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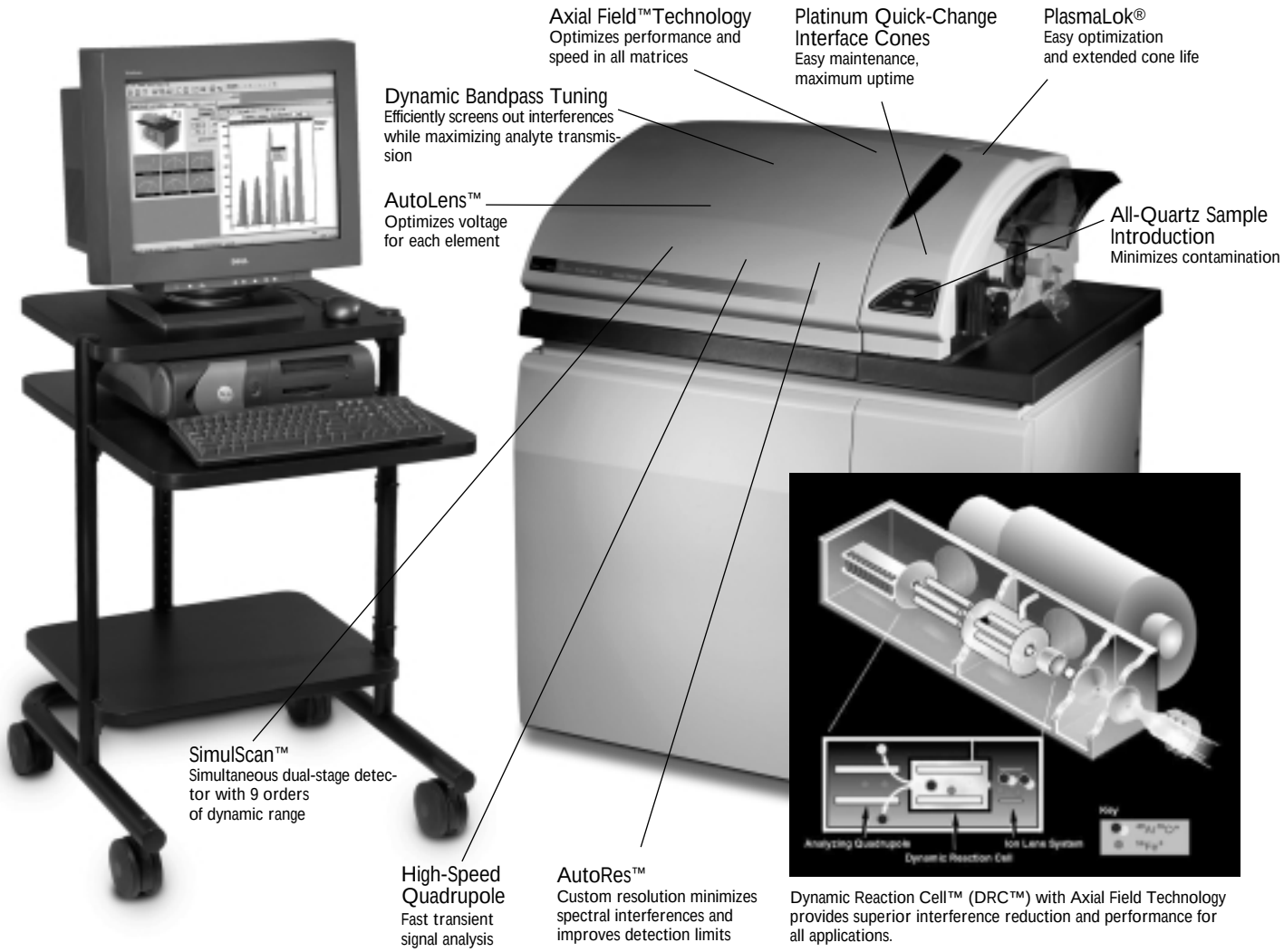

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Inductively Coupled Plasma Mass Spectrometry to Measure Multiple Toxic Elements in Urine in NHANES 1999-2000

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ABSTRACT

Inductively coupled plasma mass spectrometry (ICP-MS) has become a reliable and mature method for the measurement of both toxic and essential elements in biological matrices such as urine.We recently measured 12 elements (metals) in urine of 2,465 U.S. residents using a modified version of our previous multi-element inductively coupled plasma mass spectrometry method. The current method measures antimony, barium, beryllium, cadmium, cesium, cobalt, lead, molybdenum, platinum, thallium, tungsten, and uranium in urine. Results have been presented in The Second National Report on Human Exposure to Environmental Chemicals which can be accessed in its entirety on line at www.cdc.gov/exposurereport. Selected data will be presented in this paper. Examples of data in the report are the geometric mean for lead, with a sample size of 2465, was found to be 0.76 µg/L with a 95th percentile of 2.90 µg/L. Cadmium had a geometric mean of 0.33 µg/L, sample size of 2465, and a 95th percentile of 1.03 µg/L. Uranium, sample size 2464, had a geometric mean of 0.007 µg/L, with 0.026 µg/L 95th percentile.Tin and manganese were removed from the original panel: tin due to contamination in the ultra pure water system and manganese because of interferences in the mass spectrum. Cadmium and uranium were added to the panel. This paper describes the modifications that have been incorporated since the original method was published and presents reference ranges for the 12 elements measured.

INTRODUCTION

Except for lead and cadmium, an information gap has existed for decades on the "normal" levels of the population's exposure to a multitude of toxic elements. Therefore, the Division of Laboratory Science of the National Center for Environmental Health, and the agency of Centers for Disease Control and Prevention (CDC), uses biomonitoring techniques to accumulate metal exposure data for the U.S. population. We began by evaluating the U.S. population's exposure to 12 toxic elements. The ranges detected will help public health officials determine whether a person has had a substantial exposure to one of these metals: Sb, Ba, Be, Cd, Cs, Co, Pb, Mo, Pt, Tl, W, and U. These elements were measured in the urine of 2,465 persons participating in the National Health and Nutrition Examination Survey (NHANES 1999–2000) (1).

Urine was measured by inductively coupled plasma mass spectrometry (ICP-MS), a rapid and accurate multi-element analytical technique (2). The method is sensitive enough to screen urine specimens from subjects suspected to have been exposed to a number of clinically important toxic elements, or to help evaluate environmental or other non-occupational exposures to these elements.

Significant modifications have been made to a previously published CDC ICP-MS multi-element urine method (3) to include enhancements to the method, such as the addition to the panel of two clinically relevant elements, Cd and U. This article presents selected percentiles and geometric means of the urine levels of environmental elements in the general U.S. population from the Second National Report on Human Exposure to Environmental Chemicals 1999–2000 (1). Additionally, the ICP-MS laboratory method used to measure multiple toxic elements in urine is described and compares the current ICP-MS multi-element panel performance criteria with the panel previously described by this group; and reports the recently released multi-element panel median values from NHANES 1999–2000.

NHANES uses a stratified, multistage design to provide a representative probability sample of the civilian non-institutionalized population of the U.S., aged two months and older. The survey was conducted from March 1999 to January 2001. A detailed description of sample design specifications for NHANES 1999–2000 can be found at http://www.cdc.gov/nchs/data/

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nhanes/frequency/demodoc.pdf (4). The survey includes an interview conducted in the households and an examination at a mobile examination center. Information obtained during the interview includes individual characteristics such as sex, age, self-reported race and ethnicity, years of education completed, and income information. The examination consists of a variety of physical measurements and blood and urine collections. Urine multi-element measurements were completed on one-third of approximately 5000 people per year aged 6 years and over. (This same subset of urine collected was also analyzed for organophosphate and other non-persistent pesticides.) The one-third NHANES sample subset reported here is a probability sample designed to be representative of the U.S. civilian, non-institutionalized population. Each stage of the sample selection was randomized to ensure unbiased estimates for the U.S. population. Serum from this subset was collected to analyze for persistent pesticides, PCBs, thyroxine, and thyrotropin. A detailed description of the data collection methods for the NHANES has already been published (4) and will not be repeated here. Our 1998 paper used a "convenience" 496-sample subset, not a true representative subsample.

EXPERIMENTAL

Instrumentation

The instruments used were an ELAN® 6000 and an ELAN® 6100 (PerkinElmer SCIEX, Concord, Ontario, Canada). These instruments were equipped with a Ryton® spray chamber and a GemTip™ Cross-Flow II Ryton nebulizer for sample introduction. Table I shows the analytical parameters used in the analysis. Data acquisition was accomplished using ELAN software v. 2.4 (service pack 2) based on the Microsoft® Windows 2000® operating system.

Reagents

All reagents were of at least analytical reagent grade quality. Double-distilled nitric acid (GFS Chemicals, Columbus, OH, USA) and ≥18 MΩ cm^{-1} water from Milli-Q™ or ELIX5 / Academic A10 purification systems (Millipore Corporation, Milford, MA, USA) were used to prepare all solutions. The rinse solutions contained 0.02% Triton® X-100 (Fisher Scientific, Pittsburgh, PA, USA). The standards for the multi-element analysis of urine by ICP-MS were prepared from single-element standard reference materials (SRMs) from the National Institute of Science and Technology (NIST, Gaithersburg, MD, USA) or from certified standards obtained from SPEX Industries, Inc. (Edison, NJ, USA).

Procedure

Urine was collected using a protocol designed to minimize trace element contamination (5). Prescreened plastic containers were used for collection, aliquoting, and shipment. Specimens were frozen at ≤–20ºC until analyzed. Urine specimens for ICP-MS analysis were diluted quantitatively (1 + 9) with a solution containing 2% (v/v) double- distilled nitric acid with rhodium and iridium internal standards at 10 µg/L. The rinse solution was an aqueous solution 0.02% Triton X-100 in 5% (v/v) double-distilled nitric acid.

ICP-MS, using a matrix-matched calibration curve prepared from spiked "low-normal" urine with rhodium and iridium as internal standards, was used to determine the 12 elements in this study (6). Patient specimens were evaluated in groups of 20, which each included one blind quality control sample. Each group was bracketed by bench quality control material. The detection limits for each of the elements in the urine specimens were based on three times the concentration standard deviation of urine blanks analyzed in at least 20 separate runs. Results below the detection limit were reported as nondetectable. For statistical calculations, population measurements below the detection limit were replaced by limit of detection: LOD / $\sqrt{2}$.

TABLE I. Instrument Parameters for ICP-MS

Instrument	ELAN 6000 or ELAN 6100
Nebulizer	Cross-flow Type II
RF Power	1450 W
Argon Flow Rates	
Plasma	15 L min^{-1}
Auxiliary	1.2 L min^{-1}
Nebulizer	0.9–1.0 L min^{-1}
Sample Uptake Rate	0.65 mL min^{-1} (12 rpm)
Ion Lens Voltages	Autolens voltages optimized as needed
Detector Mode	Pulse
Measurement Parameters	180 Sweeps/Reading; 4 Replicates; 1 Reading/Replicate
Dwell Time	Be 75 ms; all others, 15 ms
Uptake and Rinse Times	
Sample Flush	48 rpm/20 sec
Read Delay and Analysis	12 rpm/30 sec
Wash	21 rpm/300 sec

RESULTS AND DISCUSSION

Since the 1998 publication of our multi-element urine method, ICP-MS technology has undergone numerous instrument enhancements allowing for improved detection abilities of trace elements in urine. In particular, a redesign of the ion optics and interface designs from the ELAN 6000/6100 have significantly increased the sensitivity of this instrument which has contributed to an improvement in the limits of detection for the multi-element urine panel.

Instrument and method parameters have also been modified since the original method was introduced. A cross-flow nebulizer replaced the Meinhard® quartz nebulizer in the sample introduction system to reduce the potential for nebulizer blockage and permit greater reproducibility of setting up the sample introduction system. The dwell time was reduced from 130 ms to 15 ms per analyte, with the exception of 75 ms for beryllium. This allowed for a larger number of sweeps of the measured masses, improving the ability to average out signal noise. Replicates were reduced from 5 to 4 in the interest of analysis time and sample throughput. Triton X–100 in the rinse solution was reduced from 0.1% to 0.02% (v/v) to allow for lower signal noise in the transition from rinse to sample solutions by minimizing surface tension differences of the solutions. Also, with improved sensitivity in the instrument, internal standard concentrations were able to be reduced from 1000 µg/L to 10 µg/L. Table II summarizes these major instrumental and method differences.

Table III lists a comparison of detection limits from our 1998 multi-element study and the current work. As evident in Table III, the limits of detection for the elements retained in the method either remained the same or were improved, with the exception of molybdenum. Tungsten, for example with a previous LOD of 0.3 µg/L, improved an order of magnitude to 0.04 µg/L. This improvement in sensitivity is significant when considering the U.S. population data 1999–2000 (Table IV). Tungsten has a 95th percentile of 0.32 µg/L. Using the previous LOD, 95% of the U.S. population would have urine tungsten levels lower than the detection limit. With the new LOD, CDC was able to provide 25th percentile urine tungsten concentrations for selected population groups, including children. The urine tungsten data provide physicians with a reference range so that they can determine whether people have been exposed to higher levels of tungsten than levels found in the general population (1). The reason for the increase in the limit of detection of molybdenum is possibly a result of using within-run limits of detection calculations, or less run-to-run reproducibility of the calibration curve intercept due to selection of higher concentration first calibrator. The increase is not significant, however, to the levels of molybdenum observed in the majority of specimens (10th percentile = 12.6 µg/L). Table III also lists the 1999–2000 highest calibrator in µg/L. All patient samples with urine metal concentrations exceeding the highest calibrator were diluted to a value within the calibration range.

TABLE II
Parameters Modified Since Original Method Published (1998)

Instrumentation	Current Parameters	Previously Published in 1998
Nebulizer	Cross-flow	Concentric
Dwell Time	75 ms Be; 15 ms all others	130 ms
Replicates	4	5
Rinse	Triton X-100 (0.02%)	Triton X-100 (0.1%)
Internal Standard	Rh and Ir (10 µg/L)	Rh and Ir (1000 µg/L)
Instrument	ELAN 6000, ELAN 6100	ELAN 500

TABLE III. Elements Measured in NHANES Urine Specimens

Analyte	Mass	LOD (µg/L)	Previous LOD (µg/L)	Internal Standard	Highest Calibrator (µg/L)
Antimony	121	0.04	0.3	^{103}Rh	6
Barium	138	0.12	0.1	^{103}Rh	40
Beryllium	9	0.13	0.1	^{103}Rh	10
Cadmium[a]	114	0.06	-[b]-	^{103}Rh	10
Cesium	133	0.14	0.2	^{103}Rh	20
Cobalt	59	0.07	0.7	^{103}Rh	10
Lead	208	0.1	0.1	^{193}Ir	20
Molybdenum	98	0.8	0.4	^{103}Rh	250
Platinum	195	0.04	0.4	^{193}Ir	6
Thallium	205	0.02	0.1	^{193}Ir	2
Tungsten	184	0.04	0.3	^{193}Ir	4
Uranium	238	0.004	-[b]-	^{193}Ir	0.5

[a] Correction equation applied (–0.026826 * ^{118}Sn).
[b] Not measured in previous study.

TABLE IV
Comparison of Previously Published Urine Metal and Current Urine Metal Selected Statistics

Metals	NHANES 1999-2000 Urine Metal Statistics				Previously Published Urine Metal Statistics	
	Sample Size	Geometric Mean	Selected Percentiles		Sample Size	Geometric Mean
			10^{th}	95^{th}		
Antimony	2,276	0.13	0.05	0.42	496	0.74
Barium	2,180	1.48	0.2	6.80	496	0.57
Beryllium	2,465	<LOD	<LOD	<LOD	496	0.14
Cadmium	2,465	0.33	0.11	1.36	-[a]-	-[a]-
Cesium	2,464	4.35	1.60	11.4	496	1.16
Cobalt	2,465	0.37	0.13	1.32	496	0.87
Lead	2,465	0.76	0.20	2.90	496	2.08
Molybdenum	2,257	45.9	12.6	178	496	46.8
Platinum	2,465	<LOD	<LOD	<LOD	496	0.92
Thallium	2,413	0.18	0.06	0.450	496	0.26
Tungsten	2,338	0.09	<LOD	0.500	496	0.70
Uranium	2,464	0.007	<LOD	0.046	-[a]-	-[a]-

[a] Not measured.

Geometric Mean and Selected Percentiles are given in µg/L.

CDC's mission is to be able to accurately detect elements in biological matrices. We are constantly re-evaluating which analytes should be measured on the basis of public health priorities. Initial changes to the panel since the last publication included the removal of manganese and tin. Manganese was removed because polyatomic interferences at m/z 55 (i.e., $^{40}Ar^{15}N$, $^{38}Ar^{17}O$, $^{36}Ar^{18}OH$, $^{38}Ar^{16}OH$, $^{37}Cl^{18}O$, $^{37}Cl^{17}OH$, and $^{39}K^{16}O$) were more significant to the measurement on standard quadrupole instrumentation than previously expected. We anticipate returning manganese to the panel by removing the interferences with the use of Dynamic Reaction Cell™ (DRC™) technology (7) in future revisions of the method. Tin was removed from the panel because of a high and variable background observed in the de-ionized water supply. This background was observed from both an ion exchange based water purification system and a system which coupled reverse-osmosis and ion exchange capabilities.

The accuracy of the method has been verified by analyzing internal and external quality control materials and by participating in the proficiency testing programs (Figure 1). The Clinical Laboratory Improvement Amendments of 1988 and Good Laboratory Practices (GLP) require that we provide external quality assurance for those elements not covered by any other program. Figure 1 represents CDC's performance in proficiency testing for Ba, Be, Co, Mo, Cd, Sb, Pt, Tl, Pb, and U. All results were found to be within CTQ acceptable limits, based upon population means of the unknown challenge samples. The coefficients of determination (r^2) range from 0.9836 (Be) to 0.9999 (Mo) demonstrating a linear relationship between the CTQ target value and the CDC submitted value. The performance data proved to be an acceptable validation of the current method, as defined by CLIA '88, receiving a score of at least 80% on any three consecutive challenges. For the remaining elements, Cs and W, we exchanged samples with two external laboratories (Figure 2). Cesium (r^2 = 0.9999) and W (r^2= 0.8910) showed reasonable correlation between the CDC laboratory and two external laboratories also using ICP-MS.

Precision was estimated on data generated from the replicate determination of four quality control pools prepared at CDC. The pool means, standard deviations of the run means, standard deviations of the single run values, and the standard deviations within the runs are given. Standard error and relative standard deviation (RSD) were calculated and are presented in Table V. The RSDs for each pool were calculated ranging from 0.93% to 9.64%. Standard errors (SE) associated with these four pools ranged from 0.0001 to 0.28.

Table IV shows selected percentiles and geometric means of the urine levels of environmental elements in the general U.S. population for 1999–2000. Percentiles are provided as additional information about the shape of the population distribution. For example, in Table IV the geometric mean of the

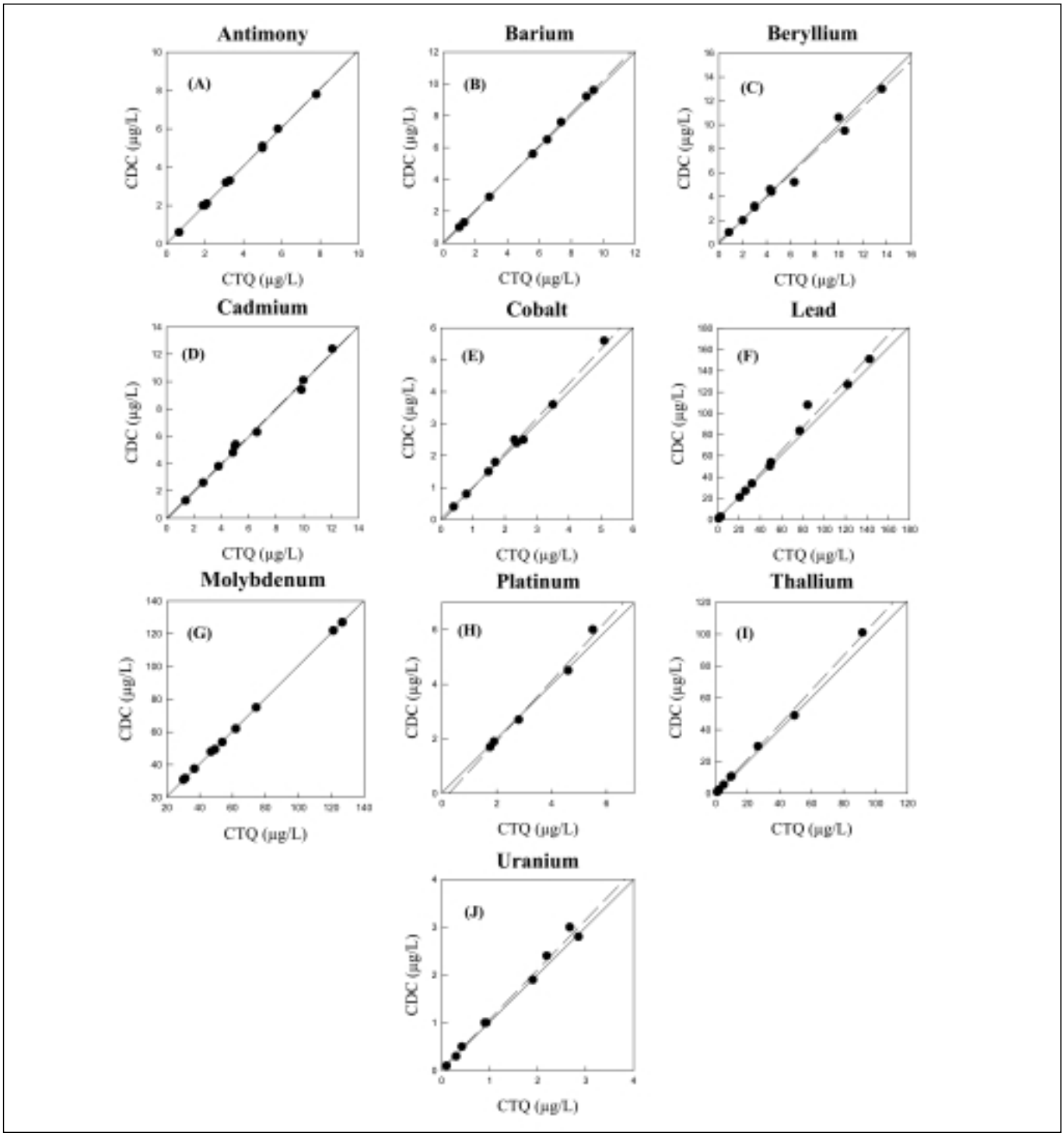

Fig. 1. CDC 1999–2002 performance results on Centre de toxicology du Quebec Round Robin for 10 elements.
(A) Antimony slope=1.01, intercept=0, r^2=0.9989; (B) Barium slope=1.03, intercept=-0.06, r^2=0.9995;
(C) Beryllium slope=0.94, intercept=0.21, r^2=0.9836; (D) Cadmium slope=1.00, intercept=-0.02, r^2=0.9943;
(E) Cobalt slope=1.09, intercept=-0.10, r^2=0.9953; (F) Lead slope=1.08, intercept=0.08, r^2=0.9902;
(G) Molybdenum slope=1.00, intercept=0.52, r^2=0.9999; (H) Platinum slope=1.10, intercept=-0.28, r^2=0.9889;
(I) Thallium slope=1.08, intercept=-0.13, r^2=0.9976; (J) Uranium slope=1.04, intercept=0.02, r^2=0.9898.

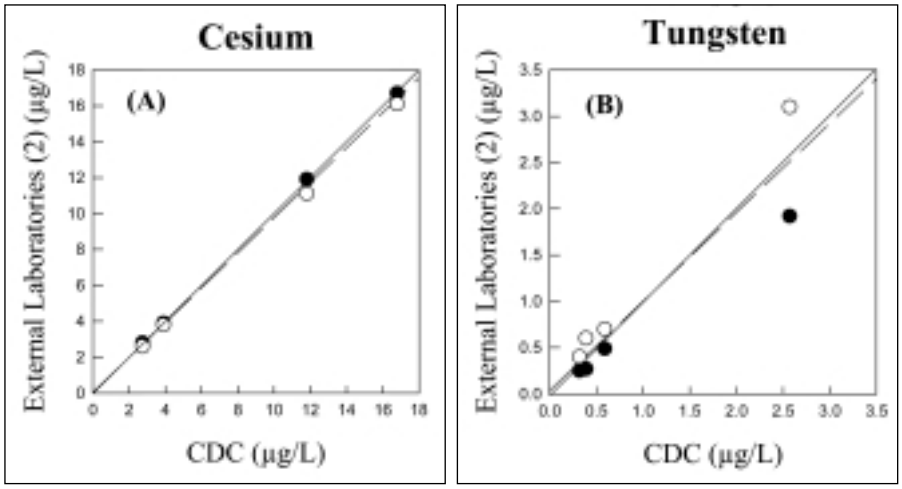

Fig. 2. CDC round-robin performance report.
(A) Cesium slope=0.99, intercept=0.06, r^2=0.9999;
(B) Tungsten slope=0.96, intercept=0.03, r^2=0.8910.

U.S. population's urine lead during 1999–2000 was 0.76 µg/L. The 10th percentile for lead was found to be 0.20 µg/L. This data allowed us to extrapolate that 10% of the study population had a urine lead concentration of less than or equal to 0.20 µg/L.

CONCLUSION

The Second National Report on Human Exposure to Environmental Chemicals set the precedence for determining reference ranges for 12 elements (Sb, Ba, Be, Cd, Cs, Co, Pb, Mo, Pt, Tl, W, and U) for an "unexposed" U.S. population. The geometric mean for lead, with a sample size of 2465, was found to be 0.76 µg/L with a 95th percentile of 2.90 µg/L. Cadmium had a geometric mean of 0.33 µg/L, sample size of 2465, and a 95th percentile of 1.03 µg/L. Uranium, sample size 2464, had a geometric mean of 0.007 µg/L, with 0.026 µg/L 95th percentile. The Second National Report on Human Exposure to Environmental Chemicals in its entirety can be accessed on-line at www.cdc.gov/exposurereport. Future reports will include more detailed assessments of exposure levels among different population groups defined by sex, race/ethnicity, age, urban/rural residence, education level, income, and other characteristics. In addition, data will be combined in future editions of the National Report on Human Exposure to provide updated national estimates of exposure.

Enhanced sensitivity afforded by the new method has contributed to improved limits of detection. Lower limits of detection increase the amount of data that is available for statistical calculations, thus decreasing the number of samples that are recorded as less than the limit of detection. The additional data provides a more detailed frequency distribution represented by the percentiles given in Table IV.

The multi-element panel has proven to be a rapid and reliable method for the evaluation of the population's exposure to various environmental elements. Future plans are to include total arsenic, manganese, chromium, nickel, and vanadium in the urine trace element panel, which will require reaction/collision cell or high-resolution sector field ICP-MS technology.

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Mention of company or product names does not constitute endorsement by the National Center for Environmental Health (NCEH), Centers for Disease Control (CDC) or the Public Health Service.

TABLE V
Precision Achieved By Analyzing Pooled Urine Quality Control Samples

Analyte	Pool	Mean[a]	STDc[b]	STDindc[c]	STDwrc[d]	N[e]	SE[e]	RSD (%)
Barium	Pool 1	1.05	0.07	0.07	0.04	192	0.0047	6.24
Barium	Pool 2	10.15	0.20	0.23	0.18	188	0.0143	1.93
Barium	Pool 3	2.03	0.08	0.08	0.04	203	0.0054	3.76
Barium	Pool 4	20.35	0.30	0.35	0.27	202	0.0208	1.45
Berillium	Pool 1	0.98	0.05	0.06	0.05	238	0.0034	5.31
Berillium	Pool 2	9.55	0.36	0.47	0.41	235	0.0237	3.80
Berillium	Pool 3	0.51	0.04	0.05	0.02	217	0.0030	8.74
Berillium	Pool 4	5.03	0.20	0.23	0.17	243	0.0130	4.02
Cadmium	Pool 1	0.99	0.04	0.04	0.02	218	0.0024	3.57
Cadmium	Pool 2	9.73	0.22	0.24	0.10	215	0.0152	2.30
Cadmium	Pool 3	0.52	0.03	0.03	0.02	225	0.0017	4.95
Cadmium	Pool 4	5.20	0.09	0.11	0.08	223	0.0061	1.74
Cobalt	Pool 1	1.02	0.04	0.04	0.03	245	0.0024	3.65
Cobalt	Pool 2	10.07	0.20	0.25	0.20	242	0.0130	2.02
Cobalt	Pool 3	0.54	0.03	0.03	0.02	235	0.0019	5.29
Cobalt	Pool 4	5.40	0.10	0.12	0.09	233	0.0068	1.93
Cesium	Pool 1	1.07	0.10	0.12	0.10	230	0.0064	9.07
Cesium	Pool 2	10.17	0.21	0.26	0.22	232	0.0137	2.06
Cesium	Pool 3	2.06	0.08	0.09	0.07	207	0.0053	3.71
Cesium	Pool 4	20.57	0.33	0.39	0.29	205	0.0229	1.59
Molybdenum	Pool 1	8.30	0.47	0.52	0.31	216	0.0319	5.65
Molybdenum	Pool 2	79.97	0.75	0.84	0.53	211	0.0514	0.93
Molybdenum	Pool 3	27.94	0.34	0.39	0.27	211	0.0237	1.23
Molybdenum	Pool 4	279.24	4.01	4.08	1.04	209	0.2772	1.44
Lead	Pool 1	1.01	0.05	0.06	0.03	233	0.0034	5.06
Lead	Pool 2	9.89	0.14	0.16	0.08	229	0.0095	1.45
Lead	Pool 3	1.07	0.04	0.05	0.03	223	0.0030	4.19
Lead	Pool 4	10.70	0.16	0.17	0.08	221	0.0110	1.52
Platinum	Pool 1	0.39	0.02	0.02	0.01	232	0.0011	4.16
Platinum	Pool 2	3.81	0.12	0.12	0.04	229	0.0078	3.08
Platinum	Pool 3	0.21	0.02	0.02	0.01	219	0.0011	7.54
Platinum	Pool 4	2.06	0.06	0.07	0.03	217	0.0043	3.05
Antimony	Pool 1	0.41	0.02	0.02	0.01	220	0.0012	4.36
Antimony	Pool 2	3.97	0.07	0.08	0.05	218	0.0047	1.75
Antimony	Pool 3	0.27	0.02	0.02	0.01	219	0.0012	6.68
Antimony	Pool 4	2.32	0.04	0.05	0.03	219	0.0028	1.81
Thallium	Pool 1	0.08	0.01	0.01	0.00	222	0.0005	8.45
Thallium	Pool 2	0.81	0.01	0.01	0.01	219	0.0008	1.42
Thallium	Pool 3	0.10	0.01	0.01	0.00	231	0.0005	7.46
Thallium	Pool 4	1.01	0.01	0.02	0.01	229	0.0009	1.35
Tungsten	Pool 1	0.39	0.01	0.01	0.01	212	0.0008	2.79
Tungsten	Pool 2	3.84	0.09	0.10	0.03	209	0.0065	2.45
Tungsten	Pool 3	0.40	0.01	0.01	0.01	225	0.0007	2.76
Tungsten	Pool 4	4.02	0.07	0.08	0.03	223	0.0050	1.86
Uranium	Pool 1	0.01	0.00	0.00	0.00	248	0.0001	9.64
Uranium	Pool 2	0.08	0.00	0.00	0.00	245	0.0002	3.71
Uranium	Pool 3	0.03	0.00	0.00	0.00	254	0.0001	5.59
Uranium	Pool 4	0.3200	0.0087	0.0099	0.0067	251	0.0005	2.72

[a] Mean = mean of the run means.
[b] STDc = standard deviation of run means.
[c] STDindc = standard deviation of single run value.
[d] STDwrc = standard deviation within run.
[e] N = sample size.
[e] SE = standard error.

The Effects of Residual Carbon on the Determination of Chromium in Blood and Tissue Samples Using Quadrupole ICP-MS

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ABSTRACT

The determination of chromium using Quadrupole ICP-MS in biological samples, such as blood and tissue, has to account for low element levels and a complex matrix. Sample preparation using acid digestion and microwave heating is widely used. Due to the residual carbon, the determination of chromium using isotopes 52 and 53 is problematic. The identification of the organic residual, using GC-MS analysis of the acid digestion solution, lead to an experiment in which carbon was added to a blank solution, and the signal from atomic masses 52 and 53 (chromium isotopes) was determined with Quadrupole ICP-MS. This analysis showed that carbon interferences produced readings that were in the range of the normal chromium levels in blood, in the absence of the presence of Cr, as determined by graphite furnace atomic absorption spectrophotometry (GFAAS). The use of Quadrupole ICP-MS is not recommended for the determination of Cr in biological samples where the expected concentration of Cr is in the µg/L range. The problem of carbon interference in biological samples can be circumvented by the use of GF-AAS or possibly sector field ICP-MS and Quadrupole ICP-MS with reaction cells or collision cells.

INTRODUCTION

Today, orthopedic implants fabricated from metallic materials are widely used to treat diseased, degenerated and/or traumatized joints. Those implants are typically well tolerated; however, there is an increasing recognition that permanent orthopedic devices may be associated with adverse local and remote tissue responses in some individuals. These adverse effects are mediated by the degradation products of these implant materials, namely wear debris, free metal ions, and organometallic complexes. Multiple studies have demonstrated chronic elevations of metal concentrations in human body fluids and tissues following total joint replacement which typically are in the parts per billion range (1–6). The known potential toxicities of elements in modern alloys (Ti, Al, V, Co, Cr, and Ni) used for implant devices are described elsewhere (7–12).

One of the current standard tools to measure metallic concentrations in the parts per billion range is graphite furnace atomic absorption spectrophotometry (GFAAS). Although this technique is widely used and is very reliable for the determination of metal contents in human body fluids and tissue samples, it is limited by the slow throughput of the process since only one element at a time can be measured. Inductively coupled plasma mass spectrometry (ICP-MS) allows simultaneous multiple element analyses within a single sample and is an attractive alternative for that reason. In addition, ICP-MS techniques can reproduce detection limits that are improved by an order of magnitude. For chromium in blood and urine, a detection limit of 0.2 µg/L using a double focusing magnetic sector ICP-MS has been reported, which is similar to a Quadrupole ICP-MS in a "carbon-free" nitric acid solution (13). Our own investigations using a simple ten-fold dilution of whole blood and sector-field ICP-MS pointed even to a lower detection limit of 0.02 µg/L, which is similar to a report by Rodushkin (14).

However, measurements of chromium in body fluids and tissue samples have to overcome certain difficulties, despite the availability of such powerful techniques as outlined above. Carbon remaining after digestion may react within the argon plasma during ICP-MS analysis. The potential generation of ArC^+ ions interferes with the readout of the chromium isotopes 52 and 53. Since the chromium levels are already low (2 µg/L and under) and further preparation techniques have to be applied to overcome matrix interferences, diluted samples with a chromium content close to the detection limit are created. This should be accounted for, and interferences with matrix residues after dilution and/or digestion need to be considered. In this study, the applicability of Quadrupole ICP-MS for the determination of clinically relevant levels of chromium in blood and tissue samples has been investigated and the results obtained with GFAAS have been compared.

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EXPERIMENTAL

Freeze-dried whole blood reference material (LOT MR9067) was obtained from Seronorm™; 5 mL of highly purified water (Mill-Q™ water) was added to the dried blood. After 30 minutes, the fluid was thoroughly, but carefully, mixed and blood samples of 1 mL were pipetted into quartz vessels. Seven different digestion protocols ('trial 1' through 'trial 7') were applied. These protocols differed with respect to the volume of the oxidizing reagents and the overall digestion time. For the oxidizing reagents, different amounts of HNO_3 (65% subboiling distillation grade) and H_2O_2 (30% "Perhydrol" grade, Merck, Darmstadt, Germany) were employed. A Multiwave® microwave digestion system (Anton Paar, Graz, Austria) housing the quartz vessels was used to heat the blood samples. The exact protocol of the seven trials is given in Table I, whereby trial 1 reflects the standard digestion protocol for organic matrices within our laboratory. After digestion, total organic carbon (TOC) was measured using a TOC analyzer (ELEMENTAR HIGH TOC) with combustion oven and Non Dispersive Infrared Detector (NDIR).

Four chromium recovery experiments of the decomposed samples after digestion protocol 'trial 1' (Table I) were carried out. To demonstrate the general ability of ICP-MS to measure precise metal concentrations in blood (apart from the difficulties of carbon-related interferences for chromium), cobalt, manganese, molybdenum, and lead were also determined. The same samples were prepared for the determination of chromium using GFAAS. Containers were opened under a clean bench after cooling to room temperature. The bench is equipped with a class 10 clean air filter to protect the samples from contamination and the operator from occasionally appearing NO_x. After transferring 4 mL of the clear solution into a 5-mL glass flask, a rhodium solution was added as the internal standard and the volume adjusted with deionized water. For ICP-MS analysis, a PerkinElmer SCIEX ELAN® 6000 quadrupole inductively coupled plasma mass spectrometer was used (PerkinElmer SCIEX, Concord, Ontario, Canada), equipped with a PerkinElmer AS-91 autosampler (PerkinElmer Life Sciences and Analytical Instruments, Shelton, CT, USA). Analysis of chromium was performed on both isotopes ^{52}Cr and ^{53}Cr, which should yield identical results. For GFAAS analysis, a graphite furnace AAS PerkinElmer SIMAA™ 6000, equipped with a PerkinElmer AS-72 autosampler, was used. The detailed protocol is outlined in Table II.

TABLE I
Digestion Protocol Displaying the Amount of Reagents, Power, Microwave Duration, and Temperature for Seven Different Trials
(Note: 5 mL liquid amounts used for every trial.)

Digestion (–)	Sample+Water (mL)	HNO_3 (mL)	H_2O_2 (mL)	Power (Watt)	Duration (min)	Temp. (°C)
1	1.0 + 3.0	1	–	600	15	206
2	1.0 + 2.8	1	0.2	700	15	265
3	1.0 + 2.0	1.8	0.2	700	15	261
4	1.0 + 2.0	1.6	0.4	700	15	259
5	1.0 + 2.0	1.6	0.4	700	25	264
6	1.0 + 1.5	2	0.5	700	25	261
7	1.0 + 1.0	2.5	0.5	700	35	267

Gas chromatography mass spectroscopy (GC-MS) was used to identify the remaining 'organic carbon' in the microwave acid digestion solution. The extraction of the organic molecules was carried out by a batching process using dichloromethane as the agent. The acid solution was neutralized with NaOH. Forty mL of this solution was triple extracted with 2 mL of dichloromethane and evaporated to 0.5 mL prior to measurement. To acquire the total ion chromatogram (TIC), a Hewlett Packard® GC 5890II with MSD 5971A was used and a mass range of 33–550 amu was scanned.

To study the quantitative effects of carbon residues on false chromium readings, a simulation model was chosen. This model utilized potassium hydrogen phthalate $C_8H_5KO_4$ within the nitric acid blank solution as the carbon source. This was done to generate highly purified samples with defined TOC, up to the maximum value determined after blood sample digestion according to Table I. The same equipment as described above was used. By means of ICP-MS the resulting false chromium levels due to polyatomic interferences of ArC^+ were measured utilizing chromium isotopes amu 52 and amu 53. Linear regression analysis was used to identify the relationships between the TOC and false chromium content.

Analytical Instruments

Elemental analyses were carried out with a Quadrupole ICP-MS, using the PerkinElmer SCIEX ELAN® 6000, equipped with a PerkinElmer AS-91 autosampler. Chromium was also measured using a GFAAS PerkinElmer SIMAA 6000, equipped with a PerkinElmer AS-72 autosampler. The carbon measurements were carried out by means of

TABLE II
Cr Measurements Using SIMAA 6000 with THGA and AS-72

GFAAS Conditions:
Element: Cr Wavelength: 357.9 nm Signal Measurement: Peak Area

Matrix Modifier: $Mg(NO_3)_2$

Calibration Standards:
Cr (µg/L): Blank / 0.5 / 1.25 / 2.50 / 3.75 / 5.00

Furnace Conditions:

Step #	Temp (°C)	Ramp Time (s)	Hold Time (s)	Internal Flow (mL/min)	Read
1	130	1	30	250	
2	180	20	90	250	
3	300	5	5	250	
4	1100	5	20	250	
5	2300	0	5	0	x
6	2450	1	3	250	

Cr, Linearity of the curve: 0.9999
Detection limit (DL): 0.08 µg/L
Cr, Limit of determination (considering the dilution): 0.22 µg/L

TABLE IV
Results for Blood (Seronorm™ Trace Elements Whole Blood, µg/L)

Element/Isotope	Reference Value	Control Range	Found (n=4)
^{52}Cr	7.1	no	13.2 – 18.0
^{53}Cr	7.1	no	9.7 – 17.0
^{59}Co	5.2	no	5.7 – 6.3
^{55}Mn	13.4	12.8 – 15.1	12.5 – 13.1
^{98}Mo	5.0	no	5.1 – 6.1
^{208}Pb	401	353 – 443	356 – 375

TOC Analyzer ("High Toc", Elementar). Sample decomposition was performed using the Multiwave microwave digestion. The GC-MS spectra where obtained using a Hewlett Packard GC 5890II with MSD 5971A.

Reagents

Elemental standard stock solutions 1000 mg/L (Merck, Darmstadt, Germany).

Internal Standard solution, 1 mg/L Rh (Merck, Darmstadt, Germany).

H_2O Millipore (Milli-Q, Millipore, Germany).

HNO_3 (65%) subboiling distillation grade.

H_2O_2 (30 %) "Perhydrol" grade, (Merck, Darmstadt, Germany).

Dichloromethane, z.R. Merck (Darmstadt, Germany).

Potassium hydrogen phthalate $C_8H_5KO_4$, p.A. Merck (Darmstadt, Germany).

TABLE III
Remaining Carbon After Microwave Digestion for the Seven Different Trials Displayed in Table I

Digestion Trial (–)	TOC (mg/L)
1	1760
2	1022
3	780
4	587
5	238
6	186
7	127

RESULTS

Carbon residues remained after each digestion protocol. Taking our laboratory standard for microwave digestion, a total carbon content of 1760 mg/L was identified. Even though TOC analysis demonstrated that this content decreases with increasing digestion time, it was observed that after 35 minutes of microwaving and the addition of further oxidizing reagents, there was still 127 mg/L carbon left in the digested sample (Table III).

With respect to the reference value provided, it was found that ICP-MS measurements yielded chromium contents that were too high. Typically, the chromium content was overestimated by a factor of two. Furthermore, the readings for both isotopes ^{52}Cr and ^{53}Cr were not identical. Table IV displays the ranges of measured chromium concentrations versus the reference value. In contrast, the measured range of Co, Mn, Mo, and Pb concentrations met the expected reference value within the expected accuracy of the standard. For two elements, Mn and Pb, the control range was provided. For those two elements, all measured values were well within the control range provided by Seronorm (see Table IV for further details).

TABLE V
GFAAS Results for Reference Blood
(Seronorm™ Trace Elements Whole Blood)

Seronorm	Reference Value (µg/L)	Control Range (µg/L)	Found (n=4) (µg/L)
Cr	7.1	no	7.4 – 8.1

TABLE VI
Chromium Concentration Measurements as a Product of Carbon-related Interferences

$C_8H_5KO_4$ as carbon source TOC (mg/L)	Polyatomic Interferences	^{52}Cr (µg/L)	Polyatomic Interferences	^{53}Cr (µg/L)
100	$^{40}Ar^{12}C^+$	0.26	$^{40}Ar^{13}C^+$	0.011
500	$^{40}Ar^{12}C^+$	0.42	$^{40}Ar^{13}C^+$	0.41
1000	$^{40}Ar^{12}C^+$	0.92	$^{40}Ar^{13}C^+$	1.67
1500	$^{40}Ar^{12}C^+$	1.2	$^{40}Ar^{13}C^+$	2.9

Table V displays the GFAAS results for chromium which were close to the reference value provided.

Gas chromatography mass spectroscopy yielded remarkable results. Theoretically, most organic molecules should have been transformed into CO_2 (or broken down into smaller components). However, nitrified organic molecules were identified, which were not expected to such an extent. Figure 1 shows the total ion chromatogram of digestion protocol 'trial 1'.

Analyzing blank solutions with increasing concentrations of potassium hydrogen phthalate caused increased false chromium readings on 52 and 53 amu (Table VI). For both isotope, a linear relationship between the TOC and false chromium concentration was obtained (adjusted R^2= 0.97; p= 0.01). These relationships, however, did not fully explain the false chromium readings of the blood samples but underestimated the total error.

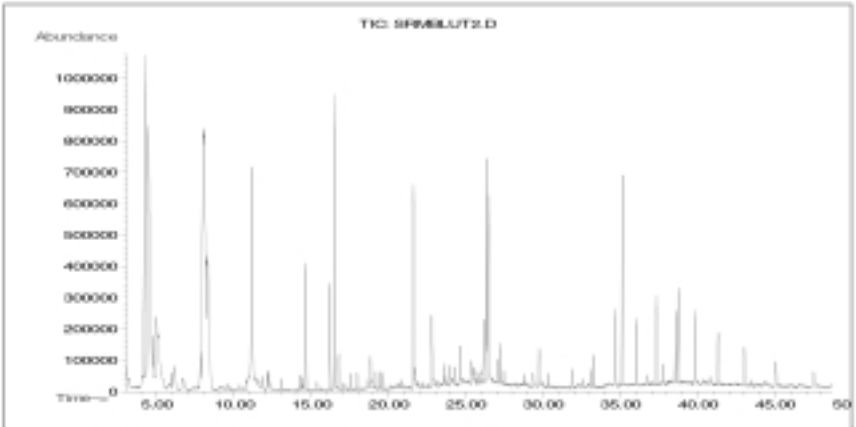
DISCUSSION

The Cr content of body fluids and tissues has emerged as a topic of increasing interest, particularly in individuals with Cr-containing orthopedic implants such as metal-on-metal bearing total hip replacements. In fact, the concentration of Cr in body fluids has been proposed as a marker to monitor the clinical performance of these devices (15). Thus, an accurate and precise methodology is needed to quantify Cr in biological samples.

Biological matrices can present technical challenges in trace metal measurements, particularly when the metal concentrations are in the parts per billion or sub-parts per billion range. Carbon-related interferences are considered to be one of the major problems in the determination of low levels of chromium in blood using the ICP-MS methodology for the determination (13). In addition, whole blood has as much as 106 mmol chloride. Therefore, the formation of chlorine-related interferences expressed as $ClOH^+$ interfere with the major chromium isotope ($^{52}Cr^+$) as well. In this paper, the focus has been on carbon-related interferences and the identification of the molecular structure of the remaining carbon in acid-digested blood samples.

As can be seen in Table IV, the Quadrupole ICP-MS-determined values for Cr in whole blood greatly exceeded (by a factor of approximately two) the reference value. In contrast, GFAAS reproduced the reference value (Table V). Both techniques have comparable detection limits for Cr (~0.2 µg/L). This discrepancy can be attributed to the residual carbon in the analyte. Even the most aggressive digestion protocols used in this study (see Table I) did not completely eliminate this residual carbon (Table III). Using potassium hydrogen phthalate as a carbon source, we have shown that the (false) ^{52}Cr and ^{53}Cr concentrations, as determined by Quadrupole ICP-MS, are a function of the total organic carbon content (Table VI). This strongly suggests polyatomic interferences by $^{40}Ar^{12}C^+$ and $^{40}Ar^{13}C^+$. The elemental correction equation as described in the literature cannot be applied to blood and tissue samples because of the extreme difference of the concentration between analyte (chromium) and matrix (carbon) (16).

Sample preparation techniques, such as microwave digestion, are critical to reduce interferences out of the matrix. Two parameters effect the decomposition: duration and oxidation power of the applied reagents. Thus H_2O_2 was added to reduce organic carbon to a minimum of at least 127 mg/L at a duration of 35 minutes (Table II). There are still carbon-related interferences remaining and the residual H_2O_2 produces a higher variation of the measured isotope signal due to the pulsating effects coming from the H_2O_2 gas bubbles. A long duration of digestion (10 hours) as described in (13) is not suitable for routine analysis.



retention time [min]	Identified molecule
4.17/4.24	**[Aldehydes/Ketones]**
4.97	2 (5H)-Furanone, 5,5-dimethyl-
6.08	[Alcohol]
7.91	**Benzaldehyde, methyl-**
7.99	**Benzaldehyde, methyl- and Benzene, nitro-**
8.22	**Pyridine, nitro-**
11.10	**Benzaldehyde, dimethyl- and [Alcohol]**
12.14	[Carbonic acid]
12.25	Isoquinoline
13.05/17.56	[iso-Alkanes]
14.25	[Benzene derivative]
14.63	[Dinitrobenzene derivative]
16.17/16/46/16.74	**Benzene, dinitro-**
	[Carbonic acids, Alkanes, Phthalate]

Fig. 1. GC-MS analysis. Total Ion Chromatogram (TIC) of a dichloro methane extract of a decomposition solution of a blood sample. Microwave acid digestion with HNO_3.

Based on these findings, quadrupole ICP-MS cannot be recommended as a reliable methodology for the determination of Cr in body fluids. In principle, however, high-resolution sector-field ICP-MS has the capability to separate the chromium isotope 52 from the isobaric interference from $^{40}Ar^{12}C$ as illustrated in reference (17). Low levels of chromium (<2 µg/L) and matrices containing high levels of carbon (>100 mg/L) make it necessary to apply the medium or even high-resolution mode to separate the chromium from the $^{40}Ar^{12}C$. Increasing the mass resolution is an elegant way to avoid the detrimental influence of this interference. The sensitivity decreases by a factor of 10, but the limit of determination is still significantly lower than Quadrupole ICP-MS. Using a medium resolution mode of 4000, a baseline separation of the analyte is accomplished. Further investigations will be necessary to determine whether the remaining carbon after microwave digestion or direct dilution of the biologic samples will result in significant polyatomic interferences in sector-field ICP-MS applied to routine chromium analysis, especially for low levels of chromium in blood and serum (1 ppb and below) (18,19). Another approach is the application of ICP-MS with reaction cells or collision cells (20). As was demonstrated, a Dynamic Reaction Cell™ ICP-MS (DRC™ ICP-MS) was evaluated for the determination of chromium in serum. The dynamic reaction cell consists of a quadrupole, which can be pressurized with a reaction gas. For the determination of chromium in serum, ammonia gas was applied and radio frequency and direct current voltages are applied to the reaction cell quadrupole to form a bandpass, which ejects $^{40}Ar^{12}C$ before entering the analyzer quadrupole. A detection limit of 0.075 µg/L was achieved (21). Considering the dilution of the serum or blood samples,

measurements near the detection limit have to be done in several cases. As for sector-field ICP-MS, the capability of the cell systems has to be demonstrated in the routine chromium determination of serum or blood in the low level region (1 ppb and below).

CONCLUSION

Biological matrices can present technical challenges in trace metal measurements of chromium, particularly when the metal concentrations are in the parts per billion or sub-parts per billion range. Carbon-related interferences are considered to be one of the major problems in the determination of low levels of chromium in blood using the ICP-MS methodology for the determination. In this study, the origin of residual carbon after microwave digestion of whole blood was studied by means of GC-MS. Due to the presence of ArC^+, Quadrupole ICP-MS gives false positive readings on chromium isotopes 52 and 53. An experiment with potassium hydrogen phthalate $C_8H_5KO_4$ as "carbon source" expresses the contribution of ArC^+ to the determination of chromium with Quadrupole ICP-MS. To overcome this problem, sector-field ICP-MS and Quadrupole ICP-MS with reaction cells or collision cells in general offer the alternative besides GFAAS which was applied in this study.

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ICP-MS Multispeciation in Certified Environmental Samples After Microwave-assisted Sequential Extraction

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ABSTRACT

A microwave-assisted sequential extraction of two NIES certified samples viz. standard pond sediment (NIES CRM No. 2) and standard vehicle exhaust particulates (NIES CRM No. 8) was developed prior to the inductively coupled plasma mass spectrometric multispeciation of 14 elements. Al, Zn, Cu, Rb, and Sr have higher environmental mobilities, whereas Ag, Cd, and Mo have the least mobility in the automobile exhaust particulates. Similar analysis of the pond sediment has shown high environmental mobility of V, Ni, Rb, and Cd, whereas Mn, Cu, Pb and Rb are mainly distributed in the residual fractions. The validity of the method was tested by comparison of the summation of individual fractions for each element with the certified values. The developed method can be recommended for routine multispeciation of environmental samples.

NTRODUCTION

The mobility, transport, and partitioning of trace metallic and metalloid species in specified matrices (such as pond sediments) and automobile exhaust, depends on the chemical forms of the element. In order to know the availability and mobility of the metals and to determine their chemical behavior and fate, it is necessary to determine their specific chemical species or different binding forms. Total analysis may give information concerning the possible enrichment of different solid samples with heavy metals, but there is not sufficient criteria to determine their environmental and ecological effects (1–3). Most of the literature on speciation work (4–12) talks about mono- or multi-species of a single element and only some authors discuss sequential determination of multielements (10,13,14).

In order to determine the mobility and bioavailability of these elements, the quantification of their different chemical forms is essential. Several sequential extraction procedures have been developed to determine the trace element mobility in different types of environmental samples. In the Tessier scheme (13), the elements were characterized in the following fractions: exchangeable, carbonate-bound, iron/manganese oxide-bound , organic matter-bound, and residual fractions. The strongly leachable parts of the elements are released in the first stage, i.e., the exchangeable portion. The carbonate-bound portion was found to be highly susceptible to changes in pH, so it was extracted at a low pH. The iron/manganese oxide-bound part is thermodynamically unstable under reducing conditions.

Multielemental analysis is now carried out by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) because this technique is a rapid, robust, and extensive method which has been extensively applied to various samples in recent years (15–21).

In this work, the multispeciation of standard pond sediment (NIES CRM No. 2) and a standard sample of vehicle exhaust particulates (NIES CRM No. 8) was developed using the microwave-assisted sequential extraction procedure in five fractions. The resultant fractions were analyzed for the determination of 14 elements by ICP-MS. The reliability of the method was established by comparison of the experimental results with the total certified values of the NIES standard reference materials.

EXPERIMENTAL

Instrumentation

An ULTRA MASS 700 Inductively Coupled Plasma Mass Spectrometer (Varian, Australia) was employed for ICP-MS analysis using yttrium as an internal standard. The instrumental operating conditions are given in Table I.

A domestic microwave oven (Samsung CE 2933), with a 2450 MHz frequency magnetron and a maximum power of 900 W, was used for the sequential extraction of fly ash samples. PTFE reactors, with 115-mL internal volume, 1-cm cell wall thickness, and hermetically sealed screw caps, were used for the sample containers in the microwave oven.

A GBC Avanta atomic absorption spectrometer was employed for the determination of selected elements. Hollow cathode lamps were used as radiation sources, with resonance lines at 324.7, 213.9, and 279.5 nm for copper, zinc, and manganese respectively. Lamp intensity and slit width were suitably adjusted in accordance with instrumental requirements. A Systronic 362 digital pH meter was used for pH measurements.

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Reagents

All reagents employed were of the highest purity available and at least of analytical reagent grade. All glassware was soaked in 10% (v/v) nitric acid for 24 hours and washed with deionized water prior to use.

Recommended Procedure

About 75 mg of each sample (NIES standards CRM No. 8 vehicle exhaust particulates and NIES CRM No.2-pond sediment) was accurately weighed; and treated subsequently with 5 mL each of 1(M) NH_4OAc (exchangeable fraction), 0.5(M) CH_3COOH (carbonate bound fraction), 0.1(M) $NH_2OH{\cdot}HCl$ (iron/ manganese oxide bound fraction), and 8.8(M) H_2O_2, followed by further extraction with 1(M) NH_2OH (organically bound fraction). The residue was treated first with 1.5 m L of 48% hydrofluoric acid, then with 4 mL of aqua regia, followed by treatment with 4 mL of H_2O_2. Finally the solutions were mixed with 20 mL of saturated boric acid solution, and heated over a hot water bath to remove any excess boric acid. The power (wattage) used and the time required for individual fractions are given in Table II. Determination of the various metals present in the different fractions was carried out by ICP-MS.

RESULTS AND DISCUSSIONS

Multispeciation of Different Elements

In the individual stages of the Tessier extraction scheme, various soluble and insoluble metal species/fractions are formed as a result of the interaction with specific reagents. The fractional distribution of different elements in the sequential extraction processes of automobile exhaust particulates and pond sediment are given in Table III and Table IV. From Table III, it is evident that Al, Zn, Cu, Rb, and Sr are mainly distributed in the early stages of the sequential extractions, (i.e., in the exchangeable and carbonate-bound fractions), which indicates their high environmental mobility compared to the other elements. Some, such as Sb, and Co have been found to be moderately distributed in all fractions. Pb is one of the most abundant elements that has been detected in the automobile exhaust particulates. However, it has been found to be mainly distributed in the latter fractions, showing its low environmental mobility. Similarly, Ag, Cd, and Mo also showed low environmental mobilities in the automobile exhaust particulates.

Analysis of the pond sediments has revealed that the maximum percentage of V, Ni, Rb, and Cd are distributed in the earlier fractions of the extraction, suggesting their high environmental mobilities in pond sediments. Table IV also shows that V, Cu , and Ni exist in their reduced forms. In the second fraction, 0.5(M) AcOH has been used to dissolve the carbonates, and thereby releases the associated trace metals into the solution. Considerable % of Cu, Mn, and Rb were found to be distributed in this fraction. The Fe/Mn oxide-bound fraction is higher in Ni, Fe, Mn, Co, and Cd, which shows their high scavenging capacity for trace metals. The $NH_2OH{\cdot}HCl$ solution was used to dissolve the manganese oxide and amorphous iron oxides in this stage, thus releasing the trace metals co-precipitated in this phase.

Degradation of organic fractions, under the oxidizing conditions causes the release of the organically bound fraction. Considerable amounts of V, Mn, Cu, and Rb are distributed in the organically bound form. On the other hand, Pb and Cd are found to be mainly distributed in the residual phase.

TABLE I
Instrumental Operating Conditions for ICP-MS

Instrumental Parameters		Scanning Parameters	
Plasma Argon Flow	20.01 L/min	Scanning Mode	Segmented Scan
Nebulizer Argon Flow	0.8 L/min	Reading Spacing	0.01 amu
Auxiliary Flow	1.0 L/min	Scans per Replicate	30
Sampling Depth	9.0 mm	No. of Replicates	5
Extraction Lens	–600 V	Sample Delay	30 seconds
First Lens	–220 V	Sample Pump Rate	25 rpm, 5 mL/min
Photon Stop	–13.2 V	Analysis Time	4 min/sample

TABLE II
Microwave Conditions for Sequential Extraction

Fraction	Power (Watt)	Time (min)
Exchangeable	180	54 (6× 9)[a]
Carbonate-bound	180	45 (5×9)[b]
Fe/Mn oxide-bound	180	45 (5×9)[b]
Organically bound	300	35 (5×7)[c]
Residual	450	14(5+5+4)[d]

[a] 6 minutes, 9 times; [b] 5 minutes, 9 times;
[c] 5 minutes 7 times; [d] 5 minutes + 5 minutes + 4 minutes.

TABLE III
ICP-MS Analysis of Sequential Extraction of Certified Automobile Exhaust into Various Fractions

Element (Conc.)[a]	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5[b]
Al (%)	1543.2±7.67	491.30±4.72	1148.00±11.08	nd	nd
V (ppm)	3.36±0.02	0.79±0.01	2.99±0.02	2.07±0.03	6.86±0.17
Cr (ppm)	12.15±0.01	0.66±0.02	1.02±0.01	1.79±0.02	6.11±0.01
Ni (%)	7.07±0.02	3.46±0.10	17.20±0.07	1.66±0.02	4.82±0.01
Co (ppm)	5.54±0.05	0.36±0.01	3.20±0.17	3.59±0.04	9.74±0.08
Cu (ppm)	13.07±0.46	10.65±0.29	24.20±0.32	7.01±0.08	9.98±0.02
Zn (ppm)	292.83±1.07	107.54±1.02	142.46±1.91	61.59±1.32	359.5±3.03
As (ppm)	0.29±0.002	0.57±0.01	0.38±0.01	0.36±0.01	0.74±0.01
Rb (ppm)	1.55±0.10	0.51±0.01	0.62±0.02	0.69±0.02	1.07±0.04
Sr (ppm)	27.39±0.02	7.37±0.11	17.17±1.02	8.56±0.12	22.04±0.91
Mo (ppm)	0.57±0.02	0.89±0.01	0.24±0.01	0.75±0.01	2.83±0.05
Ag (ppm)	nd	nd	nd	0.05±0.01	0.09±0.01
Cd (ppm)	nd	0.35±0.01	nd	0.12±0.01	0.43±0.02
Sb (ppm)	0.65±0.01	1.33±0.08	0.67±0.02	0.74± 0.01	2.27±0.01
Pb (ppm)	18.71±0.06	23.06±1.01	21.17±0.92	34.25±1.05	106.03±1.10

[a] Mean of triplicate analysis.
[b] Si was totally removed from Fraction 5 by HF treatment.

TABLE IV
ICP-MS Analysis of Sequential Extraction of Certified Pond Sediment into Various Fractions

Element (Conc.)[a]	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5[b]
Al (%)	1.36±0.03	1.67±0.02	nd	1.72±0.02	4.56±0.02
V (ppm)	63.98±1.02	86.03±1.01	37.41±0.02	32.03±1.01	6.86±0.07
Cr (ppm)	12.55±0.91	13.86±0.04	10.73±1.01	2.46±0.04	32.76±1.01
Mn (ppm)	71.64±0.62	66.08±1.81	75.22±1.57	87.06±2.52	446.08±9.75
Fe (%)	0.04±0.01	0.03±0.01	0.06±0.01	0.08±0.01	0.12±0.01
Ni (ppm)	7.07±0.04	3.46±0.01	17.2±1.01	1.66±0.01	8.82±0.12
Co (ppm)	5.54±0.01	0.36±0.02	3.20±0.01	3.59±0.01	9.74±0.13
Cu (ppm)	27.67±0.02	32.06±0.01	16.59±0.09	31.52±0.06	85.39±2.01
As (ppm)	2.71±0.01	0.49±0.01	0.08±0.01	3.18±0.01	4.82±0.01
Rb (ppm)	8.09±0.02	13.93±0.01	3.67±0.01	4.20±0.02	11.37±0.11
Cd (ppm)	nd	0.07±0.01	0.29±0.01	nd	0.37±0.01
Sb (ppm)	0.67±0.01	0.23±0.01	nd	nd	0.66±0.01
Pb (ppm)	15.51±0.41	11.17±0.36	7.93±0.08	10.11±1.05	56.24±1.67

[a] Mean of triplicate analysis.
[a] Si was totally removed from Fraction 5 by HF treatment.

Elements bound to these fractions are not expected to be released under natural conditions because they contain silicates, resistant sulphides, and refractory materials.

The distribution pattern of typical elemental species of V, Cr, Ni, Co, Cu, As, Rb, and Pb present in two certified samples are illustrated in Figures 1 and 2. An examination of the above figures reveal that the distribution of chemical species of any element depends not only on the matrix but also on the nature of the element itself.

CONCLUSION

The NIES certified samples of pond sediment and automobile exhaust have been sequentially extracted by using microwave irradiation and the speciation results of 14 elements by ICP-MS have been interpreted. The environmental mobilities of these elements have been successfully determined, and it was found that Al, Zn, Cu, Rb, and Sr have higher environmental mobilities, whereas Ag, Cd ,and Mo have the lowest mobilities in automobile exhaust particulates. Pb is one of the most abundant elements and a potential contaminant which has been detected in automobile exhaust particulates. However, Pb has been found to be mainly distributed in the latter fractions, showing its low environmental mobility. Similar analysis of the pond sediment has shown high environmental mobilities of V, Ni, Rb, and Cd, whereas Mn, Cu, Pb, and Rb are mainly distributed in the residual fraction;, hence they are less mobile. Therefore it can be concluded that the use of microwave-assisted sequential extraction coupled with ICP MS can be developed conveniently for multispeciation in various environmental matrices.

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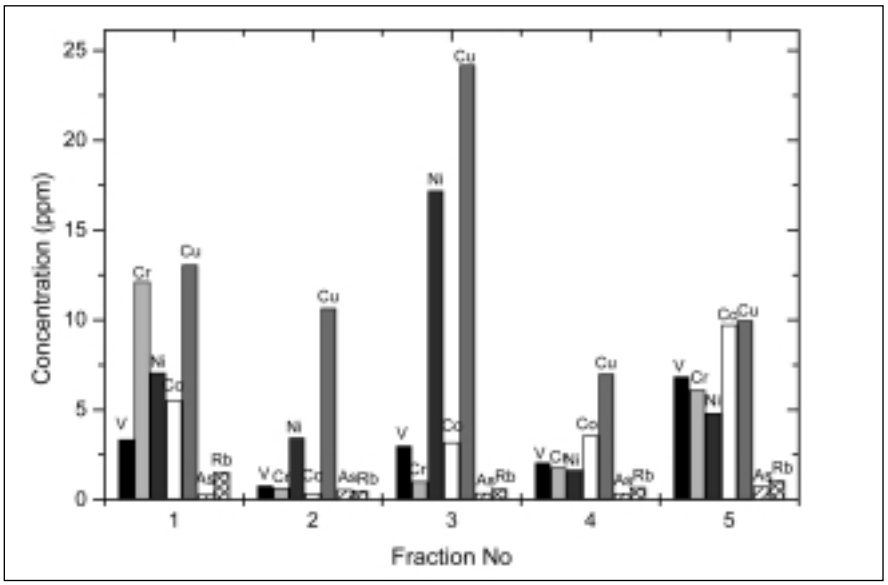

Fig. 1. Distribution of some typical metal species of certified automobile exhaust in different fractions.

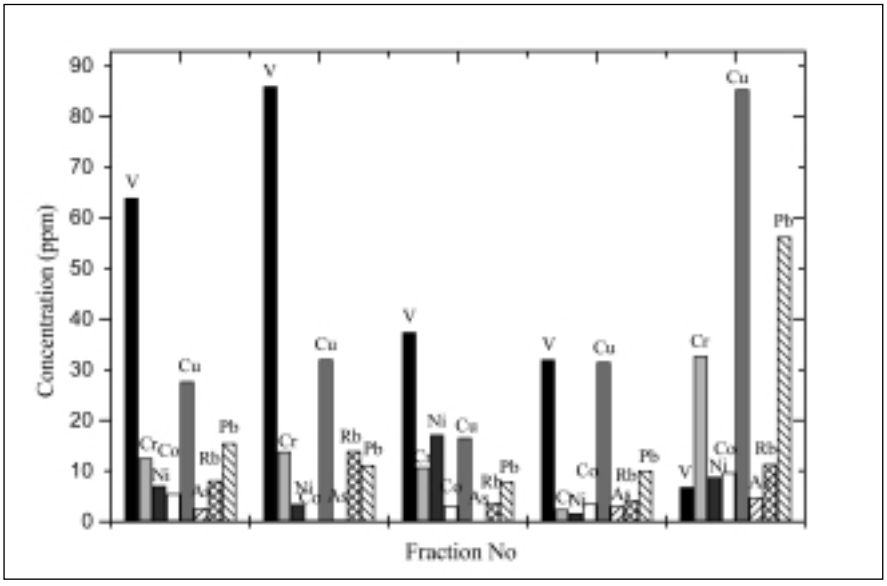

Fig. 2. Distribution of some typical metal species of certified pond sediment in different fractions.

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Evaluation of ICP-OES Applicability for Trace Element Determination in Environmental Samples

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ABSTRACT

This study deals with the application of current ICP-OES instrumentation for trace element determination in environmental samples. The concentric (Meinhard® type), cross-flow, GemCone™, and ultrasonic nebulizers as well as the cyclonic and Scott spray chambers are compared in terms of limits of detection (LODs) and plasma robustness. Under optimized conditions, LODs, in ng mL^{-1}, of As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn, in a 5% (v/v) HNO_3 aqueous medium, ranged from 1.1–16; 0.002–0.32; 0.03–1.2; 0.02–0.72; 0.03–0.82; 0.04–3.0; 0.003–0.76; 0.08–3.8; 0.22–8.9; 0.04–2.6; and 0.02–1.2, respectively, in the axially viewed plasma.

In the radially viewed plasma, the LODs, in ng mL^{-1}, for As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn ranged from 10–87; 0.01–0.91; 0.07–3.8; 0.16–4.3; 0.13–8.1; 0.16–4.3; 0.01–0.81; 0.43–7.6; 1.4–37; 0.28–6.0; and 0.77–9.5, respectively.

Methodology optimization is described and certified samples of apple leaves, water, and marine sediment as well as real samples of water, marine sediment, plant leaves, and pine needles are analyzed. In each sample, most analytes were quantified using external calibration.

INTRODUCTION

Since the early days of inductively coupled plasma optical emission spectrometry (ICP-OES), a number of improvements in the instrumentation have been made. The enhancement in sensitivity, achieved with the more recent axially viewed plasma, typically yields a 5–10-fold improvement in detection limits in relation to the radial view. Furthermore, some instruments allow both radial and axial viewing (dual-view concept), improving versatility. When using the axial view, the sensitivity is improved but matrix effects are more pronounced in comparison to the radial view. However, the matrix effects may be minimized by using robust plasma (where there is less excitation interference), such as in high RF power and low nebulizer gas flow rates (1–3). The new solid-state detectors [based on charge-coupled device (CCD) detector technology]) have good quantum efficiency, enhancing analytical performance and superior limits of detection (LODs). Other current features are simultaneous background correction and dynamic wavelength stabilization, which improve reproducibility and accuracy (4). Due to the versatility and productivity of the ICP-OES, it is used in many different applications and, nowadays, carries the workload in many laboratories.

Liquid samples, the most common means for introducing samples into plasmas, are generally dispersed into fine aerosols before being introduced into the ICP where analytical performance indices are directly affected by the quality of the aerosol generated. Pneumatic nebulizers (PNs) are easy to use but the sample transport efficiency is low (2–5%), which diminishes the sensitivity. Taking into account the sample type, the sample amount available and the target limits of detection (LODs), different nebulizers, mainly concentric (Meinhard®) nebulizers, constructed from different materials, have been developed and characterized. Concentric nebulizers were more frequently used for ICP spectrometry than devices of cross-flow design because adjustment of the gas and liquid capillaries is not crucial for nebulization quality (5). However, in the newest cross-flow nebulizers, the gas and liquid capillaries are fixed and consequently this nebulizer is now also intensely used for routine application. When spraying solutions that have high concentrations of solids, cross-flow nebulizers are generally less prone to salt buildup at the capillary tip and thus less prone to clogging. Special concentric nebulizers, either optimized for low sample consumption (6) or made with inert materials to be used in HF medium, have also been constructed (4,5).

The nebulizers above mentioned tend to clog at higher concentrations of dissolved solids. Particles floating in the sample solution (6,7) may also block the sample orifice. In view of these facts, different nebulizer types have been developed specifically for highly dissolved solid content and small particles in sample solutions (8–11). In ultrasonic nebulizer (USN) aerosol generation, extremely fine droplets are produced and the quantity of analyte transported to the plasma is increased in comparison to PN. Analyte transport efficiency is around 20% (8) when USN is used and sensitivity typically increases by a factor of about 10. Therefore, the solvent (mainly water) is partially removed (desolvation), affecting plasma conditions (4,5). In

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ICP-OES, water removal has a disadvantageous effect, since water vapor increases electron density and maintains constant excitation conditions in the plasma (12). In addition, by using USN, some types of samples may influence aerosol formation (13,14) and sensitivity, deteriorating precision and accuracy.

Scott and cyclonic spray chamber types are mostly used for removing the large droplets from the aerosol stream. It is reported that the cyclonic chamber raises the sensitivity (15) by about 50% and LODs are reduced by up to a factor of 3 when nebulizers with low sample consumption are used.

Matrix effects in ICP-OES are linked to the sample introduction system and the aerosol path to the plasma. Thus, the conditions used for ICP-OES operation and the emission measurements are important for any ICP-OES methodology. Sample analysis may require deviation from the standard operating conditions as, for instance, when analyte concentration is near the LOD and when there is a high salt and/or acid concentration in the sample.

The main purpose of this study is to investigate the applicability of a dual-view CCD detector ICP-OES for trace element (As, Cr, Cu, Ni, Pb, V, Co, Cd, Zn, Mn, and Ba) determination in environmental samples by applying the nebulizers and spray chambers commonly used.

EXPERIMENTAL

Intrumentation

Emission signal measurements were performed with an Optima™ 2000 DV ICP-OES instrument (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) based on both axial and radial viewing modes. In this instrument, both a prism (Littrow) and a grating (Echelle) are used as cross-dispersing media, while CCD is used as the detector. The PerkinElmer WinLab32™ software was used throughout this work. The instrumental operating conditions used are listed in Table I and were optimized or used as recommended by the instrument manufacturer. The USN 5000 AT$^+$ from CETAC (Omaha, Nebraska, USA), GemCone, cross-flow, and concentric nebulizers were utilized.

The concentric nebulizer was fitted either to a standard cyclonic or Scott spray chamber. The cross-flow and GemCone™ nebulizers were fitted only to a Scott spray chamber since we did not have the necessary devices to adapt them to the cyclonic spray chamber.

The following spectral lines were monitored: As(I), 188.979 nm; Co(II), 231,161 nm; Cr(II), 267.716 nm; Ni(II), 231.604 nm; Pb(II), 220.353 nm; V(II), 292.464 nm; Cd(II), 228.802 nm; Zn(I), 213.857 nm; Mn(II), 257.610 nm; and Ba(II), 455.403 nm. These emission lines were chosen according to the literature (4) or as suggested by the instrument manufacturer.

Argon 99.996 (White Martins-Praxair, SP, Brazil) was used for plasma generation as the nebulizer and as the auxiliary gas. Compressed air was used as shear gas, while N_2 5.0 (White Martins-Praxair) was used for purging the spectrometer optical system.

Chemical Reagents Solutions and Materials

For sample decomposition, either a microwave oven (Multiwave® 3000 from Anton Paar, Graz, Austria), equipped with quartz vessels, or a digestion block (Tecnal, Piracicaba, SP/Brazil) with Teflon®-capped vessels was employed.

All reagents used were of analytical grade. The acids 65% (v/v) HNO_3, 37% (v/v) HCl, 40% (v/v) HF, and 30% (v/v) H_2O_2 (from

TABLE I
Instrumental Operating Conditions

Plasma power	1300–1500 W
Plasma flow	15 L min^{-1}
Auxiliary gas flow[a]	0.1–0.4 L min^{-1}
Nebulizer gas flow[a]	0.5–0.8 L min^{-1}
Nitrogen purge	Normal
Height of observation	15 mm or 16 mm (above load coil)
	16 mm for Ni, V, Zn, and Cr in ultrasonic nebulization and radial view
Injector	Alumina (2 mm i.d.)
Spray chamber	Scott (Ryton®); cyclonic
Processing mode	Area (7 points per peak)
	3 points for Co and Cd
Resolution	High
Integration time	Auto, min 2.5 sec; max 5 sec
Sample flow	1.2 mL min^{-1}
	2.5 mL min^{-1} (ultrasonic nebulizer)
Replicates	2
Background correction	2 points

[a] Optimized according to nebulizer and spray chamber (see Figures 1 and 2).

Merck, Darmstadt, Germany) were used. HNO_3 and HCl, doubly distilled in sub-boiling quartz steam (Hans Kürner, Rosenheim, Germany) were used for sample decomposition and calibration solutions. Milli-Q™ water (Millipore Corporation, Bedford, MA, USA), resistivity of 18.2 MΩ cm, was used for all solutions and in the sample preparations. Calibration solutions were prepared by appropriate dilution of 1000 mg L^{-1} single-element stock solutions (Titrisol®, Merck) with HNO_3 solution. The HNO_3 concentration in the calibration solutions was varied in order to match that of the sample solution. Solutions of Sc, Ge, and Ga, used as the internal standards, were prepared from SPEX reagents (Matuchen, NJ, USA).

All glassware and labware used to store the solutions and samples were decontaminated by cleaning with a 10% (v/v) HNO_3 solution for 72 h. After immersion in the acid solution, all materials were thoroughly washed with distilled water and rinsed with Milli-Q water.

Sample Preparation

Certified marine sediment (PACS 2 from NRCC - National Research Council of Canada), water (NIST 1640 - National Institute of Standard and Technology, Gaithersburg, MD, USA), and Apple Leaves (NIST 1515) samples were analyzed. A real marine sediment sample (MD), collected and prepared as described elsewhere, was also analyzed (16). The marine sediment samples were solubilized according to the following procedure: 0.200 g of sample was placed into a PTFE vessel, followed by the addition of acid (7 mL HNO_3+ 6 mL HF + 3 mL HCl) (16). Subsequently, the flask was capped and placed on a digestion block at a temperature of 160ºC for 9 h. Then, the digestion block was turned off and the solution left standing to reach room temperature. Finally, the flask was opened, the sample solution transferred to a graduated polyethylene flask, which was then filled to a volume of 50 mL. In the real marine sediment sample solution, As, Cd, Co, Cu, and Pb were determined directly without further sample dilution, whereas a 20-fold dilution was employed for the determination of Ni, V, Zn, Mn, Cr, and Ba. In the certified marine sediment sample solution, a 20-fold dilution was also employed for the determination of Ni, Cd, V, Zn, Mn, Cr, Ba, Cu, and Pb, whereas Co, Cd, and As were determined directly.

The apple leaves sample was decomposed in the microwave oven during 30 min at 1400 W by using 0.200 g of sample placed in a quartz vessel to which 6 mL of HNO_3 and 0.2 mL of H_2O_2 were added. The attained apple leaves solution was diluted with water in a volumetric 50-mL flask. Non-certified bamboo leave, pine needle, and eucalyptus leave samples, collected from vegetation located in an industrial area, were analyzed. After being dried at room temperature in a clean room, they were properly ground in an agate mortar and then decomposed in the microwave oven using the same procedure as used for decomposing the apple leave sample. Black tea, placed in sachets used as a beverage, was extracted with boiling water in a decontaminated glass vessel. The obtained solution was cooled at room temperature and then transferred to a 25-mL or 50-mL volumetric flask containing HNO_3 in an attempt to reach a final 1% (v/v) HNO_3 concentration. Analyte determination in this sample solution was immediately performed in order to avoid some precipitation of tannic compounds.

Rainwater and groundwater (from a 90-m deep well) were sampled in a decontaminated polyethylene 500-mL flask containing 1 mL of HNO_3. The collected samples were then transferred to volumetric flasks and the acid concentration adjusted to 5% (v/v). Tap water was sampled directly in a 50-mL volumetric flask containing 2.5 mL of HNO_3.

Whenever necessary, the sample solutions were properly diluted. Matrix matching with HNO_3 was always performed. All samples as well as blanks were analyzed at least in triplicate.

RESULTS AND DISCUSSION

Instrumental Parameter Optimization

Initially, parameters such as plasma power and nebulizer and auxiliary gas flow rates were optimized for each nebulizer and spray chamber when using the axially viewed plasma. In Figure 1, the responses are shown with respect to plasma power and nebulizer gas flow rate. In this figure, the analytes are grouped according to their similar behavior found under the investigated conditions. For each analyte measurement, every nebulizer gas flow rate was fixed while the plasma power was varied from 1000 to 1500 W. In Figure 1a, we can observe that the highest As, Co, Cd, Pb, V, Ba, Cr, Ni, Cu, and Mn emission intensities are achieved when the nebulizer gas flow rate is 0.6 L min^{-1} and the plasma power is 1500 W by using the concentric nebulizer fitted to the cyclonic spray chamber. On the other hand, lower emission intensities of the analytes are measured if the nebulizer gas flow rate is as low as 0.3 L min^{-1} in all investigated plasma power conditions. The exception is Zn, shown separately in Figure 1b where the optimum nebulizer gas flow rate is 0.5 L min^{-1}. In addition, the behavior of Zn is also different in relation to the other investigated nebulizer gas flow rates, being the lowest emission intensities measured at 0.3 and 0.9 L min^{-1}. Figures 1c and 1d

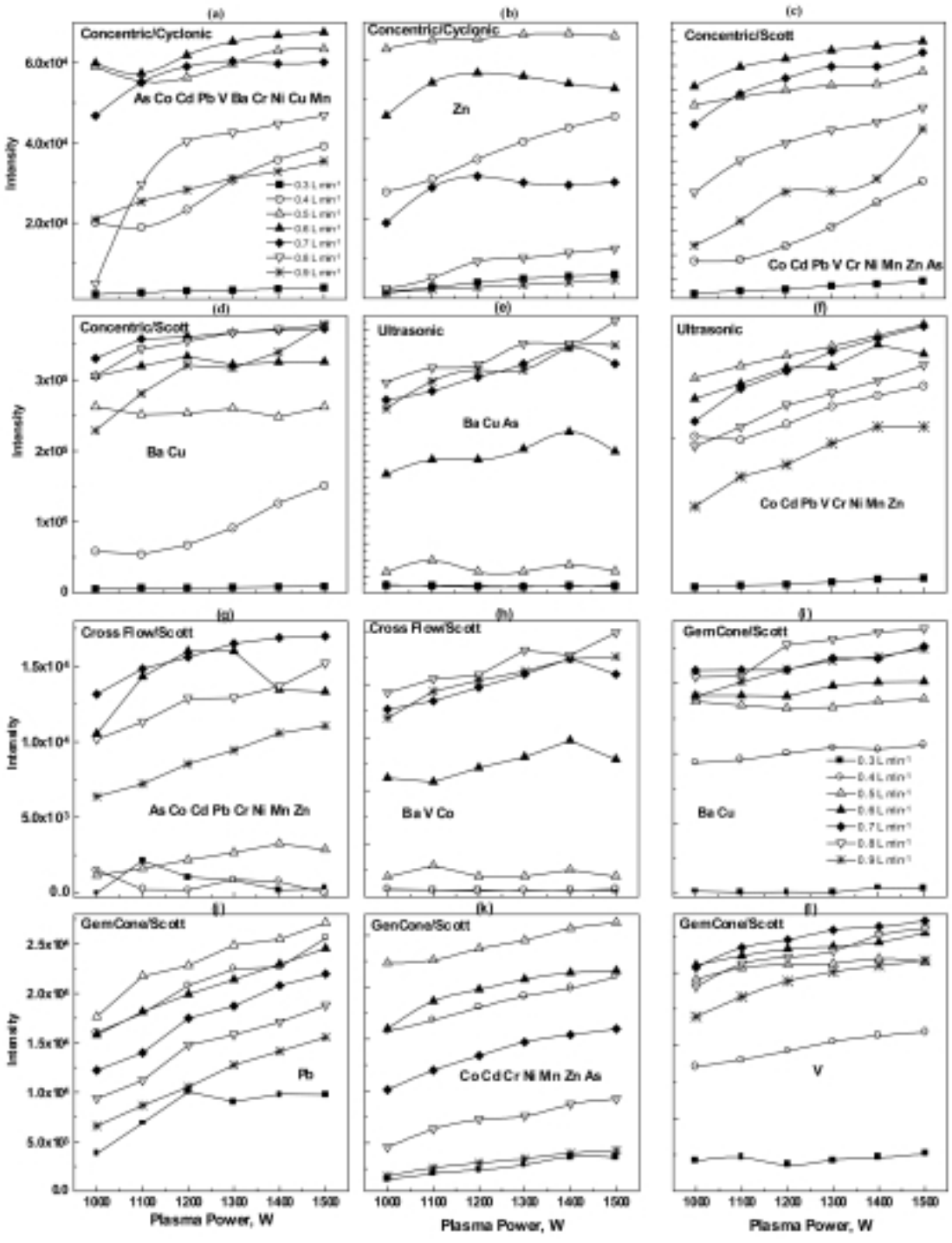

Fig. 1. Optimization of nebulizer gas flow rate and plasma power; observation height: 15 mm; plasma view: axial and auxiliary gas flow rate: 200 mL min^{-1}. Analytes in different concentrations (10.0 to 50.0 µg L^{-1} in pneumatic nebulization and 1.00 to 5.00 L^{-1} in ultrasonic nebulization) are in 5% (v/v) HNO_3. The legend for each nebulizer gas flow rate is depicted in (a); it is the same for all nebulizers and spray chambers.

depict the analyte behavior when the concentric nebulizer is fitted to the Scott spray chamber. In this case, the optimum nebulizer gas flow rate is also 0.6 L min^{-1}, but now the exceptions are Ba and Cu as shown separately in Figure 1d. For Ba and Cu, higher nebulizer gas flow rates (ranging from 0.7 to 0.9 L min^{-1}) give the highest emission intensities at 1500 W. By using the USN nebulizer, Figure 1e shows that at a power of 1500 W, the Ba, Cu, and As signals are higher when the nebulizer gas is 0.8 L min^{-1}. On the other hand, Figure 1f shows that at 1500 W, the signals for Co, Cd, Pb, V, Cr, Ni, Mn, and Zn are higher when the nebulizer gas is 0.5 L min^{-1} or 0.7 L min^{-1}. The behavior of the analytes with the cross-flow nebulizer/Scott spray chamber system as shown in Figures 1g and 1h demonstrate that a nebulizer gas flow rate of 0.7 L min^{-1} is the optimum for As, Co, Cd, Pb, Cr, Ni, Mn, and Zn (Figure 1g), while for Ba, V, and Co (Figure 1h) the optimum is 0.8 L min^{-1}. More exceptions are found when the GemCone nebulizer is used, as is shown in Figures 1 (i), (j), (k), and (l). In this case, the best nebulizer gas flow rate is 0.8 L min^{-1} for Ba and Cu; 0.5 L min^{-1} for Pb, Co, Cd, Cr, Ni, Mn, Zn and As; and 0.7 L min^{-1} for V. Lead is not grouped in Figure 1k and is shown separately in Figure 1j which is due to its different behavior in relation to the other nebulizer gas flow rates investigated. In Figure 1 it is possible to note that in almost all investigated conditions the signals increase with the increase of the plasma power and the lowest emission intensities are observed when the nebulizer gas flow rate is too low. As expected, the optimum nebulizer gas flow rate depends on the nebulizer and spray chamber employed, while the influence of the plasma power is similar in almost all conditions. If the nebulizer gas flow rate is chosen taking into account the highest sensitivity, then the plasma power can be set at 1500 W when the investigated elements are in 5% (v/v) HNO_3.

Figure 2 shows the influence of the auxiliary gas flow rate. For each element, the signals were normalized by dividing the emission intensities measured under the different conditions (at 100, 200, 300, 400, and 500 mL min^{-1}) to the highest signal of the analyte. In this study, the nebulizer gas flow rate and the plasma power were chosen and set according to Figure 1, and then the auxiliary gas flow rate was varied from 100 mL min^{-1} to 500 mL min^{-1} by using the different nebulizers and spray chambers investigated in the

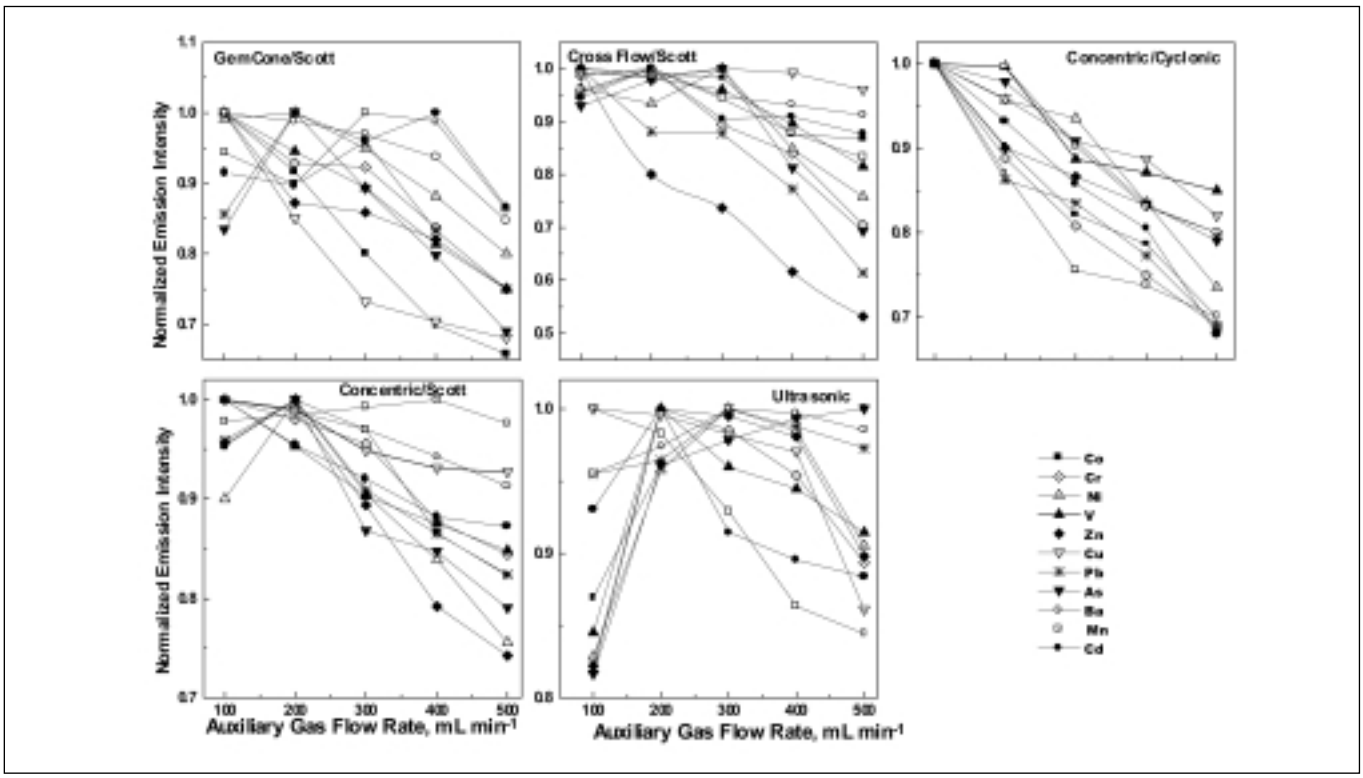

Fig. 2. Optimization of auxiliary gas flow rate; observation height: 15 mm; plasma view: axial; nebulizer gas flow rate and plasma power were set according to Figure 1. Analytes in different concentrations (10.0 to 50.0 µg L^{-1} in pneumatic nebulization and 1.00 to 5.00 L^{-1} in ultrasonic nebulization are in 5% (v/v) HNO_3.

present work. Figure 2 shows that the highest sensitivity is achieved with a flow rate of 100 mL min^{-1} or 200 mL min^{-1} in almost all cases. Remarkable exceptions are Cd and Mn measured by using the GemCone nebulizer and Cu and As by using the cross-flow nebulizer. In these cases, the auxiliary gas set at 300 mL min^{-1} gives the highest sensitivity. With respect to the ultrasonic nebulizer, we can observe that a larger range of auxiliary gas flow rates can be applied for most analytes and a nebulizer flow rate as high as 500 mL min^{-1} can be used for As and Co.

According to Figures 1 and 2, considering the highest emission intensities, the optimized parameters (nebulizer and auxiliary gas flow rates and plasma power) were established individually for each element through the software of the instrument. The above investigated parameters were also studied in radial plasma view (not shown in the text) but no important differences were observed. Hence, the same plasma power, nebulizer, and auxiliary gas flow rates adopted for the axial view were used in the radial view. In addition, the observation height was optimized (not shown in the text) and it was set as 15 mm (with the exception of Ni, V, Zn, and Cr) when using ultrasonic nebulization and radially viewed plasma. In this case, the Ni, V, Zn, and Cr emission intensities were remarkably higher at an observation height of 16 mm and this height was used for further measurements of these elements.

Analytical Parameters

The established parameters discussed previously were checked for precision and accuracy by analyzing the certified water sample (NIST 1640). The results were within the 95% confidence level of the certificate and the RSD was <2%. Next, the LODs (3 σ of 10 consecutive blank measurements) of the analytes in HNO_3 5 % (v/v) were calculated. As expected, the lowest LODs were achieved using the ultrasonic nebulizer (see Table II). With regard to the GemCone, cross-flow, and concentric nebulizers, we can see that the best LODs found were with the concentric nebulizer fitted to the cyclonic spray chamber. In Table II we can also observe that the LODs attained by using the axial view were notably lower than with the radial view in each of the examined conditions. The LODs shown in Table II are lower than others reported earlier for instrumentation available, for example, in the 1980's (4,17,18).

Plasma robustness (for the axially viewed plasma) in the optimized conditions was quantified by measuring the intensity ratio of atomic to ionic Mg lines. The atomic emission line was at 285.13 nm and the ionic emission line at 280.271 nm. The following Mg(I)/Mg(II) ratios were measured: 9.8, 14.1, 12.3, 14.0, and 14.2 for the ultrasonic, concentric/Scott, concentric/cyclonic, cross-flow and, GemCone nebulizers, respectively. Plasma is considered robust if the magnesium intensity ratio is 10 or greater (4). Thus, the plasma is robust under the investigated conditions, except in the presence of aerosol produced by the ultrasonic nebulizer. The lower plasma robustness in the employment of the ultrasonic nebulizer may be due to the higher amount of sample introduced into the plasma and as also due to the desolvation which degrades the excitation conditions.

Analyte Determination in the Samples

Initially, the analyte determinations were carried out in marine sediments whose sample matrix solution content was high in acids [14% (v/v) HNO_3 + 12% (v/v) HF + 6% (v/v) HCl] and in dissolved solids. In this case, the GemCone nebulizer (fitted to a Scott spray chamber) was employed due to the HF presence and high dissolved solids concentration. Since acids may affect aerosol formation (19), the analyte LODs in the sample blank were calculated a second time. In Table II, we can observe that the most remarkable differences are related to Ni, Cu, V, As, and Mn. However, it was verified that interference was mainly due to the sample matrix that remains in the spray chamber because the RSD for 10 consecutive runs of the sample blank, spiked with 20 mg L^{-1} of Cd and Co, was lower than 3%. In the case of the sample solution, the analyte signal increased remarkably after the 3rd run, even by using the radial view mode. However, the signal was at the expected level by washing the spray chamber with 5% (v/v) HNO_3 for at least 60 s between each run, or by diluting the sample solution 10 times. Thus, it was concluded that whenever possible the sample should be diluted at least 10 times or the spray chamber should be washed for 60 s between each sample run. However, for As detection and quantification in the marine sediment sample solution, this solution cannot be diluted. When the solution is diluted, the As concentration is below the detection limit. This may also occur with respect to the other investigated elements whose concentrations are low in real marine sediment samples. In the present work, washing of the spray chamber was the approach used for some element determinations, such as As, Co, Cu, Cd, and Pb in the real marine sediment and Co, As, and Cd in the certified sample. Internal standards (IS) were also tested, but they were not satisfactory due to the complexity of the sample matrix, which may affect each element differently. Among the IS tested, Ge and Sc were not effective at all, whereas

TABLE II
Limits of Detection (ng mL^{-1}) in 5% (v/v) HNO_3 as a Function of Different Nebulizers and Spray Chambers

Nebulizer/ Spray Chamber	Elements										
	Cr	Ni	Zn	Cu	Pb	V	As	Co	Cd	Ba	Mn
Ultrasonic Axial	0.03	0.08	0.02	0.04	0.22	0.04	1.1	0.02	0.03	0.002	0.003
Ultrasonic Radial	0.13	0.43	0.77	0.16	1.4	0.28	10	0.16	0.07	0.01	0.01
Concentric Axial/Scott	0.65	0.84	0.66	0.44	5.2	0.71	14	0.56	0.89	0.02	0.03
Concentric Radial/Scott	1.6	5.2	2.4	0.86	14	2.6	23	2.5	1.0	0.12	0.11
Concentric Axial/Cyclonic	0.33	0.75	0.55	0.41	1.5	1.4	8.2	0.41	0.22	0.02	0.05
Concentric Radial/Cyclonic	1.4	4.6	1.6	2.4	8.8	3.3	26	1.1	0.63	0.14	0.13
Cross-flow[a] Axial	0.43	2.6	0.85	0.52	3.9	1.7	16	0.52	1.2	0.02	0.05
Cross-flow[a] Radial	8.1	7.6	4.2	2.7	14	4.0	7.3	3.2	2.5	0.15	0.37
GemCone[a] Radial	4.8	14	9.5	4.3	37	6.0	87	4.3	3.8	0.91	0.81
GemCone[a] Axial	0.82	3.8	1.2	3.0	8.9	2.6	14	0.72	0.80	0.32	0.76
GemCone[a] Axial[b]	1.1	2.3	1.3	1.0	10	3.8	26	0.72	1.2	0.31	0.11

[a] Fitted to Scott spray chamber. [b] Elements in 14% (v/v) HNO_3 + 12% (v/v) HF + 6% (v/v) HCl.

TABLE III
Influence of Parameters on Cd and Co Determination in Marine Sediment (PACS-2)
(The certified values of Co and Cd are 11.5 ± 0.3 µg g^{-1} and 2.11 ± 0.15 µg g^{-1}, respectively.)

Co (µg g^{-1})	1300 W/Axial[a] Peak Area	1500 W/Axial[a] Peak Area	1300 W/Axial[a] Peak Height	1300 W/ Radial[a] Peak Height
Without spray chamber washing	10.9 ± 0.98	15.6 ± 2.8	11.8 ± 2.8	8.78 ± 1.10
RSD (%)	8.99	17.9	23.7	12.6
Spray chamber washing for 60 s	11.5 ± 0.45	10.1 ± 0.10	10.8 ± 0.13	8.41 ± 0.32
RSD (%)	3.8	0.98	1.2	3.8
Internal Standard:				
Ga 417.206	9.5 ± 0.61	–	–	–
RSD (%)	6.3	–	–	–

Cd (µg g^{-1})	1500 W/Axial[a] 0.6 L min^{-1} 3 points/peak	1500 W/Axial[a] 0.8 L min^{-1} 3 points/peak	1300 W/Axial[b] 0.6 L min^{-1}		
			Peak Area 3 points/peak	Peak Area 7 points/peak	Peak Height 1 point/peak
Without spray chamber washing	2.36 ± 0.38	2.74 ± 0.66	–	–	–
RSD (%)	16	24			
Spray Chamber Washing for 60s	1.98 ± 0.07	2.42 ± 0.03	2.05 ± 0.12	2.53 ± 0.08	2.56 ± 0.08
RSD (%)	3.5	1.2	5.8	3.2	3.1
Internal Standard:					
Ga 417.206	2.18 ± 0.09	–	–	–	–
RSD (%)	4.1	–	–	–	–

The nebulizer gas flow rate for Co was 0.6 L min^{-1}.
3 points/peak was used for Co determination. [a]: 5 consecutive runs; [b]: 3 consecutive runs.

Ga measured at 417.206 nm was effective for Cd and Co (see Table III). However, the accuracy for Co was poorest when the IS was employed.

As reported (16,20), arsenic may cause spectral interference on Cd measured at 228.802 nm. In the present work, alternative Cd emission lines could not be used due either to Fe spectral interference (at 226.502 nm) or to low emission intensity (at 214.38 nm). But, as shown in Table III, spectral interference is overcome when Cd is measured in peak area with only 3 points/peak instead of 7 points/peak. There are improvements in precision and accuracy of the Cd and Co results when the spray chamber is washed or/and a lower nebulizer gas flow rate is employed. Besides, with respect to the Co determination, considering the results shown in Table III, the use of peak area mode at 1300 W is recommended. Regarding As, not shown in Table III, only the axial view could be used as this analyte was not detected in the radially viewed plasma. In this case, accurate As results were attained using 7 points/peak in the optimized conditions, but the spray chamber needed to be washed as for the Cd and Co determinations. As previously mentioned, Pb and Cu were also directly determined in the real marine sediment sample solution. Good results were obtained if the spray chamber was washed and the radial view was used.

Regarding the other samples shown in Table IV, the nebulizers and plasma view were selected according to the expected analyte concentrations. For analyte determination in water and tea samples, the ultrasonic nebulizer was the first choice. If the analyte concentration was high in the sample, then the cross-flow or the concentric nebulizer was employed. In the case of the vegetal samples, the GemCone nebulizer was the first choice since it is less prone to clogging. When the analyte could not be quantified using the GemCone nebulizer, then the concentric nebulizer fitted to the cyclonic spray chamber was used. As a last resort, if the analyte was still not measurable, then ultrasonic nebulization was employed. Because of low concentrations, even with the use of ultrasonic nebulization and axial view, some analytes could not be detected in some samples (see Table IV). Nickel concentration in apple leaves was not detected using the pneumatic nebulizers, nor could it be accurately determined using ultrasonic nebulization, perhaps because of sample matrix interference. Acid effect (19) in the ultrasonic nebulization is mainly related to a drastic decrease in aerosol production in contrast to the effect observed for pneumatic nebulizers. Nonetheless, the HNO_3 concentration in the calibration solutions was matched with that of the apple leaves sample. Thus, the acid could not be the reason for the inaccurate result. Because the standard addition calibration technique is very time-consuming, external calibration was used throughout this investigation. Linear through zero, 5 point calibration curves with linear regression coefficients (R^2) of at least 0.99 were built.

CONCLUSION

By taking advantage of the current ICP-OES instrumentation, this study shows that low Ni, Pb, V, Cr, Co, As, Cu, Zn, Cd, Mn, and Ba concentrations can be determined in environmental samples. It can be seen that an improvement in sensitivity is achieved by using a concentric nebulizer (instead of a Scott design) fitted to a cyclonic spray chamber. By using optimized conditions and robust plasma, complex matrix samples such as marine sediment solution in a strong acid medium can be analyzed, provided the spray chamber is washed for at least 60 s. The interference of As on Cd can be overcome if Cd is measured in peak area with only 3 points/peak. Use of an ultrasonic nebulizer provides an improvement in LODs, but the plasma is less robust and consequently more sensitive to the sample matrix.

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TABLE IV
Element Concentrations in the Analyzed Samples
(Values in parenthesis are standard deviations of 3 determinations; analysis in triplicate.)

Analyte	Tap Water (µg L^{-1})	Rain Water (µg L^{-1})	Undergrd. Water (µg L^{-1})	River Water (µg L^{-1})	Apple Leaves (µg g^{-1})		Marine Sediment (µg g^{-1})			Pine Needles (µg g^{-1})	Bamboo Leaves (µg g^{-1})	Eucalyptus Leaves (µg g^{-1})	Tea[c] (µg g^{-1}[c])
					Certified	Measured	Certified	Measured	MD[d]				
Cr	0.18	0.05	0.75	2.9	0.30[a]	0.31	90.7 ± 4.6	106	75.9	0.41	0.53	0.64	0.70
	(0.01)	(0.01)	(0.01)	(0.1)		(0.02)		(6)	(2.7)	(0.03)	(0.07)	(0.004)	(0.010)
Ni	0.68	0.31	<0.081[b]	2.1	0.91 ± 0.12	0.56	39.5 ± 2.3	40.5	33.7	0.31	1.02	1.4	1.0
	(0.04)	(0.07)		(0.4)		(0.03)		(2.5)	(1.5)	(0.02)	(0.09)	(0.1)	(0.01)
V	0.08	0.047	0.22	5.7	0.26 ± 0.03	0.26	133 ± 5	147	98.4	0.22	0.24	0.09	< 0.001[b]
	(0.01)	(0.015)	(0.02)	(0.5)		(0.04)		(8)	(2.3)	(0.03)	(0.07)	(0.01)	
Cd	<0.032[b]	0.10	<0.032[b]	0.11	0.013 ± 0.0022	0.009	2.11 ± 0.15	2.71	0.29	0.12	< 0.032[b]	0.021	0.017
		(0.05)		(0.090)	(0.004)		(0.31)	(0.05)	(0.01)		(0.0010)	(0.005)	
Co	<0.024[b]	<0.024[b]	<0.024[b]	0.57	0.09[a]	0.059	11.5 ± 0.3	12.6	9.80	0.14	0.023	0.011	0.036
				(0.02)		(0.007)		(0.8)	(0.4)	(0.01)	(0.003)	(0.005)	(0.002)
Ba	60	1.6	22	64	49 ± 2	50	–	792	410	4.2	40	60	0.046
	(2)	(0.08)	(1)	(1)		(0.30)		(166)	(17)	(0.40)	(0.20)	(0.4)	(0.003)
Mn	2.3	2.60	0.062	25	54 ± 3	52	374 ± 0.23	372	614	233	302	1299	194
	(0.04)	(0.05)	(0.030)	(1)		(1)		(16)	(19)	(7)	(6)	(2)	(8)
Cu	2.7	0.30	<0.041[b]	3.8	5.64 ± 0.24	5.6	310 ± 12	377	16.9	1.89	5.3	5.7	1.22
	(0.2)	(0.02)		(0.2)		(0.060)		(11)	(1.0)	(0.08)	(0.50)	(0.07)	(0.01)
Pb	1.2	1.7	1.9	3.6	0.470 ± 0.024	0.48	183 ± 8	208	28.6	0.85	2.2	1.2	0.03
	(0.1)	(0.2)	(0.1)	(0.1)		(0.2)		(15)	(1.2)	(0.06)	(0.20)	(0.020)	(0.003)
Zn	11)	6.8	5.8	10	12.5 ± 0.3	12.7	374 ± 23	362	66	26	20	26	3.1
	(0.3)	(0.2)	(0.4)	(1)		(0.7)		(28)	(3)	(2)	(1)	(1)	(0.05)
As	<1.1[b]	<1.1[b]	<1.1[b]	1.2	0.038 ± 0.007	< 0.33[b]	26.2 ± 1.5	26.0	26.1	< 0.33[b]	< 0.33[b]	< 0.33[b]	< 0.03[b]
				(0.1)				(1.7)	(0.14)				

[a] Informed value.
[b] LODs using ultrasonic nebulization and axial view.
[c] Relative to one tea sachet containing 2 g of sample mass.
[d] Real marine sediment sample.

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Comparison of Microwave Acid Digestion With the Wet Digestion and Ashing Methods for the Determination of Fe, Mn, and Zn in Food Samples by Flame AAS

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ABSTRACT

A conventional microwave oven digestion method was evaluated for sample digestion prior to the determination of iron, manganese, and zinc in foods using nitric acid for sample dissolution in closed vessels.Under the working conditions used, the reagents [4 mL nitric acid and 3 mL (30%) hydrogen peroxide] employed to attack the samples did not significantly alter the flame atomic absorption of iron, manganese, and zinc nor were any interferences observed from other metals at their typical concentrations in food formulations. The food samples were effectively dissolved by acid treatment in the microwave oven in approximately 15 min for the rapid determination of iron, manganese, and zinc by flame atomic absorption spectrometry in beans, rice, chickpeas, and lentils.

The analytical results obtained for each 100-g sample were as follows: Beans (Fe: 79.81 µg g^{-1}, Mn: 15.33 µg g^{-1}, and Zn: 30.77 µg g^{-1}), rice (Fe: 30.96 µg g^{-1}, Mn: 8.28 µg g^{-1}, and Zn: 13.97 µg g^{-1}, chickpeas (Fe: 66.25 µg g^{-1}, Mn: 31.90 µg g^{-1}, and Zn: 29.94 µg g^{-1}), and lentils (Fe: 117.68 µg g^{-1}, Mn: 18.18 µg g^{-1}, and Zn: 44.69 µg g^{-1}).

These results are in very good agreement with the wet digestion and ashing methods, and the RSD for the microwave digestion method ranged from 2.0–5.8 %.

INTRODUCTION

Probably no other aspect of sample preparation prior to metals determination has caused more controversy than the question of how best to destroy the organic matter portion of a sample. The three most commonly used procedures are dry ashing, wet oxidation, and microwave oven (1).

The procedure used to attack the sample should ideally meet the following requirements: (a) it should result in no loss of the element(s) to be determined; (b) neither the reactants used nor the products resulting from the sample treatment should disturb the atomic absorption of the analytes; and (c) the procedure should be expeditious and convenient.

Pretreatment of foods for atomic absorption spectrometry usually involves ashing of the samples and subsequent dissolution of the ash in an acid medium or, alternatively, direct acid treatment. Sample calcination is sluggish and prone to loss of the more volatile elements (2). Table I lists several papers published on this subject (3–10) with regard to the samples analyzed, the metals determined, and the procedure used for the pretreatment of the samples.

The alternative method of electrothermal atomization of solid samples with no sample pretreatment as proposed by various authors (11–13) is still difficult to implement for routine analyses.

In this work, we developed a straightforward, rapid acid sample digestion method using a microwave oven as an alternative to the traditional calcinations, and the results are compared with the wet digestion and ashing methods. The samples chosen for our study included beans, rice, chickpeas, and lentils. We also studied the influence of the reagents and other metals present in the composition of the samples; the results are discussed in detail.

EXPERIMENTAL

Instrumentation

A PerkinElmer® Model 2100 atomic absorption spectrometer, equipped with a microprocessor, screen, and printer, was used (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). The hollow cathode lamp employed was obtained from PerkinElmer. The instrumental and operating parameters are listed in Table II.

For sample digestion, a microwave oven (Milestone Scientific, Model 1200, (Sorisole, BG, Italy) with pressure and temperature controller was used. For safety reasons, the microwave oven was placed into a room separate from the laboratory.

Reagents and Standard Solutions

Sulfuric acid, 98%; nitric acid 65%; hydrochloric acid 35%; metallic zinc; manganese (II) nitrate 4-hydrate, calcium nitrate 4-hydrate; copper (II) nitrate 3-hydrate; sodium phosphate

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TABLE I
Previous Studies Performed for Metals Determination in Foods by AAS

Food	Metals	Comments	Reference
Baby Foods	Ca, Mg, Na, K, Fe	Ashing, Microwave Oven	3
Sugar	Pb	Wet Digestion, ETAAS	4
Meat, Cereals, Fruits, Fish, and Vegetables	Pb, Cd, Zn, Cu, Ni, Cr, Mn, and Co	Ashing	5
Cereals, Cookies, Bread	Cu	Wet Digestion using ethanol/water (20%) with nitric acid (0.5%)	6
Beer, Fruits, Wine, Juice	Pb	Microwave Oven on-line with FIA-AAS	7
Milk	Mn, Zn	Microwave Oven, ETAAS with Zeeman-effect BG	8
Fish	Se	Microwave Oven, HG-AAS	9
Peanuts	Fe, Cu	Flame AAS, Wet Digestion	2
Maize	Zn, Mn	Microwave Oven, Flame AAS	10

TABLE II
Instrumental Operating Parameters

Atomic Absorption	Iron	Zinc	Manganese
Wavelength (nm)	248.3	213.9	279.5
Lamp current (mA)	15	20	10
Flame	Air-acetylene	Air-acetylene	Air-acetylene
Working range (mg/L)	0–10	0–5	0–3
Spectral slit width (nm)	0.2	0.2	0.2
Burner distance below optic (mm)	10	10	10

Microwave Oven Program

Step	1	2	3	4	5
Time (min)	1	2	5	5	5
Power (W)	250	0	250	400	500

1 hydrate and magnesium nitrate 6-hydrate from Panreac, S.A., Barcelona, Spain

Hydrogen peroxide 30%, extra-pure, Scharlau Chemie, S.A., Barcelona, Spain

Metallic iron, Merck, Darmstadt, Germany

Standard solution of iron (~1000 µg mL^{-1}):
A ~0.1-g sample of metallic iron was weighed to within 0.0001 g, placed into a beaker, and dissolved in 1% (v/v) nitric acid. The solution was transferred quantitatively into a 100-mL calibrated flask and diluted to volume with distilled water.

Standard solution of manganese (~1000 µg mL^{-1}):
A ~4.0-g sample of manganese nitrate 4 hydrate was weighed to within 0.0001 g and dissolved in distilled water. The solution was quantitatively transferred to a 1-L calibrated flask and diluted to volume with distilled water.

Standard solution of zinc (~1000 µg mL^{-1}):
A ~0.1-g sample of metallic zinc was weighed to within 0.0001 g and dissolved in 1% (v/v) nitric acid. The solution was quantitatively transferred to a 100-mL calibrated flask and diluted to volume with distilled water.

Food Samples

Beans, chickpeas, and lentils were obtained from Maravilla, S.A.Onzonilla (León), Spain, and the rice was obtained from Brazal, S.C.,Grañén (Huesca), Spain. The nutritional facts of these foods are listed in Table III.

All solutions were prepared from analytical grade reagents. More dilute solutions of iron, manganese,

and zinc were made by diluting appropriate volumes of the standards with distilled water immediately prior to use.

PROCEDURE

Sample Preparation

The food samples were crushed in an electric grinder to obtain a fine powder and stored in dry vessels at ambient temperature. The digestion vessels and covers were of Teflon® material, because they are chemically resistant to high temperatures and prevent cracking. For this study, a 250°C temperature was used and the vessels were pressed at 50 Bar. The time required for sample digestion using ashing, wet digestion, and microwave oven is listed in Table IV.

Ashing

The concentration of iron, manganese, and zinc was determined by flame AAS following the AOAC methods (14).

Approximately 1.5 g of the sample was weighed and placed into a porcelain crucible, and dried at 105°C for 5 hours after ashing the sample at 550°C for 5 h. The resultant residue was dissolved in aqua regia (5 mL) and diluted to 25 mL with distilled water.

Wet Digestion

A ~0.15-g food sample was placed into a 100-mL Kjeldhal flask. Then, 3 mL of concentrated sulphuric acid was added and heated for 20–25 min; first gently, then more vigorously. Then, 5 mL of 30% hydrogen peroxide was added drop-wise to decolorize the solution. This mixture was boiled vigorously to remove excess hydrogen peroxide, allowed to cool, and diluted to 10-mL volume with distilled water.

Microwave Oven

A ~0.5-g sample was weighed into a Teflon vessel. Then 4 mL of concentrated nitric acid and 3 mL (30%) hydrogen peroxide were added, the vessels sealed, and placed into the rotor of the microwave oven at 50 Bar of pressure. We used six vessels in each run. The samples were heated at 250 W for 1 minute, then the power was decreased to 0 W for 2 minutes, and finally increased again to 250, 400, and 500 W for 5 minutes each. After treatment, the contents were diluted to 10 mL with distilled water and subjected to flame AAS analysis.

Determination of Fe, Mn, and Zn Using Calibration Curve

The iron, manganese, and zinc standards were prepared in a series of solutions containing between 0–10 μg mL^{-1} iron, 0–3 μg L^{-1} manganese, and 0–5 μg L^{-1} zinc by diluting with distilled water. To determine these elements in foods, the solution obtained was subjected to three different treatments of nebulization. Under appropriate conditions, the reagents used to attack the sample (sulphuric acid, nitric acid, or *aqua regia*) do not alter the atomic absorption signal of iron, zinc, or manganese.

RESULTS AND DISCUSSION

Sample Digestion

Treatment of the food samples by microwave oven with 4 mL HNO_3 and 3 mL (30%) H_2O_2 is an effective way of dissolving them. Under appropriate conditions, the presence of the above reagents does not alter the atomic absorption signal of iron, zinc or manganese. It is well known that the AOAC method involving calcination and subsequent dissolution of the resultant residue in aqua regia is unduly sluggish and entails filtering before AAS determination can be performed. However, the proposed procedure permits the effective dissolution of food samples in 15 min and is faster than the wet digestion and ashing methods. As can be seen in Table IV, the time required for each sample digestion using the microwave oven is only five minutes, while for wet digestion it takes 45 min and for ashing 70 min, which is 9 or 14 times longer per sample, respectively.

TABLE III
Nutritional Information of the Food Samples

	Nutritional Information (per 100-g samples)			
Food	Carbohydrate (g)	Fat (g)	Protein (g)	Energy (Kcal)
Beans	57	1.4	21.1	325
Rice	75.3	0.4	6.2	329.9
Chickpeas	57.8	6.5	20	369.7
Lentils	56.4	2	22	331.6

TABLE IV
Time Required for Sample Digestion

Treatment	Total (min)	No. of Samples	Min./Sample
Microwave	25	5	5
Wet Digestion	45	1	45
Ashing	485	7	70

Interferences

The influence on the flame atomic absorption determination of iron, zinc, and manganese of other metals frequently used as additives in foods was also studied. The results, given in Table V, show that zinc and manganese have a high tolerance to the presence of other metals in the sample. For example, the ratio of interference of Fe or Zn : Mn is >100, while the interference of Mn or Zn : Fe is >10, which implies that interfering metal concentrations are much higher than is usual in foods (15).

Analysis of Vegetable Foods

Iron, manganese, and zinc were determined in bean, rice, chickpea, and lentil samples by preparing a calibration curve shown in Figure 1 obtained from the aqueous solutions of the three elements using the proposed microwave acid procedure. The results in Table VI show a comparison with the wet digestion and ashing methods. As can be seen, the results of the microwave acid digestion method are quite consistent with wet digestion and ashing following the Official Methods of Analysis (Assoc. Off. Anal. Chem., AOAC).

The detection limits for iron, manganese, and zinc in 100-g samples using microwave oven acid digestion were as follows (see Table VI):

Beans
(Fe: 79.81 ug g^{-1}, Mn: 15.33 ug g^{-1}, Zn: 30.77 ug g^{-1});
Rice
(Fe: 30.96 ug g^{-1}, Mn: 8.28 ug g^{-1}, Zn: 13.97 ug g^{-1});
Chickpeas
(Fe: 66.25 ug g^{-1}, Mn: 31.90 ug g^{-1}, Zn: 29.94 ug g^{-1}),
Lentils
(Fe: 117.68 ug g-1, Mn: 18.18 ug g^{-1}, Zn: 44.69 ug g-1).

TABLE V
Summary of Metal Interferences on the Atomic Absorption Signals

Interferent	Ratio of Metal : Interference		
	Fe	Mn	Zn
Calcium	1:10	1:100	1:100
Copper	1:100	1:100	1:100
Phosphorus	1:10	1:100	1:100
Magnesium	1:100	1:100	1:100
Iron		1:100	1:100
Manganese	1:10		1:100
Zinc	1:10	1:100	

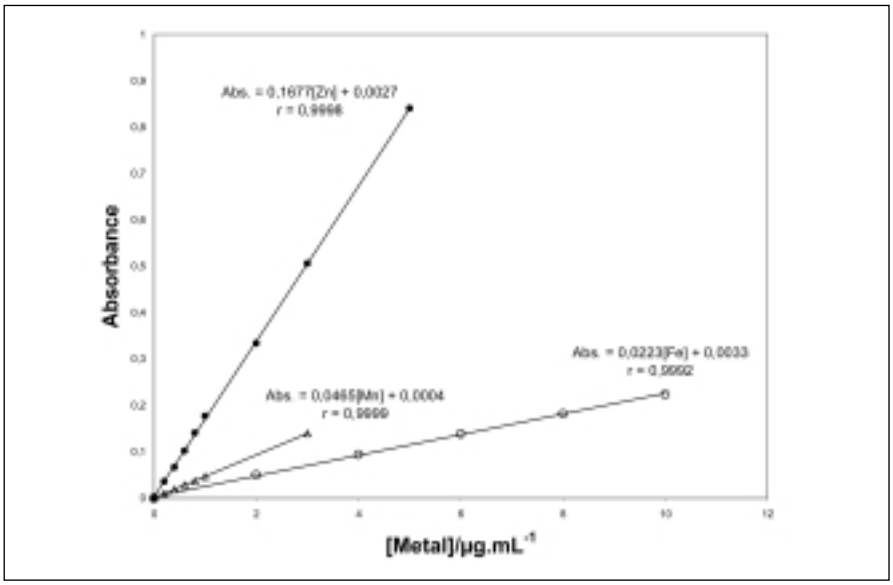

Fig. 1. Calibration curve for iron, zinc, and manganese.

These results are in very good agreement with the wet digestion and ashing methods; the RSD was in the 2.0–5.8% range for microwave acid digestion.

A comparison of the results obtained by microwave digestion with the AOAC recommended method (14) and the statistical t-test (16) revealed no significant differences ($p < 0.05$) between the average values provided by the three methods.

TABLE VI
Results Obtained in the FAAS Determination of Fe, Mn, and Zn in Vegetable Foods Using Microwave Oven, Wet Digestion, and Ashing (in 100-g samples)

	Iron			Manganese			Zinc		
Foods	Microwave	Wet Dig.	Ashing	Microwave	Wet Dig.	Ashing	Microwave	Wet Dig.	Ashing
Beans									
# Determinations	8	4	4	7	5	8	6	4	5
Avg. Value (μg g^{-1})	79.81	76.29	80.59	15.33	14.38	13.40	30.77	29.18	30.64
Range (μg g^{-1})	74.33–86.30	71.89–79.84	75.62–85.98	13.67–16.41	13.64–15.41	13.04–14.01	28.64–32.49	27.15–30.21	30.12–31.31
Std. Deviation	3.416	4.044	5.032	0.890	0.906	0.349	1.781	1.438	0.432
R.S.D. (%)	4.3	5.3	6.2	5.8	6.3	2.6	5.8	4.9	1.5
Rice									
# Determinations	6	4	4	5	4	9	6	4	4
Avg. Value (μg g^{-1})	30.96	30.22	29.11	8.28	8.29	8.04	13.97	12.86	12.05
Range (μg g^{-1})	28.68–32.36	28.69–32.30	27.2–31.53	8.00–8.86	7.31–8.88	7.52–8.35	12.15–14.23	12.11–13.15	11.38–12.76
Std. Deviation	1.598	1.868	2.199	0.351	0.852	0.283	0.276	0.503	0.691
R.S.D. (%)	5.1	6.2	7.5	4.2	10.3	3.5	2.0	3.9	5.7
Chickpeas									
# Determinations	6	4	4	6	4	6	5	4	6
Avg. Value (μg g^{-1})	66.25	66.22	66.85	31.90	30.59	30.86	29.94	31.17	30.68
Range (μg g^{-1})	64.28–67.48	63.25–67.81	62.96–74.17	30.29–33.02	28.75–31.87	29.44–32.17	28.36–30.84	30.14–32.19	30.28–31.41
Std. Deviation	1.393	2.052	6.341	0.906	1.313	1.106	1.003	1.169	0.473
R.S.D. (%)	2.1	3.1	9.5	2.8	4.3	3.6	3.4	3.8	1.5
Lentils									
#Determinations	6	4	4	5	4	6	5	4	6
Avg. Value (μg g^{-1})	117.68	111.62	105.26	18.18	19.05	17.53	44.69	47.02	38.93
Range (μg g^{-1})	114.02–121.37	109.82–113.14	102.1–114.1	17.05–18.74	18.99–19.12	15.47–18.65	42.73–46.32	45.28–48.05	37.86–39.86
Std. Deviation	3.675	1.614	5.013	0.729	0.059	1.108	1.843	1.515	0.668
R.S.D. (%)	3.1	1.4	4.8	4.0	0.31	5.2	4.1	3.2	1.7

CONCLUSION

The treatment of food samples using the proposed microwave oven acid digestion method allows the atomic absorption determination of Fe, Zn, and Mn in a faster, more convenient way than by the ashing or wet digestion methods. Under the working conditions used, the reagents employed to attack the samples [4 mL HNO_3 and 3 mL (30%) H_2O_2] did not significantly alter the flame atomic absorption of the metals; nor were any significant interferences observed from other metals at their typical concentrations in food formulations.

As a result, iron, zinc, and manganese were determined by generating a calibration curve using an aqueous solution of the three elements. The analytical results using the microwave acid digestion method are comparable to those provided by the AOAC recommended method and wet digestion method. It should be noted that the lower precision obtained in the determination of these elements (2.1% for Fe and 2.8% for Mn in chickpeas or 2.0% for Zn in rice) is offset by the greater expeditiousness of microwave oven acid digestion. The proposed method can be extended to the analysis of feedstuff, agricultural materials, fertilizers, plants, pharmaceutical products, and other cereal foods.

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Determination of Trace Copper and Nickel in Environmental and Biological Samples by Flow Injection On-line Microcolumn Preconcentration Flame AAS Using Acrylic Acid-grafted Polytetrafluoroethylene Fiber for Column Packing

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ABSTRACT

A newly prepared acrylic acid-grafted PTFE fiber sorbent (see reference 20) was extended to flow injection (FI) on-line microcolumn preconcentration - flame atomic absorption spectrometric determination of trace copper and nickel in environmental and biological samples. On-line preconcentration of trace analytes was achieved on the microcolumn packed with acrylic acid-grafted PTFE fibers, and the retained analytes were eluted with 1.0 mol L^{-1} HCl for on-line FAAS determination. The optimum pH range of the sample solution for preconcentration of Cu(II) and Ni(II) was 3.6–5.6 and 4.4–6.4, respectively. The developed sorbent exhibited fairly fast kinetics for the adsorption of Cu(II) and Ni(II), permitting the use of high sample flow rates up to at least 10.8 mL min^{-1} for the FI on-line microcolumn preconcentration system without loss of the retention efficiency.

With a preconcentration time of 45 s at a sample loading flow rate of 10 mL min^{-1}, an enhancement factor of 48 for Cu and 23 for Ni, and a detection limit (3σ) of 0.20 µg L^{-1} for Cu and 0.25 µg L^{-1} for Ni were achieved at a sample throughput of 55 h^{-1}.

Precision for 11 replicate measurements of 25 µg L^{-1} Cu and 30 µg L^{-1} Ni was 1.2 and 1.6% (RSD), respectively.

The method was successfully applied to the determination of trace copper and nickel in environmental and biological samples.

INTRODUCTION

Flame atomic absorption spectrometry (FAAS) still continues to be one of the most attractive approaches for trace element analysis in most laboratories due to its relatively low operational and instrumental costs, easy operation, and high sample throughput, although inductively coupled plasma-mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ETAAS) have sufficiently high sensitivity for element analysis in most biological and environmental samples (1). However, direct FAAS determination of trace amounts of elements is usually difficult owing to its insufficient detection power. On-line separation and preconcentration using flow injection (FI) techniques have been shown to be efficient and effective in enhancing the sensitivity and specificity of FAAS (2). In the past decades, FI on-line separation and preconcentration techniques based on sorbent extraction (3), solvent extraction (4, 5), ion exchange (6, 7), coprecipitation (8, 9), and sorption in a knotted reactor (10, 11) have been developed for FAAS determination of trace elements in a variety of matrices.

Since Olsen et al. (2) described the first application of FI on-line ion-exchange preconcentration of trace metals, many kinds of ion-exchange materials have been used as the packing material for such purposes, and this approach has proved to be an effective means of decreasing the detection limit as well as a means of removing interferences for trace element determination (12, 13). However, most ion-exchange materials swell and shrink with change in pH and/or solvent conditions or the surface properties of the resin might be irreversibly changed after having been subjected to a large number of samples, either due to contamination, deactivation, or even loss of its functional groups (14).

The chemical inertness, elevated resistibility, low friction coefficient, and good swelling resistance of polytetrafluoroethylene (PTFE) (15) make it very attractive as the substrate for column packing in FI on-line microcolumn preconcentration systems. Although the preparation of the ion-exchange materials via acrylic acid-grafted PTFE has been reported by several groups (16-19), to the best of our knowledge, only one paper on the application of the PTFE substrate to FI on-line ion-exchange microcolumn preconcentration for atomic spectrometric determination of trace metals (20) has been published so far.

The purpose of this work was to extend the newly developed acrylic acid-grafted PTFE fiber (20) to FI

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on-line ion-exchange preconcentration and separation FAAS determination of trace copper and nickel in environmental and biological samples.

EXPERIMENTAL

Instrumentation

A Model SOLAAR S2 atomic absorption spectrometer (Thermo Elemental, Waltham, MA, USA), equipped with quadline deuterium arc background correction, a universal air-cooled titanium burner, and a PTFE spray chamber with impact bead and baffle Pt/Ir PTFE nebulizer was used for all atomic absorption measurements. The AAS instrument was controlled by the SOLAAR instrumental operations software. Hollow cathode lamps (Beijing Shuguangming Electronic Light Source Instrument, Beijing, P.R. China) were used as the radiation sources at 324.7 nm for Cu and 232.0 nm for Ni with a current of 4 mA for Cu and 4 mA for Ni, and a 0.5-nm slit width for Cu and 0.2-nm for Ni. The recommended stoichiometric air-acetylene flame conditions were used (1.0 L min^{-1} acetylene for Cu, 0.8 L min^{-1} acetylene for Ni). The air flow rates were automatically adjusted to meet the stoichiometric air-acetylene flame conditions.

A Model FIA-3100 flow injection analyzer (Vital Instrumental Co. Ltd., Beijing, P.R. China) was used throughout this work. The FIA-3100 consists of two peristaltic pumps and a standard rotary injection valve (8-channel 16-port multifunctional injector). Tygon® peristaltic pump tubing was employed to propel the sample and reagent. PTFE tubing with a 0.5-mm i.d. was used for all connections. These connections were kept as short as possible to minimize dead volume.

A Model Qwave-2000 microwave digestion system (Questron Co., Missisauga, Canada) was employed to digest the samples used for validation of the developed method.

Reagents

All reagents were of at least analytical grade. All metal stock solutions (1000 mg L^{-1}) were purchased from National Research Center for Standard Materials (NRCSM, Beijing, P.R. China). The working solutions were prepared by series dilution of the stock solutions immediately prior to use. Doubly de-ionized water (DDW, 18.2 MΩ cm) obtained from a WaterPro water system (Labconco Corporation, Kansas City, MO, USA) was used throughout the experiments.

Preparation of the Acrylic Acid-grafted PTFE Fibers and the Microcolumn

The preparation of the acrylic acid-grafted PTFE fiber was described in a previous work (20), and is not repeated here. The carboxylic content of the grafted fiber was determined to be 3.06 mmol g^{-1}.

The preconcentration microcolumn was made from a PTFE tube with an effective length of 2 cm and an inner diameter of 3 mm. 136.5 mg of the acrylic acid-grafted PTFE fibers were packed into the microcolumn. The packed column was sequentially washed with DDW, 1 mol L^{-1} HNO_3 and DDW until no Cu, Ni, and Cr signals were detected with FAAS. A freshly prepared microcolumn could be used for about 2000 preconcentration cycles without significant loss of preconcentration efficiency and precision. No significant difference in preconcentration efficiency and precision was observed using repacked microcolumns containing identical amounts of the fibers.

Sample Preparation

The following certified reference materials (NRCSM) were used to check the accuracy of the developed method: GBW07605 (Tea), GBW07601 (Human Hair), GBW08571 (Mussel), GBW07405 (Soil), GBW07313 (Marine Sediment).

Sample digestion was carried out in sealed PFA (Teflon® perfluoralkoxy) vessels using a Model Qwave-2000 microwave digestion system (Questron Co., Canada). All instrumental parameters for the digestion were chosen according to the recommendations of the US Environmental Protection Agency (EPA). The clear digest was transferred into a 100-mL calibrated flask and diluted to volume with DDW. The precipitate in the resultant solution from the sediment and soil was allowed to settle and the supernatant was used for analysis.

The following amounts of certified reference materials and reagents were used for digestion:

Tea: 0.25 g of tea was mixed with 5 mL of concentrated HNO_3.

Mussel: 0.3 g of mussel was mixed with 6 mL of concentrated HNO_3 (for Cu); 1 g mussel was mixed with 10 mL of concentrated HNO_3 (for Ni).

Human Hair: 0.3 g of human hair was mixed with 6 mL of concentrated (for Cu) 1 g of human hair was mixed with 10 mL of concentrated HNO_3 (for Ni).

Soil: 0.1 g of soil was mixed with 3 mL of concentrated HNO_3 and 1 mL of concentrated HF (for Cu) and 0.5 g of soil was mixed with 8 mL of concentrated HNO_3 and 3 mL of concentrated HF (for Ni).

Marine Sediment: 0.15 g of marine sediment was mixed with 3 mL of concentrated HNO_3 and 2 mL of concentrated HF (for Ni).

Procedures for FI On-line Microcolumn Preconcentration and Separation Coupled with FAAS for the Determination of Trace Cu and Ni

The FI manifold and its operational sequence for the on-line microcolumn preconcentration and separation are shown in Figure 1 and Table I, respectively. In step 1 of Figure 1(a), the injection valve was in the fill position and pump 1 was activated so that the sample solution was loaded onto the microcolumn; the effluent from the column was flowing to waste. In step 2 of Figure 1(b), pump 2 started to work while pump 1 was stopped and the injection valve turned to the inject position to introduce diluted HCl solution for eluting the analyte retained on the column. A complete cycle of preconcentration and elution required 65 s with a sample loading time of 45 s.

RESULTS AND DISCUSSION

Effect of Sample pH

The effect of the pH of the sample solution was evaluated using 25 µg L^{-1} Cu(II) and 50 µg L^{-1} Ni(II) in the pH range of 2–10. As shown in Figure 2, the optimum pH of the sample solution was in the range of 3.6–5.6 for Cu(II), and 4.4-6.4 for Ni(II). The optimum pH ranges for the preconcentration of Cu(II) and Ni(II) were all in the weak acid range. The low absorbance of the analyte at a lower pH resulted from the low retention efficiency of the packing material due to the occupation of the active sites of the weak ion-exchanger by proton. The hydrolysis of the metal ions at higher pH probably accounted for the decrease of the absorbance of the analytes due to the diminution of free ions. Thus, the pH of the sample solution should be adjusted to optimum range before preconcentration.

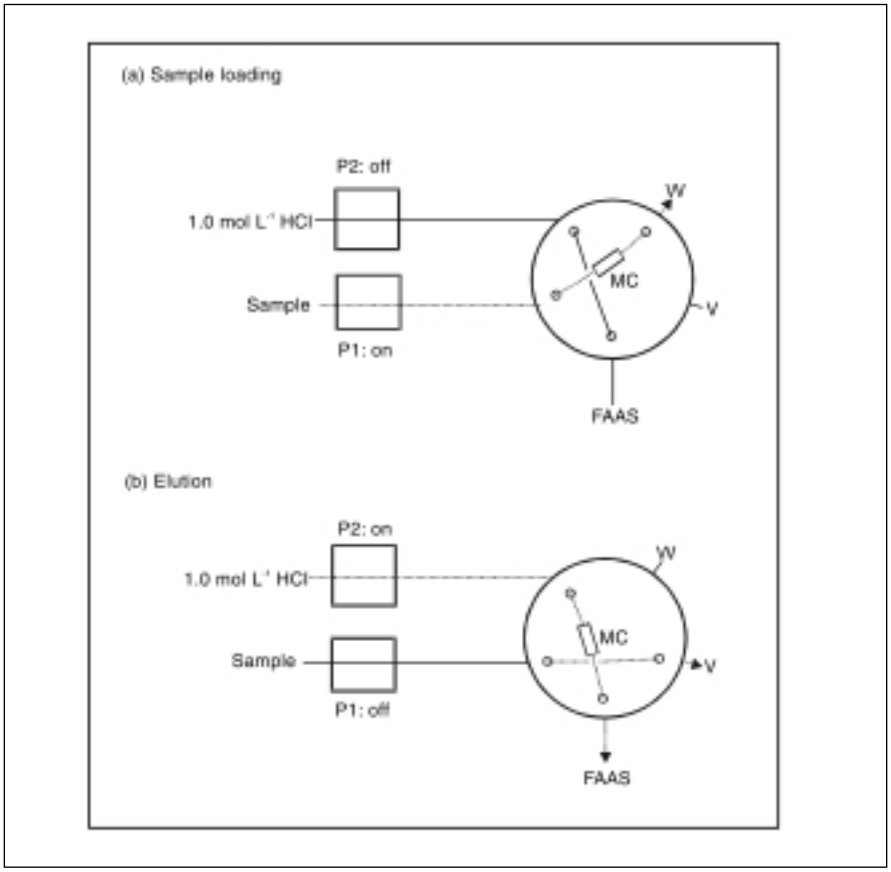

Fig. 1. FI manifold and operational sequence for the on-line column preconcentration coupled with FAAS. P1 and P2: peristaltic pump; MC: microcolumn; W: waste; V= valve; valve position: (a) fill, (b) injection.

TABLE I
Operational Sequence of the FI On-line Microcolumn Preconcentration System Coupled With FAAS for Trace Cu and Ni Determination

Step	Function	Time (s)	Pumped Medium	Flow Rate (mL min^{-1})	Valve Position
1 (Figure 1a)	Sample Loading	45	Sample Solution	10	Fill
2 (Figure 1b)	Elution	20	1.0 mol L^{-1} HCl	3.0	Injection

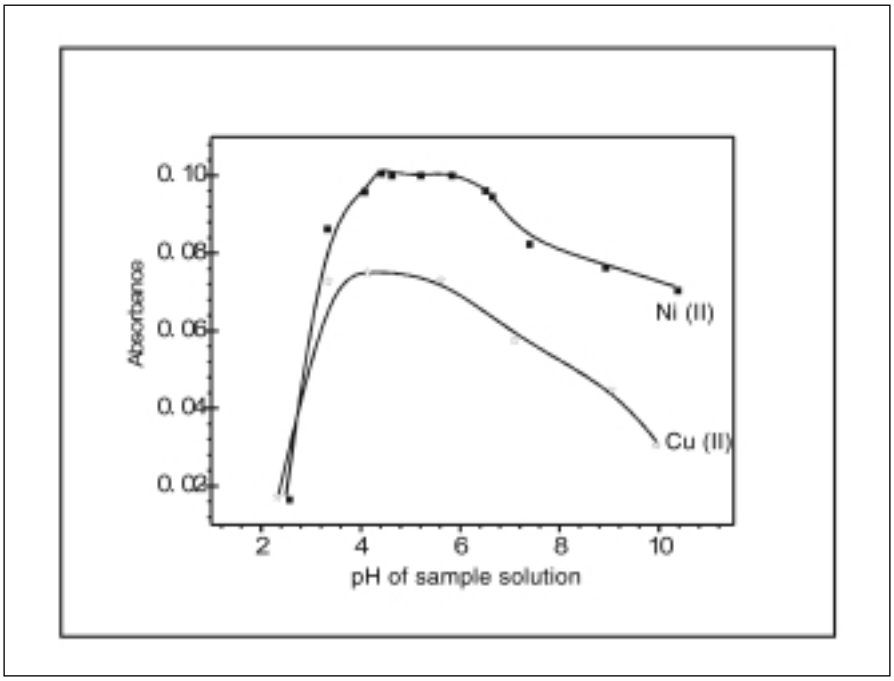

Fig. 2. Effect of pH of sample solution on the preconcentration of 25 µg L^{-1} Cu and 50 µg L^{-1} Ni. All other conditions as in Figure 1 and Table I.

Effect of HCl Concentration on Elution

Choice of a suitable eluent is important for the analytical performance of an FI on-line preconcentration system. In this work, diluted HCl solution was used as the eluent. The effect of HCl concentration on the elution of the retained Cu(II) and Ni(II) was examined at a flow rate of 3 mL min^{-1}. It was found that 1.0 mol L^{-1} of HCl solution was strong enough for quantitative elution of the adsorbed Cu(II) and Ni(II).

Effect of Sample Loading Rate and Sample Loading Time

Studies on the effect of sample loading flow rate on the 45 s preconcentration of 25 µg L^{-1} Cu(II) and 50 µg L^{-1} Ni(II) showed that the absorbance of the analytes increased linearly as the sample loading flow rate increased up to at least 10.8 mL min^{-1}. These results indicated that the kinetics for the adsorption of Cu(II) and Ni(II) on the developed sorbent was very fast. The influence of sample loading time on the preconcentration of 25 µg L^{-1} Cu(II) and 50 µg L^{-1} Ni(II) was tested at a sample flow rate of 10.8 mL min^{-1}. The results showed that the linearity was still good until a sample loading time of at least 180 s. The wide range of linearity for absorbance against sample loading time and sample loading flow rate in the developed FI on-line microcolumn preconcentration system offered great potentiality for achieving high enhancement factors by increasing sample loading rates and/or sample loading time without losing retention efficiency.

Effect of Foreign Ions

Interferences from representative alkali metal ion, alkaline metal ion, and typical transition metal ions on the preconcentration of 25 µg L^{-1} Cu(II) and 50 µg L^{-1} Ni(II) were examined. The results are shown in Table II. Up to 600 mg L^{-1} of Na(I) and 100 mg L^{-1} of Mg(II), 4 mg L^{-1} of Fe(III), 12 mg L^{-1} of Cr(III), 35 mg L^{-1} of Zn(II), 100 mg L^{-1} of Ni(II), 150 mg L^{-1} of Co(II), 3.5 mg L^{-1} of Pb(II), and 0.4 mg L^{-1} of Cd(II) had no significant interference on the determination of 25 µg L^{-1} Cu(II). The tolerable concentrations of Na(I), Mg(II), Fe(III), Zn(II), Co(II), Pb(II), Cd(II), Cu(II), and Cr(III) for the determination of 50 µg L^{-1} Ni (II) were found to be 60, 8, 4, 30, 40, 0.5, 0.3, 5.0, and 0.3 mg L^{-1}, respectively. As demonstrated later, the present system allowed the interference-free determination of trace Cu(II) and Ni(II) in the environmental and biological samples studied. Apparently, the new cation-exchange material has good ability to resist the interferences of alkali and alkali earth elements.

Analytical Figures of Merit

The analytical characteristic data for the developed FI on-line microcolumn preconcentration coupled with FAAS for the determination of trace copper and nickel under the optimum conditions are given in Table III. With a preconcentration time of 45 s at a sample loading flow rate of 10 mL min^{-1}, an enrichment factor of 48 for Cu and 23 for Ni was obtained at a sample throughput of 55 h^{-1}. The detection limits (3σ) for Cu and Ni were 0.20 and 0.25 µg L^{-1}, respectively. The precision for 11 replicate measurements of 25 µg L^{-1} Cu(II) and 30 µg L^{-1} Ni(II) was 1.2% and 1.6%, respectively.

Method Validation

To evaluate the accuracy of the developed FI on-line microcolumn preconcentration coupled with FAAS for the determination of trace copper and nickel, several certified reference materials, (CRMs), i.e., GBW 07605 (Tea), GBW 07601 (Human Hair), GBW 08571 (Mussel), GBW 07405 (Soil), GBW 07313 (Marine Sediment), and GBW 07312 (Sediment) were ana-

TABLE II
Effect of Potentially Interfering Species on the Determination of 25 µg L^{-1} Cu and 50 µg L^{-1} Ni

Interfering Ion	Conc. (µg mL^{-1})	Recovery of Cu (%)	Conc. (µg mL^{-1})	Recovery of Ni (%)
Na(I)	200	98±2	5	104±2
	500	98±1	10	106±2
	600	80±3	60	88±3
	700	74±4	80	80±2
Mg(II)	20	100±3	4	100±1
	100	88±2	6	96±2
	120	75±1	8	80±4
Fe(III)	1.0	95±2	0.5	97±1
	4.0	83±3	3.0	87±2
	5.0	70±2	4.0	84±2
Zn(II)	1.0	104±1	10	99±3
	20	99±2	15	91±2
	35	86±3	20	89±2
	40	78±3	30	80±4
Co(II)	50	100±2	0.2	98±2
	60	92±3	40	109±3
	100	92±4	50	119±2
	150	80±3		
Pb(II)	1	94±2	0.1	90±2
	3	80±3	0.5	82±2
	3.5	80±2	1.0	77±2
	4	70±2	3.0	72±2
Cd(II)	0.1	95±1	0.1	100±2
	0.2	87±2	0.2	85±4
	0.3	87±2	0.3	85±2
	0.4	86±3	0.5	76±2
	0.5	77±1		
Cu(II)			1.0	98±3
			2.0	97±2
			3.0	91±1
			5.0	86±4
Cr(III)	10	98±2	0.1	93±2
	12	82±4	0.2	86±3
	15	74±2	0.3	78±3
Ni(II)	45	94±1		
	50	93±2		
	100	85±3		
	120	78±3		

lyzed. As shown in Table IV, the determined concentrations of copper and nickel in these CRMs by the present method using simple aqueous standard solutions for calibration were in good agreement with the certified values. These results demonstrate the applicability of the developed FI on-line microcolumn preconcentration system coupled with FAAS for interference-free determination of trace Cu and Ni in the environmental and biological samples studied.

CONCLUSION

This work has demonstrated the feasibility of the acrylic acid-grafted PTFE fiber as a new packing material for FI on-line microcolumn preconcentration coupled with FAAS for the determination of trace copper and nickel. The chemical inertness, elevated resistibility, low friction coefficient, and good swelling resistance of polytetrafluoroethylene (PTFE) make it very attractive as the substrate for ion-exchangers in flow injection (FI) on-line microcolumn preconcentration systems. The developed sorbent exhibited fairly fast kinetics for the adsorption and desorption of Cu(II) and Ni(II), making the material very suitable for its application in FI on-line microcolumn preconcentration and separation systems.

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TABLE III
Analytical Performance of the FI On-line Microcolumn Preconcentration Coupled With FAAS for Trace Cu and Ni Determination

	Cu	Ni
Preconcentration Time (s)	45	45
Enrichment Factor	48	23
Sample Throughput (h^{-1})	55	55
Sample Consumption (mL)	7.5	7.5
Eluent Consumption (mL)	1	1
Precision (RSD, n = 11) (%)	1.2 (25 µg L^{-1})	1.6 (30 µg L^{-1})
Detection Limit (3σ) (mg L^{-1})	0.20	0.25
Linear Range of Calibration Graph (µg L^{-1})	2-200	1-120
Calibration Function (n = 5, A = absorbance, C in µg L^{-1})	A = 0.0056 + 0.0037C	A = 0.0009 + 0.0020C
Correlation Coefficient	0.9993	0.9997

TABLE IV
Analytical Results (µg g^{-1}, mean ± σ, n = 3) for the Determination of Trace Cu and Ni in the Certified Reference Materials (CRMs)

Sample	Cu Concentration		Ni Concentration	
	Certified	Determined	Certified	Determined
GBW 07605 (Tea)	17.3 ± 1.8	16.6 ± 1.7	4.6 ± 0.5	4.2 ± 0.2
GBW 07601 (Human Hair)	10.6 ± 1.2	10.0 ± 1.5	0.83 ± 0.19	0.80 ± 0.13
GBW 08571 (Mussel)	7.7 ± 0.5	7.4 ± 0.4	–	–
GBW 07313 (Marine Sediment)	424 ± 19	416 ± 11	150 ± 8	146 ± 4
GBW 07405 (Soil)	144 ± 9	149 ± 7	40 ± 5	38 ± 2
GBW07312 Sediment)	-	-	12.8 ± 1.9	12.1 ± 0.6

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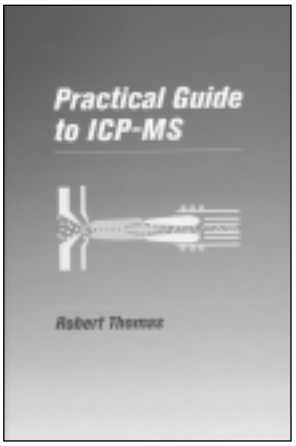

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