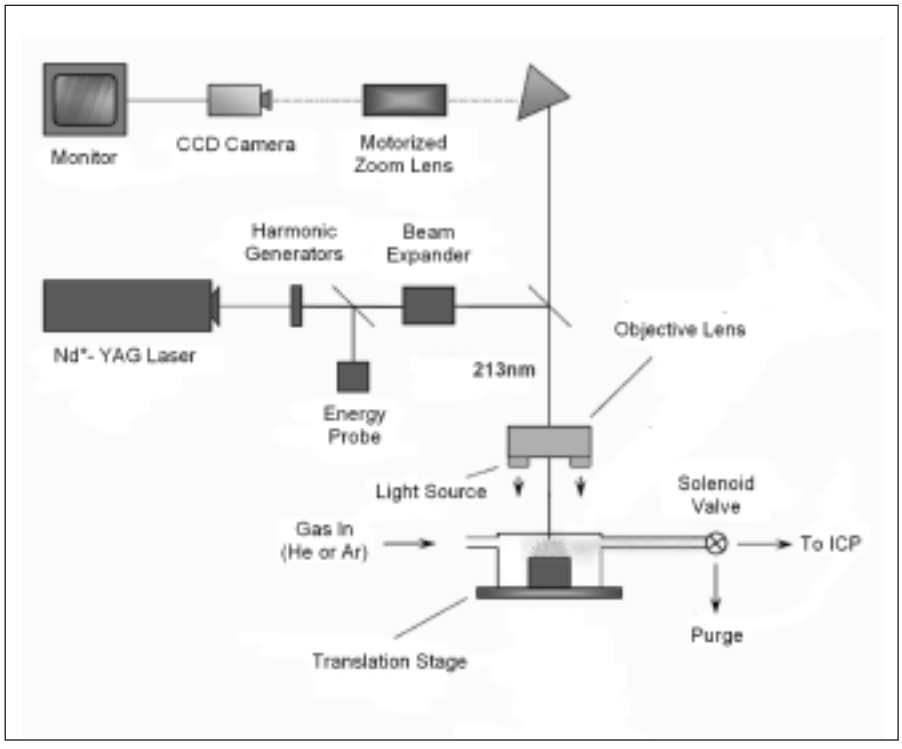

Fig. 1. Schematic of New Wave UP-213 Laser Ablation system (courtesy of NewWave Research).

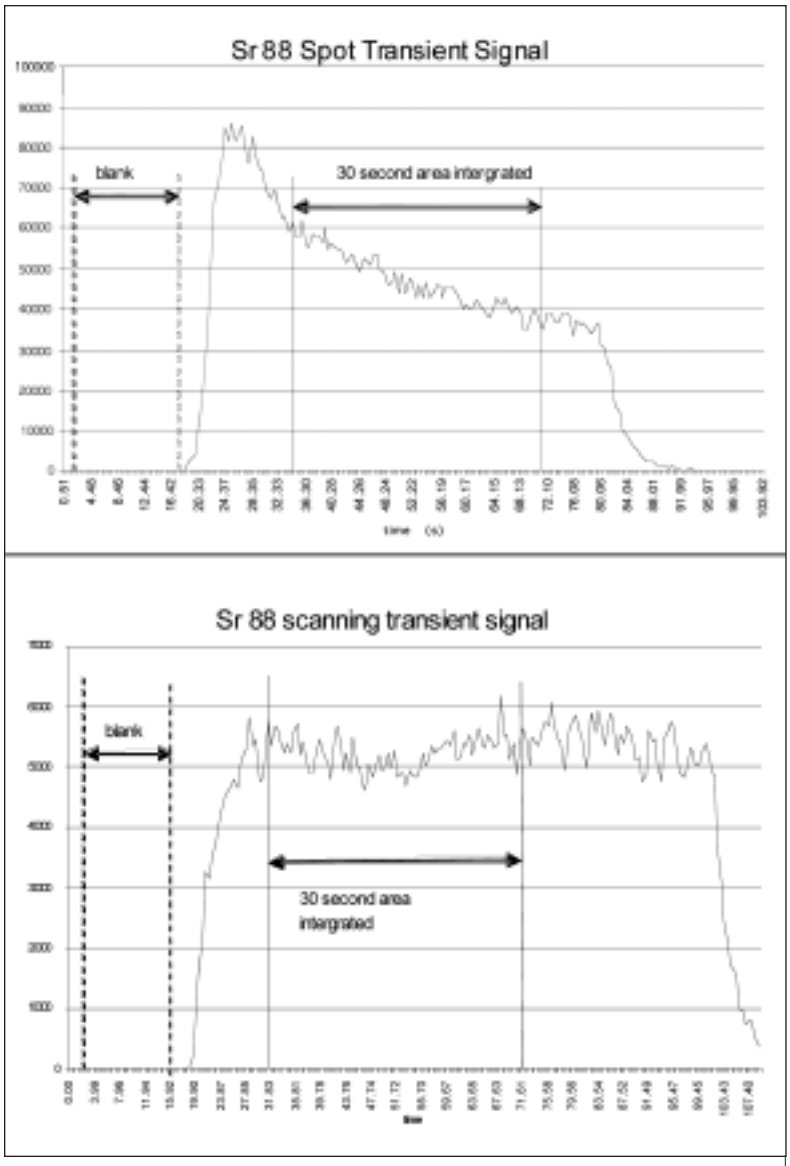

Fig. 2. Transient signals for Sr88 of a typical spot ablation (top) and a scan ablation (bottom), both containing a 20-second pre-ablation blank followed by a 60-second ablation.

suggested a typical sequence in their protocol that involves the use of calibrant at the beginning and end of the sequence in order to correct for drift over time (9). It is also recommended that the precision and accuracy of a matrix-matched control standard are monitored during the sequence, and that the values are within the quality control specifications previously set by the laboratory.

TABLE II
Expected ICP Response in Counts per Second (cps) for the Tuning With Liquid Standards

Element/Parameter	Conc. in Solution	(cps)
Mg	1 ppb	> 6,000
In	1 ppb	> 30,000
U	1 ppb	> 20,000
Background 220	N.A.	< 30
% Ba^{++}/Ba	Ba at 1 ppb	< 3%
% CeO/Ce	Ce at 1 ppb	< 3%

TABLE III
Expected ICP Response for the Tuning With SRM 612

Element / Parameter	Conc. in Glass	(cps)
^{59}Co	~40 ppm	> 10,000
^{139}La	~40 ppm	> 10,000
^{140}Ce	~40 ppm	> 16,000
^{7}Li	~40 ppm	> 2,000
^{57}Fe	~40 ppm	> 800
^{49}Ti	~40 ppm	> 1,500
Background 220	N.A.	< 2
% Ba^{++}/Ba	Ba and Ga ~40 ppm	< 3%
% CeO/Ce	Ce ~40 ppm	< 3%

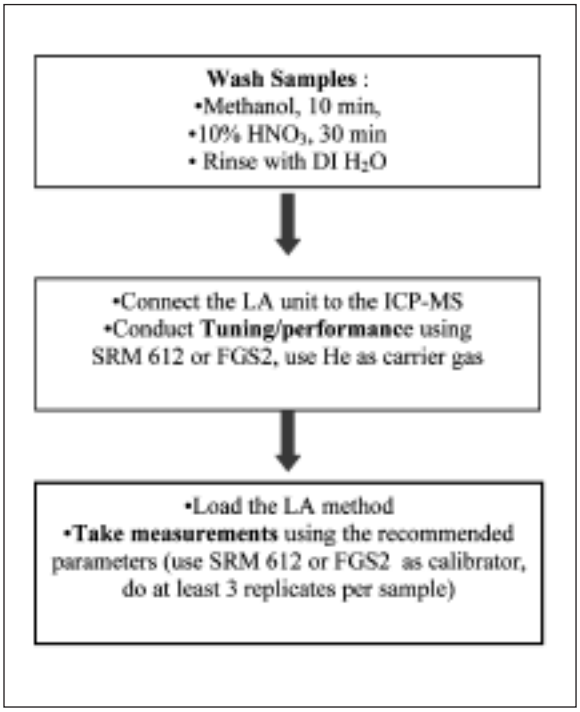

Fig. 3. Typical scheme for conducting a glass analysis by LA-ICP-MS.

Figure 4 depicts the suggested sequence of analysis recommended by the NITECRIME studies. This scheme accounts for potential instrument drift since the calibrant (NIST SRM 612) is analyzed both at the beginning and end of the sequence.

RESULTS AND DISCUSSION

Various sample analysis parameters were investigated to optimize the precision of the analysis.

Internal Standard

External calibration alone has the disadvantage that large differences in ablation yields can result from fluctuations in laser output and small differences in the target matrix. To minimize the latter, the matrix of the standards and samples should be as closely matched as possible. A combination of the use of an external reference standard with internal standardization corrects for differences in ablation yield between the sample and the calibration standard.

Internal standards can also correct for matrix effects and instrumental drift. A major component of the material, of known concentration, should be used as the internal standard. The element chosen as the internal standard should be a matrix component whose concentration has been previously determined by other techniques [i.e., electron-microprobe (EPMA), X-Ray Fluorescence (XRF) solution ICP-MS]. A general rule for selecting an internal standard is to use the least abundant isotope of the most abundant element in the material to be analyzed. Since glass has a high concentration of silicon (~70% as SiO_2), the least abundant silicon isotope (^{30}Si, 3.09%) would be selected in order to avoid saturation of the detector. However, because NO^+ produces a high background signal at m/z 30, ^{29}Si (4.7%) is a better choice to use as the internal standard for the forensic analysis of glass.

In practice, other major elements (i.e., Ca) can serve as an internal standard depending on the matrix, how closely the matrix of the sample and the standard match, and knowledge of the concentration of the element. When initially analyzing an unknown material, it is good practice to use two or more internal standards to check consistency, precision and accuracy.

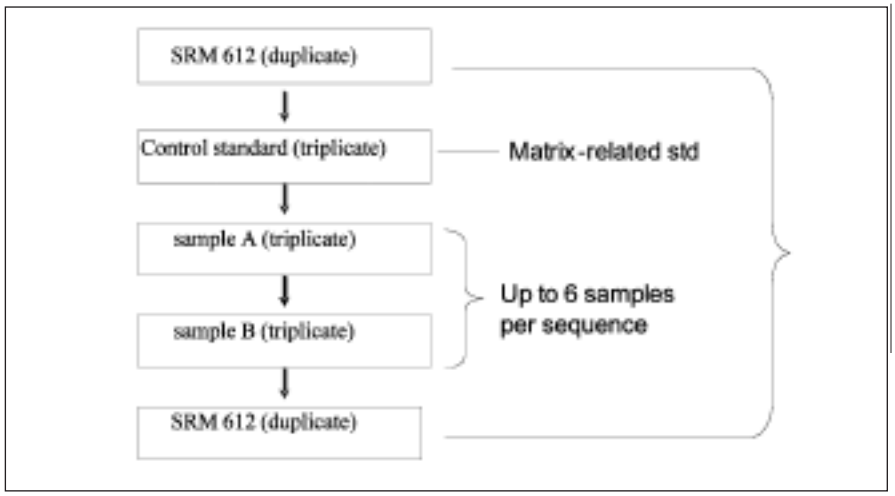

Fig. 4. Typical analytical sequence for glass analysis by LA-ICP-MS.

TABLE IV
Certified and Consensus Values (in µg/g) for Elements in the Following Standard Reference Materials: SRM 612, SRM 1831, SRM 612, FGS01, and FTGS02

Element	SRM 612 (13)	SRM 1831 (13)	SRM 621 (13)	FGS01 (9)	FGS02 (9)
Li	41.58	4.99	N.A.	6	29.2
Mg	77.63	21166	1628	23900	23400
Al	11168	6381	14555	1500	7400
K	66.38	2738	16685	920	4600
Ca	85303	58604	76542	60600	59300
Ti	48.14	113.8	83.90	69	326
Mn	38.44	12.54	22.09	43	221
Fe	56.32	802	280	580	2600
Rb	31.64	6.11	38.47	8.6	35
Sr	76.18	89.11	106	57	253
Zr	36.00	43.35	51.82	49	223
Sn	37.97	3.08	N.A.	19	94
Ba	37.79	31.51	1074	40	199
La	35.78	2.12	N.A.	4.3	18
Ce	38.36	4.53	2.18	5.2	23
Nd	35.26	1.69	N.A.	5.1	25
Hf	34.81	1.09	1.7	3.2	15
Pb	38.97	1.98	13.42	5.8	24

Scanning Versus Spot Analysis

It is known from previous work (10,11) that during a single-spot ablation with a 266-nm laser, the particle size distribution favors small and uniform particles after the initial coupling of the laser to the sample. As a result, a more reproducible signal is produced after the first 10 seconds of ablation. In comparison, scanning across a surface produces a constant supply of larger particles, which enter the plasma and lead to more pronounced matrix effects, lower precision, and a possible increase in elemental fractionation. This premise was confirmed by a study on the precision and accuracy of the analysis of geological rock and the NIST 610–616 series of glasses (12). The study stated that at 100-µm spot size, single-spot analysis produced better results and exhibited less matrix dependence. Table IV summarizes the certified and agreed upon values for the different standards employed during this study.

In the work presented here, NIST 612, 621, and 1831 were chosen to investigate the effect of scanning versus single-spot analysis on the measurement precision. The results of these analyses are summarized in Figure 5 and show a clear benefit (in terms of precision) when a single-spot analysis is used instead of a scanning pattern. The reason for this is most likely due to the particle size distributions produced by both modes: with scanning, a constant supply of larger particles enters the plasma, thus increasing the signal but decreasing the precision due to non-uniform ionization, while single-spot

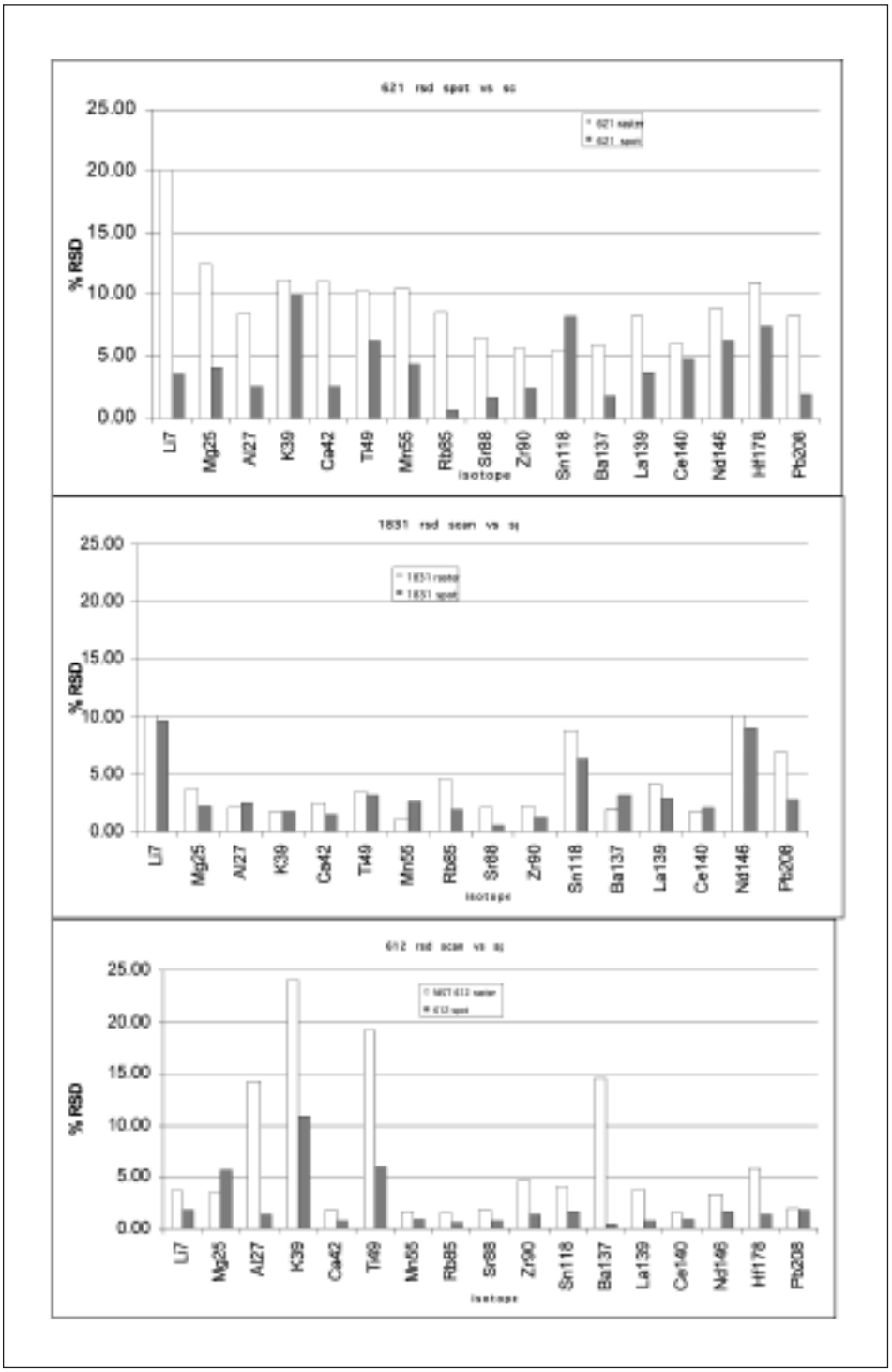

Fig. 5. Relative standard deviations for the analysis of NIST 621 (top), 1831 (middle), and 612 (bottom) analyzed by the scan method (white bar) and the single-spot method (gray bar).

analysis maintains a steady supply of smaller particles. The difference in precision is evident in Figure 2, where the integrated area of the single-spot analysis has less peak-to-peak noise than the scan analysis.

Matrix-Matched Standards

Calibrations using a single standard can be advantageous because of simplicity and time savings. However, to verify that a standard used for a single-point calibration is valid, this standard should be run as part of a multi-standard curve to establish its linear response. For example, the following NIST series of glasses give a linear calibration curve passing through the origin: 614 (~5ppm), 612 (~50ppm), and 610 (~500ppm). (The concentrations listed represent the approximate elemental levels in the glasses). Therefore, any of these standards can be used to establish a single-point calibration curve without sacrificing accuracy or precision.

For glass analysis, there are many solid standards available, such as the NIST standards (National Institute of Standards and Technology, Gaithersburg, MD, USA), the P & H Glass Standard Series (Pulles and Hanique Ltd, UK), and recently, the NITECRIME group has distributed a new series of glass standards to its members (FGS series from the BKA, Germany).

NIST SRM 612 has a composition different than that of typical float glass. Not only is its magnesium concentration lower (mg/kg versus 4% w/w), but its trace element profile contains most elements in the ~40 mg/kg range. The float glass standards FGS01 and

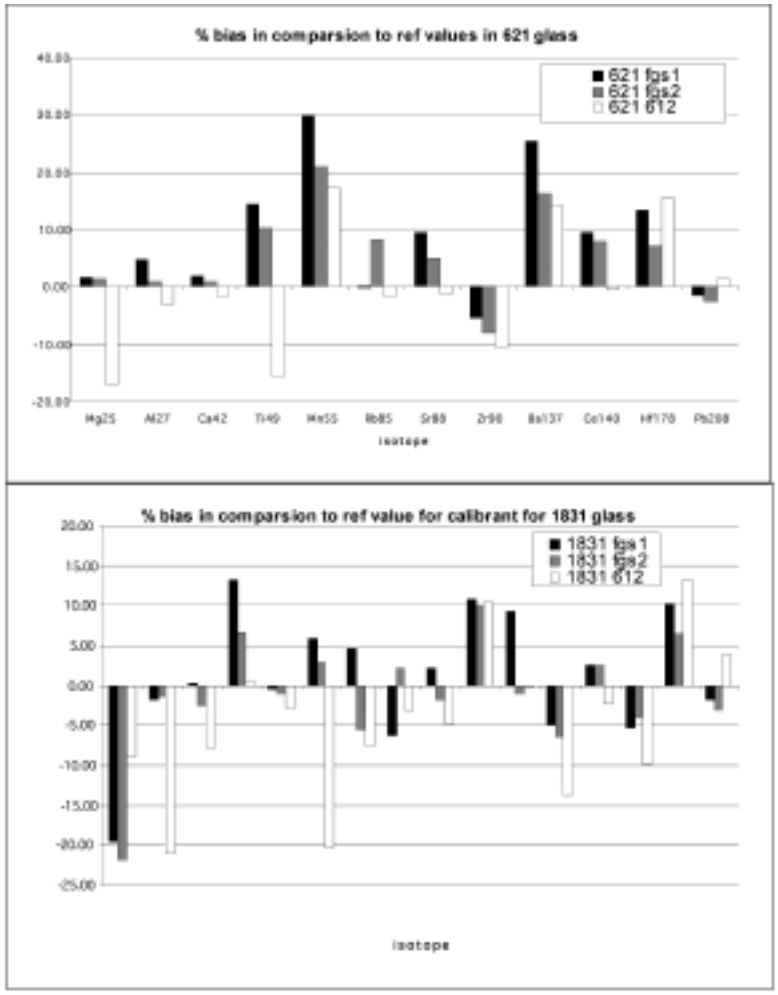

Figure 6. Percentage bias in comparison to certified or agreed value for various calibrants.

FGS02 are more representative of typical soda-lime glasses and may be better calibration standards for the analysis of float glass. However, as these standards are not certified and can only be acquired from the BKA, caution must be used in deciding to use the FGS series as sole calibrant glasses.

The effect of choosing different glass calibration standards can be seen in Figure 6, which displays the error from the certified or accepted values for the analysis of NIST 621 and NIST 1831 using NIST 612, FGS01, and FGS02 as calibration standards. The different results clearly demonstrate the importance of matrix-matching standards and samples. For each analysis, five replicates (i.e., different spots) were analyzed, and the average value was compared to the accepted values. For elements whose values were not certified, the agreed value was that taken from a round-robin analysis of the NITECRIME group. The data show that using FGS02 as a calibrant for float glasses (such as SRM 1831) produced more accurate results for some elements. However, this improvement

is less significant for the analysis of other types of glasses, such as container glass (NIST 621) where either SRM 612 or FGS02 can be used as a calibrant.

Data Analysis

Once the data has been collected, it must be corrected for drift and converted from counts per second (cps) into concentration units relative to the external standard. There are numerous packages on the market designed for this, but GLITTER (GEMOC, Macquarie University, Australia) was used in this study.

In order to validate analyses, the NITECRIME group recommends two key strategies be followed during the analysis of glass fragments:

• Matrix-matched standards should be analysed at the beginning and end of each sequence to account for drift

• At least four different spots on each sample should be analysed so that statistical tools can be applied when comparing glass fragments.

CONCLUSION

Laser ablation ICP-MS is a technique that shows potential for forensic analysis of glass samples. Benefits of this laser versus solution analysis include: high level of discrimination between glasses; minimum sample preparation and consumption; elimination of the need for complex sample handling and preparation procedures; ability to detect major, minor and trace elements with high precision and accuracy; and reducing the risk of contamination and polyatomic interferences associated with aqueous solutions.

Laser ablation has been evaluated as an analytical sampling method with more than 250 publications on the topic in the last decade encompassing fundamental studies, applications, and improved laser systems. Scientific groups such as NITECRIME have dedicated part of their efforts to validate the methodology for the quantitative analysis of glass samples.

Several authors have conducted research for the forensic analysis of glass by LA-ICP-MS, and there is an agreement in the scientific community regarding the optimum conditions to conduct this kind of analysis. For forensic applications, the following recommendations have been agreed upon:

• Using ^{29}Si as an internal standard;

• Employing single-spot (or depth profile) ablation modes at a spot size of ~50 μm;

• Disregarding at least the first 15 seconds of ablation to eliminate particle size effects;

• Using He as a carrier gas (11,14);

• Using a matrix-matched solid standard for quantification purposes (such as NIST 612 or FGS2);

• Analyzing enough different spots (minimum of three) on the sample to conduct statistical analysis.

• It is also a good practice to optimize the instrument every day and to analyze control standards to check for accuracy and precision.

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Determination of Iron in Glass by Solution and Laser Ablation DRC-ICP-MS

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ABSTRACT

The analysis of glass fragments can be useful in solving cases such as hit and run, burglaries, kidnapping and bombings. Although refractive index has been routinely used for the analysis and comparison of glass fragments for some time, the forensic community has recognized the limitations of using this technique –by itself- for the association of glass fragments. As a consequence, a range of spectroscopic techniques have been used for elemental analysis of this kind of evidence, including ICP-MS, which combines its multi-element detection capability with high sensitivity, small sample size, wide dynamic range and high sample throughput. Due to its excellent precision and accuracy, several studies have found that ICP-MS methods (solution and laser ablation) provide the best discrimination between glass fragments from different sources. Laser ablation (LA) is a state-of-the-art sample introduction technique that has simplified the analysis of solid materials, including glass samples. Laser ablation ICP-MS benefits from several key features that provide a tremendous potential for its application to forensic analysis such as, requiring minimum sample preparation and sample consumption, eliminating the need of complex procedures and handling of hazardous materials for the digestion of samples. Nevertheless, standard unit resolution ICP-MS methods suffer from polyatomic interferences including ArO^+, ArN^+, and $ArOH^+$ that compromise the measurement of trace levels of Fe, for example. This represents a drawback for the analysis of glass fragments because iron has been previously identified as a good discriminating element. The aim of this study was to perform an evaluation of the use of DRC- (dynamic reaction cell) ICP-MS for the analysis of glass samples. The use of the DRC lowers the detection limit by two orders of magnitude when compared to standard ICP-MS. The ability to measure Fe in glass using Laser Ablation DRC-ICP-MS was evaluated for detection limits and ease-of-use. The results are comparable to solution analysis, with the advantage of significantly shorter sample preparation times.

INTRODUCTION

During crime scene investigations it is often necessary to match unknown evidence found at the crime scene to known samples in order to help solve the crime. This evidence may exist in a variety of forms, including glass, hair, paint, and ink and it is usually present in only small quantities.

In the case of glass, samples are usually present in very small quantities, often less than 1 mg. Forensic glass analysis has traditionally been performed by refractive index measurements. Although a simple and rugged technique, refractive index measurements are limited in their ability to distinguish between many different glass types.

To overcome this limitation, the use of inductively coupled plasma mass spectrometry (ICP-MS) has been used recently for forensic glass analysis (1,2). ICP-MS is a very sensitive, multielement technique for inorganic measurements. By looking at minor components present in various glasses, many types of glasses can be distinguished from one another. As a result, glass samples with matching elemental profiles have a high probability of originating from the same source (3).

The more elements that can be used to distinguish glass samples, the higher the probability that a match confirms that pieces of glass originate from the same source. An element that may serve as a good marker is iron. However, Fe analysis by ICP-MS is difficult due to the presence of interferences on its major isotope: ArO^+ and CaO^+ on $^{56}Fe^+$. These interferences can be eliminated by using Dynamic Reaction Cell™ (DRC™) ICP-MS, which would allow $^{56}Fe^+$ to be used for glass analysis.

Two limitations of glass analysis by ICP-MS are the solid nature of the sample and the sample size. Because glass is a solid, it must first be digested prior to ICP-MS analysis, a procedure that can take several days. Also, the small sample sizes make digestions difficult. These limitations can be overcome through the use of laser ablation, where the ablated aerosol is transferred directly into the plasma of an

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ICP-MS. An additional advantage of laser ablation is that sample preparation takes only minutes and consists of washing the samples with acid and mounting them in the laser ablation chamber (4).

This work demonstrates the ability to measure iron in glass samples by DRC-ICP-MS, using both laser ablation and solution analysis.

EXPERIMENTAL

Sample Preparation

For laser analysis, glass shards were first washed with methanol and 5% nitric acid, then rinsed with deionized water, dried and placed in the laser ablation cell for analysis. Silicon (^{29}Si) was used as an internal standard and NIST 612 (Trace Elements in Glass, certified for 8 elements, 18 additional elements are for information only) was used as a calibration material.

For solution analysis, glass shards were digested according to the procedure described by Trejos et. al. (1) and published as ASTM standard E2330–04. The final solutions were in 0.8M HNO_3 and were spiked with scandium (^{45}Sc) as an internal standard.

Laser Conditions

The laser used for this work was a New Wave UP- 213 (New Wave Research, Fremont, CA, USA). Laser parameters are shown in Table I.

ICP-MS Conditions

An ELAN® DRC II (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) was used for all analyses. Methane was used as the reaction gas to eliminate the interferences on $^{56}Fe^+$. Table II shows the ELAN conditions used. All sample preparations and analyses were performed in a normal laboratory environment and not a clean room. As a result, iron background is present.

For the laser analysis, the signal was measured at ^{56}Fe+ for 90 seconds, although only the middle 35 seconds were used for concentration measurements. Figure 1 shows a signal trace for $^{56}Fe^+$ during a typical analysis. First, data was acquired for 15 seconds before the laser was fired to establish a background level. The laser was fired for 55 seconds and the signal recorded, although the first 20 seconds was not used for concentration calculations due to the inherent instability which results when a laser first interacts with a sample. The final 35 seconds of the laser ablation was used for analysis. After the laser was turned off, the signal was recorded for an additional 20 seconds to confirm that it returned to its blank value. The colors in the figure reflect the concentrations measured. The lighter colors signify higher concentrations, and the darker colors reflect lower concentrations.

TABLE I
Laser Parameters

Wavelength	213 nm
Spot Size	55 µm
Power	0.688 mJ
Fluence	27.25 J/cm^2
Repetition Rate	10 Hz
He Flow-through Ablation Cell	0.99 L/min

TABLE II
ICP-MS Parameters

Nebulizer Flow	1.0 L/min (Solution) 0.98 L/min (Laser)
RF Power	1500 W
Dwell Time	8.3 ms
Reaction Gas	CH_4
Reaction Gas Flow	0.5 mL/min
RPq	0.5 (Laser) 0.6 (Solution)

For solution analysis, the sample uptake rate was 1 mL/min into a quartz sample introduction system consisting of a concentric nebulizer and cyclonic spray chamber.

Data Reduction

For laser analysis, the data acquired with the ELAN was processed with GLITTER software (GEMOC, Macquarie University, Australia). Figure 1 shows a typical $^{56}Fe^+$ trace from GLITTER. For solution analysis, the data was taken directly from the ELAN software.

RESULTS AND DISCUSSION

To determine the effectiveness of the method, NIST 612 glass was analyzed. The results appear in Table III and show that that Fe can be accurately measured, thus demonstrating the effectiveness of both laser ablation and the elimination of the interferences on Fe.

Detection limits were determined for both the solution and the laser ablation techniques.

For solution analysis, the detection limit was determined by the following equation (5):

$$LOD = mean_{blank} + 3^*\sigma_{blank}$$

σ = standard deviation

For laser analysis, the following equation was used:

$$LOD = 2.3^*\sqrt{2}^*mean_{blank}$$

TABLE III
Iron Results for NIST 612 Glass in DRC Mode

Analysis Mode	Measured Conc. (mg/L)	Certified (mg/L)
Laser	51.0±1.7 (n=14)	51±2

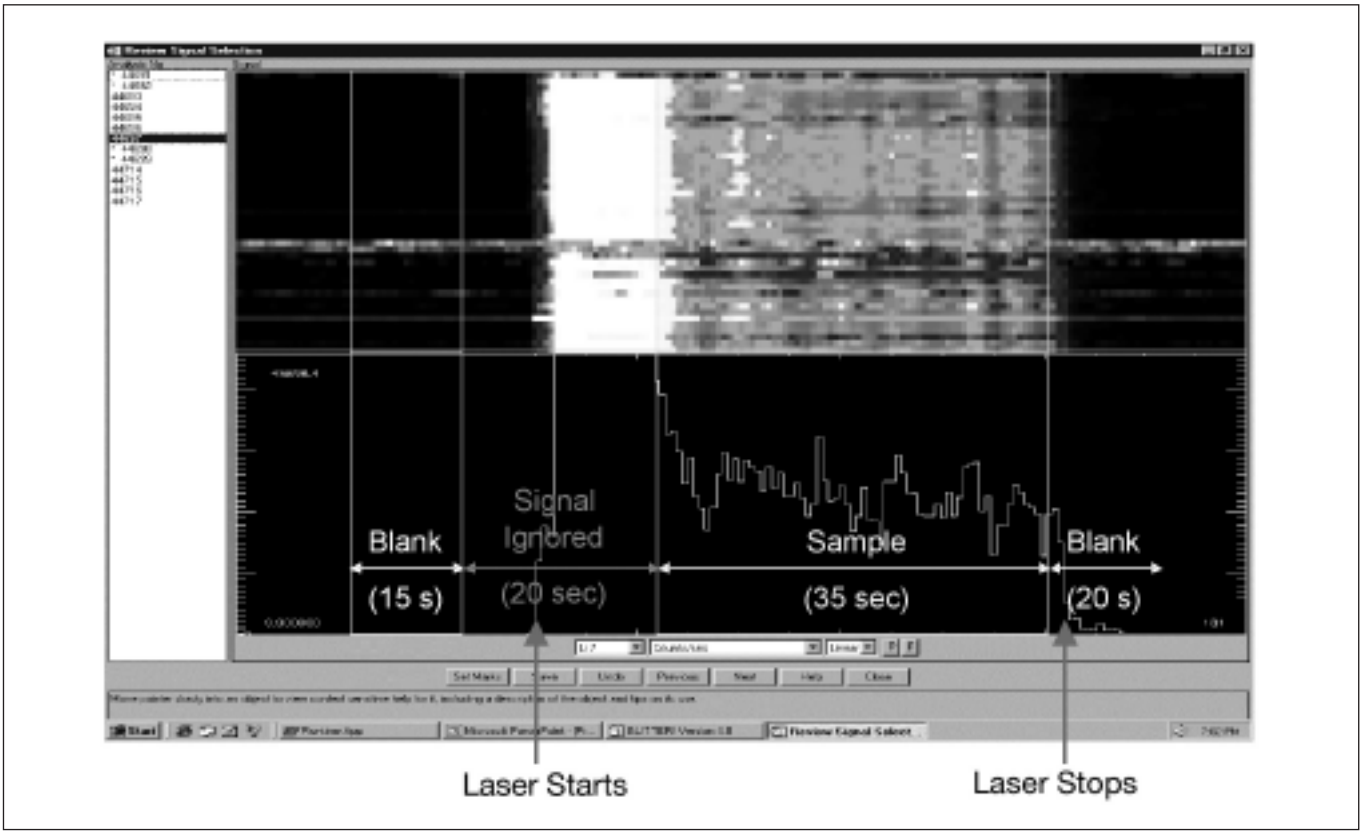

Fig. 1. A typical laser ablation signal for $^{56}Fe^{+}$, as viewed with GLITTER.

Seven blank measurements were used. Each experiment was repeated on two different days in order to check for inter-day variability. For solution analysis, the blank consisted of nitric acid (0.8 M) spiked with scandium (final concentration 50 ppb), while an acquisition without ablation was used as the blank for the laser analysis.

The detection limits appear in Table IV and demonstrate the effectiveness of laser ablation in DRC mode. The iron detection limit in DRC mode is signifcantly lower than in standard mode (0.03 vs. 9.5 µg/g) and an order of magnitude less than solution analysis in DRC mode (0.03 vs. 0.20 µg/g). This lower detection limit allows for more certainty in iron measurements at the usual concentrations found in this type of glass, which are usually very low (1 µg/g), but range from below detection limit up to 1600 µg/g (3).

CONCLUSION

This work has extended the use of ICP-MS in characterization of glass elemental composition to lower detection limits for an important element, iron, which was previously limited by interferences on its major isotope. The use of the DRC lowers the detection limit by two orders of magnitude when compared to standard ICP-MS.

TABLE IV
Iron Detection Limits in NIST 612 Glass

Laser	0.03 µg/g (With reaction gas)
Laser	9.5 µg/g (No reaction gas)
Solution	0.20 µg/g (With reaction gas)

The ability to measure Fe in glass using Laser Ablation DRC-ICP-MS was evaluated for detection limits and ease-of-use. The results are comparable to solution analysis, with the advantage of significantly shorter sample preparation times since the glass can be analyzed directly. This eliminates the need for digestion procedures, reduces the possibility of contamination, and allows lower limits of detection to be achieved.

This work has demonstrated the ability to measure Fe in forensic glass samples by laser ablation ICP-MS. By using a Dynamic Reaction Cell, interferences on Fe are eliminated, allowing for lower levels to be determined. The ability to add

Fe to a multi-elemental suite allows for better characterization of forensic glass samples.

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Determination of Total Strontium in Uruguayan Rice by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

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ABSTRACT

Strontium (Sr) is found naturally as a non-radioactive element and has 16 known isotopes. Naturally occurring Sr is found as four stable isotopes: Sr-84, -86, -87, and -88. Twelve other isotopes are radioactive. Sr-90 is the most important radioactive isotope in the environment, discovered mostly after the nuclear experiments conducted in the 1950s and 1960s.

In the present work, 86 rice samples (*Oryza sativa L.*) and 7 rice husk samples were digested by dry ashing for the purpose of determining the total Sr levels by ICP-OES. The mean concentrations found were: 0.281 μg g^{-1} for milled, 0.287 μg g^{-1} for parboiled milled, 0.564 μg g^{-1} for brown, 0.73 μg g^{-1} for parboiled brown, and 1.16 μg g^{-1} for paddy rice, and 3.44 μg g^{-1} for the rice husks. Validation of the method was conducted with a certified reference material, NIST CRM 8418 Wheat Gluten, and the recovery obtained ranged from 89–98%.

As the outer layers (aleurone, pericarp) of the grain are removed, the Sr concentration decreases. It can then be assumed that most of the Sr is stored in these layers. Although no extensive data exist for Sr levels in rice, the values obtained are in good agreement with the results reported for Sr in brown rice from Japan (0.25–0.72 μg g^{-1}) and with non-contaminated foodstuffs from other parts of the world. Thus, the Uruguayan rice has Sr levels that match non-contaminated samples and its consumption presents no health threat.

INTRODUCTION

In 1790, Adair Crawford and William Cruikshank first detected non-radioactive strontium in the mineral strontianite in Scotland; and metallic strontium was first isolated in 1808 by Sir Humphry Davy (1).

The alkali earth metal strontium has four naturally occurring isotopes: Sr-84, -86, -87, and -88. Only Sr-87 is radiogenic and is produced by decay from the radioactive alkali metal Rb-87 (2). Twelve other isotopes are radioactive and Sr-90 (half-life 28.78 years) is of greatest importance. Like many other radionuclides, it was discovered in the 1940s during nuclear experiments in connection with the development of the atomic bomb. It is a by-product of the fission reaction of uranium (^{235}U) and plutonium (^{239}Pu) in nuclear reactors and weapons. Therefore, ^{90}Sr is considered one of the most hazardous constituents of nuclear wastes. Large amounts of ^{90}Sr were produced during the nuclear weapons tests in the 1950s and 1960s, and are therefore dispersed worldwide.

In 1986, the world's worst nuclear power accident occurred at Chernobyl in the former USSR (now Ukraine), introducing a large amount of ^{90}Sr into the environment (3). The largest amount of ^{90}Sr was deposited in the Soviet Republics. The rest was dispersed as fallout over Northern Europe and all other parts of the world. Everyone is exposed to small amounts of ^{90}Sr, since it is widely dispersed in the environment and therefore in the food chain (3). Strontium-90 presents a health problem since it substitutes for calcium in bone, and prevents its removal from the body. (2)

Owing to its chemical and biochemical similarities to calcium, more than 99% of Sr is efficiently incorporated into bone tissue and teeth. Although humans can absorb Sr by inhalation (as a contaminant in dust) or dermal contact, Sr intake through diet (foodstuffs and water) is the most common pathway into humans (4).

Therefore, knowledge about total Sr in foods is of extreme importance to prevent and control contamination; mainly for those foods that are staples for millions of people.

Currently, rice is the predominant staple food for more than half of the world's population. It is used in at least 15 countries in Asia and the Pacific, 10 countries in Latin America and the Caribbean, one country in North Africa, and seven

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countries in sub-Saharan Africa. Moreover, it provides employment for millions of rice producers, processors, and traders all over the world. Recognizing the importance of rice-based systems in the fight against hunger and poverty, the United Nations General Assembly declared 2004 the International Year of Rice under the theme "Rice is Life" (5). Due to its importance, rice plays a fundamental role for world food security as well as for socio-economic development.

Uruguay is Latin America's major rice exporter and now among the world's top ten exporters. Furthermore, Uruguay is the only rice producer that designates 95% of its production to international markets. Therefore, the quality of rice produced in Uruguay is very important and well known all over the planet.

Prior to analysis, rice samples need to be converted into a liquid form. The two most widely used techniques are based on dry ashing or wet digestion. For the purposes of this work, dry ashing was selected as the solubilization technique. It is an inexpensive procedure and provides good detection limits, although it is time-consuming and strict temperature control must be observed to avoid loss of volatile elements. For the determination and quantification of Sr, inductively coupled plasma optical emission spectrometry (ICP-OES) was chosen because of the technique's versatility, precision, and accuracy. In addition, a large number of samples can be processed in a short period of time.

The aim of this work was the evaluation of total strontium concentration in samples of paddy, brown, parboiled brown, milled, and parboiled milled rice, as well as in rice husk all cultivated in Uruguay. This study is the first in performing and reporting a survey of Sr levels in Uruguayan rice. The results are of great significance for Uruguay as it is one of the biggest global exporters of rice.

EXPERIMENTAL

Instrumentation

All analyses were performed using a PerkinElmer® Optima™ 2100 DV ICP-OES (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA), equipped with a dual-view torch, Scott-type spray chamber, and GemTip™ cross-flow nebulizer. A PerkinElmer AS-90 autosampler was used for sampling. The optimum instrumental conditions determined for this study are given in the Table I.

Water purification was carried out using a Milli-Q™ Plus purifier system (Millipore Corporation, Bedford, MA, USA).

Reagents and Solutions

All chemicals used in the sample preparation and analysis were of analytical grade or better.

Working standard solutions of Sr were obtained by appropriate dilution of a Sr stock solution (1000 µg mL^{-1}) (Merck KGaA, Darmstadt, Germany), certified by the manufacturer to ± 1% (w/v) and traceable to the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

TABLE I
Instrumental Operating Conditions

Parameter	Optima 2100 DV
RF Power	1300 W
Nebulizer Flow	0.80 L/min
Auxiliary Flow	0.2 L/min
Plasma Flow	15.0 L/min
Sample Flow	1.50 ml/min
Plasma Height	15 mm
Plasma Viewing	Axial
Processing Mode	Area
Read Delay	30 sec
Rinse Delay	60 sec
Replicates	5
Wavelength	407.771 nm

All solutions were prepared with ultrapure water with a specific resistivity of 18MΩ.cm obtained by filtering distilled water through a Milli-Q™ Plus purifier system (Millipore Corporation, Bedford, MA, USA) immediately before use.

Procedure

To obtain extensive data for this work, 93 samples were collected from the main rice production areas throughout Uruguay (see Figure 1).

Prior to analysis, approximately 500 g of sample was ground in an analytical mill (Analytical Mill A10, Kinematica GAC, Luzern, Switzerland) and passed through a 1-mm sieve.

The sample (~5 g) was weighed into a beaker resistant to high temperatures and, while continuously shaking the suspension, 5 mL ashing aid solution [10% (w/v) $Mg(NO_3)_2$ in ethanol] was added. Next, the following temperature program was used: Heating at 125°C until nearly dry, then heating from 125°C to 450°C for 2 hours, and holding at 450°C for 12–14 hours. If white ashes are not obtained after this temperature cycle, 1 mL HNO_3 should be added dropwise (brown fumes should be seen) and then 1 mL 30% H_2O_2 added. Afterwards, repeat the heating cycle until white ashes are observed.

When white ashes occur from the digestion cycle, 5 mL HNO_3 (10%) is added. The solution obtained was homogenized in an ultrasonic bath and then centrifuged.

Duplicate blanks were prepared by adding 5 mL ashing aid solution for the digestion procedure.

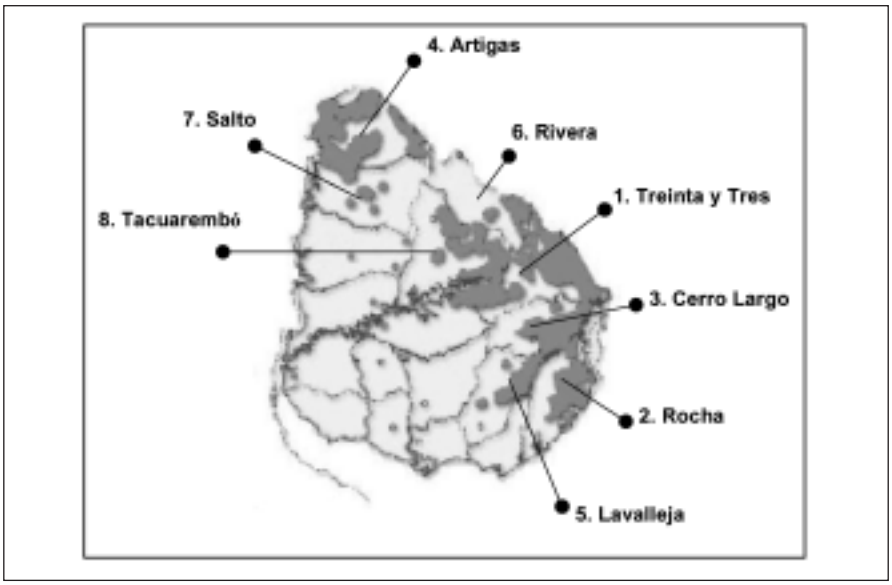

Fig. 1. The eight most important areas of rice production in Uruguay (6).

To ensure a high level of analytical reliability, recovery studies were included for each batch to estimate analytical accuracy. The rice samples were spiked with two different amounts (50 and 250 ng) of a certified solution (Merck KGaA, Darmstadt, Germany) and then submitted to the digestion procedure. Each batch also contained a certified reference material (NIST CRM 8418 Wheat Gluten).

Direct calibration against acidified standard solutions was carried out for the determination of total Sr in the solution using the dry ashing treatment. The calibration curve covers the range of 10–2000 ng mL^{-1}.

Measurement of Uncertainty

Evaluation of the measurement of uncertainty associated with a test result is now an essential requirement in many quality systems. The first step in the measurement of uncertainty, in accordance with the recommendations given by EURACHEM/CITAC (EuroAnalytical Chemistry/Co-Operation on International Traceability in Analytical Chemistry) guide (7), is to recognize all possible sources of uncertainty. Then estimate the size of the component associated with each potential source of uncertainty identified. Very useful is the construction of a cause-effect diagram (Ishikawa diagram) where the different contributions to the overall uncertainty associated with the analytical method applied are clearly visualized.

We have distinguished and evaluated the different sources of uncertainty in the quantification of total Sr in rice using ICP-OES by direct calibration. These are as follows: The analytical scale (all solutions were prepared by tared weighing), including repeatability, readability, sensitivity, and linearity of the scale; the density of solutions (density correction must be made since all solutions are prepared gravimetrically); the preparation of the stock and intermediate calibration solutions; uncertainty in the integration of peak area; uncertainty in the chemical calibration (linear least squares fitting procedure was used); the bias, etc.

To calculate the combined standard uncertainty $u_c(y)$ of the analyte concentration, we used the general relationship between $u_c(y)$ of a value y and the uncertainty of the independent parameters $x_1, x_2, \ldots\ldots, x_n$ on which it depends.

$$u(y) = \sqrt{\sum_{i=1,\,n} c_i^2\, u(x_i)^2}$$

$u(x_i)$ = uncertainty in y arising from the uncertainty in x_i

where y is a function of several parameters $x_1, x_2, \ldots\ldots, x_n$

and c_i is:

$$c_i = \frac{\partial y}{\partial x_i}$$

c_i = sensitivity coefficient.

Once the combined standard uncertainty was determined, the expanded uncertainty U was obtained by multiplying the uc(y) with a coverage factor k.

$U = k u_c(y)$

The coverage factor, k, depends on the value of the Student's t-test for the number of degrees of freedom for the confidence level of 95%.

RESULTS AND DISCUSSION

The results obtained in this work are shown in Table II. The total Sr contents were calculated on a wet basis to allow a comparison with the literature data of the product when purchased.

The methodology employed for the determination of Sr was appropriate, as can be inferred from the recovery studies performed on spiked samples, which ranged from 89–95% for the lowest concentration of Sr (50 ng) and from 91–98% for the highest concentration of Sr (250 ng). In addition, the recovery of Sr from the certified reference material (NIST CRM 8418 Wheat Gluten) was between 89.5 and 98.1±6.3%.

TABLE II
Total Sr Content in Milled (long and short grain), Parboiled Milled, Brown (long and short grain), Parboiled Brown, and Paddy Rice, and in Rice Husk

Rice Type	Grain	Total Sr Concentration (μg g^{-1})			n	LOD (ng g^{-1})	Spike Recovery (%)
		Min.	Max.	Mean			
Milled	Long	0.190±0.039	0.421±0.063	0.281±0.048	11	5	91±12 95±11
Parboiled Milled	Long	0.135±0.026	0.439±0.028	0.287±0.027	14	5	91.6±8.9 91.3±9.4
Milled	Short	0.160±0.028	0.351±0.054	0.247±0.041	13	6	89±13 89±12
Brown	Long	0.354±0.057	0.78±0.11	0.564±0.085	13	5	95.4±11.2 93.2±8.7
Parboiled Brown	Long	0.638±0.088	0.80±0.11	0.73±0.11	14	7	94.5±9.1 93±10
Brown	Short	0.554±0.089	0.605±0.097	0.578±0.092	14	6	91±12 98.2±5.7
Paddy	Long	0.97±0.14	1.41±0.19	1.16±0.16	7	7	92±10 92±11
Husk	–	2.88±0.39	3.91±0.51	3.44±0.47	7	7	90±13 93.4±9.9

Although not much data was found for the total Sr content in rice, the values obtained in the rice samples analyzed in this study are in good agreement with those reported in rice samples from Japan (8). They found levels of total strontium in brown rice ranging from 0.25 to 0.72 μg g^{-1} (as can be seen in Table III).

Table III shows the average concentration of total Sr in common natural materials. For instance, the average concentration of Sr in crustal rocks is 370 μg g^{-1}, while in soils it is 240 μg g^{-1}, but can fall below 10 μg g^{-1} or exceed 1000 μg g^{-1}. For hydrological samples (seawaters, rivers, rain) the overall mass content of Sr ranges from 0.01 to 7600 μg L^{-1} and for biological samples (wood, roots) from 8–2500 μg g^{-1} (9).

TABLE III
Average or Ranges of Sr Concentrations in Different Natural Materials (1, 4, 8–12)

Material	Sr (μg g^{-1})	Material	Sr (μg L^{-1})
Average Crust	370	Seawater	7600
Soil Minerals	240	Spring Water	20–100
Sandstone	20	Rain	1–400
Low-Ca Granite	100	Wines from:	
High-Ca Granite	440	Chile	532
Basalt	465	California	1924
Wood	8–2500	Madeira	939
Roots (Spruce)	20	Australia	1879
Conifer Needles	2–20	South Africa	722
Rice from Japan	0.25–72	Bordeaux	275

In the samples studied here, logical values of Sr were obtained from the rice's husk to the milled rice. The removal of the outer layers of the rice shows a reduction in the Sr content. Paddy rice (rice which has retained its husk after threshing) presented the highest values of Sr (0.97–1.41 μg g^{-1}). The complete elimination of the husk from the paddy rice produces brown rice; therefore, it is reasonable that the Sr content in brown rice is lower (0.35–0.78 μg g^{-1}) than in paddy rice. With the milling process of brown rice, total removal of husk and bran is performed; consequently, this type of rice presented the lowest value of Sr (0.281 μg g^{-1}).

It is generally accepted that as greater amounts of rice bran are removed from the grain during milling and polishing, more vitamins and minerals are lost (13). In the rice samples analyzed in this work, the milling process seems to have great influence on the Sr levels.

Since the Sr concentration in rice samples is highly affected by processing (milling and polishing), it can be said that most of the Sr is situated in the external layers of the grain (aleurone and pericarp).

On the other hand, prior to milling or storing, rice may be parboiled, which involves soaking the paddy rice in warm water, followed by steaming and drying (14). It has been pointed out that parboiling rice prior to cooking preserves some of the nutrient content, chiefly due to the retention of minerals and water-soluble vitamins. The higher retention of micronutrients (Ca, Cu, Fe, Zn, etc.) in parboiled rice has been assigned to their solubilization and migration from the aleurone and germ into the starchy endosperm (13,14). Paddy rice is parboiled and dehusked to obtain parboiled brown rice; once it is submitted to milling and polishing, parboiled milled rice is obtained.

From the rice samples studied here we found that the mean level of Sr in parboiled brown rice (0.73 μg g^{-1}) is higher than in parboiled milled rice (0.287 μg g^{-1}) which is in good agreement with the idea that most of the Sr in rice is situated in the outer layers. The Sr is solubilizated and introduced into the endosperm, so the milling and polishing process used to obtain parboiled milled rice causes a significant reduction in Sr concentration.

Yet, the implication of the benefits (from a nutritional or contamination point of view) of parboiled rice is still arguable, mostly due to the lack of uniform commercial processes applied in different countries. It is believed that the retention pattern of some minerals (Ca, Na, Cu, Fe, Mo, etc.) is the result of the interaction of factors such as mineral location in the grain and their solubility during soaking, different ratios of migration as well as variations in the hydrothermal process, and milling resistance of the parboiled grain (14). Hence, further studies should be carried out for a more complete understanding of mineral retention.

CONCLUSION

Since rice is the predominant staple food for more than half of the world's population, its nutritional vs. toxic composition is of special interest.

This is the first study reporting the concentration of Sr in different types of rice cultivated in Uruguay. The methodology employed for the determination of Sr was validated against a certified reference material. The good recoveries obtained make us conclude that the method used was appropriate.

Removal of the outer layers of the rice grain results in a large reduction of Sr concentration. The average concentration of Sr in paddy rice was 1.16 μg g^{-1} (which is a non-processed form of rice), while in milled rice it was 0.281 μg g^{-1}. This behavior suggests that the greater amount of Sr is situated in the non-edible parts of the grain (i.e., husk) and confirms that the highest levels of Sr found in this work were in the rice's husk (3.44 μg g^{-1}).

Although further studies should be carried out, the results here presented suggest that there is no Sr contamination from the atmosphere in rice produced in Uruguay and its consumption, therefore, presents no health threat. Unfortunately, no government or other official regulation was found to support our claim concerning limits for Sr levels in rice. However, the United States Government (15) has set a limit of 4000 μg L^{-1} of Sr in drinking water, and the Sr concentrations in all of the samples analyzed in this survey are far below this regulation (considering consumption, preferences, etc.). Therefore, we can assume that no Sr contamination has occurred in Uruguay. Since Uruguay is one of the world's top ten rice exporters, it is crucial to be able to demonstrate that its rice is of high quality with regard to Sr content.

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Speciation of Cr in Natural and Wastewaters Using Immobilized *Aspergillus niger* and Its Determination by GFAAS and FAAS

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ABSTRACT

The applicability of a fungal biomass *Aspergillus niger* immobilized on a polysilicate matrix as substrate for the separation and determination of Cr(III) and Cr(VI) in waters has been investigated. Experiments were performed with a home-made column loaded with the immobilized biomass to optimize conditions such as pH, flow rate, and eluent to achieve quantitative separation of Cr(III) and Cr(VI) and preconcentration of C (III) both at μg mL^{-1} and ng mL^{-1} levels. Cr(III) was selectively retained on the column of immobilized biomass in the pH range of 4–8 while Cr(VI) remained in solution. The retained Cr(III) was subsequently eluted with 0.8 mol L^{-1} H_2SO_4 determined by GFAAS or FAAS. Cr(VI) was obtained from the difference of total Cr determined in another aliquot on a separate column after reduction of Cr(VI) to Cr(III) and Cr(III) was determined using the first column. Cr(VI), when it is present at high levels (>100 ng/mL), was determined directly in the effluent using spectrophotometry. The sorption capacity of ~12 mg/g for Cr(III) was obtained for the immobilized biomass. The effect of various foreign ions on the sorption was also studied.

The proposed method was applied successfully for the determination of Cr(III) and Cr(VI) in natural and wastewater samples and recoveries were found to be better than 95% in all cases.

INTRODUCTION

Chromium is a ubiquitous element, not only through its occurrence in nature but also due to many anthropogenic sources resulting from its extensive use in various industrial applications such as steel works, electroplating, tanning industry, production of pigments, wood preservatives, sanitary landfill leaching, photographic material, water cooling towers, and chemical industries (1,2). Also, Cr compounds have been used as corrosion inhibitors in water pipes that constitute a potential source of Cr(VI) in the drinking water distribution system. The most prominent oxidation states of chromium that exist in nature are Cr(III) and Cr(VI). Cr(III) is an essential trace element for humans as it helps to activate insulin, whereas Cr(VI) as a strong oxidizer is highly carcinogenic, has the capability of crossing the cell membranes; and reduced to Cr(III), Cr(VI) affects lungs, liver, kidneys, and blood-forming organs (3,4). It is reported that hexavalent chromium is about 100–1000 times more toxic than the trivalent chromium (5). Therefore, total chromium determination alone is no longer acceptable for the assessment of the possible health hazards. Hence there is an obvious need for the determination of the two species individually.

Usually, the natural levels of chromium in unpolluted waters are very low and a preconcentration is often necessary prior to its determination. Various methods, such as sorption methods (6), co-precipitation with metal hydroxides (7), solvent extraction using different reagents (8,9), ion-exchange (10,11), solid phase extraction (12,13), and electrochemical separation (14,15) have been widely used for the separation of Cr(III) and Cr(VI), and a number of reviews summarize these methods (16,17). However, the application of such processes is sometimes restricted because of technical or economic constraints.

In recent years, increased attention has been focused on the eco-friendly biological technologies using various microbial species such as yeast, bacteria, fungi, and materials of plant origin as an alternative to conventional processes for the removal, preconcentration of suspended solids, dissolved nutrients, pathogens, toxic metals, and radionuclides from wastewaters (18–20), and to a lesser extent for speciation purposes (21,22). Among the various biosorbents, the fungal biomass, e.g., *Aspergillus niger* (*A. niger*) has been studied extensively for removing metals, radioactive elements, and metal cyanide complexes because of a wide range of morphological types they possess, the easy availability of large amounts, and cost-effectiveness (19,23).

Various studies have been reported on the detoxification of Cr(VI) from wastewaters using *A. niger* (24). Our earlier studies on the applicability of *Aspergillus niger* for the removal of inorganic and methyl mercury from wastewaters has demonstrated its poten-

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tial to act as a cation exchanger (20). Various other applications of *A. niger* have been reviewed by Veglio et al. (19). But so far, to the best of our knowledge, it has not been used for the speciation of chromium.

With all these considerations, we have evaluated the applicability of *Aspergillus niger* (after immobilization) as a sorbent material, for its potential applications for the speciation of Cr(III) from Cr(VI) and for the selective preconcentration of Cr(III), prior to their determination by GFAAS and flame atomic absorption spectrometry (FAAS). The optimum conditions such as pH flow rate and eluent for speciation of chromium were evaluated. The breakthrough capacity of the sorbent for Cr(III) was also studied. The method was applied to synthetic and natural waters.

EXPERIMENTAL

Instrumentation

Analytikjena (Model ZEEnit 65, Jena, Germany) graphite furnace atomic absorption spectrometer (GFAAS) equipped with an MPE 60 autosampler was used for the determination of Cr at ng mL^{-1} levels. The spectrometer was provided with both Zeeman (transverse variable magnetic field) and deuterium-HCL-based background correction systems. Zeeman background correction (2-field) was used throughout this work. A spectral band pass at 0.5 nm and a wavelength at 357.9 nm were used. Chromium was atomized directly off the wall of the pyrolytically coated tubes (part No.407–152.013). Five-point calibration curves were prepared with the autosampler by dilution in the tube. A sample volume of 20 µL was used. The furnace conditions are given in Table I(a).

Measurements with flame AAS were carried out with a GBC 932AA (Dandenong, Australia) apparatus, equipped with deuterium lamp background correction and an air-acetylene flame for µg mL^{-1} concentration levels of chromium. The analyses were performed at the most sensitive resonance line of 357.9 nm and under optimized operating conditions [see Table I(b)] providing a maximum signal-to-noise ratio.

A double beam UV-visible spectrophotometer (U-3210, Hitachi Ltd, Tokyo, Japan) with 10-mm quartz cells was used for the spectrophotmetric measurements of Cr(VI) at 540 nm. The pH adjustments were performed with dilute solutions of HCl and NaOH. All pH measurements were made with a digital pH meter.

TABLE I(a)
Instrumental and Operating Parameters Employed to Determine Cr by GFAAS

No.	Type	Temp. (°C)	Rate (oC/s)	Hold (s)
1	Drying	90	5	20
2	Drying	105	3	20
3	Drying	110	2	10
4	Pyrolysis	800	250	10
5	AZ*	800	0	6
6	Atomize	2300	1300	5
7	Cleanout	2400	500	4

* Auto-zeroing.

Reagents and Standard Solutions

All solutions were prepared using analytical reagent grade chemicals and Milli-Q™ water with >18MΩ cm resistivity (Millipore Corporation, Bedford, MA, USA). A 1,5-diphenylcarbazide (DPC) solution (0.01 mol L^{-1}) was prepared fresh daily by dissolving appropriate amounts of DPC (Merck, Darmstadt, Germany) in acetone and then diluting with water.

Stock standards of Cr(III) and Cr(VI) were prepared from $CrCl_3 . 6H_2O$ (CDH, New Delhi, India) and $K_2Cr_2O_7$ (Merck), respectively. Working solutions of Cr(III) and Cr(VI) were prepared as and when required by appropriate dilution of the stock solutions. A range of solutions of different pH values was prepared by adjusting the pH with very dilute solutions of HCl or NaOH. The solutions of Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cl^-, and NO_3^- used in the study of interferences were prepared by appropriate dilution of stock solutions.

Materials and Methods

Preparation of Bio-sorbent

The growth conditions for *Aspergillus niger*, a fungal biomass used in this study and for biosorbent preparation, were described in detail elsewhere (25). Briefly, *Aspergillus niger* mycelia was obtained after growth for 3 days, then harvested by filtration using a cheesecloth, and washed

TABLE I(b)
Instrumental and Operating Parameters Employed to Determine Cr by FAAS

Wavelength	357.9 nm
Flame	Air-acetylene
Lamp Current	10 mA
Slit Width	0.2 mm
Air Flow	5 L min^{-1}
Acetylene Flow	1.5 L min^{-1}

thoroughly with distilled water. The mycelia was pressed in-between folds of filter paper to remove excess moisture and added to a boiling KOH solution (5%) and left standing for 15–20 min. After cooling to room temperature, the biomass was washed extensively with distilled water to a neutral pH and stored under cold conditions (around 4°C) until further use. The yield of the biosorbent was about 20% of the initial weight of the mycelia.

*Immobilization of **A. niger** on Sodium Silicate*

Microbial biomass (*A. niger*) consists of small particles with low density, poor mechanical strength, and little rigidity. Immobilization of the biomass on solid support allows the preparation of a material with suitable size, good mechanical strength, and a rigidity and porosity necessary for use in columns for the fast separation of metal ions from solution (26). Immobilization can also yield beads or granules that can be regenerated and re-used in a manner similar to ion exchange resins.

A polysilicate matrix as a support material was used in this study to immobilize the biomass. The method adopted for immobilization of material was similar to that reported by Gardea et al. (27). A volume of 75 mL of 5% H_2SO_4 was mixed with sufficient sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. Then, 5 g of powdered fungal biomass was added to the silica solution and stirred for 15 minutes. The pH was then raised slowly by the addition of 6% sodium silicate to reach pH 7.0. The polymer gel formed was washed thoroughly with water until all sulphate was removed. This was further confirmed by the addition of a few drops of barium chloride ($BaCl_2$). The polymer gel with immobilized A. niger was dried overnight at 45–50°C, then ground using an agate mortar and pestle, and sieved to obtain 50–100 mesh sizes.

Column Preparation

A homemade PTFE column (60 mm × 3.0 mm i.d) with end caps was used for the present studies. Five hundred milligrams of immobilized biomass was filled into the column plugged with a small portion of glass wool at both ends and capped. The column was washed thoroughly with Milli-Q water and then conditioned to the desired pH before passing the chromium-containing solutions.

General Procedure for the Speciation of Cr(III) and Cr(VI) and Their Determination

The pH of the metal-containing solution plays a crucial role in passive biosorption. The retention behavior of Cr(III) and Cr(VI) ions of the column loaded with immobilized biomass as a function of pH was investigated separately as well as together. The procedure developed for the separation of individual species and their determination was as follows:

- 100 mL of sample solution spiked with 500 ng/mL of Cr(III), Cr(VI), or a mixture of both species was used. Then the pH of the spiked sample solution was adjusted to 4 and passed through the column at a constant flow rate (2 mL/min) using a peristaltic pump. The retained Cr(III) was eluted with 10 mL of 0.8-mol L^{-1} H_2SO_4 and determined by GFAAS or FAAS. In another aliquot of sample (100 mL), Cr(VI) was initially reduced to Cr(III) by adding 0.2 mL of 10% aqueous ascorbic acid solution, then Cr(III) was sorbed onto the column (using a separate column) and eluted to determine total chromium [Cr(III) + Cr(VI)]. The difference of total chromium and Cr(III) was calculated and taken as Cr(VI).

However at high levels of Cr(VI) (>100 ng/mL) and after separation of the Cr(III) species, Cr(VI) in the effluent could be determined using the DPC spectrophotometric method.

RESULTS AND DISCUSSION

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

A critical parameter in achieving the speciation of Cr(III) and Cr(VI) is the pH of the solution. In the initial experiments, the effect of pH on the retention of Cr(III) and Cr(VI) on the column was investigated. The pH of the sample solutions was varied in the range of 2 to 10 and the solution passed through the column at a flow rate of 2 mL/min. The effect of change of pH on the speciation of the Cr(III) and Cr(VI) species is shown in Figure 1. As can be seen from Figure 1, the retention of Cr(III) by the biomass is pH-dependent, with maximum retention (>95%) occurring between pH 4–8. However, Cr(VI) showed negligible retention (<3%) throughout the investigated pH range. This behavior made it possible to separate Cr(III) and Cr(VI) from their mixtures.

This trend in pH-dependent binding of Cr(III) with maximum retention in the pH range of 4–8 suggests that carboxylic groups play an important role in the retention mechanism of various cationic species of Cr(III) by the biomass. It is well known that carboxylic acids are deprotonated at pH >4 and readily retain positively charged Cr species such as Cr^{3+}, $Cr(OH)^{2+}$, and $Cr(OH)_2^{+}$. Similar pH-dependent behavior for removal of various metal ions on different biomasses and plant materials having carboxylic acid groups, have earlier been reported (21). Our earlier studies also showed that the binding of both inorganic and methyl mercury on biomass significantly decreased from 95% to 40% on

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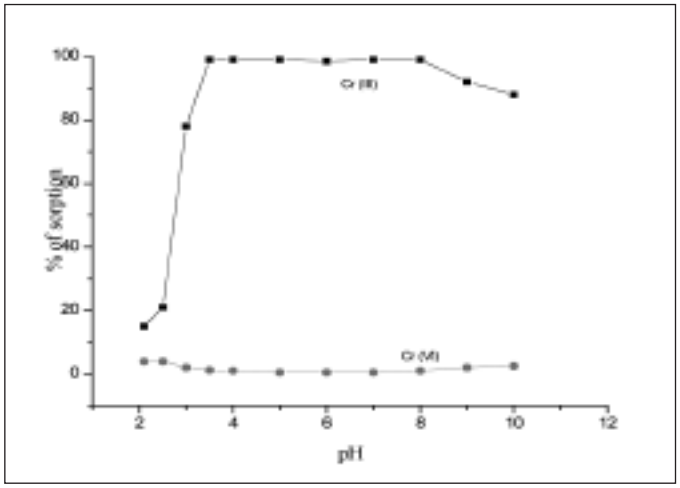

Fig. 1. Effect of pH on the separation of Cr(III) and Cr(VI).

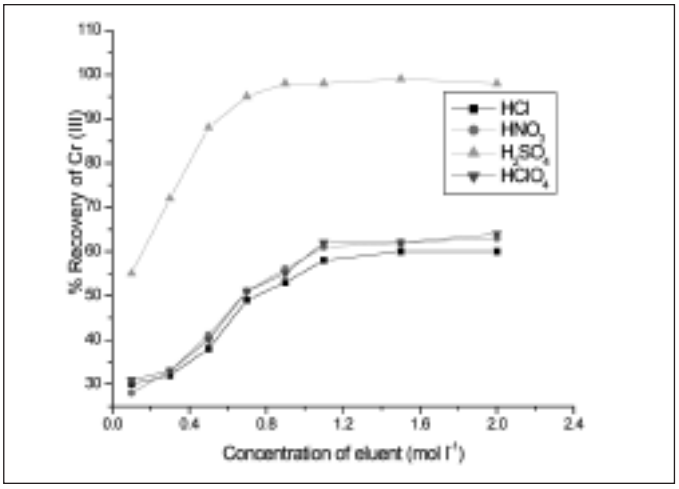

Fig. 2. Effect of type of eluent on the recovery of Cr(III).

esterification which suggested that sorption behavior might be an ion-exchange process involving the carboxylate groups (20).

Elution of Cr(III)

The desorption of the retained Cr(III) could be achieved by the use of an appropriate eluent capable of effectively stripping the ion from the biomass. It is desirable that the least possible deterioration in properties of the biomass occurs so as to allow its repeated use in sub-sequent loading-elution cycles.

Table II presents elution data for the chromium species after preconcentration from a 50-mL solution with 500 ng/mL of Cr(III). Various acids HCl, HNO_3, H_2SO_4, and $HClO_4$ at different concentrations were tested for their abilities to elute the bound Cr(III) from the column. As can be seen from Table II, the optimum elution (>95%) was obtained with 10 mL of 0.8 M H_2SO_4. Figure 2 shows that elution of the bound Cr(III) was poor (50–60%), even with higher concentrations (up to 3 M) of HCl, HNO_3, and $HClO_4$. Gardea-Torresdey et al. (28) observed similarly that Cr(III) bound on the silica-immobilized biomass alfalfa was difficult to desorb even with high concentrations of HCl (up to 2 M) and reported a 45–50% recovery of Cr(III). In the present studies, quantitative elution (>95%) of retained Cr(III) could be achieved with 10 mL of 0.8 M H_2SO_4 and the same was used as eluent in subsequent experiments.

Effect of Flow Rate of Sample Solution

The flow rate of the sample solution is also an important parameter for the quantitative separation of Cr(III) from Cr(VI) on the sorbent and for the duration of the complete analysis. Therefore, the effect of the flow rate of the sample solution was examined using the general procedure under optimum conditions. The flow rates were varied in the range of 0.5–3 mL min^{-1}. Maximum retention (98%) of Cr(III) occurred up to a flow rate of 2 mL min^{-1} while >95% of Cr(VI) remained in the effluent. At higher flow rates (>2 mL min^{-1}), retention of Cr(III) decreased gradually due to a decrease in the adsorption kinetics at higher flow rates. Hence, a flow rate of 2 mL min^{-1} was used for all subsequent experiments.

TABLE II
Effect of Volume of Eluent on Recovery of Bound Cr(III) From Biomass Column

Eluent	Volume of Eluent (mL)	[a]Recovery (%)
HCl		
0.8 mol L^{-1})	5	35±3
	10	53±2
	15	58±2
HNO_3		
(0.8 mol L^{-1})	5	33±2
	10	57±3
	15	60±4
H_2SO_4		
(0.8 mol L^{-1})	5	90±3
	10	98±2
	15	99±3
$HClO_4$		
(0.8 mol L^{-1})	5	37±2
	10	58±4
	15	61±5

Volume of sample solution = 50 mL, concentration of sample (Cr(III)) = 500 ng/mL.

[a]Mean of three determinations, analyzed by FAAS.

Evaluation of Cr(III) Retention Capacity of the Immobilized Biomass

The maximum amount of Cr(III) retained by the immobilized *A. niger* column was evaluated by using breakthrough (BT) capacity studies. A feed solution of Cr(III) having a concentration of 5 µg/mL was passed through a column loaded with immobilized biomass at an optimized flow rate of 2 mL/min. After attaining 100% BT, the retained Cr(III) was eluted and determined by GFAAS or FAAS. From these studies it was estimated that the biomass *A. niger* was able to accumulate up to 12 mg/g of Cr(III).

Limit of Detection and Precision of the Method

The detection limit was evaluated as the concentration corresponding to three times the standard deviation of the blank signal. The detection limit for Cr was found to be 0.2 ng mL^{-1} and 145 ng mL^{-1} for GFAAS and FAAS, respectively. For the DPC spectrophotometric method, the limit of detection value achieved was 5 ng mL^{-1}.

The precision of the determination of Cr(III) was evaluated under the optimum conditions mentioned above. For this purpose, three successive retention and elution cycles with 50 mL of sample solution containing a 500-ng/mL mixture of Cr(III) and Cr(VI) were performed. These studies indicate that the recovery of Cr(III) from the column was 96 ± 1%, while the recovery of Cr(VI) in the effluent was 98%±3%.

Effect of Foreign Ions

The interference of different metal ions on the sorbent is in general attributed to the strength of the interaction between the metal ion and the adsorbent. The effect of different ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cl^-, SO_4^{2-}, and NO_3^-, which are commonly encountered in waters on the speciation of Cr(III) and Cr(VI), was evaluated to assess the selectivity of the method. A 25-mL portion of synthetic water spiked with various ions at different concentrations (each 10–100 µg/mL), and containing 0.5 µg/mL of Cr(III) and Cr(VI) each, was passed through the column. These studies showed that the speciation of Cr(III) and Cr(VI) from the synthetic sample solutions was not affected by the presence of cations (such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and Fe^{2+}) and anions (such as Cl^-, SO_4^{2-}, and NO_3^-) in the specified concentration range.

Analysis of Synthetic Samples and Recovery Studies

In order to demonstrate the reliability of this proposed speciation method, experiments were carried out with synthetic water samples prepared by adding known amounts of Cr(III) and Cr(VI) to Milli-Q water and applying the general procedure previously described. As shown in Table III, synthetic sample solutions were prepared by spiking different amounts of Cr(III) and Cr(VI) in Milli-Q water. The recoveries of both Cr(III) and Cr(VI) are presented in Table III. Quantitative sorption of Cr(III) occurred in all samples while the sorption of Cr(VI) was <3%. The recoveries of Cr(III) and Cr(VI) in all of the cases were found to be better than 95%.

Analysis of Natural Water Samples

Muncipal, lake, and ground water samples were collected in pre-cleaned polyethylene bottles in Hyderabad, India, and also one ground water sample near chromite mines in Orissa, India. These water samples were passed through the column loaded with immobilized biomass without adjustment of sample pH as acidification would change the chemical species. The concentrations of Cr(III) and Cr(VI) determined in these samples using the proposed method are given in Table IV. In the absence of a reference standard, known amounts of Cr(III) and Cr(VI) were added to these samples and the recoveries obtained were found to be above 95% (Table IV).

Analysis of Wastewater Samples

Two wastewater samples containing both Cr(III) and Cr(VI) were obtained from a tannery and a bulk-chemical industry located in Hyderabad, India. These wastewater samples were passed through the column loaded with immobilized biomass and the proposed method was applied under optimal experimental conditions as described. As can be seen in Table V, the concentrations of Cr(III) and Cr(VI) obtained by the present method were in good agreement with those of our earlier reported method (21). The results show that the immobilized *A. niger* is therefore well-suited for the quantitative separation and determination of Cr(III) and Cr(VI) from wastewaters.

CONCLUSION

The applicability of *Aspergillus niger*, immobilized on a silica matrix, for the speciation of Cr(III) and Cr(VI) in natural and wastewaters was investigated. These studies indicate that Cr(III) was quantitatively retained on the column in the pH range of 4–8 while Cr(VI) remained in the solution. Quantitative separation was achieved with the column even from low ppb level solutions. The capacity of the immobilized biomass was found to be ~12 mg/g for Cr(III). The proposed method was applied successfully for the determination of Cr(III) and Cr(VI) in various spiked, natural, and wastewater samples, and the recoveries were found to be better than 95% in all the cases. It was found

TABLE III
Determination of Cr(III) and Cr(VI) in Spiked Sample Solutions

Synthetic Sample	Added (ng/mL) Cr(III)	Added (ng/mL) Cr(VI)	Found (ng/mL) Cr(III)	Found (ng/mL) Cr(VI)	Recovery (%) Cr(III)	Recovery (%) Cr(VI)
1[a]	50	0	49.3±1.3	0.0±0.1	98±3	
2[a]	25	25	24.3±1.1	24.1±0.9	97±4	96±4
3[a]	0	50	0.0±0.1	48.5±0.8		97±2
4[b]	500	0	490±10	0.0±0.5	98±2	-
5[b]	250	250	246±6	240±8	98±3	96±4
6[b]	0	500	0.0±0.2	487±12	-	97±3

TABLE III Footnotes:

[a] Sample volume 100 mL; Cr(VI) obtained by determining total Cr using another column after reduction to C(III); Cr(III) determinations were done by GFAAS after elution; ± Standard deviation (n=3).

[b] Sample volume 50 mL; Cr(VI) obtained by DPC-spectrophotometric method in the effluent; Cr(III) determinations were done by FAAS after elution; ± standard deviation (n=3).

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

Sample	Added (ng/mL) Cr(III)	Added (ng/mL) Cr(VI)	Found (ng/mL) Present Method Cr(III)	Found (ng/mL) Present Method Cr(VI)	*Recovery% **Reported Method (Ref. 21) Cr(III)	*Recovery% **Reported Method (Ref. 21) Cr(VI)	Total Cr (ng/mL)
Muncipal Water[a] (pH=7.5)							
	0	0	0.9±0.1	0.3±0.1	-	-	1.2±0.1
	10	10	11.2±0.2	10.5±0.2	*103±2	*102±2	21.7±0.4
Lake Water[a] (pH=7.3)							
	0	0	7.5±0.2	3.3±0.2	-	-	10.8±0.2
	10	10	17.2±0.2	12.8±0.3	*96±2	*96±3	30.1±0.4
Ground Water[a] (pH=6.4)							
	0	0	2.6±0.2	1.3±0.2	-	-	3.9±0.2
	10	0	12.2±0.2	10.9±0.3	*97±2	*95±3	23.1±0.4
Ground Water[b] (Near Chromate Mines) (pH=7.1)							
	0	0	35±3	168±11	**32±3	**174±9	190±10

TABLE IV Footnotes:

[a] Sample volume 100 mL; Cr(VI) obtained my determining total Cr using another column after reduction to C(III); Cr(III) determinations were done by GFAAS after elution; ± standard deviation (n=3).

[b] Sample volume 50 mL; Cr(VI) obtained by DPC-spectrophotometric method in the effluent; Cr(III) determinations were done by GFAAS after elution; ± standard deviation (n=3).

TABLE V
Speciation of Cr(III) and Cr(VI) in Real Wastewater Samples

Sample	Found Present Method (ng/mL) Cr(III)	Found Present Method (ng/mL) Cr(VI)	Found Present Method (ng/mL) Total Cr	Reported Method (ng/mL) (Ref. 21) Cr(III)	Reported Method (ng/mL) (Ref. 21) Cr(VI)	Reported Method (ng/mL) (Ref. 21) Total Cr
Bulk-chemical Industrial Wastewater[a] (pH = 7.4)						
	51±3	70±5	121±10	54±4	72±6	118±7
Tannery Effluent Water (pH = 7.3)[b]						
	420±18	4600±200	4900±240	460±50	4300±200	4800±300

TABLE V Footnotes:

[a] Sample volume 100 ml, Cr(VI) obtained by determining total Cr using another column after reduction to C(III); Cr(III) determinations were done by GFAAS after elution; ± standard deviation (n=3).

[b] Sample volume 50 mL, Cr(VI) obtained by DPC-spectrophotometric method in the effluent; Cr(III) determinations were done by FAAS after elution; ± standard deviation (n=3).

that immobilized *Aspergillus niger* functions as a cation-exchange resin and can be recycled. Other advantages include its use in a wide pH range, and it is cost-effective and eco-friendly.

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Determination of Antimony in Mouse Serum by Electrothermal AAS Using Zirconium Plus Rhodium as Permanent Modifier

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ABSTRACT

The object of the present study was the development of a safe, fast, and efficient methodology for the direct determination (without previous digestion of the sample) of antimony in mouse serum using eletrothermal atomic absorption spectrometry (ETAAS). The methodology developed was used to evaluate a first oral antimonial drug for treatment of leishmanioses. Serum samples were diluted 1+4 with 1% (v/v) nitric acid containing 0.02% of trycetil methyl ammonium chloride (TMAC) to prevent the formation of carbonaceous residues inside the graphite furnace.

The method was tested in several ways: with and without modifier. Without modifier and with Pd+Mg the results were negative over the whole temperature range. With Ru as the permanent modifier, a plateau was obtained instead of a peak, which did not return to baseline even with a 15-second atomization. Using Zr as the permanent modifier, the results were satisfactory but the peak was still very irregular. Only the mixture of Zr+Rh gave positive results. The peak was well defined, returning to baseline in less than 5 seconds and at the pyrolysis and atomization temperatures of 600 and 1900°C, respectively.

Matrix-matched calibration curves (all curve points contained 200 µL of serum in 1000 µL solution) presented an r higher than 0.999. The characteristic mass and the absolute detection limit were 30 pg and 60 pg, respectively. Serum samples spiked with different antimony levels and analyzed on three different days resulted in recoveries close to 100%. The proposed ETAAS method using Zr+Rh as the permanent modifier was applied successfully in investigating the absorption of a first oral antimonial drug developed for the treatment of leishmaniasis (international patent pending).

INTRODUCTION

Leishmaniasis is a group of diseases caused by the invasion of a parasite of the Leishmania genus into the reticuloendothelial system of a vertebrate host. These diseases are a significant cause of morbidity and mortality in various countries around the world. The parasite is found as a motile promastigote in the shadfly. It transforms into an amastigote when engulfed by host macrophages and lives in the acid environment of secondary lysosomes (1). The treatment of choice for all forms of leishmaniasis depends on drugs containing pentavalent antimony [Sb(V)], such as meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostan). Unfortunately, these drugs have to be given parenterally and often show severe side effects (2). Recently, novel liposome- and cyclodextrin-based formulations of these drugs have been proposed (3,4). However, detailed characterization of the pharmacokinetics of antimony following administration of these formulations is still needed. Such evaluations require an accurate and simple method for the determination of low concentrations of antimony in the serum of experimental animals.

In one study, antimony was determined with a hydride generator coupled to an atomic absorption spectrometer (HG-AAS) (5). However, this methodology requires the previous destruction of the matrix by solubilization methods. In addition, interference of other elements present in the sample has been reported.

Alternatively, Sb has also been determined by electrothermal atomic absorption spectrometry (ETAAS) (6). However, since the sample was analyzed using a low wavelength, background problems were detected.

The use of palladium, iridium, or rhodium as modifiers in graphite furnace atomic absorption spectrometry (GFAAS) was investigated to determine antimony in eluent fractions of high-performance liquid chromatography (HPLC) in

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the separation of clinical samples (7). The separation of albumin and transfer at a physiological pH was carried out by ion chromatography on a Cosmogel DEAE column. As an eluent, 0.01 mol L^{-1} Tris-HCl in NaCl 1 mol L^{-1} gradient was used. Several fractions of 0.5 mL each were collected and antimony concentration was determined off-line by ETAAS. Palladium, iridium, and rhodium stabilize antimony in an aqueous standard solution effectively. Palladium and iridium stabilize antimony up to 1500°C in a matrix containing proteins, Tris-HCl buffer, and NaCl efficiently, while rhodium does not.

Costa et al. (8) determined Sb levels in serum, liver, spleen, and skin of hamsters submitted to Sb therapy by instrumental neutron activation analysis (INAA).

Another ETAAS methodology was developed by Subramanian (9) for the determination of Sb in drinking water, red blood cells, and serum using transversely heated graphite furnace (THGF) resulting in a limit of detection (LOD) of 2.6 µg/L.

Human exposure to Sb due to high soil contamination was established by analyzing blood and scalp hair samples by ETAAS after microwave digestion (10). LODs of 0.5 µg/L and 5 µg/Kg were obtained for blood and scalp hair samples, respectively.

In a previous study by Silva et al. (11), Sb and other metals were determined using rhodium (500 µg) as the permanent modifier, which resulted in good sensitivity and in agreement with the results obtained for biological certified samples.

In this work, the determination of Sb in mouse serum by ETAAS was investigated using the following methods: without modifier, with Pd+Mg universal mixture, with Ru (500 µg), with Zr (500 µg), and with Zr+Rh (500 + 250 µg) as the permanent modifier.

EXPERIMENTAL

Instrumentation

The integrated absorbances were obtained using a PerkinElmer® Model AAnalyst™ 100 atomic absorption spectrometer, equipped with a Model HGA®-800 graphite furnace, AS-72 autosampler, and deuterium arc lamp continuum background corrector (PerkinElmer Life Sciences and Analytical Instrumentation, Shelton, CT, USA). Except for the conditions optimized in this work, the instrument was operated under the conditions recommended by the manufacturer.

Integrated absorbance (peak area) was used exclusively for signal evaluation. The Sb hollow cathode lamp (Hitachi, Part No. 207–2006, wavelength 217.6 nm) was operated at 12.5 mA with a slit width of 0.7 nm. Sample and calibration solution volumes of 20 µL were added to the graphite tubes. The tubes were treated similar to a previously described method (6), i.e., employing 100 µL of noble metal solution (1000 mg L^{-1}) and submitting the tube to the temperature program. This procedure was repeated five times in order to obtain a deposit of 500 µg of the noble metal. Argon, 99.996% (White Martins, Belo Horizonte, MG, Brazil), was used as sheath gas. Pyrolytically coated graphite tubes without platform (Hitachi, Part No. 190-6003) were used for all studies. The graphite furnace temperature program was optimized for the determination of Sb (see Table I).

Reagents and Solutions

All chemicals used were of analytical reagent grade unless specified. Water was de-ionized in a Milli-Q™ system (Millipore, Bedford, MA, USA).

The following 1000 µg mL^{-1} stock solutions were used: Antimony Titrisol® from Merck (Darmstadt, Germany), ruthenium (Fluka, Buchs, Switzerland, No. 84033), rhodium (Fluka, No. 83722), and zirconium (Aldrich, Milwaukee, WI, USA, No. 27,497-6), all in 1 mol L^{-1} hydrochloric acid. Magnesium nitrate solution (Merck, No. B593213 431) and palladium nitrate solution (Merck, No. B936989 710) for graphite furnace AAS analysis were also used.

All glassware was washed thoroughly with a detergent solution, rinsed with de-ionized water, maintained in a bath of 50% (v/v) nitric acid for a period of at least one hour, and finally rinsed several times with de-ionized water prior to use. The autosampler cups were submitted to the same treatment.

TABLE I
Temperature Program for the Determination of Sb in Mouse Serum Samples Using Zr+Rh as Permanent Modifier

Step	Temp. (°C)	Ramp (s)	Hold (s)	Air Flow Rate (mL min^{-1})
1	90	5	40	250
2	140	5	20	250
3	600	5	20	250
4a	1900	0	5	0
5	2500	1	5	250
6	20	1	5	250

[a] Reading in this step.

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Procedure

The serum samples were diluted 1+4 with 1% (v/v) nitric acid containing 0.02% of tricethyl methyl ammonium chloride (TMCA). The use of TMCA is necessary to prevent carbonaceous residues inside the graphite furnace (10). Quantification was performed with matrix-matched calibration solutions containing 5, 10, 20, 40, 60, 80, and 100 µg L^{-1} of antimony on the graphite wall. All curve points contained 200 µL of serum in 1000-µL volume of diluent. For verification of the accuracy of the methodology proposed, recovery of the spiked serum samples with 30, 50, and 70 µg L^{-1} of antimony was performed. The recovery studies were performed on three different days against matrix-matched calibration curves. The limit of detection (LOD, µg L^{-1}) was calculated using the equation LOD = 3 x S_{BL}/b, where S_{BL} is the standard deviation of 10 measurements of the blank [200 µL of serum plus 1% (v/v) nitric acid containing 0.02% (v/v) of TMCA (from Aldrich, Milwaukee, WI, No. 29273-7) in a total of 1000-µL volume].

RESULTS AND DISCUSSION

Pyrolysis and Atomization Temperature Curves

The thermal behavior of antimony was evaluated in mouse serum through pyrolysis and atomization temperature curves. They were studied without the use of a modifier, with a universal mixture of Pd+Mg in solution, with Ru permanent modifier (500 µg), with Zr permanent modifier (500 µg), and with Zr+Rh permanent modifier (500 and 250 µg, respectively).

Without modifier and with the universal mixture of Pd+Mg, the signals were negative and had an excessively high background (up to 2.5 units of absorbance) in every temperature range studied.

With permanent Ru, the peak formed a plateau instead of returning to base line, even when using 15 seconds of atomization. This fact had already been reported (10) in the determination of chromium in human serum using the same sample dilution in the same equipment and with the same tube type without platform. However, in the study of chromium in serum, the peak did not return to base line for any of the investigated permanent modifiers of iridium, ruthenium, zirconium, and a mixture of iridium and rhodium (250 mg of each) (10).

With permanent zirconium, the peak was better defined, but had a very irregular profile even with the best atomization temperature of 2400°C, and the tail returned to base line only at 9–10 seconds.

With rhodium (250 µg) applied to the zirconium-treated tube, the peak became well defined. It returned to base line in less than 5 seconds with corrected background and at the best pyrolysis and atomization temperatures of 600 and 1900°C, respectively (see Figure 1), and with a characteristic mass of 30 pg (recommended for aqueous solutions of 20 pg).

Analytical Figures of Merit

Matrix-matched calibration curves were constructed following the procedure described in the Experimental section and applying the temperature program given in Table I. The analytical characteristics for the determination of antimony without modifier are listed in Table II.

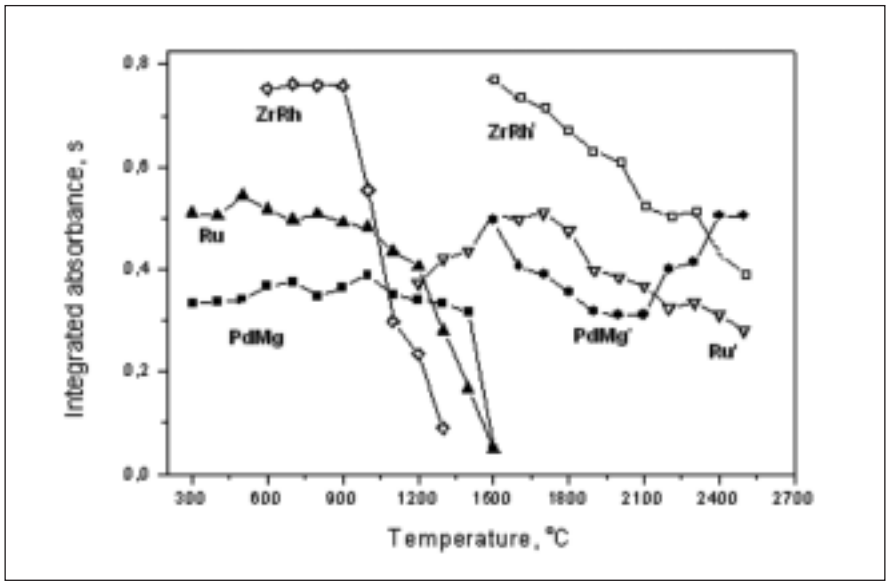

Fig. 1. Pyrolysis and atomization temperature curves for 50 pg of antimony in diluted serum sample using Zr+Rh as the permanent modifier.

TABLE II
Analytical Characteristics for the Determination of Sb in Serum Samples by ETAAS With Zr+Rh as Permanent Modifier

Modifier	Mo[a]	LOD	RSD (%) (n=3)	r^2	Calibration Range (µg L^{-1})
Zr+Rh (500 + 250 mg)	30	60	<5.0	0.99987	10–100

[a] Recommended characteristic mass for aqueous solutions was 50 pg.

Recovery experiments were carried out with the optimized pyrolysis and atomization temperatures and the proposed methodology (using Zr+Rh as permanent modifier). Diluted serum samples spiked with Sb amounts of 30, 50, and 70 μg L^{-1} were measured against matrix-matched calibration curves. The recoveries were made in triplicates and on three different days (shown in Table III).

The results show that the recoveries obtained on the three different days ranged from 85.7–113.3%, indicating good accuracy. In addition, the standard deviations were compatible with the graphite furnace analysis and similar to data reported in the literature for serum samples submitted to a microwave digestion procedure with acids.

In our study it was also verified that more than 500 determinations can be performed using the same graphite tube without causing analyte signal alteration or crust formation.

The methodology proposed was to establish whether cyclodextrin promotes oral absorption of antimony in mice. In vivo evaluations against cutaneaus leishmaniasis showed the effectiveness of this new formulation of the drug by oral route (4), which was the first report of an orally active formulation for an antimonial drug.

TABLE III
Recovery of Sb in Spiked Serum Samples Using Zr+Rh as Permanent Modifier

Expected Value (μg L^{-1})	Determined Value (μg L^{-1})	Recovery (%)	RSD (n=3)
1st day			
30	32.0	106.6	1.5
50	44.0	88.0	1.5
70	60.0	85.7	3.3
2nd day			
30	28.3	94.3	3.7
50	46.6	93.2	3.4
70	77.0	110.0	5.0
3rd day			
30	34.0	113.3	4.3
50	56.0	112.0	5.0
70	74.3	106.0	2.4

CONCLUSION

Antimony can be directly determined in mouse serum by ETAAS without prior sample digestion when a mixture of zirconium plus rhodium is used as the permanent modifier. The results presented good accuracy and precision by using 1% (v/v) nitric acid and 0.02% (v/v) of TMCA as the diluent in pyrolytically coated graphite tubes without platform and a deuterium arc lamp continuum background corrector. Since sample digestion is not necessary, the speed of the analysis was increased. The recoveries ranged from 85.7–113.3% of serum samples spiked with antimony thus providing good accuracy. The characteristic mass of 30 pg and an LOD of 60 pg showed that the methodology presented good sensitivity. Tube lifetime was established with at least 500 atomization cycles.

All other elements tested as modifiers (universal Pd+Mg, Mg in solution, permanent Ru or Zr, and a mixture of Ir+Rh as permanent modifier) did not show promising results.

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Determination of Mercury Levels in Blood and Urine of Occupationally Exposed Workers Using Flow Injection With Cold Vapor AAS

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ABSTRACT

The high volatility and easy skin absorption of mercury make it a toxic substance that is difficult to control. A flow injection system for the determination of mercury based on a cold vapor atomic absorption spectrometry technique (CVAAS) is used to determine mercury in blood and urine samples.

The study was carried out for four groups of workers exposed to elementary mercury: Dentists, dental assistants, laboratory and chloralkali workers and compared to a non-occupationally exposed group. The results show that only the dentist and chloralkali group showed mercury exposure.

The safe levels of exposure indicated by ACGIH, Biological Exposure Indices (BEI), are 35 µg/g creatinine (CR) for urine mercury (UHg) and 15 µg/L for blood mercury (BHg). However, because of the immunological etiology found in experimental studies on animals, where inorganic mercury may induce autoimmune glomerulonephritis in the absence of dose-response studies for groups of immunologically sensitive individuals, it is not scientifically possible to set a level for mercury below which (in individual cases) mercury-related symptoms will not occur. The most exposed group, chloralkali, has mean levels of UHg and BHg of 19.4 µg/g CR and 14.4 µg/L, respectively. The dentist group shows a mean mercury concentration of 9.6 µg/L in blood and 3.9 µg/g CR in urine. This is significantly higher than the mercury concentrations found in the control group (7.4 µg/L in blood and 2.0 µg/g CR in urine) or in the dental assistant group (7.1 µg/L in blood and 1.9 µg/g CR in urine). There is no significantly statistical difference in these two last groups. For the chloralkali group, clearly exposed to vapor mercury, there appears to be a statistically significant correlation between blood and urine mercury (N=42; rho=0.586; p<0.001).

INTRODUCTION

The principal source of inorganic mercury for the general population is the vapor released from amalgam fillings, whereas the principal source of organic mercury is fish and shellfish (1–4). The most important source of mercury comes from ingestion of fish (5). The principal route of occupational exposure to metallic mercury is pulmonary as a consequence of vapor inhalation (6).

The occupational risk of mercury poisoning appears in workers involved in the following occupations: miners, jewelers, odontology and laboratory personnel (1). The latter group, together with those involved in the preparation or use of organomercurial fungicides, presents the biggest risk of Hg poisoning due to organic compounds (1).

Chronic exposure to elementary mercury usually occurs in industrial workers involved in processes of catalysis in chloralkali plants, electrical equipment, painting, manufacture of fungicides, and in mines where metal extractions are performed using mercury (7–11). The industries thought to be the principal pollution source are chloralkali plants (electrodes of mercury), followed by paper mills where mercurial compounds are used as fungicide for wood pulp (12).

One of the most controversial topics is dental amalgam containing mercury and used by dentists (13–14). Dental amalgams can cause Hg exposure to dentists, dental assistants, and also to patients (2). Low Hg exposure levels are common in dental practices and it has also been observed in the general population due to release of mercury from dental amalgams (15).

Dentists and their assistants can be exposed to high concentrations of mercury during extraction (16-17), restoration, and polishing of amalgam fillings (17). The risk is especially high when suitable protection measurements are not taken (18–21) such as the use of water and suction (16–17). It has been reported that the concentration of mercury in the area where the dentists breathe decreases ten times with appropriate protection proce-

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dures such as coolants or adequate aspiration techniques (20).

The most common form of chronic poisoning is as a consequence of vapor inhalation (11) and is referred to as hydrargyrism (22). The medical profile characterizes this condition as the neurological - psychiatric, renal and stomatologic syndrome (11), also called "asthenic vegetative syndrome" (23).

Current evidence suggests that this nephrotic syndrome has an immunotoxic origin. It affects the kidneys at exposure levels lower than those related to the appearance of signs and symptoms of the nervous central system (2). The safe levels of exposure indicated by ACGIH, Biological Exposure Indices (BEI), are 35 µg/g creatinine (CR) for urine mercury (UHg) and 15 µg/L for blood mercury (BHg) (24). Experimental studies in animals show that mercury compounds have an immunomodulator activity, where an autoimmune response and a membranous nephropathy is induced (25). One of the consequences of the immunological etiology is that, in the absence of dose-response studies for groups of immunologically sensitive individuals, it is not scientifically possible to set a level for mercury in blood and urine below which, in individual cases, symptoms related to mercury will not occur (2).

EXPERIMENTAL

Instrumentation

A PerkinElmer® Model FIMS™-400 flow injection mercury system, based on cold vapor atomic absorption spectroscopy (CVAAS), was used (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). For creatinine determination, a Beckman Synchron CX-5 autoanalyzer was used (Beckman Coulter, Fullerton, CA, USA). The method of Lopez-Colon (26) was used for the determination of mercury in blood. Thus, 1 mL of 0.2% Triton® X-100 solution and 0.5 mL of blood sample or calibration solution were placed in a 50-mL polypropylene tube with 0.1 mL of stabilizing solution of 0.5% $K_2Cr_2O_7$ in 50% (v/v) HNO_3. Nitric (1 mL) and sulfuric (2 mL) acids were added and the mixture heated at 60ºC overnight to digest the sample. After cooling to room temperature, the solution was diluted to 10 mL with diluent solution [10% (v/v) HNO_3 and 20% (v/v) H_2SO_4 in water].

For the determination of urine mercury, the same method was used as for blood but using 1 mL of urine (instead of 0.5 mL) and 0.5 mL of Triton X-100 solution 0.2% (instead of 1 mL).

Creatinine in urine was determined using the method recommended for use with the Synchron CX5 system which provides an analytical range between 10 to 400 mg/dL (884 to 35.360 µmol/L).

Procedures and Methods Used

The study was carried out for four groups of workers exposed to elementary mercury (dentistis, dental assistants, laboratory and chloralkali workers) and the results were compared to a nonoccupationally exposed group, referred to as the control group.

The control group consisted of 186 volunteers (113 men and 73 women between the ages of 15 and 84 years) with an average age of 45 years, a median age of 44 years, and a mode of 35 years. The statistical variable age in the control group follows a normal distribution (Z-Kolmogorov-Smirnov=0.778, p=0.58).

Two groups were established for personnel employed at dental clinics. The group of dental assistants consisted of 22 women and 2 men, ranging in age between 24 and 58 years, an average age of 44 years, and a median of 43 years. The dentist group consisted of 15 men and 15 women, ranging in age between 24 and 51 years, an average age of 37 years, and a median of 41 years.

The laboratory group consisted of 7 men and 7 women, employed at an analytical laboratory for environmental samples where mercury is determined frequently, with ages between 23 and 51 years, an average age of 30 years, and a median of 28 years.

The chloralkali group consisted of 54 workers, all men, and working at factories where an electrode of mercury is used to obtain chlorine for electrolysis. Their age range was between 22 and 62 years, with an average age of 48 years, and a median of 50 years.

A questionnaire was asked to be filled out by every participant which included their age, height, weight, smoking habits and frequency, frequency of fish consumption, number of amalgam fillings, and whether they had a disease that could alter the results of this study by affecting kidney function. In any case, when the values of serum creatinine and urea were above the reference intervals (0.6–0.9 mg/dL serum creatinine in women, 0.8–1.2 mg/dL serum creatinine in men and 8–21 mg/dL of urine nitrogen (BUN)) (27), the results were not taken into account in order to eliminate participants with a renal dysfunction.

Since it is difficult to quantify fish consumption (28), Mushroom et al. used the following distribution in a study they performed in 1994: more or 1 time a day, 3–4 times a week, 1 time a week, 1 time a month, never (29). In 1991, Brune et al. used the same configuration for their study to assess fish consumption. We used a similar configuration for the present study as follows: Zero consumption, 1 to

2 times a week, 3 to 4 times a week, 5 to 6 times a week, more than 6 times a week (30).

Blood specimens were collected by venipuncture with a vacuum blood collection tube. Venoject® tubes (Model VT-109SAS, Terumo Medical Products, Hangzhou, P.R. China) of 9 mL with gel and clot activator and Venoject® lithium heparin tubes (Model VT-050SHL) of 5 mL were used for serum and blood collection, respectively.

Urine samples were taken from first urination in the morning in order to unify the schedule and to prevent circadian cycles as well as to obtain lower factors of dilution. The values of urine mercury were divided by urine creatinine values to correct the different urine flow and dilution in accordance with the recommendations published by the Spanish National Institute for Occupational Security and Hygiene (31). Urine samples that were too diluted or concentrated were rejected. For instance, urine with creatinine values lower than 0.5 g/L or higher than 3 g/L (28) were rejected since both high and low speeds of urine production result in a poor correlation between values of 24-hour urine mercury and those values corrected with creatinine (32).

The specimens were refrigerated between 2–7ºC and stored for a maximum of two days. When longer storage was required, the samples were frozen to –20ºC.

Sample analyses were always carried out in duplicate. Seronorm™ Trace Elements Whole Blood, levels I and II, (Nycomed AS, Oslo, Norway) and Biorad Lyphocheck Urine Chemistry Control, levels 1 and 2, were used as internal controls (Bio-Rad Laboratories, Hercules, CA, USA). Our laboratory takes part in the intercomparative program of mercury in blood and urine, referred to as the "Interlaboratory Comparison Program" of the Toxicology Centre of Quebec (CTQ), National Institute of Public Health, Quebec, Canada.

RESULTS

Mercury in Blood

Since a person's age is related to the amount of concentration of mercury found in blood and in order to compare the mercury-exposed groups with each other and then with the control group, only the results obtained for the ages ranging from 23 years (lowest age in dental assistant group) to 58 years (highest age in dentist group) were selected. The age groups are listed in Table I. The statistical indexes of blood mercury for these groups are shown in Table II.

Because a variance homogeneity test indicates a statistically significant difference between the groups, the non-parametric test of Kruskal-Wallis (33) was used to compare the groups listed. This test allows to reject, with a probability of 89%, the null-hypothesis of equality among the groups (χ^2=0.8946; df=2; p=0.011). In order to verify which group causes the significant result, the groups were compared with the non-parametric U-test of Mann-Whitney (33), resulting in statistically significant differences between the dentist group and the control group (p=0.005), and between the dentist group and the dental assistant group (p=0.046). The null-hypothesis of equality cannot be rejected between the control group and the dental assistant group (p=0.289).

There is no statistically significant difference between the lab group and the control group when the U-test of Mann-Whitney was applied (p=0.816). Moreover, as expected, the latter test did not show a significant difference between the lab group and the dental assistant group (p=0.375). The chloralkali group, where the average blood mercury concentration was highest (14.4 µg/L), supports

TABLE I
Age of Control Group, Dental Assistants, and Dentists

Statistical	Control Group	Dental Assistants	Dentists
Sample Size	121	28	23
Mean	41.5	44.3	37.0
Standard Deviation	9.46	8.75	10.48
Minimum	23	24	24
Maximum	58	58	51
Median	41	43	40

TABLE II
Blood Mercury (in µg/L) for the Different Groups With Ages Ranging From 23 and 58 Years

Groups	N	Average	Std. Dev.	Median	Max.	Min.
Control	121	7.4	5.87	6.1	33.9	0.7
Dentists	28	9.6	4.80	8.5	23.0	3.0
Dental Assistants	23	7.1	2.38	6.7	14.5	2.5
Laboratory	15	6.1	2.56	6.0	10.4	0.7
Chloralkali	36	14.4	6.65	14.0	32.0	4.0

N=number of participants.

the statistically significant differences with the rest of the groups.

The distribution of the different group variants is represented with box plots in Figure 1.

Since the consumption frequency of fish influences the concentration of blood mercury, all groups were compared with each other using the Chi-square test (33). A statistically significant difference between the groups was not observed (χ^2=9.422; p=0.308) and, therefore, the differences in blood mercury cannot be attributed to fish consumption

Urine Mercury

Statistical indexes of urine concentration corrected with creatinine for the different groups are listed in Table IV. The distribution of the different group variants is represented with box plots in Figure 2. This plot shows, apart from the expected higher level of urine mercury in the chloroalkali group, a little difference between the dentist group and the rest of the groups, indicating a possible exposure to mercury in this group.

Statistical variants are transformed to log variants to comply with normal distributions. Normality tests, Kolmogorov-Smirnov and Shapiro-Wilks (33), of the different groups are shown in Table V.

There is a statistically significant difference between the control, dental assistant, and dentist groups when the non-parametric Kruskal-Wallis test was performed (p<0.001). This difference remains when the cases are restricted to the same age interval as was applied for blood.

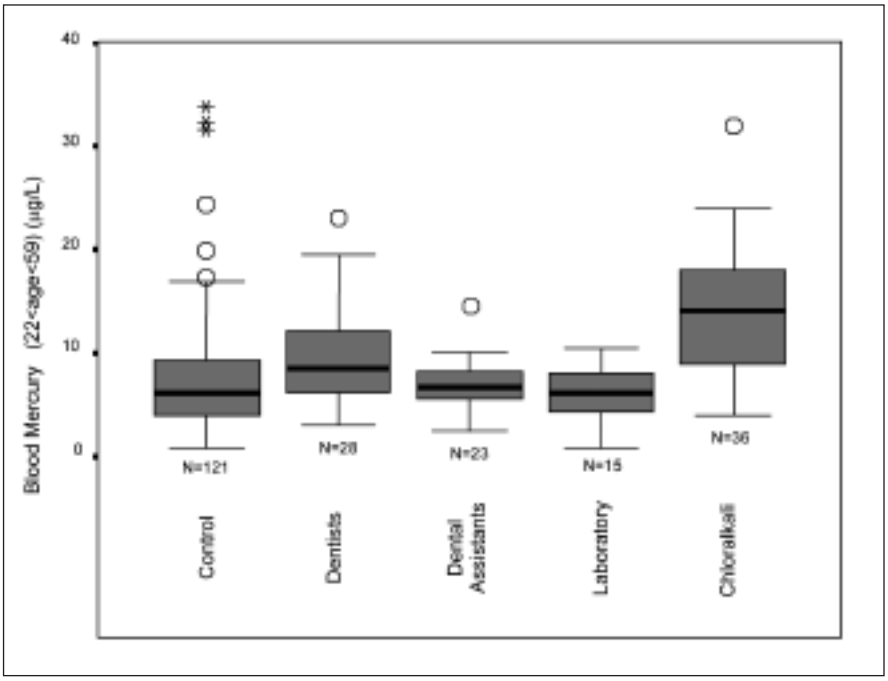

Fig. 1. Box plots representing variant distribution in all groups within the 23 and 58 year age range.

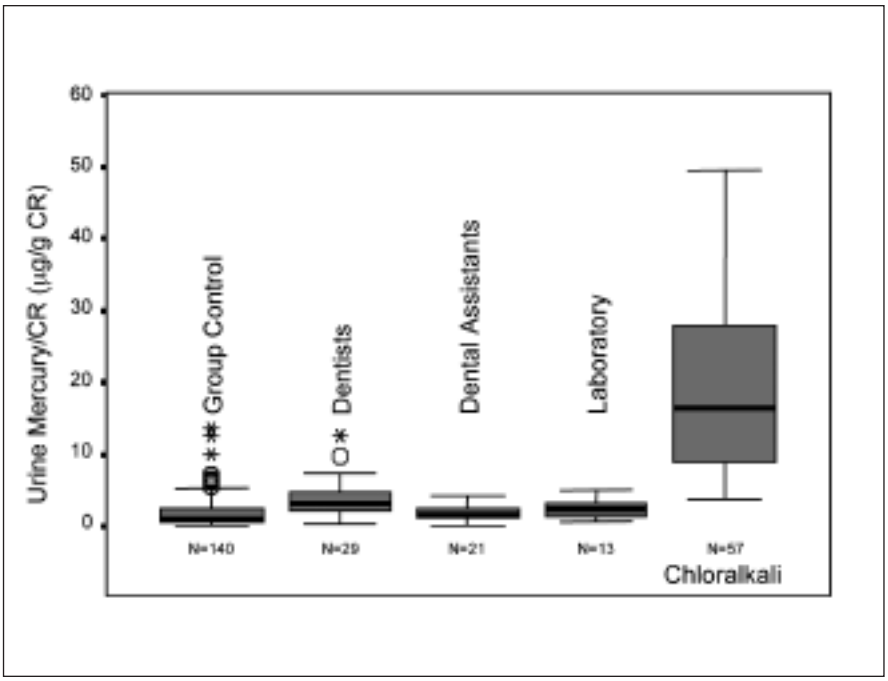

Fig. 2. Box plots representing variant distribution in all groups.

TABLE III
Fish Consumption Frequency in the Different Groups Analyzed (N=Number of Participants)

Fish Consumption (times per week)	Control Group N	%	Dentists N	%	Dental Assistants N	%	Laboratory N	%	Chloralkali N	%
Zero Consumption	5	2.8%	1	3.4%					2	4.8%
1 to 2	94	52.5%	14	48.3%	8	34.8%	11	73.3%	26	61.9%
3 to 4	56	31.3%	13	44.8%	13	56.5%	2	13.3%	11	26.2%
5 to 6	18	10.1%	1	3.4%	2	8.7%	1	6.7%	3	7.1%
More than 6	6	3.4%					1	6.7%		

TABLE IV
Statistical Indexes of Urine Concentration Corrected With Creatinine in the Different Groups (µg of Hg per gram of Creatinine) (N=Number of Participants)

Groups	N	Mean (µg/g CR)	Std. Deviation	Median (µg/g CR)	Maximum	Minimum
Control	140	2.0	2.168	1.1	0.1	13.6
Dentists	29	3.9	2.593	3.1	3.4	12.3
Dental Assistants	21	1.9	0.949	1.6	0.1	4.2
Laboratory	13	2.4	1.265	2.3	0.8	5.0
Chloralkali	57	19.4	13.139	16.5	3.7	49.6

TABLE V
Kolmogorov-Smirnov and Shapiro-Wilks Normality Tests for the Different Statistical Variants

Variant	Group	Kolmogorov-Smirnov Statistic	fd	Sig.	Shapiro-Wilks Statistic	fd	Sig.
Urine Mercury µg/Hg / g Creatinine	Control	0.191	140	<0.001[a]			
	Dentists				0.834	29	<0.010[a]
	Dental Assistants				0.961	21	0.527
	Laboratory				0.946	13	0.519
	Chloralkali	0.142	57	0.006[a]			
Log Urine Mercury	Control	0.049	140	0.888			
	Dentists				0.935	29	0.091
	Dental Assistants				0.802	21	<0.010[a]
	Laboratory				0.953	13	0.607
	Chloralkali	0.079	57	0.871			

([a]) Null-hypothesis is rejected with a significance p<0.01.

To compare the control, dentist, and dental assistant groups, a non-parametric Mann-Whitney test was used. This test shows a statistically significant difference ($p<0.001$) between the control and dentists groups, and between the dentist and dental assistants groups. However, there is no significance when it comes to comparing the control and dental assistant groups (see Table VI). The same result remains when the age range is restricted to 23 to 58 years. A Student's t-test also reflects a statistically significant difference ($p<0.001$) in the log variants of the control and dentist groups which still remains when the cases are restricted to age ranges from 23 to 58 years.

The higher urine mercury concentration in the dentist group cannot be imputed to a higher fish consumption since, as can be observed in Table III, the fish consumption is frequently higher for the dental assistant group than for the dentist group. Moreover, a Chi-square test (33) does not show any statistically significant differences in the number of amalgam fillings between all groups ($\chi^2=6.852$; df=8; $p=0.553$).

In the laboratory and control groups, the U-Mann-Whitney test does not reflect a statistical difference between urine mercury variants nor between the same groups with ages ranging from 23 to 58 years.

As was expected, there is a statistically significant difference between the urine mercury log variants of the control and chloralkaly groups when the Student's t-test was applied ($p<0.001$).

RESULTS AND DISCUSSION

The results show that, of all groups, only the dentist and chloralkali groups were found to be exposed to mercury (Tables II and IV), although the exposure for the dentists is really low.

Numerous studies (17–18,21, 34–45) have been published investigating mercury exposure from dental amalgams. However, the declining use of amalgams and the implementation of hygiene protocols, such as using encapsulated amalgams and automatic triturators, has reduced exposure to this toxic element (11). It has been reported that encapsulated amalgams result in lower levels of urine mercury exposure than with semi-open methods (21,45).

The results of different studies indicate a progressive reduction over the years of urine mercury concentration in personnel from dental clinics. For instance, a study by Joselow et al. in 1968 (34) reported 40 ug/L CR, Naleway et al. carried out a study between 1975-1983 with 4272 U.S. dentists and reported 14.2 µg/L CR (41). In 1991, Akesson et al. reported the lowest levels of 3.4 µg/g CR (45), which is similar to the levels found in dentists studied in the present work (3.9 µg/g CR).

The dentist group has blood and urine mercury levels significantly higher than those found in the control or the dental assistant group, without a significant difference between the last two groups. This difference cannot be attributed to other factors (such as fish consumption, amalgam fillings, and age) that can increase mercury concentration. Firstly, the dentist group has a mean age lower than the rest of the groups (41.5 years in control group, 44.3 years in dental assistant group, and 37.0 years in dentist group). Secondly, there is no statistically significant difference ($\chi^2=9.422$; $p=0.308$) in the frequency of fish consumption between these groups. And finally, a Chi-square test does not show any statistically significant differences in the number of amalgam fillings between all groups ($\chi^2=6.852$; $p=0.553$).

In the chloralkali group, clearly exposed to vapor mercury, there appears a statistically significant correlation between blood and urinary mercury (N=42; rho=0.586; $p<0.001$). This correlation is indicated also in the bibliography (43-44). Gothe, studying the concentration of mercury in blood and urine of 185 exposed workers, finds a significant correlation between both variants with a coefficient of Pearson's correlation of

TABLE VI
Non-parametric U Mann-Whitney Test
to Compare the Control, Dentist, Dental Assistant, and Laboratory Groups

Groups	U Mann-Whitney	W Wilcoxon	Z	Significance
Dental Assistants and Dentists	119	350	–3.646	Less than 0.001[a]
Dental Assistants and Control	1163	11033	1.541	0.123
Dentists and Control	858	10728	–4.885	Less than 0.001[a]
Laboratory and Control	613	5385	–1894	0.52

([a]) Null-hypothesis is rejected with a significance $p<0.001$.

0.51 (46). Mazarrasa et al. studied a group of 58 exposed workers and found a coefficient of Pearson's correlation of 0.67 (N=58; $p < 0.01$) (47). Both correlation coefficients are similar to the coefficient obtained in this work (0.586).

CONCLUSION

In this study, mercury in blood and urine was determined using a flow injection system based on a cold vapor atomic absorption spectrometry technique (CVAAS). Four groups of occupationally exposed workers were selected: dentists, dental assistants, laboratory and chloralkali workers. The results were compared with a control group. It was found that the chloralkali group showed the highest Hg exposure (14.4 µg/L in blood and 19.4 µg/g CR in urine) while the dentist group showed the lowest Hg exposure (9.6 µg/L in blood and 3.9 µg/g CR in urine).

The mercury concentration found in dentists is still high and cannot be attributed to a contaminated environment in dental clinics since dental assistants do not show significant differences in urine and blood mercury levels in comparison to the non-exposed population. Therefore, this high Hg concentration found in dentists must result from their specific work and a lack of proper safety measurements used. A further study would be required to assess this question of high Hg exposure of dentists and how to eliminate this contamination.

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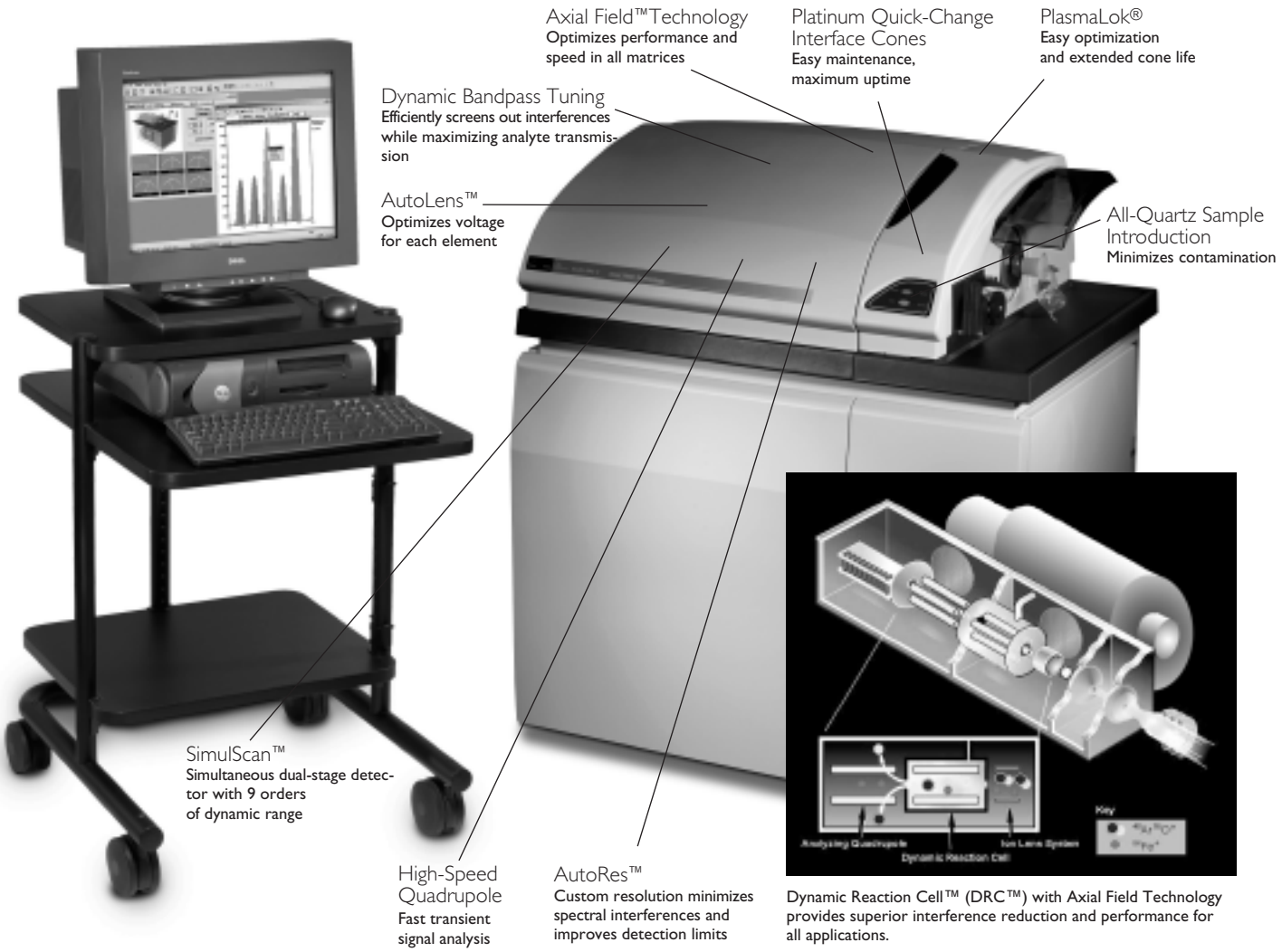

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