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ICP-MS Determination of Trace Elements in Serum Samples of Healthy Subjects Using Different Sample Preparation Methods

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ABSTRACT

The concentration of some trace elements of clinical importance was studied in blood serum samples of healthy subjects (n=19, mean age 22±4.5 years) to determine reference ranges for the healthy urban population in Eastern Hungary. A sample preparation method has been developed using closed-vessel microwave digestion with an oxidizing acid mixture (nitric acid and hydrogen peroxide) for multielemental analysis and atmospheric wet digestion with the aqueous solution of trimethyl amine (TMAH) for the measurement of aluminum. Contamination of the samples with Al was observed during acidic microwave digestion because of the chemicals used. Trace element concentrations in the samples were measured with ICP-MS. The reference ranges obtained were (in µg/L): Al 0.98-1.74, Cr 42.8-59.3, Mn 2.27-5.05, Fe 1282-2050, Co 0.22-0.88, Ni 0.03-16.33, Cu 691-1003, Zn 591-1217, Sr 30.37-47.37, Mo 0.73-1.19, Cd 0.02-0.62, and Pb 0.02-2.70. The results were compared to the reference ranges actually used in the clinical practice, some of which are available in the literature. The effect of the applied digestion methods on the obtained individual results and average concentration ranges was also studied.

INTRODUCTION

The rapid development of industrial and agricultural activity is a major source of environmental pollution. Pollutants originating from various human activities may also influence the concentration of essential and toxic elements in the human body. Hence, it is important to check trace element concentrations in the body of healthy subjects regularly, especially in order to prevent and recognize the possible accumulation of toxic elements or the low level of essential elements in some patients (1).

Clinical reference ranges and recommendations are available in the literature for comparison (2-4), but trace element concentrations in serum of healthy subjects may differ in different countries or regions because of different environmental conditions, living standards, eating habits, etc. Another problem, observed in the literature, is that the reference ranges for some analytes are significantly different in different publications.

Different kinds of samples are used to determine trace element concentrations in the human body, including scalp hair (5), nails (5), blood (6-8), plasma (9), serum (8,10-12), and urine (13). Human hair is a metabolic end product; hence, major, minor, and trace elements, available through the circulatory system, are accumulated and fixed in its organic matrix. By analyzing hair samples, the integrated concentration levels over recent months can be determined, avoiding the effect of day-to-day fluctuations. Despite its advantages, the major problem with hair analysis is that no sample preparation method has yet been published that can remove exogenous contamination from the surface of hair samples without changing the endogenous trace element content (5). Hence, human blood, plasma, and serum samples are usually used to determine trace element levels in the human body.

The presence of a biological matrix requires careful sample preparation prior to analysis. Dry ashing is sufficient for removing the organic matrix content of the sample, but contamination and loss of volatile analytes are possible because of the open system and the high temperature applied. Atmospheric wet digestion can be slow for some sample types, and may also cause contamination during the sample preparation process. For some years, high-pressure closed-vessel microwave digestion has been applied successfully to destroy the organic matrix of biological samples in a relatively short digestion procedure (15-20 minutes) (14). In the closed vessels of the digestion unit, sample contamination and analyte loss can also be prevented.

The concentration of most essential and toxic trace elements is low in human samples (µg/L - fg/L range); hence, a sensitive analytical method is required for analysis. Non-destructive methods such as neutron activation analysis (NAA) (15) or particle-induced X-ray emission (PIXE) (16), and destructive methods such as inductively coupled plasma optical emission spectrometry (ICP-OES) (8), graphite furnace atomic absorption spectrometry (GFAAS) (10,17), and inductively coupled plasma mass spectrometry (ICP-MS) (7,9,14) are used for this purpose. Of these techniques, ICP-MS has excellent sensitivity, detection limits, high sample throughput and multielemental capabilities, which enables accurate and precise analysis of a high number of samples in a short period of time (1,18,19).

In the present work, an analytical method has been developed for sample preparation and the multi-

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elemental analysis of human serum samples of healthy subjects. The main goals of this study were to develop and compare alternative methods for sample preparation of human serum samples, to study the effect of the applied sample preparation methods on the obtained individual results and average concentration ranges, to determine reference ranges for some selected trace elements in the serum of healthy subjects living in the central-eastern region of Hungary, and to compare the obtained concentration ranges to those found in the literature.

EXPERIMENTAL

Instrumentation

Multielemental analysis of the prepared samples was made with an ELAN® 6000 quadrupole-based ICP-MS instrument (PerkinElmer SCIEX, Concord, Ontario, Canada). The original sample introduction system of the instrument was replaced with a microconcentric nebulizer (AR30-1-FN02, Glass Expansion, Australia) and minicyclonic spray chamber (Glass Expansion, Australia) to decrease the sample solution uptake rate and to obtain higher nebulization efficiency. Instrumental parameters are shown in Table I. Method development and validation using small amounts of different certified reference materials is described elsewhere (14).

Samples

The blood samples were collected after overnight fasting from 19 healthy male volunteers (mean age: 22±4.5 years) and placed into Vacutainer tubes (Beckton-Dickinson, USA). The serum was separated from the native blood by centrifugation (4000 r/min, 4°C). The samples were stored at -70°C until analysis.

TABLE I
Instrumental Parameters

ELAN 6000 ICP-MS	
RF power	1300 W
Coolant Ar flow	14 L/min
Auxiliary Ar flow	1.2 L/min
Nebulizer Ar flow	0.93 L/min
Sample uptake rate	0.087 cm^3/min
Nebulizer	Micromist
Spray chamber	Minicyclonic
Sampler cone	Nickel, 1.1 mm orifice diameter
Skimmer cone	Nickel, 0.9 mm orifice diameter
Mass resolution (m/Δm)	300
Scanning mode	Peak hopping
Dwell time/amu	200
Sweeps/replicate	10
Replicates	3

Sample Preparation

The serum samples were prepared using microwave digestion. This method effectively decreases the amount of organic compounds in the sample, thus reducing the formation of molecular ions which may interfere with the measured analytes during the analysis. In addition, high amounts of organic material may also affect the efficiency of the sample introduction. A 500-µL serum sample was mixed with an oxidizing acid mixture containing 1 cm^3 nitric acid, 0.5 cm^3 hydrogen peroxide, and 0.2 cm^3 hydrogen fluoride in Teflon® crucibles (6 cm^3 volume, screw cap, Savillex Corp., USA). The crucibles were closed and placed into the vessels (XP-1500) of the microwave digestion unit (MARS-5, CEM Corp., USA). High-purity deionized water (10 cm^3) was poured into the vessels outside the crucibles to provide nearly equal pressure inside and outside the crucibles, thus avoiding their opening during digestion. The samples were digested using the following program: (a) 150 W for 10 min; (b) 0 W for 2 min; (c) 300 W for 10 min. The samples were transferred to plastic sample tubes and filled to 10 cm^3 with high-purity deionized water. For ICP-MS measurements, sample dilutions with 2% acid content were prepared and rhodium was added as the internal standard element in a final concentration of 10 µg/L. Because of possible contamination with aluminum during sample preparation, a different method was applied for the aluminum measurements. The serum samples (500 µL) were mixed with 500 µL trimethyl amine (TMAH) aqueous solution (25%, containing 0.2% ethylene-diaminetetraacetic acid, EDTA) and stored at room temperature for 30 min. The samples were then diluted to 5 cm^3 with high-purity deionized water and rhodium was added as the internal standard element (10 µg/L final concentration).

Chemicals

Suprapur® nitric acid [65% (m/m), Merck, Germany], Suprapur hydrogen fluoride [40% (m/m), Merck, Germany], and Suprapur hydrogen peroxide [30% (m/m), Merck, Germany] were applied for microwave digestion. Suprapur nitric acid was further purified by using subboiling-point distillation. For alternative

sample preparation, semiconductor-grade TMAH [25% (m/m)] aqueous solution (Tamapure-AA, Tama Co. Ltd., Japan) was used. For external calibration, mono- and multielemental standard solutions were used (1000 mg/L, Merck, Germany). Calibrating solutions were prepared in a matrix similar to that of the samples, i.e., in 2% nitric acid in the case of microwave-digested samples and in 2.5% TMAH (containing 0.02% EDTA) for the TMAH-digested samples. For sample dilution, high-purity deionized water was used (prepared with a Millipore Milli-Q™ Plus water purification system).

RESULTS AND DISCUSSION

In addition to sample collection and storage (20), one of the most critical steps in the analytical process is careful sample preparation to avoid sample contamination and analyte losses. Therefore, recovery of the selected analytes was studied using pooled human serum samples and monoelemental standard solutions.

The recoveries of the concentrations added to the microwave-digested serum samples are shown in Table II. In general, excellent recoveries of the added concentrations were observed for most analytes, even in the case of 0.5 µg/L additions. High recoveries of Fe are due to isobaric interferences of the molecular ions $Ar^{14}N^+$ and $Ar^{16}OH^+$ on the studied isotopes ^{54}Fe and ^{57}Fe, respectively. The low recovery of Cu (0.39±0.01 instead of 0.5 µg/L) is due to possible analyte loss in the case of that particular sample. However, for the other additions of Cu, the recovery values were satisfactory. Another observation during the study was that the recovery of aluminum is higher than the expected value for all additions. The difference between the measured and expected concentrations ranged from 1.34 µg/L to 2.24 µg/L. Separate solutions of the applied digestion agents were prepared and their aluminum concentration was measured to identify the source of contamination. It was shown that Suprapur nitric acid and hydrogen fluoride were responsible for the contamination.

TABLE II
ICP-MS Results of Serum Standard Addition Experiments After Microwave Digestion (Concentrations in µg/L, n=10)

Analytes	Recovery of the added concentrations of 0.5 µg/L	1 µg/L	5 µg/L	10 µg/L
Al	1.90±0.13	2.34±0.09	7.05±0.19	12.24±0.20
Cr	0.5±0.02	1.00±0.05	5.12±0.17	10.09±0.15
Mn	0.48±0.02	0.99±0.04	5.08±0.15	10.19±0.29
Fe	0.97±0.45	2.19±1.23	6.27±1.17	11.17±0.43
Co	0.48±0.01	1.00±0.03	5.07±0.15	10.26±0.25
Ni	0.48±0.02	1.00±0.04	5.09±0.12	10.18±0.13
Cu	0.39±0.01	0.95±0.02	4.96±0.14	10.05±0.13
Zn	0.49±0.01	1.04±0.03	4.88±0.16	10.17±0.16
Sr	0.51±0.02	1.05±0.06	5.09±0.19	10.45±0.44
Mo	0.48±0.01	1.01±0.03	5.04±0.17	10.22±0.24
Cd	0.50±0.02	1.01±0.03	5.06±0.06	10.07±0.23
Pb	0.49±0.02	0.99±0.03	5.06±0.21	10.11±0.30

TABLE III
Concentration Ranges Measured From the Serum Samples of Different Digestions (Concentrations in µg/L, n=19)

Analytes	Microwave digestion	TMAH digestion
Al	8.0-55.2	0.98-1.74
Cr	42.8-59.3	(88.58-99.90)
Mn	2.27-5.05	1.45-2.39
Fe	1282-2050	not measured
Cu	0.22-0.88	0.01-0.08
Ni	0.03-16.33	3.21-3.73
Cu	691-1003	778-1124
Zn	591-1217	not measured
Sr	30.37-47.37	26.7-41.26
Mo	0.73-1.19	0.72-1.34
Cd	0.02-0.62	0.01-0.15
Pb	0.02-2.70	0.08-0.50

In order to obtain reliable results on the aluminum concentration of the samples, an alternative digestion method was applied. In this method, an aqueous solution of TMAH was used to dissolve the organic matrix of the human serum samples and to avoid their precipitation in the sample introduction system and in the torch of the ICP-MS instrument. Ethylene-diaminetetraacetic acid (EDTA) was added to the TMAH solution as a complexing agent to keep the various metals dissolved in the EDTA complex even at the alkaline pH of the TMAH-digested sample solutions.

Serum samples of healthy subjects were digested using the above-detailed methods. Multielemental determination of the selected analytes was performed with the ICP-MS method. The comparison results of the two digestion methods are shown in Table III. The analytical results of the two digestion meth-

ods were similar in the case of Cu, Sr, and Mo. For Cr, the result was lower after microwave digestion than after TMAH digestion. This is due to the high background and isobaric interferences of $Ar^{12}C^+$ and $Ar^{13}C^+$ at masses m/z=52u and 53u from the TMAH matrix. In the case of Mn, Ni, and Pb, most of the results from the microwave-digested samples were significantly higher than from the TMAH-digested serum samples. This is due to the precipitation of these metals from the alkaline sample solutions. The recoveries of these metals were good in the standard addition experiment made with microwave-digested samples; hence, these results were used to establish the reference ranges. For aluminum, the microwave-digested samples gave extremely high recoveries due to their contamination; hence, the concentration of aluminum was measured from the samples digested with the TMAH solution. For this analyte, the results obtained from the TMAH-digested samples were used, which fits well into the clinical reference range.

The detection limits of the selected analytes are presented in Table IV. Detection limits were estimated using the 3σ criteria, i.e., the concentration of the analyte which yields a signal equivalent to three times the standard deviation of the blank signal. Detection limits were determined in the sample blank solutions and calculated for the human serum samples according to the sample dilution factors. The detection limits obtained from the TMAH matrix were similar to or higher than those from the microwave-digested acidic matrix, with the exception of aluminum, where the detection limit was better in the TMAH matrix.

Because of the biological variation in the normal concentrations of the selected analytes and the differences observed among the inhabitants of different countries or regions, it is essential to determine reference ranges for the studied analytes. The results obtained for healthy subjects (all from Eastern Hungary) are shown in Table V. Some reference ranges, available in the literature, are also included. Comparing the reference ranges from the literature and from the clinical practice, it can be seen that these ranges are similar for some analytes, e.g., for Fe, Cu, or Zn. However, in most cases, these values or ranges are quite different and can be as high as some orders of magnitude, e.g, for Al, Co, Ni, Mo, Cd, or Pb. For the present study, the ranges given in the Fischbach manual (3) are considered to be adequate. These ranges were determined in 1990 and are officially used in the clinical practice. The concentration of aluminum is under the recommended limit in the serum samples of the subjects examined. Similar trends were observed in the case of Co, Ni, Cd,

TABLE IV
Detection Limits of ICP-MS Measurements
(Determined in the serum samples, concentrations in µg/L)

Analytes	Microwave digestion	TMAH digestion
Al	0.125	0.095
Cr	0.030	0.067
Mn	0.005	0.050
Fe	0.406	not determined
Co	0.003	0.175
Ni	0.023	0.040
Cu	0.017	0.015
Zn	0.120	not determined
Sr	0.014	0.012
Mo	0.003	0.020
Cd	0.002	0.004
Pb	0.008	0.008

TABLE V
Comparison of Reference Ranges and Measured Concentrations in Human Serum Samples (Concentrations in µg/L)

Analytes	Iyengar (2)	Fischbach (3)	WHO (4)	Measured
Al	110-780	4	1-5	0.98-1.74
Cr	2-20	14	0.1-0.2	42.8-59.3
Mn	0.54-61	4-11.4	0.5-1	2.27-5.05
Fe	870-1870	500-1600	800-1200	1282-2050
Co	0.2-62	1.2-2	0.1-0.3	0.22-0.88
Ni	7.8-58	26	1-2	0.03-16.33
Cu	970-1640	700-1400	800-1100	691-1003
Zn	670-1830	550-1500	800-1100	591-1217
Sr	< 46	not available	not available	30.37-47.37
Mo	6-27	5-30	0.1-0.5	0.73-1.19
Cd	2.3-12	1-5	0.1-0.3	0.02-0.62
Pb	16-130	< 400	< 1	0.02-2.70

All data from the literature refer to males.

and Pb. The concentrations of Cu and Zn fit into the middle of the specified reference range, while the concentration of manganese is at the bottom of the reference range. The concentration of Cr found in the serum samples was higher than the recommended level because the samples were contaminated with this analyte during the sampling procedure from the alloy of the needles used (20).

CONCLUSION

The developed microwave digestion method was successfully applied for the preparation of human serum samples. This sample preparation can avoid sample losses and contamination during sample treatment. However, the chemicals used for the digestion must be carefully tested prior to use because they might be a source of contamination. This problem can be overcome by using high-purity chemicals, a further purification process, or an alternative sample preparation method. In the present study, digestion with TMAH solved the problem of aluminum contamination. The analytical method was tested with standard addition using a microwave-digested human matrix. The recoveries were in good agreement with the expected concentrations. In the control serum samples, the concentration of most analytes agreed well with the concentration ranges referred in the clinical practice.

In the future, the number of healthy controls will be increased and the established control values will be applied as "healthy references" in a study related to patients with chronic renal failure and undergoing regular haemodialysis treatment.

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ICP-OES Determination of Elements in Volatile Organic Compounds and Anesthetics

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INTRODUCTION

The administration of inhaled anesthetics requires the use of a vaporizer, which is composed of a heated stainless steel vessel that allows for more convenient vaporization of the chemical agent. The inhalation agent is often administered as a gas with nitrous oxide (N_2O) (1). However, in this environment, the stainless steel vessel may slowly leach elements such as chromium, iron, copper, and zinc into the gas stream and potentially expose the patient to an unnecessary health risk (2). Therefore, it would be important to determine whether these exposures are taking place.

The simultaneous determination of several elements within volatile organic compounds has proven to be a difficult task. Studies have been performed to determine wear metals in oil, using kerosene as a solvent (3). In addition, studies have been carried out to determine specific elements in gasoline (4). However, few articles have been written that address the need to determine elements in highly volatile matrices using ICP-OES (3 and 4). The objective of this study was to determine the parameters necessary to perform the simultaneous determination of several elements within a highly volatile organic matrix. Subsequently, these parameters will allow for the analysis of inhaled anesthetics to determine whether or not a health concern exists.

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ABSTRACT

The determination of several trace elements, including calcium, chromium, copper, iron, lead, magnesium, silver, and zinc in an extremely volatile matrix was performed by ICP-OES. Trichlorotrifluoroethane was used as the primary solvent due to its similarity to the inhaled anesthetic agents, with high volatility and low combustibility. The parameters discussed allowed for a correlation coefficient of each calibration curve for the elements determined to be within the range of 0.99-1.0. Good recovery of spiked samples was obtained, allowing for the determination of elements within a volatile matrix.

EXPERIMENTAL

Instrumentation

A PerkinElmer Optima 4300™ DV ICP-OES (PerkinElmer, Shelton, CT USA), equipped with AS-90plus autosampler, was used. Modifications to the ICP-OES system were as follows:

1. The GemCone™ low-flow nebulizer was replaced with the Meinhard™ nebulizer to achieve smaller sample throughput (part number 00472020).
2. The cyclonic spray chamber was replaced with a baffled cyclonic spray chamber to allow for finer aerosol droplets (part number N077-6053).
3. The standard 2.0-mm i.d. alumina injector was replaced with a 0.8-mm i.d. alumina injector (part number N077-5227), which required the use of an injector adapter (part number N077-0603).
4. The baffled cyclonic spray chamber and peristaltic sample tubing were packed in ice chips to reduce the temperature and volatility of the volatile organic samples (setup is shown in Figure 1).

Reagents

Calibration standards were made from Conostan 5000-ppm hydrocarbon oil standards (Conostan Division, Conoco Inc., Ponca City, Oklahoma USA). The standards were made in the range of 0.1 ppm, 1.0 ppm, 10.0 ppm, 15.0 ppm, and 25.0 ppm containing calcium, chromium, copper, iron, lead, magnesium, silver, and zinc blended together and diluted in trichlorotrifluoroethane (Aldrich, 99.9% pure). The sample matrices (provided by Texas Tech University School of Medicine) were: Halothane anesthetic (2-bromo-2-chloro-1,1,1 trifluoroethane, manufactured by Halocarbon Laboratories, River Edge, NJ USA) and Forane anesthetic (isoflurane 1-chloro-2,2,2 trifluoroethyl difluoromethyl ether, manufactured by Baxter Healthcare Corporation, Deerfield, IL USA) which, for the purpose of this work, were spiked with known concentrations of metal solutions. In addition, trichloro-trifluoroethane-spiked samples were used for comparison purposes.

Sample and Standard Preparation

All standards were prepared by serial dilution of the 5000-ppm Conostan metal hydrocarbon oil standards. First a 100-ppm metal solution was prepared by adding 2 mL each of the metal standards to a 100-mL volumetric flask and then bringing to volume with trichlorotrifluoroethane. From the 100-ppm solution, the 10.0-ppm, 15.0-ppm, and 25.0-ppm metal standards were prepared using trichlorotrifluoroethane as a diluent. From the 10.0-ppm standard, the 0.1-ppm and 1.0-ppm metal standards were made using trichloro-trifluoroethane

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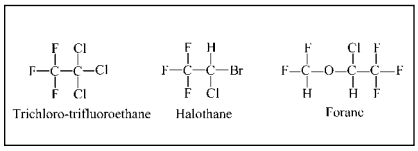
as a diluent. Samples of trichloro-trifluoroethane, Halothane, and Forane were prepared at concentrations of 0.5 ppm and 5.0 ppm. The 0.5 ppm metal solution was prepared by adding 5 mL of the 10-ppm standard to the sample and diluting with the appropriate diluent to 100 mL. The 5.0-ppm metal solution was prepared by adding 5 mL of the 100-ppm standard to the sample and diluting with the appropriate diluent to 100 mL. All samples and standards were kept in glass containers and capped with glass stoppers, sealed with Teflon® tape until analysis to prevent volatilization of the sample. In order to reduce the volatility of the organic samples, which would extinguish the torch, the spray chamber and sample tubing were packed in ice.

RESULTS AND DISCUSSION

Trichloro-trifluoroethane, more commonly known as FREON, was used for this study, because of its resemblance to the anesthetic Halothane, low cost, and reduced risk of exposure to prescription analgesic agents. Below are the chemical structures for trichloro-trifluoroethane, Halothane, and Forane:



In addition, trichloro-trifluoroethane has low reactivity with the analgesic agents and hydrocarbon oil standards, and proved to be a good solvent for this study. However, environmental concerns regarding the use of this chemical agent should be noted. Table I displays the parameters used for the calibration of the PerkinElmer Optima 4300 DV ICP-OES (dual-view inductively coupled plasma optical emission spectrometer).

TABLE I
Instrumental Operating Parameters

Parameter	Axial Setup
Plasma Gas	17.0 L/min
Auxiliary Gas	0.2 L/min
Nebulizer Gas	0.2 L/min
Power	1500 Watts
Nebulizer Type	Minehard
Spray Chamber	Baffeled Cyclonic
Pump Rate	1.0 mL/min

TABLE II
Data Collection Conditions

Element	Read Delay (s)	Read Time (s)	Wavelength (nm)
Ag	180	20	338.289
Ca	180	20	317.933
Cd	180	20	214.440
Cr	180	20	267.716
Cu	180	20	324.752
Fe	180	20	239.562
Mg	180	20	279.553
Pb	180	20	217.000
Zn	180	20	206.200

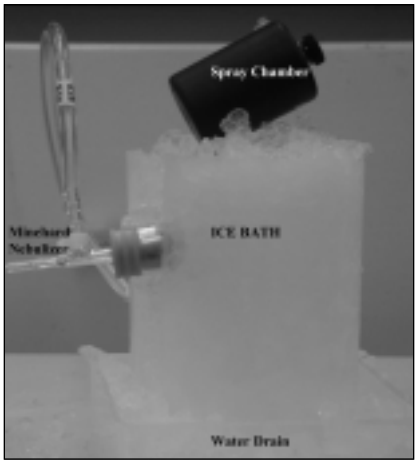


Fig. 1. Photo of ice bath and water drain for spray chamber.

Trichloro-trifluoroethane was used as the wash solvent, which rinsed the spray chamber for 180 seconds between each sample and standard. Sample read delay times of 180 seconds were maintained to ensure adequate stabilization of the sample. The baffled cyclonic spray chamber was packed in ice using a modified polystyrene bottle to help reduce volatility of the sample (shown in Figure 1). In addition, the sample pump tubing was packed in ice to decrease sample volatility prior to nebulization of the sample to prevent extinguishing the plasma and to improve the precision of the analysis due to the volatility of the matrix. The increased optimal plasma flow was found to be 17.0 L/min, which ensured stabilization of the torch discharge. The auxiliary gas and nebulizer gas flows were reduced to limit the amount of sample introduced into the plasma. As more sample is introduced, the volatility of the matrix quickly overloads and extinguishes the plasma. In addition, a 0.8-mm alumina injector, baffled cyclonic spray chamber, and Minehard nebulizer were utilized to control the sample throughput, thus preventing the torch from extinguishing. The torch was set to 1500 watts as the optimal power, which was found to provide best sensitivity for the analysis. The recommended wavelengths suggested by the manufacturer were utilized for this study and are listed in Table II, along with the

sample read delay and analysis times. Calibration of the instrument using these conditions allowed for a correlation coefficient of 0.99 or greater for all of the elements determined. The data are shown in Table III. Two spiked sample concentrations were chosen for this study, one for low concentrations (0.5 ppm) and one for higher concentrations (5 ppm). Five replicates of each sample were read to ensure accuracy (results are displayed in Table IV and Table V). No sample memory effects were observed while using the 180-second read delay. However, due to the volatility of the matrix, some samples proved to have poor recoveries as can be seen in Table IV. While good recoveries were obtained, the sample matrix evaporated and resulted in higher recoveries (see Table V). Poor recoveries may have been affected by the buildup of carbon on the injector and torch assembly. To reduce this, the samples should be placed in ice and opened to the atmosphere for a bare minimum of time. Also, carbon buildup may be reduced by a shorter analysis time of 6-8 hours. Operation longer than eight consecutive hours results in skewed results due to carbon buildup. The suggested operating time is four hours or less.

CONCLUSION

This study has shown that the determination of elements in highly volatile organic matrices is possible using the Optima 4300 DV ICP-OES. Chilling of the sample, tubing, and spray chamber is essential for this type of analysis. Excellent calibration of the instrument was achieved; however, volatility of the matrix affected recoveries of the sample concentrations. Shorter analysis times will reduce carbon buildup on the injector and torch assembly and provide better precision and sensitivity.

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TABLE III
Calibration Results

Element	Wavelength (nm)	Correlation Coefficient (%)
Ag	328.068	0.996
Ca	317.933	0.992
Cd	214.440	0.991
Cr	267.716	0.994
Cu	324.752	0.997
Fe	239.562	0.993
Mg	279.553	0.992
Pb	217.000	0.999
Zn	206.200	0.992

TABLE IV
Spiked Sample Results (0.5 ppm)

Sample	Element	Spike (ppm)	Found Concentration (ppm)	Recovery (%)
Freon	Ag	0.5	0.298	-40.4
	Ca	0.5	0.446	-10.8
	Cd	0.5	0.344	-31.2
	Cr	0.5	0.261	-47.8
	Cu	0.5	0.3	-40
	Mg	0.5	0.4	-20
	Pb	0.5	0.397	-20.6
	Zn	0.5	0.244	-51.2
Forane	Ag	0.5	0.509	1.8
	Ca	0.5	0.419	-16.2
	Cd	0.5	0.36	-28
	Cr	0.5	0.535	7
	Cu	0.5	0.618	23.6
	Mg	0.5	0.464	-7.2
	Pb	0.5	0.639	27.8
	Zn	0.5	0.205	-59
Halothane	Ag	0.5	0.447	-10.6
	Ca	0.5	0.547	9.4
	Cd	0.5	0.504	0.8
	Cr	0.5	0.451	-9.8
	Cu	0.5	0.616	23.2
	Mg	0.5	0.546	9.2
	Pb	0.5	1.33	166
	Zn	0.5	0.381	-23.8

TABLE V
Spiked Sample Results (5.0 ppm)

Sample	Element	Spike (ppm)	Found Concentration (ppm)	Recovery (%)
Freon	Ag	5.0	6.74	34.8
	Ca	5.0	7.12	42.4
	Cd	5.0	7.25	45
	Cr	5.0	6.79	35.8
	Cu	5.0	6.41	28.2
	Fe	5.0	6.71	34.2
	Mg	5.0	7.21	44.2
	Pb	5.0	6.34	26.8
	Zn	5.0	6.96	39.2
Forane	Ag	5.0	6.11	22.2
	Ca	5.0	6.83	36.6
	Cd	5.0	6	20
	Cr	5.0	5.94	18.8
	Cu	5.0	6.71	34.2
	Fe	5.0	5.93	18.6
	Mg	5.0	5.93	18.6
	Pb	5.0	6.83	36.6
	Zn	5.0	5.82	16.4
Halothane	Ag	5.0	5.78	15.6
	Ca	5.0	4.56	-8.8
	Cd	5.0	4.91	-1.8
	Cr	5.0	4.95	-1
	Cu	5.0	6.73	34.6
	Fe	5.0	4.94	-1.2
	Mg	5.0	5.26	5.2
	Pb	5.0	5.52	10.4
	Zn	5.0	4.85	-3

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On-line Preconcentration and Speciation of Chromium Using Solid-phase Extraction and Detection by Flame AAS

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ABSTRACT

A method for on-line separation and simultaneous preconcentration of Cr(III) and Cr(VI) using Flame AAS analysis of water samples was developed. The method uses solid-phase extraction with an anionic exchanger and previous formation of an anionic compound of Cr(III) with EDTA or CDTA. Conditions of derivatization and separation were studied and tolerance limits established. The influence of sample conductivity was also studied. A comparison was carried out between derivatization with EDTA and CDTA. Derivatization with CDTA results in higher sensitivity, but EDTA provides greater selectivity. For the analysis of samples with high conductivity such as wastewaters, derivatization with EDTA is recommended. Reproducibility was 8.2% (n=5) for Cr(VI) and 1.2% (n=5) for Cr(III); recovery was 100% (n=3) for Cr(VI) and 55% (n=3) for Cr(III). The detection limit was 0.15±0.03 µg (n=9) for Cr(VI), depending on the sample volume when it is expressed in concentration units. For Cr(III), the detection limit is dependent on the conductivity of the sample.

INTRODUCTION

The development of procedures for chromium speciation has increased due to the toxicity of Cr(VI) and 80% of the studies published since 1980 were performed during the last decade. These procedures can be classified into two groups: one is based on the analysis of one oxidation state and the determination of total chromium after oxidation or reduction; the other is based on previous separation of Cr(III) and Cr(VI) and detection of both oxidation states, which can be carried out by using solid-phase extraction (1-7). Sperling et al. (7) proposed the use of on-line separation of Cr(III) and Cr(VI) by using a micro column of activated alumina and sequential sorption of Cr(III) and Cr(VI). After elution, the detection was carried out with flame atomic absorption spectrometry (FAAS).

The procedures based on previous separation and detection of both oxidation states include the chromatographic (8-12) and electrophoretic (13, 14) methods. These procedures generally imply previous formation of an anionic compound of Cr(III). Derivatization can be carried out using several reagents as complexones (CDTA or EDTA). However, the kinetic inertness of Cr(III) (5) complicates these procedures, and the published studies show no consensus with respect to derivatization conditions such as pH, temperature, and heating time (Table I).

The aim of this work was to achieve speciation of chromium using FAAS. Since this technique is not sufficiently sensitive, preconcentration is required. For this purpose, an on-line solid-extraction using an anionic exchanger, after the formation of an anionic complex of Cr(III), is proposed. The use of on-line separation decreases the sample preparation time and increases sample throughput. Moreover, the separation and preconcentration steps are simultaneous.

Optimum conditions of derivatization of $Cr(CDTA)^-$ were established and compared with EDTA and CDTA in terms of selectivity and sensitivity. The limits of conductivity of the samples were also studied in order to establish the limitations of the procedure in the analysis of samples that have high conductivity, such as wastewater.

EXPERIMENTAL

Instrumentation

A PerkinElmer Model 5000 atomic absorption spectrometer (PerkinElmer, Shelton, CT USA), equipped with a chromium hollow cathode lamp, was used for absorbance measurements. Absorbance measurements were carried out as peak height at 357.9 nm using a 15-mm burner height and a $Q_{acetylene}/Q_{air}$ ratio of 0.09. A microwave IGNIS AKL-540 was employed to heat the solutions in the derivatization process. A conductimeter Crison microCM 2200 was used for conductivity measurements.

Figure 1 shows the flow injection (FI) manifold employed for on-line separation of Cr(III) and Cr(VI). An Omnifit column with an internal diameter of 10 mm, length of 50 mm, and volume of 1.7 mL, with $SAX[-Si-CH_2-CH_2-CH_2N^+-(CH_3)_3]$ (Merck) as the solid phase, was used. The sample or eluent was pumped (Perimax 12 peristaltic pump) through the column using a 3-way valve, the column connected to a FAAS, and the peaks recorded.

Reagents

Distilled water (Milli-Q™ system, Millipore) was used to prepare all solutions.

Stock solutions of 1000 mg/L of Cr(III) and Cr(VI) were prepared from $Cr(NO_3)_3$ and K_2CrO_4, respectively.

Stock solution of ethylenediaminetetraacetic acid (EDTA) 0.1 mol/L was prepared from their sodium salts, and a stock solution of

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TABLE I
Chromium Speciation Studies

Derivatization conditions	Stability of Cr(VI)	Detection	Separation	Measured	Limit of detection	Interferences	Reference
EDTA:Cr 1000:1 80°C 10 min or 75 s in a microwave oven pH=4.5	Stable	Flame AAS	Solid-phase extraction (anionic phase)	Cr(III) Cr(VI)	0.4 µg/L 1.1 µg/L	Studied interferences: Fe, Mn, Ca, Mg, Sn, Ba, Al, NO_3^-, I^-, Br^-,Cl^-, F^-, SO_4^{2-}, HCO_3^-, PO_4^{3-}	6
EDTA:Cr 10:1 40°C 5 min pH=6	Unstable at pH<2	Spectrophotometry	Ion-pair chromatography	Cr(III) Cr(VI)	0.02 mg/L	Studied interferences: Fe, Pb, Mn, Ni, Co, Cu, Zn, Cd, Sn, Ca, Mg, NO_3^-, I^-, Br^-,Cl^-, SO_4^{2-}	8
CDTA:Cr 5:1 60°C 10 min pH=6.5	----------	Spectrophotometry	Ion-pair chromatography	Cr(III) Cr(VI)	8 mg/L 35 mg/L	Studied interferences: Fe, Ni, Co, Cu, Zn, Ca, Mg	9
EDTA:Cr 10:1 60°C 1 h pH=5	----------	Spectrophotometry and Flame AAS	Ion chromatography anionic exchange	Cr(III) Cr(VI)	0.08 mg/L 0.2 mg/L	----------	10
EDTA:Cr 80°C 15 min	----------	Spectrophotometry with post-column derivatization with diphenylcarbazide	Ion chromatography anionic exchange	Cr(III) Cr(VI)	---------	----------	11
CDTA:Cr (CDTA 2 mM) 80°C 15 min	Unstable when (CDTA)>2 mM	Spectrophotometry with post-column derivatization with diphenylcarbazide	Ion chromatography anionic exchange	Cr(III) Cr(VI)	4.5 ng/L 1.5 ng/L	Studied interferences: Ca, Mg, Fe	11
EDTA:Cr 50:1 60°C 5 min pH=7	Unstable when T>60°C	ICP	Ion chromatography anionic exchange	Cr(III) Cr(VI)	81 ng/L 88 ng/L	Studied interferences: ArC^+, ClO^+	12
CDTA:Cr 20:1 70°C 5 min pH=6.5-7.0	Unstable when T>70°C	Photometric	Capillary electrophoresis	Cr(III) Cr(VI)	50 µg/L 10 µg/L	Studied interferences: Transition metals	13
CDTA:Cr 20:1 70°C 5 min pH=6.5-7.0	----------	Photometric	Capillary zone electrophoresis	Cr(III) Cr(VI)	50 µg/L 10 µg/L	Fe and Co interfere	14

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trans-1,2-diamine-cyclohexane-N,N,N',N' tetraacetic acid (CDTA) 0.01 mol/L was prepared by addition of NaOH to the acid.

HAc/Ac⁻ buffer, pH 4.5, and solutions of interferents (10,000 mg/L) were prepared from analytical grade reagents.

The eluent solutions were prepared from their sodium salts and filtered with a Vidra Foc 682-D system.

Procedure

Solutions of Cr(III) and Cr(VI) between 0 and 2 mg/L, the conductivity controlled by addition of NaCl, were prepared.

HAc/Ac⁻ buffer (1:10 v/v) and an adequate aliquot of the derivatization agent were added to the samples and standard solutions. All solutions were heated for 75 s in a microwave oven and introduced into the column. Cr(III) and Cr(VI) were eluted and their peak height was measured.

RESULTS AND DISCUSSION

Cr(CDTA)⁻ Derivatization Conditions

The Cr(III) derivatization conditions were optimized and the stability of Cr(VI) was tested simultaneously. For this purpose, solutions containing 0.6 mg/L of Cr(III) and Cr(VI) were prepared.

The heating conditions were studied using a microwave oven at 400 W. One mL of CDTA 0.01 mol/L was added to 5 mL of chromium solution at pH 4.5 and different heating times between 10 and 90 s were employed. The solutions were then introduced into the SAX column and eluted using a solution of Na_2SO_4 0.1 mol/L.

The Cr:CDTA molar relation was tested by addition of a 1-mL solution of different concentrations of CDTA at pH 4.5.

Finally, to study the pH, HCl or NaOH solutions were used to adjust the pH from 2 to 9.

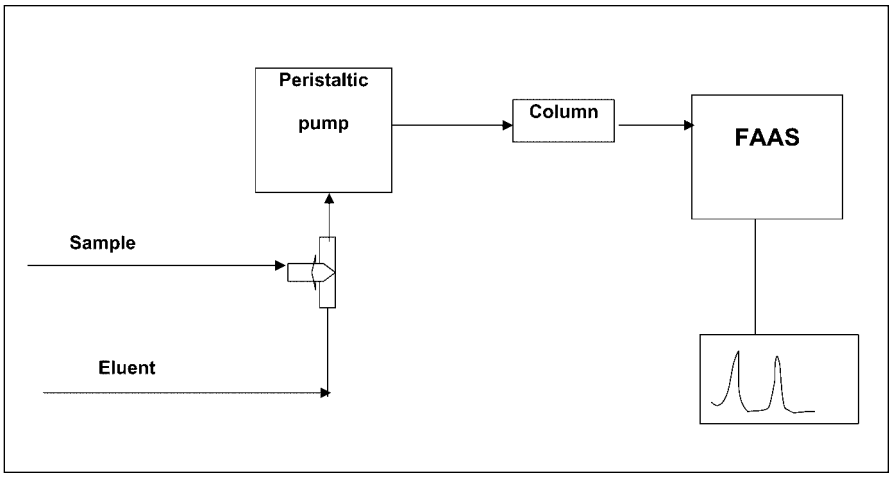

Fig. 1. On-line system for separation and preconcentration of Cr(III) and Cr(VI).

From the study carried out, it can be concluded that a heating time of 60 s at pH 4.5 was required, with a CDTA:Cr molar relation of 40 for the quantitative formation of the Cr(CDTA)⁻. When a shorter heating time or lower CDTA concentration was used, the Cr(III) was not quantitatively derivatized. Moreover, working at a pH<4 or pH>5, the Cr(III) signal decreased. For this reason, HAc/Ac⁻ buffer at pH=4.5 was considered to provide the best conditions for the formation of Cr(CDTA)⁻.

On the other hand, Cr(VI) remains unaltered when the pH is higher than 4; but at a lower pH, the signal decreases because under these conditions Cr(VI) is unstable.

Retention and Elution Conditions

Solutions containing 0.6 mg/L of Cr(VI) and 0.6 mg/L of Cr(III) derivatized with CDTA and EDTA (6) were introduced into the column. The influence of flow, temperature, pH, nature, and the concentration of counter-ions for elution conditions was studied.

The influence of temperature on the ionic exchange was studied by immersion of the column in a water bath. Our results showed that temperatures between 20 and 90°C do not affect the separation process. Similar results were obtained working with eluent solutions at pH 3.8-5.7.

On the other hand, among counter-ions tested (NO_3^-, Cl^- and SO_4^{2-}), best results were obtained with the counter-ion of highest selectivity (SO_4^{2-}) with a concentration of 0.1 mol/L (Figure 2). Finally, maximum separation between Cr(III) and Cr(VI) was obtained with a flow rate of 3.9 mL/min when Cr(III) was derivatized with CDTA; with EDTA, a flow rate of 3.3 mL/min was required for separation. When NO_3^- or Cl^- was used, larger concentrations of counter-ions or higher flow rates were required to obtain good separation and sensitivity.

The recovery of the base line was fast using NO_3^- and SO_4^{2-}; however, when Cl^- was used, the base line was recovered more slowly.

Interferences

The influence of other ions on the separation and determination of Cr(VI) and Cr(III) in water samples was studied. When derivatization was carried out with EDTA, only concentrations larger than those studied in a previous work (6) were tested. However, the conductivity of all solutions was measured with the aim to prepare a solution of chromium having the same conduc-

tivity (same concentration of chromium and interferent) in order to establish if the variation of the signal is due to an interference or due to the variation in conductivity. The tolerance limit obtained, when EDTA was used, increased for Ba(II) (1 mg/L), I^- (100 mg/l), and SO_4^{2-} (1000 mg/L), because the amount of solid phase was larger.

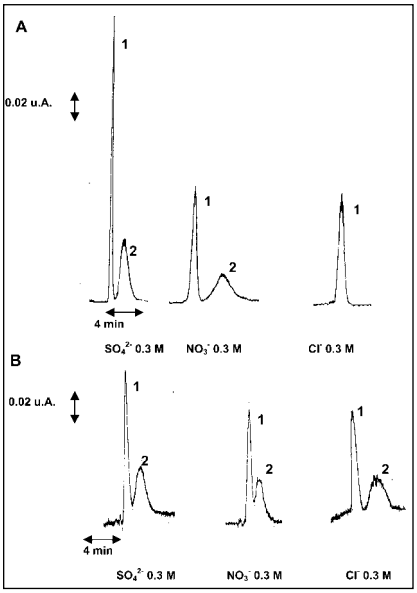

Fig. 2. Influence of the nature of counter-ions in the separation process: 1 = 0.6 mg/L of Cr(III) as (A) $Cr(CDTA)^-$ and as (B) CrY^- 2 = 0.6 mg/L of Cr(VI). Flow rate (A) 3.9 mL/min and (B) 3.0 mL/min. Volume of sample 5 mL.

TABLE II
Tolerance Limits

Interferent	Tolerance Limit (mg/L)	
	Cr(III) Derivatization with CDTA	Cr(VI)
Al(III)	10	100
Mn(II)	20	100
Mg(II)	122	600
Ca(II)	0.4	300
Ba(II)	0.4	50
I^-	60	60
Br^-	40	600
F^-	0.4	600
SO_4^{2-}	1100	5800
PO_4^{3-}	1	650

Table II shows the results obtained for Cr(VI) and Cr(III) when CDTA was used for the derivatization. Derivatization with CDTA provided higher sensitivity; but when EDTA was used, higher tolerance limits were obtained. The use of a higher concentration of CDTA to increase selectivity is not possible because of its low solubility. Therefore, EDTA is recommended for speciation of chromium in samples, such as wastewater, containing high concentrations of ions.

Calibration Curves of Cr(III) and Cr(VI), Detection Limit, Recovery, and Reproducibility

The influence of the conductivity of samples was studied from calibrations obtained with different conductivities by addition of NaCl. The calibrations obtained using identical volumes of solution with different concentrations and identical concentrations but different volumes were also studied. Finally, calibration was examined with multicomponent solutions [containing Cr(III) and Cr(VI)] and individual solutions [containing Cr(III) or Cr(VI)].

Table III shows the sensitivity and detection limit obtained for different conductivities and different volumes of solutions. It can be seen that for Cr(VI) the sensitivity is independent of the conductivity and increases when larger volumes of sample are used, but the sensitivity remains constant in absolute units. Therefore, a preconcentration of Cr(VI) is simultaneous with separation. The sensitivity enhancement factor compared to conven-

TABLE III
Sensitivity and Detection Limit of Cr(VI) and Cr(III) Under Various Conditions

For calibration curves of Cr(VI)

Conductivity (µS)	Volume (mL)	Sensitivity ± S_b[a] ($L\ mg^{-1}$)	Sensitivity ± S_b[a] (μg^{-1})	Detection Limit (µg)
1350	10	0.079±0.010	0.0079±0.0010	0.16
2000	3	0.0290±0.0014	0.0097±0.0005	0.14
3700	1	0.0080±0.0011	0.0080±0.0011	0.17
3700	3	0.0220±0.0005	0.0073±0.0002	0.18
5100	3	0.0260±0.0005	0.0087±0.0002	0.15
9400	3	0.0215±0.0016	0.0072±0.0005	0.20
10000	5	0.0435±0.0014	0.0087±0.0003	0.15
10000	5	0.0441±0.0014	0.0088±0.0003	0.15
16000	3	0.028±0.003	0.0069±0.0010	0.17

For calibration curves of Cr(III)

Conductivity (µS)	Derivatization agent	Volume (mL)	Sensitivity ± S_b[a] ($L\ mg^{-1}$)	Sensitivity ± S_b[a] (μg^{-1})	Detection Limit (µg)
100	—[b]	1	0.058±0.003	0.058±0.003	0.02
1350	EDTA	10	0.079±0.004	0.0078±0.0004	0.17
2000	EDTA	3	0.0567±0.0014	0.0189±0.0005	0.06
3700	—[b]	1	0.0542±0.0015	0.0542±0.0015	0.02
3700	—[b]	3	0.0707±0.0010	0.0236±0.0003	0.06
5100	EDTA	3	0.0543±0.0014	0.0181±0.0005	0.07
9400	EDTA	3	0.044±0.002	0.0146±0.0007	0.10
10000	EDTA	5	0.074±0.003	0.0149±0.0006	0.09
10000	EDTA	5	0.074±0.003	0.0149±0.0006	0.09
16000	CDTA	3	0.058±0.003	0.0193±0.0010	0.07

[a] Standard deviation of slopes of calibration curves obtained from four different concentratons of chromium.

[b] Without derivatization agent.

tional continuous nebulization was 0.2V (mL). However, for Cr(III), the sensitivity does not increase linearly with an increase in sample volume but is dependent on conductivity. It is therefore necessary to work with standards having the same conductivity as the samples and having identical volume of samples and standards.

The calibration curves obtained working with constant and variable volumes are linear for Cr(VI) in both cases; but for Cr(III) they are only linear with volume constant.

The intercept and slope of the calibration curves obtained from multicomponent and individual solutions (Figure 3) of Cr(III) and Cr(VI) were statistically comparable. The separation between two species of chromium was adequate, which clearly shows that it is possible to work with multicomponent solutions.

Recovery, established from solutions with a conductivity of 100 µS and by measuring the chromium not retained in the solid phase, was 100% (n=3) for Cr(VI) and 55±8% (n=3) for Cr(III) when derivatization was carried out with EDTA and 87±9% (n=3) with CDTA.

The reproducibility, obtained as the coefficient of variation, was 8.2% (n=5) for Cr(VI) and 1.2% (n=5) or 0.7% (n=5) for Cr(III) when derivatization was carried out with EDTA and CDTA, respectively.

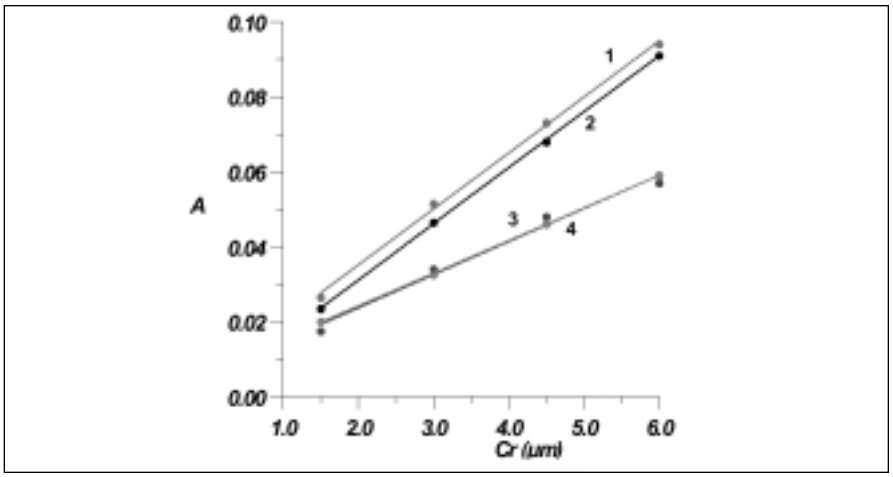

Fig. 3. Calibration curves obtained for multicomponent and individual solutions. 1 = CrY^- in multicomponent solutions, 2 = CrY^- in individual solutions, 3 = Cr(VI) in multicomponent solutions, and 4 = Cr(VI) in individual solutions.

TABLE IV
Analysis of Samples

Sample	Conductivity (µS)	Cr added (mg/L) V (mL)	Cr added (mg/L) Cr (III)	Cr added (mg/L) Cr (VI)	Cr obtained (mg/L) Derivatized with EDTA	Cr obtained (mg/L) Derivatized with CDTA	Cr obtained (mg/L) Reference method
M-1	1350	10	3	4	Cr(III) 3.2±0.3[a] Cr(VI) 4.3±0.5[a]	—	—
M-2	100	3	—	—	Cr(III) 19±2[a] Cr(VI) 8.1±0.8[a] Total Cr 27±2[c]	Cr(III) 18.1±0.2[a] Cr(VI) 7±2[a] Total Cr 25±2[c]	Cr(III) 20±2[b] Cr(VI) 7±2[a] Total 27.1±0.7[a]
M-3	44000	1	1	1	Cr(III) 1.0±0.2[a] Cr(VI) 1.02±0.10[a]	Cr(III) 0.47±0.05[a] Cr(VI) 1.22±0.12[a]	—

[a] $ts/\sqrt{n}$ with a confidence level of 95% (n=3).
[b] Obtained from difference between total chromium and Cr(VI).
[c] Obtained as Cr(VI)+Cr(III).

Analysis of Samples

The procedure suggested above was applied to the analysis of two synthetic samples and one real sample. Synthetic samples 1 and 3 were prepared by addition of chromium to tap water and Mediterranean Seawater, respectively, and sample 2 was an industrial wastewater.

The reference method involved reaction with diphenylcarbazide for the determination of Cr(VI) (14) and the determination of total chromium by FAAS.

All samples were analyzed in triplicate and Table IV lists the results obtained. As can be seen, the results obtained by the proposed procedure with EDTA as the derivatization agent are in all cases in agreement with the reference or theoretical values. However, the determination of Cr(III) after derivatization with CDTA did not provide accurate results.

CONCLUSION

The results of this study show that good separation of Cr(III) and Cr(VI) and simultaneous preconcentration are obtained by using on-line solid-phase extraction. This preconcentration is independent of sample conductivity for Cr(VI); but in the case of Cr(III), it was dependent on sample conductivity and volume introduced. On the other hand, derivatization with CDTA showed greater sensitivity, while derivatization with EDTA allows speciation of chromium in samples with a higher ion content. Therefore, a procedure involving EDTA as the derivatization agent and an on-line separation by a strong anionic solid phase (SAX) can serve as an alternative for the speciation of chromium in wastewater samples with a frequency of injection of 12 samples/hour.

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Flow Injection Flame AAS Determination of Ascorbic Acid Based on Permanganate Reduction

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ABSTRACT

An indirect flow injection method for the determination of ascorbic acid in fruit juices is proposed. Permanganate in an acid medium was injected into an ascorbic acid stream which reduced Mn(VII) to Mn(II). The Mn(II) formed was retained on-line, proportional to the ascorbic acid concentration in the sample, on a poly(aminophosphonic acid) chelating resin, which is only selective for this oxidation state. The non-reduced Mn(VII) was determined by flame atomic absorption spectrometry. The proposed method allows the determination of ascorbic acid in the 0.2-34.5 µg/mL range with a relative standard deviation of 2.2%, detection limit of 0.06 µg/mL, and sample throughput of 90 samples/h. The results were consistent with those obtained by the reference method (AOAC).

INTRODUCTION

Ascorbic acid, commonly known as Vitamin C, is one of the most important water-soluble vitamins. Vitamin C is an important antioxidant, helps protect against cancer, heart disease, and stress, is part of the cellular chemistry that provides energy, and is essential for making the collagen protein involved in the building and health of cartilage, joints, skin, and blood vessels. Vitamin C helps in maintaining a healthy immune system, aids in neutralizing pollutants, is needed for antibody production, acts to increase the absorption of nutrients in the alimentary canal, and thins the blood. It is also used as a food additive for antioxidant purposes (1). It is therefore necessary to have fast, selective, and automatic methods for its determination, particularly for routine analyses in the pharmaceutical and food industries.

Flow injection (FI) has been widely applied to the determination of ascorbic acid in several matrices (2) using spectrophotometric, electroanalytical, and chemiluminescence analysis. Various methods based on amperometry (3-9), spectrophotometry (10-16), and chemiluminescence (17-18) have recently been developed.

Atomic absorption spectrometers are analytical instruments used in most analytical laboratories and are suitable for the indirect on-line analysis of organic compounds (19). Until the present time, only two methods have been proposed that make use of an indirect methodology with atomic absorption for ascorbic acid determination. Yebra-Biurrun et al. (20) were first to develop an indirect FI atomic absorption spectrometric method (FI-AAS) for ascorbic acid determination. This solid-phase extraction method is based on reducing the properties of ascorbic acid. The cationic complex formed between Fe(II) [reduced from Fe(III) by ascorbic acid] and o-phenanthroline forms an ion pair with the picrate anion which is adsorbed on-line on Amberlite XAD-4, proportional to the amount of ascorbic acid in the sample. The unadsorbed iron was determined by FAAS. Zhang et al. (21) proposed another indirect methodology based on the reduction of chromate to Cr(III) by ascorbic acid in an acid medium. The Cr(III) formed was retained by a cation exchange resin, which was then eluted with 3 mol/L nitric acid into the nebulizer-burner system of a flame atomic absorption spectrometer. This system is comprised of two micro columns and two peristaltic pumps. However, the use of concentrated nitric acid, introduced in the flow by the FI system, can damage the nebulizer and its sample throughput is much lower (20 samples/h).

In this work we propose a more simple, indirect reversed-flow injection atomic absorption spectrometric determination of ascorbic acid, in which the reagent [Mn(VII)] is injected into the sample carrier (ascorbic acid). This method involves the reduction of Mn(VII) to Mn(II) by ascorbic acid. The Mn(II) formed was quantitatively retained on-line on the poly(aminophosphonic acid) chelating resin (PAPhA) (22), and the unretained manganese [unreduced Mn(VII)] was measured by flame atomic absorption spectrometry (FAAS). The analyte concentration is directly related to the absorbance decrease in the injected Mn(VII) solution caused by Mn(II) retention on the PAPhA chelating resin. Using this method offers the advantages of the AAS technique, which is one of the most effective and sensitive techniques for the determination of micro amounts of elements. The procedure is simple, sensitive, rapid, and cost-effective, does not require a sample preparation step, and has been applied to the determination of ascorbic acid in fruit juices.

EXPERIMENTAL

Instrumentation

A PerkinElmer Model 5000 atomic absorption spectrometer (PerkinElmer, Shelton, CT USA), equipped with a manganese hollow cathode lamp and deuterium lamp

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background corrector, was used in this study. An air-acetylene flame (21.0/2.0 L/min) as the atomizer was used as the detector for Mn. Peak height was measured at 279.5 nm. The aspiration flow rate of the nebulizer was adjusted to be the same as the flow rate of the FI channel. The spectrometer output was connected to a PerkinElmer 50 servograph recorder with a 5-mV range. The signals measured were the height of the absorbance peaks.

Manifold System

The flow injection system consisted of a Gilson® Minipuls-3 peristaltic pump, fitted with poly(vinyl chloride) tubes, Rheodyne injection valve, Rheodyne selection valve, and an ion-exchange mini column manufactured from PVC tubing (85 mm - 1.6 mm i.d.) packed with 20-30 mesh poly(aminophosphonic acid) chelating resin. The tube ends were fitted with glass wool to retain the resin beads in the tube. For the coils, PTFE [poly(tetrafluoroethylene)] tubes of 0.5 mm i.d. were employed.

Reagents and Solutions

All chemicals were of analytical reagent grade. Ultrapure water of 18.3 MΩ.cm resistivity, obtained from a Milli-Q™ water purification system (Millipore), served for dilution and washing.

The standard solutions of ascorbic acid (1000 µg/mL) were prepared fresh daily by dissolving 0.1000 g ascorbic acid (Merck) in water and diluting to 100 mL in a volumetric flask. A solution of 1000 µg/mL Mn(VII) was prepared by dissolving 1.4386 g of $KMnO_4$ (Merck) in water and diluting with water to 500 mL in a volumetric flask. Then, 5 mL of this solution was diluted with 2 x 10^{-3} mol/L sulfuric acid to 50 mL in a volumetric flask. Poly(aminophosphonic acid) chelating resin with macroreticular support (20 –30 mesh) was synthesized as described earlier (22).

Sample Preparation

Natural or commercially available fruit juices were filtered. An appropriate volume of the filtrate was diluted to 25 mL in a volumetric flask. Fresh juices were prepared immediately before measurement.

Procedure

A one-channel, reversed-FI configuration (Figure 1) was used to reduce Mn(VII) to Mn(II) by ascorbic acid. The Mn(VII) solution, 100 µL of 100 µg/mL at pH 2.5, was injected into the carrier stream (blank, standard of ascorbic acid, or sample solutions), reducing Mn(VII) to Mn(II) in the reaction coil by ascorbic acid. Mn(II) was retained quantitatively on the chelating PAPhA resin contained in a mini column. The unretained Mn(VII) was determined by FAAS. When the blank was pumped instead of the sample or ascorbic acid standard solution, Mn(VII) was not reduced to Mn(II) because of the absence of ascorbic acid and thus, the maximum signal was obtained since Mn(II) was not formed. The presence of ascorbic acid causes a weakening of the analytical signal proportional to its concentration.

Washing of Mini Column

The total lifetime of the mini column corresponds to about 15-20 sample determinations because the PAPhA resin exceeds its sorption capacity. Then, it must be washed with 1 mol/L hydrochloric acid before it can be used again.

RESULTS AND DISCUSSION

The separation device used was the poly(aminophosphonic acid) chelating resin (PAPhA), which is selective for Mn(II) ions, but does not retain the Mn(VII) ions. Thus, a separation between the two oxidation states of manganese is possible [unreduced Mn(VII) and Mn(II) reduced by ascorbic acid].

Chemical and Flow Optimizations

The chemical variables were varied, while keeping the FIA variables constant. They were optimized by continuously introducing a standard solution containing 10 µg/mL ascorbic acid into the system. The pH of the Mn(VII) solution and sample was studied. The optimum pH of the permanganate solution was tested in the 0-6 range and maximum absorbance difference (quantitative reduction) was obtained at a

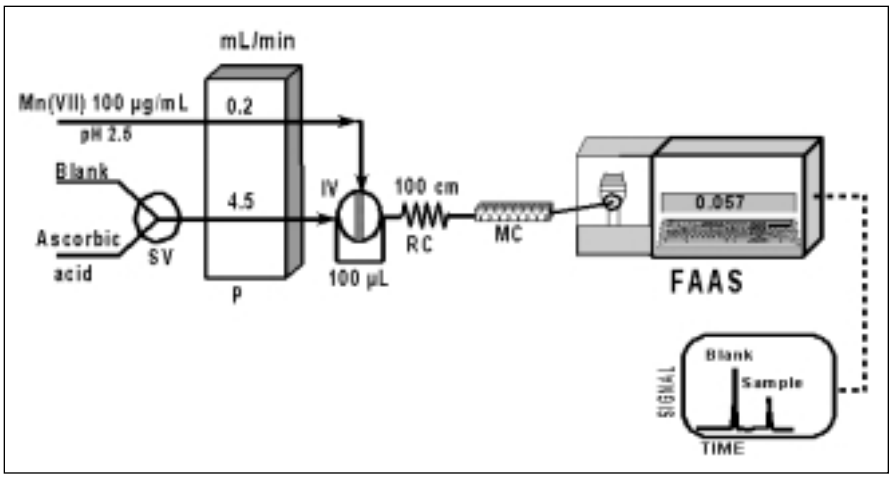

Fig. 1. Schematic diagram of FI manifold and optimum working conditions for ascorbic acid determination. P = peristaltic pump; IV = injection valve; RC = reaction coil; SV = selecting valve; MC = mini column; FAAS = flame atomic absorption spectrometer.

pH lower than 3. Therefore, a permanganate solution at pH 2.5 was chosen.

The effect of the sample pH was studied in the 1-6 range using the oxidant reagent under optimum conditions. The absorbance difference vs. the pH curve was plateau-shaped over the pH range tested and a sample pH of 5.8, obtained directly without any adjustment, was therefore chosen. The concentration influence of the oxidant reagent solution [Mn(VII)] was tested and a concentration of 50 µg/mL was found to be sufficient for maximum response. Increasing the Mn(VII) concentration up to 125 µg/mL did not affect the net signal. A concentration of 100 µg/mL Mn(VII) was chosen for further experiments.

The flow variables studied were the injection volume of the Mn(VII) solution (100 µg /mL), the reaction coil length, and the flow rate.

The effect of the injection volume on peak shape at a constant sample flow rate of 4.0 mL/min and an ascorbic acid concentration of 10 µg/mL was studied. The absorbance increased with the amount of Mn(VII) injected into the carrier flow. Injection volumes higher than 110 µL could not be used, because the blank signal falls outside the linear response of the detector. The injection volume could be increased by diluting the stream with water, but it decreases the atomic signal before each measurement. This increases the linear range of the method, but the PAPhA resin can exceed its sorption capacity. Thus, an injection volume of 100 µL was chosen as a compromise. Increasing the flow rate of the ascorbic acid carrier between 2.0-6.0 mL/min gave rise to increasing absorbance differences, resulting in an increase in the amount of available sample. However, the absorbance differences decreased dramatically at flow rates higher than 4.7 mL/min. This is due, on the one hand, to the residence times at higher sample flow rates being too short for quantitative Mn(VII) reduction and, on the other hand, being too short for quantitative retention of Mn(II) on the chelating resin. Therefore, a sample flow rate of 4.5 mL/min was selected.

The influence of the reaction coil length of 50 to 200 cm was studied. Best system performance was obtained with a 100-cm long reaction coil. Longer and shorter reactors resulted in decreased absorbance differences by an increase (higher dispersion) or decrease (incomplete reduction) in the residence times of the plug.

Interference Study

A study of potential interferences in the determination of ascorbic acid in fruit juice was performed. An absorbance variation of less than 10% was considered to be within the range of experimental error. Potential interfering substances were added individually to a solution containing 10 µg/mL ascorbic acid and the FI procedure was applied. The results and the concentrations tested are summarized in Table I and, as can be seen, no interferences could be observed using the test parameters.

Features of the Method

Under the optimized working conditions shown in Figure 1, the calibration curve was run (n=7). The representative equation was absorbance difference = 0.003 + 5.51×10^{-3} C, with C in µg/mL (the absorbance of the blank was 0.160). Therefore, it was linear over the 0.2–34.5 µg range of ascorbic acid/mL. The detection limit (0.06 µg/mL) was calculated as three times the standard deviation of the peak height for 30 injections of the water blank. The precision of the flow injection method obtained for 11 standard solutions containing 10 µg/mL ascorbic acid was 2.2%, expressed as the coefficient of variations. The throughput achieved under the optimized experimental conditions was about 90 determinations per hour.

TABLE I
Interference Study

Substances	Concentration (µg/mL)	(%) Recovery
Aspartame	500	98
Citric acid	500	100
Fructose	500	102
Glucose	500	96
Lactic acid	500	102
Malic acid	500	98
Phosphoric acid	500	100
Saccharin	500	98
Sodium benzoate	500	100
Sodium chloride	500	102
Tartaric acid	500	98

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Determination of Ascorbic Acid in Fruit Juices

The method described was applied to the determination of ascorbic acid in fruit juices. The results obtained in three individual determinations of ascorbic acid and their standard deviations are shown in Table II. The same batch of samples listed in Table II was also analyzed by the AOAC standard method (23) based on 2,6-dichloroindophenol. The paired *t*-test was applied to the results obtained by the proposed and the reference method. It showed that the calculated *t* value (t=0.35) was lower than the tabulated *t* value (t=2.57, n=5, P=0.05). This suggests that at the 95% confidence level, the difference between the results obtained by the proposed method and the reference method was statistically not significant. Thus, the proposed method can be successfully applied to real samples.

TABLE II
Determination of Ascorbic Acid in Real Samples

Fruit Juices	Concentration found[a] (µg/mL)	
	Proposed method	Official method
Orange[b]	340.7 ± 1.8	338.2 ± 2.0
Orange[c]	257.6 ± 1.3	259.6 ± 1.9
Lemon[b]	290.7 ± 1.6	295.9 ± 1.7
Peach[c]	59.5 ± 0.4	57.1 ± 0.6
Pineapple[c]	80.2 ± 0.7	78.7 ± 0.7
Grape[c]	10.7 ± 0.2	11.1 ± 0.3

[a]Average of three determinations ± S.D.

[b]Natural juices.

[c]Commercial juices.

CONCLUSION

The results of the proposed indirect FI determination of ascorbic acid clearly show that the method is simple and easy to operate. Sample analysis was realized without prior chemical treatment, which decreases the number of sample handling steps and increases sample throughput. The method offers high precision, high sample throughput, and high accuracy, and the detection limit is enough to determine ascorbic acid. Sample analysis is not hampered by coloration of the fruit juices, which can interfere using visible spectrophotometric procedures. The applicability of the method to real samples has been demonstrated and the results compare well with the Official Methods (23). Compared with previous FI-AAS methods, this FI methodology is simple and offers lower detection limits.

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ETAAS Determination of Essential and Potentially Toxic Trace Elements in Antarctic Krill Certified Reference Material: Evaluation of Two Microwave Digestion Procedures

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ABSTRACT

Seven potentially toxic and/or environmentally relevant trace elements, namely Cd, Cr, Cu, Fe, Mn, Ni, and Pb, were determined by electrothermal atomic absorption spectrometry (ETAAS). Two microwave (MW) acid-assisted digestion methods (with HNO_3/H_2O_2 and HNO_3/HF) were tested to mineralize the certified reference material MURST-ISS-A2, Antarctic Krill. The effect of power and time parameters was tested to investigate the influence of the matrix on analyte recovery. The use of the mixture of Pd and $Mg(NO_3)_2$ as the matrix modifier in the ETAAS determination increased the thermal stabilization of Cd and Pb significantly. The detection limits ranged between 3 and 35 ng g^{-1} and the dynamic range of the method spared one order of magnitude. The precision was better than 5% in all cases when peak area absorbance was used. The results obtained were in good agreement with the certified values.

INTRODUCTION

Owing to their potential environmental accumulation and harmful biological effects, heavy metals such as Cu, Cr, Fe, Ni, and Pb are of growing public concern. The determination of essential and toxic elements in biota is a challenge with atomic spectroscopy techniques due to the low concentrations required to be quantified. Essential elements include Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Se, V, and Zn (1), which play a variety of roles in biochemistry, often as enzyme cofactors. Marine organisms are considered to be among the greatest bioaccumulators of trace elements. Different kinds of marine organisms can reflect levels of trace metals and are thus the most widely used samples to monitor trace elements in coastal waters (2).

Over the last years, there has been a growing interest in the determination of trace metals in biota and different techniques and analytical methodologies have been developed to determine its elemental composition. Atomic spectroscopy techniques are among the most sensitive and selective techniques for trace element determination. Flame atomic absorption spectrometry (FAAS) was employed by Moreno et al. (3) to evaluate levels of essential and non-essential heavy metals in Antarctic organisms. A great number of couplings and methodologies for the determination of Cd, Co, Cu, and Pb in a variety of matrices have been reported in the literature (4, 5). Maher (6) described a method for the determination of total Sb in marine organisms by stibine generation and AAS detection. Concentrations of Sb between 0.010 and 0.193 µg g^{-1} in different kinds of marine samples were found. Norheim (7) used FAAS to determine trace metals in liver and kidney samples obtained from 92 seabirds of 10 different species from Spitsbergen and the Antarctic. Madrid et al. (8) studied a hydride generation AAS (HG-AAS) method for the determination of Pb in mussels, sardines, Atlantic bluefin tuna, anchovy, and Atlantic pomfret. Concentrations of Pb up to 1.98 µg g^{-1} were detected. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was employed by Lau and co-workers (9) to evaluate heavy metal concentrations in tissues and shells in freshwater mollusks. High-performance liquid chromatography (HPLC) coupled to hydride generation has been used in speciation studies in conjunction with AAS and ICP-AES. Caroli et al. (10) reported the on-line speciation of six arsenic compounds in fish and mussel extracts by HPLC-ICP-AES. The detection power of the overall system allowed each As form to be detected at concentrations ranging from 0.0013 to 0.0027 µmol L^{-1}. An important contribution to biota analysis is the paper by Morales-Rubio and de la Guardia (11) who, during the period from 1983-1994, reviewed flow injection atomic spectrometry methods to determine the elemental composition of biota samples.

Inductively coupled plasma mass spectrometry (ICP-MS) combines the advantages of low detection limits, multielement capability, and high sample throughput. ICP-MS was employed by Chamberlain and co-workers (12) to reach the detection limits necessary to determine 17 trace elements in fishes collected from the Columbia River (State of Washington, USA).

Electrothermal atomic absorption spectrometry (ETAAS) appears to be one of the most successful instrumental techniques for biota analysis owing to a combination of advantages: high sensitivity, good accuracy, adequate precision for most trace element determinations, large elemental coverage, and low sample volume requirements (5-50 mL). The determination of Cd and Cu in seals, penguins, and skuas (13); Ni in tea leaves (14); and Pb in biological materials (15) are some of the applications reported in the literature. A combination of HG and

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graphite furnace AAS (GFAAS) for the determination of Pb in mussels was authored by Aroza et al. (16). A detection limit of 4 ng g^{-1} was achieved using three different mineralization procedures. A rapid slurry atomization procedure for the determination of trace elements in oyster tissues using transversely heated electrothermal atomic absorption spectrometry (TH-ETAAS) was reported by Meeravali and Jai Kumar (17).

Based on the above applications and advantages, it was considered of interest to investigate the capabilities of ETAAS in the determination of trace elements in a certified reference material, Antarctic Krill, and to evaluate the influence of two microwave (MW) sample pre-treatment procedures on trace element recovery. This sample material resulted from the combination of three different catches (Ross Sea, Marguerite Bay, and Levingston Island, respectively) (18) and was processed at the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) into a homogeneous and dried powder. ETAAS was one of the nine instrumental techniques employed in the certification of Antarctic Krill.

EXPERIMENTAL

Instrumentation

A PerkinElmer Model 5100 ZL atomic absorption spectrometer (PerkinElmer, Shelton, CT, USA), equipped with a PerkinElmer Model THGA™ graphite furnace, PerkinElmer Model AS-71 autosampler, and longitudinal Zeeman-effect background corrector, was used for the atomic absorption measurements. Electrodeless discharge lamps (EDL, PerkinElmer) were the sources of radiation used for Cd and Pb determination. Hollow cathode lamps (PerkinElmer) were used as the radiation source for Cr, Cu, Fe, Mn, and Ni. Pyrolytically coated graphite tubes with inserted pyrolytic graphite L´vov platforms were employed. High-purity Ar (flow rate: 300 mL min^{-1}) was used to purge air from the graphite tubes, except during the atomization step, where stopped-flow conditions were used. The analytical measurements were based on peak area. Autosampler volumes of 20 µL of sample followed by 5 µL of chemical modifier were employed for all studies. Each analysis was repeated at least three times to obtain the average value and its relative standard deviation (%RSD). The program was optimized using the digested krill samples.

A Model MLS/2000 (Milestone-FKW, Sorisole, Bergamo, Italy) microwave apparatus, equipped with Teflon® vessels, was used to decompose the krill samples. The main ETAAS operating conditions and matrix modifier used are summarized in Table I.

Reagents

All chemicals used were of analytical reagent grade unless otherwise stated. Deionized water (Barnstead, Dubuque, IA, USA) was used throughout. All solutions were stored in high-density polypropylene bottles. Plastic bottles, autosampler cups, and glassware were cleaned by soaking in 20% (v/v) HNO_3 for 24 h. The material was then rinsed three times with deionized water. Commercially available 1000 mg L^{-1} Cd, Cr, Cu, Fe, Mn, Ni, and Pb standard solutions (Merck, Darmstadt, Germany) were prepared daily by serial dilutions of the stock solutions. Analytical reagent nitric acid (Merck) was used after additional purification by sub-boiling distillation in a quartz still. Palladium solution (0.10% m/v) was prepared by dilution of a 10 g L^{-1} Pd standard solution (Merck). A 0.06% (m/v) $Mg(NO_3)_2.6\ H_2O$ (Merck) solution was prepared by dissolving the salt in deionized water. High-purity Ar was used to purge air from the graphite tubes.

Safety

Hydrofluoric acid is a corrosive reagent that should be handled with appropriate safety precautions to avoid personal damage.

Blanks

Additional purification of the modifiers from Cd and Pb was not necessary because low blanks were obtained. Blank signals were in all cases lower than 0.004 absorbance units for Cd and Pb in peak area measurements when 5 µL of the chemical modifier was injected in each firing.

Samples

A certified reference material (CRM), MURST-ISS-A2, Antarctic Krill, was used for this study. The

TABLE I
Operating Conditions for the Determination of Cd, Cr, Cu, Fe, Mn, Ni, and Pb by ETAAS

Analytical wavelengths (nm)	Cd: 228.8; Cr: 357.9; Cu: 324.8; Fe: 248.3; Mn: 279.5; Ni: 232.0; Pb: 283.3
Slit width (nm)	0.7 (Cd, Cr, Cu, Pb) ; 0.2 (Fe, Ni, Mn)
Lamp current (mA)	Cr: 25; Cu: 15; Fe: 30; Mn: 20; Ni: 25
Electrodeless discharge lamp power	Cd: 4 W; Pb: 7 W
Injection volume (sample)	20 µL
Injection volume (modifier)	5 µL
Matrix modifier	Pd 0.10 % + $Mg(NO_3)_2$ 0.06 % (m/v) (only for Cd and Pb)
Measurement mode	Peak area
Purge gas (flow rate)	300 mL min^{-1} (Ar)

certified concentrations in μg g^{-1} were Cd: 0.731±0.083, Cu: 64.1±5.1, Fe 56.6±2.8, Mn: 4.12±0.16, Ni: 1.28±0.13, and Pb: 1.11±0.11. The informative concentration for Cr was 0.73±0.14. Further information on the preparation and certification of this material has been reported (18).

Sample Preparation Procedure

Two microwave sample pretreatment procedures were set up and compared in order to establish which method would allow the complete destruction of the organic material contained in the samples. The experiments were performed in triplicate. In all cases, a set of digestion blanks was prepared together with each microwave digestion procedure. The sample pre-treatments to digest the krill samples are detailed below.

Microwave Digestion Procedure 1 (MW1)

0.5 g of freeze-dried krill was transferred into closeable Teflon® vessels and 5 mL of concentrated HNO_3 (Merck, 70%) and 1.0 mL of HF (Merck, 40%) were added. The average power applied during the digestion program varied from 300 to 600 W and the complete mineralization cycle was less than 30 min. The operating conditions for the microwave attack of the krill samples are summarized in Table II. Optimized power and time parameters are required in the microwave digestion in order to achieve complete dissolution of the samples. After cooling, the digest solutions were transferred into 25-mL graduated flasks. Five mL of saturated H_3BO_3 solution was added to eliminate any remaining HF. The final solution was diluted to 25 mL with deionized water.

Microwave Digestion Procedure 2 (MW2)

0.5 g of freeze-dried krill was transferred into closeable Teflon vessels and 5 mL of concentrated HNO_3 (Merck, 70%) and 1.5 mL of H_2O_2 (Merck, 40%) were added. The heating program shown in Table II was run to completion. The mineralization cycle lasted 17 min. The digest solutions were allowed to cool and then transferred into 25-mL graduated flasks and diluted to the mark with deionized water.

Calibration

The calibration curves were obtained with calibration standards prepared in the same acid medium as the krill samples. In spite of the complexity of the matrix analyzed, no standard addition calibration was necessary. The calibration was checked every 30 measurements with solutions of the analytes varying between 1 and 5 ng mL^{-1}.

TABLE II
Microwave Digestion Programs (MW 1 and MW2) to Mineralize Krill Samples

	MW1	MW2	
Sample weight	0.25 g	0.25 g	
Reagents	5 mL HNO_3 + 1.0 mL HF	5 mL HNO_3 + 1.5 mL H_2O_2	
Final volume	25 mL	25 mL	
Microwave program:			
Applied power (W)	Time (min)	Applied power (W)	Time (min)
300	5	250	1
0	1		0 1
350	5	250	5
0	1	400	5
300	5	600	5
0	1		
400	5		
600	3		

TABLE III
Graphite Furnace Temperature Program

Parameter	Drying	Pyrolysis	Atomization	Conditioning
Temperature (ºC)	1st. Step: 110 (all elements) 2nd. Step: 130 (all elements)	1000 (Cd, Pb) 1500 (Cr) 1200 (Cu) 1400 (Fe) 1100 (Mn, Ni)	2000 (Cd) 2300 (Cr, Ni) 1900 (Cu) 2100 (Fe) 1800 (Mn) 2200 (Pb)	2400
Ramp time (s)	1st. Step: 1 (all elements) 2nd. Step: 15 (all elements)	10 (all elements)	0	1
Hold time (s)	1st. Step: 30 (all elements) 2nd. Step: 30 (all elements)	20 (all elements)	5 (all elements)	2
Ar flow rate (mL min^{-1})	300	300	0 (read)	300

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ETAAS Determination

Trace element concentrations were determined by ETAAS by injecting 20-µL aliquots of digested krill samples and 5 µL of chemical matrix modifier at each firing into pyrolytically coated tubes and applying the operating conditions and the temperature program given in Tables I and III, respectively. The signals were registered as peak area. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Chemical Modifiers

The use of a chemical modifier in the determination of Cd and Pb in a complex matrix such as krill is mandatory. The chemical modifier provides thermal stabilization in the pyrolysis step of volatile elements such as Cd and Pb and also allows a temperature increase of several hundred degrees without loss of analyte. In general, mixed modifiers are advantageous in ETAAS because they often exhibit more versatile effects and higher efficiency to analytes and matrices, having pronounced synergetic effects and moderating some negative effects of individual modifiers. A previous study on krill showed that a mixed modifier containing Pd and $Mg(NO_3)_2$ was suitable for the determination of Pb and Cd in marine tissues (19). Mg is retained in the furnace as MgO up to a temperature of 2000°C and thus inhibits the production of volatile carbides. On the other hand, Pd stabilizes the analytes during the graphite furnace cycle. This transition metal forms solid solutions and/or compounds with the analytes, which are entrapped within the bulk elemental modifier. For this reason, an early reduction of the modifier is essential in order to transform the modifier into an adequate and reactive elemental form. Lima et al. (20) reported that modifiers containing noble metals were more effective for Cd determination in fish slurries. The optimum amount of modifier for Cd and Pb was found to be 5 µg Pd and 3 µg $Mg(NO_3)_2$. Higher modifier masses did not provide significant improvements in the recoveries or in the maximum temperature.

Optimization of Graphite Furnace Heating Programs

The acidic krill solutions achieved after microwave attack were employed for the optimization of the graphite furnace temperature program. Each program was established from the construction of pyrolysis and atomization curves. A two-step temperature program (100 and 130°C) provided an efficient and uniform drying of the krill samples.

To obtain the pyrolysis curve, the drying and atomization temperatures were fixed and the temperature of pyrolysis was increased in steps of 100ºC. A similar procedure was employed to obtain the atomization curve. At each temperature, three sample aliquots of 20 µL were injected and a mean value of peak area was obtained. The maximum peak area with a good peak shape was used as the criteria to select the optimum temperature. Figures 1 and 2 show the pyrolysis and atomization curves for Cd and Pb with and without matrix modifier.

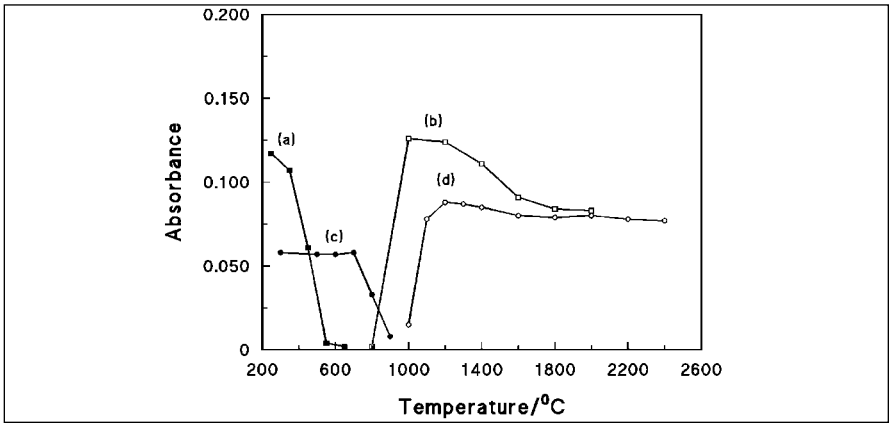

Fig. 1. Pyrolysis and atomization curves for Cd in krill samples: (a) pyrolysis curve without modifier, (b) atomization curve without modifier, (c) pyrolysis curve with modifier, (d) atomization curve with modifier. Cd concentration: 2.0 ng mL^{-1}; Matrix modifier: Pd 0.10 % + $Mg(NO_3)_2$ 0.06 % (m/v).

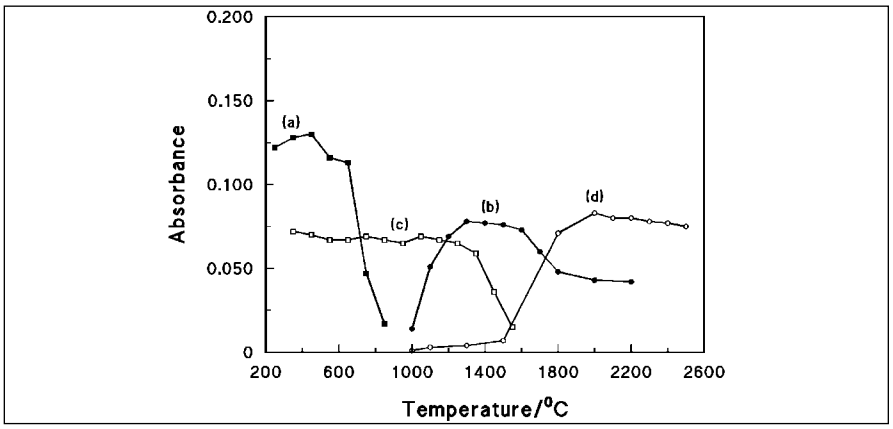

Fig. 2. Pyrolysis and atomization curves for Pb in krill samples: (a) pyrolysis curve without modifier, (b) atomization curve without modifier, (c) pyrolysis curve with modifier, (d) atomization curve with modifier. Pb concentration: 20 ng mL^{-1}; Matrix modifier: Pd 0.10 % + $Mg(NO_3)_2$ 0.06 % (m/v).

Evaluation of Microwave Sample Digestion Procedures

Strong oxidation conditions were necessary to destroy the organic matter present in the samples. Prior microwave digestion studies (21) demonstrated that a multicycle program using maximum power during the last stage was beneficial for better digestion of the krill samples. It is attributed to the fact that the rate of the digestion reaction and the oxidation power of the acids are increased with higher powers. Under the optimized conditions listed in Table II, no visible residual solid particles were observed. The addition of peroxide to the nitric acid was necessary to increase the efficiency of the oxidation of fat tissues. Addition of HF was tested to remove siliceous components, which are present in the caparace (shell) of krill. Cooling steps were included in the microwave program to ensure that the reaction was brought under control. The digests were prepared using microwave-optimized conditions and diluted to 25 mL to bring the concentration of the analytes in the sample within the normal working range. The addition of H_3BO_3 did not produce any effect on the heating temperature program in the graphite furnace.

The two microwave digestion procedures were compared based on three criteria that would ensure that the krill samples are mineralized: (a) recovery; (b) reproducibility; and (c) duration. Both procedures resulted in good recoveries in the certified range of the CRM (93 to 111%) for the elements tested (Cd, Cr, Cu, Fe, Mn, Ni and Pb) as can be seen in Table IV. Also, a good level of reproducibility was achieved with both attacks. Considering the duration, the time necessary to complete the digestion was 26 and 17 min for MW1 and MW2, respectively. Using these three criteria and also to avoid the use of HF, MW2 was found to be the best alternative and was subsequently used for the analysis of the krill samples.

TABLE IV
Average Recovery±Standard Deviation of Cd, Cr, Cu, Fe, Mn, Ni, and Pb Using Two Microwave-assisted Digestion Procedures

Element	Recovery (%) MW1	Recovery (%) MW2
Cd	97	100
Cr	95	111
Cu	99	100
Fe	99	103
Mn	104	107
Ni	96	93
Pb	101	108

TABLE V
Determination of Trace Elements in the CRM MURST-ISS-A2, Antarctic Krill, by ETAAS (Concentrations in µg g^{-1})

Element	Certified values	Found values[a]
Cd	0.731±0.083	0.734±0.008
Cr	0.73±0.14[b]	0.81±0.05
Cu	64.1±5.1	64.1±3.0
Fe	56.6±2.8	58.3±1.3
Mn	4.12±0.16	4.43±0.11
Ni	1.28±0.13	1.19±0.10
Pb	1.11±0.11	1.20±0.07

[a]Mean value ± standard deviation (n=3).
[b]Indicative value.

Analytical Performance

The detection limits were calculated following the IUPAC rules (22) on the basis of the 3σ criterion for 10 replicate measurements of the blank signal. The detection limits for Cd, Cr, Cu, Fe, Mn, Ni, and Pb were: 0.1, 0.3, 0.9, 0.5, 0.5, 1.0, and 1.0, respectively.

The relative standard deviations (%RSD) for 10 successive measurements of a sample containing a final concentration of 1 ng mL^{-1} (Cd) and 5 ng mL^{-1} (Cr, Cu, Fe, Mn, Ni and Pb) of each analyte were: 3, 1, 2, 1, 1, 2, and 4% for Cd, Cr, Cu, Fe, Mn, Ni, and Pb, respectively.

Determination of Cd, Cr, Cu, Fe, Mn, Ni, and Pb in Antarctic Krill

Table V lists the results obtained when Cd, Cr, Cu, Fe, Mn, Ni, and Pb were determined in Antarctic Krill by ETAAS using MW2 procedure to attack the samples. The results are in concordance with the certified (Cd, Cu, Fe, Mn, Ni, and Fe) or indicative (Cr) values. The measured concentrations are the mean and the standard deviation of the determination of five independent digestions of krill samples.

CONCLUSION

Both sample pre-treatments are appropriate to digest krill samples. However, the MW2 procedure (HNO_3/H_2O_2) is recommended as the best alternative since it requires less time for sample pre-treatment, minimizes vessel leakage, and also does not require the use of hydrofluoric acid. The use of a matrix modifier was essential to obtain accurate and reproducible results for the Cd and Pb measurements. The type and amount of modifier employed was effective in stabilizing the more volatile analytes during the pyrolysis step. Despite the complexity of the matrix analyzed, the results obtained using the calibration curve and the standard additions method were consistent. For this reason, the MW2 procedure was selected since the time required for sample analyses was shorter. Good agreement was achieved between the results of the method proposed and the certified values.

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Determination of Au, Pd, and Pt in Copper Ores and Concentrates by GFAAS and ICP-OES

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ABSTRACT

A method has been developed for the determination of Au, Pd, and Pt in copper ores and concentrates by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The method involves the decomposition of samples by aqua regia digestion, followed by Hg/Hg_2Cl_2 precipitation, to collect Au, Pd, and Pt, and the measurement of these metals by GFAAS and ICP-OES. The method has been applied to three international standard samples (CCRMP), PTC-1, PTM-1, and PTM-1a and the results agreed well with the recommended values. The method is very useful for the determination of platinum-group elements in copper-rich samples as the Fire Assay method cannot be applied to these types of samples.

INTRODUCTION

Gold and the platinum-group elements (PGEs) are known to occur in certain sulphide ores at very low concentrations (ppb). It is extremely difficult to quantify such low levels of these elements with existing instrumental analysis techniques. Because of their global scarcity and economic importance, the presence of gold and PGEs at such low levels cannot be ignored and an accurate method for their determination is very important.

The determination of low levels of Au and PGEs essentially involves two major steps: (a) preconcentration by fire assay (1-2) or chemical methods (3); (b) instrumental measurements by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (4) or Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (5). Other, but more expensive, techniques include Neutron Activation Analysis (NAA) (6) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (7). Fire Assay either as Lead Fire Assay or Nickel Sulphide Fire Assay is the most preferred preconcentration technique for the determination of gold and PGEs in geological materials, but both of these fire assay techniques have severe limitations for copper-rich materials such as copper ores (8). In Lead Fire Assay, the presence of large amounts of copper inhibits the separation of the lead button and also causes severe interference in cupellation. In the Nickel Sulphide Fire Assay, all copper passes into the NiS phase; thereby no enrichment of gold and PGEs occurs.

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Acid dissolution followed by precipitation using tellurium (9) or mercury (10-11) as the carrier stream has been established as an alternative separation technique for noble metals. However, tellurium has a tendency to form insoluble copper telluride (from a high concentration of copper solution) (7), which will interfere in the measurement of precious metals. Some authors overcome the problem by employing either solvent extraction (12) or Se collection (13) for coprecipitation of Au and PGEs in copper-rich materials. For organic solvents, the solvent extraction method is highly disadvantageous in either GFAAS or ICP-OES analysis. Se as the carrier for coprecipitation has been studied very little and there is uncertainty about complete precipitation of Au and PGEs. All of these problems do not arise using the mercury coprecipitation method.

In this work, a method has been developed for the determination of precious metals (Au, Pd, and Pt) in copper ores and concentrates. The method involves acid digestion and mercury coprecipitation followed by the determination of Au, Pd, and Pt by GFAAS and ICP-OES. Three international standards (CCRMP) PTC-1, PTM-1, and PTM-1a, and two Geological Survey of India (GSI) in-house standards, CC-1 and CC-2, were analyzed both by GFAAS and ICP-OES following the above method. The results were compared with those of the recommended values.

EXPERIMENTAL

Reagents and Standards

All acids and reagents used were of analytical reagent grade. Au, Pd, and Pt stock solutions (1000 µg/mL) were prepared from corresponding metals. Working standards of 100 ng/mL and 1000 ng/mL were prepared by dilution of the stock solutions with deionized water. Stannous chloride solution (20%, W/v) was prepared by dissolving solid $SnCl_2$, $2H_2O$ in concentrated HCl and diluting with deionized water to produce a 1 mol/L acid solution. Mercuric chloride solution was prepared by dissolving $HgCl_2$ in HCl and diluting with deionized water to obtain 0.1 mol/L solution with respect to HCl.

INSTRUMENTATION

GFAAS Analysis

A UNICAM SOLAR 929 AAS, equipped with a GF 90 graphite furnace and FS 90 Plus furnace autosampler, was used; integrated absorbance (peak area) values were used in the measurements. All elements were determined using hollow cathode lamps. Standard

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TABLE I
Parameters for GFAAS Analysis

		Temperature Program					
		Drying		Ashing		Atomization	
Element	Wavelength	Temp (ºC)	Time (s)	Temp (ºC)	Time (s)	Temp(ºC)	Time (s)
Au	242.8 nm	120	30	800	20	1900	3
Pd	247.6 nm	120	30	1100	20	2300	3
Pt	265.0 nm	120	30	1200	20	2500	3

pyrolytically coated graphite tubes were used for the determination of Au, Pd, and Pt. Nitrogen was used as the furnace purge gas. The operating conditions for GFAAS are given in Table I.

ICP-OES Analysis

A Jobin Yvon Model JY-38 sequential-type ICP-OES instruments was used with the operating conditions as listed in Table II. The wavelengths used for Au, Pd, and Pt were 242.795, 340.438, and 214.423 nm, respectively.

Recommended Procedure

Twenty grams of powdered sample was roasted in a furnace at 600ºC for 3 hrs. The roasted material was transferred to a 500-mL beaker (A) and treated with 50 mL concentrated HCl. The mixture was heated to boiling for about half an hour, then diluted with deionized water to about 70 mL, and allowed to settle. The supernatant was decanted into a 500-mL beaker (B) leaving the undissolved materials and remaining hydrochloric acid in the original beaker (A). Five mL nitric acid and 15 mL hydrochloric acid were added and the mixture heated to incipient dryness. Finally, the mass was dissolved in 50 mL 2 mol/L HCl and filtered. The filtrate was mixed with the original solution in the beaker (B) and the residue transferred to a Teflon® beaker. Five mL of HF and 2 mL of $HClO_4$ were added to the filtrate and the beaker placed on a hot plate until the acids were evaporated to dryness. The dried mass was treated with 10 mL (1 : 1) HCl and the solution obtained was transferred to the beaker (B), and diluted to about 200 mL with deionized water.

TABLE II
ICP-OES Operating Conditions

ICP-OES instrument	Jobin Yvon JY-38, sequential, C-T scanning monochromator, holographic grating, 3600 grooves/mm, 1.0 m focal length
RF generator	56 MHz, 2 KW working power
Plasma torch	Quartz, 28 mm outer diameter
Gas flow	Plasma gas 20 L/min
	Cooling gas 0.4 L/min
	Carrier gas 0.35 L/min
Nebulizer	Pneumatic
Sample uptake	1 mL/min
Observation height	14 mm above load coil
Flush/integration time	10 sec each

The solution was neutralized with dilute sodium hydroxide until a permanent precipitate formed. Five grams of hydroxylamine hydrochloride was added to the precipitate resulting in a clear solution. Ten mL mercuric chloride solution was added and the mixture heated to boiling. Stannous chloride solution was added drop by drop until a permanent precipitate appeared. Ten mL stannous chloride solution was added in excess and the Hg / Hg_2Cl_2 precipitate allowed to settle for one hour. It was then filtered and washed with hot water. The precipitate was transferred to a small beaker and dissolved in aqua regia, evaporated to about 0.5 mL volume, diluted to 5 mL with 1 mol/L HCl, and measured for Au, Pd, and Pt by GFAAS and ICP-OES. The calibration solutions and blanks were prepared using the same procedure.

RESULTS AND DISCUSSION

Extraction of Au and PGEs by treatment with aqua regia was performed by many workers with varying degrees of success. In the case of silicate rocks, especially those containing chromite, extraction of PGEs by aqua regia is far from satisfactory (14). Complete dissolution with HF and $HClO_4$ followed by aqua regia treatment is the only alternative. Unfortunately, complete dissolution of large amounts of silicate materials (10-25 g) with such a mixture of acids is a very cumbersome process and, obviously, fire assay techniques are employed by all commercial laboratories. In the case of copper sulphide ores, however, the aqua regia method poses no problems. The only prerequisite is that the material be roasted before treatment with aqua regia to remove the sulphur.

Extraction of Precious Metals by Coprecipitation

Gold and platinum metals are known to be separated completely from matrix elements by reductive coprecipitation either with Te or Hg. Both methods have been studied in order to test the suitability in the presence of high concentrations of copper, nickel, and iron. Standard solutions of Au, Pd, and Pt are added to a mixture of concentrated solutions of copper, nickel, and iron (~30%) and both Te and Hg coprecipitation methods are applied.

It was found that recovery of the elements is seriously affected in the Te coprecipitation method. Te retains high amounts of copper (5000 ppm), which probably hinders the extraction of Au and PGEs. In comparison, the Hg precipitate retains very little copper, iron, and nickel (<5ppm) and extraction of Au, Pd, and Pt is found to be almost 100% (Table III).

Interference Study in GFAAS Analysis

In the GFAAS measurement of Au, Pd, and Pt, interferences are expected only from Cu, Ni, Fe or from the elements themselves. It was found that the presence of more than 200 ppm of Cu slightly enhances the absorption signal of precious metals. However, both Ni and Fe, if present above 100 ppm, decrease the absorption values of these elements. As mentioned earlier, the Hg precipitate retains < 5 ppm of Cu, Ni, or Fe; therefore, interferences from these elements are very negligible (Table IV). Interelement interferences from Au, Pd, and Pt are significant only if they are present above the 2000-ppb level.

Preliminary Investigation and Interference Study in ICP-OES Analysis

The emission lines of 242.795, 340.438, and 214.423 nm for Au, Pd, and Pt, respectively, were selected after a thorough study of the different lines. Although the above-mentioned Au and Pd lines have been used by different workers, the Pt line has seldom been used (15). However, we found that this particular line is interference-free. The mutual interference of Au, Pd, and Pt and the interference effects of copper, nickel, and iron on Au, Pd, and Pt were studied (Table V). Taking analyte concentrations of 1 ppm and 1000 ppm of the interferent showed that there is little interference effect on the analytes in these lines.

Calibration of the instrument was accomplished by aspirating three standard solutions (0.1, 1, 10 ppm) and a blank. Each sample solution was analyzed in duplicate and an average was taken.

TABLE III
% Recovery of Au, Pd, and Pt From Mixture of Concentrated Solution of Cu, Ni, and Fe (5 mL 100-ppb solution of Au, Pd, and Pt added; after extraction, volume was adjusted to 5 mL)

	Hg Coprecipitation	Te Coprecipitation
Au	98%	25%
Pd	97%	15%
Pt	97%	30%

TABLE IV
Interference in GFAAS System Interferent (µg/mL)

	Cu (µg/mL)			Ni (µg/mL)			Fe (µg/mL)		
	50	100	200	50	100	200	50	100	200
Au (20 ng/mL)	20.1	20.1	20.2	20.2	19.0	9.0	20.0	19.0	13.5
Pd (20 ng/mL)	20.2	20.1	20.2	20.1	19.1	8.5	20.1	18.5	14.2
Pt (100 ng/mL)	100.2	99.3	101.5	99	95	45	100.2	96.3	65.0

In the presence of Cu, Ni, and Fe (50 µg/mL, 100 µg/mL, and 200 µg/mL), the changes in the analyte concentrations (20 µg/mL of Au, 20 µg/L of Pd, and 100 µg/mL of Pt) in GFAAS measurements are shown.

TABLE V
Interferences in ICP-OES System (expressed in terms of µg/mL analyte)

Analyte (1 µg/mL)	Interferents (1000 µg/mL)					
	Au	Pd	Pt	Cu	Ni	Fe
Au	-	0	0	0	0	0.2
Pd	0	-	0	0	0	0.2
Pt	0	0	-	0	0	0.4

In the presence of 1000 µg/mL of interferents (Au, Pd, Pt, Cu and Ni), the analyte (Au, Pd, and Pt) concentration did not show any change in ICP-OES measurements. Only in case of Fe (1000 µg/mL), the analyte concentration increased to the extent of 0.2, 0.2, and 0.4 µg/mL for Au, Pd, and Pt, respectively.

Analysis of Standard Reference Samples (CCRMP)

Three international standard reference samples, PTC - 1 (Sulphide Concentrate), PTM-1 (Nickel-copper Matte), and PTM-la (Nickel-copper Matte) were analyzed for Au, Pd, and Pt by GFAAS and ICP-OES following the proposed method. The results presented in Table VI, obtained by both techniques, compare favorably and are in good agreement with those of the recommended values. Because of the limited availability of standard reference samples, only 10-g samples were taken for the analysis of

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TABLE VI
Results for Standard Reference Samples (PTC-1, PTM-1, PTM-1a) and In-house Standards (CC-1 and CC-2) (Values are in µg/g for standard reference samples and ng/g for in-house samples.)

	Au			Pd			Pt		
	GFAAS	ICP-OES	Certified Value	GFAAS	ICP-OES	Certified Value	GFAAS	ICP-OES	Certified Value
PTC-1[a]	0.60	0.63	0.65	12.5	12.4	12.7	3.10	3.20	3.0
PTM-1[a]	1.72	1.73	1.80	7.85	7.90	8.10	5.72	5.75	5.80
PTM-1a[b]	3.35	3.40	3.30	10.20	10.25	10.01	7.35	7.40	7.31
C C-1	250	260	-	88	92	-	42	45	-
C C-2	630	640	-	190	195	-	185	190	-

[a] W.S. Bowman, CCRMP certified reference materials, CANMET - 90 - 1E, p. 63 - 65 (1990).

[b] W.S. Bowman, CCRMP certified reference materials, CCRMP - 94 - 1E, p. 46 (1994).

PTC-1 and PTM-1. However, a 20-g sample was taken for PTM-1a. The precision study was carried out with only one sample (PTM-1-a) and the RSD found for Au, Pd, and Pt was 1.6%, 1.4%, and 1.2%, respectively.

Analysis of GSI In-house Standards, CC-1 and CC-2

CC-1 and CC-2 are two copper concentrates, prepared at the Geological Survey of India as in-house standards. The copper concentration (%) of the two samples was 21.74 and 22.50, respectively.

The proposed method was applied to these samples and the results obtained by both techniques compared well with each other. These samples were analyzed five times each and an average value was taken.

CONCLUSION

The proposed method is well suited to copper-rich materials such as copper ores and concentrates. As large amounts of sample can be used, low detection limits can easily be obtained. Mercury coprecipitation is very clean and interference-free by either GFASS or ICP-OES. Matrix modifiers were not used for the GFAAS measurements.

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PLEASE NOTE CORRECTION for July/August 2000 issue of Atomic Spectroscopy, Volume 21(4), article entitled "Enhancement of Thallium Response by Flow Injection Hydride Generation AAS Using Palladium and Rhodamine B," by Zhu Daan and Xu Shukun:

The units in the captions of Figures 2, 4, and 5, pp. 138-140, should read "200 ng/mL thallium" instead of 200 mg/L.

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