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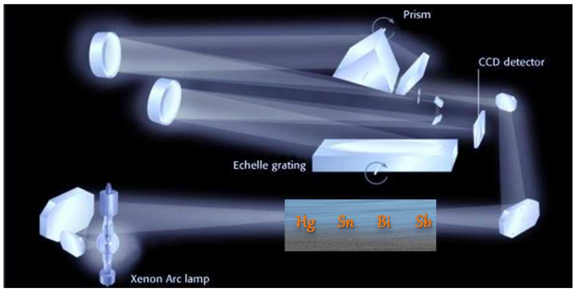

The multielement determination of Sb, Bi, Sn and Hg by the FI-SPE-CVG-CS-ETAAS system is rapid, sensitive, selective, automatic and green.

High resolution continuum source atomic absorption spectrometry and solid phase extraction for the simultaneous separation/preconcentration and sequential monitoring of Sb, Bi, Sn and Hg in low concentrations

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A rapid method was developed for separation, pre-concentration and sequential determination of antimony, bismuth, tin and mercury by flow injection solid phase extraction coupled with on-line chemical vapor generation electrothermal atomic absorption spectrometry. The system is based on chelating/cationic retention of the analytes onto a mini-column filled with a mesoporous silica functionalized with 1,5 bis (di-2-pyridyl) methylene thiocarbohidrazide. Several variables (sample flow rate, eluent flow rate, eluent concentration and reductant concentration) were considered as factors in the optimization process. Interactions between analytical factors and their optimal levels were investigated using three central composite designs. The optimized operating conditions were: sample pH=2.2, sample flow rate 3 mL min^{-1}, eluent flow rate 2.5 mL min^{-1}, eluent 3.1 % HCl for Sb, Bi and Sn, and 4.6 % thiourea for Hg, and reductant 0.6% $NaBH_4$. The optimum conditions established were applied to the determination of Sb, Bi, Sn and Hg in sea water and river water by flow injection solid phase extraction coupled with on-line chemical vapor generation high resolution continuum source electrothermal atomic absorption spectrometry (FI-SPE-CVG-CS ETAAS). For the quality control of the analytical performance and the validation of the newly developed method, the analysis of two certified samples, TM 24.3 and TMDA 54.4, Fortified Lake Waters were addressed. The results showed good agreement with the certified values.

Keywords: Antimony, Bismuth, Tin, Mercury, High resolution continuum source electrothermal atomic absorption spectrometry, Chemical vapor generation, Mesoporous silica, Water analysis.

1. Introduction

The trace elements are important environmental contaminants and their diffusion across the Earth has already been extensively studied on various environmental matrices.[1] Trace elements are released into the environment by a large number of natural mobilisation processes, and also during the last few centuries by a number of uncontrolled human activities.[2] A huge variety of environmental samples, such as marine and lake sediments, snow, and ice, have been analysed.[3] In our case, we have focused our attention on natural waters because of the importance of these matrices in the global transport of trace elements.[4,5] Among the trace elements with greater concern for the general public and therefore also for environmental agencies in the majority of states are Sb, Bi, Sn and Hg.[6] The monitoring and control of these trace elements in the environment and in food sources require processing large number of samples to accurately characterize their abundance and to reach reliable conclusions.

In this paper, a new ETAAS technique based on a high resolution continuum source (HR-CS-ETAAS) is used. The HR-CS-ETAAS represents the most significant advance experienced by this technique in various decades. [7] An advantage derived from the use of a continuum source is higher multi-element capabilities. This device is based on a high-pressure xenon short-arc lamp operating at brightness temperatures of approximately 10.000ºC capable of providing a high intensity in the visible and UV region, an optical system based on an echelle monochromator dispersing radiation in two steps (by using a prims first and an echelle grating afterwards) and a linear CCD array for detection.[8] This device provides significant advantages in comparison to traditional line source devices, such as improved signal stability, superior background correction potential, capabilities to monitor narrow molecular "lines" which permit the determination of non-metals, and improved linearity by making use of side pixels.[9-12]

Nevertheless, ETAAS has some disadvantages, high concentrations of matrices, such as Na, K, Mg and Ca, in sea water, can cause serious damage to the graphite furnace. One of the most used techniques for

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isolating trace of elements from liquid samples before analytical determination is solid phase extraction (SPE), especially when coupled to a flow injection (FI) system on-line. In the last years, mesoporous silica has been proposed as a novel SPE, these materials are characterized by their high surface areas, uniform and controllable pore sizes, and the periodic order of their pore packing.[13] The modification with organic chains has become a large focus of interest in such applications. The primary adsorption mechanism of ions to mesoporous silica is perceived to be surface complexation with functional groups. In this case, it can be avoided the problem of salt effect by providing selective extraction of such analytes.

Furthermore, chemical vapor generation (CVG) is other alternative technique to reduce salt effect on the analytical signal. The determination for hydride elements forming in several samples by HG-ETAAS has been thoroughly documented. The purpose for the present article was to find the critical variables affecting the Sb, Bi, Sn, and Hg FI-SPE-CVG-CS ETAAS procedure, for this, one needs to adjust some variables in order to establish optimum pre-concentration conditions for the determination of the analytes. A fruitful way to conduct experimental designs and optimization is according to surface-response methodology (SRM). This includes a group of mathematical-statistical techniques that were designed to optimize the analytical response by producing a model in which a response function corresponds to several variables called factors. In this work, four variables: concentration of eluent, concentration of reductant, sample flow rate and eluent flow rate were considered as factors in the optimization process. Interactions between analytical factors and their optimal levels were investigated using central composite designs (CCD).[8,14] On this way, a simple and sensitive method for determination of Sb, Bi, Sn and Hg in complex samples such as sea water and river water by FI-SPE-CVG-CS ETAAS has been developed. For the quality control of the analytical performance and the validation of the newly developed method, the analysis of two certified samples, TM 24.3 and TMDA 54.4, Fortified Lake Waters were addressed. The results showed good agreement with the certified values.

2. Experimental

2.1 Apparatus

All the experiments in this work were carried out using a HR-CS-ETAAS, ContrAA 700, commercially available from Analytik Jena AG (Jena, Germany) and equipped with both graphite furnace and flame atomizers. The optical system comprises a Xenon short-arc lamp (GLE, Berlin, Germany) operating in “hot-spot” mode as the radiation source, a high-resolution double echelle monochromator (DEMON) and a linear CCD array detector with 588 pixels, 200 of which are used for analytical purposes (monitoring of the analytical signal and BG correction), while the remainder are used for internal functions, such as correcting for fluctuations in the lamp intensity. More details on this type of instrumentation can be found elsewhere.[15] The HR CS ETAAS instrument is equipped with a transversely heated graphite tube atomizer and both solid sampling and liquid sampling autosamplers.

A four-channel peristaltic pump (Minipuls 3, Gilson, Villiers le Bel, France) was used for the continuous-flow vapour system with a Rheodyne Type 50 six-port rotary valve and a gas-liquid separator. Schematic diagrams of the continuous flow systems used for determination of the different CV forming elements studied are shown in Figure 1.

The mini-column was a glass tube (3 cm×3mm i.d.), packed with the chelating resin to a height of 0.7cm. At both ends of the mini-column, polyethylene frits (Omnifit, Cambridge, UK) were fixed to prevent material losses. The mini-column was initially flushed with 2 M nitric acid; subsequent use of the eluent in each operating cycle was sufficient to make it ready for re-use. Tygon pump tubings were used to deliver sample, reagents and withdraw waste. The reaction coil and connections were made of 0.8 mm i.d. PTFE tubing. The element vapours generated in the reactor was transported to the graphite tube through a PTFE transfer line connected to a gas/liquid separator with a PTFE membrane of 0.5 μm pore diameter. The FI system and the ETAAS instrument were coupled and operated completely synchronously.

Argon with a purity of 99.99 % was used as the carrier gas for vapours and as the purge and the protective gas for the graphite atomizer.

2.2 Reagents and samples

High purity reagents were used in all experiments. All plastic and glassware were cleaned with hot concentrated nitric acid and stored soaked in 10% (wt/wt) nitric acid, they were rinsed several times with water immediately before use. Doubly de-ionized water (18 MΩ cm) obtained from a Milli-Q water system (Millipore, Bedford, MA, USA) was used throughout.

The mini-column was packed with 1,5 bis (di-2-pyridyl)methylene thiocarbohidrazide bonded to mesoporous silica (DPTH-ms). The synthesis and

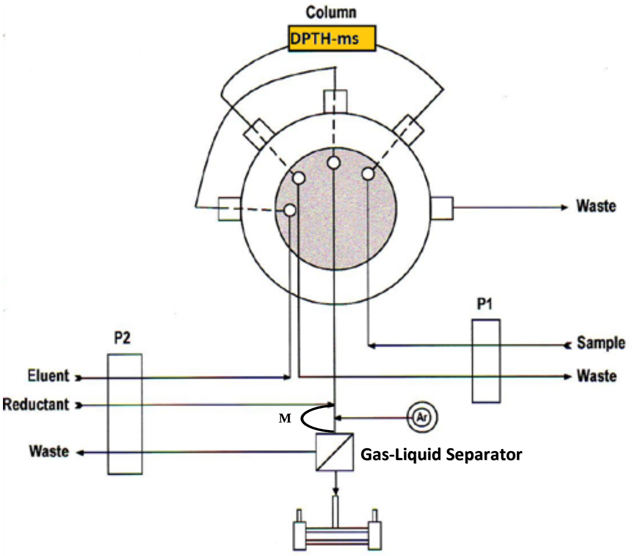


Figure 1. Schematic diagram of FI system for the pre-concentration and separation of antimony; P1 and P2, peristaltic pumps; M, mixing coil and Ar, stream of argon.

characterization of this resin was described in the paper published previously.[16]

Sb (III), Bi (III), Sn (II) and Hg (II) stock standard solutions 1000 mg L^{-1} (Merck, Darmstadt, Germany) were used. A standard 1000 mg L^{-1} Ir(III) solution (Perkin Elmer pure, Atomic Absorption Standard, Darmstadt, Germany) was used to coat the graphite furnace platform. A pH 2.2 buffer was prepared by mixing 25 mL of 0.2 M glycine (Merck, Darmstadt, Germany) with 22 mL hydrochloric acid 0.2 M (Merck, Darmstadt, Germany) and dilute to 100 mL with de-ionized water. A 0.6 % (wt/vol) sodium tetrahydroborate (III) ($NaBH_4$) (Merck, Darmstadt, Germany) solution prepared in 0.1% (wt/vol) NaOH (Merck, Darmstadt, Germany) was used as reductant and a 4.6 % (wt/vol) thiourea (Merck, Darmstadt, Germany) and 3.1 % (wt/wt) hydrochloric acid were used as eluents, in order to elute Hg and Sb, Bi and Sn, respectively.

The certified reference materials (CRMs) analyzed to determine the accuracy of the proposed procedure were from National Research Council of Canada (NRCC): TMDA 54.4 and TM 24.3, Fortified Lake Waters. In order to study the applicability of the method, sea and river water samples were collected in polypropylene bottles (previously cleaned by soaking for 24 h in 10 % (wt/wt) nitric acid and finally rinsed thoroughly with ultrapure water before use). Samples were immediately filtered by using a membrane of 0.45 µm pore size cellulose nitrate filters from Millipore (Bedford, MA, USA). After that, the samples were acidified to 0.1% (vol/vol) by the addition of concentrated HNO_3 and were stored in low density polypropylene bottles at 4º C as recommended by Method 3010B from the Environmental Protection Agency (USA), for less than 3 days until analysis. For the analysis of these samples, aliquots of 20 mL of sample were placed in volumetric flasks of 25 mL, then 2.5 mL of buffer pH 2.2 and de-ionized water were added up to the mark.

2.3 Treatment of the graphite tube with a permanent modifier

Pyrolytically coated graphite tubes were pretreated by injection 50 µL of a 1000 mg L^{-1} Ir standard solution into the tube, and submitting it to a temperature program based on a device program.

Each injection was dried slowly by heating the atomizer at 100 ºC with a ramp rate and hold times of 2 ºC s^{-1} and 170 s, respectively. Then, thermally treated at 1200ºC with a ramp rate and hold times of 37 ºC s^{-1} and 30 s was used. Following this, a reduction step at 2150ºC was applied during 20 s. This procedure was repeated 21 times, resulting in a total mass of 1050 µg of the modifier deposited on the tube graphite wall. A tube treated in this manner can pass through about 500 firing cycles.

2.4 Pre-concentration and elution procedure

The FI manifold used for on-line pre-concentration and elution is shown in Figure 1. The FI system was operated as follows: during the 60 s sample loading period, valve in the "fill" position, a 3.0 mL min^{-1} flow of sample (standard or blank) at pH 2.2 was pumped (via pump P1) through the mini-column (located in the loop of the valve). The Sb, Bi, Sn and Hg analytes were retained on the sorbent mini-column while the sample matrix components were directed to waste.

At the beginning of the 45 s elution stage, the valve position was changed to the inject position and the sample pump P1 was stopped. The eluent passes through the mini-column. Thus, the accumulated target analytes were eluted at an elution rate of 2.5 mL min^{-1} and merged with 2.5 mL min^{-1} flow of reductant flow in the mixing coil, M, where direct generation of antimony, bismuth, tin and mercury CV take place. The gas generated and the solvent were then passed into the gas-liquid separator; the liquid was drained and the vapour is swept into the graphite furnace by a stream of argon (100 mL min-1) during 45 s. With this procedure, the FI system and the ETAAS instrument were coupled and operated completely synchronously. Peak areas were used for analytical measurements. The furnace temperature

Table 1. Optimized furnace program used for Sb, Bi, Sn and Hg determination.

Sb (217 nm)

Step	Temperature /ºC	Ramp time /ºC s^{-1}	Hold time /s	Argon flow rate /L min^{-1}
Conditioning	300	300	2.0	0
Drying	305	50	26.0	2
Atomization	2200	1000	7.0	0
Cleaning	2200	1000	4.0	2

Bi (223 nm)

Step	Temperature /ºC	Ramp time /ºC s^{-1}	Hold time /s	Argon flow rate /L min^{-1}
Conditioning	300	300	2.0	0
Drying	305	50	26.0	2
Atomization	2000	1000	5.0	0
Cleaning	2150	500	4.0	2

Sn (286 nm)

Step	Temperature /ºC	Ramp time /ºC s^{-1}	Hold time /s	Argon flow rate /L min^{-1}
Conditioning	300	300	2.0	0
Drying	305	50	26.0	2
Atomization	2250	600	7.0	0
Cleaning	2250	1000	4.0	2

Hg (253 nm)

Step	Temperature /ºC	Ramp time /ºC s^{-1}	Hold time /s	Argon flow rate /L min^{-1}
Conditioning	150	300	2.0	0
Drying	150	0	26.0	2
Atomization	900	800	7.0	0
Cleaning	2100	1000	5.0	2

program for each element determination is shown in Table 1.

2.5 Optimization strategy

There are a large number of parameters that are relevant to the optimization, which might be classified as parameters relating to: (1) elution of Sb, Bi and Sn in the manifold and reaction conditions for CVG (reagent concentrations); (2) elution and reaction conditions for mercury (reagent concentrations); and (3) FI parameters of the four elements.

In this work, three rotable uniform central composite designs (CCDs)[14,17] were performed giving three response surfaces, one for each type of parameters. In design (1) the variables to be optimized were concentrations of $NaBH_4$ and HCl. The lower and upper values given to each factor were 0.1 and 1.0 % for $NaBH_4$ concentration and 2.0 and 5.0 % for HCl concentration. In design (2) the variables to be optimized were thiourea and $NaBH_4$ concentrations for elution and Hg cold vapour generation. The lower and upper values given to each factor were 1.0 and 4.0 % for thiourea concentration and 0.1 and 1.0 % for $NaBH_4$. In design (3) all variables commented above were kept constant at the optimum values found previously and the sample and elution flow rates were optimized. The lower and upper values given to the factors were 1.0 and 5.0 mL min^{-1} and 1.0 and 3.0 mL min^{-1} for elution pump and sample pump, respectively.

The CCDs used in this investigation included a total of 2^k+2k+n runs, where k is the number of studied factors, (k=2, in the three designs), 2^k are the points from the factorial experiments carried out at the corners of the cube and $2k$ are the points carried out on the face centered star. The repetition of the center point was used to estimate the experimental error (n=2, 2, 3 in design 1, 2 and 3, respectively). The resulting 10, 10 and 11 experiments were randomly performed.

The experimental data were processed by using the STATGRAPHICS program (version 16.1.11 for Windows).[18] For CCD, the significance of the effects was checked by analysis of the variance (ANOVA) and using *p*-value significance levels. This value represents the probability of the effect of a factor being due solely to random error. Thus, if the *p*-value is less than 5% the effect of the corresponding factor is significant.

3. Results and discussion

The determination of trace elements in aqueous environmental samples is difficult not only due to the low analyte concentrations present but also due to the complexity of the samples. Thus, the proposed on-line column pre-concentration CVG procedure describes the simultaneous separation and pre-concentration of the target analytes from the matrix before quantification by ETAAS. Therefore, several problems related to interfering species were decreased or eliminated. For this purpose a chelating resin, DPTH-ms, synthesized by us,[16] was used. This chelating resin has good chemical properties as a general chelating reagent, with good adsorption capacities, is stable over a wide pH range (2.0-11), besides being stable for a very long time, and the mini-column has a practically unlimited lifetime without the necessity for regenerations, hence, the material can be stored and applied conveniently.

3.1 Optimization of the experimental variables

The configuration of the FI-system and the effect of different parameters on the accumulation and recovery of Sb, Bi, Sn and Hg were studied in order to optimize conditions for achieving the best results. For this purpose, most efforts were focused on the conditions for sample loading and Sb, Bi, Sn and Hg elution from the mini-column, as well as the flow system which was coupled on-line with the pre-concentration and separation unit in order to obtain highly sensitive, accurate and reproducible results. For measurements to be useful, it was considered that a relative standard deviation (R.S.D.) of about 5 % was acceptable. The best signal-to-noise (S/N) ratios between a blank and a standard solution of 5.0 µg L^{-1} Sb (III), Bi (III), Hg(II) and 10.0 µg L^{-1} Sn, were chosen as the optimization criterion. Peak areas were used for analytical measurements. The scanning of each sample was repeated four times. As has been described above, central composite designs were used for most of the variables optimization; however, a one-at-a-time method was also used for optimization of some variables.

3.1.1 Optimization using one-at-a-time method

Furnace program. The trapping time onto the iridium coated graphite tube for Sb, Bi, Sn and mercury CV was investigated; the results obtained showed that the signal increased with the trapping time up to 45 s, above this time the signal remained constant. This indicates that this time is necessary to elute the target analytes from the minicolumn and to ensure that all of the Sb, Sn, Bi and Hg CV had been stripped from the solution and transferred into the atomizer. Hence, the above-mentioned trapping time was selected for subsequent experiments.

The atomization temperatures (using hold times between 5 and 8 s depending of the element) were investigated. This temperature was varied in experiments conducted with a 5.0 µg L^{-1} solution of each analyte, while running a blank in parallel. Thus, for Sb, with a hold time of 7s, the temperature was varied over the range 2000-2200 ºC, the optimum atomization temperature was determined to be 2200 ºC because at lower temperature, broader peaks were observed; for Bi, the temperature was varied over the range 1900-2100 º C, and the optimum was 2000ºC, with a hold time of 5s; for Sn the temperature was studied between 2150 -2250 ºC, and the optimum temperature was 2250ºC with a hold time of 7s, and for Hg, the temperature was varied over 800-1000ºC, being the optimum temperature 900ºC and a hold time of 7s.

The effects of the conditioning temperature and hold times, on the efficiency of trapping analytes onto the coated graphite tubes were also examined and it was found that these parameters have little influence on the analytical signal within the ranges 20 - 420 ºC and 1 - 20 s, respectively. The optimized conditions for trapping onto Ir-coated graphite tubes and atomization of the vapors generated are shown in Table 1.

Effect of pH on the collection of the analytes. Since the solution pH affects the extent of complexation with DPTH-ms, which in turn determines the percentage of analyte retained by the resin, the pre-concentration of traces of Sb, Bi, Sn and Hg ions from solutions buffered at different pH was studied. The pH was varied between 2.0 and 9.0. The pH from 2.0 to 5.0 was adjusted using glycine-HCl or sodium acetate-acetic acid buffer and from 5.0 to 9.0 using borax-boric acid buffer. As can be seen in Figure 2, in order to accomplish the sequential determination of the target elements, a pH value of 2.2 was chosen as overall optimum.

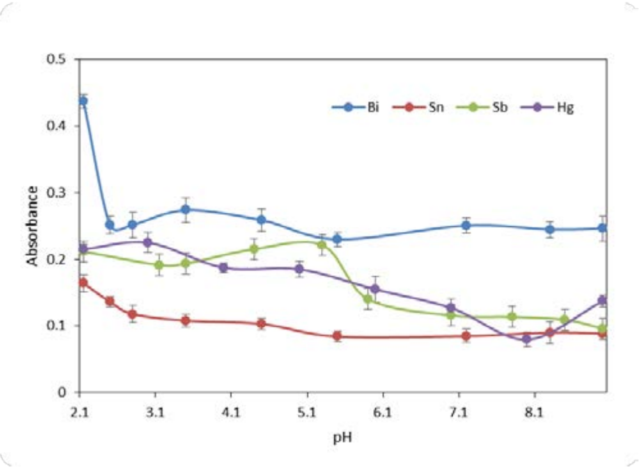


Figure 2. Influence of pH on the pre-concentration of antimony, bismuth, tin ad mercury (area peak).

Selection of eluent. In order to minimize the time needed for quantitative elution of the analytes, the selection of a suitable eluent is very important. Strong acids are effective in dissociating complexes and releasing free metal ions. Furthermore, CVG is most frequently facilitated in acidic medium. For this reason, hydrochloric acid and nitric acid were tested as eluents, also thiourea (which form a very stable complex with Hg) was tested. In all experiments, the eluent was passed through the mini-column in a reverse flow relative to the pre-concentration step. The results obtained showed that HCl was capable of removing the adsorbed analytes (Sb, Bi and Sn) from DPTH-ms resin better than HNO$_3$ and moreover, it provides the highest S/N ratios. Thiourea was the eluent which offered the best mercury signal.

3.1.2 Optimization using factorial designs

Three independent experimental designs were developed in order to obtain the best conditions to determine the lowest concentrations of the analytes studied.

Effect of NaBH$_4$ and HCl on elution and Sb, Bi and Sn CVG. The concentrations of NaBH$_4$ and HCl were considered to be dependent variables and the effects of these were studied by applying a CCD involving 10 runs in order to obtain the optimum conditions. The three dimensional representation is presented in Fig. 3. The data indicate that interactions usually occur between principal factors. This means that the response surfaces in the factorial space are curved in the domain of the experimental design. As result of these observations, the optimum conditions for each element and for the multiple response of the three target analytes, were obtained (Table 2). However, due to the fact that tin signals are the lowest, and in order to improve the sensitivity for this element, the Sn optimum conditions were chosen as working conditions. Hence, as results of all these experiments, the following Sb, Bi, Sn optimum working conditions were chosen: 3.1 % (wt/wt) HCl and 0.6% (wt/vol) NaBH$_4$.

Effect of NaBH$_4$ and Thiourea on elution and Hg CVG. The second CCD involving 10 runs was performed in order to obtain the optimum conditions with the two continuous variables found to be potentially significant for Hg determination. The variables to be optimized were: eluent concentration and reductant concentration. The response surface has been represented in Figure 4a.

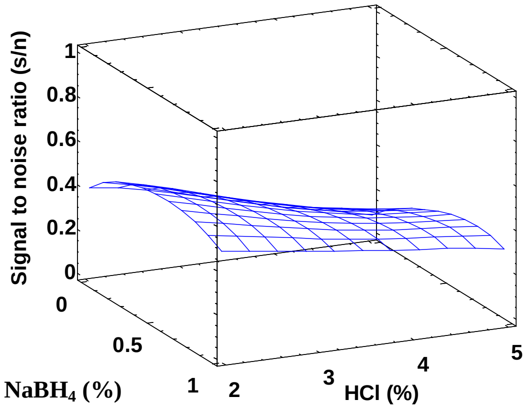


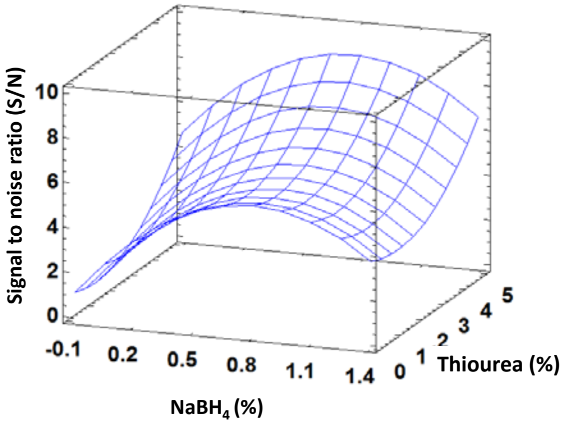
Figure 3. Response Surface of the three analytes Sb, Bi and Sn.

Table 2. Optima conditions for each target analyte

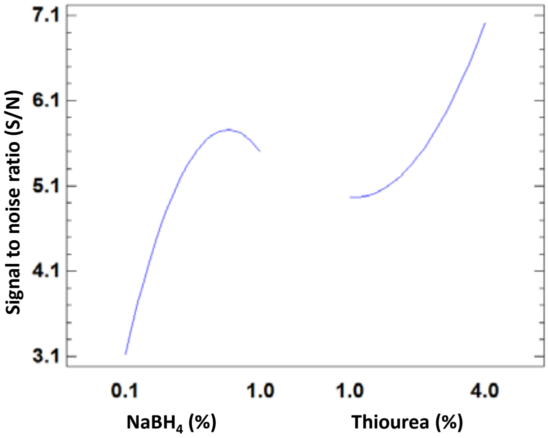
Element		Optimum conditions		
		Eluent (HCl) /wt/wt	Eluent (Thiourea) /wt/vol	Reductant (NaBH$_4$) /wt/vol
Sb	Single response	1.00	0.00	0.10
Bi		1.15	0.00	1.30
Sn		3.10	0.00	0.60
Multiple response (Sb, Bi, Sn)		1.00	0.00	0.54
Hg		0.0	4.62	0.77

Furthermore, in Figure 4b, the ANOVA results produced the graphs showing the influence of main effect. It can be deduced that the two factors were significant. This representation showed that the signal increased when NaBH$_4$ concentration increased up to 0.77 % (wt/vol), and the concentration of thiourea increased, at least, up to 4.0 %. From this study, one deduces the necessity of continuing the optimization of the thiourea concentration. It was obtained that the optimum thiourea concentration was 4.6 %. A concentration of thiourea higher than 4.6 % produces larger back pressures from the system due to the deposit of this reagent inside the tubes and mini-column, and consequently, making the system less favorable to the Hg determination. As results of all these experiments, the following Hg optimum working conditions were obtained: 0.77 % (wt/vol) NaBH$_4$ and 4.6 % thiourea (wt/vol). However as it is commentated in order to improve Sn signals (which have the less sensitivity) when the sequential determination of the four analytes takes place a 0.6 % NaBH$_4$ solution was selected.

In order to maintain the stability of the whole system during CVG and optimize the



(a)



(b)

Figure 4 Graphs showing (a) Response Surface of the Hg; (b) Influence of the main effect on Hg hydride signal. The lines indicate the magnitude and sign (increase or decrease) of the variation of the area peak with the factor level.

performance of the overall system, it is mostly desirable to employ a single HCl concentration that is favorable to both the retained analyte elution from the column and the CVG process. For this purpose, the HCl concentration used was carefully considered, and the results showed that a HCl concentration of 3.1 % (wt/wt) can, to a large extent, fulfil both purposes. Thus, a 3.1 % HCl solution was employed for carrying the eluate/concentrate to meet the alkali $NaBH_4$ solution and facilitate the ensuing CVG.

On the other hand, the experimental results showed that when employing a fixed $NaBH_4$ concentration (0.6%), an improvement in the S/N ratios was observed with the increase of NaOH concentration up to 0.1% (wt/vol), while afterward a drop of the signals was recorded with further increase of the NaOH concentration when exceeding 0.1%. The reason for this observation might be attributed to the fact that the reduction by $NaBH_4$ is preferentially performed in acidic medium, the employment of NaOH solution is simply to stabilize the $NaBH_4$ by avoiding its adverse reaction with water during the storage. The excessive increase of NaOH concentration will definitely decrease the acidity of the reaction mixture and hence, the reaction rate of CVG. Besides, the heat generated by the neutralization between H^+ and OH^- will very likely also contribute to the reaction and thus have certain influence on the recorded signals. For further experiments, a NaOH concentration of 0.1% (wt/vol) was adopted throughout.

Selection of FI variables. The third CCD involving 11 runs was used in the optimization of the FI parameters for the all four analytes. First, the flow rates of the samples and eluent were varied by changing the speed of the peristaltic pumps (P1 and P2). The lower and upper values given to these factors were 1 and 5 mL min^{-1} for P1, and 1 and 3 mL min^{-1} for P2, respectively. This study was made using 1.14 mm id Tygon peristaltic pump tubing. The three dimensional representation is showed in Figure 5, as can be seen, the optimum conditions were: sample flow rate 3.0 mL/min and eluent flow rate 2.5 mL/min.

It is well known[19] that, in general, CVG of classical chemical vapor forming elements is negatively affected by the presence of some transition and noble metals that may produce severe interferences. For this reason the influence of the mixing coil length was examined to ensure homogeneous mixing of the sample and $NaBH_4$ solution and to ensure short residence time of the CV of Sb, Bi, Sn and Hg in the reaction medium. For this purpose Tubing coils (1.0 mm id) of different lengths (5-20 cm) were tested. It was found that CV of the elements were successfully generated even by using a 5cm long tubing. The coil length selected was 8 cm. This ensured both homogeneous mixing of the elements and $NaBH_4$ solutions and as well as a short residence time of the vapors in the reaction medium, thus minimizing their contact with the nascent transition metals or their borides by immediate separation of the formed vapors from the reaction medium. With this action the signals for the elements were increased and thus the slope of the calibration curves.

On the other hand, in time-based pre-concentration systems the loading time indicates the pre-concentration time and affects directly the enrichment factor. Thus, the loading time was investigated in the range between 30 s and 540 s for each analyte, using the optimum conditions described above. The absorbance was practically linear up to 540s, 540s, 420s and 360s for Bi, Hg, Sb and Sn, respectively. Although sensitivity increases on increasing the sample loading time, the loading time was set at 60 s in order to achieve high sample frequency with a reasonable sensitivity. Longer loading times can be used for samples with low concentrations of Sb, Bi, Sn and Hg. Also, preliminary tests showed that the sample volume was a not important factor when the mass of analyte arriving at the mini-column was kept constant.

3.2 Performance of the Method

Under the optimum conditions described above, performance data of the on-line SPE-HG-FI-CS ETAAS system for sequential determination of antimony, bismuth, tin and mercury were obtained. For 1 min pre-concentration time and a sample flow rate of 3 mL min^{-1}, the linear calibration graph from 0.025 μg L^{-1} - 2.5 μg L^{-1} of each analyte are shown in Table 3. A complete cycle of the FI-operation is ca. 120 s per analyte, as the HR-CS-ETAAS permits the sequential determination of the analytes with the same source, the four analytes in a sample can be determined with a throughput of about 7.5 h^{-1}.

The detection (LOD) and quantification limits (LOQ), defined as 3s (n= 11) and 10s (n =11) respectively; the precision for aqueous standards, evaluated as the relative standard deviation obtained after analyzing a standard of 1 μg L^{-1} of Sb, Bi, Sn and Hg (11 replicates); and the enrichment factor EF, defined as the ratio of the slopes of the linear section of the calibration graphs with and without pre-concentration (changing the mini-column by another unfilled) are shown in Table 3. The EF and determination limit can be improved by increasing the pre-concentration time which can be increased at least up to 6 min.

Although is difficult to compare the figures of merit for the developed method directly with results from other workers, because of different experimental conditions such as column dimensions, sample flow rate, etc., some estimations can be made. A comparison of the precisions, detection limits and recoveries of the DPTH-ms procedure with some of the reported ones with detection by various spectrometric techniques are summarized in Table 4. As can be seen, generally, the detection limit for Sb and Bi and the precision of the developed method were better that those reported in the literature. The obtained recoveries for analytes were also comparable to or better than other proposed methods.

3.3 Analytical applications

The accuracy and applicability of the proposed method to the analysis of environmental water samples were studied by examining two certified reference materials, CMR TMDA 54.4 and TM 24.3 Fortified Lake Waters, and sea and river water samples collected locally. The certified sample solutions were quantified by external calibration, by comparing the net signals against those of multi-element standards. For this purpose, calibration plots were constructed from aqueous standard solutions containing the four target analytes within the concentration ranges of 0.2 – 1.5 μg L^{-1} for Bi, 0.5 - 1.5 μg L^{-1} for Sb, 1.0 -3.0 μg L^{-1} for Sn and 0.5 -3.0 μg L^{-1}for Hg. Correlation coefficients of 0.99 or

Table 3. Analytical performance

Figure of merits	Sb	Bi	Sn	Hg
Enrichment factor	4	18	7	9
Detection limit/ μg L^{-1}	0.009	0.001	0.18	0.17
Determination limit/ μg L^{-1}	0.17	0.16	0.47	0.47
Precision /%	2.4	2.0	2.2	1.9
Calibration functions	y=0.2216x+0.0469	y=0.3621x+0.1298	y=0.0478x+0.0476	y=0.0923x+0.0575
Correlation coefficients	0.999	0.993	0.992	0.997
Blanks/peak area	0.034±0.005	0.106±0.008	0.050±0.002	0.061±0.004

Table 4. Comparison of analytical performance data with other data reported in the literature.

Sample	Technique	Pre-concentration technique or sorbent	Analytes	Detection limit / µg L^{-1}				Precision / %	Recovery /%	Ref.
				Sb	Bi	Sn	Hg			
Atmospheric particulate matter	HG-ETAAS	Quartz fiber filters (PM_{10}, $PN_{2.5}$)	As, Bi, Sb	0.030	0.015	0.041	--	5.3-9.1	36-96	29
Sea water and estuarine water	ICP-MS	Fe-PDC	Sb, Bi, Sn and 39 more elements	0.042	0.0039-0.0050	0.022-0.0023	--	2.64-16.9	67.07-104.6	30
Standard solutions	HG-ETAAS	Headspace single drop microextration	As, Sb, Bi, Ge, Sn, Se, Te, Hg	0.14	0.13	1.62	0.21	1.1-6.8	--	31
Fortified lake water	HS-SDME and ETV-ICP-MS	--	As, Sb, Bi, Pb, Sn, Hg	0.04	0.01	0.09	0.8	4.6-8.2	82.84-103.58	32
Biological and environmental materials	HP-MIP-AES	--	As, Bi, Ge, Sb, Se, Sn, Hg, Ca, Fe, Mg, Mn, Zn	0.10	0.29	0.14	--	9.0-10.0	91.3-98.8	33
Alloy, sediments river water coal fly ash garlic, soil river water	HG-IAT-FAAS	Atom trapping or silica tubes trap	Ge, Sn	--	--	8.00	--	8.0-10.0	95-104	34
Fortified lake water and sea water	FI-HG-ICP-MS	DPTH-gel	Sb, Bi, Sn	0.011	0.002	0.142	--	1.1-1.5	97.0-107.5	35
Whole blood	ICP-MS		Sb, Bi, Mn and Zn	1	1	--	--	4.9-10.7	96-104	36
Whole blood	ICP-QMS		Bi, Sb, Hg and others	0.04 0.02	0.04	--	0.01	<11	96.7-109.1	37
Cosmetic lotion and certified cosmetic cream	VG-ICP-MS	slurry	Ge, As Cd, Sb, Hg and Bi	0.002	0.0006	--	0.003	<18	--	38
Biological and environmental materials	USN/MSIS-MIP-OES	--	Bi, Sb, Sn, Hg and others	0.5	1.5	0.6	0.8	5-7	--	39
Real biological and environmental materials	MIP-OES	--	Bi, Sb, Sn, Hg and others	16	22	20	15	3-5	--	40
Fortified lake water, river and sea water	FI-HG-ETAAS	DPTH-ms	Sb, Bi, Sn and Hg	0.009	0.001	0.18	0.17	1.9-2.4	91.2-103.1	This work

better were obtained for all graphs, indicating good linearity for the working ranges aforementioned. The results, as the average of three separate determinations, are shown in Table 5. No significant differences were observed, for *p*=0.05 when comparing the values obtained by the proposed method and the certified values (t-test for a 95 % confidence interval). Thus, recovery values from 91.2 to 103.1 % were obtained. Due to the fact that these standard reference samples have included trace elements such as transitions metals, it can be said that there is no interference from these metals at ng mL^{-1} concentrations.

For the sea water and river water collected locally, an alternative verification process using the method of standard additions was conducted also a recovery study was developed on spiked samples containing known additions of Sb, Sn, Bi and Hg. From the results in Table 5, it was found that recovery values from 94.6 to 110.0 % of the metals added to water samples were obtained. The accuracy achieved for the spiked samples demonstrated that the method is not affected by high salinity (approximately 35 g L^{-1} in sea water). In addition, no significant interferences were observed in the analysis of these samples from the transition metal or CV forming elements and consequently offers accurate sequential determination of analytes in sea water and river water samples. On the other hand, the concentrations found are well within the normal concentration ranges for the target elements in these samples.[5,32]

4. Conclusions

In developing a method for a sequential analysis procedure, one needs to adjust some variable in order to establish optimum conditions for the determination of Sb, Bi, Sn and Hg. The design of experiments has proved to be an excellent tool to optimize process in order to find the main factors affecting the CVG efficiency of the analytes studied. In this study has been able to establish the optimum conditions for the proposed procedure by using several CCD designs.

The pre-concentration and separation system using DPTH-ms as a sorbent material has been evaluated and demonstrated to be promising for routine determination of the trace amounts of Sb, Bi, Sn and Hg in sea and river water samples. Furthermore, the HR-CS ETAAS instrument has permitted the sequential determination of the four elements, saving time of analysis and achieving a sample throughput of about 7.5 h^{-1}.

The method proposed in this work is rapid, easy to use, automatic and selective with a good sensitivity

Table 5. Analytical applications

	Certificate / µg L^{-1}				Added / µg L^{-1}				Found^a / µg L^{-1}				Recovery / %			
Samples	Sb	Bi	Sn	Hg	Sb	Bi	Sn	Hg	Sb	Bi	Sn	Hg	Sb	Bi	Sn	Hg
TMDA 54.4	25.7±0.5	17^b	48.2±1.0	--	--	--	--	--	26.500±0.001	15.50±0.04	49.25±0.17	21±1	103.1	91.2	102.2	--
TM 24.3	3.36±0.03	2.37±0.1	3.72±0.05	--	--	--	--	--	3.38±0.09	2.33±0.04	3.47±0.09	3.54±0.02	100.6	98.3	93.3	--
Sea water					0	0	0	0	0.320±0.003	0.16±0.02	1.150±0.003	< LOD^c	--	--	--	--
					1	1	1.5	2.08	1.30±0.08	1.26±0.05	2.57±0.02	2.01±0.02	98.0	110.0	94.6	99.5
					1.5	1.5	2	3.16	1.89±0.02	1.65±0.03	3.12±0.03	3.08±0.18	104.6	99.3	98.5	99.3
River water					0	0	0	0	0.02^b	0.003^b	< LOD^c	< LOD^c	--	--	--	--
					0.5	1	1	1	0.53±0.01	1.05±0.07	1.08±0.04	1.0±0.5	102.0	105.0	108.0	100.0
					0.75	1.5	1.5	1.5	0.76±0.03	1.49±0.17	1.56±0.07	1.56±0.07	98.6	99.1	104.0	104.0

[a] Mean ± SD for three replicate measurements of three individual preparations. [b] Indicative value. [c] Below detection limit

(compared with other methods) for the determination of Sb, Bi, Sn and Hg. The method is a relatively inexpensive approach and can be considered as a green analytical method because requires low volume of sample and reagents, reducing the residue production in the laboratory which reaches figure of merit equivalent to ICP-MS. Furthermore, the FI-SPE-CVG-CS ETAAS system has the advantage of being able to be fully automated without complicated hardware and software; in fact modification of the software of the spectrometer and the use of additional computers were not necessary.

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