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# Speciation of mercury in fish samples by flow injection catalytic cold vapour atomic absorption spectrometry

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ABSTRACT

A rapid flow injection catalytic cold vapour atomic absorption spectrometric (FI-CCV-AAS) method is described for speciation and determination of mercury in biological samples. Varying concentrations of $NaBH_4$ were employed for mercury vapour generation from inorganic and mixture of inorganic and organic (total) Hg. The presence of $Fe^{3+}$, $Cu^{2+}$ and thiourea had catalytic effect on mercury vapour generation from methylmercury (MeHg) and, when together, $Cu^{2+}$ and thiourea had synergistic catalytic effect on the vapour generation. Of the two metal ions, $Fe^{3+}$ gave the best sensitivity enhancement, achieving the same sensitivity for MeHg and inorganic $Hg^{2+}$. Due to similarity of resulting sensitivity, $Hg^{2+}$ was used successfully as a primary standard for quantification of inorganic and total Hg. The catalysis was homogeneous in nature, and it was assumed that the breaking of the C—Hg bond was facilitated by the delocalization of the 5*d* electron pairs in Hg atom. The extraction of MeHg and inorganic mercury (In-Hg) in fish samples were achieved quantitatively with hydrochloric acid in the presence of thiourea and determined by FI-CCV-AAS. The application of the method to the quantification of mercury species in a fish liver reference material DOLT-4 gave 91.5% and 102.3% recoveries for total and methyl mercury, respectively. The use of flow injection enabled rapid analysis with a sample throughput of 180 $h^{-1}$.



## 1. Introduction

Mercury species present in the environment differ greatly in their bio-physico-chemical properties, particularly in their toxicity, solubility, and rate of bioaccumulation by organisms [1–3]. For example, organomercurials are generally more toxic than inorganic mercury compounds [4,5]. Yet organic mercury species, such as monomethyl mercury (MMeHg or MeHg), dimethyl mercury (DMeHg), ethyl mercury (EtHg), and phenyl mercury (PhHg) are often detected in environmental samples, such as sediment, water and soil [6–8]. Of these, MeHg is the only compound that is bioaccummulated and biomagnified in the food chain and is also by far the most toxic mercury compound which represents a major health risk [9]. For this reason, the determination of total Hg (T-Hg) concentrations in samples does not offer adequate information on potential risk of mercury exposure, particularly with respect to the presence of organic mercury. A knowledge of mercury species present is therefore more relevant in gaining better understanding of the impact of mercury pollution, and information on MeHg content in environmental and biological samples has special significance [10].

The majority of instrumental analytical methods developed for the determination of MeHg to date are based on chromatographic separation, such as GC [11–27], HPLC [28–37], and IC [38], hyphenated with different detection techniques including ECD [23,25], UV [30,32], FAAS [33], CVAAS [11,22,26,29,37,38], CVAFS [12,13,16–18,21,24,36], MS [27], ICP-AES [20,32], and ICP-MS [14,15,21,31,34,35]. In particular, GC hyphenated with spectroscopic detection is one of the most popular techniques. An obvious advantage of chromatographic methods is the ability to distinguish between different mercury species in samples. However, a common disadvantage in these methods is that they involve complex and tedious pre-treatment procedures for separation of organic mercury (MeHg in biological samples) from the sample matrix by different methods, such as distillation, alkaline digestion-solvent extraction and acidic digestion-solvent extraction [19,23]. The separation of Hg species from sample matrix is one of the most critical steps and, for biota and sediments, almost certainly the most critical.

The extraction of Hg compounds from biological samples can be accelerated with the aid of microwave [39–41] or ultrasound [23,36], but the whole analytical procedures when employed with chromatographic methods are still tedious and time-consuming. In addition, hyphenated analytical methods, such as GC-AAS, GC-AFS, HPLC-AAS/AFS are not commercially or readily available, but often assembled for specific use only in laboratories. Also, the formation of extraneous MeHg during the analytical procedure is a problem often encountered. In particular, when distillation method

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is applied to sediments, it may result in a significant bias in measurements [42]. Methylation artefacts were also observed during hot alkaline digestion and supercritical fluid extraction [42].

Due to these various disadvantages, non-chromatographic methods have attracted significant interest in recent decades and numerous methods have been reported [14,43–64]. Typically, discrimination between In-Hg and MeHg was realized by the direct reduction of In-Hg with a weak reductant and reduction of total mercury (T-Hg) after digestion or in-line or off-line oxidation, and MeHg concentration can be calculated by the difference between In-Hg and T-Hg concentrations [43–49]. Selective extraction of MeHg from biological samples has also been reported [50–54]. For example, MeHg can be selectively extracted by using 2 M HCl without extracting In-Hg [50,51]. In recent years, in-line separation method has been reported based on the different retention properties of In-Hg and MeHg [14,55–58]. An additional benefit of this approach is the ease of analytes preconcentration which enabled achievement of improved sensitivity [58].

Without digestion or oxidation, direct determination of T-Hg in acid extracts by vapour generation-spectroscopic techniques has proven to be difficult because the vapour generation efficiencies of In-Hg and MeHg are different [43]. However, sequential determination of In-Hg and T-Hg in the same acid extracts by CVAAS has been reported [50,60–62]. These were based on the discrimination between In-Hg and MeHg by using different $NaBH_4$ concentrations, but the application of this approach is seldom considered.

It was reported that in the presence of $Cu^{2+}$, alkaline $SnCl_2$ solution gave the same sensitivity for MeHg and $Hg^{2+}$, enabling the determination of In-Hg and T-Hg in the same sample solution without digestion or oxidation of MeHg [63]. However, this was disregarded because of the formation of precipitate in the sample solution and associated contamination problems [61]. The catalytic effect of $Fe^{3+}$ was also employed to achieve improved sensitivity with $NaBH_4$ as the reductant for MeHg [65,66], but no detailed optimization of catalyst concentration or explanation of the catalytic mechanism was reported.

In this study, we investigate the catalytic effect of $Cu^{2+}$, TU and $Fe^{3+}$ on the vapour generation of MeHg with $NaBH_4$ as the reducing agent and an attempt is made to explain the possible mechanism involved. The possibility of achieving rapid ultrasound extraction of In-Hg and MeHg with HCl in the presence of thiourea (TU) will also be investigated. Furthermore, the application of the method to the determination of In-Hg and MeHg in fish samples by FI-CCV-AAS will be considered.

## 2. Experimental

### 2.1. Reagents and apparatus

All chemicals were of analytical reagent grade unless stated otherwise. 12.5 mg of methylemercury chloride (Merck, Darmstat, Germany) was dissolved in 20 mL of 5 M HCl and diluted to 100 mL with 2 M HCl to give a MeHg concentration of 125 mg $L^{-1}$ (100 mg Hg $L^{-1}$). This stock solution was stored in the fridge at 4 °C. A diluted solution (1 mg $L^{-1}$) was prepared fortnightly and working standard solutions were prepared daily. $Hg^{2+}$ standard solution of 1000 mg $L^{-1}$ was purchased from BDH Chemicals (Australia) Pty Ltd. (Kilsyth, Victoria, Australia) and adequate dilution was made prior to use. Milli-Q water was used throughout the study.

A 1% $NaBH_4$ solution was prepared by dissolving 1 g of $NaBH_4$ in 20 mL of 0.1% NaOH solution and diluted to 100 mL with Milli-Q water. Dilution of this solution for the vapour generation of $Hg^{2+}$ or T-Hg was made with 0.1% NaOH solution. A 1% $Fe^{3+}$ solution was prepared by dissolving 2.9 g of $FeCl_3$ in 100 mL of 0.5 M HCl. A 1% antifoaming solution was prepared by dissolving 0.25 g of antifoam SE-15 (Sigma–Aldrich, St. Louis, USA) in Milli-Q water. A 1% sodium tetraethylborate ($NaBEt_4$) solution and a 1% sodium tetraphenylborate ($NaBPh_4$) solution were prepared by dissolving 0.25 g of solid $NaBEt_4$ (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 0.25 g of $NaBPh_4$ (Cros Organics, New Jersey, USA), respectively, in 25 mL of Milli-Q water. Methylethyl mercury (MeEtHg) and methylphenylmercury (MePhHg) were prepared by aqueous phase ethylation and phenylation of MeHgCl at pH 4.5 with $NaBEt_4$ and $NaBPh_4$, respectively. Dimethyl mercury (DMeHg) and dibutyl mercury (DBuHg) were synthesized through the Grignard reagents [67] and their structure was confirmed with a GC-MS system which comprised of a Hewlett Packard 6890 GC and a Hewlett Packard 5970 mass selective detector.

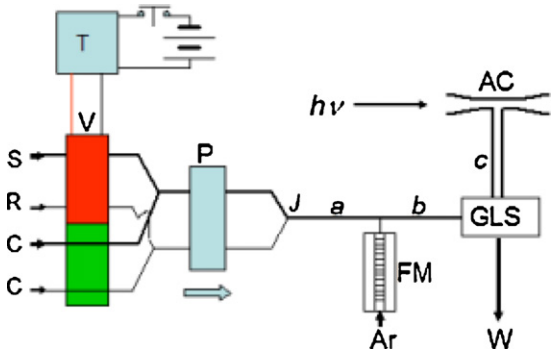


**Fig. 1.** FI-CCV-AAS system used for Hg determination. T: time delay relay; V: pinch valve; P: peristaltic pump; GLS: gas–liquid separator; AC: absorption cell; FM: flow meter; *J*: joining point; *a* and *b*: tubular reactors; *c*: gas delivery tube; S: sample; R: reductant ($NaBH_4$ solution); Ar: argon gas; C: carrier stream; W: waste.

A Perkin-Elmer 3030 atomic absorption spectrometer (Perkin-Elmer Pty Ltd., Rowville, Victoria, Australia) was used with Hg hollow cathode lamp as the irradiation source for the measurement of Hg. The instrument was operated in background correction mode with a deuterium lamp as the continuum irradiation source. The detection was carried out at 253.7 nm with a bandpass width of 0.7 nm. An absorption cell was assembled in our laboratory, as described previously [68]. The transient signals were recorded with a Perkin-Elmer 56 chart recorder.

The sample extraction was carried out in a Soniclean 160 T ultrasound bath (Extech Equipment Pty. Ltd., Wantirna South, Victoria, Australia). To perform the vapour generation, a flow injection (FI) system was assembled in our laboratory as described previously [69], except that in the present case we have included a time delay relay and a cone-shaped absorption cell, as illustrated in Fig. 1. Tygon pump tubing of 1.6 mm i.d. and 0.32 mm i.d. (Elkay Products, Inc. Worcester, Massachusetts, USA) were used to deliver the sample and reductant solutions, respectively. Flexible pump tubing and PTFE tubing of 1 mm in diameter (Cole-Parmer Instrument Company, Verson Hills, Illinois, USA) were used for the connection and liquid delivery. The tubular reactors *a* and *b* were 1 mm i.d. and 10 cm long. The pump was operated at 48 rpm, achieving flow rates of 9.6 and 3.2 mL $min^{-1}$ for the sample and reductant, respectively. A U tube was used as the gas–liquid separator (GLS).

The operational procedure of the FI-CCV-AAS system composed of two steps. In the first step, sample and reductant solutions were loaded, while in the second step, the sample and reductant bands were injected into their channels and merged at downstream of the pump and vapour generation reaction occurred in the tubular reactor *a*. The gas–liquid mixture was carried into the GLS where the gas phase was separated and swept into the absorption cell, whereas the liquid phase was discarded.