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Determination of Selenium in Biological Samples Using ICP-QMS

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INTRODUCTION

Selenium is an important essential trace element and of increasing interest in the study of nutrition and biochemical processes. It is known to be toxic at high levels and a Se deficiency has been related to a various physiological disorders (1). Selenium intake differs from region to region, as shown by the blood selenium levels measured in the population of different countries (2). This is in part due to a widespread soluble discharge of selenium from agriculture and urban industrial sources (3,4), which makes the study of selenium behavior and its accumulation in the environment essential.

The quantitative determination of Se in biological samples requires sensitive analytical methods because of its relatively low concentration. Inductively coupled plasma mass spectrometry (ICP-MS) offers an excellent possibility for the measurement of trace and ultra-trace elements in biological materials, because of the ability to achieve low detection limits, high sensitivity, and good precision (5). However, selenium determination in solid material is difficult due to problems such as loss of selenium during sample digestion because of the volatility of selenium compounds. In order to minimize selenium loss, closed-vessel microwave digestion has been applied for the treatment of coal and ash reference materials (6), water, biological tissue, and sediments samples (7), plant and grain reference materials (8), biological reference materials (9) and plankton (10). In general, closed-vessel microwave digestion for total Se determination showed

ABSTRACT

A method for the determination of selenium traces in plant tissue samples is described. Freeze-dried and homogenized biological samples were decomposed with HNO_3, HF and H_2O_2 by using closed-vessel microwave digestion under temperature and pressure control. NIST SRM 1577b Bovine Liver and NIST SRM 1547 Peach Leaves reference materials were investigated to optimize the analytical procedure. Selenium concentrations were measured with quadrupole-based inductively coupled plasma mass spectrometry (ICP-QMS) using external calibration and the isotope dilution method. A special solution introduction device combining pneumatic nebulization with hydride generation in the thin liquid film on the walls of the minicyclonic spray chamber was employed for sample introduction into the ICP-MS, which allowed the sensitivity for Se to be increased by up to one order of magnitude without increasing the memory effects. SRMs were doped with different amounts (0, 0.1, 0.2, 0.5 and 1.0 µg/g) of enriched ^{78}Se spike (98.58% of ^{78}Se) before digestion to study the method performance and selenium losses during sample preparation. For a given matrix selenium losses were reproducible as follows: 9.9±1.6% for Bovine Liver SRM, 15.8±3.6% for Peach Leaves SRM and 20.0±4.5% for the real plant tissue samples. The detection limit for selenium calculated for solid plant tissue was 0.2 µg/g (3σ-criteria, m/z=82, digestion 1:1000) using conventional pneumatic nebulization for solution introduction and 0.03 µg/g for a combination of pneumatic nebulization with hydride generation. Applying the method developed, a large number of plant tissue samples were analyzed to study selenium behavior and accumulation in the environment.

good results, although losses during the sample preparation procedure could not be completely ruled out (6). Other analytical problems for Se determination include a relatively low sensitivity of ICP-MS due to the high ionization potential of selenium (9.8 eV) and limitations caused by isobaric interferences of the major selenium isotopes (m/z= 76, 78, and 80 u) with the argon dimers ($^{40}Ar^{36}Ar^+$, $^{38}Ar_2^+$, $^{40}Ar^{38}Ar^+$ and $^{40}Ar_2^+$). Hence, only the less abundant isotopes (m/z= 74, 77, and 82) are used for quantification in ICP-QMS instruments (7, 11). All of the above-mentioned factors affect the sensitivity and accuracy of ICP-MS measurements.

One of the well-known sample introduction techniques used for increasing the sensitivity of selenium determination with ICP-MS is based on hydride generation (12-15). It allows selenium to be separated from the sample matrix, thus achieving Se preconcentration from the sample. This method has been successfully applied for the determination of selenium in various matrices including marine certified reference materials (14), radioactive waste materials (15), geological material (16,17), natural water and seawater (13,18,19), as well as for trace element speciation (20). However, the formation of volatile hydrides can be suppressed by transition metals (21) if they are not separated from the analyte before the measurement is performed. Non-volatile selenium species are produced by a reaction between selenium hydride and interfering metals (22). A low-volume "movable reduction bed hydride generator" was proposed by Tian et al. (23) to minimize suppression effects, where the volatile products were immediately

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removed from the reaction surface by a flow of gas. Some studies (24,25) have also focused on the use of very short reaction times (less than 100 ms) and rapid separation of the gaseous products. To this end, an additional inner capillary was mounted in a concentric nebulizer (for simultaneous introduction of the sample and reductant) and a spray chamber (for gas-liquid separation of the product) was used. In a recent paper (26), the sample introduction system consisting of a cross-flow nebulizer and a modified Scott spray chamber was applied for the determination of Se and other hydride-forming elements in biological materials. A short reaction time was required and rapid separation of the reaction products was achieved by mixing the acidified sample and sodium boro-hydride solution at the tip of a cross-flow nebulizer.

In the present work, an analytical method was developed for the determination of selenium in plant tissue samples. The most important goal was to improve the sensitivity and detection limits for selenium while maintaining the advantages of conventional sample introduction, based on pneumatic nebulization, and to study and minimize possible selenium losses during sample preparation. For this purpose, a closed-vessel microwave digestion procedure under temperature and pressure control was applied. The selenium loss during sample preparation was studied by using standard reference materials and real plant tissue samples spiked with high-enriched ^{78}Se. A solution introduction system was studied, consisting of a microconcentric nebulizer and a minicyclonic spray chamber for hydride generation in the thin liquid film formed on the inner walls of the water-cooled minicyclonic spray chamber together with pneumatic nebulization. This approach allowed the fast extraction of volatile hydrides from

the solution, thus overcoming the reaction between selenium hydride and transition metals and resulting in reduced memory effect due to the short wash-out time.

EXPERIMENTAL

Instrumentation

A quadrupole-based ICP-MS, the ELAN® 6000 (PerkinElmer SCIEX, Ontario, Canada), was used for selenium measurements. The gas input was controlled by built-in mass flow controllers. For solution introduction into the inductively coupled plasma, a microconcentric MicroMist nebulizer (Model MicroMist AR30-1F02) with a solution uptake rate of 0.3 mL min^{-1} attached to a Cinnabar minicyclonic spray chamber (both from Glass Expansion Pty. Ltd., Camberwell, Victoria, Australia) was used. Solution aspiration was performed with a peristaltic pump (Perimax 12, Spetec GmbH, Erding, Germany).

In order to raise the sensitivity for selenium and to improve the conventional solution introduction system, selenium hydride generation was performed directly in the spray chamber. The thin liquid film, which is formed from the aerosol waste by the argon flow on the inner wall of the minicyclonic spray chamber, was used as the reaction volume for hydride generation. The advantage of this approach is the ability of a fast extraction of the volatile selenium hydrides and a short wash-out time, thus reducing memory effects (see Figure 1). The aerosol droplets precipitating on the spray chamber wall were mixed with 0.4% NaBH$_4$ in 0.4% NaOH solution in Milli-Q™ water, which was introduced on the wall opposite the nebulizer with an additional pump at a solution uptake rate of 0.05 mL min^{-1}. This provided the extraction of other elements that also form volatile hydrides from the aerosol waste. The spray chamber was placed in the cavity filled with

water at a temperature of 18°C, resulting in improved stability and yield of hydride generation.

Samples and Standards

The samples represented different parts of plants (Typha latifolia L. and Phragmites australis) as well as sediments samples from an outdoor "constructed wetland" (aquatic model vegetation facility). The plants had been watered with a solution spiked with selenium in the form of sodium salt, Na$_2$SeO$_4$. NIST SRM 1577b Bovine Liver and NIST SRM 1547 Peach Leaves were used for the method development and quality assurance of the analysis. The high-enriched ^{78}Se solution, consisting of ^{74}Se (0.06±0.02)%, ^{76}Se (0.11±0.02)%, ^{77}Se (0.17±0.02)%, ^{78}Se (98.58±0.08)%, ^{80}Se (1.0±0.02)%, ^{82}Se (0.08±0.02)%, was obtained from Oak Ridge National Laboratory.

Reagents

Suprapur® nitric acid, hydrogen peroxide, and hydrogen fluoride (all from Merck, Darmstadt, Germany) were used for the sample digestion. The nitric acid was further purified by subboiling-point distillation. The calibrating solutions were diluted from a 1000 µg/L single-element selenium standard solution (Merck). The acid content of the calibrating solutions was set to 2% with subboiled nitric acid. High-purity deionized water (18 MΩ, produced with a Milli-Q Plus water purification system) was applied for the dilution of the samples and calibrating solutions.

Sample Preparation

A microwave oven digestion procedure was developed for the Se determination in plant samples, using SRM 1547 Peach Leaves. The 100-mg biological samples were digested in closed vessels (XP-1500) in a microwave oven (Microwave Activated Reaction System Mars 5, CEM Corporation, USA) under temperature and pressure control using

a mixture of 2 mL concentrated Suprapur nitric acid, 0.5 mL 40% HF, and 0.2 mL 30% hydrogen peroxide (analytical grade purity). Some samples were spiked with 0.1–0.5 μg ^{78}Se (^{78}Se 98.58%) before digestion for isotope dilution analysis.

The plant tissue samples were freeze-dried and milled before digestion and prepared as mentioned above. Sample aliquots of ~100 mg were digested as described above. The digested samples were transferred to clean polypropylene tubes (15 mL, Falcon) and made up to 10 mL with Milli-Q water.

Measurement Procedure

The experimental parameters of the ELAN 6000 ICP-QMS (see Table I) were optimized with respect to the maximum ion intensity of ^{82}Se$^+$. The ICP-MS was flushed with a 2% HNO$_3$ solution for 5 min between aliquot measurements to reduce the memory effect of selenium. SRM 1547 Peach Leaves was used for the development of the digestion procedure and for quality control during routine analysis of the plant samples. Isotope dilution analysis and external calibration using ^{103}Rh as an internal standard was applied for ICP-MS measurements.

Instrumental Neutron Activation Analysis (INAA)

Sample irradiations were carried out at the FRJ-2 reactor. The use of the k$_0$ method of INAA (as applied in the Central Department for Analytical Chemistry at the Research Center Jülich (27)] allowed the accuracy to be improved and did not require the use of standards, thus opening up the possibility of absolute measurements. Irradiation of the samples with neutrons was carried out in the in-core irradiation system (central channel) of the FRJ-2 reactor with the density of the thermal neutron flux being $\Phi = 8 \cdot 10^{13}$ cm^{-2}s^{-1} for one hour.

TABLE I
Experimental Parameters Used for ELAN 6000 ICP-QMS

PerkinElmer SCIEX ELAN 6000 ICP-MS	
RF Power	1375 W
Coolant Ar Flow Rate	17 L/min.
Plasma Ar Flow Rate	1.2 L/min
Nebulizer Ar Flow Rate	0.95 L/min
Sample Uptake Rate	0.32 mL/min
Nebulizer Type	Microconcentric Micromist
Spray Chamber	Minicyclonic Cinnabar
Sampler Cone	Nickel, 1.1 mm Orifice Diameter
Skimmer Cone	Nickel, 0.9 mm Orifice Diameter
Dwell Time	200 ms
Replicates	5
Sweeps/Replicates	10
Isotopes Measured	^{76}Se, ^{77}Se, ^{78}Se, ^{82}Se
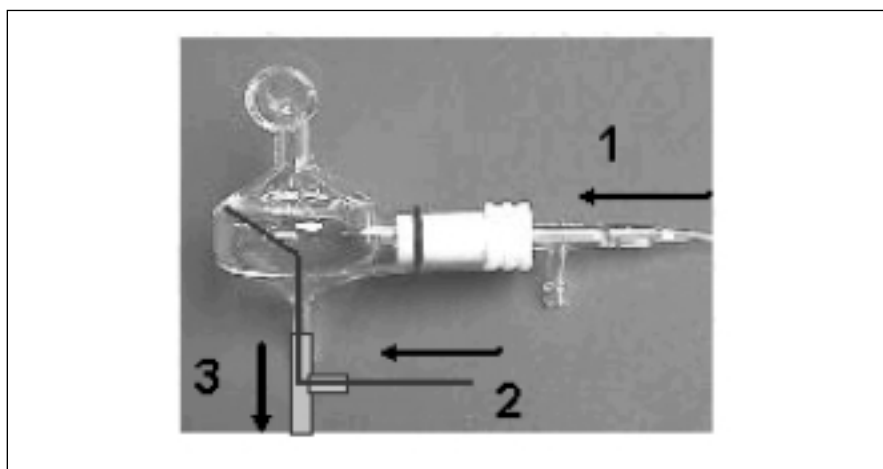
Fig 1. Sample introduction system: 1 – sample solution introduced by a MicroMist nebulizer, 2 – solution of 0.4% NaBH$_4$ in 0.4% NaOH in Milli-Q water introduced on the inner wall of the spray chamber opposite the nebulizer, 3 – waste solution.

A Genie-PC Spectroscopy System (Model S400) with coaxial semiconductor Ge-detectors from Canberra Packard was used to measure the induced γ-activity.

RESULTS

Optimization of Solution Introduction Into the ICP

In order to improve the selenium sensitivity, the performance of a solution introduction system (Figure 1), combined with pneumatic nebulization with hydride formation, was studied. Application of a minicyclonic spray chamber allows the production of a thin liquid film on the inner walls of the spray chamber, in which additional chemical reactions are possible. The sample solution was nebulized in a minicyclonic spray chamber and the waste mixed on the inner spray chamber wall with NaBH$_4$ in 0.4% NaOH solution in Milli-Q water. This solution was introduced by an additional pump on the wall opposite the nebulizer. As a result, a thin liquid film formed in which hydride generation of Se was very

effective. This approach also allowed the fast extraction of volatile hydrides from the solution and the reduction of memory effects.

Examples of the dependence of Se sensitivity on solution uptake rate at different concentrations of $NaBH_4$ are presented in Figure 2. At lower $NaBH_4$ concentrations (0.1% $NaBH_4$ in 0.4% NaOH), the maximum ion sensitivity of analytes is shifted to higher solution uptake rates. However, higher $NaBH_4$ solution uptake increases the thickness of the liquid film formed on the walls, which in turn decreases the yield of volatile selenium hydrides. Optimal conditions with maximum ion intensity of analytes were found at a concentration of 0.4% $NaBH_4$ at a flow rate of 0.05 mL min^{-1}. Further increasing the concentration to values higher than 0.5% $NaBH_4$ led to a relative decrease of sensitivity. The temperature of the spray chamber walls was found to be crucial to the stability of hydride generation. To obtain a stable signal, the spray chamber was placed in a cavity filled with water at a temperature of 18°C.

The device allowed the simultaneous determination of hydride-forming elements as well as all other elements. Figure 3 shows the behavior of the $^{82}Se^+$ and $^{103}Rh^+$ intensities measured in solution containing 1 µg L^{-1} Rh and 10 µg L^{-1} Se. When the $NaBH_4$/NaOH solution was delivered to the inner wall of the spray chamber, the $^{82}Se^+$ intensity increased. Cooling the spray chamber to 18°C improved the sensitivity and stability for the Se$^+$ ion signal. However, an investigation of the dependence of these parameters on the spray chamber temperature will be subject of a future work. The procedure developed allowed the sensitivity for Se to be improved by approximately one order of magnitude due to the extraction of additional volatile selenium hydrides from the waste
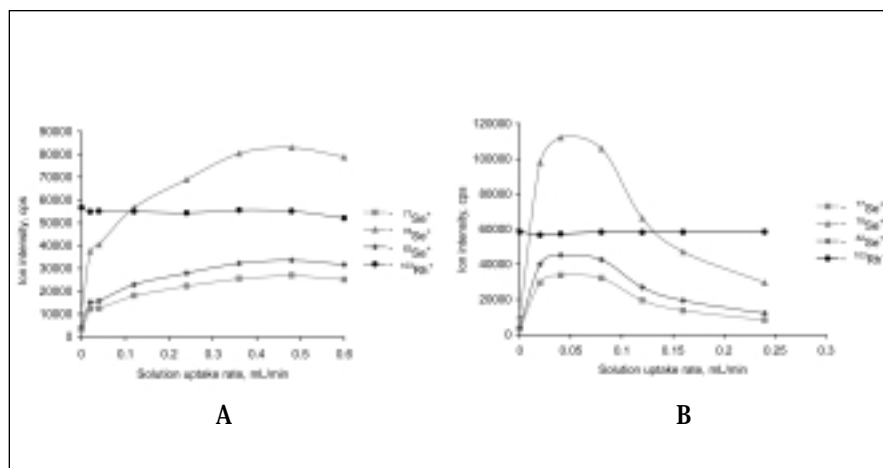
Fig. 2. Dependence of ion intensities of $^{77}Se^+$, $^{78}Se^+$, $^{82}Se^+$ and $^{103}Rh^+$ on the uptake rate of $NaBH_4$/NaOH solution (1 µg L^{-1} Rh and 10 µg L^{-1} Se solution was used, spray chamber was cooled to 15°C, experimental parameters of the ICP-MS are summarized in Table I):
(A) with 0.1% $NaBH_4$ in 0.4% NaOH solution,
(B) with 0.4% $NaBH_4$ in 0.4% NaOH solution.
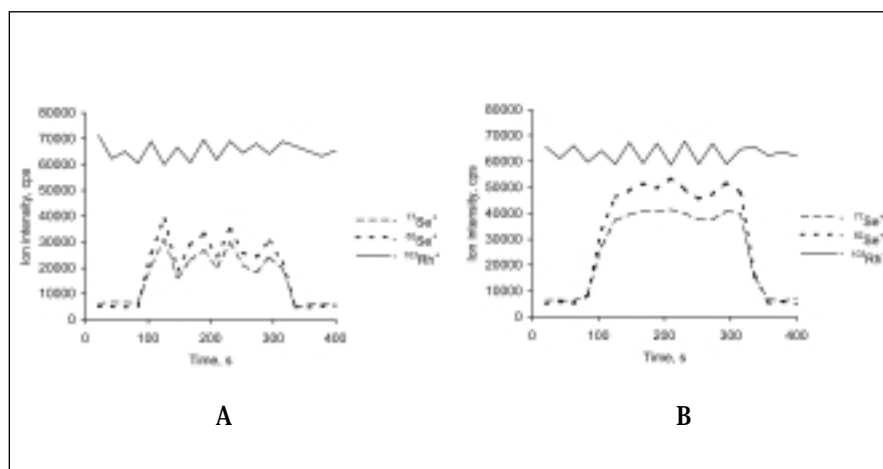
Fig. 3. Behavior of ion intensities of $^{77}Se^+$, $^{82}Se^+$ and $^{103}Rh^+$ while turning the pump delivering $NaBH_4$ / NaOH solution to the microconcentric spray chamber on/off (1-µg L^{-1} Rh and 10-µg L^{-1} Se solution was delivered continuously, 0.4% $NaBH_4$ in 0.4% NaOH solution was introduced at solution uptake rate of 0.05 mL/min, dwell time 20 ms, sweeps number 10, other parameters are summarized in Table I):
(A) at a room temperature of 24°C
(B) at a temperature of 18°C in the water-cooled spray chamber

without loss in sensitivity for other elements (see Rh in Figure 3). Moreover, the memory effect was not an issue, because the spray chamber was flushed as usual with the aerosol delivered by the nebulizer and the waste was immediately pumped off.

In spite of the fact that about 90% of the selenium ion intensity was measured due to volatile hydride formation, while rhodium was nebulized via conventional pneumatic nebulization, the optimum nebulizer gas flow rate was found to be nearly the same for

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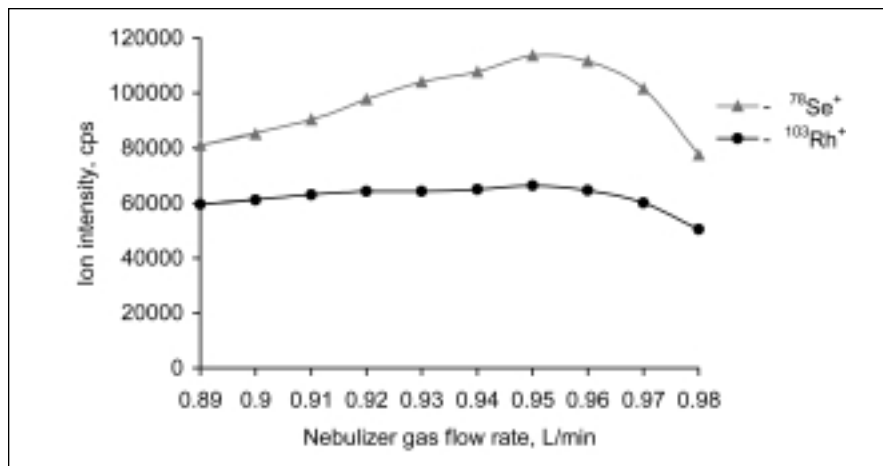
Fig. 4. Dependence of ion intensities of $^{78}Se^+$ and $^{103}Rh^+$ on nebulizer gas flow rate (1-µg L^{-1} Rh and 10-µg L^{-1} Se solution). 0.4% $NaBH_4$ in 0.4% NaOH solution was introduced at solution uptake rate of 0.05 mL/min, spray chamber was cooled to 18ºC, other ICP-MS parameters are summarized in Table I.

both elements. The dependence of sensitivities for $^{82}Se^+$ and $^{103}Rh^+$ on the nebulizer gas flow near the optimum value is shown in Figure 4. Optimization of the nebulizer gas flow rate with respect to maximum ion sensitivity depends both on the plasma conditions and on the yield of pneumatic nebulization/hydride formation. Changing the nebulizer gas flow rate had more influence on the selenium sensitivity than on the rhodium sensitivity. However, close investigation of these effects was not the aim of this work. In any case, approximately the same behavior of different elements

makes it easy to optimize experimental conditions for multielement determination by applying simultaneous pneumatic nebulization/ hydride formation. Furthermore, the plateau of the optimization curves decreases the measurement errors due to uncertainty of the experimental parameters.

Sample Digestion

Because of the volatility and possible losses of selenium during digestion, the dependence of digestion quality on temperature was tested using SRM 1547 Peach Leaves. The temperature for

microwave digestion was changed from 150ºC to 200ºC. After sample digestion, the selenium content was measured by ICP-MS using external calibration and the isotope dilution method in (1:200 – 1:1000) diluted solution (depending on sample matrix – sediment or plant tissue – and on analyte concentration in the original samples). The results of both methods were compared with the certified values. The selenium concentration using the external calibration method (Table II) and-measured via ^{77}Se was systematically higher than the concentration obtained via ^{82}Se. Thus, ^{82}Se was chosen as the analytical isotope for selenium measurement in biological samples since ^{77}Se can be affected by $^{40}Ar^{37}Cl^+$ ions.

At a temperature of 150ºC, the selenium concentrations measured by external calibration were lower than those certified. Probably, digestion was not complete at this temperature, although visual control of the digested sample did not cast any doubt on the digestion quality. However, the results obtained using the isotope dilution method were higher than the certified values. The best agreement of values measured by both methods with certified values was observed at a temperature of 175ºC. The results of the isotope dilution method agreed with certified values

TABLE II
Measured Concentrations and RSD (n=3) of Se in SRM 1547 Peach Leaves Using Microwave Digestion Under Different Experimental Conditions

Digestion Parameters			External Calibration		Isotope Dilution	
Temperature (ºC)	Pressure (psi)	Isotope	Concentration (ng/g)	RSD (%)	Concentration (ng/g)	RSD (%)
150	47	^{82}Se	88	14	149	28
		^{77}Se	92	30	137	45
175	110	^{82}Se	101	3.6	129	3.2
		^{77}Se	114	2.5	124	2.3
200	210	^{82}Se	83	11	105	22
		^{77}Se	99	11	107	24
Certified concentration (ng/g)			120±9			

within experimental errors. The results of the external calibration method using [82]Se as an analyte were consistently lower than the certified concentration by 15.8%. These values were used later for correction of the measured selenium concentrations in the analyzed samples. At a temperature of 200ºC, the experimental values obtained with both methods were lower than the certified concentrations.

The minimum deviation of the measured values in three independent measurements, including three digestions in different vessels in the same digestion cycle (1 control vessel for temperature and pressure measurement and 2 vessels without control), was obtained at a temperature of 175ºC (Table II). At 150ºC and 200ºC, the deviation of experimental results was much higher, possibly due to the non-controlled deviations of experimental conditions from vessel to vessel. However, these deviations did not strongly influence the measurement results in the medium temperature range, which makes a temperature of about 175ºC most advantageous for selenium determination.

Plant Sample Analysis

Using the method described, a wide variety of plant tissue samples were analyzed to study the selenium behavior and its accumulation in the environment. The

detection limit for selenium calculated for solid plant tissue was 0.2 µg/g (for liquid sample 0.2 ng/mL) using pneumatic nebulization and 0.03 µg/g (for liquid sample 0.03 ng/mL) using a combination of pneumatic nebulization with hydride generation. The detection limits in biological samples were determined from the mass spectrum of the (matrix-matched) blank solution using the 3σ criterion (the detection limit is given by $m_b+3\sigma_b$, where m_b is the mean value of the blank measurements and σ_b is the standard deviation of five independent measurements of the blank value). For a given matrix, the selenium losses were reproducible as follows: $9.9\pm1.6\%$ for Bovine Liver SRM, $15.8\pm3.6\%$ for Peach Leaves SRM, and $20.0\pm4.5\%$ for the real plant tissue samples. Recovery of the results of the isotope dilution method ranged from 101.2% to 98.5% by changing the quantity of the [78]Se isotopic standard added from 0.1 µg to 0.5 µg. Instrumental neutron activation analysis was applied to compare the measurements, because it does not require any sample pretreatment, thus overcoming most problems of contamination and losses. However, the rather high limit of selenium detection provided by INAA only allowed an analysis of samples with selenium concentrations higher than 1 µg/g. Table III shows the results of selenium measurement in

different parts of plants using ICP-MS and INAA. The results obtained by different methods were reproducible and agreed within experimental errors, taking into account the correction of selenium losses during digestion, which was necessary in the case of ICP-MS with external calibration. The measured selenium content differed from sample to sample depending on plant type and plant part.

CONCLUSION

ICP-QMS was applied for the routine selenium determination in a large number of biological samples to study selenium behavior and its accumulation in the environment. A sample digestion procedure in closed vessels was developed using a microwave oven operated under temperature and pressure control, thus minimizing Se losses and enabling reproducible results to be achieved. Measurements of NIST standard reference materials and comparison with neutron activation analysis showed good accuracy of the ICP-QMS method.

A special device combining pneumatic nebulization and hydride generation in the minicyclonic spray chamber was employed for sample introduction into the ICP-MS, which allowed the sensitivity for Se to be increased by up to one order of magnitude without increasing the memory effects. The solu-

TABLE III
Concentrations of Se in Different Parts of Selected Biological Samples Determined With ICP-MS
(Relative error of the measured value ±10%, INAA results are given in square brackets)

Sample	1	2	3	4
Sample Part	Concentration (µg/g)			
Leaf	1.80 (2.1)	0.22 (<1)	1.14 (1.2)	0.25 (<1)
Stem	0.64	<0.03	0.9	0.26
Rhizome	0.44	0.29	1.92	0.35
Fine Root	0.88	0.94	4.92	1.28
Fine Material	1.27	1.09	6.0	1.01

tion introduction system can be used for Se determination as well as other hydride-forming elements such as As, Bi, Hg, Sb, and Sn. This procedure also improves the sensitivity for hydride-forming elements with simultaneous multielemental analysis.

Another possibility of improving the sensitivity and precision of Se determination is the application of ICP-QMS instruments equipped with a gas-filled collision cell. In this case, the most important mass spectrometric interferences (including argon-based molecular ions $^{36}Ar^{40}Ar^+$ and $^{36}Ar^{40}Ar^+$) affecting Se determination can be reduced or even eliminated via ion/molecule reactions in collision cells filled with collision gases. Hence, the use of the most abundant selenium isotopes is possible for detection. Further work will consider this approach for selenium determination.

ACKNOWLEDGMENTS

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New Approaches to the Direct Analysis of Highly Saline Samples by FIA-ICP-MS

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INTRODUCTION

Since its introduction at the beginning of 1980, ICP-MS has become a widely used technique mainly due to its high sensitivity, great speed, excellent detection limits, multi-elemental capacity (which includes most of the elements in the periodic table), and the possibility of performing isotopic measurements (1,2). One of the most interesting fields of application is the determination of trace elements in matrices with a high saline content. In seawater, these trace elements are present at low concentrations and in certain brines used in the chlor-alkali industry, high concentrations of certain cations may reduce the lifetime of the most recently used membrane cells (3). However, a number of interferences were identified in earlier studies dealing with ICP-MS (4,5). Many studies were subsequently carried out to identify and characterize these interferences, as can be seen in the literature (6-8).

It is well known that spectroscopic interferences are caused by atomic or molecular ions having the same nominal mass as the analyte of interest. Even when nearly all possible spectroscopic interferences are theoretically known (9), the degree of formation of these species is unpredictable, especially if the samples have complex matrices, as for saline samples. The determination of analytes (particularly below 80 m/z) in the presence of these species is problematic and the analyte mass of the polyatomic ion species cannot be resolved when conventional

ABSTRACT

The direct FIA-ICP-MS determination of Al, As, Cr, Mn, Mo, Ni, Pb, Rh, Sb, Te, and V in saline matrixes with different total dissolved solids content (TDS) (0.03-30%) was studied. Sample introduction by FIA permits the nebulization of matrixes with higher TDS in the ICP-MS. In this way, the direct determination of Mo and Mn in a certified seawater sample (CASS-3) was successfully carried out and the results obtained were in good agreement with the certified values. The other elements studied were either available in very low concentrations or do not have certified values for the CASS-3 sample. The study of the influence of the instrumental parameters, such as nebulizer gas flow and plasma power, showed that the intensity obtained for the different elements studied decreases with matrix concentrations of 3% TDS or more. The higher the saline concentration, the smaller a flow is needed to minimize the interferences caused by the matrix. With regard to plasma power, it was observed that an increase in power reduces both spectroscopic and non-spectroscopic interferences. A study of the recoveries showed that Al, As, Cr, Mn, Mo, Ni, Pb, Rh, Sb, Te, and V can be determined directly by FIA-ICP-MS depending on the dissolved solids content. Mn and Mo can be determined directly in matrixes with 30% TDS or less; Sb and Pb with 15% TDS or less; Rh and Te with 3% TDS or less; Al and Cr with 0.3% TDS or less; and all elements cited can be determined directly in matrixes with 0.03% TDS.

quadrupole mass filters are used. Reduction of the polyatomic ion species can be achieved by optimization of the instrumental parameters, particularly the carrier gas flow rate and forward rf power (10). The possibility of adding inert gases or replacing one or more of the three gas flows of the ICP to reduce the polyatomic ions has also been studied. For example, the addition of nitrogen (11,12) hydrogen (13), xenon (14,15), and methane (16) has been studied. It was generally found that they had an attenuating effect on specific interfering species. Other methods for limiting or reducing spectroscopic interferences require the use of additional instrumentation, such as electrothermal vaporization (17), hydride generation (18), or laser ablation (1), although these methods require longer preparation or longer analysis times. Alternatively, instrumentation such as high-resolution magnetic-sector ICP-MS (although at high capital cost) or the use of alternative skimmer cones (19) could be employed.

Non-spectroscopic interferences refer basically to matrix effects and can also be divided into two categories: reversible and irreversible (20). Whereas irreversible effects are well known, reversible effects have not been fully characterized and may be due to changes in the transmission of ions to the MS. Although non-spectroscopic interferences caused by a saline matrix of sodium chloride have been studied since the ICP-MS was first introduced (21-23), the extent of matrix effects for the same concentration (0.1% Na) and the same elements reported in the literature ranges from a decrease of 60% in the signal

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to an increase of 100%. Attempts have been made to better understand the mechanisms involved that give rise to these differences (24-26), but there is no consensus of opinion, although all studies agree that the extent of the matrix effects depends on the operating conditions.

Most of the papers published recommend the use of off-line and on-line column preconcentration systems to reduce and eliminate non-spectroscopic interferences in samples with a high saline content (6,27,28). Systems for separating analytes from the matrix by hydride generation (29) and coprecipitation systems (30,31) have also been employed.

The purpose of the present paper was to investigate the possibility of the direct FIA-ICP-MS multi-elemental analysis of matrices with a different saline content (higher than most studies reported in the literature). To this end, the behavior of certified seawater (CASS-3) and different synthetic saline matrices with a total TDS ranging from 0.03% to 30% was studied, because very few papers deal with saline matrices (brines) with a TDS of more than 3%. The direct determination of 21 elements covering the entire range of masses (Al, As, Au, Be, Co, Cr, Cu, Hf, Ir, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Te, V, and Zn) with ionization potentials ranging from 5.986 eV (Al) to 9.394 eV (Zn) was studied. Only the quantitative determination of some elements was possible as will be discussed.

EXPERIMENTAL

Instrumentation

All experiments were performed with a PerkinElmer SCIEX ELAN® 6000 ICP mass spectrometer (Toronto, Canada), using nickel cones.

The sample solutions were aspirated into the argon plasma via a peristaltic pump (Gilson® Minipuls 3). Flow injection was used as the method of sample introduction in order to minimize the effects of nebulizer and cone blockage (see Figure 1) (32,33). In some circumstances it may actually be better to allow the sampling orifice to partially clog, thereby achieving a pseudo steady-state situation where the rate of deposition is equalled by the rate of dissociation (6). In fact, FIA-ICP-MS has already been successfully used for the direct determination of Mn, Mo, and U in reference seawater sample NASS 4 without using a preconcentration or separation step (34). The sample was injected via an automatic injection valve with 6 channels (Eurosas). The injected sample volume was 500 μL and the carrier solution was 2% HNO_3.

The instrumental operating conditions and measurement parameters are given in Table I. Prior to any experiment, the ICP-MS instrument was optimized for routine multi-elemental analysis following the manufacturer's instructions. The nebulizer gas flow rate, oxides, lens voltage, and daily performance of the instrument was optimized by aspirating a solution containing Rh, Mg, Pb, Ba, and Ce (10 μg L^{-1} each), and the autolens calibration was optimized by aspirating a solution of Be, Co, In, and U (10 μg L^{-1} each).
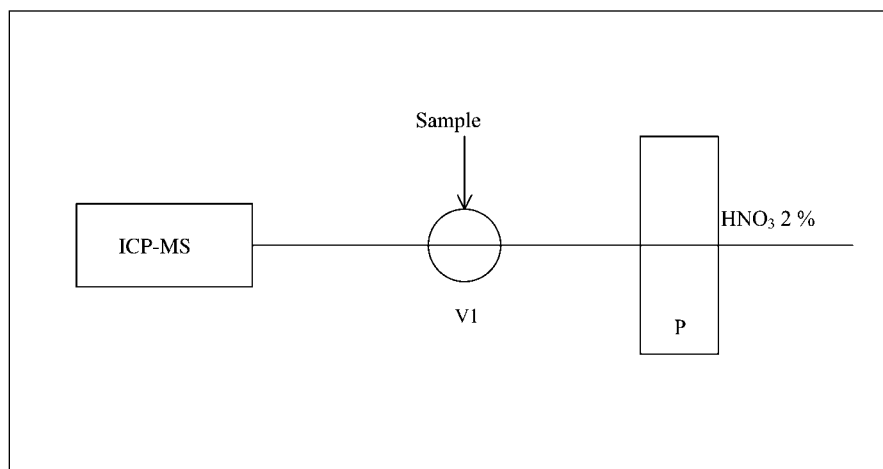
Fig. 1. Scheme of FIA setup: P: peristaltic pump, V1: automatic injection valve.

TABLE I
Instrumental Operating Conditions and Measurements Parameters for FIA-ICP-MS

Forward Power	1000 W
Sample Uptake Rate	1 mL.min^{-1}
Coolant Argon Flow	14 mL.min^{-1}
Sweeps/ Reading	1
Readings/Replicate	30
Replicates	1
Dwell Time	20 ms
Scan Mode	Peak Hopping
Injection Volume	0.5 mL

As it is well known the ICP-MS, the operating conditions may have a big influence on the analytical performance. The effect of the nebulizer gas flow rate and plasma power on the direct FIA-ICP-MS multi-elemental analysis in matrices with very high saline content was studied and the results will be discussed.

Reagents, Standards, and Sample Preparation

Nitric acid (suprapur, Baker Instra Analyzed, J.T. Baker B.V., Deventer, Holland) was used. NaCl (suprapur, Merck, Darmstadt, Germany), KCl (pro analysi, Merck, Darmstadt, Germany), $CaCl_2$ (suprapur, Merck, Darmstadt, Germany), $MgCl_2$ (pro analysi, Merck, Darmstadt, Germany) and Na_2SO_4 (Scharlau) were used for the preparation of synthetic brines (see later section). Three different aqueous stock solutions with different metal concentrations were used (see Table II): Standard 1 (Baker Instra-analyzed, J.T. Baker, Phillipsburg, NJ USA; Standard 2 (Baker Instra-analyzed, J.T. Baker, Phillipsburg, NJ USA; Standard 3 (ICP-MS Standard AccuStandard, New Haven, CT USA. Intermediate solutions of 10 mg L^{-1} were prepared from standards 1 and 2 and the different multi-elemental solutions used in the study were prepared with these intermediate solutions and standard 3: multi-elemental solution in 2% HNO_3 (containing 10 μg L^{-1} of all the elements from Table II except Al (50 μg L^{-1}), V (25 μg L^{-1}), Cd (0.25 μg L^{-1}), and Hg (0.05 μg L^{-1}); different multi-elemental solutions with 10 μg $L^{-1}$of all the elements from Table II, except Al (50 μg L^{-1}), V (25 μg L^{-1}), Cd (0.25 μg L^{-1}), and Hg (0.05 μg L^{-1}) with different TDS (0.03,0.3, 3, 15, and 30%) were prepared as later described. Sc, In, and Bi single-element standard solutions (1000 mg L^{-1}) (Baker Instra-analyzed, J.T.Baker, Phillipsburg, NJ USA) were used. From these standard solutions of Sc, In, and Bi, a 10-mg L^{-1}

intermediate solution of the three was prepared. All the samples were spiked with this solution so that the final concentration of Sc, In, and Bi was 10 μg L^{-1} which was used as the internal standards in the calibration system employed.

All solutions were prepared using ultrapure water, with a resistivity of 18.2 MΩ obtained from a Milli-Q™ water purification system (Millipore, Saint Quentis Yvelines, France). All polyethylene material was decontaminated with nitric acid (10% v/v) for at least 72 h, rinsed with ultrapure water and dried.

A seawater reference material, CASS-3 (National Research Council of Canada, Ottawa, Canada) was used. For recovery studies, this solution was also spiked with standards 1, 2, and 3 (containing 10 μg $L^{-1}$of all the metals from

Table II, except Al (50 μg L^{-1}), V (25 μg L^{-1}), Cd (0.25 μg L^{-1}), and Hg (0.05 μg L^{-1}).

The different synthetic brines with a different TDS were prepared according to the recommendation of Schulz et al. (35). In fact, two synthetic brines were prepared (15% TDS and 30% TDS for which the amounts given in Table III were weighed. These solutions were kept in the refrigerator. Other brines with a TDS of 0.03%, 0.3%, and 3% were prepared by dilution of these two synthetic brines.

RESULTS AND DISCUSSION

The aim of the present paper was to investigate a simple and rapid method for the multi-elemental FIA-ICP-MS determination of Al, As, Au, Be, Co, Cr, Cu, Hf, Ir, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Ru, Sb, Te, V, and Zn in saline matrixes with

TABLE II
Standard Solutions Used

Standard	Elements (*)
Standard 1	Al (5000), As (1000), Be (1000), Cd (250), Cr (1000), Co (1000), Cu (1000), Fe (1000), Pb 1000), Mn (1000), Hg (50), Ni (1000), V (2500), and Zn (1000)
Standard 2	Ba (500), Ca (500), Mg (1000), Mo (500), K (100) and Na (500)
Standard 3	Sb (10), Au (10), Hf (10), Ir (10), Pd (10), Pt (10), Rh (10), Ru (10) and Te (10)

(*) Values in parentheses indicate the concentration in mg L^{-1}.

TABLE III
Artificial Brines Composition

Salt*	TDS 15 g/100 mL	TDS 30 g/100 mL
NaCl	12.8803	25.7419
$CaCl_2$	1.6023	3.2029
$MgCl_2$	0.3786	0.7473
KCl	0.1081	0.2020
Na_2SO_4	0.0493	0.0993
Total	15.0186	29.9934

*Salts were dissolved in 100 mL of 2% HNO_3.
TDS: Total dissolved solids.

a high content of dissolved solids and thus to establish the critical matrix concentration at which interferences become more marked.

The determination of these elements at trace levels is not only important in seawater samples but also in brines with a higher saline content. Little work has so far been carried out pertaining to the analysis of concentrated brines (more than 3% TDS). Since flow injection was used in this study for sample introduction in order to minimize the effects of the nebulizer and cone blockage (32,33), the recoveries of the different analytes were previously calculated (Table IV) for different dilutions of an undiluted certified seawater sample (CASS-3) to which 10 μg L^{-1} of the elements listed in Table II were added, except Al (50 μg L^{-1}) and V (25 μg L^{-1}). The direct analysis of an undiluted seawater sample, CASS-3, without a spike was also carried out. A study was made of the recoveries of the synthetic matrixes with different TDS (Table 5), which were prepared as previously described. To this end, each synthetic brine was spiked with the above-mentioned elements at a concentration of 10 μg L^{-1}, except Al (50 μg L^{-1}) and V (25 μg L^{-1}). All samples analyzed also contained 10 μg L^{-1} each of Sc, In, and Bi, used as the internal standards. For the recovery studies in seawater and the synthetic saline matrixes, a comparative study of the recoveries of the same elements in a solution without a matrix was carried out. To this end, a standard solution was prepared containing the same elements at the concentration of 10 μg L^{-1}, except Al (50 μg L^{-1}) and V (25 μg L^{-1}) in 2% HNO$_3$, which was analyzed in the same way by injecting 500 μL of the solution into the carrier stream (also 2% HNO$_3$) that reached the ICP-MS. Tables IV and V list only the elements with recoveries of 100% ± 10. The rest of the elements

TABLE IV
Recoveries (%) in CASS-3 for FIA-ICP-MS Multielemental Analysis

Element	Standard in HNO$_3$ 2% Rec.	CASS-3+St. (1:99) Rec.	CASS-3+St. (1:9) Rec.	CASS-3+St. Rec.	CASS-3 * Rec.
Al	113	91	104	63	-
V	101	90	129	195	-
Cr	92	103	108	235	2119
Mn	96	98	99	105	90
Ni	91	93	119	256	2868
As	104	109	175	511	5562
Mo	95	98	91	109	90
Rh	94	97	102	93	-
Sb	98	90	96	97	-
Te	105	96	94	91	-
Pb	97	113	114	120	-

*If there is no value in CASS-3 unspiked and without dilution it is because that element is not present in the sample or its concentration is too low to be determined directly by FIA-ICP-MS.

TABLE V
Recoveries (%) in Synthetic Brines for FIA-ICP-MS Multielemental Analysis

Element	Standard in HNO$_3$ 2% Rec.	Brine 0.03%TDS Rec.	Brine 0.3%TDS Rec.	Brine 3%TDS Rec.	Brine 15%TDS Rec.	Brine 30%TDS Rec.
Al	105	109	95	64	48	19
V	98	107	124	276	661	1003
Cr	96	95	108	541	1506	306
Mn	99	92	99	95	97	94
Ni	97	96	129	324	825	500
As	102	105	186	642	3269	7737
Mo	90	94	103	101	104	91
Rh	90	94	92	91	75	66
Sb	93	102	97	92	94	67
Te	110	104	94	89	61	46
Pb	100	97	104	107	109	115

studied are strongly affected by spectroscopic and non-spectroscopic interferences and show very low recoveries even with matrixes of 0.03 % TDS.

The results in Tables IV and V suggests that practically all of the elements in the seawater sample (CASS-3) display the same behavior as in synthetic brines with the same TDS, although slight differences

may exist. The elements most affected by spectroscopic interferences were As, Cr, Ni, and V. Some of these possible spectroscopic interferences are listed in Table VI. The effect is first observed for both the CASS-3 and synthetic saline matrixes with a TDS of 0.3%. Since the Mo concentration in CASS-3 is 8.95±0.26 μg L^{-1}, it can be determined directly by FIA-ICP-MS with-

TABLE VI
List of Possible Spectroscopic Interferences in ICP-MS Caused by a Saline Matrix.

Analyte isotopes	Interfering species
^{51}V	ClO^+
^{52}Cr	$ClOH^+$
^{53}Cr	ClO^+
^{55}Mn	ClO^+, $ClOH^+$
^{58}Ni	CaO^+, ArO^+
^{59}Co	CaO^+,$CaOH^+$, $MgCl^+$, $ArNa^+$
^{60}Ni	CaO^+, $NaCl^+$, $CaOH^+$
^{62}Ni	Na_2O^+, NaK^+
^{63}Cu	$ArNa^+$
^{65}Cu	$CaOH^+$, $COCl^+$, Ba^{2+}
^{64}Zn	CaO^+
^{67}Zn	ClO_2^+
^{68}Zn	$ClOO^+$
^{70}Zn	$ClCl^+$, $ClOO^+$
^{75}As	$ArCl^+$
^{74}Se	$ClCl^+$
^{77}Se	$ArCl^+$

out previous preconcentration or dilution. A concentration of 8.05±0.8 µg L^{-1} was obtained in this study, corresponding to a recovery of 90%. The recovery for Mo was good (91%) even in the synthetic brine with 30% TDS. Mn can also be determined directly in the seawater sample (CASS-3) using the proposed method, because the certified value is 2.51±0.36 µg L^{-1}. A value of 2.18±0.04 µg L^{-1} was obtained in our study. For matrixes with TDS higher than 3% (seawater), the recoveries of Mn was also good (97% and 94% for 15% and 30% TDS, respectively). Sb and Pb appear to be affected by relatively high matrix concentrations (about 15% TDS), although it should be pointed out that while the effect for Sb is negative (the signal decreases), the effect for Pb is positive (increase in signal). In principle, this effect should not be due to a spectroscopic interference caused by a saline matrix because a possible spectroscopic interference for

the ^{208}Pb isotope would be $^{192}Pt^{16}O^+$ in spite of ^{192}Pt being a low-abundance isotope. This phenomenon, however, has been observed by other authors (6) studying the matrix effect on different elements and sometimes report a signal enhancement or suppression. Te seems to be affected by matrix concentrations higher than 3% TDS, showing signal suppression (recoveries of 61 and 46% for matrixes with a TDS of 15% and 30%, respectively).

There is a relationship between the mass of the elements and non-spectroscopic interferences. For example, if elements with a different mass and similar ionization potentials are compared, it can be seen that for any concentration in the synthetic saline matrixes or in CASS-3, the recoveries are better for the smaller element. This was reported before (36) and appears to be due to an accumulation of a larger number of electrons in the

central zone of the plasma which cause an increase in ambipolar diffusion and which affects elements with a low mass more than heavier elements. This leads to a build-up of the lighter elements outside the central channel of the plasma where the temperature is higher due to the presence of fewer electrons, resulting in an increase in the degree of ionization of the lighter elements. However, in our study, some smaller elements had lower recoveries than heavier elements with the same ionization potential. ^{59}Co (IP=7.86 eV), for example, had lower recoveries (75, 67, 61, 56, and 49% for matrixes with 0.03, 0.3, 3, 15, and 30%, respectively) than ^{208}Pb (IP=7.416 eV) for any concentration of the synthetic brine (97, 104, 107, 109, 115%) or CASS-3 (101, 105, and 108%) owing to the charge effects that appear in the ionic beam. In the ion-focusing process, the ionic optics only focuses on positive ions, the beam loses electrons and acquires a positive charge, and the ions repel each other. In the presence of an excess of heavy ions, the light ions are repelled by the ionic beam due to their reduced kinetic energy and displaced from their original path; thus, their transmission to the analyzer is worse. Consequently, elements such as Co cannot be determined by the proposed method.

Figures of Merit

In order to determine the influence of matrixes with a higher TDS than usually studied, the detection limits were calculated and the reproducibility carried out on the basis of the relative standard deviation (RSD %). The stability of the FIA-ICP-MS system for the direct FIA-ICP-MS analysis of saline matrixes with a different TDS was also verified by performing 10 replicates of each blank corresponding to each synthetic brine with a different saline content (0.03%, 0.3%, 3%, 15%, and 30%). For the

TABLE VII
Analytical Figures of Merit for Multielemental FIA-ICP-MS Analysis

	Standard/ HNO₃ 2%			Brine TDS 0.03%			Brine TDS 0.3%			Brine TDS 3%			Brine TDS 15%		
	D.L. $\mu g\,L^{-1}$	RSD %	Rec %	D.L. $\mu g\,L^{-1}$	RSD %	Rec %	D.L. $\mu g\,L^{-1}$	RSD %	Rec %	D.L. $\mu g\,L^{-1}$	RSD %	Rec %	D.L. $\mu g\,L^{-1}$	RSD %	Rec %
Al	1.64	11	103	0.73	6.5	105	14.45	3.2	92		3	68		6.8	55
Sc		8			4.9			2.7			2			3	
V	1.49	6	100	0.29	5.1	99	1.87	3.8	197	8.88	6	288	175.54	3	670
Cr	4.57	7	94	0.37	6.7	91	0.90	15.3	174	4.78	61	560	1919.51	45.2	1663
Mn	0.44	6	94	0.07	5.2	98	0.11	3	97	0.44	2	91	7.72	8.7	94
Ni	1.59	8	95	0.36	6.5	94	0.95	4.6	172	3.06	11	265	1152.70	13.1	773
As	0.29	3	102	0.40	5.6	110	0.33	2	179	11.64	15	587	1106.07	59.4	3412
Mo	0.04	6	93	0.05	6.4	98	0.10	3	99	0.19	10	104	2.98	8.9	97
Rh	0.006	7	90	0.03	5	91	0.01	2	88	0.02	1	81	0.10	11.6	68
In		5			5.6			2.4			2			4.9	
Sb	0.01	4	102	0.04	3.8	105	0.02	1.4	105	0.09	1	94	0.37	3.8	73
Te	0.03	4	108	0.06	3.2	105	0.05	3.9	104	0.12	3	88	3.21	8.7	60
Pb	0.08	13	96	0.10	8.8	92	0.41	2.5	90	0.29	1	100	1.22	5.2	111
Bi		10			6.7			2.2			1			2.9	

measurement of synthetic brines with a 3% TDS more, five minutes were allowed between each replicate for cleaning the ICP-MS with 2% HNO₃. The cones were thoroughly cleaned after every 20 measurements. They were removed and cleaned in an ultrasonic bath for a period of 3 minutes using a 2% Citranox solution. The results obtained are given in Table VII which shows which elements can be determined directly in the presence of a specific matrix concentration or the amount of dilution necessary for their determination. The study of the RSD of the brines with a different TDS and the RSD of the standard without a matrix yielded important information regarding the influence of the dissolved solids on method reproducibility. Calculation of these RSDs makes it possible to determine the extent at which the signal remains constant in the presence of the matrix and the crucial concentration after which the signal reproducibility of an element deteriorates. The RSD for Sc, In, and Bi (10 $\mu g\,L^{-1}$) are also given in Table VII. It can be observed that

the RSD of these three elements is lower than 10% (a very acceptable value for measurements by FIA-ICP-MS) for any matrix concentration and even lower than the RSD value when the standard without a matrix was measured. More or less the same applies to Al, Rh, Sb, Te, and Pb, with RSD values in all cases 10% or lower for any matrix concentration, and in many cases lower than the RSD obtained for the standard in 2% HNO₃. V, Mn, and Mo have RSD values of less than 10% for a matrix concentration of 3% or lower (seawater). The elements that posed most reproducibility problems were Cr, Ni, and As, which are affected by spectroscopic interferences. Non-spectroscopic interferences appear to have less effect on reproducibility, possibly due to the fact that, in certain circumstances, partial clogging of the sample orifice may be an advantage as a pseudo steady-state situation is achieved and where the rate of deposition on the cones is equalled by the dissociation rate (6). The detection limits for each matrix concentration were calculated according to the criterion 3 σ_{n-1},

where σ_{n-1} is the standard deviation of 10 blanks. Apart from the sensitivity of the method, blank contamination and memory effect have a significant influence on the detection limits. No serious blank contamination was found due to the purity of the reagents used. With regard to memory effects, as previously reported (36), five minutes were allowed between consecutive measurements for cleaning with 2% HNO₃, which flowed continuously to the ICP-MS. The detection limits for some elements in the absence of a matrix were even lower than one hundredth of a $\mu g\,L^{-1}$. Only Cr had higher detection limits (4.5 $\mu g\,L^{-1}$), probably due to memory effects. The average recovery values obtained from the 10 measurements carried out to calculate the RSD are also given in Table VI. It should be noted that Mo and Mn, which had been determined directly by FIA-ICP-MS after a 5-fold dilution of the seawater sample (34), can be determined directly by FIA-ICP-MS without any dilution, even in matrixes with 30% TDS; Sb and Pb in matrixes with 15% TDS or less; Rh and Te in matrixes with 3% TDS or

less; Al and Cr in matrixes with 0.3% TDS or less; and all the elements cited can be determined directly in matrixes with 0.03% TDS.

Furthermore, the analytical parameters studied provided good results for Mn, Mo, Sb, Rh, Te, and Pb for higher matrix concentrations (15% TDS or less) with detection limits lower than 10 µg L^{-1}, an RSD of around 10%, and recoveries close to 100%.

Influence of the ICP-MS Operating Conditions

As is well known, the operating conditions may have a great influence on multi-elemental ICP-MS analysis. Laborda et al. (11) studied the effects of instrumental parameters (power, nebulizer gas flow, and temperature of the spray chamber) on spectroscopic interferences caused by polyatomic ions. Rodushkin et al. (36), who studied the effects of a saline matrix with 0.6% TDS on non-spectroscopic interferences, reached the conclusion that non-spectroscopic interferences may vary considerably depending on the instrumentation used. He also suggested that researchers should determine the behavior of interferences in their own instrument employed. In the present paper, for which the sample was introduced by FIA, the effect of the nebulizer gas flow and plasma power on multi-elemental ICP-MS analysis of saline matrixes with a TDS higher than 0.6% (up to 15% TDS) was studied. To this end, a standard containing 10 µg L^{-1} of As, Cr, Mn, Mo, Ni, Pb, Sb, and Te; 50 µg L^{-1} of Al and 25 µg L^{-1} of V was analyzed without a matrix and with matrixes with a different TDS (0.03%, 0.3%, 3%, 15%, and 30%) by FIA-ICP-MS using the procedure described. The intensity values were obtained for different nebulizer gas flow values (0.75, 0.8, 0.85, 0.9, and 0.95 L min-1). These values include the optimum nebu-
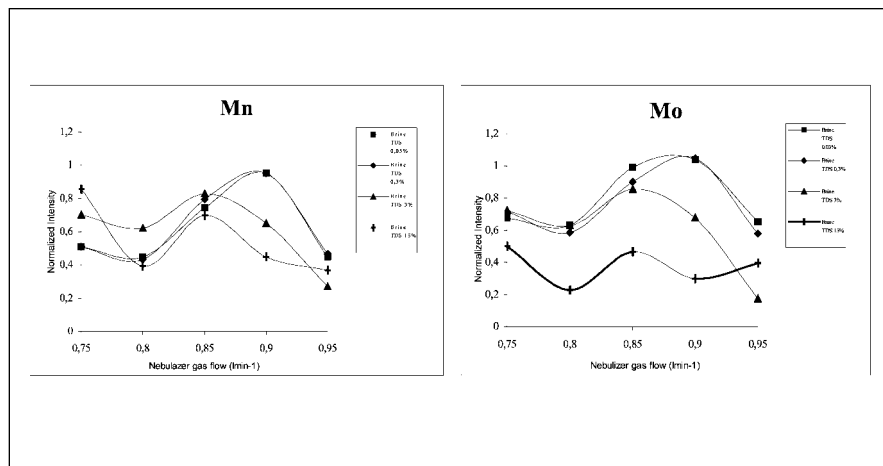

Fig. 2. Influence of nebulizer gas flow on Mn and, Mo analysis by FIA-ICP-MS with different matrix concentration.

lizer gas flow values obtained on different days, when the measurements were performed during the general optimization of the instrumentation, when criteria of maximum intensity were used for a solution of Rh (10 µg L^{-1}), and an oxide level below 3%. Five-minute pauses were intercalated between consecutive measurements to avoid blockage of the cones and to improve precision. For a better interpretation of the results, the normalized intensities obtained were calculated as the quotient between the intensity of the element in the corresponding saline matrix and the intensity of the same element in the standard without a matrix for the different nebulizer gas flows. The optimum nebulizer gas flow rate was practically the same for all elements for the same saline concentration. In general, the matrix effect is more marked when the nebulizer gas flows are higher. The greater the saline concentration, the smaller the maximum nebulizer gas flow obtained; that is to say, the smaller the flow needed to minimize the matrix interferences produced. In principle, this is in agreement with the results obtained for more dilute saline matrixes (35) and is due to the deposition of salts on the cones, which reduces cone

diameter and results in a loss of ions entering the mass spectrometer. Low nebulizer gas flows compensate for this effect by increasing the temperature in the central channel of the plasma. Providing the results obtained for all elements, matrix concentrations, and flows would result in a proliferation of numerical data. However, as an example, Figure 2 provides the normalized intensities obtained for Mn and Mo.

The powerful influence of highly saline matrixes on the analytical performance of multi-elemental ICP-MS analysis was also studied. To this end, a standard containing 10 µg L^{-1} As, Cr, Mn, Mo, Ni, Pb, Rh, Sb, and Te; 50 µg L^{-1} of Al and 25 µg L^{-1} of V without a matrix, and the same standard in the presence of matrixes with a different TDS (0.03%, 0.3%, 3%, and 15%), were analyzed by FIA-ICP-MS. Intensity values were obtained using different plasma power (800, 1000, 1100, and 1200 W). Five-minute pauses were allowed between consecutive measurements. The normalized intensities obtained were calculated as the quotient between the intensity of the element in the corresponding saline matrix and the intensity of the same element in the standard without a matrix for the

different power values. In general, the normalized intensity increases as the power applied increases. That is to say, the intensities of the different elements with a matrix approach those of the elements in the standard without a matrix as the power increases. This could be due to an increase in power by raising the plasma temperature, which makes it possible to compensate for the reduction of energy caused by the easily ionizable elements in the matrix. In that case, the use of higher powers would partially reduce reversible non-spectroscopic interferences. For the sake of simplicity, the normalized intensity values obtained are plotted against the different plasma powers (see Figure 3) for Mn and Mo, as an example.

CONCLUSION

In this paper, the possibility of the direct FIA-ICP-MS multi-elemental analysis of matrixes with a different saline content (0.03-30% TDS) was investigated, which is higher than for most studies reported. The direct introduction of a certified seawater sample (CASS-3) with a TDS of approximately 3% by the proposed method permitted the determination of Mo and Mn with results very close to the certified values. For elements such as Pb, Rh, Sb, and Te, which are present in very low concentrations or do not have certified values for the seawater samples (CASS-3), the recoveries of 108, 93, 97, and 91% were obtained.

This study of the influence of the nebulizer gas flow and the plasma power shows that the intensity obtained for the different elements studied decreases when the TDS of the matrix is 3%. However, the study of the recoveries of the different elements and matrix concentrations shows that for any matrix concentration, Mo and Mn have similar recoveries to those of the standard without a matrix. However, the recoveries for Rh, Sb, Te,
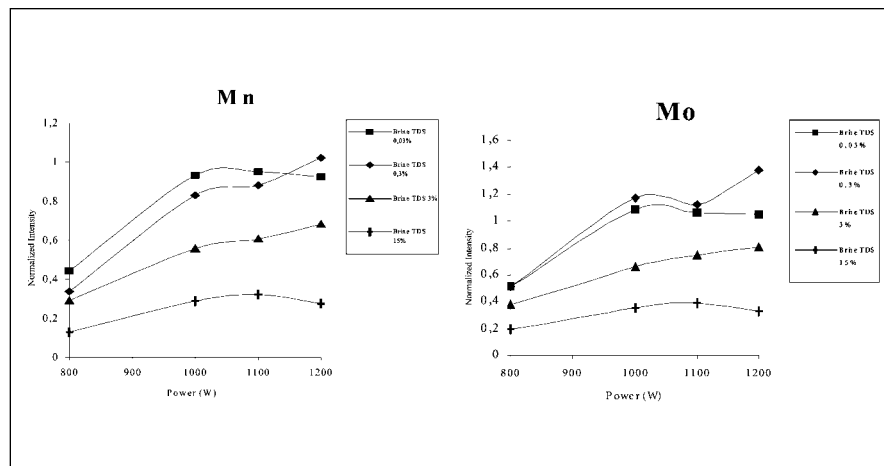

Fig. 3. Influence of Rf power on Mn and Mo analysis by FIA-ICP-MS with different matrix concentration.

TABLE VIII
Possibility of Direct Determination by FIA-ICP-MS in Highly Saline Matrix

Element	Standard in HNO$_3$ 2%	Brine 0.03%TDS	Brine 0.3%TDS	Brine 3%TDS	Brine 15%TDS	Brine 30%TDS
Al	OK	OK	OK	No	No	No
V	OK	OK	No	No	No	No
Cr	OK	OK	OK	No	No	No
Mn	OK	OK	OK	OK	OK	OK
Ni	OK	OK	No	No	No	No
As	OK	OK	No	No	No	No
Mo	OK	OK	OK	OK	OK	OK
Rh	OK	OK	OK	OK	No	No
Sb	OK	OK	OK	OK	OK	No
Te	OK	OK	OK	OK	No	No
Pb	OK	OK	OK	OK	OK	No

and Pb differ significantly from those of the standard without a matrix when the matrix TDS is 15%. This may be due to the fact that with the FIA system of sample introduction, a pseudo steady-state situation is achieved in which the rate of deposition on the cones is equalled by the dissociation rate. This can also be observed in the reproducibility values with RSD values for Al, Mn, V, Mo, Rh, Sb, Te, and Pb, which are, in all cases, 10% or lower for any matrix concentration and, in many cases, less than the RSD obtained for the standard

in 2% HNO$_3$. Ni and As have RSD values lower than 10% when the matrix TDS is less than 3%. Table VIII lists which of the elements studied can be determined directly by FIA-ICP-MS and in which matrix concentration.

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Influence of Additives on the Carryover Effects of Hg During ICP-OES Analysis of Immunoglobulines Samples

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INTRODUCTION

The [(o-carboxyphenyl)thio]ethylmercury sodium salt, a chemical compound known as thimerosal, is widely used tincture for first-aid home use, a preservative in cosmetics, including make-up removers, eye moisturizers, and mascaras. It may be found in soap-free cleansers; nose, eye, and ear drops; topical medications and antiseptic sprays, among others. It is also used as a preservative in vaccines, antitoxins, tuberculin tests, and desensitization solutions as well as a preservative added to the immunoglobuline.

The multiple uses of thimerosal require specific analytical methods of analysis for mercury determination in this type of sample. The methods of analysis for mercury-containing drugs are generally nonspecific. However, the development of a general method is complicated by the existence of a large variety of drug preparations and by the wide variation in their mercury concentrations, ranging from parts per million to percent levels. The atomic absorption spectrometic method is the method usually recommended (1). The choice of flame or electrothermal atomization depends on the concentration of mercury. Most laboratories routinely use cold vapor atomic absorption spectroscopy (CVAAS) with a gold trap preconcentration step. A digestion procedure is gen-

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ABSTRACT

A method for the determination of mercury in immunoglobulines samples by inductively coupled plasma optical emission spectrometry (ICP-OES) is described, in which sample dilution with some additives was the only pretreatment prior to analysis. Different chemical reagents, such as deproteinization agents, and other additives, such as surfactants, were proven as potentially useful. Residual mercury signal after samples analysis was one of our main concern. Among the diluents studied, EDTA and Triton® X-100 showed more accurate results. The Hg concentration in the samples, using the proposed method, are in good agreement with those obtained of samples analyzed by the official method using Cold Vapor Atomic Absorption Spectrometry (CV-AAS) with partial and total destruction of the sample.

erally considered mandatory before the reduction step to convert all mercury species present to a form amenable to reduction before analysis. Digestion methods typically involve combinations of strong acids, oxidants, elevated temperatures and pressures, and UV irradiaton (2,3). Most of those digestion procedures are based on the use of a mixture of nitric and suphuric acids. The oxidizing properties of these mixtures are sometimes insufficient, and oxidation must be implemented with the aid of catalysts and/or with the addition of a stronger oxidant to finish mineralization. Further concern exits regarding the ability of the various pretreatment procedures to provide

accurate total mercury concentrations, some methods having been shown to give low recoveries (4).

It is also well known that after a sample containing mercury is nebulized into a conventional spray chamber, significant residual mercury signal can be detected many minutes afterwards (5). The mercury signal fails to return to baseline and all subsequent samples appear to contain mercury, whether or not they do. Retention of mercury in spray chambers and long washout times with nitric acid have been documented (6,7). Mercury either adsorbs onto the spray chamber walls or is retained as vapor in the dead volume of the spray chamber (8,9). With nitric acid alone in solution, mercury adsorption onto the container walls is prevented but does not prevent mercury volatility (10). The adsorption-vaporization phenomena are observed with mercury solutions in a variety of matrices.

Several solutions to the problem of mercury memory in the spray chamber have been published. Flow injection and mercury reduction followed by gold amalgamation has been used to eliminate aerosol spray chamber memory (11). Another solution has been the use of direct injection nebulizer (DIN). Conventional spray chamber-pneumatic nebulization has been used with diluents such as tetramethyl ammonium hydroxide (TMAH) (8), ethylenediamine-tetraacetic acid (EDTA) in TMAH (12), HCl-Cisteine (13), or gold (7,9, 14–16) and HBr (6) to modify samples, thus reducing the memory effect. The addition of gold or dichromate has been reported (10) to prevent volatiliza-

tion and adsorption losses of mercury. Nixon et al. (17) describe the use of dichromate in 2% HCl as a diluent for mercury determination in whole blood and urine by ICP-MS. In addition, to the combination of dichromate with HCl, gold was evaluated for effectiveness with biomedical samples. They concluded that dichromate plus hydrochloric acid as a diluent in the wash solution is effective at minimizing mercury memory. This mixture was found to be superior to gold.

There are other reagents that have been used to stabilize the ppb concentration of mercury in water, and to remove the memory effects in glass or plastic spray chamber. These reagents include hydro-bromic acid or potassium bromide (18), hydroxylamine (19), Triton® X-100 or Briji 35 (20), $(NH_4)_2H_2EDTA$ (21).

The development of a method in which sample treatment can be reduced to a minimum and which will allow total mercury determination in this type of biological samples is of analytical interest. For clinical applications, as in the immunoglobulines, the simple dilution approach for the determination of mercury, coupled with conventional pneumatic sample introduction, is desirable.

A method for the determination of mercury in immunoglobuline by inductively coupled plasma optical emission spectrometry (ICP-OES) is described. A study of different chemical reagents potentially useful for sample dilution as the only pretreatment of the immunoglobuline samples prior to mercury determination is proposed. These diluents were chosen on the basis of their uses as deproteinization agents in clinical samples and additives, such as surfactants, to emulsify samples for metals determination by spectroscopic methods.

EXPERIMENTAL

Instrumentation

A PerkinElmer (Norwalk, CT USA) Model Optima 3000™ inductively coupled plasma optical emission spectrometer was used. The instrumental and operating parameters are listed in Table I. A standard demountable type quartz plasma torch was used throughout. The i.d. of the alumina injector was 1.5 mm. A ten-roller peristaltic pump was used to feed the nebulizer with the sample solution. All functions of the plasma were computer controlled.

Reagents

All reagents were of the highest available purity. Ultrapure water was obtained from a Milli-Q™ system (Millipore). The standard solutions were freshly prepared from a 1000-mg L^{-1} stock solution (BDH Chemical L.T.D.). The working standard solutions used for calibration purposes were prepared by suitable dilution of the stock standard solution. All glassware was cleaned in nitric acid prior to use. High-purity (99.95%) argon was utilized.

Sample

Immunoglobuline samples were donated by QUIMBIOTEC, a bio-derivatives production plant for the national market, of the Caribbean and the Andean Region.

Sample Preparation

Immunoglobuline samples were simply diluted with the respective diluent. A 0.5-g sample was weighted and diluted to 25-mL with deionized water.

Destruction of the sample for its analysis by CVAAS was carried out by weighting the immunoglobuline samples (0.1 g). They were left stand overnight and then mechanically shaked for half an hour prior to analysis.

RESULTS AND DISCUSSION

Official Method

The recommended method for the determination of mercury in mercury-containing drugs, as for thimerosal, is atomic absorption spectrometry using a conventional air –acetylene flame for high levels of mercury and cold vapor for low levels of mercury. For this, total

TABLE I
ICP Operational Parameters

|	
RF generator	40 MHz
Operating power	1300 W
Nebulizer	Meinhard
Spray chamber	Scott Type
Sample delivery	Peristaltic pump
Pump uptake rate:	1.5 mL/min
Nebulizer flow rate	0.60 L/min
Plasma gas flow	12 L /min
Auxiliary gas flow	0.2 L /min
Plasma viewing	Axial
Observation height above r.f. coil	5 mm
Background correction	Automatic
Integration time	200 msec
Working wavelength: Hg (I):	253.652 nm
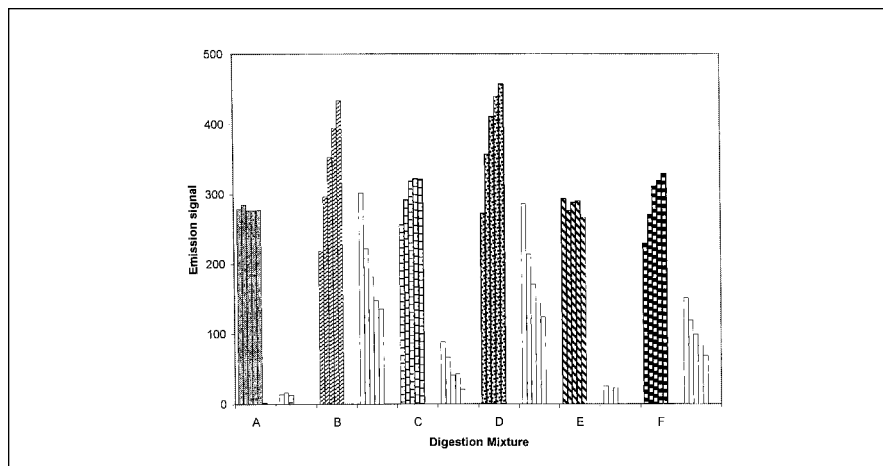
Fig. 1. Mercury carryover effect after sample digestion.

$A = H_2O + HCl + HNO_3$

$B = HCl$

$C = HNO_3$

$D = H_2SO_4 + H_2O_2$

$E = HNO_3 + H_2SO_4$

$F = H_2O$

$\square = water$

destruction of the organic matter is impractical because a prolonged digestion can result in significant losses of the volatile mercury. It has been reported (1) that in determining mercury in this type of compound, hydrocholric acid is not convenient, since it does not dissolve those compounds where mercury is bound to sulfur atoms, like in the thimerosal molecule. A mixture of hydrochloric-nitric acid has instead been recommended. Holak et al. (1) reported that difficulties in obtaining a steady AAS signal were experienced and explained that the cause was the reduction of Hg (II) to Hg (I) or to elemental Hg, partially solving the problem by the addition of potassium dichromate.

Based on these findings, and since we were using an ICP-OES for the determination of mercury in immunoglobuline, we decided to investigate the effect of different diluents on this particular determination further.

Sample Treatments With Different Oxidizing Mixtures

Initially, all samples of immunoglobuline were digested using different oxidizing mixtures and the mercury emission signal from each digested solution was registered. After aspiration of the digested sample solutions, water was run and the carryover effect measured. Figure 1 shows the emission signal reproducibility, expressed as the %RSD and the % carryover effect, taken from five replicates readings. It can be seen that the highest mercury retention effect was shown by sample treatments B, D, F, C (69.3, 57.8, 42.2, and 21.7%, respectively). The carryover effect was less pronounced when sample treatment involved the acid mixtures of nitric-suphuric and nitric-hydrochloric acids (retention effect 8.1 and 5.2%, respectively). However, sample dissolution was only partial with the mixture of nitric and hydrochloric acids (the recommended procedure). The mixture of nitric-sulphuric acid

dissolves the sample completely; however, the procedure is time-consuming (digestion time about five hours at temperatures less than 60ºC) and also a very high acid concentration is required to assure the complete decomposition of the organic matter. Thus it is advisable to use alternative methods for this particular application.

Surfactant Effect

Surfactants, such as Triton X-100, complexes the mercury ion and reduces the interaction of the Hg ion with glassware (20). Because these surfactants form emulsions, they are applicable in the pharmaceutical, plastics, and oil industries, among others. From an analytical point of view, the application of surfactants is for the preparation of the samples prior to their analysis by spectrophotometric methods. It is desirable that analytes in viscous samples, such as immunoglobulines, can be quantified using aqueous standards for calibration purposes. The viscosity of the samples and standards should match as closely as possible. For this reason, we studied the possibility of using surfactants to dilute samples such as immunoglobuline, simplyfying sample treatment before analysis, avoiding sample digestion prior to element determination, and using aqueous standards for calibration. A qualitative study with several surfactants was carried out to assess the carryover effect after aspiration of the immunoglobulines samples for mercury determination. Also, signal reproducibility was taken into account. The surfactants were Triton X-100, ethoxynonylphenol, Tween-80, Deltex, and Corexit, a commercial product, used for decontamination purposes by the oil industry in oil spills. These non-ionic surfactants were chosen due to their easy availibility and have been used by the authors for emulsifying edible oils (22). An aqueous solution of the sample was run for comparison. Figure 2 shows the

influence of the different surfactants (2% v/v) on the mercury emission signal in the immunoglobuline sample (0.5 g immunoglobuline in 25 mL). As can be seen, the highest memory effect is shown by the aqueous solutions of the sample (~42.2 %). The mercury signal retention memory effect was calculated from ~9.4 to 21.2 %. Signal reproducibility (shown in Figure 2 as %RSD) is improved when Corexit (1%), Triton X-100 (2.7%), and Tween 80 (3.7) were used as diluents. Signal sensitivity is not the main concern in this work, given that the samples have a relatively high mercury concentration (~50 mg/L). Corexit and Triton X-100 produced the best results with regard to signal reproducibility and Triton X-100 with lesser memory effects (9.4%). However, corexit is a commercial product which is not easily available and this limits its use. Triton X-100 is more often found in the chemicals laboratories.

Gold in Combination With Other Additives

Because gold amalgamates mercury to a certain degree, it minimizes the adsorption of Hg to all glass/quartz parts of the sample introduction system. For this reason, mercury was measured in solutions of immunoglobulines and thimerosal spiked with 5 ppm gold. An aqueous standard (1.6 ppm) of mercury containing gold was analyzed for comparison purposes. Figure 3 shows a pronounced carryover effect, produced when an aqueous solutions of mercury is aspirated (16.0%). The addition of gold to this solution decreases the signal intensity markedly, and the carryover effect was less than 3%. However, the addition of gold to thimerosal, did not improve either the signal reproducibility or carryover effect. A similar effect was observed with the immunoglobuline sample.
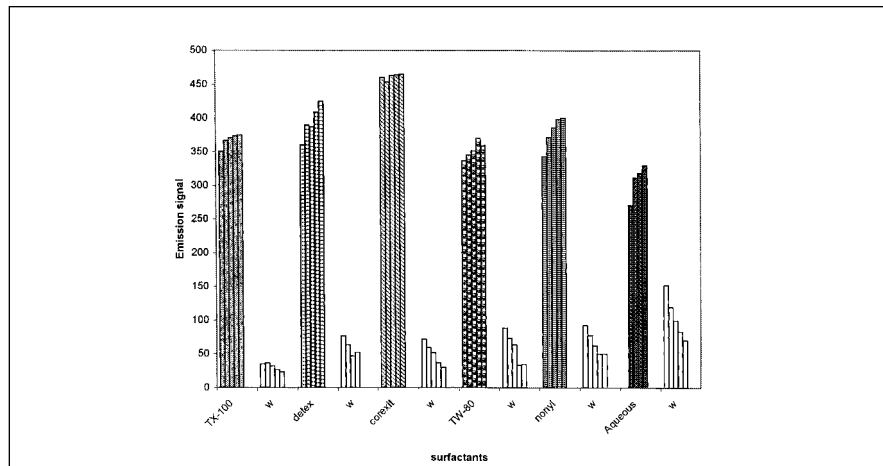

Fig. 2. Effect of different surfactants on the Hg emission signal in immunoglobuline.
TX-100: Triton X-100   Detex and Corexit: commercial surfactants
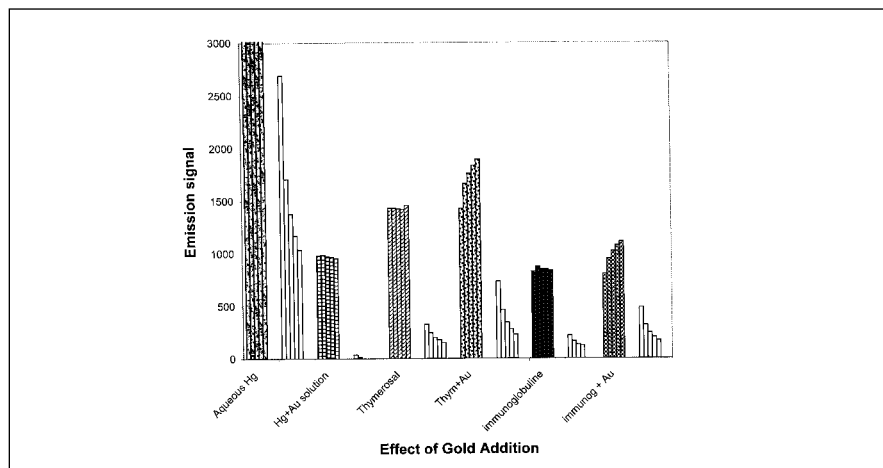TW-80: Tween 80   nonyl: ethoxy nonyl phenol
□: water

Fig. 3. Effect of gold addition on mercury emission signal.
Aqueous Hg solution   HG+Au solution
Thymerosal   Thymerosal+Au solutio
Immunoglobuline sample   Immunoglobuline+Au solution
□: water

In addition to the results obtained due in the presence of gold, it was decided to test the combination of gold with hydrochloric and sulphuric acid and with Triton X-100. Solutions of immunoglobulines with these additives were measured for mercury, and the reproducibility and memory effects were compared with those obtained from an aqueous standard of mercury (1.6 ppm) with the same additives. The signal reproducibility and carryover effects were worse for immunoglobuline compared with the emission signals obtained from the aqueous standards.

For protein purification of the heterogeneous group of serum proteins, reagents such as trifluoracetic acid (TFA) and ethylenediamine tetraacetic acid (EDTA) were used at concentrations usually recommended for this purpose. Addition of TFA acid to the aqueous solution of mercury did not improve the Hg signal reproducibility or its retention effect. However, its presence is advantegous to the immunoglobuline samples. The opposite effect was observed with the additon of gold (5ppm): gold stabilizes the mercury emission signal in the aqueous matrix, but not in the immunoglobuline matrix. These results are shown in Figure 4.

Addition of EDTA improves signal reproducibility in both aqueous and physiological matrices and reduces carryover memory effect for the aqueous sample (Figure 5). Addition of gold to the above solutions shows the same tendency as before, i.e., improved precision and carryover effect in the aqueous system but no advantages to the physiological fluid.

Some authors (18) reported that the presence of excess bromide ions in acidic solutions form stable mercury solutions of the type $(HgX_4)^{-2}$, where X is a halide, and that the stability of $(HgX_4)^{-2}$
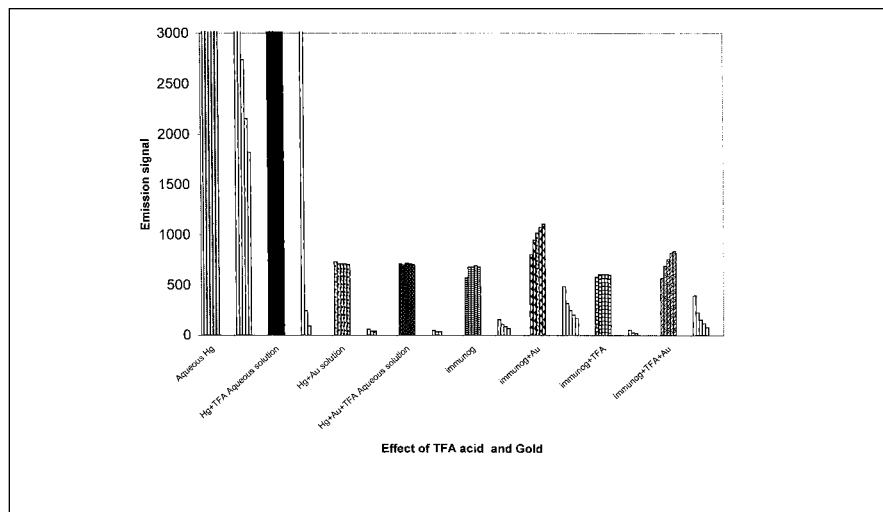

Fig. 4. Effect of trifluoracetic acid (0.4 % v/v) and Gold on Hg emission signal.
   Aqueous Hg solution   Hg+TFA solution   HG+Au solution
   Hg+Au+TFA solution   Immunoglobuline   Immunogl.+Au
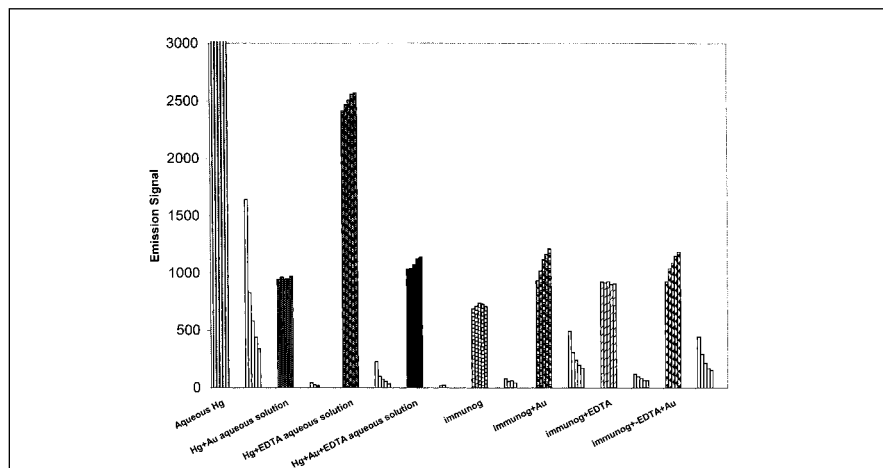   Immunogl.+TFA   Immunogl.+TFA+Au   □: water

Fig. 5. Effect of EDTA (0.4 %w/w) and Gold (5 ppm) on the Hg emission signal from aqueous and immunoglobuline solutions.
   Aqueous Hg solution   HG+Au solution   Hg+EDTA solution
   Hg+Au+EDTA solution   Immunogl.   Immunogl.+Au
   Immunogl.+EDTA   Immunogl.+EDTA+Au   □: water

increases with the size of the anion. They tested chloride unsuccefully and suggested to work with iodide to determine how well it works for mercury. So, aqueous solutions of mercury containing different iodide contents were run and were then compared with the signal obtained from the solutions containing bromide. The solutions containing gold at the recommended concentrations were also run and compared (Figures 6a and 6b for the aqueous and immunoglobulines samples, respectively).

The value of acidified dichromate solutions in preventing loss of mercury has been known since the mid-1970s (23,10). It prevents loss of mercury by preventing adsorption onto the walls of storage containers (an effect of the acid) and by preventing its volatilization as Hg vapor (an effect of the oxidizing action of acidified dichromate) (10). Potassium dichromate solutions were also tested at different concentrations and the effect on the Hg emission signal was registered. Other researchers used hydroxylammonium chloride (1%) to clean the sample aspirating cell. The results using these solutions are shown in Figures 6a and 6b. No significant improvement in signal reproducibility and carryover effect was observed for the two matrices.

Quantitative Determination of Mercury in Immunoglobuline

Based on the results reported in the previous section, the quantitative determination of mercury in immunoglobulines was carried out by ICP-OES by simple dilution with EDTA, Triton X-100, and TFA. The results of these determinations were compared with those obtained from the official method (1) and also from a total digested sample with a mixture of acids using cold vapor atomic absorption spectrometry (see Table II). The significance t-test applied for comparing the mean revealed that there
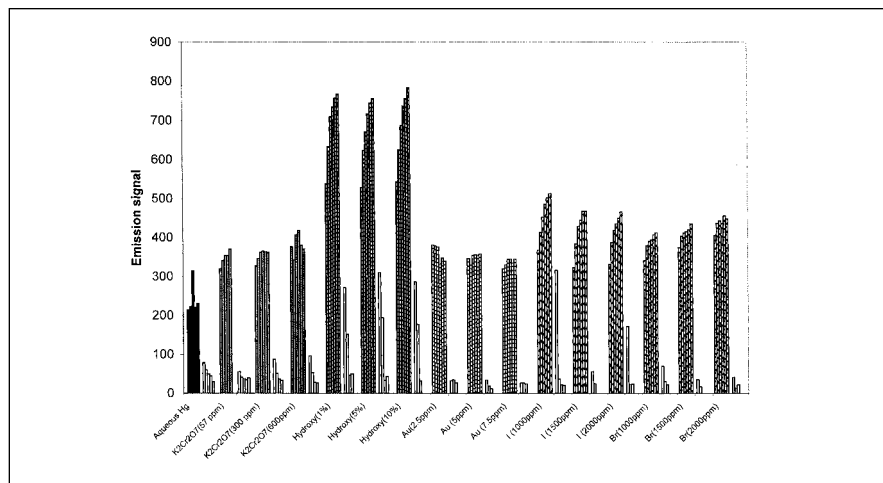

Fig. 6a. Effect of $K_2Cr_2O_7$, $HONH_3Cl$, I^- and Br^- on the Hg emission signal from an aqueous standard. Gold was run for comparison.
Aqueous Hg solution
$K_2Cr_2O_7$ solution (57, 300, and 600 ppm
Hydroxylammonium chloride solution (1, 5, and 10% v/v)
Au solution (2.5, 5, and 7.5 ppm)
I^- solution (1000, 1500, and 2000 ppm)
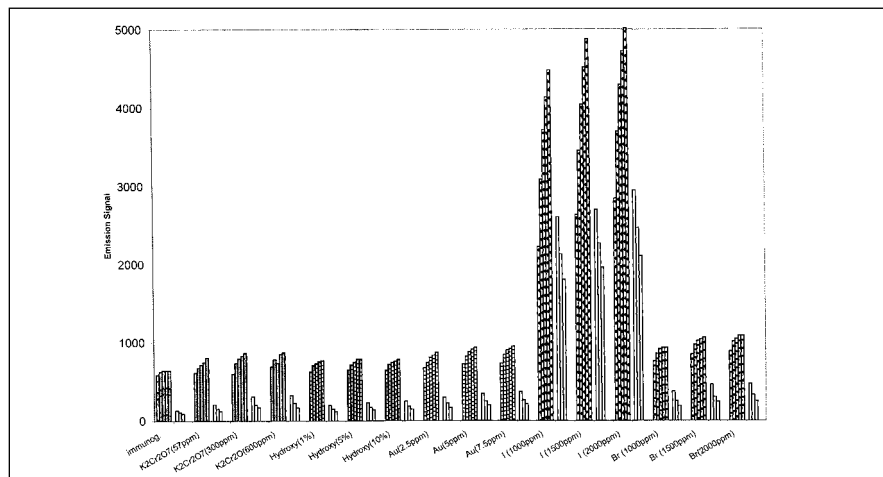*Br^- solution (1000, 1500, and 2000 ppm)*
□: water

Fig. 6b. Effect of $K_2Cr_2O_7$, $HONH_3Cl$, I^- and Br^- on the Hg emission signal from an immunoglobuline sample. Gold was run for comparison.
Immunoglobuline Hg solution
$K_2Cr_2O_7$ solution (57, 300, 600 ppm)
Hydroxylammonium chloride solution (1, 5, and 10% v/v)
Au solution (2.5, 5, and 7.5 ppm)
I^- solution (1000, 1500, and 2000 ppm)
Br^- solution (1000, 1500, and 2000 ppm)
□: water

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is no significant difference at the 95% confidence level using EDTA or Triton X-100 with ICP-OES analysis or when using cold vapor AAS. In addition, the results obtained by applying the ICP-OES method developed are no different from those obtained when the sample is digested totally and determined by CVAAS.

CONCLUSION

The inductively coupled plasma optical emission spectrometry determination of mercury in immunoglobuline using either EDTA or Triton X-100 to dilute the samples has been found to be an alternative method for this particular application. Gold addition to the aqueous standard solutions is necessary in order to stabilize the signal. Mercury carryover effect, usually as a result of sample aspiration, is reduced to a minimumn with the method developed in this work. Good accuracy is found when compared with the CVAAS method for mercury determination.

TABLE II
Comparison of Results

	ICP-AES			CV-AAS	
	EDTA	Triton X-100	TFA	Official Method	Total Digestion
Hg Concentration (µg/L)	52.37	55.03	60.81	51.62	52.29
Standard Deviation (µg/L)	0.97	0.94	0.78	1.37	1.45

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ICP-OES Determination of Rare Earth Elements in Silicate Rocks Using Ultrasonic Nebulization and On-line Ion-exchange Iron Separation

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INTRODUCTION

The concentration of rare earth elements (REEs) in silicate rocks for geochemical studies is well documented (1,2). The determination of REEs is important, since they are sensitive indicators of the geological processes by which silicate rocks have formed. REEs are also used in quantitative petrogenetic modeling (3). They are usually employed as a group in a suitably normalized way, of which the REE distribution shape is the most important indicative criterion. However, a smaller, appropriately chosen number, can also be used for obtaining the general shape of the REE normalized distribution curve in geological materials (4).

REEs are used individually along with other trace elements to build discrimination diagrams for the identification of magma sources and differentiation of magmatic processes in certain specific tectonic environments (5,6).

The distribution of REEs in geological materials, and particularly silicate rocks, is generally very susceptible to the changes suffered by these materials in their evolution. Since these elements are present at the trace level, it becomes necessary to use techniques that are accurate and sensitive enough so as to clearly detect such changes.

For many years, the most common analytical techniques for measuring REEs in silicate rocks have been neutron activation analysis (NAA) (7) and isotope dilution

ABSTRACT

Rare earth elements (REEs) were determined in geological samples by inductively coupled plasma optical emission spectrometry (ICP-OES) with on-line iron separation using flow injection (FI) with an ultrasonic nebulization system. The anion-exchange separation method of a hydrochloric system was applied to the separation of REEs from iron. The iron-chlorocomplexes were retained on an anion-exchange (Dowex 1X-8) micro-column (3.0 mm i.d. x 50.0 mm bed length), while the analytes were introduced into the ultra-sonic nebulization system. After iron removal, a 700-μL sample was injected into a water carrier stream. The system was found to have a detection limit of La 0.30 μg/L ; Ce 0.93 μg/L; Nd 0.51 μg/L; Sm 0.25 μg/L; Eu 0.045 μg/L; Gd 0.72 μg/L; Er 0.12 μg/L; Yb 0.01 μg/L; Lu 0.006 μg/L. The application of the method on Reference Materials GS-N (ANRT); AC-E (IGW), G-2, RGM-1, AGV-1 and SDC-1 (USGS) demonstrated that results were statistically indistinguishable from published values.

mass spectrometry (IDMS) (8). However, NAA does have a long turn-around time and sophisticated instrumentation is required, which is generally not available in most analytical laboratories.

Inductively coupled plasma mass spectrometry (ICP-MS) has proved ideally suited as an alternative approach for the determination of REEs in various matrices due to its high sensitivity, selectivity, and sample throughput (9–11).

Inductively coupled plasma optical emission spectrometry (ICP-OES) constitutes an alternative technique for the determination of most elements at different concentration levels due to a very wide linear response range of more than five orders of magnitude.

In rock analysis, and particularly in REE determination by ICP-OES, some spectral interferences can occur, of which the most severe are commonly generated by Fe (12–14). This interference remains a serious analytical problem since Fe, a major element in silicate rocks, has more than 1,000 lines between 200 and 300 nm (15), which makes it a very problematic interferent.

Elimination of line interference due to coincidence or overlap can be achieved by choosing an alternative analytical line. However, this may lead to a sensitivity loss, which becomes critical when working at trace levels.

With the purpose of minimizing line interference, certain analytical methodologies using ionic exchange resins have been used in a batch system (16–19). These have the disadvantage of being tedious, labor-intensive and, to some extent, dependent on operator skill.

The possibility of developing a method that enables interferent separation and REE determination at a single on-line step in a continuous flow system is currently being investigated in our laboratory. Flow injection on-line separation techniques have become increasingly popular in the last decade for the determination of trace elements in different types of samples (20–22).

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In conjunction with the use of an ultrasonic nebulizer, it can provide a 5–50 fold improvement in detection limits (23-25).

The present paper reports on a flow injection system for the determination of REEs in silicate rocks using ICP-OES with ultrasonic nebulization. In this system, iron interference is removed on-line by using a microcolumn of an ion-exchange resin Dowex 1X-8 chloride form in a continuous-flow system.

EXPERIMENTAL

Reagent

The anion exchange resin was Dowex 1X8 100-200 dry-mesh, chloride form 8% cross linkage (Aldrich Chemical Co.). Before analysis, the resin was washed with deionized water plus HCl drops in order to separate the fine particles by sedimentation. The use of smaller resin particles could improve retention capacity, but this would increase back-pressure of the microcolumn and the flow rate would have to be reduced, with a consequent increase in pre-concentration time. The resin was oven-dried overnight at 80°C. Standard stock solutions (1,000 mg/L) of the REEs were prepared by dissolving accurately weighed amounts of high-purity oxides (99.99 %, Aldrich Co., Milwaukee, WI, USA). All other solvents and reagents were of analytical reagent grade or better.

Instrumentation

The measurements were performed with a sequential inductively coupled plasma spectrometer (Baird ICP 2070). The 1m-Czerny-Turner monochromator had a holographic grating with 1800 grooves/mm. An ultrasonic nebulizer U-5000 AT (CETAC Technologies) was used. The ICP and ultrasonic nebulizer operating conditions are listed in Table I. The flow injection system used is shown

TABLE I.
ICP and Ultrasonic Nebulizer Instrumental Parameters

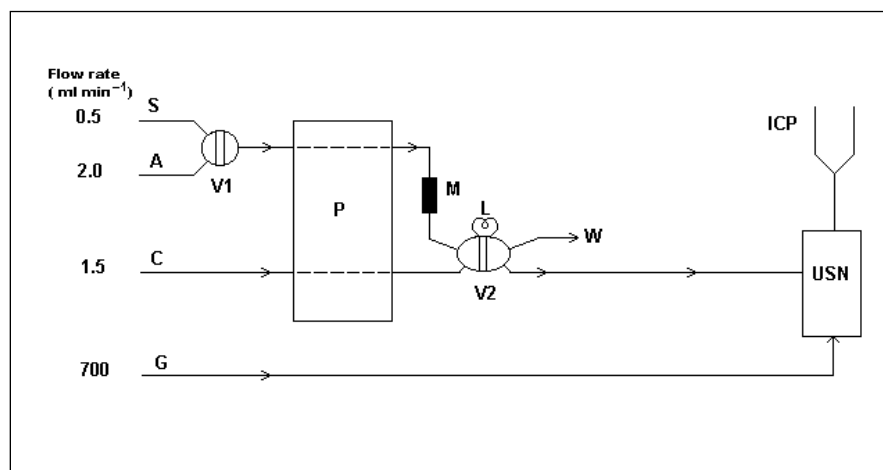| ICP conditions	
RF generator power	1 kW
RF generator frequency	40.68 MHz
Plasma gas flow rate	8.5 L/min
Observation height (above load coil)	15 mm
Ultrasonic nebulizer conditions	
Heater temperature	140°C
Condenser temperature	5°C
Nebulizer gas flow rate	0.7 L/min

Fig 1. Schematic diagram of the instrumental setup. S: sample (0.5 mL/min); A: HNO$_3$ 2 mol/L (2.0 mL/min); C: carrier (water, 1.5 mL/min); G: argon (700 mL/min); P: peristaltic pump; V1: two-way valve; V2: injection valve; M: microcolumn; W: waste; USN: ultrasonic nebulizer.

in Figure 1. The sample injection was achieved using a Rheodyne Model 50 four-way rotary valve. A microbore glass column (50 mm length, 3 mm internal diameter), fitted with porous 25-µm glass frits, was used as the resin holder. The measurements of the FI system were expressed as peak height emission, which was corrected against the reagent blank.

Sample Preparation

The ICP-OES is a solution-based technique, which requires that the elements to be measured are taken quantitatively into solution. The digestion method used in this work was an open acid digestion for silica elimination as volatile SiF$_4$ in order to minimize the level of total dissolved solids (TDS) and to achieve low blank levels.

The method adopted was as follows: 0.5 g of sample (minus 200 mesh) were placed in a 100-mL PTFE beaker and moistened with a drop of deionized water. After addition of 1 mL HNO$_3$, the mixture was evaporated to dryness. Then, 10 mL HF, 3 mL HClO$_4$, and 2 mL HNO$_3$ were added. The mixture was allowed to digest and evaporate to incipient dryness. The HF-HClO$_4$-HNO$_3$ digestion was repeated three times and finally 2 mL HNO$_3$ were added and again evaporated to dryness. The final residue was taken up in 10-mL water with gentle heating and addition of concentrated HCl.

When digestion was complete, more concentrated HCl and water was added until 50 mL of 6 mol/L HCl solution was obtained. Any insoluble residues in this step were removed by filtration and fused with minimal amounts of flux (NaOH). The resulting cake was dissolved with HCl and added to the original filtrate (26).

Procedure

The microcolumn was filled with anionic resin. Prior to use, the resin was conditioned by pumping a solution of 2 mol/L HCl through the column for 2 min at a flow rate of 1 mL/min.

The injection volume was 700 µL, the loop charge flow rate 0.5 mL/min, and the carrier flow rate 1.5 mL/min. After triplicate injections, the microcolumn was regenerated using 2 mol/L nitric acid, which was pumped continuously through the microcolumn at a flow rate of 2 mL/min for 60 s, after which the sample line was washed with analytical-reagent grade water. The standard solutions of REEs were measured with the same flow injection system as the samples. The flow injection system measurements were expressed as peak height emission, which was corrected against the reagent blank. The operating conditions were established and the determination was carried out.

The breakthrough capacity of the ionic exchange resin widely exceeded the needs of Fe elimination in silicate rocks so it was possible to perform three successive injections.

Limits of Detection

The limits of detection (LoD) were calculated as the amount of REEs required to yield a net peak that was equal to three times the standard deviation of the background signal (3σ) using the system depicted in Figure 1. The lines chosen for the determination and the corresponding limits of detection are listed in Table II.

The choice of analytical lines depended on the abundance of individual REEs in the sample and on the concentration level of matrix elements in the final solution after ion exchange. The most sensitive lines were employed, taking into account the presence of interferents other than Fe.

RESULTS AND DISCUSSION

In this work we used a microcolumn filled with an anionic resin (Dowex 1X-8) which had the capacity to retain iron (as anionic chloro-complexes) and not REEs when the concentration of the hydrochloric acid solution was between 3.5 and 9 mol/L (Figure 2). When the concentrations were below 3.5 mol/L, iron was not quantitatively retained due to the deficiency in the chloro-complexes formation. Consequently, a concentration of 6 mol/L was adopted, since, with this concentration, optimum iron-chloro-complexes formation was ensured. Also, in order to demonstrate non-retention of REEs in the microcolumn, the recovery of these elements in the eluate was evaluated using a synthetic solution of the REEs in front of excess of iron

TABLE II
Limits of Detection (LoD) for the Determination of REEs by ICP-OES With Ultrasonic Nebulization

Elements	Wavelength (nm)	LoD (µg/L)[a]	LoD (ng/g)[b]
La	398.852	0.30	30
Ce	418.660	0.93	93
Nd	430.358	0.51	51
Sm	359.260	0.25	25
Eu	381.967	0.045	4.5
Gd	342.247	0.72	72
Er	390.631	0.12	12
Yb	328.927	0.01	1
Lu	261.542	0.006	0.6

[a]in solution.

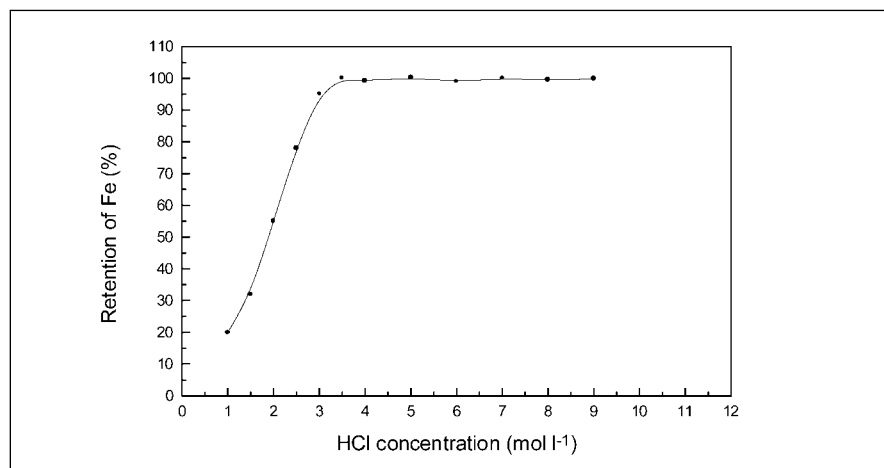[b]in solid (0.5 g of sample in a final volume of 50 mL).

Fig 2. Effect of hydrochloric acid concentration on the retention of iron in the microcolumn. Sample flow rate 0.5 mL min[1]. Iron concentration 1000 mg/L.

and in a 6-mol/L hydrochloric acid medium. The recovery percentage was between 98.4 and 100 % (see Table III).

The optimum flow rate for sample introduction into the ICP system was found to be 1.5 mL/min. This rate was optimized by loading the loop (700 µL) with standard solutions of the REEs and injecting this volume at different carrier (water) flow rates. Figure 3 shows the results obtained for Lu. Similar results were obtained for the remaining REEs.

However, if the sample was run through the column at this rate, iron was not quantitatively retained. This problem was solved by using an FI system for introduction of the sample. This system allowed the separation of iron in the ion exchange column at a flow rate of 0.5 mL/min; subsequently, the sample-containing loop was injected into the carrier at 1.5 mL/min.

Each measurement represents the mean of results from three 700-µL injections, after which the resin was regenerated by running 2 mol/L nitric acid for 60 s and then 2 mol/L hydrochloric acid for 60 s. In general, increase of the volume favors sensitivity (27). Figure 4 shows the effect of loop volume on the sensitivity for Gd determination. Similar results were obtained for the remaining REEs. A 700-µL injection volume was chosen because it is the minimum volume with which maximum sensitivity is obtained.

The ultrasonic nebulization system, coupled to the iron interference separation on-line, allowed the determination of rare earth elements in silicate rocks to values compatible with the REE contents in the sample.

The effects of representative potential interferent species were tested. Thus, Al could be tolerated

TABLE III
Recovery of REEs in the Eluate, After Passing Through the Micro-column Loaded With the Anionic Resin (Fe): 1000 mg/L, HCl: 6 mol/L

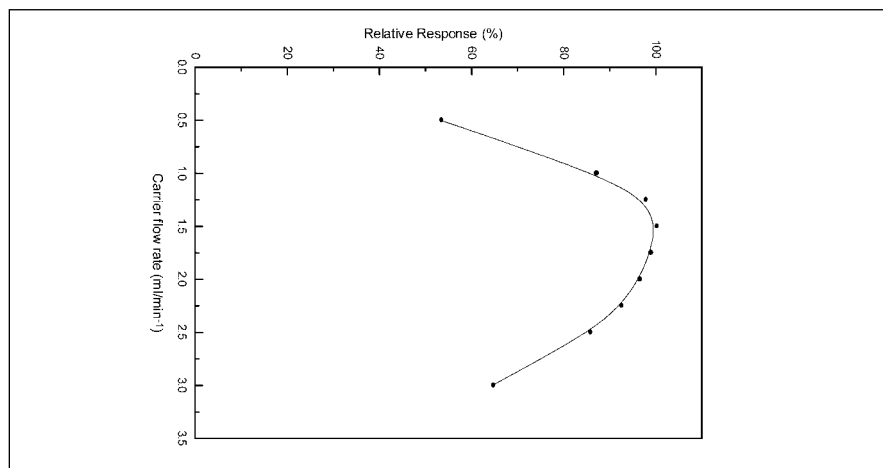| Element	Quantity added (µg/L)	Quantity found (µg/L)	Recuperation (%)
La	50.0	49.4	98.8
Ce	50.0	50.0	100.0
Nd	50.0	49.2	98.4
Sm	50.0	49.6	99.2
Eu	50.0	50.0	100.0
Gd	50.0	49.5	99.0
Er	50.0	49.8	99.6
Yb	50.0	49.3	99.6
Lu	50.0	49.5	99.0

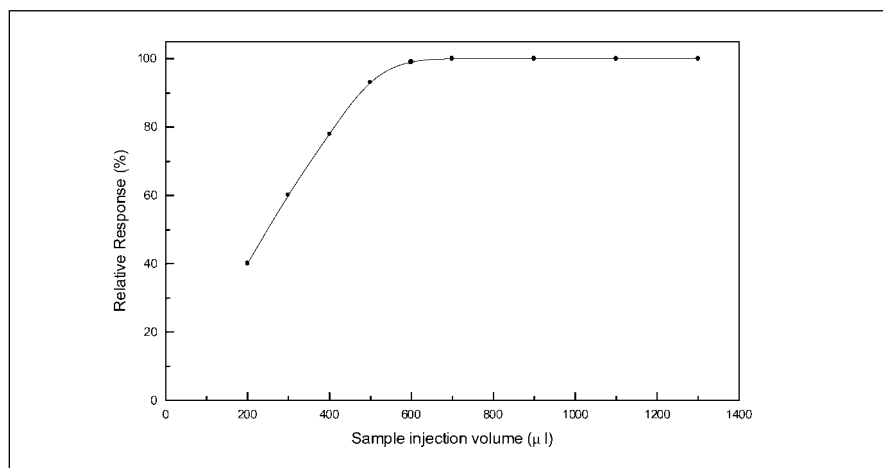Fig 3. Dependence of relative response for Lu with carrier flow rate.

Fig 4. Effect of sample injection volume on the response of Gd.

up to at least 1,000 mg/L, and Mg, Ca, Ba, Ti, and Sr up to at least 500 mg/L.

For the concentration relation in the solution coming from the silicate rock samples, the interferences between REEs were negligible for the lines used. This is in agreement with results reported by Roelandts et al. (16).

In order to test the procedure, the rare earth element contents of some reference materials were determined. The results are listed in Tables IV and V. In general, the agreement between reference and measured concentration appeared to be satisfactory.

Values were plotted relative to chondrites in Figures 5 and 6. The values used for normalization are from Nakamura (28). With the exception of Eu (due to the Eu geochemical anomaly), our data exhibit a smoothed curve for the elements determined in this work, which constitutes a quality criterion for our procedure.

CONCLUSION

The results obtained in the analysis of the six reference materials show that it is possible to determine some REEs accurately in silicate rocks, using ICP-OES with an ultrasonic nebulization system, and with prior iron elimination. Iron was removed on-line by means of a rapid and simple FI method using an anion exchange procedure. This method was applied to the determination of REEs in silicate rocks of reference

TABLE IV.
Concentration (µg/g) of the REEs Reference Materials as
Determined by ICP-OES With Ultrasonic Nebulization

Element	G-2 This work*	Gladney et al.(29)	GS-N This Work*	Govindaraju (30)	AC-E This Work*	Govindaraju (30)
La	87±2.5	89±8	73±0.8	75±2.7	59.2±0.9	59±2
Ce	152±6	160±10	145±1	135±7	158±3.1	154±4.7
Nd	54±0.5	55±6	48±0.3	49±1.5	91±1.7	92±6
Sm	6.9±0.2	7.2±0.7	8.2±0.01	7.5±0.22	25.6±0.6	24.2±0.8
Eu	1.42±0.02	1.4±0.12	1.8±0.01	1.7±0.06	1.8±0.05	2±0.09
Gd	4.3±0.4	4.3±0.8	5.1±0.05	5.2±0.3	28.1±0.5	26±1.5
Er	0.9±0.06	0.92±0.18	1.6±0.03	1.5±0.15	18±0.5	17.7±1.2
Yb	0.82±0.02	0.8±0.17	1.6±0.02	1.4±0.15	18.1±0.3	17.4±0.5
Lu	0.12±0.01	0.11±0.02	0.19±0.01	0.22±0.03	2.5±0.03	2.45±0.11

*Mean values ±2 standard deviations of analytical results calculated for five determination of the same sample solution

Fe content as Fe_2O_3 (%) for the reference materials used: **G-2**: 2.66; **GSN**: 3.75 and **ACE**: 2.53. Source: Govindaraju, K (31).

TABLE V.
Concentration (µg/g) of the REEs Reference Materials as
Determined by ICP-OES With Ultrasonic Nebulization

Element	AGV-1 This work*	Gladney et al. (29)	RGM-1 This Work*	Gladney et al. (32)	SDC-1 This Work*	Gladney et al. (32)
La	37.4±0.4	38±3	22.8±0.3	24.0±1.1	40.8±0.4	42±3
Ce	68.2±0.9	67±5	48.2±0.5	47±4	89.2±1.2	93±7
Nd	31.8±0.4	33±3	20.0±0.3	19±1	41.3±0.5	40±4
Sm	5.8±0.2	5.9±0.4	4.2±0.1	4.3±0.3	8.16±0.2	8.2±0.5
Eu	1.70±0.01	1.64±0.1	0.64±0.01	0.66±0.08	1.68±0.03	1.71±0.12
Gd	5.2±0.1	5.0±0.5	4.12±0.03	3.77±0.4	7.4±0.3	7.2±0.4
Er	1.84±0.03	1.7±0.2	2.46±0.02	2.6±0.3	3.6±0.2	4.1±0.7
Yb	1.72±0.02	1.72±0.2	2.50±0.02	2.6±0.3	3.8±0.1	4.0±0.7
Lu	0.26±0.01	0.27±0.03	0.40±0.01	0.41±0.03	0.48±0.02	0.53±0.11

*Mean values ±2 standard deviations of analytical results calculated for five determination of the same sample solution

Fe content as Fe_2O_3 (%) for the referece materials used: **AGV-1**: 6.77; **RGM-1**: 1.86 and **SDC-1**: 6.90. Source: Govindaraju, K. (31)
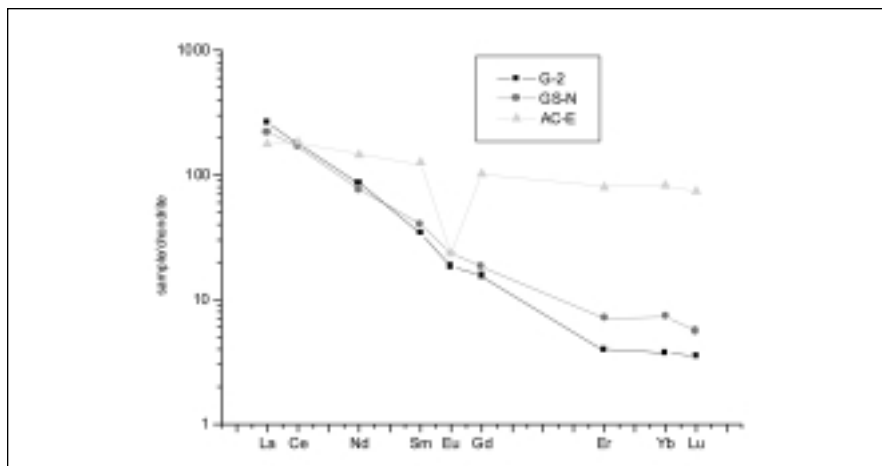
Fig 5. Diagram showing normalized values with chondrites for G-2, GS-N, and AC-E.
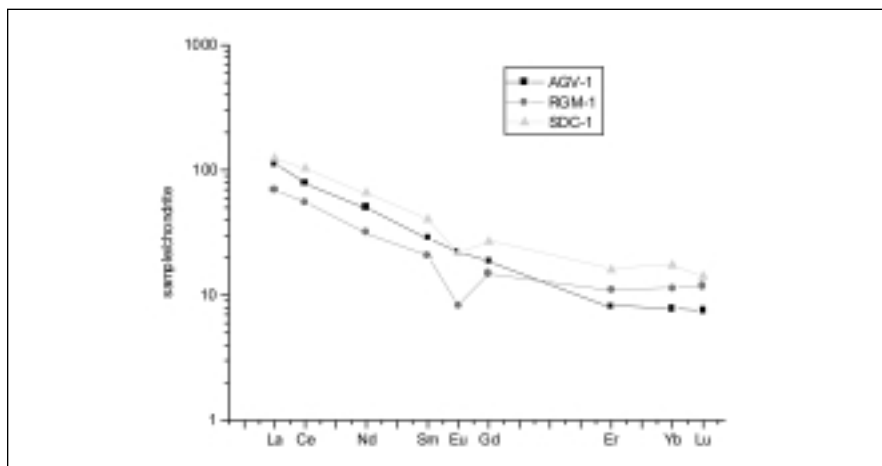

Fig 6. Diagram showing normalized values with chondrites for AGV-1, RGM-1, and SDC-1.

materials. The detection limits of the proposed method are compatible with values commonly found in silicate rocks, with the advantage of requiring less time and less complex than preconcentration methods.

ACKNOWLEDGMENTS

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Determination of Cd, Co, Hg, and Ni in Seawater After Enrichment on Activated Carbon by Slurry Sampling Electrothermal AAS

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INTRODUCTION

Activated carbon (AC) has been widely used for many purposes both in laboratory and industrial settings, due to its ability to adsorb organic compounds and organic metal complexes. Since its introduction in analytical chemistry, enrichment of trace metals using AC has been favorably performed with very high concentration factors in different matrices (1-8). Generally, sorption of dissolved metal ions on AC can be improved in the presence of chelating or precipitating agents (9-13).

The techniques used for subsequent analysis of the samples include x-ray fluorescence (4,9), neutron activation analysis (4), as well as atomic absorption spectrometry (10-13). Some of the advantages of the first two techniques listed were their simultaneous multielement and direct solid introduction possibilities. Earlier works using either flame (10-15) or nonflame (16-18) atomic absorption spectrometry systems required prior acid leaching of the adsorbed metals from AC.

Okutani et al. applied slurry introduction into the electrothermal atomizer for AC after enrichment from sediment digests (19-21) and water samples (22-24). The method was documented for thallium using xanthogenate complex (19), nickel and cobalt using 1,2-cyclohexanediondioxime (20), and silver and gold using dithizone (21). Use of acetylacetone was also

ABSTRACT

An enrichment of trace metals using the activated carbon method was investigated for the preconcentration of Cd, Co, Hg, and Ni in seawater after complexing with 150 mg mL^{-1} 8-hydroxyquinoline, pH 6-10. The adsorbed activated carbon was prepared as a slurry in glycerol or in boiled tapioca suspension before electrothermal atomic absorption spectrometric measurement. A contact time of 5 minutes was adequate for quantitative adsorption. Slurry concentration between 1-10 % could be used. An enrichment factor of up to 20,000-fold was achieved, with recoveries ranging from 84–114%. At 3% slurry concentration, the detection limits for Cd, Co, Hg, and Ni in seawater were 0.0003, 0.007, 0.12 and 0.007 ng mL^{-1}, respectively.

studied for beryllium (22) and indium (23) in water samples. In addition, impregnation of AC with 2,4,6-tri-2-pyridyl-1,3,5-triazine prior to determination of ruthenium was also investigated for several water samples (24).

The application of electrothermal atomic absorption spectrometry (ETAAS) to the direct solids analysis, without previous dissolution steps, has been studied by a number of analysts (25-32). The direct introduction of solids into the ETAAS atomizer offers many advantages over conventional techniques in terms of speed, simplicity, small reagent consumption, reduced risk of sample contamination, and improved detection limits. Solid samples may be introduced in the form of a slurry suspension.

With the slurry method, the samples can be transferred directly into the atomizer using the conventional liquid sampling procedure without the need for atomizer modification. Homogenization procedures, however, are required to ensure injection of a representative slurry sub-sample into the atomizer. Homogenization can be done by suspending fine solid powder in an appropriate medium with subsequent thorough mixing. Bendicho et al. (33) summarized and categorized the application of a slurry sampling technique in four types of slurry media. These media are glycerol (34,35), Triton® X-100 (36-38-31), sodium hexametaphosphate (34,37,39,30,32), and Viscalex (40-43), a thixotropic thickening agent. In our laboratory, a boiled tapioca flour suspension, hereafter referred to as TAPIOCA, was successfully used as a slurry medium for rice flour (44), as well as soil and sediment, with relatively low blank contribution. In this work, TAPIOCA is also employed for AC powder slurry preparation.

The present work combines the merits from a high concentration factor of AC enrichment of heavy metals from aqueous solution and the merits from a simple direct slurry sampling ETAAS analysis for the determination of trace amounts of Cd, Co, Hg, and Ni in seawater. The stability of AC powder slurry, maximum allowable slurry concentration, effect of AC on atomic absorption signals, and characteristics of multielement trace enrichment on AC are investigated and optimized.

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EXPERIMENTAL

Instrumentation

A PerkinElmer Model 3100 atomic absorption spectrometer, equipped with a deuterium arc background corrector and an HGA®-600 electrothermal atomizer, was used. A PerkinElmer Model AS-60 autosampler was employed to dispense 20-μL aliquots of the sample solutions or slurry with 5 μL of chemical modifier when needed. Wall atomization with standard pyrolytically coated graphite tubes (PerkinElmer) was used and the measurements were based on peak height. Cathodeon hollow cathode lamps (Cathodeon Cambridge, U.K.) were used. The instrumental parameters and operating conditions are given in Table I. The atomization temperatures used for Co and Ni were in accordance with the manufacturer's recommendations. For Hg and Cd, atomization using Pd as the modifier was studied and the atomization temperatures that gave best analytical precision and sensitivity were used.

Reagents

Activated carbon (AC) was purchased from Fluka Chemicals, Switzerland. Prior to use, the AC was further purified by treatment with HF and HCl, then washed with deionized water to remove impurities. The cleaned AC was then sieved to particle sizes of 53–106 μm. All stock standard solutions of 1000 μg mL^{-1} were from Merck, Darmstadt, Germany. An analytical reagent grade 8-hydroxyquinoline (Oxine) was purchased from the British Drug Houses, Poole, England. TAPIOCA was prepared freshly before use by suspending a suitable amount of tapioca flour (from a local supermarket) in deionized water; the mixture was then brought to boiling while at the same time vortex mixing.

TABLE I
Instrument Operating Conditions

	Hg	Cd	Co	Ni
Wavelength (nm)	253.7	228.8	240.7	232.0
Lamp Current (mA)	6	5	10	12
Ar Flow (mL/min)	300	300	300	300
	(flow interrupted at atomize stage)			
Injection Volume (μL)				
- sample	15	15	20	20
- modifier (1000 ppm Pd)	5	5	-	-
Heating ProgramTemperature (ramp time, hold time)				
- Dry	100 (20,20)	100 (20,20)	100 (20,20)	100 (20,20)
- Ash	350 (10,20)	500 (10,20)	1000 (10,20)	1000 (10,20)
- Atomize	2000 (1,3)	1750 (0,3)	2100 (0,3)	2300 (0,3)

Purification of Activated Carbon and Particle Size Selection

The AC was acid-washed (50 g of AC was successively washed with 150 mL HF and 150 mL HCl) and rinsed three times with deionized water to remove impurities. Even so, a high blank still remained for elements such as Pb, Cu, and Cr (410, 1610, and 2900 ng g^{-1}, respectively). As can be seen, it is not practical to use this method for such elements. A particle size of 53–106 μm was obtained by sieving through stainless steel sieves. This particle size range was selected because it constituted the largest portion of the AC purchased. Furthermore, it provided adequate stability in the slurry medium used.

Procedure for Metal Enrichment on Activated Carbon and Slurry Preparation

An accurately weighed AC was added to an appropriate volume of solution or seawater sample. Oxine solution was then added to obtain 150 μg mL^{-1} in the solution. After thorough stirring of the mixture for 10 min, the AC was separated from the aqueous phase by filtration, and left standing in a desiccator overnight until dry. Three

percent AC slurry was prepared by vortex mixing of a known amount of the dried adsorbed AC in 0.5 - 2.0 % TAPIOCA.

RESULTS AND DISCUSSION

Stability of Activated Carbon Slurry

In our laboratory TAPIOCA has been successfully used as a slurry medium to stabilize powder samples such as flours (44), soil, sediment, seaweed and seafood samples before introduction of the sample into the ETAAS atomizer. To evaluate the stabilization ability of TAPIOCA to suspend the adsorbed AC for quantitative and reproducible transfer of 20-μL amounts of slurry samples, metal-adsorbed AC in 2% TAPIOCA was sampled and measured at various times. The widely used stabilization medium glycerol was also investigated for comparison. Figure 1 demonstrates that both media can stabilize AC for at least 15 min.

Stabilization was greatly dependent on the solid powder particle size. Therefore, the AC used must be of narrow range, because different particle sizes can affect the stabilization time of the slurry.
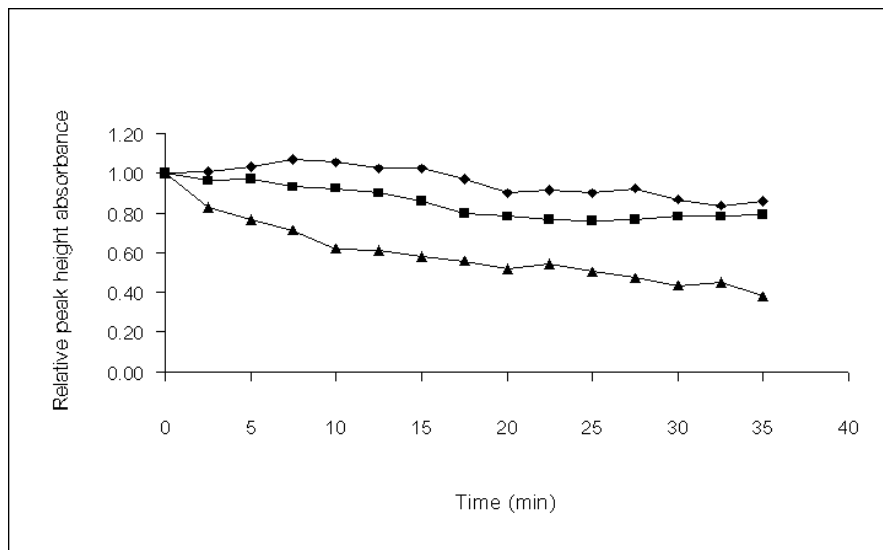
Fig 1. Stability of activated carbon (53-106 mm) in 0.5% TAPIOCA (▲), 2% TAPIOCA (◆) and glycerol (■) as evaluated by changes in ETAAS signals of the Cd-adsorbed activated carbon slurry (at 3% slurry concentration).
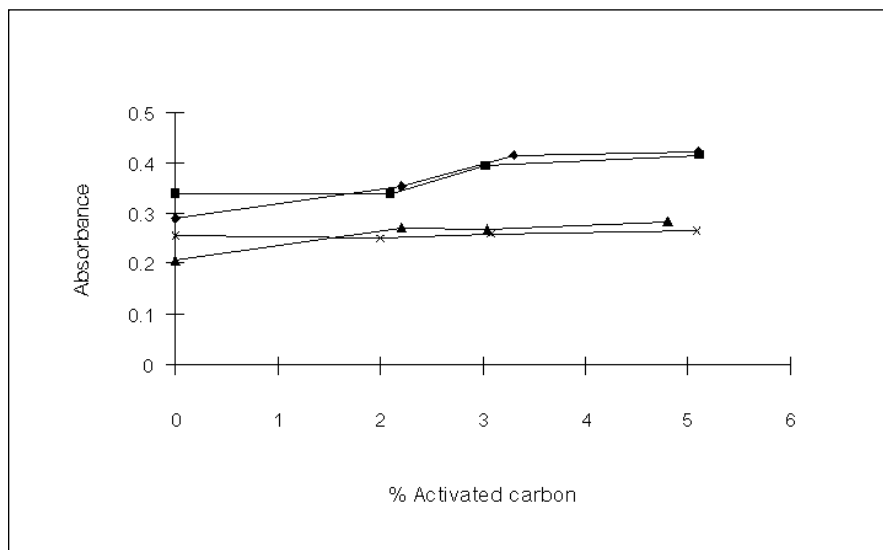

Fig 2. Effect of activated carbon on atomic absorption signals of Cd (✕), Co (■), Hg (▲), and Ni (◆). Pd was used as modifier for Hg and Cd, 0.5% TAPIOCA was used as stabilization medium.

The TAPIOCA not only helps to stabilize the AC slurry, but also assists the thorough mixing of small AC particles. However, it was found to cause high background absorption for Hg determination because only low temperature ashing could be used. At 2.0% TAPIOCA, a sharp background peak (approximately 0.8 absorbance unit at peak maximum) appeared prior to the atomic peak. At the atomic peak maximum, the background signal contributed to about 0.05 absorbance unit. Although this contribution could be corrected for by the background corrector, it may cause poor analytical precision. Therefore, only 0.5% TAPIOCA was used for the determination of Hg, which resulted in less than 5-min stability. In such cases, the sample slurry was vortex-mixed just prior to sampling.

Effect of Activated Carbon on ETAAS Signals

To perform slurry introduction of adsorbed AC for ETAAS measurement, it is necessary to investigate the effect of AC on the atomic absorption signals for the elements studied. Atomic absorption signals from the standard solutions of Hg, Cd, Co, and Ni in 0.5% TAPIOCA with the addition of various amount of AC were monitored. The results in Figure 2 indicate a small enhancement with increasing amounts of AC. The authors in another work found that AC enhanced the Hg signal considerably when Pd was used as the modifier (45). Other researchers reported the same effect from ascorbic acid (46). It was explained that the reduction of Pd by such a modifier facilitated the amalgamation of Hg with Pd, resulting in improved atomic absorption signals. In this experiment, only a slight change could be attributed to the fact that carbon residue from the pyrolysis of TAPIOCA provided sufficient reducing power for Pd. Thus, the use of additional AC has no significant effect. The mechanism of a carbon-enhancing atomic absorption signal in the graphite furnace atomizer was reported by Ashton (47), among other researchers.

Maximum Slurry Concentration for ETAAS Measurement

Use of high slurry concentration can improve the detection limits of the analytical procedure. The maximum allowable slurry concentration was examined by preparing various slurry percentages from the same adsorbed AC with subsequent
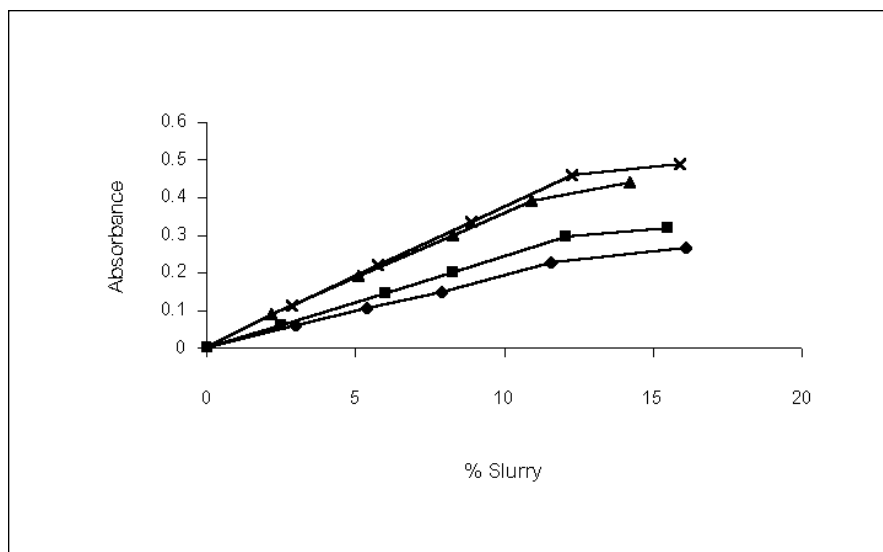
Fig 3. Relationship between atomic absorption signals of Cd (✕), Co (■), Hg (▲), and Ni (◆) and percentage of activated carbon slurry concentration.

TABLE II
Concentrations of Some Impurities in Activated Carbon (before and after acid leaching) and in Tapioca Flour

Elements	Concentration in AC (ng/g)		Concentration in Tapioca Flour (ng/g)
	Before purification	After purification	
Cd	n.d. <0.18	n.d. <0.18	n.d. <0.18
Co	n.d. <1	n.d. <19	n.d. <19
Cr	6400	2900	n.d. <10
Cu	3090	1610	n.d. <15
Hg	n.d. <86	n.d. <86	n.d. <86
Ni	47	n.d. <19	n.d. <19
Pb	830	410	n.d. <30

n.d. = not detectable.

ETAAS measurement. The atomic absorption signals of a 20-μL slurry at various concentrations are illustrated in Figure 3.

Figure 3 demonstrates that slurry concentrations up to 10% could be used. However, to avoid the problem from carbon build-up in the atomizer, which can lead to inaccuracy in the subsequent sample introduction, 3-4% slurry concentration is recommended considering also that the effect of carbon on AA signals is negligible at this range (see Figure 2). If high slurry concentrations are necessary to improve the method detectin limit, air or oxygen ashing can be employed to slow down or eliminate the carbon build-up.

Contamination of Metals in Activated Carbon and its Reduction

To obtain as low a blank signal as possible, the purest AC is required. Even the purest available AC contains rather high levels of some elements, therefore further purification by acid leaching was attempted using the procedure proposed by Vanderborght (48). Some metal concentrations in the AC before and after acid leaching as determined by slurry ETAAS are presented in Table II. The efficiency of removal was found to be approximately 50%, which is similar to the results of Vanderborght. Repetitive acid leaching can only slightly reduce the metal content further, probably because of the purity of acids and the cleanliness of glassware being used. An additional acid leaching can be worthwhile only under very careful control of contamination sources. The content of Cr, Cu, and Pb in the purified AC was 2.9, 1.6, and 0.4 $\mu g\ g^{-1}$, respectively. These levels can result in high blank readings and poor analytical performance. As a consequence, these elements were excluded from further studies.

Enrichment of Heavy Metals by Activated Carbon

The optimum pH and oxine concentration for chelating metal ions as well as the effect of contact time for efficient adsorption of metal oxinates on AC were studied in order to optimize the enrichment procedure.

Contact Time for Adsorption

The results of the effect of contact time are shown in Figure 4. Clearly, less than 5 minutes were required to reach a constant reading of the atomic absorption signal of the metal oxinates studied.

Effect of Oxine Concentration and pH

The use of 8-quinolinol (8-hydroxyquinoline), oxine, as a complexing agent to chelate metals has long been documented (4). The adsorption of metal on AC tends to increase when the metal is complexed with organic ligands, owing to the fact that metal complexes are less soluble in water
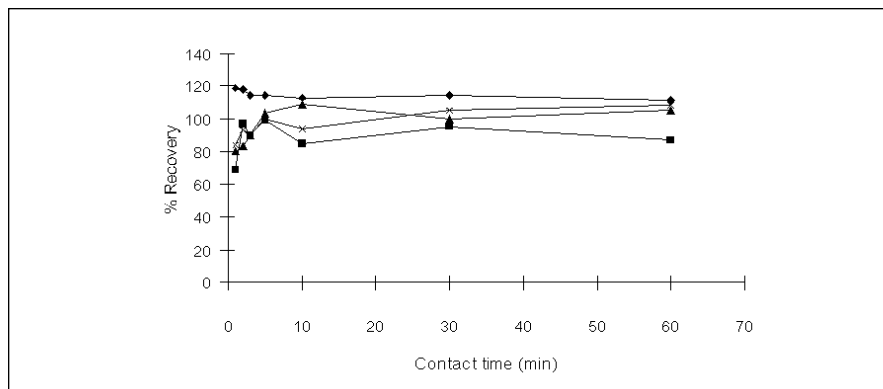
Fig 4. *Effect of contact time on the recovery of Cd (✕), Co (■), Hg (▲), and Ni (◆). The 100.0 mL solution contains 10 ng mL^{-1} of Hg, 0.008 ng mL^{-1} of Cd, 1 ng mL^{-1} of Co and 3 ng mL^{-1} of Ni in 150 μg mL^{-1} oxine. Activated carbon 50 mg was used. The solution was buffered at pH 7.*
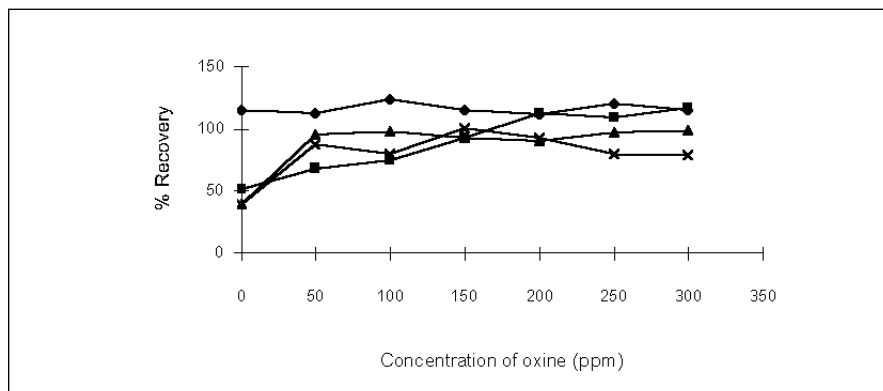

Fig 5. *Effect of oxine concentration on the recovery of metal oxinates [Cd (✕), Co (■), Hg (▲), and Ni (◆)]. The 100.0 mL solution contains 10 ng mL^{-1} of Hg, 0.08 ng mL^{-1} of Cd, 1 ng mL^{-1} of Co, and 3 ng mL^{-1} of Ni. Activated carbon 50 mg was used. The contact time was 5 min. The solution was buffered at pH 7.*
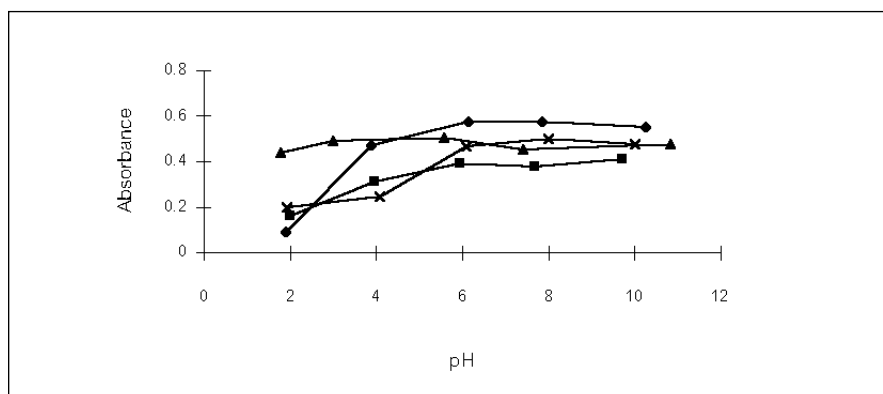

Fig 6. *Effect of pH on the adsorption of metal oxinates. [Cd (✕), Co (■), Hg (▲), and Ni (◆)]. The 100.0 mL solution contains 10 ng mL^{-1} of Hg, 0.08 ng mL^{-1} of Cd, 1 ng mL^{-1} of Co, and 3 ng mL^{-1} of Ni in 150 μg mL^{-1} oxine. Activated carbon 50 mg was used. The contact time was 5 min.*

resulting in higher adsorption on AC. The effect of oxine concentration and pH on the enrichment of metal oxinates were studied with results shown in Figures 5 and 6. Oxine concentration of 150 μg mL^{-1} was considered to be sufficient for quantitative recovery of metals studied. The optimum pH was found to be at 6-10 which allowed natural waters to be determined without any pH adjustment.

Amount of Activated Carbon and Concentration Factor

The effect of the AC amount on adsorption efficiency was examined. Figure 7 shows that only 0.02 g of AC was adequate for the quantitative recovery of Cd, Co, Hg, and Ni under the conditions studied. To increase the concentration factor of enrichment, the sample volume was increased to obtain the maximum concentration factor. Figure 8 shows that a 1000-mL sample volume can be used without a significant decrease in the recovery of the metals. Since a 0.05-g amount of AC was used, the concentration factor can be estimated to be 20,000-fold.

Application to Seawater Analysis

The proposed combination of AC enrichment with slurry sampling-ETAAS was applied to the analysis of 1000 mL of seawater and 100 mL of 3.5% saline solution using 0.05 g of AC (20,000-fold and 2,000-fold enrichment, respectively). The results are given in Table III. The recovery of the four elements studied was found to be satisfactory, indicating that major and minor cations in seawater such as Na, Mg, and Ca produced insignificant effects on the adsorption efficiency of the trace metals studied. The detection limits of the proposed method as determined by the concentration that gives a signal equal to three times the standard deviation of five determinations of near blank levels for Cd, Co, Hg,
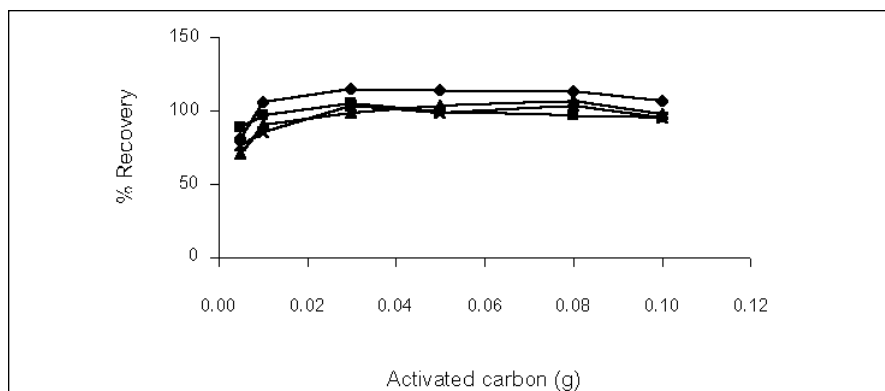
Fig 7. Effect of amount of activated carbon on the recovery of metals oxinates [Cd (✕), Co (■), Hg (▲), and Ni (◆)]. The 100.0 mL solution contains 10 ng mL^{-1} of Hg, 0.08 ng mL^{-1} of Cd, 1 ng mL^{-1} of Co and 3 ng mL^{-1} of Ni in 150 µg mL^{-1} oxine. The contact time was 5 min. The solution was buffered at pH 7.
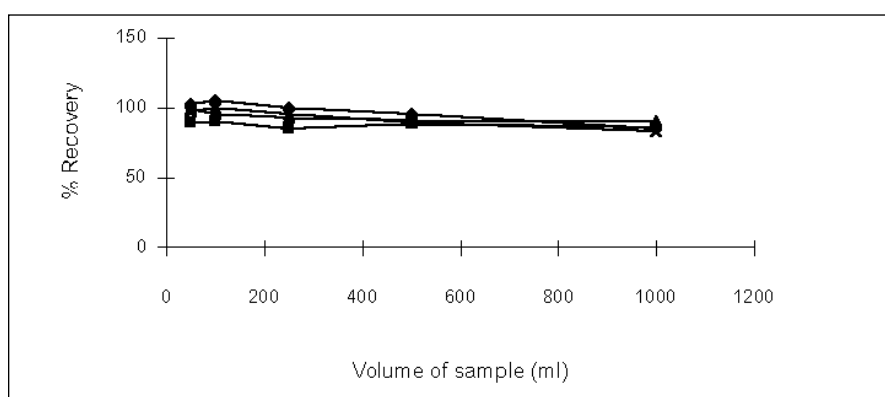

Fig 8. Effect of sample volume on the recovery of metal oxinates [Cd (✕), Co (■), Hg (▲), and Ni (◆)]. All solutions contain 1 mg of Hg, 0.008 mg of Cd, 0.1 mg of Co and 0.3 mg of Ni in 150 µg mL^{-1} oxine. Activated carbon 50 mg was used. The contact time was 5 min. The solution was buffered at pH 7.

TABLE III
Results and Recovery of Hg, Cd, Co, and Ni From Deionized Water, 3.5% Saline and Seawater

Elements	Sample	Added (ng)	Found (ng)	Recovery (%)	RSD[a] (%)
Hg	Purified water (1000 mL)	1	0.97	97	9.37
	3.5% saline (100 mL)	0	0.315		
		1	1.16	86	8.86
	Seawater (1000 mL)	0	n.d.		
		1	1.14	114	7.52
Cd	Purified water (1000 mL)	0.008	0.0078	97	8.12
	3.5% saline (100 mL)	0	0.0003		
		0.008	0.0079	95	7.85
	Seawater (1000 mL)	0	0.0037		
		0.008	0.1116	94	8.87
Co	Purified water (1000 mL)	0.1	0.089	89	7.96
	3.5% saline (100 mL)	0	0.014		
		0.1	0.087	86	9.45
	Seawater (1000 mL)	0	0.018		
		0.1	0.105	88	10.67
Ni	Purified water (1000 mL)	0.3	0.31	102	7.72
	3.5% saline (100 mL)	0	0.012		
		0.3	0.328	105	10.14
	Seawater (1000 mL)	0	1.25		
		0.3	1.5	97	9.50

[a] Relative standard deviation (n=5).

n.d. = not detectable.

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and Ni were found to be 0.0003, 0.007, 0.12, and 0.007 ng mL^{-1}, respectively.

CONCLUSION

To determine trace metals at sub-ppb levels using ETAAS, a preconcentration technique has to be introduced in the sample preparation step. The use of AC to adsorb encapsulated metals in combination with slurry sampling ETAAS offers a high concentration factor without the need of desorption to bring the adsorbed metals into solution for measurement. A 2% TAPIOCA suspension can stabilize the AC slurry for 15 min. By optimizing the enrichment conditions, quantitative adsorption of many of the elements on AC at the same time was achieved. Although the maximum allowable concentration of AC slurry was about 10%, 3-4% slurry was recommended to avoid a rapid build-up of carbon residue in the atomizer. The proposed enrichment method was resulted in a concentration factor up to 20,000-fold.

In this report, the preconcentration method was applied to seawater analysis for only four elements; Cd, Co, Hg and Ni, although many more elements can be adsorbed onto AC and determined in the same manner. Provided that better purity of AC is available, elements such as Pb, Cr, and Cu and many others (4) can be simultaneously enriched. Wall atomization was used for this work (instead of the more widely accepted approach of platform atomization) due to previously observed problems associated with reproducibly pipetting of the slurry slution onto the flat L'vov platform (44). However, with the relatively new integrated platform and peak area measurements, precision and accuracy of the proposed method should further be improved.

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Flame AAS Determination of Copper in Urine Using a Flow Injection On-line Preconcentration System Based on a Polyamine Chelating Ion Exchange Column

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INTRODUCTION

Copper is an essential trace element whose determination in urine is of particular importance for the diagnosis of Wilson's disease, where a significant increase of copper renal excretion is found (1). Copper determination in urine is further important for the diagnosis of other disease states (2), the monitoring of Wilson's patients receiving chelation therapy (3), the detection of environmental/occupational exposure (3), and for nutritional studies (2).

Since the typical copper concentrations in urine are less than 40 µg/L (4), graphite furnace atomic absorption spectrometry (GFAAS) is the instrumental technique of choice (4). This analytical procedure is widely described in the literature (2,3,5–9). However, the determination of copper in serum (which is also very important for the assessment of problems related to copper metabolism, particularly in Wilson's disease) is usually carried out by flame AAS (4), since the typical copper serum levels (700 – 1550 µg/L) are within the analytical capabilities (regarding sensitivity) of this technique. From a practical point of view, this means that the utilization of two instrumental techniques (usually two different instruments) is required to carry out the two determinations (copper in serum by flame AAS and copper in urine by GFAAS) (4).

ABSTRACT

A flow injection system incorporating a polyamine chelating ion exchange column for the flame atomic absorption determination of copper in urine is proposed. At a 5-mL sample volume, the procedure yielded a detection limit of 1 µg/L and a sample frequency of 20/h. Repeatability (%RSD, n=10) was 0.9% at a 100-µg/L level and 1.4% at a 20-µg/L level. Reproducibility (%RSD; n=3) was 5% for a mean sample concentration of about 33.8 µg/L. For maximum accuracy, a rapid wet sample digestion with H_2O_2 had to be performed. Results obtained by the proposed methodology (n=20) were in good agreement with those provided by graphite furnace atomic absorption spectrometry.

The possibility of determining copper in both types of samples (serum and urine) strictly by Flame AAS is very attractive. Nevertheless, considering the typical concentration levels referred to before, the determination of urinary copper by flame AAS requires measurements close to the detection limit (DL) of the technique (2,10), unless procedures enhancing the analytical sensitivity are employed or a preconcentration step is carried out (11-15). This work describes an on-line preconcentration system for the determination of copper in urine by flame AAS incorporating a mini-column filled with a chelating resin of the polyamine type (Metalfix® Chelamine®) in which copper in a first step is directly retained. In a second step, the elution is done with 2M HNO_3 directly into the nebulizer-burner system of the atomic absorption spectrometer.

Chelamine, which is a resin produced by immobilization of a pentamine ligand (tetraethylene pentamine) in an organic polymer (reticulated polyacrylamide), was selected as the packing material for the column because of its reported advantages over other resins (16,17). With respect to the purposes of the present work (copper preconcentration in urine, which is a well-known complex matrix), the resin's high capacity [1.0 + 0.1 mmol of Cu(II) per each resin gram, at pH 5] (16) and high selectivity for the transition metals regarding the alkali and alkaline earth metals are its major advantages. Even considering only the transition metals (it must be stressed that urine has great amounts of Zn), Chelamine presents a better complex formation constant with Cu(II) (16).

EXPERIMENTAL

Instrumentation

For flame AAS determinations, a PerkinElmer Model 5000 air-acetylene flame atomic absorption spectrometer attached to a Model E 586 Labograph recorder (Metrohm AG) was used. The flow injection (FI) system (Figure 1) used for the preconcentration step was based on a Gilson® Minipuls 3 peristaltic pump with Gilson pumping tubes, two 6-port Rheodyne rotary valves and Omnifit PTFE tubes (0.8 mm i.d.) with Gilson end-fittings and connectors.

For GFAAS determinations, a PerkinElmer Model 4100 ZL atomic absorption spectrometer, equipped with a transversely heated graphite
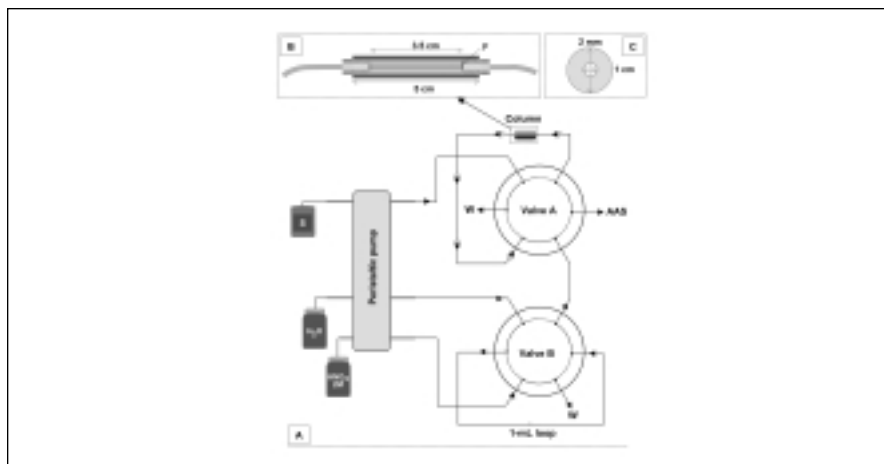
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Fig 1. Schematic diagram of the FI on-line preconcentration system for flame AAS determination of copper in urine (A).
Detail (B) and transverse perspective (C) of the preconcentrating column.
S = sample; AAS = flame atomic absorption spectrometer; W = waste; F = 35-μm Teflon filter, contact time of 5 min. The solution was buffered at pH 7.
Flow rates: sample = 2.0 mL/min; H_2O = 5.4 mL/min; 2M HNO_3 = 1.75 mL/min.
Operating mode: 1) Sample loading and filling of the loop with nitric acid (2.5 min.): valve A in dotted line and valve B in straight line; 2) Column washing with H_2O and filling of the loop with nitric acid (10 s): valve A in straight line and valve B in dotted line; 3) Elution (20 s): valve A in straight line and valve B in straight line.

TABLE I
Instrumental Parameters and Graphite Furnace Program

Instrumental parameter:	GFAAS	FI-Flame AAS
Lamp current	10 mA	10 mA
Wavelength	324.8 nm	324.8 nm
Slit with	0.7 nm, low	0.7 nm, high
Background correction	Longitudinal Zeeman effect	None
Measurement mode	Peak area (A.s)	Peak height (A)
Injection volume	20 μL*	5 mL
Number of replicates	2	2

Furnace Time / Temperature Program

Step	Temp. (°C)	Ramp (s)	Hold (s)	Argon gas flow (mL/min)	
1	110	1	20	250	
2	130	5	30	250	
3	1200	10	40	250	
4	2000	0	5	0	Read
5	2400	1	5	250	

*Samples and standards were previously diluted 1+1 with a solution containing 0.1% (v/v) Triton X-100, 0.03% (w/v) Mg(NO$_3$)$_2$ and 0.05% (w/v) Pd (as nitrate).

atomizer incorporating end-capped graphite tubes with integrated L'vov platform (PerkinElmer Part. No. B300-0655) and an AS-70 autosampler was used. All equipment was controlled with PerkinElmer software installed in a personal computer and the results were printed on an HP® DeskJet® printer. A copper hollow cathode lamp (S&J Juniper) was used as the radiation source for both instruments. Other instrumental conditions and the graphite furnace program used are summarized in Table I.

Microwave-assisted wet digestions were made in a Milestone MLS 1200 microwave oven equipped with an HPR-1000/10 S rotor.

Reagents and Standard Solutions

Copper standard solutions were prepared by two-step dilution of a 1000-mg/L Spectrosol stock solution (BDH) with 0.2% (v/v) nitric acid. Except for nitric acid, which was *Suprapur®* quality (Merck), all other reagents (hydrogen peroxide, sodium acetate, and acetic acid) were *pro analysis* quality (Merck). The latter reagents were used for the preparation of a buffer solution with pH 5.9 by mixing 64 mL of 2M sodium acetate with 36 mL of 2M acetic acid, which were diluted to a final volume of 1000 mL (18).

To prepare the preconcentrating column, the chelating resin Metalfix Chelamine (Fluka), which is commercially available as particles ranging from 40 to 80 μm diameter, was used. For the GFAAS determinations, a matrix modifier solution prepared from commercial solutions of magnesium nitrate and palladium nitrate (Merck) and Triton® X-100 (also from Merck) was used. All solutions were prepared with high-purity water (18 MΩ•cm) obtained with a Milli-Q™ system (Millipore).

Precautions Against Contamination

All laboratory ware, including the microwave oven vessels, was cleansed by soaking in diluted (1+5) nitric acid for 24 hours and rinsing thoroughly with Milli-Q water just before use. The measurement of low volumes was carried out with automatic pipettes (Gilson), with disposable plastic tips. Autosampler cups for the GFAAS equipment were used as supplied by the manufacturer and discarded after each utilization.

Samples

The samples consisted of a 24-h urine, collected into plastic vessels that were cleaned as described. After collection, the samples were acidified (0.4% of Suprapur nitric acid, Merck) and frozen until analysis. For comparison purposes, some samples were spiked with different amounts of copper nitrate.

Procedure

Column Preparation

The preconcentrating column was prepared by packing a Perspex home-made small tube (3.5 cm long, 2 mm i.d.; see Figure 1) with approximately 0.2 g resin previously slurried in de-ionized water. This was accomplished by placing a Teflon® filter with 35-μm pores (MoBiTec) on one of the column ends and then introducing the resin slurry with a syringe through the opposite end, which was finally covered with a filter of the same type. Both column ends were threaded, thus enabling the connection to the flow system by means of a threaded connector.

Sample Pretreatment and Copper Determination

The analytical procedure after experimental optimization consisted in (a) placing 10-mL samples (urine previously homogenized by vigorous stirring) in the microwave vessels; (b) adding 2 mL H_2O_2; (c) performing the digestion procedure
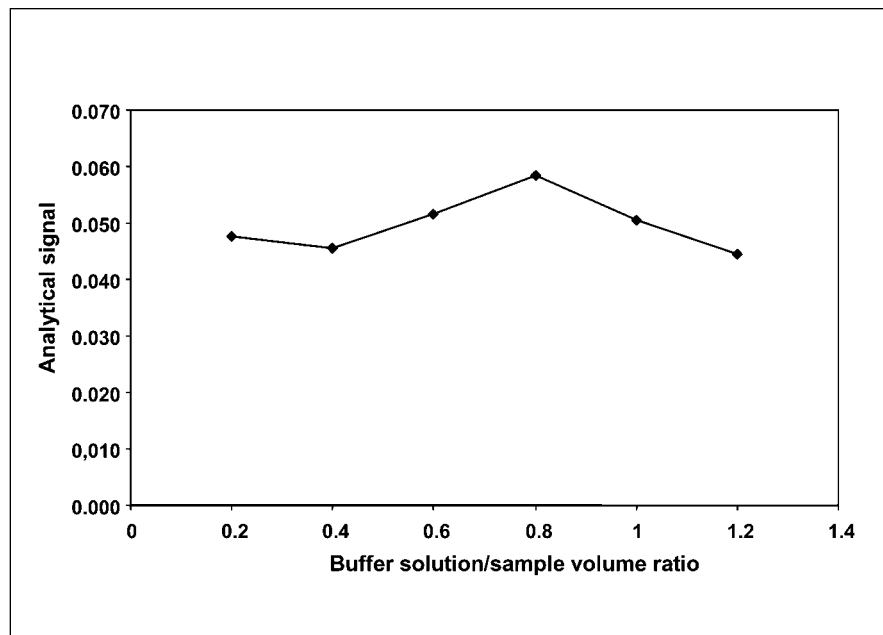

Fig 2. Dependence of the analytical signal on the buffer solution/sample volume ratio.

for 5 min at 500 W; (d) after cooling, adding 8 mL buffer solution; (e) preconcentrating copper by passing the solutions through the column for 2.5 min at a 2.0-mL/min flow rate; (f) performing elution with 2 M HNO_3 and recording the analytical signal thus obtained.

RESULTS AND DISCUSSION

Optimization of Experimental Parameters

Preconcentration Procedure

According to manufacturer's instructions, Chelamine resin can be used within a pH range of 1 to 12. However, the pH must be of a value that ensures maximum uptake changes for a specific metal ion (16,17). In this work, the assessment of the best experimental conditions for copper preconcentration started with the study of the effect of pH over copper retention. Within the range studied (4.4–9.5), the pH variation did not produce a significant effect on that retention, although a 12% difference between the maximum and minimum values was found. Therefore, knowing that

even under normal conditions the pH of urine may vary significantly (4.6 – 8.0) (19), adjustment of the sample pH was considered necessary and an acetic acid/sodium acetate buffer solution (pH 5.9; composition already described) was used. The relationship between sample volume and buffer solution volume enabling the highest analytical sensitivity was also assessed. Hence, 2 mL of H_2O_2 and increasing volumes (2, 4, 6, 8, 10 and 12 mL) of the buffer solution were added to a 10-mL urine sample. The results obtained (Figure 2) showed that after correction to the same final volume, the ratio of an 8-mL buffer solution to 10-mL sample provided the highest sensitivity, which is most likely due to the effect of the ionic solution. This ratio was selected because the absolute signal values did not change signficantly and eventual matrix effects were minimized. For further optimization of the experimental parameters, the effect of the sample loading flow rate on copper retention was assessed. Studying the flow rate ranging from 0.3 to

2.8 mL/min showed that there was a decrease in sensitivity, although not very significant for flow rates higher than 2.0 mL/min (Figure 3); thus, this flow rate was selected for this study.

With respect to the operating mode of the developed FI system, it must be noted that the introduction of step 2 (see caption for Figure 1), consisting of rinsing the column with water for several seconds between the preconcentration and elution steps, showed some influence over precision. This step was favorable in two ways: the matrix components retained in the column after preconcentration were removed and there was a redistribution of the resin. This seemed to improve the precision of the subsequent elution step.

Sample Pretreatment

Sample pretreatment procedures for the determination of copper in urine vary from simple sample acidification (2,13,15,20) and filtration (17,21) to its somewhat drastic digestion (22–24) even when GFAAS analysis (24) is intended. In this work, the direct sample analysis (pumping of the urine sample through the preconcentration column without sample pretreatment)

proved inadequate since the rate of copper retention in the column changed from sample to sample varying from 68% to 95%. This may mainly be due to the large amounts of material suspended in some urine samples (such as cells and crystals) which hinder copper bonding to the resin to a certain extent. Hence, prior treatment of the samples was considered necessary.

The preconcentration of metals in urine using Chelamine resin (and a subsequent determination by plasma atomic emission spectrometry) was accomplished by some authors (17) by simple filtration through a membrane filter (0.45 μm pore size). However, the copper recovery rate was only 82%. Moreover, this is a time-consuming and rather impractical procedure prone to contamination. Some difficulties were reported regarding agreement of the results using GFAAS and an extraction method (2). This problem was considered to be related to copper precipitation in urine. In fact, analyzing the precipitate separately, the same authors found large amounts of copper. Other authors (21) studying this aspect reported similar results and mentioned that even in acidified urine samples,

some precipitation may still occur, producing negative errors throughout the analysis.

Based on the above findings, in the present work a sample digestion was used to ensure maximum analytical accuracy. The results of the experiments proved that by simply boiling the sample with H_2O_2 for 1 to 2 minutes, the recovery rates were close to 100% and the results in agreement with those obtained by GFAAS. Nevertheless, to prevent the samples from possible losses or contamination, digestion in a microwave oven was also performed. Application of 500 W power for 5 min was sufficient to achieve clear and transparent solutions as well as adequate precision and accuracy of the results.

Evaluation of the Analytical Procedure

Linearity, Sensitivity, and Detection Limit

Under the experimental conditions described above, there was a linear relationship (r = 0.999) between the detector response and the sample concentration up to ~240 μg/L (120 μg/L for final solutions, considering the 1+1 dilution of the samples with the buffer solution and H_2O_2). This is an adequate interval for the usual copper concentrations found in urine or even partly for the high levels that may occur during occupational/environmental exposure or with Wilson's disease.

Still, within the scope of the calibration procedure and for some practical interest, the need of standard solutions (as well as samples) to be submitted to the same treatment in a microwave oven was eliminated. In effect, the comparison between the mean values of the slopes (S) from four calibration curves obtained with the buffer solutions, treated the same as the samples, and those from four calibration curves obtained without this treatment (S = 0.26876 and
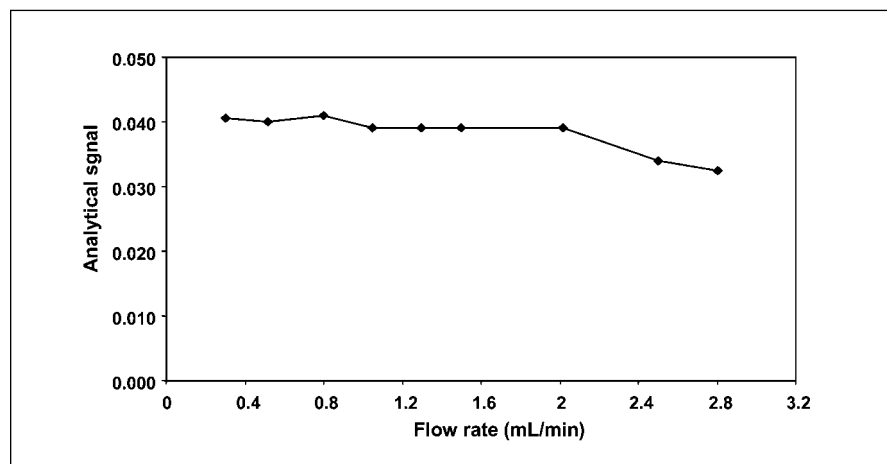

Fig 3. Effect of sample loading rate on the analytical signal for a 25 μg/L copper solution.

S = 0.26192, respectively) did not show significant statistical differences (Student's *t*-test). Therefore, during routine analytical work, the establishment of the calibration curve can be made while waiting for sample digestion (5 min) and cooling (15 to 20 min), thus increasing analytical output.

The relationship between the analytical signal and sample loading time was also assessed. A 160-µg/L standard solution (80 µg/L after addition of 2 mL H_2O_2 and 8 mL buffer solution to a 10-mL standard solution) was preconcentrated during different time intervals varying from 0.5 to 3.5 min. The results, plotted in Figure 4, show that copper retention increased linearly up to 2.5 min (corresponding to a preconcentration of approximately 5 mL of solution). In practice, this means that the establishment of a calibration curve up to 160 µg/L, which covers the majority of sample concentrations, enables any sample that would eventually be of a higher concentration to be reanalyzed by using a shorter preconcentration time without requiring further dilution .

With respect to sensitivity, it must be noted that this procedure also demonstrated long-term stability. In fact, a set of 8 calibration curves obtained during the final trials of method validation (about 15 days) provided a mean slope value of 0.2653 for the 0 – 200 µg/L interval, with a standard deviation of 0.009497, corresponding to 3.6% RSD. Linearity was always acceptable, with a mean value of $R^2 = 0.9993$.

Using the conditions described, a detection limit of 2.5 µg/L (n=20; 3σ) was achieved. However, the blank level reached a significant value (about 24 µg/L) and it was found that this depended nearly always on the buffer solution alone. Nevertheless, when this solution was previously passed through a
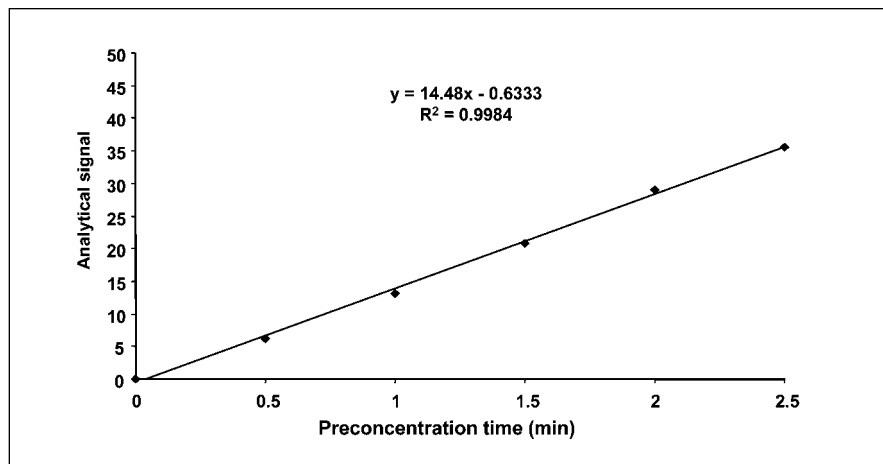

Fig 4. Effect of sample loading time on the analytical signal for a 80 µg/L copper solution.
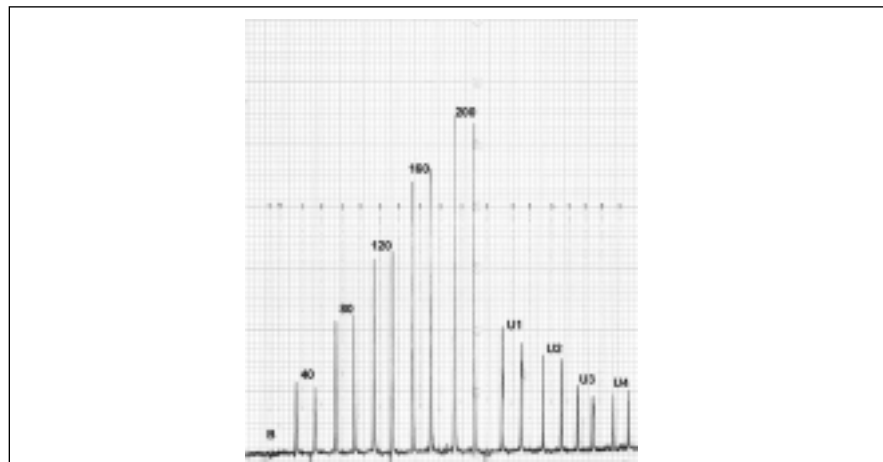

Fig 5. Recorder output corresponding to a calibration curve and to the analysis of 4 urine samples (recorder was stopped during the preconcentration step). The values presented correspond to the calibrating solutions concentration before the 1+1 dilution (10 mL of standard solution + 2 mL H_2O_2 + 8 mL buffer solution).

column similar to that used in the preconcentration system, blank levels indistinguishable from the baseline noise were consistently attained (see Figure 5) and a detection limit of about 1 µg/L (2 µg/L for real samples).

Enhancement Factor, Concentration Efficiency, and Retention Efficiency

Evaluation of the performance of the proposed system using the criteria suggested by Fang (25) led to the attainment of an enhancement factor of about 23.5 and a concentration efficiency of 9.4/min. The column efficiency, as calculated by Hartenstein et al. (26), was close to 96%.

Precision and Accuracy

Precision was assessed for both repeatability of consecutive injections of the same solution and reproducibility of the results obtained by replicate analysis of the same samples. As to repeatability, the determination of the analytical signal corresponding to 10 consecu-

tive injections of a standard solution with a 100-μg/L final concentration provided a 0.85% RSD. The same assessment was made with a solution containing 20 μg/L, which resulted in 1.35% RSD.

The within-run reproducibility, evaluated by the threefold analysis (digestion of three aliquots of the same sample with double determination of the analytical signal corresponding to each solution obtained) gave a 5.0% RSD for a mean concentration of 33.8 μg/L. Similarly, the replicate (n=2) analysis of a set of five samples with concentrations ranging from 33 to 208 μg/L (also with double determination of the analytical signal for each solution) provided a < 5% RSD each time. Both evaluations proved that the sample pretreatment procedure is not a factor of considerable analytical variation. The between-run precision was evaluated by independently analyzing (n=2) the same set of six samples, with concentrations ranging from 20 to 70 μg/L. The results obtained showed no significant statistical difference (Student's *t*-test).

Accuracy was assessed by comparing the results from the analysis of 20 urine samples by the analytical procedure developed and by GFAAS. The statistical evaluation of the results obtained (Figure 6) showed no significant difference, indicating that this procedure has enough accuracy for the determination of copper in urine.

CONCLUSION

The FI on-line preconcentration system proposed proved to be robust. Within reasonable limits, the variation of the main experimental parameters did not affect the results significantly. Furthermore, not even small pulses or flow instability caused by the peristaltic pump affected the precision, since preconcentration occurred for a long time (2.5 min), which was enough for minimizing these effects. In particular, the Chelamine mini-column showed good behavior with respect to both copper retention efficiency and short- and long-term stability.

Moreover, though being robust and fulfilling the main requirements referred to by Fang (27) using flame AAS preconcentration systems, the system is quite simple and therefore easily implemented as a routine procedure in clinical chemistry laboratories.

The good performance of the preconcentration procedure developed requires sample pretreatment but, as described before, it is very fast, simple, and safe (digestion with peroxide hydrogen), in addition to being totally compatible with the preconcentration step. In addition, maximum reliability of the determinations is assured when the problems due to possible precipitation of copper in the samples between sample collection and analysis are eliminated.

Compared to other studies (15), it must be stressed here that the proposed methodology does not require a complexation reagent and elution is made with a diluted acid instead of methanol, which is well known for its toxicity.

Regarding the alternative GFAAS method generally used for the determination of copper in urine, the preconcentration system described allows copper determination to be performed by flame AAS, thus taking advantage of the economical and practical aspects of this technique. Moreover, this determination is accomplished with an equivalent analytical throughput (about 20 measurements/h). In effect, a detection limit of 1 μg/L was attained using a 2.5-min preconcentration time. About 30 sec was sufficient to wash and fill the system and insert a new sample before the next preconcentration step.
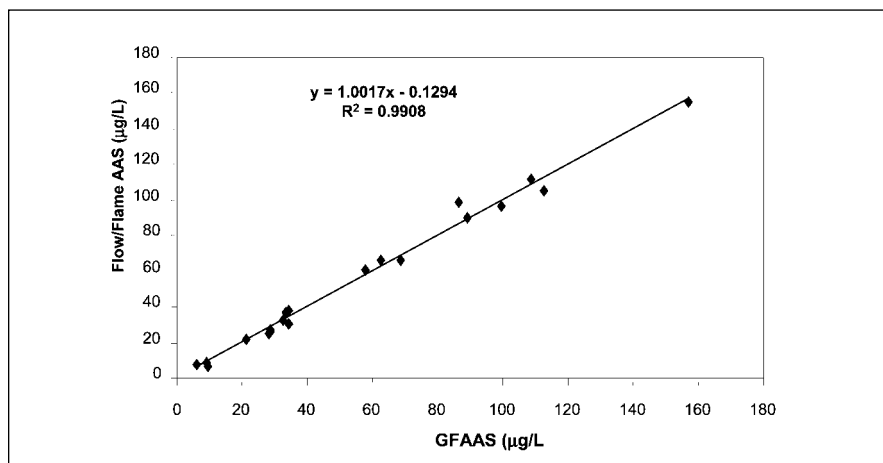

Fig 6. Comparison of the results (mg/L) obtained in the determination of copper in 20 urine samples by GFAAS and by the developed procedure (Flame AAS with an on-line preconcentration step). Linear regression analysis: Y = 1.0017 X - 0.1294 (R^2 = 0.9908). Paired Student's t-test: mean flow/FAAS = 55.5; mean GFAAS = 55.4; variance flow/FAAS = 1712.7; variance GFAAS = 1734.5; calculated t value = 0.03918; critical t value = 2.09302.

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