On-line Ion Exchange Preconcentration in a Sequential Injection Lab-on-valve Microsystem Incorporating a Renewable Column With ETAAS for the Trace Level Determination of Bismuth in Urine and River Sediment

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ABSTRACT

A sequential injection system for on-line ion exchange separation and preconcentration of trace level amounts of metal ions with ensuing detection by electrothermal atomic absorption spectrometry (ETAAS) is described. Based on the use of a renewable microcolumn incorporated within an integrated lab-on-valve microsystem, the column is initially loaded with a defined volume of beads of an SP Sephadex C-25 cation exchange resin. After having been exposed to a metered amount of sample solution, the loaded bead suspension is precisely manipulated within the valve to allow reproducible elution of the retained analyte by 30 µL nitric acid (1:16,v/v) which, via air segmentation, are then transported into the graphite tube for quantification. The content of the used column is afterwards discarded and new column material is aspirated for the next run. The ETAAS determination is performed in parallel with the preconcentration process of the ensuing sample. The performance of the system is demonstrated for the determination of bismuth. With 2.4-mL sample loading, an enrichment factor of 33.4, a detection limit of 27 ng L^{-1}, along with a sampling frequency of 10 h^{-1} was obtained. The relative standard deviation was 2.3% for the determination of 2.0 mg L^{-1} Bi (n = 7). The procedure was validated by determination of bismuth in a certified reference material CRM 320 (river sediment) and by bismuth spike recoveries in two human urine samples.

INTRODUCTION

The determination of low or trace level amounts of metals by electrothermal atomic absorption spectrometry (ETAAS) often requires the use of suitable preconcentration and/or separation procedures in order to attain the necessary sensitivity and selectivity.

Such schemes are advantageously executed in flow injection (FI) or sequential injection (SI) systems which, in addition to reducing sample and reagent consumption, allow all manipulations to be made on-line under enclosed and strictly controlled conditions, thereby minimizing the risk of contamination from the environment.

Various separation/preconcentration procedures have been suggested and applied, such as liquid-liquid extraction (1), (co)precipitation (including the use of knotted reactors) (2-4), adsorption (5,6), hydride generation (7), or ion exchange (8-10). The latter is possibly the most versatile and used approach, relying on incorporating small column reactors containing suitable resin materials into the FI/SI-system. However, ion exchange column reactors suffer from some inherent drawbacks. First is the volume change that many ion exchange resins undergo when they are converted from one form to another, i.e., from the acidic to basic form. If a micro column, therefore, is exclusively subjected to a unidirectional flow, the ensuing swelling or shrinking of the resin will cause a progressively tighter packing and, hence, increased flow resistance, leading to impaired performance or even resulting in blocking of the flow (11,12). This can be alleviated by using countercurrent flow during the loading and elution sequences, but similar problems may arise over extended periods of operation. The second problem is that 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 functional groups (13). These problems are especially serious when using ETAAS as the detection device, because the limited accommodation volume of the graphite tube requires that the retained analyte must be eluted completely (or at least to a reproducible degree) within a very small volume of eluent. Irregularities might consequently lead to risks of carryover in long-term operations.

All of these problems might be readily overcome by using an approach where the ion exchange material is discarded and renewed after each measuring cycle. Such a system was recently proposed by the present authors using the so-called lab-on-valve system (9,14) which in its design only consumes very minute amounts of ion exchange material. The cost per assay is amply compensated for by superior performance. In fact, two approaches are conceivable: (a) The analyte-loaded ion exchange beads are transported directly into the graphite tube where they are pyrolized and the analyte is atomized and quantified (9) or (b) the loaded beads can be eluted and the eluate forwarded to the ETAAS instrument for measurement (14). Both approaches have been tested with satisfactory results, although the latter appears to yield the lowest RSD values.

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Atomic Spectroscopy
Vol. 22(3), May/June 2001

In the present communication, the applicability of the novel format encompassing elution and renewal of the resin material is communicated and demonstrated for the assay of low levels of bismuth. Precise and accurate analytical methods for this potentially toxic element are in great demand because bismuth increasingly has found use in the production of pharmaceuticals and cosmetics (15). Furthermore, assay of bismuth is of interest in biological and environmental samples where the presence of interfering matrix constituents ordinarily might cause severe signal depression (16) and deteriorate the detection limit (17).

EXPERIMENTAL

Instrumentation

A PerkinElmer AAnalyst™ 600 atomic absorption spectrometer, equipped with Zeeman background corrector, AS-800 autosampler, and THGA™ graphite furnace, was used. The bismuth hollow cathode lamp (S&J Juniper & Co.) was operated at 25 mA, using a wavelength of 232.0 nm and a spectral bandpass 0.2 nm. Pyrolytically coated graphite tubes (PerkinElmer, part No. B3 000641) were employed. Integrated absorbance with a read time of 3 s was used for evaluating the results. The ETAAS was coupled with a FIAlab-3500 flow injection/ sequential injection system (Alitea, USA), equipped with an external 6-port selection valve (SV) mounted with an integrated "lab-on-valve" microsystem (14), syringe pump (SP, 2.5 mL), and auxiliary peristaltic pump. The syringe pump and valve were controlled by a separate computer, independent of the spectrometer. As detailed previously (3), the computer for the ETAAS was made a "slave" of the computer of the FIAlab-3500, whereby all unit operations could be effectively synchronized. Zeeman background correction was used for all measurements.

The "heart" of the system, that is, the integrated micro conduit is shown in Figure 1. Mounted vertically on a selection valve, the conduit is manufactured by Perspex (diameter 50 mm, thickness 10 mm), and contains 7 micro channels (i.d. 1.66 mm, length 12.0 mm), drilled to correspond with the orifice of the selector in the center. Two of the channels (port 4 and the central port) served as micro columns for accommodating the ion exchange bead resin material (details are given in Figure 2 and below). Small pieces of PEEK tubing (i.d. 1.60 mm, length 3.5 mm) with an internal channel (17.8 µm) were inserted into these two channels in order to hold the beads within the cavities of the micro columns and preventing them from escaping. Other characteristics of the "lab-on-valve" system have been previously described in detail (9).

All externally used tubes shown in Figure 1 were made of PTFE. The holding coil (HC) was made from 141.5 cm (i.d/o.d = 1.50 mm/2.10 mm) tubing, corresponding to 2.5 mL. The line from HC to the micro column in the central port was made from (i.d/o.d = 0.50 mm/1.70 mm) tubing. The delivery tube from port 4 to the graphite tube (VG

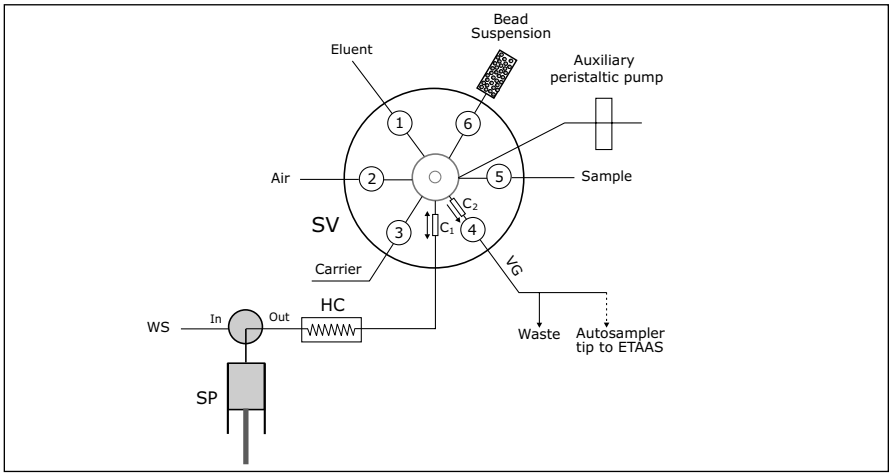

Fig. 1. Manifold for the sequential bead injection-elution on-line ion exchange preconcentration. SV = 6-port selection valve mounted with the integrated micro conduit; SP = syringe pump; HC = holding coil; C_1, C_2 = micro columns within the lab-on-valve micro system; VG = communicating tube between the micro conduit and the ETAAS.

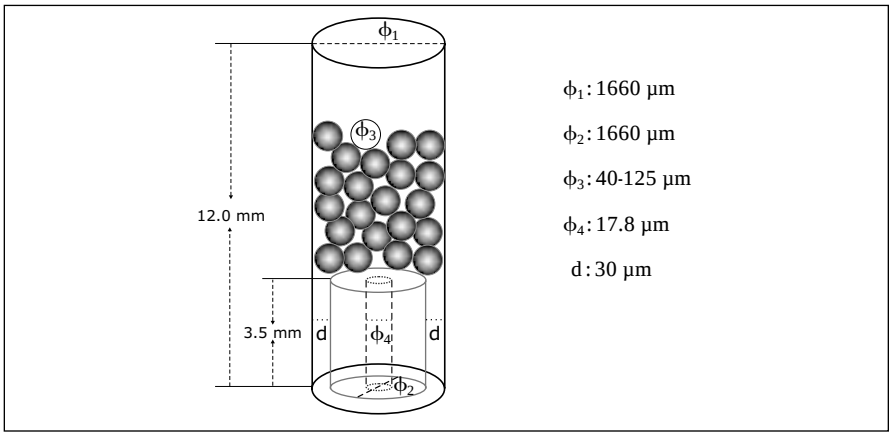

Fig. 2. Schematic diagram of the micro column.

line), which was inserted into the arm of the autosampler, was made from (i.d/o.d = 0.50 mm/1.60 mm) tubing, the length being 30 cm, corresponding to 60 µL.

Reagents

All reagents used were of analytical grade or better, and Milli-Q™ water was used throughout. Working standard solutions of bismuth were obtained by step-wise dilution of a 1000-mg L^{-1} standard solution (PerkinElmer) with a 0.10 M acetate buffer of pH 3.7. The cation exchange resin, SP Sephadex™ C-25 (Amersham Pharmacia Biotech AB, dry bead size: 40-125 µm, Na^+ form), was converted to the H^+ and K^+ forms, as described previously (9), and suspended in an appropriate amount of Milli-Q water. The practical suspensions used in these experiments were 1:10-1:20 (w/v).

Other chemicals used were: HNO_3 (65%, Merck), $HClO_4$ (70%, Merck), HF (40%, Merck), acetic acid glacial (100%, Merck), and potassium acetate (BDH).

Samples and Sample Pretreatment

River Sediment

Community Bureau of Reference (BCR) CRM-320 (river sediment): 0.1 g of CRM 320 was weighed and transferred to a PTFE beaker to which 3.0 mL of 65% nitric acid and 2.5 mL of 40% HF were added. The sample was soaked for 1 hour, then heated gently on a hot plate until fumes appeared and the solution had nearly dried. After cooling, 1.0 mL of perchloric acid was added. The content was heated to near dryness and 5.0 mL of Milli-Q water added; heating continued to near dryness again. The content was dissolved in 0.10 M acetate buffer solution (pH 3.7) by gentle heating and diluting to 100 mL with the buffer. As the content of iron in the final digestion solution greatly exceeds the tolerable limit, and since it is not possible to dilute further in order to maintain the bismuth content within the measuring range, the majority of the iron was eliminated by extraction with ether.

Urine Samples

A 20-mL urine sample was transferred into a PTFE beaker and 15 mL of 65% nitric acid was added. The mixture was heated gently on a hot plate to near dryness. After cooling, 1.0 mL of perchloric acid was added and the solution again heated to near dryness. Then 5.0 mL of Milli-Q water was added and heated to near dryness. The content was dissolved in 0.1 M acetate buffer solution (pH 3.7) and diluted to 50 mL with the buffer. A further 10-fold dilution was performed before actual assay in order to alleviate any matrix effects from dissolved salts.

Operating Procedure

The FIA/SIA-system used is shown in Figure 1. The cation exchange bead suspension is first aspirated into a 1.0-mL plastic syringe, which is mounted vertically on port 6 of the valve. Operation is not initiated until the beads have settled on the bottom of the syringe. At the same time, the whole system is conditioned by making SP aspirate 2000 µL washing solution (0.02% nitric acid), which is subsequently propelled forward through port 4 to waste.

Port 5 has two lines, which are connected to the sample solution and the auxiliary peristaltic pump, respectively. On changing sample, the pump is started to aspirate the sample solution past the central flow-through port, whereupon it is stopped.

A separation/preconcentration cycle runs through four steps, which are as follows:

Step 1: Preconditioning.
SP is set to aspirate 600 µL of the carrier solution through port 3 (flow rate 150 µL s^{-1}), 550 µL of which is directed subsequently via the central port to port 4 (100 µL s^{-1}) to rinse the micro column C2 and the VG line. 50 µL of carrier solution is thus left in the holding coil.

Step 2: Preconcentration.
The central port is directed to port 5, and 2400 µL of sample solution is aspirated (150 µL s^{-1}) and stored in HC. Thereafter, 15 µL of bead suspension is very slowly (3 µL s^{-1}) aspirated into column C1 through port 6. The central port is now directed to port 4 and while SP moves slowly forward (12 µL s^{-1}), the beads are transported to column C_2 along with the sample solution, that is, the preconcentration is taking place. The 50 µL of the previously stored carrier solution is also propelled to wash the column after the preconcentration. 500 µL carrier and 480 µL air are then aspirated into HC through port 3 and port 2, respectively, at a flow rate of 100 µL s^{-1}. 240 µL air is afterwards propelled through port 4 (24 µL s^{-1}), thereby leaving the carrier solution in HC, 60 µL of air in the VG line, and 240 µL of air between the holding coil and the central port. At the same time, the ETAAS program is activated.

Step 3: Eluting the beads and transporting the eluate to the furnace.
30 µL of nitric acid (1:16, v/v) is aspirated through port 1 into C_1 (5 µl s^{-1}). Afterwards, the central port is directed to port 4, and SP is directed to move slowly forward (8 µl s^{-1}), whereby the analyte-loaded micro column C_2 is eluted and the eluate, sandwiched by air, is transported into the graphite tube via the autosampler tip.

Step 4: Discarding the used beads and returning to the preconditioning step.
In this step, advantage is taken of the fact that the resin beads are soft and therefore, at high flow rates, can become squeezed and can pass through the narrow exit channel of

Atomic Spectroscopy
Vol. 22(3), May/June 2001

column C_2 (14). Thus, with the central port still in the position of port 4, and after the autosampler tip has moved out of the graphite tube, SP is set to move forward to propel the previously aspirated carrier solution at a flow rate of 100 µL s^{-1}, whereby all the beads in C_2 are flushed out and directed to waste. At the same time the VG line is rinsed.

The ETAAS program was set up so that when one sample was processed in the FIA/SIA system, the previously handled sample was quantified in the furnace.

RESULTS AND DISCUSSION

ETAAS Parameters

The effects of the pyrolysis and the atomization temperatures were investigated thoroughly and the results are shown in Figures 3 and 4. These studies showed that at a pyrolysis temperature lower than 250ºC, unacceptably high background signals were obtained. Well-shaped and maximum signals were obtained at a pyrolysis temperature around 400ºC and by fixing the atomization temperature at 1200ºC. At higher pyrolysis temperatures, bismuth tended to be lost, i.e., about 20% was lost at 600 ºC and 85% at 800 ºC. Furthermore, by increasing the holding time from 10 to 40 s, the background was decreased and the analyte signal enhanced. A pyrolysis temperature of 400ºC, along with a holding time of 40 s, was therefore used.

The experimental results also showed that the integrated absorbance value increased when the atomization temperature was increased from 900 to 1200ºC, the analyte peak being broadened at lower atomization temperatures, i.e., lower than 1000ºC. Therefore, only results recorded above 1100ºC are given in Figure 4. The signal reached maximum at about 1200ºC and changed merely marginally within the 1100-1300ºC range, yielding well-shaped peaks. Further increases in the atomization temperature led to significantly decreasing integrated absorbance values, i.e., at 1500ºC the integrated absorbance dropped to only about 60% of the value at 1200ºC. As a compromise, an atomization temperature at 1200ºC was chosen. The temperature program for the graphite furnace is summarized in Table I.

TABLE I
Graphite Furnace Program for the Determination of Bismuth

Steps	Temperature (ºC)	Ramp (s)	Hold (s)	Argon flow rate (mL min^{-1})
Preheating	70	5	10	250
Drying	130	5	20	250
Pyrolysis	400	20	40	250
Atomization	1200	0	3	0
Cleaning	2500	1	2	250

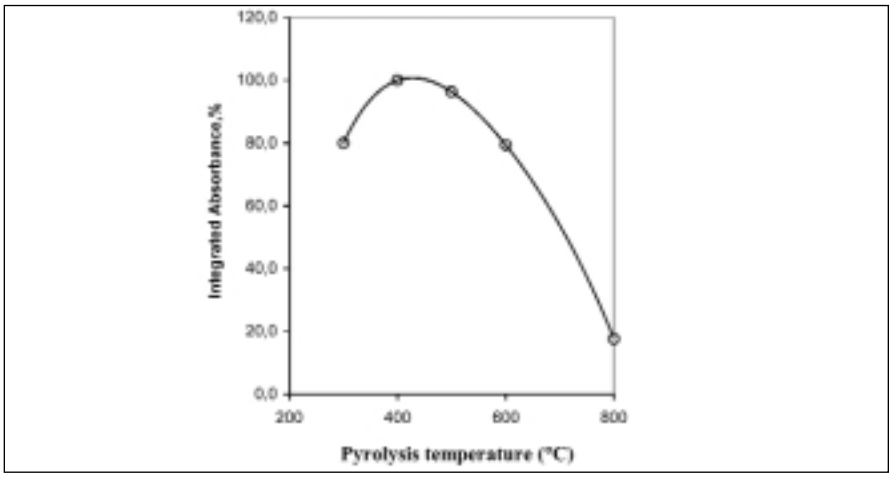

Fig. 3. Effects of pyrolysis temperature as recorded at the following conditions: 2.0 µg L^{-1} Bi^{3+}; sample flow rate 12 µL s^{-1}; buffer pH 3.7; sample loading time 200 s; holding time 40 s; and atomization temperature 1200ºC.

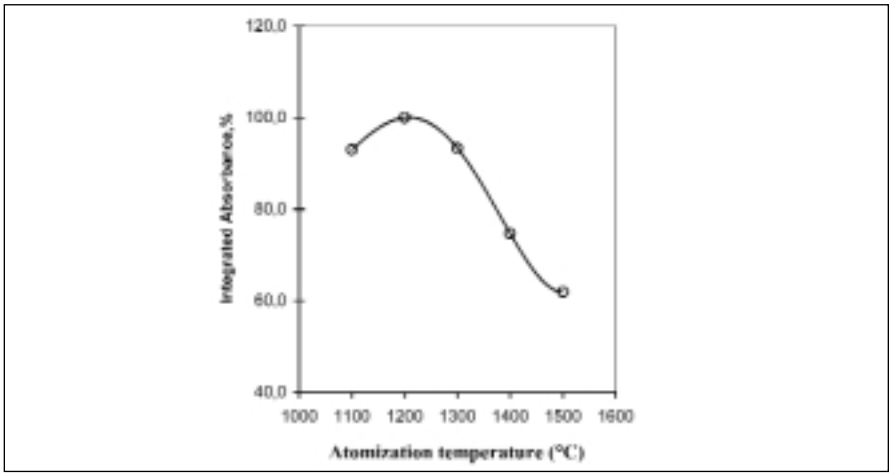

Fig. 4. Effects of atomization temperature as obtained at the following conditions: 2.0 µg L^{-1} Bi^{3+}; sample flow rate 12 µL s^{-1}; buffer pH 3.7; sample loading time, 200 s; pyrolysis temperature 400ºC; and holding time 40 s.

It is interesting to note that both the pyrolysis and atomization temperatures are lower than the recommended values (18). This might be attributed to the fact that no chemical modifiers were used in the present study.

Effects of Acidity and Ion Exchanger Forms

Studies of the effects of sample acidity showed that the enrichment efficiency of bismuth was optimal within a pH range of 3.0-4.8, using either the H^+ form or the K^+ form of the SP Sephadex C-25 resin. Furthermore, both forms yielded approximately similar enrichment efficiencies. The blank level for the K^+ form resin, however, was found to be about 0.05-0.06 integrated absorbance, which is much higher than that for the H^+ form resin, which gave values around 0.01 integrated absorbance. The H^+ form of the SP Sephadex C-25 resin was therefore used in the ensuing experiments, and the acidity of the sample was controlled by using a 0.10 M acetic acid-potassium acetate buffer solution at pH 3.7.

Effects of Eluent Concentration and Its Corresponding Volume

Nitric acid was used as the eluent since the present preconcentration procedure is aimed to be used later in conjunction with ICP-MS. Figures 5 and 6 show the effects of the concentration and the volume of the eluent, respectively. It is obvious from Figure 5 that the integrated absorbance increased rapidly when increasing the concentration of nitric acid up to 6.25% (1:16 v/v), but a further increase in the acidity resulted in very limited signal enhancement. It can also be seen from Figure 6 that by flowing 30 µL of 6.25% nitric acid through the micro column, the majority of the retained bismuth can be eluted (ca. 85%). Considering that the furnace can only safely accommodate this volume in one operative step, very little is gained by eluting the last 15%, because this would require a total of 50-60 µL of eluent.

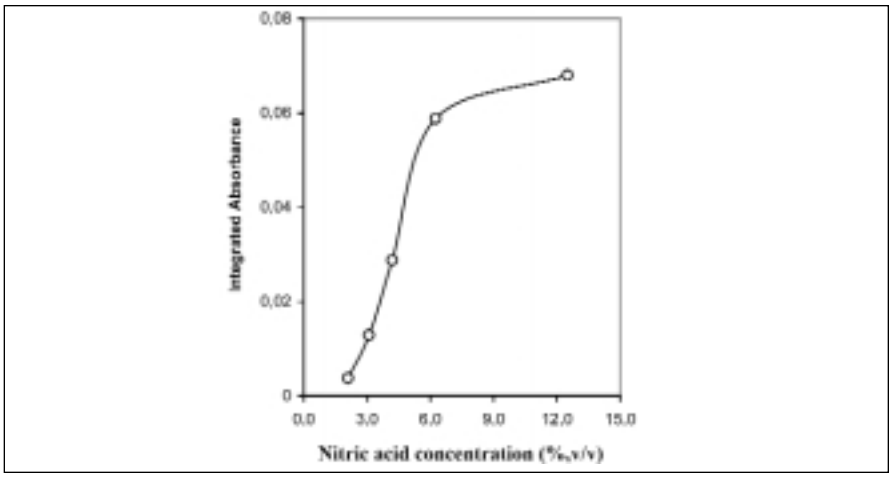

Fig. 5. Effects of eluent (nitric acid) concentration obtained at the following conditions: 2.0 µg L^{-1} Bi^{3+}; sample flow rate 12 µL s^{-1}; buffer pH 3.7; sample loading time 200 s; pyrolysis temperature 400ºC; and atomization temperature 1200ºC.

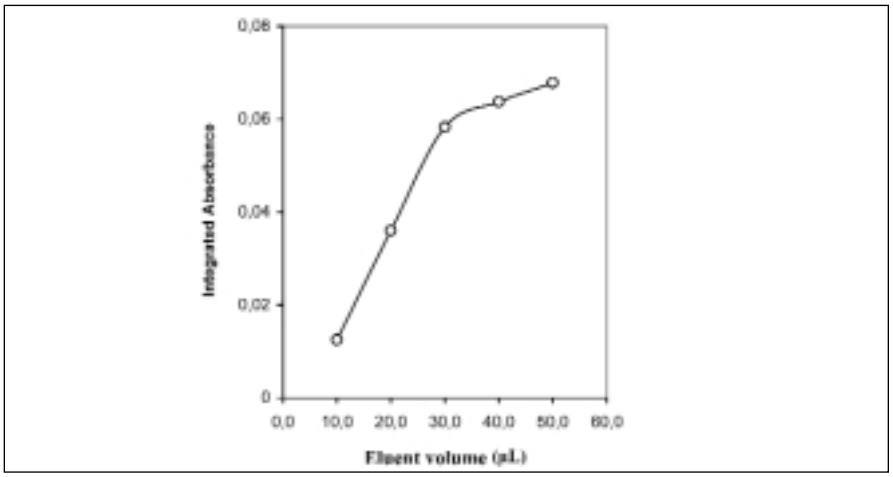

Fig. 6. Effects of eluent (nitric acid) volume obtained at the following conditions: 2.0 µg L^{-1} Bi^{3+}; sample flow rate 12 µL s^{-1}; buffer pH 3.7; sample loading time 200 s; pyrolysis temperature 400ºC; and atomization temperature 1200ºC.

Effects of Sample Loading Time, Sample Flow Rate, and Eluent Flow Rate

The experiments showed that the enrichment factor of bismuth increased linearly with increasing sample loading time. No breakthrough was observed by loading 2.4 mL sample within the concentration range of 0.10-3.0 µg L^{-1}. The sample loading time was fixed at 200 s, corresponding to 2.4 mL of sample, as a compromise between the sampling frequency and the enrichment efficiency.

It is very critical to control the sample flow rate within a certain range in order to obtain the optimal enrichment efficiency. The results indicated that the enrichment factor increased with the sample flow rate within a range of 4-12 µL s^{-1}, which is in contrast to the previous study for nickel preconcentration, where the enrichment efficiency was improved by decreasing the sample flow rate within the same

range (14). At higher flow rates, a slow decline in the enrichment factor was observed; yet at sample flow rates exceeding 30 µL s^{-1}, a very rapid drop was recorded. This is attributed to the fact that at high flow rates, the beads tend to become squeezed and lost via the space between the inner wall of the channel and the small piece of PEEK tubing. A sample flow rate of 12 µL s^{-1} was therefore chosen throughout.

The investigations on the effect of eluent flow rate indicated that the enrichment factor of bismuth was increased by decreasing the eluent flow rate, although the gain is not significant within the range of 4-16 µL s^{-1}. A substantial decrease of the signal was observed at higher flow rates exceeding 30 µL s^{-1} (loss of beads, as indicated above). An eluent flow rate of 8 µL s^{-1} was adopted for further studies.

Interferences

The potential interferences from substances or ions that might often be encountered in biological and environmental samples were studied.

The results showed that at a bismuth concentration level of 2.0 µg L^{-1}, and within a ±5% error range, 1.0 mg L^{-1} Co^{2+}, Cd^{2+}, and Mn^{2+}; 5.0 mg L^{-1} Cu^{2+}; 10.0 mg L^{-1} Zn^{2+}, Fe^{3+} and Pb^{2+} do not interfere with the determination of bismuth. The common matrix cations, such as Na^{+}, Ca^{2+}, and Mg^{2+} do not interfere up to a level of 2.3 g L^{-1}, 200 mg L^{-1}, and 120 mg L^{-1}, respectively. For most biological and environmental samples, the content of coexisting heavy metals will not exceed the concentration levels listed here; therefore, in most cases, no masking agent are actually needed.

Performance of Procedure

The characteristic performance data for the sequential bead injection on-line ion exchange preconcentration procedure for bismuth are presented in Table II. An enrichment factor of 33.4 was obtained in comparison with direct introduction of 20 µL sample solution into the graphite tube.

The procedure was validated by the analysis of a certified reference material (CRM 320, river sediment) and two human urine samples. The recovery tests for the urine samples were made by spiking the original sample solutions with 2.0 µg L^{-1} bismuth before digestion. The digest of the CRM 320 mentioned in the sample pretreatment section was determined directly, and the urine digests were further diluted by a factor of 10 before actual analysis. The results are listed in Table III.

CONCLUSION

On-line ion exchange separation and preconcentration, as facilitated by a sequential injection lab-on-valve micro system incorporating a renewable column, is demonstrated for the determination of low levels of bismuth with detection by ETAAS. As the ion exchange beads of the micro column can be renewed and discarded at will (after one or possibly several runs), the problems associated with long-term operations of conventional ion exchange columns can be effectively eliminated. Even when renewing the column for each sample cycle, the consumption of ion exchange material is minimal. This novel approach therefore promises to possess unique advantages in practical assays. As for the actual assay of bismuth, the detection limit obtained is one of the lowest encountered in the literature (6, 19-21).

TABLE II
Characteristic Performance for the SI Bead Injection On-line Ion Exchange Preconcentration for Bismuth

Linear calibration range	0.05-3.0 µg L^{-1}
Regression equation	$AA = 0.0698C_{Bi} - 0.0008$
Correlation coefficient	r = 0.9996
Sampling frequency	10 h^{-1}
Detection limit (3σ, n=7)	27 ng L^{-1}
Precision (R.S.D, n=7)	2.3 % (2.0 µg L^{-1})
Sample consumption	2.4 mL
Bead consumption	15 µL
Enrichment factor[a]	33.4

[a]The enrichment factor was obtained by comparing with direct introduction of 20 µL sample solution into the graphite tube.

TABLE III
Determination of Bismuth in CRM 320 Certified Reference Material and Human Urine Samples (n=3)

Sample	Indicative Value (µg g^{-1})	Found Value (µg g^{-1})	Spiked (µg L^{-1})	Recovery (%)
CRM 320	0.2 ± 0.5	0.29 ± 0.06		
Urine A[a]		1.04 ± 0.41	2.00	103.0
Urine B[a]		1.43 ± 0.34	2.00	96.3

The results were obtained at 95% confidence level.
[a] in µg L^{-1}.

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