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ORIGINAL PAPER

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On-line mercury and methylmercury pre-concentration by adsorption of their dithiophosphoric acid diacyl ester chelates on a C_{18} column and cold-vapor atomic-absorption detection

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Abstract The pre-concentration of mercury(II) and methylmercury by adsorption of their dithiophosphoric acid diacyl ester (DDTP) chelates on a C_{18} column, then detection with cold-vapor atomic-absorption spectrometry was investigated. Conditions such as sample pH, reductant and chelating agent flow and concentration, and eluent and carrier gas flow were optimized. Optimization was performed by use of evolutionary operation with a proper factorial design. At a sample flow of 5.3 mL min^{-1} and a loading time of 4.5 min, column adsorption efficiency ranged from 88 to 93% for both species. Detection limits down to 10 ng L^{-1} were obtained at a sample throughput of 12 h^{-1}. There was good agreement between found and certified values in the analysis of certified reference materials after their microwave-assisted mineralization with HNO_3 and H_2O_2.

Introduction

Mercury is important environmentally and toxicologically [1, 2]. Consequently, much effort has been focused on the development of reliable methods for its determination at trace levels. Among numerous procedures described in the literature [3, 4], mercury detection by cold-vapor atomic-absorption spectrometry (CVAAS) has been the most popular [5, 6, 7, 8, 9]. CVAAS accessories are easily attached to commercial atomic-absorption apparatus, although special equipment is also available [10, 11, 12]. Although other AAS methods, e.g. inductively-coupled-plasma atomic-emission spectrometry (ICP–AES) [13, 14], inductively-coupled plasma mass spectrometry (ICP–MS) [15, 16], and atomic-fluorescence spectrometry (AFS) [17, 18] can also be used, directly or after vapor generation, CVAAS is a very good compromise between sensitivity and operating costs, if Hg concentrations in the most relevant environmental and clinical samples can be simply assessed by this technique. Cold-vapor generation is performed well in flow-injection systems [19, 20, 21], although batch systems can lead to better detection limits because of the large amount of sample they can use [22]. Despite the low limits of detection of CVAAS, the analysis of some samples might require a pre-concentration step. Among the different pre-concentration techniques available [23, 24, 25, 26, 27, 28, 29, 30], amalgamation of the mercury vapor on an Au/Pt trap should be emphasized [31, 32, 33, 34]. Column pre-concentration has also been used extensively [35, 36]. For this technique very low pH should usually be avoided, although acidic samples are very common in mercury determination. It is, on the other hand, well known that dithiophosphoric acid diacyl ester (DDTP) chelates are formed even at low pH [37, 38, 39, 40]. The aim of this work was to investigate an alternative on-line mercury and methylmercury pre-concentration procedure based on adsorption of their DDTP chelates on a C_{18} column.

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Experimental

Apparatus

A Perkin–Elmer model 1100B atomic-absorption spectrometer, equipped with a Perkin–Elmer FIAS 200 flow-injection accessory were used for the measurements. A mercury hollow-cathode lamp (Hamamatsu Photonics) was operated at 6 mA, at the 253.7 line. A T-shaped 16 cm long quartz tube (Perkin–Elmer #B0507486) with quartz windows was used as absorption cell. The C_{18} column was from Perkin–Elmer (#B0504047) as was the gas–liquid separator (#B0193772). A closed system (CEM Mars 5) was used for microwave-assisted digestion.

Reagents and solutions

The DDTP-NH_4 (Ega Chemie, Steinheim Albuch, Germany) solution (0.05% m/v) was obtained by dissolving the salt in Milli Q water. Analytical $HgCl_2$ solutions were prepared daily by convenient dilution of a 1000 μg mL^{-1} Titrisol stock solution (Merck, Darmstadt, Germany). The 1000 μg mL^{-1} CH_3Hg^+ stock solution was prepared by dissolving the chloride salt (Pfaltz and Bauer, CT,

USA) in 50% ethanol (Vetec, Rio de Janeiro, Brazil) and the respective analytical solutions were prepared daily by convenient dilution of this solution. Other reagents were of analytical reagent grade and Milli Q water was used throughout.

The FIAS 200 pre-concentration manifold – set-up and operation

The flow-injection manifold for the on-line pre-concentration procedure is shown in Fig. 1 (valve in the inject position); Table 1 shows the respective FIAS-200 program. With the valve in the injection position the sample and the chelating agent were carried by pump 2 and directed to the pre-concentration column (step 2). Step 2 was performed 1, 2, or 3 times for loading times of 1.5, 3.0, and 4.5 min, respectively. Elution occurred at step 3 (valve in the fill position), when the eluent (ethanol) was carried by pump 1 to the column. The direction of elution was opposite to that of adsorption flow [41], to minimize analyte dispersion through the column. Concomitantly 1.0×10^{-4} mol L^{-1}HCl (Hg^{2+}) or 0.5 mol L^{-1}HCl+0.01% $FeCl_3$ (CH_3Hg^+) merged with the eluate flow, followed by the reductant ($NaBH_4$) stream. Reduction occurred and the Hg vapor was separated at the gas–liquid separator and carried by the argon flow to the absorption cell. Steps 1 and 4 were necessary for cleaning. For comparison, a conventional FIA montage, without any pre-concentration step, and following the conditions proposed by the manufacturer [42] was also used; in this experiment a 500-µL sampling loop was employed. Table 2 shows the FIAS program used for this set-up. All measurements were of peak height.

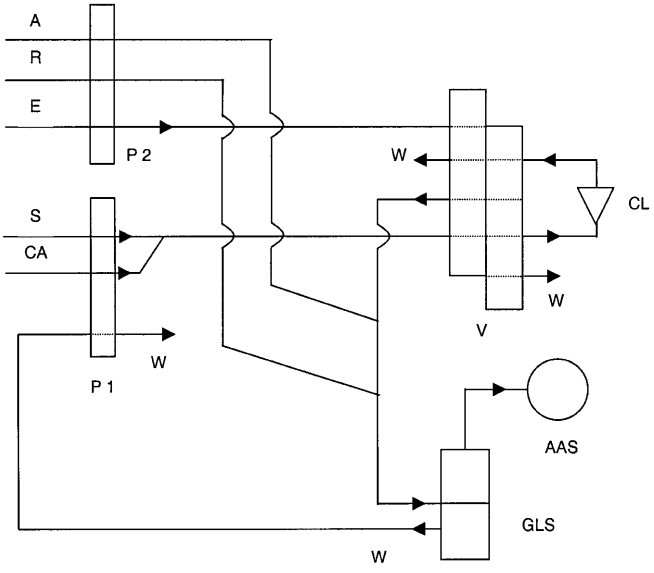

Fig. 1 The FIA manifold used for on-line pre-concentration of Hg^{2+} and CH_3Hg^+ by adsorption of their DDTP chelates sorption on a C_{18} column. The valve is in the inject position. *A*, HCl; *R*, $NaBH_4$; *E*, ethanol; *S*, sample; *CA*, DDTP; *P1* and *P2*, peristaltic pumps; *W*, waste; *Cl*, column; *V*, valve; *GLS*, gas-liquid separator; *AAS*, atomic absorption spectrometer

Table 1 FIAS 200 program used for on-line pre-concentration of Hg and CH_3Hg^+ by adsorption of their DDTP chelates on a C_{18} column

Step	Time (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve position	Read
1	1	80	60	Fill	No
2[a]	90	80	0	Inject	No
3	40	80	60	Fill	Yes
4	1	80	0	Fill	No

[a]Step 2 is performed 1, 2, or 3 times for loading times of 1.5, 3.0, and 4.5 min, respectively

Table 2 FIAS 200 program used for conventional (without pre-concentration) flow injection Hg determination (sample loop, 500 µL)

Step	Time (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve Position	Read
1	20	80	100	Fill	No
2	30	0	100	Inject	Yes

Table 3 Microwave heating program for digestion of the certified reference materials

Step	NIST 1572 citrus leaves		MESS-3 marine sediment	
	Time (min)	Power (W)	Time (min)	Power (W)
1	5	300	2	240
2	0.5	600	5	300
3	4	240	2	0
4	4	0	1	600
5	-	-	16	300

Certified reference materials digestion procedures for total Hg determination

NIST 1572 citrus leaves (300 mg) were mineralized with conc. HNO_3 (5 mL) plus 30% H_2O_2 (2 mL). MESS-3 marine sediment reference material (250 mg) was digested with HNO_3 (2 mL) plus 30% H_2O_2 (2 mL). In both experiments mineralization was performed by microwave-assisted heating in a closed system. After digestion the clear solution was filtered through a Whatman no. 41 filter paper and the volumes were diluted to 50 mL with Milli Q water. The microwave heating programs are shown in Table 3.

Certified reference material extraction procedure for methylmercury determination

Methylmercury was extracted by mechanically shaking the sample (Dolt-2, Dogfish liver, National Research Council, Canada; 100 mg) with HCl (3 mol L^{-1}, 10 mL) for 10 min, in a screwed-capped, conical ended, 50-mL plastic tube. The resulting suspension was filtered through a Whatman no. 41 filter paper and diluted to 50 mL with Milli Q water.

Results and discussion

Optimization of the pre-concentration flow-injection conditions

The conditions used for the flow injection procedure were optimized by evolutionary operation (EVOP) [43], with two levels factorials. Table 4 shows the conditions and their optimized values, obtained by use of 1 µg L^{-1} analytical solution. pH was adjusted by using HCl solutions of appropriate concentration as diluent. Different chemical con-

Table 4 Optimized conditions used for on-line pre-concentration of Hg^{2+} and CH_3Hg^+ by adsorption of their DDTP chelates on a C_{18} column

Parameter	Optimized values
Analytical solution pH	0–5
Chelating agent concentration	0.05%
Acidifying solution for the reduction	1.0×10^{-4} mol L^{-1} HCl (Hg^{2+}) and 0.5 mol L^{-1}HCl +0.01% $FeCl_3$ (CH_3Hg^+)
Reductant ($NaBH_4$) concentration	0.1% in 0.1% NaOH (Hg^{2+}) and 0.3% in 0.1% NaOH (CH_3Hg^+)
Argon flow	80 mL min^{-1}
Sample flow	5.3 mL min^{-1} (1.52[a])
Chelating solution flow	2.2 mL min^{-1} (0.76[a])
Reductant solution flow	1.9 mL min^{-1} (0.76[a])
Acid solution flow	3.3 mL min^{-1} (1.14[a])
Eluent flow	1.9 mL min^{-1} (0.84[a])
Elution time	40 s

[a]Values in parenthesis indicate the respective pump tube inner diameter, in mm

ditions are necessary for reduction of the species. A more concentrated reducing solution and the presence of Fe^{3+} was necessary to reduce the organic species. Increasing the concentration of $NaBH_4$ and the presence of Fe^{3+} did not change the Hg^{2+} response. Thus, both species could be reduced equally well reduced under the conditions used for CH_3Hg^+.

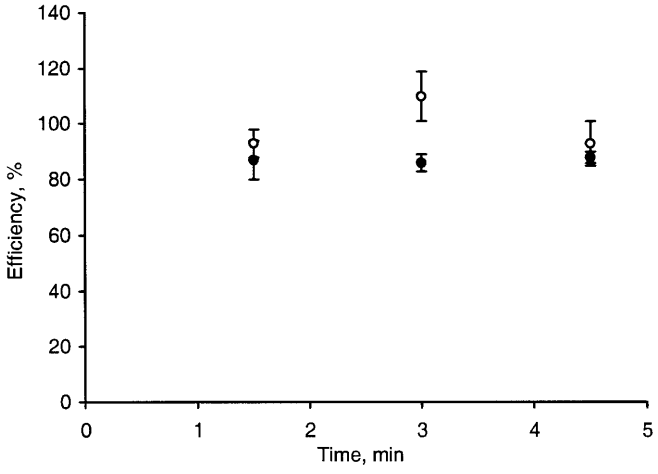

Fig. 2 Dependence of column adsorption efficiency on adsorption time for Hg^{2+} (*filled circles*) and CH_3Hg^+ (*open circles*). Analytical conditions as listed in Table 4

Adsorption efficiency

The adsorption efficiency was studied for different elution times (1.5, 3.0, and 4.5 min) using a 10 μg L^{-1} solution, under the optimized conditions shown in Table 4. For assessment of this efficiency the ethanolic eluates were recovered and analyzed by use of conventional FIAS 200. A 500-μL loop was used and the analytical curve was obtained by use of the same medium as for the eluate. Figure 2 shows the results; average efficiencies >86% were always obtained for all the loading times studied.

Analytical figures of merit

Analytical figures of merit for different loading times are shown in Table 5. For comparison, data obtained by conventional FIAS using a 500 μL loop are also shown. The detection limits are calculated from 3×s/b where s is the standard deviation from ten analytical solution blank measurements, and b is the slope of the respective calibration plot. The calibration plots were always linear, with correlation coefficients >0.99, for the concentration range studied (0.05–5 ng mL^{-1}).

The same sensitivities are observed for both species, and the limits of detection were close. Pre-concentration factors up to 20 were obtained, if calculated from the ratio of the pre-concentration procedure characteristic concentrations to that of the conventional FIAS procedure. Com-

Table 5 Analytical figures of merit

Proposed Procedure							Conventional FIAS procedure	
Loading time (min)	1.5		3.0		4.5		-	
Species	Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+
Limit of detection (ng L^{-1})	38	44	15	31	10	18	200	517
Characteristic concentration (ng mL^{-1})	0.51	0.50	0.27	0.25	0.19	0.17	3.7	6.4
Characteristic mass (ng)	4.0	4.0	4.3	4.0	4.5	4.1	1.8	3.2
Sample throughput (h^{-1})	27	27	16	16	12	12	72	72

Table 6 Results (μg g^{-1}, n=6) from the determination of Hg in certified reference materials by the proposed procedures. Analytical conditions as listed in Table 4

Certified reference material	Found	Certified
NIST 1572, citrus leaves[a]	0.09 ±0.02	0.08 ±0.02
MESS-3, marine sediment[a]	0.093±0.013	0.091±0.009
DOLT-2, dogfish liver[b]	0.690±0.010	0.693±0.053

[a]Total mercury
[b]Methylmercury

parison of the limits of detection (a more realistic evaluation of the potential of the method [38]) shows the improvement to be as much as 20 and 29 times for Hg^{2+} and CH_3Hg^+, respectively. As expected for the system, the characteristic masses do not depend on the loading time; this is also consistent with the similar adsorption efficiencies observed for the different loading times. The lower characteristic mass of the conventional FIA procedure is also expected, because of the lower Hg dispersion obtained by use of this procedure, considering the measurement mode (peak height). Loading times >4.5 min did not lead to significant improvement of the analytical performance.

The determination of total mercury in certified reference materials

To check the applicability of the procedure to real samples, two certified reference materials were analyzed – NIST 1532 citrus leaves and NRC MESS-3 marine sediment. After microwave-assisted digestion, as described above, Hg was determined using the proposed system. Aqueous analytical solutions at the same pH as the final sample solution were used for calibration. The loading time was 3.0 min. The results, displayed in Table 6, show the good agreement between found and certified values.

The determination of methylmercury in certified reference material

The applicability of the method for methylmercury determination was investigated by analysis of the Dolt-2 dogfish liver certified reference material (National Research Council, Canada). The methylmercury was extracted as described above and the resulting 50-mL solution was used for the analysis. Quantitative methylmercury extraction was achieved, but inorganic mercury was co-extracted. Thus, in an initial run the total mercury content of the solution was determined using a mixture of 0.3% $NaBH_4$ and 0.01% $FeCl_3$ as reductant. Inorganic mercury was determined in a second run, using solely 0.1% $NaBH_4$ as reductant [44]. Methylmercury was then determined by difference. All other conditions were kept as shown in Table 4, and a 1.0 min loading time was used. Aqueous analytical solutions prepared with the respective mercury species, at the same acid concentration as that of the final sample solution were used for calibration. The result is shown in Table 6; agreement between certified and found values was excellent.

Conclusions

On line pre-concentration of Hg^{2+} and CH_3Hg^+ has been achieved by chelation with DDTP and adsorption of the chelates on a C_{18} column; adsorption efficiencies ranged from 86 to 110% for the different species and loading times studied. Ethanol was the eluent of choice and reduction of the species occurred even in ethanolic medium. Similar limits of detection and characteristic concentrations were observed for both species. As already verified for other elements, both Hg chelates could be formed and adsorbed even in very acid media, eliminating the need for buffering in the analysis of samples of low pH. This facilitates the analysis of acid-digested or extracted samples, a very common situation in trace analysis of Hg. This feature was used in the analysis of certified reference materials, and good agreement was obtained between found and certified values. The results indicate the potential of the method for the accurate analysis of similar samples treated in the same manner. The limit of detection was improved by up to 20 times compared with that of a direct FIAS procedure. The same C_{18} column was used throughout the development of the whole work, and the cleaning steps of the FIAS program were sufficient to maintain column performance, although strictly off-line filtration of the digested or extracted samples was necessary to prevent clogging of the column. By introduction of a membrane filter before the column this step also could be automated.

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