Cent. Eur. J. Chem. • 10(4) • 2012 • 1175-1182
DOI: 10.2478/s11532-012-0027-0

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Central European Journal of **Chemistry**

Mercury determination and speciation analysis in surface waters

Research Article

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Received 10 December 2011; Accepted 8 February 2012

Abstract: A sorbent L-cysteine grafted silica gel has been evaluated for separation and enrichment of dissolved inorganic i-Hg(II) and methylmercury CH_3Hg(I) from surface waters at sub-μg L^{-1} concentrations. Chemical parameters for mercury species enrichment and separation have been optimized. Analytical schemes for the determination of Hg species, using selective column solid phase extraction (SPE) with continuous flow chemical vapor generation atomic absorption spectrometry (CF-CVG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) were developed. Possibilities for *on-site* SPE enrichment were demonstrated as well. The limits of quantification were 1.5 and 5 ng L^{-1} for dissolved i-Hg(II) and CH_3Hg(I) by CF-CVG-AAS and 1 and 2.5 ng L^{-1} by ICP-MS with relative standard deviations between 7 – 12% and 7 – 14%, respectively. The chemically modified SPE sorbent has demonstrated high regeneration ability, chemical and mechanical stability, acceptable capacity and good enrichment factors. Results for total dissolved mercury were in reasonable agreement with those from independent analyses by direct ICP-MS determinations for river waters and for estuarine water certified reference material.

Keywords: *L-cysteine modified sorbent • Mercury speciation • Surface water analysis*

1. Introduction

Mercury is a priority hazardous pollutant for aquatic environment, therefore remarkably stringent environmental quality standards (EQS) of 0.05 and 0.07 μg L^{-1} mean annual and maximum allowed total Hg concentrations, respectively have been defined by European Commission for the evaluation of chemical status of water bodies [1]. The limits of quantification (LOQ, 10σ) on analytical procedures applied for total Hg determination should be equal or lower to a value of 30% of the relevant EQSs [2]. Therefore, procedural LOQs should be in the low ng L^{-1} range (0.015 ng L^{-1}) and the limits of detection (LOD, 3σ) should not exceed an impressive figure of ca. 5 ng L^{-1}. In surface waters background total dissolved mercury concentrations are very low: from 0.5 to 3.0 ng L^{-1}in the open ocean, from 2.0 to 15 ng L^{-1}in coastal estuaries and rainwater, less than 5 ng L^{-1} for fresh waters and up to 10 or 20 ng L^{-1}in humic-rich lakes (mostly as particulate mercury) [3,4]. Mercury levels established for the aquatic system in Antarctica ranged from 0.45 to 1.9 ng L^{-1} [5]. It is also well recognized that mercury toxicity and mobility depend on its chemical speciation [6]. Methylmercury CH_3Hg(I), one of the most toxic mercury species, represents special concern because of its penetration through biological membranes, high stability and accumulation in the animal and human food chain [7]. Dissolved inorganic Hg(II) and monomethyl mercury(I), denoted as i-Hg(II) and CH_3Hg(I), are major Hg species in aquatic environment. However, typically CH_3Hg(I) represents less than 10% of the total mercury content in water [8,9]. Development of analytical procedures (of few ng L^{-1} levels) for reliable and accurate determination and speciation of Hg in water samples is a difficult analytical task, even by using highly sensitive hyphenated methods based on chromatographic separations and quantification by inductively coupled plasma mass spectrometry (ICP-MS), cold vapor (CV) atomic fluorescence spectrometry (CV-AFS) or CV atomic absorption spectrometry (CV-AAS) [9-11].

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Widely used approaches in routine analytical practice are based on preliminary separation and enrichment of Hg species by solid phase extraction (SPE). An ideal SPE procedure should fulfill several requirements: (i) to permit simultaneous sorption and retention of both Hg species; (ii) to permit selective sequential or simultaneous determination of target species, without extra steps; (iii) to ensure preservation of the original nature of each of the species; (iv) to be compatible with automated analytical systems; and (v) to allow *on-site* enrichment/separation during field sampling. An effective way to accomplish these requirements depends mostly on the features of the SPE sorbent and on the design of developed analytical scheme. Available schemes for i-Hg(II) and $CH_3Hg(I)$ speciation analysis are based on: selective sorption of i-Hg(II) with subsequent CV-AAS analysis of eluate, and calculation of $CH_3Hg(I)$ as a difference between the total Hg content after sample digestion and the i-Hg(II) content [12]. Otherwise, by sorption of both inorganic and methylated species followed by selective elution [13-15] or selective chromatographic separation of mercury species after enrichment [16]. Silica gel (SiG) is a well known inert support with high mechanical and chemical stability, easily modified by reaction of particular molecule with the siloxane (nucleophilic substitution at the Si atom) or silanol (direct reaction with the hydroxyl group) [17]. Chelating agents containing N and S atoms are usually used as surface modification agents, for example: ammonium pyrrolidine dithiocarbamate (APDC) [13], sodium diethyldithiocarbamate (SDDC) [16], polyaniline [15], 2-mercaptobenzimidazole [14], 1,5-bis(di-2-pyridyl) methylene thiocarbohydrazide [18], and dithizone-anchored polymeric microbeads [19].

This study focuses on the evaluation of L-cysteine grafted silica gel as an efficient SPE sorbent for Hg speciation analysis and determination in surface waters. L-cysteine immobilized on silica has been applied for the removal of Hg vapor from flue gasses [20]. Sorption of Hg(II) from aqueous solutions has been achieved by polythiol-functionalized alumina membranes [21] or cysteine functionalized controlled pore glass [22], but without possibilities for Hg speciation. The aim of the study was to develop a reliable method for quantitative sorption of both dissolved i-Hg(II) and $CH_3Hg(I)$ on the newly synthesized sorbent followed by selective elution of each species and measurements by CVG-AAS or ICP-MS. Analytical procedure has been applied for Hg determination in fresh and sea waters in Bulgaria in the low ng L^{-1} concentration range. Results for total Hg contents are confirmed by analysis of certified reference material (CRM) and independent parallel analyses by direct ICP-MS.

2. Experimental procedure

2.1. Instrumentation

CF-CVG-AAS measurements were carried out with a Varian AA 240 atomic absorption spectrometer equipped with a continuous flow VGA-77 Vapor Generation Accessory and externally heated quartz tube atomizer. Measurements were carried out at room temperature for i-Hg(II) and total Hg(II), and at 920°C for $CH_3Hg(I)$ (in eluates), controlled with an ETC-60 Electrothermal Temperature Controller. For convenience and better sensitivity of measurements, eluate solutions were introduced via *both* peristaltic pump channels (nominally indicated and utilized as ‘sample channel’ and ‘acid channel’ and used at flow rates of 7 and 1 mL min^{-1}, respectively), yielding in this case a combined flow rate of sample/eluate solutions of 8 mL min^{-1}. Reductant solution, 0.2% m/v $NaBH_4$ for i-Hg(II) (and 0.6% m/v $NaBH_4$ for $CH_3Hg(I)$) in 0.2% m/v NaOH, was introduced at a flow rate of 1 mL min^{-1}. The optimal instrumental parameters for Hg species determination by CVG-AAS are compiled in Table S1 (supporting information).

A microprocessor pH meter (Hanna Instruments, Portugal) was used for pH measurements.

Conventional infrared (IR) spectra were measured on a Bomem Michelson 100 FTIR spectrometer within 4000–400 cm^{-1}, with 2 cm^{-1} resolution; 200 scans.

Elemental analysis was carried out with a universal CHNOS elemental analyzer Vario EL III (Elementar Analysensysteme GmbH, Germany).

On site experiments for filtration and enrichment were performed with a home-made appliance for filtration and propelling liquids using positive external pressure from a small argon tank.

In column mode of SPE/elution experiments, liquid flows were pumped through SPE columns with ISM 846 peristaltic pump (ISMATEC SA, Glattbrugg, Switzerland).

2.2. Reagents

All reagents were analytical-reagent grade and all aqueous solutions were prepared in high-purity water (Millipore Corp., Milford, MA). Stock standard solutions for Hg were: inorganic Hg(II), stock standard solution for AAS, Trace CEPT™, 998 µg mL^{-1} in 2 mol L^{-1} HNO_3 (Sigma-Aldrich). Methylmercury stock solutions (100 mg L^{-1}) were prepared by dissolving the appropriate amount of methylmercury(II) chloride, PESTANAL®, analytical standard, Fluka (33368) in 5 mL of methanol and diluting to 100 mL with water. All stock solutions were stored in dark glass bottles at 4ºC. Working standard solutions for calibration were freshly (daily) prepared by successive dilution and contained reagents used for sample preparation or elution; they are further referred as 'reagent-matched standard solutions', *e.g.* in 4 mol L^{-1} HCl or in 0.1 mol L^{-1} thiourea – 1 mol L^{-1} HCl, *etc.*

Reductant solution of sodium tetrahydroborate(III) $NaBH_4$ (Merck) in 0.1% m/v NaOH was prepared fresh daily and was used without filtration.

L-cysteine (0.1 mol L^{-1}) and thiourea (0.05-0.1 mol L^{-1}) solutions were prepared fresh daily from (Merck, Darmstadt, Germany) reagents.

Silica gel 60, glutaraldehyde (25% m/m, Sigma – Aldrich, Germany), 3-aminopropyltrimethoxysilane, (APS, Fluka, Germany) were used to prepare the cysteine-functionalized silica gel sorbent (SiG-CYS).

Buffer solutions used for pH adjustment were: citrate/HCl for pH 3; CH_3COONa/CH_3COOH for pH 4 – 6; NaH_2PO_4/Na_2HPO_4 for pH 6 – 7. Doubly distilled water was used in all of the experiments.

For validation purposes a BCR®-505 Estuarine Water CRM stabilized with HNO_3 at pH 1.6, with 'Additional material information' value of 0.69 nmol kg^{-1} was used.

2.3. Procedures

2.3.1. Synthesis of L-cysteine grafted silica gel (SiG-CYS)

The scheme of L-cysteine modified silica gel preparation is shown in Supplementary Scheme 1 (see Supporting Information) [23]. Briefly amino groups were introduced onto silica surface by treatment of surface silanol groups with 3-aminopropyltrimethoxysilane, then L-cysteine was immobilized *via* a bifunctional reagent glutaraldehyde. The surface chemistry of cysteine modified adsorbent was proved by FTIR spectrometry and elemental analysis.

2.3.2. Column solid phase extraction procedures

2.3.2.1. Sorption and desorption studies

A small syringe (2 mL volume, 8 mm i.d., 60 mm length) was filled with 50 mg of dried SiG-CYS using aqueous slurry method to form a hydrated sorbent bed of ca. 8−10 mm height. A piece of frit was placed on the bottom of the syringe to fix the sorbent, while the slurry of SiG-CYS (50 mg) in water was passed under small pressure and then blocked above with another piece of frit. This procedure ensured even distribution of sample solution during column conditioning, sampling pumping through the column, and elution. A suitable volume of sample/test solution (50–200 mL), adjusted to a desired pH or acidity, was passed through the column at a flow rate of approximately 5 mL min^{-1}, which was found to be optimum for further applications. Various type of elution solutions were pumped through the column with flow rates of 1−2 mL min^{-1}. Mercury species were quantified in column effluates after sorption and in eluates by CVG-AAS or ICP-MS. The degree of sorption D% was calculated:

$$D\% = (m_i - m_{eff})/m_i \times 100,$$

where m_i [µg] is the initial mass of i-Hg(II) or $CH_3Hg(I)$ in sample solution and m_{eff} [µg] is their mass in effluent solution. The degree of elution E% is calculated as:

$$E\% = m_{el}/m_s \times 100,$$

where m_s [µg] is the mass of Hg(II) or $CH_3Hg(I)$ sorbed on SiG-CYS and m_{el} [µg] is their mass in eluate solution.

2.3.2.2. Analysis of surface waters

A. Determination of total dissolved Hg. According to the recommended procedures for sampling water, samples were filtered through a 0.45 µm membrane and preserved with $K_2Cr_2O_7$ (50 mg L^{-1} Cr(VI) in 0.05 mol L^{-1} HNO_3) until laboratory measurements. In this way the total Hg only could be determined. Water sample 200 mL was pumped with a flow rate of 5 mL min^{-1} through the column to retain the total Hg(II) which was then eluted with 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl at a elution

Table 1. Effect of sample solution pH and acidity on the degree of sorption of Hg(II) and $CH_3Hg(I)$ (mean of 3 measurements ± sd.).

Sample solution, acidity/pH	Degree of sorption (%)	
	Hg(II)	$CH_3Hg(I)$
2 mol L^{-1} HCl	> 99	< 1
1 mol L^{-1} HCl	> 99	< 5
0.5 mol L^{-1} HCl	> 99	< 5
0.2 mol L^{-1} HCl	> 99	< 10
0.1 mol L^{-1} HCl (pH 1)	> 99	30 ± 6
0.01 mol L^{-1} HCl (pH 2)	> 99	82 ± 7
pH 3	> 99	80 ± 8
pH 4	> 99	83 ± 7
pH 5	> 99	> 99
pH 6	95 ± 3	96 ± 3
pH 7	96 ± 3	97 ± 3

flow rate of 2 mL min^{-1} . Subsequently the sample was measured by CF-CVG-AAS *versus* calibration by means of reagent-matched standard solutions.

B. Determination of i-Hg(II). Water sample 200 mL was filtered through a 0.45 µm filter and preserved with 1 mol L^{-1} HCl until laboratory measurements. Sample was pumped through the column at a flow rate 5 mL min^{-1}. The retained i-Hg(II) was then eluted with 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl at a flow rate of 2 mL min^{-1} , and measured by CF-CVG-AAS *vs.* reagent-matched standard solutions.

C. Determination of Hg(II) and $CH_3Hg(I)$. During sampling water, sample 200 mL was filtered through a 0.45 µm filter. Filtrate was then pumped through the column filled with SiG-CYS. Columns were individually packed in plastic bags and transported to the laboratory for elution and quantification within 48 h from sampling/enrichment. In the laboratory, $CH_3Hg(I)$ was first eluted with 2 mL of 4 mol L^{-1} HCl, then Hg(II) was eluted with 2 mL 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl and Hg species were measured in eluates by CVG-AAS or ICP-MS *vs*. reagent matched standards.

3. Results and discussion

3.1. Optimization of parameters for Hg species separation and preconcentration

3.1.1. Effect of pH and sample acidity

The pH value and acidity of sample solution play an important role in the adsorption of ionic Hg species onto the L-cysteine modified silica gel. The influence of pH and HCl acid concentration on the degree of sorption of i-Hg(II) and $CH_3Hg(I)$ species is shown in Table 1.

Quantitative sorption for i-Hg(II) (95 – 99%) is achieved in a broad range of pH 1 – 7 and also in the presence of 0.1 – 2 mol L^{-1} HCl; quantitative sorption for $CH_3Hg(I)$ is found within the range of pH 5 − 7. The HCl acid concentration in sample solution would exhibit two side effects: first, protonation of amino and imino groups of the chelating molecules, and second, ensuring formation of chloro complexes on both i-Hg(II) and $CH_3Hg(I)$ [24,25]. However the chloro complexes of i-Hg(II) and methylmercury differ in their stability constants, charges and geometry. In acidic medium, retention of complex species of both inorganic and methylated species on the surface of SiG-CYS could be expected. This process is due to ion association between negatively charged chloro complexes and protonated SiG-CYS binding sites (see Supplementary Scheme 2, Supporting Information). However, the quantitative sorption of chloro complexes of monomethyl mercury(I) species, $[CH_3HgCl_2]^-$, would be prevented because of their larger size, lower charge and lower stability, particularly when large sample volumes are pumped through the sorbent [26]. At elevated pH values, efficiency of sorption would depend on the deprotonation of binding sites of chelating agent and complex formation on the surface of the SiG-CYS sorbent. The later factor would be in competition with possible interaction of mercury species with hydroxide ions in solution due to hydrolysis or hydroxo complexes formation. Strong retention of Hg(II) in the whole examined pH interval could be explained by high affinity of Hg(II) towards −SH groups and possibility for complex formation as shown in Supplementary Scheme 2 and Supplementary Scheme 3 (see Supporting Information). As seen in these schemes, methylmercury also could be retained on the SiG-CYS through complex formation of $CH_3Hg(I)$ with −SH groups, yet with lower stability constants *versus* i-Hg(II) ions [27]. Due to the strength of the S−Hg bond, complex formation through SH groups could be expected for i-Hg(II) and $CH_3Hg(I)$ existing as chloro compelexes in sea waters and in high-salt near-shore lake waters; therefore these species are quantitatively sorbed at pH values typical for sea water (see Supplementary Scheme 3, Supporting Information).

3.1.2. Optimization of elution conditions

For quantitative and selective elution of Hg species, solutions with different acid/base, redox or complex forming properties were evaluated. Volumes and flow rates of eluents were optimized in such a way to yield a few mL of eluent required for performing CVG-AAS or ICP-MS measurements, *e.g.* 2 – 5 mL (Supplementary Table 2, Supporting Information). As seen from these results, the i-Hg(II) species are strongly retained on SiG-CYS and are quantitatively eluted by acidic solution of complex yields such as thiourea. Both complexing agents L-cysteine and thiourea could elute up to 95% of the sorbed i-Hg(II) species, however for L-cysteine quantitative elution is achieved at pH ~ 5−6, whereas acidic medium (pH < 1) is required for thiourea. Eventually 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl has been adopted as more suitable eluent, taking into account a 20% signal depression by L-cysteine in CV-CVG-AAS measurements.

3.1.3. Effect of flow rate on the degree of sorption

Sample flow rate has been varied between 1 and 7 mL min^{-1}, considering optimal time of contact analyte/solid and the backpressure of SPE sorbent in column. The effect of sample flow rate on the degree of sorption was studied with model solutions spiked with 100 ng L^{-1} i-Hg(II) and $CH_3Hg(I)$. Experiments were carried out for i-Hg(II) in the presence of 1 mol L^{-1} HCl and for both i-Hg(II) and $CH_3Hg(I)$ in sample solutions with pH 6. Quantitative sorption ($D > 97\%$) was achieved for i-Hg(II) species at flow rate 7 mL min^{-1}, while $CH_3Hg(I)$ was not readily retained on the column and required lower flow rates. The recommended compromise sample flow rate for quantitative sorption of both Hg species was 5 mL min^{-1}.

3.1.4. Breakthrough volume

The breakthrough volume of the column is an important parameter for evaluating the application and extraction efficiency for real samples. Model breakthrough experiments were performed for both Hg species at concentration range 50 − 100 ng L^{-1}, added to ultrapure (Milli-Q) water. Different sample volumes (50 − 200 mL) were passed through the column at optimal flow rate; elution was performed with 2 mL or 5 mL of recommended eluent solutions (0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl for i-Hg(II) and 4 mol L^{-1} HCl for $CH_3Hg(I)$). Recoveries are summarized in Table S3 (supporting information), indicating minor R% impairment between 20, 100 and 200 mL; the largest sample volume of 200 mL was used in further studies for better enrichment factors.

3.1.5. Sorbent capacity

Sorbent capacity of the SiG-CYS is defined as the amount of the adsorbed i-Hg(II) and $CH_3Hg(I)$ (mmol) per gram of sorbent. It was determined by sorption from solutions containing individual Hg species at pH value 6.0 for CH_3HgCl, and in the presence of 1 mol L^{-1} HCl for i-Hg(II). For this purpose, a known amount of Hg species was passed through the column at flow rate of 5 mL min^{-1} and concentration of Hg remained in effluent solution was determined by ICP-MS. If necessary this process was repeated until column was saturated. The amount of adsorbed mercury species was calculated by using the following expression:

$$q = (n_i - n_{eff})/m,$$

where n_i [mmol] is the amount of substance of Hg species in initial solution, n_{eff} [mmol] is the amount of substance of Hg in effluent solution and m is the mass of sorbent (50 mg). Sorbent capacity was estimated at 0.7 mmol per gram sorbent for i-Hg(II) and 0.4 mmol per gram sorbent for CH_3HgCl. These capacity values (ca. 4 – 5 mg of Hg per 50 mg of sorbent) are considered high enough for practical work.

3.1.6. Stability of the column filled with SiG-CYS

The stability of the column has been evaluated for a period of 6 months. Experimental results have demonstrated fair high stability: the degree of sorption and elution of both Hg species under optimal chemical conditions has not changed after 6 months of storage. The column is characterized by high reusability and chemical stability. The efficiency of separation and preconcentration of both Hg species has not changed after at least 100 sorption/desorption cycles. The stability of inorganic and methylated Hg species loaded on the column was also tested. Different SPE columns were loaded with 5 ng each of Hg(II) and $CH_3Hg(I)$ and their elution was carried out 1, 2 and 3 days later after storage of wet columns (in sealed plastic bags) in a refrigerator at 4ºC. It was found that both Hg species could be eluted after 2 days of ageing. Reduced recoveries < 70% were observed for longer storage periods.

3.2. Analytical application of the sorbent SiG-CYS

3.2.1. Laboratory experiments

In order to test the applicability of the proposed SiG-CYS sorbent for the separation and preconcentration of i-Hg(II) and $CH_3Hg(I)$, unpolluted river (Kamchija River) and Black sea (Kaliakra Cape) waters were spiked individually with inorganic and methylated Hg(II) species at concentration levels in the range 10 − 50 ng L^{-1}. Optimized chemical conditions were applied for selective determination of i-Hg(II) in three types of water samples: i) with additions of HCl (final concentration in the sample 0.5 mol L^{-1} HCl), ii) in water samples adjusted to pH 6 with acetate buffer and iii) in waters at their natural pH (typical pH 7 – 7.9 for river waters and pH 8 – 8.2 for Black sea and salt-lakes waters). Same experiments for $CH_3Hg(I)$ were carried out at pH 6 as well as at natural pH values of water samples. Recoveries achieved by using 200 mL sample volumes and 5 mL eluent solutions are presented in Table 2.

It can be seen that the SiG-CYS sorbent offers quantitative recoveries for both Hg species under optimal chemical conditions. Thus, the sorbent could be successfully incorporated in analytical procedure for i-Hg(II) and $CH_3Hg(I)$ determination in surface waters. The sorbent has also been applied for the determination of total Hg in real water samples preliminary preserved (during sampling) with different stabilizing agents such as HNO_3 (100 µL per 200 mL sample) or a mixture of 0.05 mol L^{-1} HNO_3 + $K_2Cr_2O_7$ (50 mg L^{-1} Cr(VI)). Water samples (river and Black sea) after preservation were spiked with 50 ng L^{-1} i-Hg(II) and without any additional treatment were passed through the SiG-CYS column. Recoveries for total Hg varied within 93 – 99% for both preservation agents and results are comparables with those by direct ICP-MS measurements.

Table 2. Recovery test for spiked surface water samples (mean of 3 measurements ± sd.). Eluents: 4 mol L^{-1} HCl for $CH_3Hg(I)$; 0.1 mol L^{-1} thiourea in 0.1 mol L^{-1} HCl for i-Hg(II).

Sample	pH of sorption	Added species (ng L^{-1})	Found (ng L^{-1})	Recovery(%)
Kamchija River	0.5 mol L^{-1} HCl	i-Hg(II) 20	19 ± 1	95 ± 5
	pH 6.0	i-Hg(II) 50	48 ± 3	96 ± 6
	pH 6.0	$CH_3Hg(I)$ 10	10.2 ± 0.8	102 ± 7
	pH 7.8	i-Hg(II) 50	49 ± 4	98 ± 8
	pH 7.8	$CH_3Hg(I)$ 10	9.7 ± 0.7	97 ± 7
Black sea, Kaliakra Cape	0.5 mol L^{-1} HCl	i-Hg(II) 20	21 ± 1	105 ± 5
	pH 6.0	i-Hg(II) 50	48 ± 3	96 ± 6
	pH 6.0	$CH_3Hg(I)$ 10	9.6 ± 0.8	96 ± 8
	pH 7.8	i-Hg(II) 50	49 ± 3	98 ± 6
	pH 7.8	$CH_3Hg(I)$ 10	9.6 ± 0.9	96 ± 9

Table 3. Figures of merit.

Parameter	CVG-AAS			ICP-MS		
	Total dissolved Hg	i-Hg(II)	$CH_3Hg(I)$	Total dissolved Hg	i-Hg(II)	$CH_3Hg(I)$
RSD (%)	8 – 12	7 – 11	9 – 14	7 – 10	7 – 11	8 – 12
LOQ (ng L^{-1})	2.5	2.5	5	1	1	1.5

3.2.2. On-site enrichment/separations

The possibility for field treatments for speciation determination and enrichment of dissolved i-Hg(II) and MeHg(II) during sampling of surface waters, followed by subsequent laboratory measurement of eluted Hg species has been examined. Surface water sample 200 mL spiked with both 20 ng L^{-1} i-Hg(II) and 10 ng L^{-1} $CH_3Hg(I)$ was first filtered through a 0.45 μm membrane, and filtered again directly through the column filled with 50 mg SiG-CYS. The whole enrichment procedure was performed at the sampling site without any previous preservation or acidification of the sample avoiding probable changes/redistributions in analyte speciation. The column was kept wet in a sealed plastic bag and transported to the laboratory for selective elution of the sorbed Hg species followed by CF-CVG-AAS measurements. Recoveries for river waters and Black sea water varied between 92% and 96%, provided that the period between sampling/column loading and (laboratory) elution did not exceed 48 hours.

3.2.3. Figures of merit

The accuracy and precision of this analytical procedures have been evaluated by the determination of total dissolved Hg (after sample treatment with HNO_3), dissolved i-Hg(II) and dissolved $CH_3Hg(I)$ in spiked (10 – 50 ng L^{-1} i-Hg(II), 5 – 10 ng L^{-1} $CH_3Hg(I)$) unpolluted river and Black sea water. Three different columns filled with SiG-CYS under optimal chemical conditions (200 mL sample solution; 5 mL min^{-1} sample flow rate; 5 mL eluent) were used. Eluted Hg has been measured by CF-CVG-AAS or ICP-MS. Results were used for the calculation of quantification limits (LOQ, 10σ criteria) and relative standard deviations (RSD%) as shown in Table 3.

For partial validation of procedure, a CRM Estuarine Water BCR® – 505 was analyzed. Three sample aliquots of 50 mL (prepared in 0.5 mol L^{-1} HCl) were pumped through the SiG-CYS column and eluted Hg(II) was measured by CF-CVG-AAS. The result of 0.63 ± 0.06 nmol L^{-1} Hg(II) was in reasonable agreement with the (‘additional material information’) value of 0.69 nmol kg^{-1} Hg. The content of $CH_3Hg(I)$ species was below the LOQ (< 5 ng L^{-1} by CF-CVG-AAS and < 1.5 ng L^{-1} by ICP-MS). Several real river and near-shore high-salt lake water samples have been analyzed by CF-CVG-AAS after enrichment for i-Hg(II) and $CH_3Hg(I)$, and by direct ICP-MS without enrichment, after dilution in external laboratory (Table 4). The agreement between methods for river waters is acceptable, while the direct ICP-MS lacked sensitivity (LOQ < 100 ng L^{-1}) with high-salt water samples. Low methylmercury content (< 5 ng L^{-1}) in examined (top 20-cm) surface water samples is an expected finding.

4. Conclusion

Sorbent based on silicagel grafted with L-cysteine has been characterized for efficient solid phase extraction and speciation determination of Hg in surface waters.

Table 4. Comparative analyses of river and Black sea water samples by proposed procedure and by direct ICP-MS without enrichment, ng L^{-1} (mean of 3 measurements ± sd.).

Sample	SPE (SiG-CYS) CVG-AAS		Direct ICPMS*
	i-Hg(II)	$CH_3Hg(I)$	Total dissolved Hg
Provadijska River	< 2.5	< 5	< 100
Vranja River	17.1 ± 1.5	< 5	< 100
Ajtoska River	10.2 ± 1.1	< 5	< 100
Kamchija River	< 2.5	< 5	< 100
Beloslav Lake	17.6 ± 1.3	< 5	< 100
Varna Lake West	14.1 ± 1.3	< 5	< 100
Maritsa River	48 ± 4	< 5	50 ± 5
Struma River	18.1 ± 1.8	< 5	20 ± 3
Tundja River	42 ± 3	< 5	40 ± 4

Direct ICPMS results from an external laboratory, GAMA Centre, University of Plovdiv.

Enrichment procedure is simple and fast and could be performed *on site* during sampling. Performance characteristics such as limits of quantification and precision for dissolved inorganic mercury(II) and methylmercury(II) species are in compliance with requirements of Directive 105/2009/EC and 2009/90/EC, which renders analytical procedures applicable to River Basin monitoring programs.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the National Scientific Fund, Project DDVU 02/77. Thanks are due to Dr. V. Kmetov (Faculty of Chemistry, GAMA Center, University of Plovdiv) for independent measurements by ICP-MS.

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