The Analyst

Determination of total mercury in biological tissues by flow injection cold vapour generation atomic absorption spectrometry following tetramethylammonium hydroxide digestion

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A simple, rapid and reliable method was developed for the determination of total mercury in biological samples. Samples were solubilized using tetramethylammonium hydroxide (TMAH). The organically bound mercury was cleaved and converted to inorganic mercury by on-line addition of $KMnO_4$. The decomposed mercury together with inorganic mercury originally present in samples was determined by flow injection cold vapour atomic absorption spectrometry after reduction to elemental mercury vapour using $NaBH_4$. A sample throughput of 100 measurements per hour was achieved after a 30 min dissolution with TMAH. The relative standard deviation for 20 μg l^{-1} Hg was 1.3% (n = 11) and the limit of detection was 0.1 μg l^{-1} (3σ). The proposed method was validated by the analysis of a suite of certified marine biological reference materials, DORM-2 (dogfish muscle), DOLT-2 (dogfish liver) and TORT-2 (lobster hepatopancreas), with calibration against simple Hg^{II} standards.

Keywords: *Mercury; biological samples; tetramethylammonium hydroxide digestion; flow injection; cold vapour atomic absorption spectrometry*

There is increasing demand for rapid and sensitive techniques for the determination of toxic elements in biological materials. Mercury is of considerable interest because of its toxic nature and ability to bioaccumulate in many organisms. For the determination of mercury in biological samples, cold vapour atomic absorption spectrometry (CVAAS) is one of the most commonly used methods, owing to its high sensitivity and its ease of operation.[1] However, an important prerequisite is that mercury is present in the +2 oxidation state in order for reduction to elemental mercury (Hg^0) to occur by a suitable reductant such as tin(II) chloride.[2–5] Quantitation can then be performed by sweeping the mercury vapour formed into a quartz atomizer for atomic absorption detection. Organomercury compounds, however, are not reduced to metallic mercury by $SnCl_2$ or not completely by $NaBH_4$ and so quantitation is impossible unless suitable pre-treatment of the sample is undertaken.[4–11] For the analysis of biological samples by CVAAS, the pre-treatment must achieve two objectives. Firstly, the organic matter in the sample must be sufficiently oxidized to liberate the mercury species from the sample matrix, and secondly, the liberated mercury must be fully oxidized to Hg^{II}. Two types of decomposition methods have been employed for this purpose, *i.e.*, wet digestion and dry ashing. A bewildering variety of combinations of strong acids (HCl, H_2SO_4, HNO_3), oxidants (H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, $K_2S_2O_8$), elevated temperatures, UV irradiation and microwave exposure have been used and recommended.[2–12] However, due to the well-known problems associated with the mobility of this element and the inherent risk of contamination, volatilization and adsorption losses, care must be taken during the sample pre-treatment. Recently, systems were reported for the on-line digestion of biological samples with or without microwave assistance and determination of mercury by CVAAS, which provided an automated, contamination-free enclosed sample handling system.[5,13–17] They have been successfully applied to the determination of mercury in fluid samples, such as blood, urine and saliva. Although solid samples could also be run by the above systems, they must be slurried and homogenized prior to the introduction to the system. This required tedious slurry preparation, which is prone to contamination errors. Therefore, hitherto, these systems were limited to the analysis of liquid samples.

Tetramethylammonium hydroxide (TMAH) has been used as a 'tissue solubilizer' for various biological samples prior to analysis for major and minor inorganic elements by flame,[18–21] furnace atomic absorption spectrometry,[21–25] and inductively coupled plasma (ICP) atomic emission spectrometry,[26,27] and recently electrothermal vaporization ICP-mass spectrometry.[28] This alkaline digestion with TMAH offers a rapid and simple approach to the preparation of a homogenized sample solution, which is a distinct advantage over conventional slurry preparation methods.

In the present work, a rapid and simple method is presented for the analysis of total mercury in solid biological samples. TMAH was used to solubilize the samples and mercury was then determined by flow injection CVAAS with on-line decomposition of organomercury using $KMnO_4$.

Experimental

Instrumentation

A Perkin-Elmer (Norwalk, CT, USA) Model 4100ZL atomic absorption spectrometer in conjunction with a Perkin-Elmer FIAS-400 flow injection system and an AS-90 autosampler was used in this study. A Perkin-Elmer mercury electrodeless discharge lamp operated at 180 mA was used as the line source. The mercury absorbance was measured at 253.6 nm.

The flow injection system is shown in Fig. 1, which consists of a 4 : 5 port injection valve, two peristaltic pumps, a reagent-sample mixing chemifold and a glass gas–liquid separator (Part No. B09193772). A quartz cell with a path-length of 160 mm and a diameter of 7 mm was used as atomizer. The cell was heated to 200 °C to prevent condensation of moisture. Tygon pump tubings were used to deliver sample, reagents and withdraw waste. The reaction coils and connections were made of 0.9 mm id PTFE tubing. Sample and reagent flow-rates, including the concentrations of the reagents and argon stripping

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gas are also shown in Fig. 1. The flow injection program used is shown in Table 1.

Reagents and standard solution

All chemicals used were of analytical-reagent grade unless specified otherwise. Deionized distilled high purity water (18 MΩ cm) was obtained from a Nanopure system (Barnstead–Thermolyne, Dubuque, IA, USA)

TMAH (30% in methanol, Aldrich, Milwaukee, WI, USA) was used to solubilize the samples. An antifoaming agent (defoamer product No. 4528R158), obtained in the form of a household carpet cleaning additive from a local hardware store (Home Hardware Stores, Burford, Ontario, Canada), was used. Although any silicone-based antifoaming agent, such as Dow Corning DB110A, could be equally effective, this antifoaming agent is more cost-efficient. Due to its high viscosity and thus difficulties to pipette, the agent was diluted ten times (w/v) before use. $NaBH_4$ (0.2%, m/v) solution was prepared daily (Alfa Chemicals Inc., Newburyport, MA, USA, 01950, caplets) in 0.05% (w/v) NaOH. To 200 ml of this solution, 1 ml of the diluted antifoaming agent was added. $KMnO_4$ (0.2%, m/v) was also prepared daily in 15% sub-boiling nitric acid produced in-house from reagent grade feedstocks. This solution was kept in a dark brown bottle to prevent it from decomposing. Nitric acid (0.1 mol l^{-1}) was used as carrier.

Mercury standard solution was prepared by dissolution of $HgCl_2$ (gold star, Alfa Chemicals) in dilute nitric acid. A methyl mercury standard was prepared by dissolving MeHgCl (Alpha Division, Danvers, MA, USA) in an appropriate amount of propan-2-ol. Ethylmercury and phenylmercury stock solutions were prepared as described elsewhere.[4] Working solutions were prepared daily by serial dilution with high purity water. The final solutions contained 4% (v/v) TMAH.

National Research Council of Canada (NRCC) certified reference materials, DORM-2 (dogfish flesh), DOLT-2 (dogfish liver) and TORT-2 (lobster hepatopancreas) were used to assess the accuracy of the method.

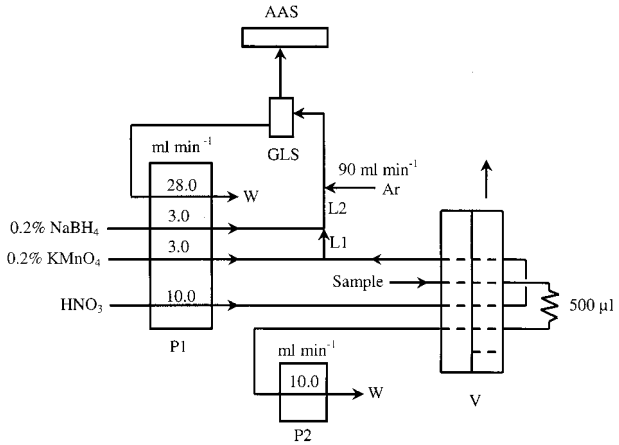

Fig. 1 Flow injection manifold for CVAAS with on-line $KMnO_4$ oxidation. GLS, gas-liquid separator; L1, L2, reaction coils, 15 and 50 cm long, respectively; P1, P2, peristaltic pumps; V, injection valve; W, waste.

Table 1 FIAS-400 programme

Step. No.	Time/s	Pump 1	Pump 2	Valve position	Read
Prefill	15	Off	On	Fill	
1	10	On	On	Fill	
2	15	On	Off	Inject	Read

Sample preparation

Nominal 0.25 g sub-samples of reference material biological tissues were weighed into 50 ml pre-cleaned screw-capped poly(propylene) bottles and 4 ml of TMAH added. Following the reaction of the tissue with the TMAH for approximately 5 min, high purity water was added to bring the volume to 25.0 ml (mass basis). Blanks were processed through an identical procedure. The resulting samples were ready to be analyzed in 30 min. Samples of DORM-2 were further diluted 4 times prior to analysis.

Hg *measurement*

The procedures for flow injection CVAAS are shown in Fig. 1 and Table 1. After the sample loop (500 µl) was filled with sample in step 1, the injection valve was switched to the inject position to introduce sample into the carrier stream (0.1 mol l^{-1} HNO_3) where it was then mixed with $KMnO_4$ and $NaBH_4$ solutions in sequence in the chemifold. The decomposition of organomercury by $KMnO_4$ occurred in reaction coil L1 and mercury vapour was generated in reaction coil L2. The mercury vapour formed was separated in the gas–liquid separator and transferred by the argon carrier into the quartz cell assembly for detection. The peak height measurement mode was used. Quantitation was achieved by comparison of response against a simple calibration curve prepared from Hg^{II} standards processed in the same manner as above.

Results and discussion

Optimization of experimental parameters

TMAH is an efficient reagent for solubilizing tissue samples. Compared to conventional slurry preparation methods, which require ultra-sonication or long-term stirring, this method is relatively simple, fast and less prone to contamination. In the present work, the fish muscle, liver and lobster tissues could be solubilized and homogenized in a few minutes, although the resulting sample 'digest' is neither clear nor colorless. On standing, the digest becomes less cloudy in appearance but no difference in final results was found for samples prepared 30 min or 3 months prior to determination. In addition, such TMAH digested solutions have been found to be stable for at least 1 year after preparation in the usual laboratory environment.[29]

After the sample is digested in TMAH, the mercury is retained in its original species. Although $NaBH_4$ can reduce both inorganic and organic forms of mercury, their resulting CVAAS sensitivities were different.[30] This was also confirmed in this study. The slopes of methyl- and ethylmercury calibration curves were about 75% and 60%, respectively, of that for inorganic Hg whereas the phenylmercury slope was about 35% of the inorganic mercury slope, even when the quartz tube atomizer was heated to 800 °C. The possible reasons for lower sensitivities for organomercury could be a slower reduction process, an accompanying reduced rate of mercury release, and lower atomization efficiency. It was thus impossible to measure the total mercury if the sample was directly run after TMAH digestion. Therefore, it was necessary that all forms of mercury in the sample be oxidatively converted to Hg^{II} prior to reduction to elemental Hg. In the present work, $KMnO_4$ was used to decompose the organomercury species. The decomposition was achieved by on-line addition of $KMnO_4$. Since the sample was dissolved in alkaline TMAH media, nitric acid was added to the $KMnO_4$ so as to acidify the sample digest and provide a favorable environment for decomposition of organomercury. The effects of the $KMnO_4$ and nitric acid concentration on the decomposition efficiency of organomercuric species are shown in Fig. 2 and Fig. 3, respectively.

Methyl-, ethyl- and phenyl-mercury were chosen to investigate the decomposition efficiency. It should be mentioned that these organic compounds used in the decomposition test cannot represent all of the organic mercurials that may possibly exist in marine biological samples, although methyl-, ethyl- and phenyl-mercury are the most commonly reported organomercurials.[31–33] Of these, methylmercury is the predominant and most toxic organic species in biological samples. Thus, the decomposition test should be representative of the majority of cases. As shown in Fig. 2, increasing the concentration of $KMnO_4$ resulted in increased decomposition of the organomercury species. Complete decomposition was obtained using a concentration of 0.2% or higher, while the sensitivity for inorganic mercury remained constant. Therefore, 0.2% $KMnO_4$ was chosen for further study. In addition to its effective decomposition of organomercurials, $KMnO_4$ enhanced the Hg^{II} signal by about 20% (Fig. 2), in agreement with observations by Guo and Baasner.[15,16] Thus, despite the dilution of the sample plug in the carrier stream, which was unavoidable during the on-line addition process, both the peak height and area of the signal increased rather than decreased when $KMnO_4$ was added.

Fig. 3 shows the effect of nitric acid concentration (in $KMnO_4$) on the sensitivities of inorganic and methyl mercury. Since the ethyl- and phenyl- mercury followed the same trend as methylmercury, only methylmercury was selected for illustration. As seen in Fig. 3, 15% nitric acid (in 0.2% $KMnO_4$) provides favorable conditions for both decomposition and cold vapour generation.

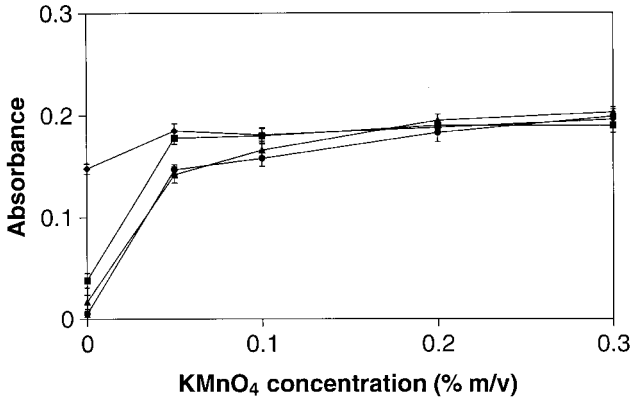

Fig. 2 Effect of $KMnO_4$ concentration on the mercury signal for Hg^{II}, methyl-, ethyl-, and phenyl-mercury. The concentrations of these four species were 20 μg l^{-1} Hg. The concentration of HNO_3 in $KMnO_4$ was 15%. Other conditions are the same as shown in Fig. 1. ◆ Hg; ■ methyl Hg; ▲ ethyl Hg; ● phenyl Hg.

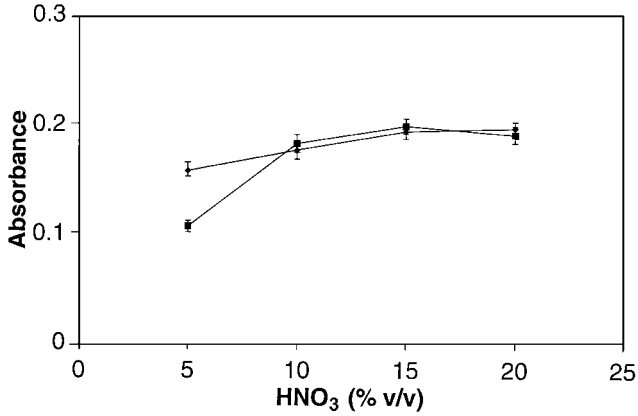

Fig. 3 Effect of HNO_3 concentration on the mercury signal for Hg^{II} and methylmercury. The concentration of $KMnO_4$ was 0.2%. Other conditions are the same as shown in Fig. 1. ◆ Hg; ■ methyl Hg.

The effect of $NaBH_4$ concentration on the mercury sensitivity was also investigated. As shown in Fig. 4, the response remained almost constant beyond 0.2%. Due to the organic matter which still remained in the sample after TMAH digestion, the reduction reaction became so vigorous when the concentration of $NaBH_4$ was higher than 0.3% that excessive foam was created. This made the gas–liquid separation ineffective. A 0.2% $NaBH_4$ solution was thus chosen for further study.

In the present system, the time for the oxidation reaction between $KMnO_4$ and the organomercuric species was determined by the reaction coil, L1 in Fig. 1. This reaction was very fast, permitting a short length of 15 cm to be used while ensuring complete decomposition.

Figures of merit

The system was calibrated with a series of Hg^{II} standards having concentrations up to 30 μg l^{-1}. Calibration graphs obeyed the equation $H = 9.82 \times 10^{-3}\ C + 1.15 \times 10^{-3}$ (correlation coefficient $r^2 = 0.999$), where H is peak-height absorbance and C is the mercury concentration in μg l^{-1}. A blank, limited by instrumental noise of 0.3 ± 0.04 μg l^{-1} was obtained, yielding a limit of detection of 0.1 μg l^{-1} (based on 3 σ of a blank TMAH solution). The relative standard deviation of the signal at a level of 20 μg l^{-1} Hg was 1.3% (n = 11). After the sample is digested, sample throughput is about 100 h^{-1}, *i.e.*, about 30 samples per hour measured in triplicate.

Accuracy

The accuracy of the method was evaluated by analyzing a suite of certified marine biological reference materials, *i.e.*, DORM-2, dogfish flesh material; DOLT-2, dogfish liver tissue; and TORT-2, lobster hepatopancreas. The determined values for total mercury agree with the certified values (see Table 2).

Conclusions

It has been demonstrated that FI–CVAAS with on-line decomposition of organomercury is a fast and reliable method

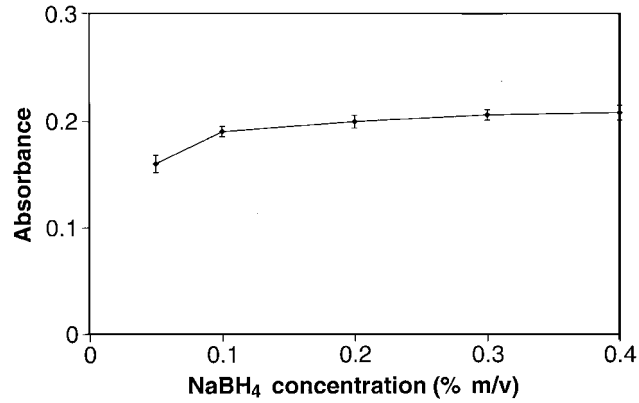

Fig. 4 Effect of $NaBH_4$ concentration on the inorganic mercury signal. Other conditions are the same as shown in Fig. 1.

Table 2 Analytical results for certified reference materials (μg g^{-1})

Sample	Certified	This work*
DORM-2	4.64 ± 0.26	4.53 ± 0.072
DOLT-2	2.14 ± 0.28	2.04 ± 0.052
TORT-2	0.27 ± 0.06	0.27 ± 0.014

* Mean values ± standard deviations (n = 3).

for the routine analysis of total mercury in solid biological samples. Due to the simplicity of slurry preparation using TMAH and the fully automated measurement of mercury by FI–CVAAS, the risks of analyte loss and contamination are considerably reduced and large batches of samples can be rapidly processed.

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Paper 8/02000K
Received February 13, 1998
Accepted April 3, 1998