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On-line speciation of mercury and methylmercury by cold vapour atomic absorption spectrometry using selective solid phase extraction

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A novel non-chromatographic on-line cold vapour atomic absorption spectrometric (CV-AAS) method for sequential mercury speciation at the ng l^{-1} level was developed based on the selective retention of inorganic mercury on polytetrafluoro ethylene (PTFE) using a dual manifold. A column packed with PTFE turnings was used for inorganic mercury separation from the sample solution *via* the efficient retention on the sorbent material of the pyrrolidine dithiocarbamate complex $Hg(PDC)_2$. On the other hand, the PDC complex of methylmercury (CH_3HgPDC) is barely adsorbed, thus facilitating its direct determination after reduction by $NaBH_4$ and subsequent on-line thermal dissociation of the resulting hydride. Inorganic mercury in the presence of methylmercury species is determined in a parallel manifold due to the fact that the later one cannot be reduced by $SnCl_2$. The recovery of the proposed method was evaluated for drinking water, sea-water and urine samples.

Introduction

Various selective hyphenated techniques have been applied for trace mercury speciation, which combine chromatographic separation and spectrometric detection.[1–6] However, due to the complexity and high cost of instrumentation, a number of non-chromatographic speciation schemes have also been reported for mercury speciation employing CVAAS.[7–9] Subtraction between total and inorganic mercury fraction is a common indirect speciation approach found in the literature. Without any prior decomposition procedure, inorganic mercury is selectively determined, otherwise total mercury is determined. Another approach is the use of the SPE technique and the successive elution of the species.[8,10]

Our previous research has proved the ability of PTFE turnings to act as a strong absorber of the pyrrolidine dithiocarbamate (PDC) complex of inorganic mercury.[2] In contrast to inorganic mercury, we observed that the retention of the pyrrolidine dithiocarbamate complex of methylmercury is negligible, and to our knowledge, this discrimination has not been applied for mercury speciation purposes.

The aim of this work was to develop a novel on-line CVAAS method for mercury speciation based on the different retention times of the two species on the PTFE sorbent material.

Experimental

Instrumentation

A Zeiss PMQ3-MQ3 model UV/VIS spectrophotometer was used as the detector and a Perkin Elmer mercury electrodeless discharge lamp was used as an intense line source at 253.7 nm. This configuration allowed the employment of a laboratory manufactured cylindrical glass gas flow-through atomic absorption cell (AAC, 300 mm length; 7 mm id) with quartz windows. A flow-through electrically heated quartz tube (HT, 200 mm length; 7 mm id) was used for on-line thermal dissociation of the methylmercury hydride and it is located before the AAC, as is illustrated in Fig. 1.

The FI on-line cold vapour generation dual manifold and its operation are shown schematically in Fig. 1. It consists of three peristaltic pumps (Watson-Marlow Model 205U/BA, Gilson Model Minipuls 3), two six-port two-position injection valves, IV1 and IV2 (Reodyne, USA) with a separation column (SC) on IV1, three eight-port selection valves, SV1, SV2 and SV3 (Valco, C252) and two integrated gas–liquid separators/reactors, GLS-1 and GLS-2. Tygon pumping tubes were used for aqueous solutions delivery.

The separation column (SC), 100 mm length, 4 mm id, which was packed with PTFE turnings (*ca.* 0.1 mm width, 1.3 g), was produced in our laboratory as described previously.[2] The advantages of using PTFE turnings as packing material are the very good stability and resistance to strong chemicals and the excellent affinity for dithiocarbamate metal complexes, as is reported elsewhere.[11] In addition the geometry of turnings allows high sample flow rates with low back pressure, facilitating the high sampling frequency.

The integrated gas–liquid separator/reactor (GLS), 100 mm length and 26 mm id, has been introduced and described previously.[12] On the outlets of the two GLSs, a selection valve SV3 was elaborated to facilitate the sequential transportation of CH_3HgH and Hg^0 to the AAC through the HT. With this manifold the released volatile compounds were collected into the upper part of each GLS until SV3 is opened.

Reagents

All chemicals used were of analytical reagent grade and were supplied by Merck. Water from a Milli-Q water system (Millipore, Bedford, MA, USA) was used throughout. All working standard solutions were prepared prior to use by stepwise dilution of a 1000 mg l^{-1} Hg^{2+} stock standard solution. A stock solution of 1000 mg l^{-1} CH_3HgCl was prepared by dissolving a suitable amount of CH_3HgCl in a minimum volume of methanol and diluting to the required μg l^{-1} levels. The $SnCl_2$ reducing solution, 2.5% m/v, was prepared fresh daily by dissolving 3.0 g of $SnCl_2 \cdot 2H_2O$ (<0.000 001% Hg) in 5 ml of concentrated HCl and diluting to 100 ml. The $NaBH_4$ solution, 3% m/v, was prepared daily by dissolving 3.0 g of $NaBH_4$ (<0.000 005%Hg) in 10 ml of 2.5 mol l^{-1} NaOH and diluting to 100 ml. 1-Octanol was used as an antifoaming agent.

† Part of his PhD Thesis.

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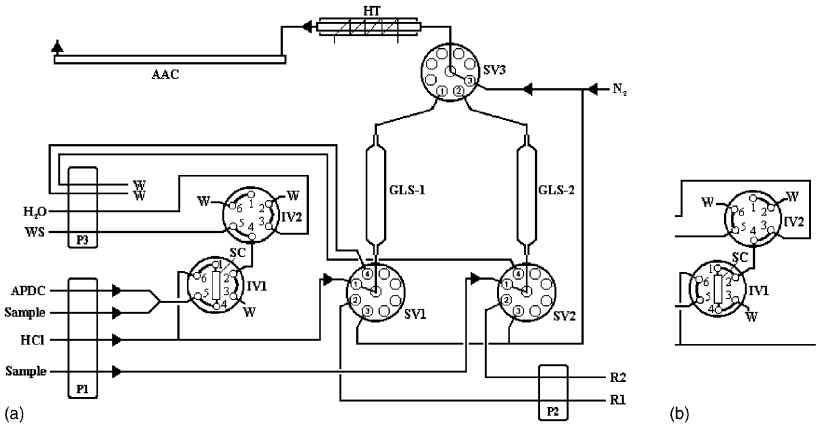

Fig. 1 FI on-line determination of mercury species by CVAAS; APDC, 0.05% m/v APDC solution; HCl, 1.5 mol l^{-1} HCl solution; R1, 3% m/v $NaBH_4$ solution; R2, 2.5% m/v $SnCl_2$ solution; WS, washing solution, 1.5 mol l^{-1} HCl; W, waste; SC separation column; P1, P2, P3 peristaltic pumps; IV1, IV2, injection valves; SV1, SV2, SV3, selection valves; GLS-1, GLS-2, gas–liquid separator/reactor; HT, electrically heated tube; AAC, atomic absorption cell. (a) Injection valves IV1 and IV2 in "A" position. (b) Injection valves IV1 and IV2 in "B" position.

Procedure

The operation sequence for the on-line determination of mercury species is summarized in Table 1 and runs in two parallel lines, through six steps. Methylmercury is reduced in GLS-1, inorganic mercury in GLS-2.

In step 1 (Fig. 1), sample and APDC streams were merged together towards the separation column (SC), where only $Hg(PDC)_2$ complex could be retained. Thus, the GLS-1 was filled with a solution containing only $CH_3Hg(PDC)$. In the same time an equal sample volume was pumped directly into the GLS-2. Meanwhile, the heated tube (HT) and the atomic absorption cell (AAC) were purged by a continuous nitrogen stream. During step 2, $NaBH_4$ and $SnCl_2$ solutions were loaded to the GLS-1 and GLS-2, respectively, in order to reduce the two mercury species. During this step the outlets of the two GLSs were closed, thus resulting in the collection of CH_3HgH and Hg^0 in GLS-1 and GLS-2, respectively. In step 3 the generated (in GLS-1) methylmercury hydride vapour was separated from the liquid mixture and transported to the AAC through HT by the stream of N_2. In the HT the hydride was thermally dissociated to elemental mercury vapour. During this step the absorbance of methylmercury was measured. In step 4, SV3 was turned to position "2" and the released mercury vapour in GLS-2 was transported to AAC and the absorbance measured.

Sample treatment

The aqueous samples of natural waters were filtered through a 0.45 μm membrane filter, and acidified to pH 2.5 ± 0.2 with dilute HNO_3. Urine samples (250 ml) taken from two healthy persons, filtered and acidified to a pH of *ca.* 2.5 with dilute HNO_3. A suitable portion of urine sample was transferred to a 50 ml volumetric flask where 1 ml of Hg^{2+}–CH_3Hg^+ mixed standard solution was added. No dead volume remained at the top of the flask, in order to prevent any volatilization of methylmercury. The flask was closed and the mixture left to be equilibrated in a water bath for 2 h at 37 °C. Owing to foaming in the urine, a small quantity of 0.5 ml of antifoaming reagent was introduced into the GLSs prior to mercury reduction.

Results and discussion

Speciation scheme

The speciation of inorganic and methylmercury was based on the observation that, in contrast to inorganic mercury, the retention of the pyrrolidine dithiocarbamate complex of methylmercury in the PTFE column is not significant. A likely cause for this different behavior is probably that $CH_3Hg(PDC)$ is more polar than (PDC)Hg(PDC). A study on the retention capability of the PTFE column proved that less than 5% of $CH_3Hg(PDC)$ was retained, while for $Hg(PDC)_2$ the retention was quantitative using individual solutions of the two species at a concentration of 1.0 μg l^{-1}.

According to the above observation, a determination of the two mercury species using $SnCl_2$ as the selective reductant for Hg^{2+} in the presence of CH_3Hg^+ and $NaBH_4$ for CH_3Hg^+ after a preceding separation of Hg^{2+} is feasible. In order to minimize the time for the determination of the two species and to avoid problems of subsequent use of the two reductant solutions in the same manifold, a simultaneous determination using a dual manifold was preferred, as is described above (Fig. 1, Table 1). Preliminary experiments showed

Table 1 Operating sequence of the FI on-line speciation of mercury and determination by CVAAS

		Valve positions					Pumps			Delivered	Flow	
Step	Time/s	IV1	IV2	SV1	SV2	SV3	P1	P2	P3	medium[a]	rate/ml min^{-1}	Operation
										Sample[b]	12.0	
1	50	A	A	1	1	3	ON	OFF	OFF	APCD	0.6	Sample loading
										HCl	1.2	
2	10	A	A	2	2	3	OFF	ON	OFF	$NaBH_4$	4.8	Reductant loading and reduction
										$SnCl_2$	6.0	
3	10	A	A	3	2	1	OFF	OFF	OFF	N_2	200	Methyl mercury measurement
4	10	A	A	4	3	2	OFF	OFF	OFF	N_2	200	Inorganic mercury measurement
5	30	B	A	4	4	3	OFF	OFF	ON	HCl	10	Washing of column and GLS evacuation
										Waste	24	
6	10	B	B	4	4	3	OFF	OFF	ON	H_2O	10	HCl removing from column

[a] In all steps except 3 and 4, the flow through cell is purged by N_2 at 200 ml min^{-1} flow rate. [b] Two separate sample lines were used simultaneously with a flow rate of 12.0 ml min^{-1} in each one.

Table 2 Analytical performance of the on-line CVAAS speciation method

Parameter	Hg^{2+}	CH_3Hg^+
Sample volume	10 ml	10 ml
Sampling frequency	30 h^{-1}	30 h^{-1}
Linear range	0.07–5.0 μg l^{-1}	0.12–7.0 μg l^{-1}
Regression equation ([Hg] in μg l^{-1})	0.0392[Hg^{2+}] + 0.0037	0.0183[CH_3Hg^+] + 0.0002
Correlation coefficient (r)	0.9996	0.9993
Detection limit (c_L)	0.04 μg l^{-1}	0.08 μg l^{-1}
Precision (s_r, $n = 10$, 1.5 μg l^{-1})	2.7%	7.1%

that it is more convenient to liberate and measure the methylmercury hydride first, in order to avoid a pressure increase due to the produced nascent hydrogen and the subsequent restriction to mercury vapour evolution.

Another point to mention is the formation of volatile hydride CH_3HgH from the reduction of the CH_3Hg(PDC) by $NaBH_4$ in acidic medium instead of atomic mercury vapour, which is produced from the reduction of $Hg(PDC)_2$ by $SnCl_2$. For this reason, in order to measure methylmercury species a thermal decomposition of the CH_3HgH is necessary. [5] The decomposition/atomization temperature was studied and the minimum temperature for efficient atomization of the methylmercury hydride was found to be in the range 650–700 °C, thus 700 °C was adopted for further study. At this temperature, no significant decrease in the sensitivity of inorganic mercury determination was observed. Finally, with the proposed speciation procedure, the use of strong acids or volatile organic solvents for analyte elution before the reduction is avoided and an extra oxidation step is not necessary.

Optimization of the method

The chemical and flow variables of the used dual manifold, which affect both separation and mercury vapour generation, were optimized using 1.0 μg l^{-1} Hg^{2+} and 1.0 μg l^{-1} CH_3Hg^+ species, respectively.

The optimum pH range for $Hg(PDC)_2$ formation was between 2.0 and 3.1. Thus, the effect of the sample pH on the retention of CH_3Hg(PDC) complex was studied just in the range between 2.0–3.1. At this range the retention of methylmercury was less than 4–7%, consequently the sample pH was adjusted to 2.5. A 0.05% m/v APDC solution was used throughout, in order to ensure complete retention in case of the presence of other metals.

According to previous work[2] a 12.0 ml min^{-1} sample flow rate was selected for the separation of CH_3Hg^+ in the GLS-1 for high sensitivity and sufficient sampling frequency. The same sample flow rate was used for sample loading in the GLS-2.

The effect of the $NaBH_4$ concentration was investigated in the range 0.3–3.0% m/v and maximum signal was obtained at 3% m/v $NaBH_4$. A volume of 0.8 ml of $NaBH_4$ was adequate for a 10 ml sample as was proved in a preliminary experiment. Thus, a 4.8 ml min^{-1} $NaBH_4$ flow rate for 10 s was adopted. The flow rate of $SnCl_2$ was fixed at 6.0 ml min^{-1} for 10 s in order to introduce 1 ml, which is a sufficient volume for the reduction.

Analytical performance characteristics. The performance characteristics of the proposed on-line separation CVAAS method of inorganic and methylmercury speciation, under optimum conditions, are summarized in Table 2. The precision of inorganic mercury determination is very good ($s_r = 2.7\%$), while the corresponding value of methylmercury determination was higher ($s_r = 7.1\%$).

The linear range of inorganic mercury is not limited by the presence of methylmercury because the latter cannot be reduced by $SnCl_2$. However, the linear range of methylmercury is practically not affected in the presence of inorganic mercury up to 2 μg l^{-1} concentration levels. At a higher concentration of Hg^{2+} the retention of inorganic mercury in the PTFE column is not quantitative, so the samples should be diluted at the expense of methylmercury sensitivity. The determination of inorganic mercury species by $SnCl_2$ as reducing agent is not affected by the presence of Zn^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+} and Al^{3+} up to 5 mg l^{-1}. On the other hand, if an ion affects the complexation and retention of inorganic mercury, it may also affect the determination of CH_3Hg^+ species. It was found that concentrations up to 2 mg l^{-1} of Zn^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+} and Al^{3+} did not produce significant interference.

Recovery studies and applications

The performance of the proposed method was tested for the analysis of artificial mixtures of mercury and methylmercury prepared in properly acidified (pH *ca.* 2.5) matrices like drinking water and sea-water, collected from Northern Greece, and a human urine sample prepared as described under *Sample treatment*. The results are presented in Table 3.

Table 3 Determination of mercury and methyl mercury in water and urine samples

	Added/μg l^{-1}		Found/μg l^{-1} [a]	
Sample	Hg^{2+}	CH_3Hg^+	[Hg^{2+}] ± s	[CH_3Hg^+] ± s
Drinking water				
	0.00	0.00	<0.04	<0.08
	1.50	1.50	1.44 ± 0.09	1.58 ± 0.14
	1.00	2.00	1.03 ± 0.05	1.84 ± 0.15
Sea-water				
Coastal sea-water I	0.00	2.00	<0.04	2.05 ± 0.21
	2.00	0.00	2.16 ± 0.09	<0.08
	2.00	2.00	2.10 ± 0.11	1.97 ± 0.18
	2.00	5.00	2.12 ± 0.11	4.81 ± 0.48
Coastal sea-water II	0.00	0.00	0.05	<0.08
Estuarine water	0.00	0.00	0.09 ± 0.01	<0.08
Human urine				
Sample A	0.00	2.00	0.10 ± 0.01	2.25 ± 0.20
	2.00	0.00	1.99 ± 0.12	<0.08
	2.00	2.00	2.10 ± 0.08	2.28 ± 0.23
	2.00	5.00	2.05 ± 0.10	4.72 ± 0.42
Sample B	0.00	0.00	0.22 ± 0.03	<0.08

[a] Mean ± s based on five replicates.

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