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Determination of mercury in fish otoliths by cold vapor generation inductively coupled plasma mass spectrometry (CVG-ICP-MS)☆

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ABSTRACT

A method based on cold vapor generation inductively coupled plasma mass spectrometry (CVG-ICP-MS) has been developed for determination of inorganic mercury, Hg(II), and total mercury in fish otoliths. Sodium borohydride ($NaBH_4$) was used as the only reducing agent and its concentration was optimized across an acidity gradient to selectively reduce Hg(II) without affecting methylmercury, $CH_3Hg(I)$. Inorganic Hg was quantitatively reduced to elemental mercury (Hg^0) with 1×10^{-4}% (m/v) $NaBH_4$. $CH_3Hg(I)$ required a minimum of 0.5% (m/v) $NaBH_4$ for complete reduction. Increasing the HCl concentration of solution to 5% (v/v) improved the selectivity toward Hg(II) as it decreased the signals from $CH_3Hg(I)$ to baseline levels. Potassium ferricyanide solution was the most effective in eliminating the memory effects of Hg compared with a number of chelating and oxidizing agents, including EDTA, gold chloride, thiourea, cerium ammonium nitrate and 2-mercaptoethylamine chloride. The relative standard deviation (RSD) was less than 5% for 1.0 $\mu g\,L^{-1}$ Hg(II) solution. The detection limits were 4.2 and 6.4 $ng\,L^{-1}$ (ppt) for Hg(II) and total Hg, respectively. Sample dissolution conditions and recoveries were examined with ultra-pure $CaCO_3$ (99.99%) spiked with Hg(II) and CH_3HgCl. Methylmercury was stable when dissolution was performed with up to 20% (v/v) HCl at 100 °C. Recoveries from spiked solutions were higher than 95% for both Hg(II) and $CH_3Hg(I)$. The method was applied to the determination of Hg(II) and total Hg concentrations in the otoliths of red emperor (CRM 22) and Pacific halibut. Total Hg concentration in the otoliths was $0.038 \pm 0.004\ \mu g\,g^{-1}$ for the red emperor and $0.021 \pm 0.003\ \mu g\,g^{-1}$ for the Pacific halibut. Inorganic Hg accounted for about 25% of total Hg indicating that Hg in the otoliths was predominantly organic mercury (e.g., methylmercury). However, as opposed to the bioaccumulation in tissues, methylmercury levels in otoliths was very low suggesting a different route of uptake, most likely through the deposition of methylmercury available in the water.

1. Introduction

Fish otoliths are calcium carbonate minerals (as aragonite) in the head of fish that aid in balance and hearing to the fish [1,2]. These aragonite minerals grow throughout the life of fish by deposition of calcium carbonate in concentric layers on a proteinaceous matrix. In the meantime, trace elements from the surrounding water successively incorporate into the newly forming aragonite layer. The aragonite polymorph is not susceptible to resorption. Therefore, the temporal concentrations of the trace elements (so-called fingerprints) remain unchanged throughout the fish's lifetime, and consequently integrate over the fish's life history when a whole is dissolved [1–7].

Trace elements and heavy metals make up less 1% (by mass) of an otolith. With the exception of Sr, their concentrations range from low $ng\,g^{-1}$ to a few $\mu g\,g^{-1}$ [3,8–14]. The utilization of trace elements as robust biological tags in otolith micro-chemical analysis has been largely due to the use of inductively coupled plasma mass spectrometry (ICP-MS) as a highly sensitive tool. Nevertheless, accurate determination of most trace elements and heavy metals, with the exception of relatively abundant Mg, Cu, Mn and Zn, from the otoliths is still a challenging task by direct analysis [3,4,6,8–10]. Various analytical methods, including isotope dilution [4,15], solvent extraction [16], solid phase extraction [6,17–20], co-precipitation [21] and hydride generation [22] have been developed to overcome the difficulties associated with low elemental concentrations in a highly saline calcium matrix.

Mercury (Hg) in the aquatic ecosystems mainly originates from the deposition of atmospheric Hg released from the anthropogenic

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activities [23–25]. Inorganic Hg in water is converted by bacteria to highly toxic methylmercury that accumulates in the sediments [23,25–27]. While microorganisms, such as phytoplankton and zooplankton ingest methylmercury (CH_3Hg) from water, dietary uptake is the major route of exposure of fish to methylmercury [25,28–30]. It is now well-documented that most Hg in fish tissue is in the form of methylmercury, although total body burden could vary with geological, biological and physiological differences among species [31–33].

The concentrations for a number of minor and major elements (Al, Na, Cl, Sr, Ca, Si) in bluefin tuna otoliths were reported to vary with total Hg body burden [34]. Further, laboratory exposures conducted with different fish indicate that the uptake of Hg into otoliths is related with its concentration in the water [35]. To date, however, Hg has not been considered as a biological tracer in otolith microchemistry, which is due in part to measurement difficulties and relatively low sensitivity of solution-based ICP-MS to this element. Thus, there is no information about the chemical forms of Hg in fish otoliths and whether otolith Hg could aid in population studies or not.

In this study, we have developed a cold vapor generation method for determination of Hg(II) and total Hg in otoliths by ICP-MS in an attempt to elucidate the chemical forms of Hg in fish otoliths, and to provide an insight about its source and utility in otolith microchemistry. Sodium borohydride ($NaBH_4$) was used as reducing agent to discriminate between the Hg(II) and total Hg levels. Studies were performed with a number of chelating and oxidizing reagents to eliminate the memory effects. Effects of acid dissolution on the stability and recoveries of Hg species were examined by spiking Hg(II) and CH_3HgCl to ultra-pure calcium carbonate. The method was applied to the determination of Hg(II) and total Hg in otolith samples from two different oceanic fish species, red emperor and Pacific halibut.

2. Experimental

2.1. Reagents and solutions

Deionized water produced by Barnstead™ E-Pure system with minimum resistivity of 17.8 MΩ cm was used throughout. A 1.0 μg mL^{-1} Hg(II) solution was prepared from a 1000 μg mL^{-1} standard solution (Sigma Aldrich) and stored in 5% (v/v) HNO_3 (Trace metal grade, Fisher Scientific). Methylmercury chloride (CH_3HgCl) solution (1000 μg mL^{-1} in water) was purchased from Alfa Aesar (99.99%). A 1.0 μg mL^{-1} CH_3HgCl stock solution was prepared and stored in water. All experimental solutions and calibration standards were prepared from these standard solutions. Trace metal grade hydrochloric acid (HCl, BDH Chemicals) was used for dissolution of samples and preparation of experimental solutions. Sodium borohydride ($NaBH_4$, 99.9%, Sigma Aldrich) was used as reducing agent. Aqueous solutions of $NaBH_4$ were stabilized in 0.1% (m/v) NaOH (99.9%, Sigma Aldrich). All other reagents, including ethylenediaminetetraacetic acid (EDTA), potassium ferricyanide [$K_3Fe(CN)_6$], thiourea, L-cysteine, 2-mercaptoethyamine chloride, cerium(IV) ammonium nitrate [$(NH_4)_2Ce(NO_3)_6$], and gold chloride ($HAuCl_4{\cdot}3H_2O$) were reagent grade.

2.2. Instrumentation

A Varian 820MS ICP-MS instrument (Varian, Australia) was used. The instrument was equipped with a peltier-cooled double-pass glass spray chamber, micromist nebulizer, standard one-piece, low flow, ball-and-socket connection quartz torch, standard Ni sampler and skimmer cones, patented Collision Reaction Interface (CRI), a unique 90° ion mirror delivering exceptional sensitivity, all-digital detector; Discrete Dynode Electron Multiplier (DDEM, Model AF250, ETP Australia) providing nine decades of linear dynamic range. Samples were introduced manually. The instrument was optimized daily for sensitivity, doubly charged ions (<2%) and oxides (<3%) with 5 μg L^{-1} ^{138}Ba, ^{25}Mg, ^{115}In, ^{140}Ce, ^{208}Pb solution. Data collection was achieved by ICP-MS Expert software package (version 2.2 b126). The operating parameters of the instrument are summarized in Table 1.

Table 1
Operating parameters for Varian 820-MS ICP-MS instrument for cold vapor generation.

ICP-MS	
RF power (kW)	1.4
Plasma Ar flow (L min^{-1})	18
Auxiliary Ar flow (L min^{-1})	1.8
Nebulizer Ar flow (L min^{-1})	1.2
Sheath Ar flow (L min^{-1})	0.1
Sampling depth (mm)	6
Pump rate (rpm)	25
Stabilization time (s)	50
Spray chamber temperature (°C)	3
Scan mode	Peak hopping
Dwell time (ms)	20
Points/peak	3
Scans/peak	5
Scans/replicate	10
CRI gas flow	0
Isotopes measured	^{200}Hg and ^{202}Hg
Vapor generation	
Sample acidity (% (v/v) HCl)	5.0
Sample flow rate (mL min^{-1})	2.0
$NaBH_4$ flow rate (mL min^{-1})	1.0

Schematic diagram of the cold vapor generation manifold is illustrated in Fig. 1. The stand-alone spray chamber with 100 mL inner volume was used as gas–liquid separator. The nebulizer housing was fitted with a T-adaptor through which the sample line was extended into the spay chamber (Fig. 1). Nebulizer argon was introduced as carrier gas through the T-inlet. Tygon pump tubings were used for sample (1.14 mm i.d., red/red) and $NaBH_4$ (0.76 mm i.d., black/black). The waste line (2.79 mm i.d., purple/white) was on a separate peristaltic pump (Ismatec). The reaction line (e.g., transfer line) was 30 cm long PTFE tubing (0.8 mm i.d.) extending from the mixing point of sample solution and $NaBH_4$ lines into the spray chamber.

2.3. Otolith samples

Fish otolith certified reference material (CRM 22) was purchased from the National Institute of Environmental Studies (NIES), Japan. The material was prepared from saggittal otoliths

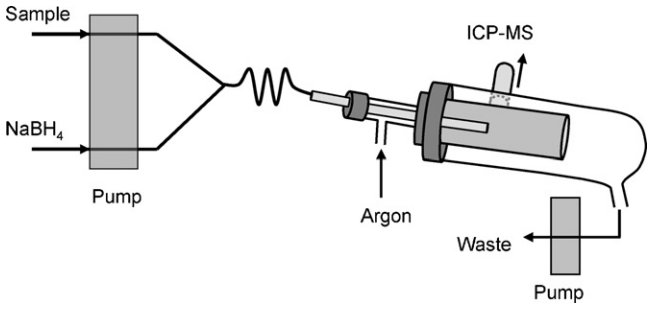

Fig. 1. Schematic diagram of cold vapor generation manifold and gas–liquid separator (GLS). The GLS is the stand-alone double pass spray chamber. Optimum CVG conditions: sample acidity = 5% (v/v) HCl; $NaBH_4 = 1 \times 10^{-4}$% (m/v) for Hg(II) and 0.5% (m/v) for total Hg.