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On-line Speciation of Mercury and Methylmercury in Aqueous Samples by Chromatography-Atomic Fluorescence Spectrometry after Hydride Generation

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A method is described for the determination of picogram amounts of inorganic mercury(II) and methylmercury(II) compounds in seawater. These compounds are first volatilized from the water samples by reduction to mercury (Hg^0) and formation of methylmercury hydride (CH_3HgH) by means of a sodium borohydride ($NaBH_4$) solution and cryogenically focused onto a chromatographic stationary phase. Both mercury compounds are eluted according their boiling points and are passed through a pyrolysis tube, at 900 °C, before entering the atomic fluorescence detector. Absolute detection limits (3σ) are 1 and 2 pg for, respectively, inorganic mercury and methylmercury. For seawater analysis, detection limits of 0.1–1.0 ng l^{-1} can be achieved.

Keywords: Organometallic speciation, mercury, methylmercury, seawater, hydride generation, gas chromatography, atomic fluorescence spectrometry

INTRODUCTION

Mercury is one of the contaminants that is best known because of the occurrence and high toxicity of methylmercury ($MeHg^+$). Mercury accumulates in the food chain principally as $MeHg^+$.[1] For analytical determination of $MeHg^+$ in environmental samples considerable efforts have been made. Most chromatographic applications have been focused on the analysis of biological tissues but other environmental matrices, e.g. water and sediment, have received some attention. In comparison with other organometallic species, the method developments related to mercury speciation are not completely satisfactory and most of them are cumbersome to apply.[2]

Traditionally, gas-chromatography analysis has been performed with electron capture detection (ECD).[3,4] Classical methods for extracting and separating organomercurials from the organic matrices for ECD detection are well established and commonly derive from the Westöö method.[4,5] Other detectors capable of detecting organomercurials after chromatographic separation are microwave-induced plasma/atomic emission spectrometry (MIP/AES),[6] atomic absorption spectroscopy (AAS)[8-10] and atomic fluorescence spectroscopy (AFS).[11,12]

AAS and AFS detection systems are often used on-line with derivatization/cryogenic focusing and gas-chromatographic separation. Derivatization of organomercurials can be carried out by means of aqueous-phase ethylation with sodium tetraethylborate ($NaBEt_4$).[9,10,12] Quite recently it was found that derivatization by means of hydride generation with sodium borohydride ($NaBH_4$) also yields volatile derivatives: elemental mercury and methylmercury hydride, respectively, for inorganic and methylmercury species.[10,13] Simultaneous determinations of Hg(II), $MeHg^+$, Me_2Hg and Et_2Hg with detection limits of 50 pg for organomercury compounds and 110 pg for Hg(II) have been described recently.[14] This sensitive hydride method is sufficiently rapid to analyse approximately five samples per hour. Hydride generation/cryogenic trapping/gas chromatography/atomic spectrometry (HG/CT/GC/AS) is nowadays a well-established technique for speciation analysis.[2] This technique was first

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used in 1975 for the determination of methylated forms of selenium in fresh water.[14] Since then, it has been used for the speciation of arsenic,[16,17] antimony,[17] selenium,[18] germanium,[19] tin[19] and recently mercury.[9,11,13,14] This HG/CT/GC/AS technique combines four basic stages: on-line derivatization of the analytes; pre-concentration by cryofocusing; chromatographic separation; and detection by atomic spectrometry. The detection systems most frequently used in the particular case of mercury are atomic absorption spectroscopy (AAS) and atomic fluorescence spectroscopy (AFS). Hyphenation of the different analytical stages is simple, since after the derivatization—or reduction to elemental mercury in the case of inorganic mercury—the volatile analytes are processed and introduced into the detector in a gaseous state.

In the case of methylmercury, hydride generation will yield methylmercury hydride, which was found to be sufficiently stable during the analytical determination.[13,20,21] The half-life of methylmercury hydride at room temperature was found to be approx. 2 h.[13] The existence of methylmercury hydride has been confirmed by mass spectrometry[20] and by Fourier transform infrared (FTIR) spectroscopy.[13] In a certification exercise under the auspices of the Community Bureau of Reference (BCR) of the Commission of the European Communities (EC), several techniques have been proved capable for the determination of methylmercury in biological samples.[22,23] Separation was generally performed with packed gas chromatography or capillary chromatography columns while final detection was by neutron activation analysis with radiochemical separation (RNAA), electron capture detection (ECD), cold-vapour AAS or electrothermal AAS. Hydride generation/gas chromatography/FTIR determinations for this certified reference material were in good agreement with the certified value for methylmercury,[19] indicating that hydride generation is a suitable technique for derivatization.

Determination of ethylated mercury compounds by AFS has been described previously[12] so the hyphenation of hydride generation/gas chromatography with atomic fluorescence detection should be possible.

This paper describes the hyphenation of hydride generation/gas chromatography with atomic fluorescence detection for the determination of inorganic mercury and methylmercury in seawater at the low nanograms-per-litre level.

EXPERIMENTAL

Reagents

Sodium acetate (anaytical grade), glacial acetic acid (Ultrex II), ethanol (analytical grade), sodium borohydride (analytical grade) and a 1000 mg l^{-1} mercury(II) solution (Baker, Instra-Analyzed, prepared by dissolution of mercury metal of 99.99% spectral purity in 1.0 M HNO$_3$) were obtained from Baker Chemicals, Deventer, The Netherlands. A 40 g l^{-1} sodium borohydride solution was prepared freshly in Milli-Q water. This solution was stable for at least one day. Methylmercury chloride (Pestenal®) was obtained from Riedel de Hahn, Seelze, Germany. A 1 M acetate buffer was prepared by dissolving 8.2 g sodium acetate in 1 M acetic acid to a final volume of 100 ml.

Calibrants

Inorganic mercury calibrant was prepared from a 1000 mg l^{-1} stock solution. A working calibrant solution of 821 pg ml^{-1} was prepared in 1 M nitric acid. The methylmercury chloride calibrant was prepared by dissolving the pure compound in ethanol, to give a working calibrant solution of methylmercury chloride of 3.700 ng ml^{-1} (as Hg). Calibrant solutions were stored at room temperature during the whole experimental phase (six months). In this period no reductions of the GC–AFS response was observed.

Apparatus

An analytical HG/CT/HG/AFS set-up, as shown in Fig. 1, was used throughout the study: it was described earlier for AAS detection.[16,19] All tub-

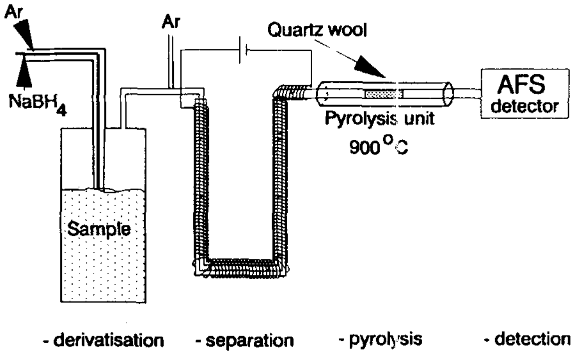

Figure 1 Hyphenated system for the speciation of mercury (Nafion dryer tube not shown). For a detailed description, see text.

ing between the different parts were made from PTFE. The reaction vessel was made from borosilicate glass and had a volume of 250 ml. This volume of the reaction flask was found suitable, especially when regarding detection limits, during organotin and organoarsenic speciation.[16] Argon was used as stripping gas and as carrier gas during the volatilization and chromatographic separation respectively. Water was removed before cryogenic trapping by a Perma Pure® dryer (model MD-250-24P) (see also the next section). The pyrolysis tube was heated to 900 °C in a MHS-20 quartz furnace heating block (from Perkin–Elmer, Gouda, The Netherlands). A 10.023 Merlin fluorescence detector (PS Analytical, Sevenoaks, Kent, UK), equipped with a heat-resistant introduction chimney, was used for the detection of mercury.

Water removal in the gas stream to the column

Water was removed before the analytes were trapped on the column; this was necessary because otherwise the water would freeze in the column and block it after several minutes. Removal of the water was performed by using a Nafion drying tube. Nafion is a copolymer of Teflon and perfluoro-3,6-dioxa-4-methyl-7-octenesulphonic acid. The Nafion molecule has an exposed sulphonic acid group which endows it with unusual desiccant properties. Sulphonic acid has a very high affinity for water, absorbing up to 13 water molecules per sulphonic acid group.[24] The Nafion dryer incorporated one or more strands of Nafion tubing within a sealed shell, with fittings to supply a countercurrent of dry purge gas. Water vapour extracted from the sample was carried away by the dry purge gas. The residence time of the sample in the Nafion drying tube was short (approx. 1 s) but it was more than enough to remove all the water vapour in the sample. In fact, it took 100–200 ms to dry the sample.

Analytical procedure

To approx. 160 ml of seawater 0.5 ml 1 M acetate buffer was added, resulting in a pH of 4.7 ± 0.2. While the chromatographic column was immersed in liquid nitrogen the solution was purged for 2 min with a steam of argon (250 ml min^{-1}). Addition of approx. 10 ml of sodium borohydride solution (40 g l^{-1}) to the sample was performed by means of a peristaltic pump. Inorganic mercury and methylmercury in the solution reacted with this sodium borohydride solution to produce volatile elemental mercury and methylmercury hydride. These volatile mercury species are removed from the aqueous phase by an argon stream (10 min; 250 ml min^{-1}) and subsequently were cryogenically trapped on the chromatographic column (U-shaped PTFE column filled with 250 mg Chromosorb GNAW 60/80 mesh, coated with 10% SP-2100 held in place by glass-wool plugs), which was placed in liquid nitrogen (−196 °C).

The next step was the separation of the mercury species by removing the liquid nitrogen, switching over to dry argon, and heating the chromatographic column during 10 min (by applying a voltage of 20 V over a Ni–chrome wire). The carrier gas was passed sequentially through a 900 °C thermal decomposition tube and the AFS cell. The thermal decomposition tube consisted of a 25 cm length of 6 mm (o.d.) × 4 mm (i.d.) quartz tubing with the central 4 cm packed with quartz wool. This thermal decomposition was necessary to render all eluted species to elemental mercury, the only form of mercury detectable by AFS.

The following parameters were optimized: pH, amount of sodium, borohydride and the pyrolysis temperature and purge time at a constant purge flow rate. Calibration curves for both mercury species were made in seawater because it was found that Milli-Q water and tapwaters contained more mercury than did seawater.

RESULTS AND DISCUSSION

Purge efficiency

In Fig. 2 the relative yield for both mercury species is plotted vs the stripping time. For this experiment 160 ml Milli-Q water was spiked with 821 pg of mercury(II) and 688 pg of methylmercury chloride (as Hg). From this graph it is clear that a reaction and stripping time of at least 10 min is necessary to derivatize and remove elemental mercury and methylmercury hydride quantitively (>95%) from the aqueous solution.

Pyrolysis tube

It was observed that decomposition of methylmercury hydride to elemental mercury did not occur when quartz wool was absent from the

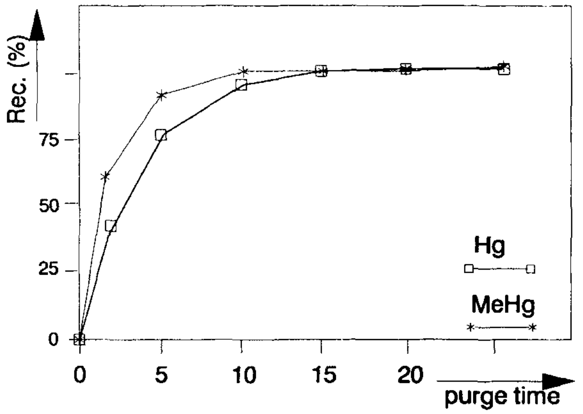

Figure 2 The recovery of inorganic mercury and methylmercury as a function of the reaction and purge time.

tube. At temperatures between 20 and 500 °C decomposition of methylmercury hydride was not as efficient as at 900 °C; therefore the pyrolysis tube temperature was set at 900 °C.

Influence of pH and sodium borohydride

Several efficiency experiments were carried out at different pH values (1.0, 3.8, 4.7, 6.0). It was concluded that pH 4.7 gave optimum results. This pH has been used before to derivatize methylmercury in water.[20] From addition of different amounts (ranging from 1 to 10 ml) of sodium borohydride (40 g l^{-1}) it was learned that 1 ml sodium borohydride solution was itself sufficient to reduce/derivatize the mercury species present in the sample. For normal procedures 10 ml sodium borohydride (40 g l^{-1}) was always added. The hydride efficiency was not known in this case, but would be nearly quantitative because addition of another portion of 10 ml sodium borohydride (40 g l^{-1}) to the already-purged sample did not yield any elemental mercury or methylmercury hydride.

Blank

Testing the argon, acetate buffer, sodium borohydride solution and water used for the experiments, it was shown that the blank levels were always as low as the detection limit except for the water used in all the tests. Three different tapwaters (from different locations in the Netherlands), demineralized water and Milli-Q water always contained inorganic mercury at levels between 1.0 and 2.2 ng l^{-1}.

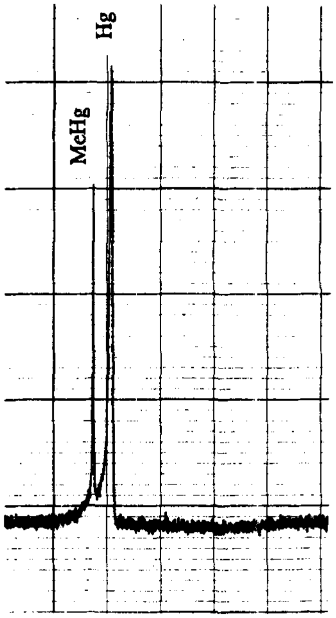

Figure 3 GC–AFS separation of mercury and methylmercury hydride in seawater (approx. 0.6 ng Hg l^{-1} for both inorganic mercury and methylmercury; for separation conditions, see the text.

Separation

There is a base-line separation between the two mercury species; retention times are 2.66 and 4.07 min for elemental mercury and methylmercury hydride respectively (Fig. 3). No other peaks occur in the chromatograms when analysing different types of water.

Calibration curves

These were performed in seawater (North Sea, Dogger Bank) and were linear up to at least 5 ng l^{-1} and 20 ng l^{-1} (as Hg) for inorganic mercury and methylmercury, respectively. With these calibration curves the levels of both mercury species in the seawater used can be extrapolated. It was deduced that this type of seawater contained 0.65 ng l^{-1} of inorganic mercury and 0.38 ng l^{-1} (as Hg) of methylmercury. The GC–AFS response for both mercury compounds in seawater were higher than in Milli-Q water by a factor of 1.6. The GC–AFS response in seawater for derivatized methylmercury was 18% lower than the response for inorganic mercury. This difference in GC–AFS response for derivatized inorganic mercury and methylmercury in seawater indicates small losses of methylmercury during the whole process. Most probably the efficiency of decomposition of methylmercury hydride in the pyrolysis tube was not 100%. Absolute detection limits (3σ of the noise) were 1 and 2 pg (as Hg) for inorganic and methylmercury respectively.

Reproducibility

The reproducibilty at environmentally significant levels (50 ng l^{-1} as Hg) was better than 8% ($n=7$) when using the standard addition technique.

CONCLUSIONS

From the authors' knowledge this is the first time that the commonly known hydride generation/cryogenic trapping/gas chromatography system has been used in combination with atomic fluorescence spectrometry for the determination of mercury(II) and methylmercury in seawater. This technique has a high potential, especially for seawater analysis, allowing detection of concentrations as low as 0.1 ng l^{-1} (as Hg), while absolute detection limits are in the picogram range. The technique is simple, fast and reproducible for the determination of inorganic mercury and methylmercury in seawater.

There is much interest in determining methylmercury in biological tissues. With this analytical technique and a suitable digestion method (e.g. with 4.5 mol l^{-1} KOH in methanol under ultrasonic conditions) it should be possible to determine methylmercury at the low picogram-per-gram level.

Because mercury(II) is reduced to elemental mercury there is no differentiation between elemental mercury and ionic mercury in the sample. Dimethylmercury was not found in seawater, but when this compound is present in the sample it may be separated from both other environmentally significant species and subsequently detected by the AFS detector.

The apparatus described here has great potential for the determination of methylmercury in sediments and biological samples. The determination of mercury species in fish and sediments will be tested in the near future.

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