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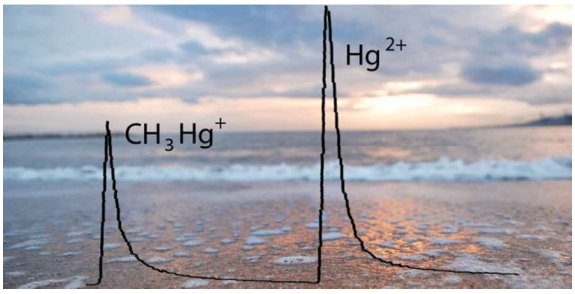

The FI-CV-ICP-MS system using PMTH-mesoporous sorbent has demonstrated to be promising for routine determination of mercury species in sea-water samples

Use of a new enrichment nanosorbent for speciation of mercury by FI-CV-ICP-MS

I. Sánchez Trujillo, E. Vereda Alonso*, J.M. Cano Pavón, A. García de Torres

A new enrichment nanosorbent based on mesoporous silica functionalized with 1,5 bis (2-pyridyl) methylene thiocarbohidrazide was synthesized and characterized. From the study of its adsorption capacity toward metal ions, Hg^{2+} was observed to be one of the most retained 173.1 µmol g^{-1} at pH 5. Thus, a flow injection solid phase extraction and cold vapor generation method for its determination and speciation based on the use of this new chelating nanosorbent was optimized. The method developed has showed to be useful for the automatic pre-concentration and sequential determination of mercury and methylmercury in environmental and biological samples. The system was based on chelating retention of the analytes onto a mini-column filled with the new nanosorbent and their sequential elution by using two different eluents, 0.2 % HCl for CH_3Hg^+ and 0.1 % thiourea in 0.5 % HCl for Hg^{2+}. The determination was performed using inductively coupled plasma mass spectrometry. Under the optimum conditions and 120 s preconcentration time, the enrichment factors were 4.7 and 11.0, the detection limits (3σ) were 0.002 and 0.004 µg L^{-1}, the determination limits (10σ) were 0.011 and 0.024 µg L^{-1}, and the precisions (calculated for 10 replicate determinations at a 2 µg L^{-1} standard of both species) were 2.8 and 2.6 % (RSD); for CH_3Hg^+ and Hg^{2+}, respectively. Linear calibration graphs were obtained for both species from the determination limits to at least 70 µg L^{-1}. For the quality control of the analytical performance and the validation of the newly developed method, the analysis of two certified samples, LGC 6016 estuarine water and SRM 2976 mussel tissue were addressed. The results showed good agreement with the certified values. Also the method was successfully applied to the speciation of mercury in sea-water samples collected in the Málaga Bay.

Keywords: Mercury, speciation, solid phase extraction, nanosorbent, cold vapour, inductively coupled plasma mass spectrometry, biological samples, sea water samples.

Introduction

Mercury is one of the most toxic environmental pollutants and its effects on human and ecosystem health are well known. All mercury species are toxic, being organic mercury compounds generally more toxic than inorganic species. A wide range of mercury species exists within our environment and the chemical form of mercury controls its bioavailability, transport, persistence and impact on the human body.[1] Amongst these compounds, methylmercury is the most abundant and also the most toxic form of mercury in the environment. Methylmercury is the only mercury compound that is bioaccumulated and biomagnified in the food chain.[2] Nowadays it is well know that any mercury released into the environment undergoes biogeochemical transformation processes and can be converted into the most toxic methylmercury form.

Water is probably the most studied environmental sample and, in fact, the major part of speciation studies has been carried out in waters. The main dissolved mercury species in aquatic ecosystems are elemental mercury (Hg^0), complexes of Hg(II) with various inorganic and organic ligands, and organic Hg forms, mainly methyl and dimethyl mercury. Mercury is naturally present in waters at very low levels, being mercury speciation largely dominated by organic ligands such as humic and fulvic acids. In seawater, however, the proportion of Hg(II) bound to humics is decreased due to chloride ion competition.[3] So, the ratio of organic mercury concentrations in the marine environment is lower than 5% of total mercury, although in the

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case of the Mediterranean seawater the proportion is higher, up to 30% of total mercury.[4]

It is well known that, generally, the determination of the total concentration of an element is important but not sufficient to evaluate its toxicity and bioavailability. Hence, speciation analysis provides very useful additional information. Speciation analysis always implies the determination of very low concentrations of minor species and in the case of mercury speciation in waters it means that concentrations in a range of ng to pg L^{-1}. The most common technique for mercury speciation in natural waters is gas chromatography (GC) coupled to either atomic fluorescence spectrometry (AFS) or inductively coupled plasma mass spectrometry (ICP-MS) detection.[1] However these approaches have the disadvantages of requiring a derivatisation step to obtain volatile derivatives. On the other hand, high performance liquid chromatography (HPLC) has the advantage of simplified sample preparation,[5] but it is significantly less sensitive than GC procedures and the introduction of large amount of organic solvent to detectors from reverse phase separation needs to be settled.[6] Chromatographic techniques (GC, HPLC) coupled to element specific detectors, are able to separate mercury species in order to elucidate mercury transformation and transport processes where the determination of all mercury species is desirable. However, in practice, especially in sampling campaigns for sea water analysis where a large number of samples are collected over a longer period of time, a combination of methods is usually applied to accurately determine the most toxic mercury species. These include non-chromatographic methods based on the different chemical and/or physical behavior of the mercury species. These non-chromatographic methods can be less time consuming, more cost effective and available, and present competitive limits of detection.[7] Especially when mercury cold vapor (CV) generation technique coupled to ICP-MS detection is employed, this reduces salt effect on the analytical signal and improves the sensibility. However, a significant and difficult problem to overcome when using ICP-MS for mercury determination is the severe memory effect, which has been attributed to a combination of sample introduction, spray chamber and nebulization effects[8]. The consequences of these effects include non-linear calibration graphs, long washout times, decreasing sensitivity with time, and signals dependent on the matrix. A number of different approaches have been used with ICP-MS analysis to eliminate the mercury memory effect. By using a combination of flow injection (FI) sample introduction and a sulfur-containing compound in the carrier solution, it was possible to decrease the memory effect of mercury.[8]

Among non-chromatographic methods, solid phase extraction and microextraction (SPE and SPME) which is becoming increasingly popular for sample preparation in organic analysis, found its way to speciation analysis of organometals. SPE/SPME is the most popular sample preconcentration method for its simplicity, high enrichment factor, low or no consumption of organic solvents and feasibly to be automated (e.g., by FI). Also, the use of in-situ SPE methods overcomes problems with sample stability at the point of collection (e.g., on board ship) and during transport and storage.[9] The analytes are extracted by sorption, eluted with a small amount of eluent and derivatized or directly detected. Speciation analysis takes place by selective sorption[10,11] or selective elution[12-14]. The typical sorbents for mercury speciation are derived from chemical or physical immobilization of suitable organic agents to different solid surfaces on solid supports. With few exceptions, sulfur containing molecules as ligands are preferred for mercury preconcentration because of their high affinity for mercury species leading to high preconcentration factors and high selectivity for Hg^{6}. The most common support materials have been alumina and silica.[9,15,16] On the other hand, the exploration of new materials, especially nanometer sized materials, as the support phase is another active research area in SPE/SPME for mercury determination. The use of nanoparticles leads to higher extraction capacity/efficiency and rapid dynamics of extraction originated from the higher surface area to volume ratio and short diffusion route.[6,17] In the last years, mesoporous silica has been proposed as a novel SPE support, these materials are characterized by their high surface

areas, uniform and controllable pore sizes, and the periodic order of their pore packing.[18] The primary adsorption mechanism of ions to mesoporous silica is perceived to be surface complexation with functional groups.[19,20]

The purpose for this study has been the development of a simple, sensitive and automatic "on-line" procedure for the preconcentration and speciation of Hg^{2+} and CH_3Hg^+ in environmental and biological samples (including sea-water) by the use of a novel chelating sorbent based on mesoporous silica functionalized with 1,5 bis (2-pyridyl) methylene thiocarbohidrazide (PMTH-ms). This new sorbent was packed into a minicolumn and placed in the sample loop of the injection valve of a FI system coupled to an ICP-MS instrument. The system was based on chelating retention of the analytes onto the mini-column filled with the new nanosorbent and their sequential elution by using two different eluents, 0.2% HCl for CH_3Hg^+ and 0.1% thiourea in 0.5% HCl for Hg^{2+}. In this work the new nanosorbent was successfully synthesized and characterized. Also the study of its adsorption capacity toward metal ions was addressed. For the quality control of the analytical performance and the validation of the newly developed method, the analysis of two certified samples, LGC 6016 estuarine water and SRM 2976 mussel tissue were addressed. The results showed good agreement with the certified values. The method was successfully applied to the speciation of mercury in sea-water samples collected in the Málaga Bay.

Experimental

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. For the synthesis of PMTH-ms, γ-aminopropyltrimethoxysilane, and diglutaric aldehyde were purchased from Fluka (Buchs, Switzerland). Brij 76C18EO10, tetraethoxysilane (TEOS), thiocarbohydrazide and di-2-pyridil aldehyde were supplied by Aldrich Chemie (Steinheim, Germany). Methanol, ethanol, toluene and glacial acetic acid were obtained from Carlo Erba (Milano, Italy). A standard 1000 mg L^{-1} for Hg (II) solution Merck (Darmstadt, Germany) was used. The 1000 mg L^{-1} standard for CH_3Hg^+ was prepared from CH_3HgCl Carlo Erba (Milano, Italy). Standards of working strength were made immediately prior to use by appropriate dilution as required. A pH 5 buffer was prepared by mixing 14.8 mL of acetic acid 0.2 M Merck (Darmstadt, Germany) and 35.2 mL sodium acetate 0.2 M Merck (Darmstadt, Germany) and diluting to100 mL with de-ionized water. A 1.25% (wt/vol) sodium tetrahydroborate (III) (THB) Merck (Darmstadt, Germany) solution, prepared in 0.5% (wt/vol) NaOH Merck (Darmstadt, Germany), was used as reductant; and 0.2 % HCl Merck (Darmstadt, Germany) and 0.1% (wt/vol) thiourea Merck (Darmstadt, Germany) solution in 0.5% (wt/wt) HCl were used as eluents for CH_3Hg^+ and Hg^{2+}, respectively.

The certified reference materials (CRMs) analyzed to determine the accuracy of the proposed procedure were from United Kingdom Accreditation Service (UKAS): LGC 6016 Estuarine Water and from National Institute for Standard & Technology (NIST): SRM 2976 mussel tissue. The mussel tissue was prepared as follow: first, the sample was dried in accordance with the instructions of the analysis certificate, after which an accurately weighed amount of 1.00–2.00 g was subjected to microwave digestion. In order to dissolve the sample without destroying the organic mercury, 10 ml of 4.0 M HNO_3 was added (methylmercury is stable up to 4.0 M HNO_3 and degraded at higher concentrations, according to Mizanur Rahman and Skip Kingston[21]) and the mixture was subjected to 28 min of microwave irradiation (power 500W; temperature 45 ºC). Then, the sample was placed in a centrifuge tube and sonicated for 5 min. After extraction, the suspension was centrifuged at 3500 rpm for 10 min and the supernatant was taken for further analysis. Then, the pH of the solution was adjusted to 5.0 with concentrated NaOH and

buffer solution and, finally, the sample was diluted to adequate volume with de-ionized water in a calibrated flask. Samples were analysed, in triplicate, immediately after preparation by introducing them into the manifold described below. Blanks were prepared in parallel.

Seawater 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 mm pore size cellulose nitrate filters from Millipore (Bedford, MA, USA). After that, the samples were stored in low density polypropylene bottles at 4ºC 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 of pH 5 and de-ionized water were added up to the mark.

Synthesis of chelating resin

Mesoporous silica phase was synthesized at room temperature by using a non-ionic surfactant as the structure-directing agent. In a typical preparation, as this, 4.0 g of Brij 76 was dissolved in 20 g of water and 80 g of 2 M HCl solution with stirring. Then 8.80 g of TEOS was added to that homogeneous solution with stirring at room temperature for 20 h. The solid product was recovered, washed, and air-dried at room temperature. The synthesis is described elsewhere.[22] Yields are typically 95% (based on silicon). Mesoporous silica was refluxed with 6 M HCl for 3 h to remove metals. Washed with de-ionized water and dried in an oven at 180 1C for 24 h. Activated mesoporous silica (ms) (10 g) was suspended in 100 mL of 10% (vol/vol) ɣ-aminopropyltrimethoxysilane in dry toluene. The resulting product, ɣ- aminopropyl mesoporous silica (AP-ms), was filtered off and washed consecutively with toluene and methanol. This product was mixed with 100 mL of 3% (vol/vol) diglutaric aldehyde in de-ionized water and the reaction mixture was refluxed for 4 h. The solid obtained glutaric aldehyde ɣ- aminopropyl mesoporous silica (GlutAP-ms) was filtered off, washed with de-ionized water and mixed with 1.5 g of thiocarbohidrazide previously dissolved in 100 mL of de-ionized water; five drops of glacial acetic acid were added. After boiling and refluxing for 24 h the corresponding derivative thiocarbohidrazide glutaric aldehyde ɣ-aminopropyl mesoporous silica (TCHGlutAP-ms) was obtained and suspended in 180 mL of 2% (wt/vol) 2-pyridil aldehyde in ethanol. After refluxing for 24 h, the resulting product 1,5 bis (2-pyridyl) methylene thiocarbohidrazide (PMTH-ms) was filtered off, washed with ethanol and dried in an oven at 50ºC. The reaction for PMTH-ms formation is shown in Fig. 1.

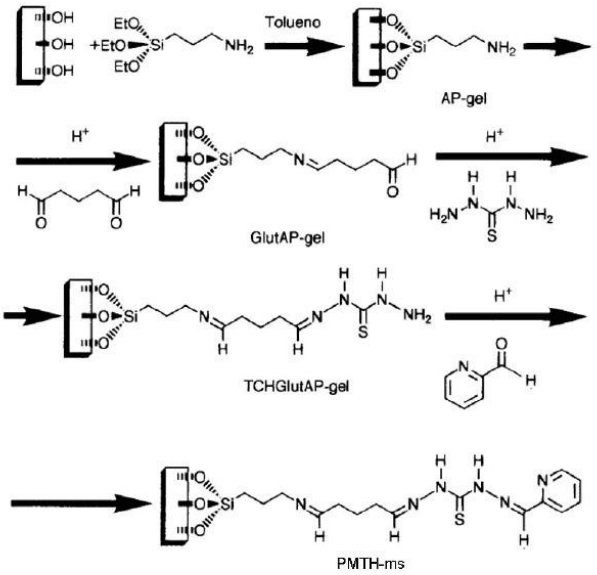


Fig. 1 Schematic diagram of the synthesis of PMTH-ms

Instrumentation

Elemental analysis was performed with a LECO CHNS-932 Elemental Analyzer (LECO, Michigan, USA). The Mass spectrum was obtained with a Trace DSQ Mass spectrometer from Thermo Electron Corporation (Massachusetts, USA), the sample was introduced by means of a DIP (direct introduction probe). IR Spectra were recorder on a Perkin Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer, Concord, Canada), samples were measured by

using potassium bromide pellets, in which the concentrations for the samples were 2% (wt/wt) approximately. The adsorption capacity of the resin was determined with a Perkin-Elmer Optima 7300 DV inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer SCIEX Instruments, Concord, Canada).

A Perkin-Elmer ELAN DRC-e inductively coupled plasma mass spectrometer (Perkin-Elmer SCIEX Instruments, Concord, Canada) equipped with an autosampler AS-91 was used throughout. The instrument, with standard nickel sampler and skimmer cones, was optimized daily and operated as recommended by the manufacturer. The nebulizer gas flow-rate was adjusted so that the CeO^+/Ce^+ ratio and Ba^{++}/Ba^+ were less than 3%. The optimum operation conditions are summarized in Table 1. A Perkin-Elmer FIAS-400AS system, which consists of two peristaltic pumps with PVC tubings of various diameters and a five-port rotary way valve, was used as the flow injection accessory controlled by the ELAN DRC-e software. Also, a six-port rotary way valves manually controlled was used. The FIAS-400 AS system was connected directly to the ELAN DRC-e by means of a 35 cm length of PTFE tubing (1.75 mm i.d.), the least possible distance to avoid sample dispersion. The minicolumn packed with the chelating resin was placed in the sample loop of the five-port rotary valve. The minicolumn was a glass tube (3 cm x 3 mm i.d.), the resin was packed to a height of 0.5 cm, at both ends of the minicolumn, polyethylene frits (Omnifit, Cambridge, UK) were fixed to prevent material losses.

Table 1 Operating parameters for ICP-MS system

Instrument	Elan DCR-e (Perkin-Elmer)
Nebulizer gas flow rate/L min^{-1}	Ar 0.98
Auxiliary gas flow rate/L min^{-1}	1.2
Plasma gas flow rate/L min^{-1}	15
Autolens voltage/V	6.75-7.50
Incident power/kW	1.1
Analogical phase voltage/kV	-2000
Digital phase voltage/kV	1000
Scanning mode	Peak jumping
Dwell time/ms	50
Number of points/reading	30
Number of readings/replicate	200
Number of replicates	3
Reaction gas (CH_4) flow rate/mL min^{-1}	0.2
Distance from FIAS-400 to ICP-MS/cm	35
Element	mass
Hg	202

Operating procedure of the FI-SPE-CV-ICP manifold

The operating procedure of the FI manifold and its operational sequence are represented in Figure 2 and Table 2, respectively. Daily, the system was cleaned with eluent 2 (thiourea 0.1% in HCl 0.5%) by processing of two replicates (504 s, necessary time for plasma stabilization), in which the eluent is used as sample and as eluent; after, the system was washed by MQ water. Then, blank, standards and samples were measured as follows: (A) during the 2 min sample loading period, valves 1 and 2 in the *fill* position, the sample solution (standard o blank) at pH 5 was delivered at 2.3 mL min^{-1} by peristaltic pump P1 to pass through the PMTH-ms chelating resin packed mini-column. CH_3Hg^+ and Hg^{2+} in sample solution were retained on the surface of the resin while sample matrix components were directed to waste and the peristaltic pump (P2) pumped eluent to the CV generator. Before the elution step, MQ water is pumped through the mini-column for 1 min to remove salt residues from the sample matrix and then the sample pump P1 is stopped. (B) The valve 1 is turned to the *elution* position and the eluent 1 (HCl 0.2%) passes through the minicolumn at 1.6 mL min^{-1} in a

reverse flow relative to the pre-concentration step (avoiding the continuous increase in column compactness). Thus, the ions CH_3Hg^+ are eluted for 177 s and mixed with a 0.8 mL min^{-1} flow of reductant ($NaBH_4$ 1.25% in NaOH 0.5%) in the mixing coil (RC) (length 30.6 cm, i.d. 0.8 mm) where direct generation of mercury vapor takes place. The gas generated and the solvent are forwarded to the gas–liquid separator which provides a separation of gases from liquid. The liquid is drained and the vapor is swept into the ICP by a stream of argon. (C) After that, the valve 2 is turned and the eluent 2 (thiourea 0.1% in HCl 0.5%) passes through the minicolumn at 1.6 mL min^{-1} to elute the Hg^{2+} ions for 147 s and mix them with the reductant solution in the mixing coil where direct generation of mercury vapor takes place. The gas generated and the solvent are forwarded to the gas–liquid separator which provides a separation of gases from liquid. The liquid is drained and the vapor is swept into the ICP by a stream of argon. Two signals due to CH_3Hg^+ and Hg^+ were registered during 324 s. Finally, the two valves turn again to *fill* position for a new sample loading period. This process is repeated three times, number of replicates programmed in the method. By using instrument software, the transient signals were measured as peak height maximum with 50 ms dwell time and 200 readings per replicate. An example of the elution peaks is shown in Fig. 3. At the end of the working day, the system in the load position, was cleaned with the eluent 2 during two minutes and after washed by HNO_3 4% to avoid reagents precipitation into the FIAS tubes and valves during the night.

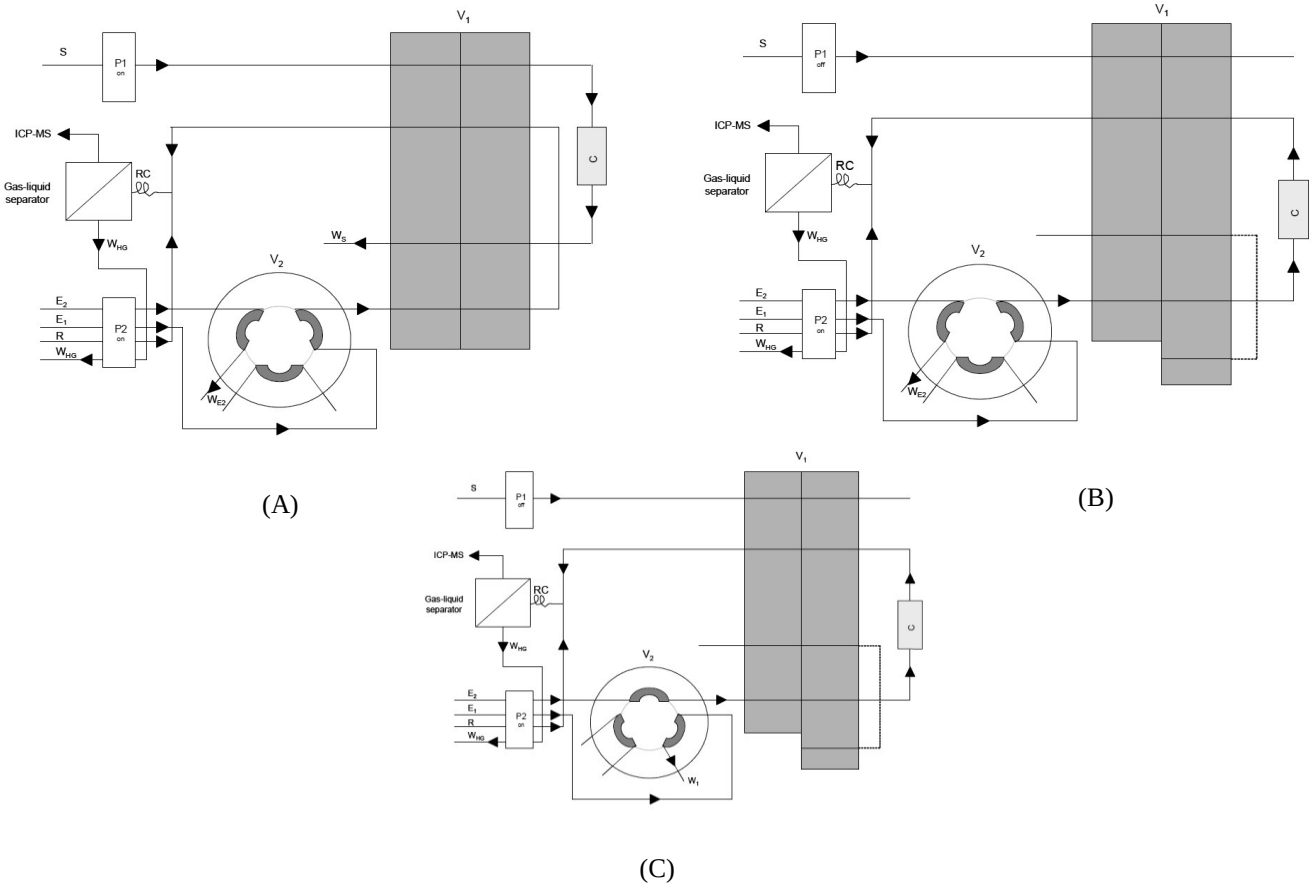


Fig. 2 Schematic diagram of FI system for the pre-concentration and separation of CH_3Hg^+ and Hg^{2+}; C: minicolumn of PMTH-ms; P1 and P2: peristaltic pumps; V_1: five-port rotary valve; V_2: six-port rotary valve; RC, mixing coil; S: sample; E_1: eluent 1; E_2: eluent 2; R: reductant solution; W: waste. (A) preconcentration step; (B) CH_3Hg^+ elution step; (C) Hg^{2+} elution step

Table 2 Optimum operating condition for FIAS system

Step	Solution	Valve posi-tion	Flow rate /mL min^{-1}	Time /s	Way
Prefill	MQ water	1	2.3	45	Waste
Sample loading	Sample pH 5	1	2.3	120	Waste
Washing	MQ Water	1	2.3	60	Waste
Elution 1 (P1 off)	HCl 0.2%	2	1.6	177	ICP-MS
Elution 2 (P1 off)	HCl 0.5% Thiourea 0.1%	2	1.6	147	ICP-MS
Reduction (P1 off)	$NaBH_4$ 1.25% NaOH 0.5%	2	0.8	324	ICP-MS

Results and discussion

Characterization of synthesized resin

The contents of carbon, hydrogen, nitrogen and sulfur in the PMTH-ms and its precursor, AP-ms were measured with the CHNS elemental analyzer, being 53.74% C; 4.083% H; 29.37% N; and 10.16% S for PMTH-ms and 16.43% C; 5.494% H; 7.334% N; and 0.0% S for AP-ms. The presence of sulfur and the increased percentage of N confirmed that the synthesis could to have been effective. Else, it is interesting that the nitrogen-to-sulfur ratio is quite similar than that predicted by theory. The infrared spectrum (KBr pellets) is complicated because the large mass of silica and the aromatic portion of the molecule produce numerous bands, the overlap of which makes detailed assignments difficult. However, in the mid-infrared region

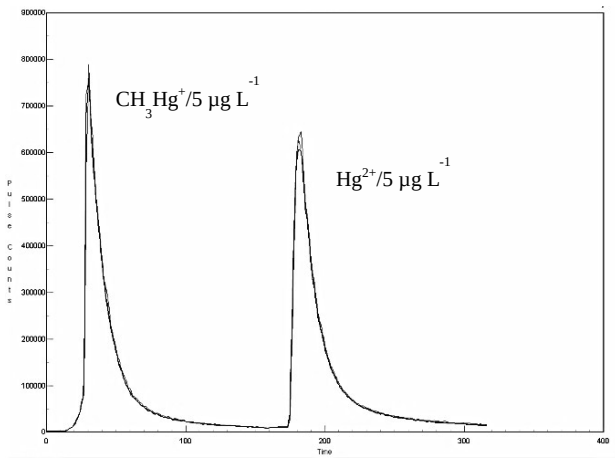


Fig. 3 Elution profiles of three repeated replicates for a $CH_3Hg^+ + Hg^{2+}$ standard of 5 µg L^{-1} in each specie

some assignments can be done. Comparing with the IR spectrum of the reagent 1,5 bis (2-pyridyl) methylene thiocarbohidrazide (PMTH), several intense bands appear in the region between 1340-1130 cm^{-1} that can be attributed to N-CS-N; several bands medium-weak intensity appear in the region between 1600-1460 cm^{-1} corresponding to the bending vibration of N-H; and finally, in the region between 3080-2990 several low intensity bands appear due to the aromatics C-H. The fingerprint region (~1200-600 cm^{-1}) serves to identify compounds by comparison of their spectra. In Figure 4 can be seen the matching between bands for the IR-spectra of PMTH-ms and PMTH in the fingerprint region. The mass spectrum has been recorded by evaporation until 250 ºC from PMTH-ms and the attribution of some peaks are showed in Table 3.

Adsorption capacity of the PMTH-ms resin

Adsorption capacity is an important factor to evaluate the sorbents, because it determines how much PMTH-ms is required for a given solution. The sorption capacity of the resin was determined by the batch process by equilibrating about 300 mg of PMTH-ms resin with solutions of 25 mL of 50 mgL^{-1} of individual metal ions for 24 h at pH 5 and pH 8 at 20 ºC (without shaking). After this,

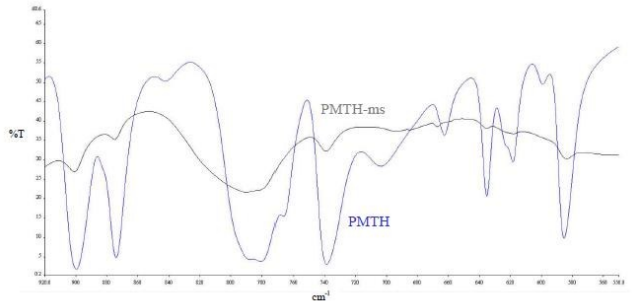


Fig. 4 IR spectra of PMTH-ms and PMTH in the fingerprint region

the PMTH-ms was filtered from the standard and the analytes remaining in solution was determined by ICP-OES. The sorption capacity of the resin for each metal ion was calculated from the difference between the metal ion concentrations in the solution before and after sorption. The absorption capacity calculated for Co, Cr, Ni, Cd, Mn, Zn, Cu, Pb, Hg, As and Sb are shown in Table 4. As can be seen, mercury is one of the elements with higher adsorption capacity on the PMTH-ms, 41.6 mgL^{-1} of mercury were retained (83.2 %) from the original 50 mgL^{-1} at pH 5. On the other hand, the resin is stable over a wide pH range and the micro-column packed with this resin has a practically unlimited lifetime without the demand for regeneration, hence, the material can be stored and applied conveniently. Thus, PMTH-ms was chosen to develop a new method for mercury speciation.

Table 3 Identification of some peaks of the MS of PMTH-ms

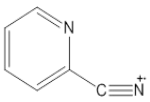
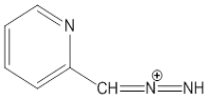
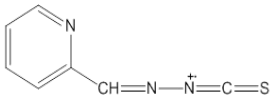
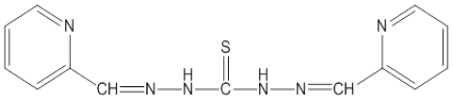
Peak m/z	Attributions
79	
104	
120	
163	
284	

Table 4 Adsorption capacity of the resin at two different pH

Element	pH 5 /μmol g^{-1}	pH 8 /μmol g^{-1}
Co	286.0	175.8
Cr	99.3	115.4
Ni	140.0	102.2
Cd	86.1	38.5
Mn	158.5	62.2
Zn	90.1	56.2
Cu	178.4	124.7
Pb	20.8	18.1
Hg	173.1	98.5
As	24.5	32.9
Sb	124.4	105.4

Optimization of the experimental variables

The configuration of the FI-system and the effect of different parameters were studied in order to obtain highly sensitive, accurate and reproducible results. To optimize the system, most efforts were focused on the conditions for sample loading and selective elution of the two different species of mercury from the column, as well as the conditions to generation of the mercury vapor. 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} of methylmercury and mercury were chosen as the optimization

criterion. Peak heights were used for analytical measurements, because the detection and determination limits were better than those calculated with peak areas. A one-at-a-time method was used for optimization of the variables.

Preliminary studies. In order to establish the configuration of FI system for the simultaneous determination of methylmercury and mercury, first the retention of both species was corroborated by processing separate standards solutions containing Hg^{2+} or/and CH_3Hg^+. On the base of the paper by Krishna and col[23] who only achieved quantitative elution of Hg^{2+}, avoiding high acid concentrations, when the complexing agent thiourea was added to HCl solutions, a FI system was tested which used only one minicolumn placed in the sample loop of the automatic five-port rotary valve of FIAS-400 where the simultaneous preconcentration of both species occurred. For the elution of CH_3Hg^+ a flow of HCl was passed through the minicolumn and for the elution of Hg^{2+} a flow of HCl + thiourea. Switching between the two flows was achieved by means a selection valve. $NaBH_4$ in NaOH was used as reductant for the vapor generation. This FI system was tested with good results. Thus, a FI system with two peristaltic pumps, two valves and a gas/liquid separator was employed for the development and optimization of the method for mercury speciation.

Effect of pH. **Selection of the buffer.** Since the solution pH affects the extent of complexation with PMTH-ms, which in turn determines the percentage of analyte retained by the resin, the pre-concentration of traces of CH_3Hg^+ and Hg^{2+} ions from solutions buffered at different pH was studied. The pH was varied between 2.0 and 10.0. The pH from 2.0 to 5.0 was adjusted using glycine-HCl or sodium acetate-acetic acid buffer and from 5.0 to 10.0 using borax-boric acid buffer. As can be seen in Figure 5(A), in order to accomplish the sequential determination of the target ions, a pH value of 5.0 ± 0.5 was chosen for the sequential determination of both analytes. For subsequent experiments a sodium acetate/acetic acid buffer was used to adjust this pH.

Optimization of the concentration of the reagents involved in the system. $NaBH_4$ was chosen as reductant. The $NaBH_4$ concentration was varied from 0.1 to 1.0% (w/v). As can be seen in Figure 5(B), the higher S/N ratios were obtained for a concentration of 0.25 %, higher concentrations produced a decrease of these ratios. On the other hand, the NaOH concentration to stabilize $NaBH_4$ solution was also studied. The experimental results showed an improvement in the S/N ratios with the increase of NaOH concentration up to 0.5% (wt/vol) (Figure 5(C)).

In order to minimize the time needed for quantitative elution and good separation 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, CV is most frequently facilitated in acidic medium. For this reason, hydrochloric acid and nitric acid were tested as eluents; also thiourea (which forms 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 obtained results showed that HNO_3 favored the elution of Hg^{2+} while HCl favored the elution of CH_3Hg^+. Because CH_3Hg^+ is more concerning, HCl and its mixture with thiourea were chosen as eluents.

To the optimization of the concentrations of HCl and thiourea, the concentration of HCl was varied between 0.1-0.5%, the best S/N ratios were obtained for a concentration of 0.5% (Figure 5(D)). At this concentration was corroborated, by means recovery studies, that some of Hg^{2+} was eluted together with CH_3Hg^+. So that, the HCl concentration for the elution of CH_3Hg^+ was decreased while the HCl concentration for the elution of Hg^{2+} was retained constant in 0.5%. It was observed a good elution of CH_3Hg^+ without elution of Hg^{2+} for a HCl concentration of 0.2%. On the other hand, as was said before[23], the total elution of Hg^{2+} only is achieved when the

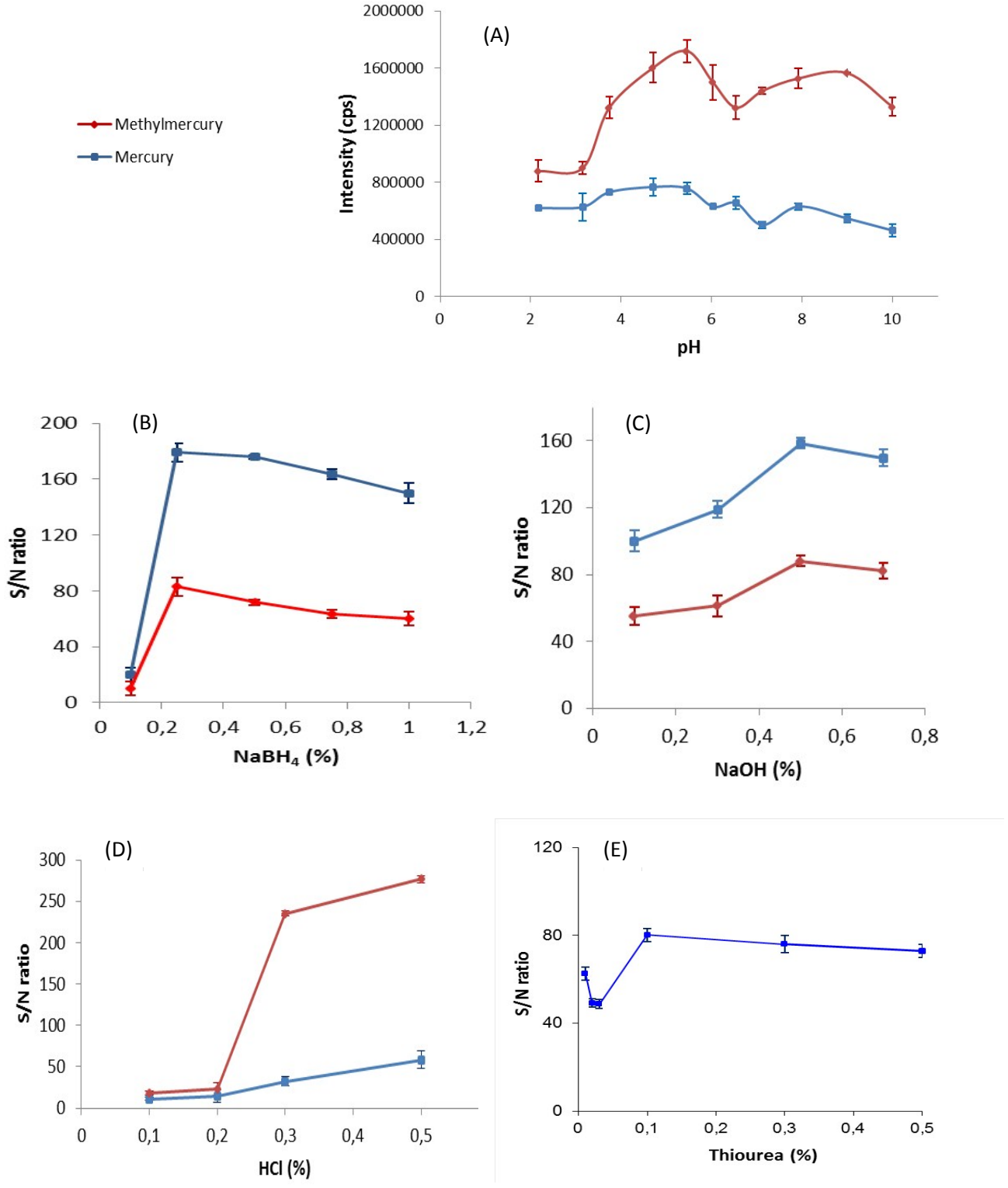


Fig. 5 (A) Effect of pH on the retention of CH_3Hg^+ and Hg^{2+}

(B) Effect of $NaBH_4$ concentration on the CVG

(C) Effect of NaOH concentration on the CVG

(D) Effect of the HCl concentration

(E) Effect of thiourea concentration on the Hg(II) elution

complexing agent thiourea was added to HCl solutions. Thus, keeping constant the HCl concentration in 0.5%, the thiourea concentration was varied from 0.01 to 0.5%. The best S/N ratios for Hg^{2+} were obtained for a thiourea concentration of 0.1% (Figure 5(E)). Hence, HCl 0.2% was employed to CH_3Hg^+ elution and thiourea 0.1% in HCl 0.5% was employed to Hg^{2+} elution.

Selection of FI variables. The analytical figures of merit of the determination of the volatile species are strictly related to the rate of gas–liquid separation and transport.[24] So, the conditions of the gas–liquid separation unit were also studied. For this, membranes with different pore sizes (0.2, 0.5 and 1.0 µm) were tested; with each membrane, the flow-rate of Ar was changed between 0.9 and 1.2 L min^{-1}. The best S/N ratios were obtained with the membrane of 0.2 µm pore size and a flow-rate of Ar of 1.0 L min^{-1}. With higher flow-rate of argon, the membrane was wet and the gas–liquid separation was poor. On the other hand, the length of the PTFE tube for connecting the plasma with the gas–liquid separation unit was shortened at the least possible distance, 35 cm. With this action, the signals for the two analytes were increased.

It is well known[25] that, in general, CV generation 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 Hg in the reaction medium. For this purpose tubing coils (1.0 mm id) of different lengths (10-300 cm) were tested. The coil length selected was 31 cm. This ensured a very 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.

The optimization of the flow-rate for sampling and elution of the elements is also important. The sample flow-rate should be optimized to ensure quantitative retention along with minimization of the time required for sample processing. For quantitative desorption of the retained analytes in a small eluent volume, which is needed for a high enrichment factor, a low elution flow-rate should be used, providing sufficient time for equilibrium between the solid-phase and the eluent. On the other hand, the flow-rate of the reductant is also very important in order to obtain a high performance in the vapor generation. The effect of elution flow-rate was studied between 0.4 and 2.3 mL min^{-1}. Besides the best S/N ratio, the elution profiles for the two species were observed. The best results (narrower peaks with little tail together with higher S/N ratios) were obtained with an elution flow-rate of 1.6 mL min^{-1} and this value was selected for subsequent experiments. Both flow-rates were delivered through the peristaltic pump P2, thus, the flow-rate of the reductant was also studied and optimized, by changing the pump tube diameter of the reductant, in 0.8 mL min^{-1}. The effect of the sample flow-rate was investigated in the range 0.4–2.7 mLmin^{-1}, using a constant sample volume of 1.8 mL. An improvement in the signals was observed with increasing the loading flow-rate up to 2.3 mL min^{-1}, while afterward a decline was recorded when exceeding this value to 2.7 mL min^{-1}. For further experiments, a sample loading flow-rate of 2.3 mL min^{-1} was thus employed.

The pre-concentration time affects directly the enrichment factor. Thus, the loading time was investigated in the range between 2 and 9 minutes, using the optimum conditions described above. The signal increased linearly up to at least 9 min preconcentration time for two Hg species; however, when the sample loading time is longer it results in lower sampling frequency, in addition, it is less cost-effective since it will require the use of larger quantities of chemicals. Taking this into consideration, a pre-concentration time of 2 min was selected to achieve good sensitivity and sampling frequency. A longer loading time can be employed for samples with low concentrations of the analytes.

When sea-water is analyzed by conventional ICP-MS one or more of the polyatomic species

may overlap with the analyte signals. Many of these problems can be reduced or eliminated by using a dynamic reaction cell (DRC). Thus, to improve the accuracy of analysis, the flow-rate of the reaction gas (methane) was optimized according to the manufacturer instructions, for that a blank and a standard of 10 µg L^{-1} of CH_3Hg^+ and Hg^{2+} were prepared. Besides, the S/N ratios were measured for a blank and a sea water sample spiked with 5 µg L^{-1} of both mercury species. The methane flow-rate was studied between 0-1.4 mL min^{-1}. The best S/N ratios were obtained for a methane flow-rate of 0.2 mL min^{-1}. On the other hand, detection limits in the DRC mode are significantly better than for standard mode primarily because the blank in the standard mode is significantly higher than in DRC mode. In addition, the standard deviation of the background is also higher.

Effects of matrix elements

Residues from the matrix sample, such as Na^+, K^+, Ca^{2+} or Mg^{2+} that are not adsorbed on the resin, could be present in the dead volume of the minicolumn and possibly pose a potential source of error. For removing these matrix elements from the minicolumn, after the seawater sample spiked with 5 µg L^{-1} of CH_3Hg^+ and Hg^{2+} was loaded on the resin, the minicolumn was washed with MQ water or buffer solution at different washing times between 1 and 3 min. The S/N ratios were studied and the best results were obtained after washing with MQ water for 1 min.

The synergetic effects between both species were also studied, so that calibration curves were prepared by spiking 0; 1.0; 2.0; 3.0 and 5.0 µg L^{-1} of both mercury species to sea water. After, spiked sea water samples with both analytes to different ratios between them (from 1:4 to 4:1) were analyzed. The results obtained are shown in Table 5. Recovery values from 92.0 to 106.7 % were obtained, indicating that no synergetic effects were observed, at least to a ratio 1:4 of each specie.

As mentioned, a significant and difficult problem to overcome when using ICP-MS for mercury determination is the severe memory effect. However, as can be seen in Figure 3, the mercury profiles tailed properly, the signal reaches the baseline before the next injection, and the three repeated replicates showed similar signal intensities. These observations indicate no memory effects, so thiourea + HCl is a suitable eluent for the determination of mercury at this concentration level.

Table 5 Synergetic effects

	Added/ µg L^{-1}		Found/ µg L^{-1}		Recovery/%	
Ratio	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}
1:2	1.00	2.00	0.97	2.10	97.0	105.0
2:1	2.00	1.00	1.96	0.93	98.0	93.0
1:3	1.00	3.00	0.98	3.2	98.0	106.7
3:1	3.00	1.00	3.00	0.92	100.0	92.0
1:4	1.00	4.00	1.04	3.94	104.0	98.5
4:1	4.00	1.00	3.96	0.97	99.0	97.0

Performance of the Method

Under the optimum conditions described, the quality parameters of the on-line FI-CV-ICP-MS system for the speciation of mercury were obtained. With the use of 2 min pre-concentration time and 1 min washing time, the total time employed for a measurement is 504 s, thus the sampling frequency was 7.1 h^{-1}. For the two species, a linear calibration range was obtained, from the determination limit (0.011 µg L^{-1} for CH_3Hg^+ and 0.024 µg L^{-1} for Hg(II) to at least 70.0 µg L^{-1} of both mercury species. The enrichment factors, detection and determination limits, correlation coefficients and precisions are given in Table 6. The low detection and determination limits (LOD and LOQ), high regression coefficients (R>0.999), as well as the good precision obtained confirmed that signals were independent on the matrix and there were no memory effects. The enrichment factors were calculated as the ratio of the slopes of the linear sections of the calibration graphs with and without preconcentration (changing the minicolumn by a 500 µl sample loop). The

Table 6 Performance of the method

Specie	Calibration equation[a]	Regression coefficient	Detection limit/ μgL^{-1}	Determination limit/ μgL^{-1}	Precision/ % RSD	Enrichment factor
CH_3Hg^+	y=268938x+5511.9	0.999	0.002	0.011	2.8	4.7
Hg^{2+}	y=388657x+17438	0.999	0.004	0.024	2.6	11.0

[a] y, signal/cps; x, concentration.

detection and determination limits were calculated as the concentration of analyte giving signals equivalent to three and ten times, respectively, the standard deviation of the blank plus the net blank intensity. The precisions were determined as the relative standard deviation of ten determinations of a standard with 2 µg L^{-1} of CH_3Hg^+ and Hg^{2+}. Peak height was used in all cases because the detection and determination limits were better than those obtained with peak area. 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 with other non-chromatographic methods can be made. A comparison of these methods is shown in Table 7. In general, the detection limits and precisions obtained by the present procedure are better than those reported in the literature for the determination and speciation of mercury.

Analytical applications

The accuracy and applicability of the proposed method were studied by examining two certified reference materials, LGC6016 Estuarine Water and SRM 2973 mussel tissue; and three sea water samples collected locally. Mercury is not certified in the sample LGC6016, however total mercury was determined in a previous paper.[35]

All samples were quantified by external calibration. On the other hand, the recovery tests of spiked samples were also examined. The results, as the average of four separate determinations, are shown in Table 8. 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 94.5 to 112.0 % 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 µg mL^{-1} concentrations. The accuracy achieved for the spiked sea-water samples demonstrated that the method is not affected by high salinity (approximately 35 g L^{-1} in sea water).

Conclusions

The synthetized nanosorbent PMTH-ms has a high adsorption capacity of transition metals including mercury due to its high surface area, uniform and controllable pore size, and the periodic order of their pore packing. In general, the use of nanoparticles leads to higher extraction capacity/efficiency and rapid dynamics of extraction originated from the higher surface area to volume ratio and short diffusion route. The pre-concentration and separation system using PMTH-ms as a sorbent material has been evaluated and demonstrated to be promising for routine sequential determination in the same sample of the trace amounts of CH_3Hg^+ and Hg^{2+} in biological and sea-water samples. Furthermore, the FI-CV-ICP-MS method has permitted the sequential determination of the two species of mercury, saving time of analysis and achieving a sample throughput of about 7.1 h^{-1}. The method proposed in this work is rapid, easy to use,

Table 7 A comparison of analytical performance data with other non-chromatographic methods described in the literature

Species	Preconcentration	Separation	Detection	Detection limit/ ngL^{-1}	Precision/ % RSD	Reference
Hg^{2+} Hg_{total}	Preconcentration in a minicolum filled with 1,5-bis(2-pyridyl)3-sulfophenyl-methilen-thiocarbohidrazide immobilized on controlled pore glass (PSTH-cpg)	Selective preconcentration of Hg^{2+}. Hg_{total} is determined prior oxidation of the organic mercury. The organic mercury is obtained by the difference between Hg_{total} and organic mercury	CV-AAS CV-ETAAS	Hg^{2+}: 10 Hg_{total}: -- Hg^{2+}: 6 Hg_{total}: --	Hg^{2+}: 3.4 Hg_{total}: -- Hg^{2+}: 3.5 Hg_{total}: --	10
Hg^{2+} CH_3Hg^+	Retention of Hg^{2+} and CH_3Hg^+ in a minicolumn filled with Sttrepto-coccus pyogenes immobilized on Dowex Optipore SD-2	Sequential elution of CH_3Hg^+ and Hg^{2+} with HCl 0.1 and 2 mol L^{-1}, respectively	CV-AAS	Hg^{2+}: 2.1 CH_3Hg^+: 1.5	Hg^{2+}: <7 CH_3Hg^+: <7	14
Hg^{2+} CH_3Hg^+	Retention of Hg^{2+} and CH_3Hg^+ in a minicolumn filled with Chlorella Vulgaris immobilized on Silicagel	Sequential elution of CH_3Hg^+ and Hg^{2+} with HCl 0.03 and 1.5 mol L^{-1}, respectively	CV-AAS	Hg^{2+}: 500 CH_3Hg^+: 4000	Hg^{2+}: -- CH_3Hg^+: --	26
Hg^{2+} CH_3Hg^+	Retention of Hg^{2+} and CH_3Hg^+ in a minicolumn filled with L-cysteine grafted in cellulose fiber	Determination of Hg^{2+} by CV-AFS and determination of Hg_{total} by flame-AFS	AFS	Hg^{2+}: 1 CH_3Hg^+: 3	Hg^{2+}: 1.5 CH_3Hg^+: 2.6	27
Hg^{2+} CH_3Hg^+	Tungsten coil atomizer	Hg^{2+} was measured at room temperature and CH_3Hg^+ at 500ºC	CV-AAS	Hg^{2+}: 60 CH_3Hg^+: 89	Hg^{2+}: 3.1 CH_3Hg^+: 2.6	28
Hg^{2+} Hg_{total}	Selective retention of Hg^{2+} in polymethacrylate microbeads linked to a specific ligand of Hg(II) (TAN)	Determination of Hg^{2+} in non-mineralized samples and determination of Hg_{total} in the mineralized samples	CV-AAS	Hg^{2+}: 6 Hg_{total}: --	Hg^{2+}: 5-9 CH_3Hg^+: 5-9	29
Hg^{2+} Hg_{total}	Retention of Hg^{2+} and CH_3Hg^+ in a minicolumn filled with polyaniline (PANI)	Two procedures: 1) Selective retention of Hg^{2+} and CH_3Hg^+ at different pH; 2) Sequential elution of CH_3Hg^+ and Hg^{2+} with HCl 2% and thiourea 0.02% in HCl 2%, respectively	CV-ICP-MS	Hg^{2+}: 25 Hg_{total}: 32	Hg^{2+}: 2.0 Hg_{total}: 2.5	30
Hg^{2+} Hg_{total}	Preconcentration in an ion exchange membrane	Retention of the mercury tetrachloride complexes while the organic mercury is not retained	CV-AFS	Hg^{2+}: 14 Hg_{total}: 16	Hg^{2+}: 1.14 Hg_{total}: 1.28	31
Hg^{2+} Hg_{total}	Selective retention of CH_3Hg^+ in membrane disc of modified silica with MPMP	Hg^{2+} is determined without preconcentration	CV-AAS	Hg^{2+}: 3.8 Hg_{total}: --	Hg^{2+}: 3.1 Hg_{total}: --	32
Hg^{2+} CH_3Hg^+	Retention of in a minicolumn filled with native sheep wool	CH_3Hg^+ is determined with a previous treatment with a oxidant	FI-AFS	Hg^{2+}: 10 CH_3Hg^+: 10	Hg^{2+}: 2.9 CH_3Hg^+: 3.1	33
Hg^{2+} CH_3Hg^+	Selective retention of CH_3Hg^+ in a knotted reactor	The selective retention is achieved by using different chelating agents for Hg^{2+} and CH_3Hg^+, diethyl dithiophosphate and dithizone (DZ), respectively	CV-AFS	Hg^{2+}: 3.6 CH_3Hg^+: 2.0	Hg^{2+}: 2.2 CH_3Hg^+: 2.8	34
Hg^{2+} CH_3Hg^+	Preconcentration in a minicolumn filled with PMTH-ms	Sequential elution of CH_3Hg^+ and Hg^{2+} with HCl 0.2 % and thiourea 0.1% in HCl 0.5%, respectively	FI-CV-ICP-MS	Hg^{2+}: 4 CH_3Hg^+: 2	Hg^{2+}: 2.6 CH_3Hg^+: 2.8	This work

Table 8 Results of the analysis of certified reference and sea water samples

ımple	Certified/ µg L^{-1}			Added/ µg L^{-1}			Found/ µg L^{-1}			Recovery/ %		
	CH_3Hg^+	Hg^{2+}	Hg_{total}	CH_3Hg^+	Hg^{2+}	Hg_{total}	CH_3Hg^+	Hg^{2+}	Hg_{total}	CH_3Hg^+	Hg^{2+}	Hg_{total}
RM	28.09 ± 0.31	--	61.0 ± 3.6	--	--	--	28 ± 2	36 ± 3	64 ± 4	99.7		104.9
)76[a]				25	--	25	56 ± 4	34 ± 6	90 ± 7	112.0		104.0
				50	--	50	76 ± 6	36 ± 6	112 ± 8	96.0		96.0
GC	--	--	27 ± 2[b]	--	--		12.3 ± 0.3	13.5 ± 0.7	25.8 ± 0.8			95.6
)16				50	50		65 ± 3	66.9 ± 0.8		105.4	106.8	
				75	75		84 ± 2	92 ± 1		95.6	104.7	
ea-	--	--	--	--	--		--	0.028±0.004		--	--	
ater 1				0.1	0.1		0.10 ± 0.01	0.13 ± 0.01		100.0	102.0	
				0.3	0.3		0.30 ± 0.02	0.338±0.001		100.0	103.3	
ea-	--	--	--	--	--		--	0.031±0.008		--	--	
ater 2				0.2	0.2		0.21 ±0.02	0.22 ± 0.01		105.0	94.5	
				0.3	0.3		0.31 ±0.01	0.34 ± 0.01		103.3	103.0	
ea-	--	--	--	--	--		--	0.025±0.002		--	--	
ater 3				0.1	0.1		0.10 ± 0.02	0.120±0.005		100.0	94.5	
				0.3	0.3		0.30 ± 0.03	0.33 ±0.03		100.0	101.6	

[a] µg Kg^{-1}

[b]Total mercury concentration found in a previous paper[34]

automatic and selective with a good sensitivity and precision compared with other non-chromatographic methods for the mercury speciation. 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.

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