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High yield synthesis and characterization of isotopically enriched monoethylmercury chloride ($C_2H_5{}^{201}HgCl$)

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An important but commercially unavailable compound isotopically enriched monoethylmercury chloride ($C_2H_5{}^{201}HgCl$), has been synthesized from commercially available ^{201}HgO (98.11% enriched isotopic purity) and tetraethyltin. The required synthesis time is 1 h at 90 °C, and the product is the single product of monoethylmercury chloride, yielding more than 95% as ^{201}Hg in $C_2H_5{}^{201}Hg^+$ (98.19 ± 0.22% enriched isotopic purity). The synthesized product was analyzed with high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) to determine its concentration, isotopic composition and purity. The synthetic isotopically enriched monoethylmercury synthesized can be used in speciated isotope dilution mass spectrometry (SIDMS) and isotope dilution mass spectrometry (IDMS) analyses as a standard.

KEYWORDS: ethylmercury chloride; isotopically enriched; isotope dilution mass spectrometry (IDMS); speciated isotope dilution mass spectrometry (SIDMS).

INTRODUCTION

The interest in the toxicology of ethylmercury has been raised significantly based on a letter sent by Lowell *et al*. claiming that ethylmercurythiosalicylate preservative (product name: Thimerosal, Thiomersal, Merthiolate) in hepatitis B immunoglobin caused severe ethylmercury intoxication in infants and small children.[1] Most likely as a reverberation of this 1999 letter, the Public Health Services, US Department of Health and Human Services and the American Academy of Pediatrics (AAP) published a joint statement[2] in which they identified Thimerosal as a widespread source of organic mercury exposure in infants/small children and recommended that it should be reduced or eliminated from childhood vaccines. Eventually it was hypothesized that the administration of Thimerosal-containing vaccines was associated with neuro-developmental disorders such as autism and speech delay.[3] According to Ball *et al*., an infant might be exposed to approximately 200 μg of Hg (as $C_2H_5Hg^+$) during the first 6 months of life through vaccinations.[4] Ball *et al*. also mentioned that the effect of childhood ethylmercury ($C_2H_5Hg^+$) exposure has not been systematically studied, but the qualitative effect is thought to be similar to that of monomethylmercury.[4] On the other hand, Pichichero *et al*. reported that, when a vaccine containing Thimerosal was administered in the recommended dose, no harmful toxic effects were observed but when administered at a massive overdose then toxic effect was observed.[5] Pichichero *et al*. also pointed out that the administered monoethylmercury eliminated from the blood via stool within 7 days. In 2001, the Immunization Safety Review Committee of the US Institute of Medicine concluded in a hypothesis[3] that the exposure to Thimerosal-containing vaccines causing neurodevelopmental disorders has not been established but is biologically plausible. The Committee recommended that Thimerosal-containing vaccines should not be administered to infants, small children and pregnant women.[6] Monoethylmercury (fungicides, Thimerosal in vaccines and γ-globulin) also causes renal and central nervous system toxicity and is deposited in the liver, kidneys, skin, brain, spleen and plasma. Monoethylmercury,

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like monomethylmercury, is metabolized to the inorganic mercury form and accounts for 50% of the mercury eliminated in urine and feces. Monoethylmercury may actually be converted to inorganic mercury in the tissues in greater amounts and more rapidly than monomethylmercury.[7] Therefore, the fate and true concentration of ethylmercury species in the human body or in the environment needs to be evaluated through accurate analysis.

Typically, analysis of mercury species involves a succession of analytical steps which are amenable to alkylation or de-alkylation. The extent of alkylation or de-alkylation is dependent on sample matrices and on sample preparation techniques used.[8,9] In order to determine the original concentration or the fate of ethylmercury, corrective measures, such as species-specific isotope dilution mass spectrometry (SSIDMS) or the EPA Method 6800 (elemental and speciated isotope dilution mass spectrometry, SIDMS)[10] need to be applied. Both require isotopically labeled monoethylmercury as a tag or spike. A major advantage of these techniques is that chemical separations need not be quantitative for accurate ratio measurement. Moreover, ratios can be measured very reproducibly, therefore, the post-spike interconversions and degradation are traceable and can be corrected leading to highly accurate and precise concentration determinations.[11–13]

The commercial unavailability of the isotopically labeled monoethylmercury standard has meant that the only way to obtain this standard has been for each laboratory to produce its own standard following the literature. A number of methods for synthesis of naturally abundant monoethylmercury have been found in the literature,[14–19] whereas only one method was available for the synthesis of isotopically enriched monoethylmercury chloride.[20]

Ol'dekop *et al*.[14] reported optimization of the monomethylmercury (C_2H_5HgCl) synthesis procedure, and the best conditions were found to be the reaction of 0.01 g Hg, 0.1 mol $C_2H_5CO_2H$, 0.0006 mol HNO_3 and 0.012 mol H_2O_2, followed by 0.002 mol $C_2H_5CO_2Na$, 0.21 mol $(C_2H_5CO)_2O$ and 0.03 mol H_2O_2 at 97–98 °C, yielding up to 92% C_2H_5HgCl. The entire synthesis procedure requires more than 6 h. Zupancic and Kumelj[15] investigated the synthesis of C_2H_5HgCl using the Grignard reagent. The purification process was simplified by the aqueous medium, yielding higher purity (94.6%), but the reaction time was 8–9 h. Ol'dekop and Maier[16] synthesized C_2H_5HgCl (61.5%) by decarboxylation of 10.4 g $(C_2H_5CO_2)_2Hg$ by mixing over 2 h with 8.75 g $(C_2H_5CO_2)_2$ in 140 ml of $C_2H_5CO_2H$ with continuous heating for 1.5 h at 97–98 °C followed by distillation of volatile materials and treatment with aqueous KCl. Rumpf[17] also applied the Grignard reagent method but obtained less pure C_2H_5HgCl (80%) with a shorter reaction time (5 h). During this synthesis process, 140 g of $HgCl_2$ were added to the freshly prepared Grignard reagent and boiled for 4 h on a water bath. The product was then added to 800 ml of 1% HCl and ether-distilled, and then the residue was filtered and dried. Whelen[19] synthesized C_2H_5HgCl from mercuric chloride and tetraethyllead. The ethanolic solution of $(C_2H_5)_4Pb$ was added gradually to a suspension of $HgCl_2$ in ethanol and the whole mixture was heated for 4 h at 65 °C in a water bath. The reaction mixture was then filtered and cooled to yield 72% C_2H_5HgCl.

Previously, the more toxic diethylmercury had been used to synthesize the mono-ethylmercury chloride. Qvarnström *et al*.[20] followed the Snell *et al*.[21] protocol, wherein the synthesis was based on ethylation of ^{199}Hg-enriched mercuric chloride with Grignard reagent. A mass of ^{199}HgO was dissolved in concentrated HCl to convert the oxide to mercuric chloride. The solution was then evaporated at 90 °C, leaving a dry mercuric chloride powder, which was subsequently dissolved in toluene. A diethylmercury stock solution was prepared by reacting mercuric chloride with ethylmagnesium chloride in tetrahydrofuran at 0 °C for 0.5 h. The mixture was then centrifuged, and the organic phase was retained, resulting in diethylmercury which was mixed with an equimolar amount of mercuric chloride in toluene and then refluxed overnight at 120 °C to yield $C_2H_5{}^{199}HgCl$ (93%) in toluene. Considering the high toxicity of diethylmercury,[22] this method was not suitable for the synthesis of C_2H_5HgCl in our work.

Therefore, the goal of this study was to investigate and to optimize the synthesis of highly pure isotopically enriched monoethylmercury chloride, while avoiding formation of the more toxic diethylmercury, so as to achieve higher yield and shorter reaction time. For this purpose, the monomethylmercury synthesis procedure described in Rahman *et al*.[23] was modified to permit micro-scale synthesis.

EXPERIMENTAL

Instrumentation

A ConstaMetric 4100Bio/MS polymeric inert pump (Thermo Separation Products, Riviera Beach, FL, USA) and a 5 μm Supelcosil LC-18 HPLC column with a Pelliguard LC-18 guard column (Supelco, PA, USA) were used in this study to separate inorganic and monoethylmercury. A six-port injection valve (Valco Vicci) was used between the pump and column. Because no special interface is required between the LC-18 column and the ICP-MS, one outlet of the column is directly interfaced to the nebulizer of the ICP-MS with a piece of perfluoroalkoxy (PFA) tubing, and the other end is connected to a 50 μl TEFZEL™ sample loop (CETAC Technologies, Omaha, NE, USA). Figure 1 shows a typical separation of mercury II (Hg^{2+}) and $C_2H_5Hg^+$ using this system at a flow rate of 1.0 ml min^{-1}. The mobile phase was buffered 30% methanol (refer to Reagent Section).

An HP 4500 ICP-MS (Agilent Technologies, Palo Alto, CA, USA and Yokogawa Analytical System Inc., Tokyo, Japan) was used in this study. The sample delivery system consisted of a peristaltic pump and quartz spray chamber with concentric nebulizer and quartz torch. The instrument was fitted with platinum sampler and skimmer cones and optimized daily using 10 ppb tuning solution (Agilent

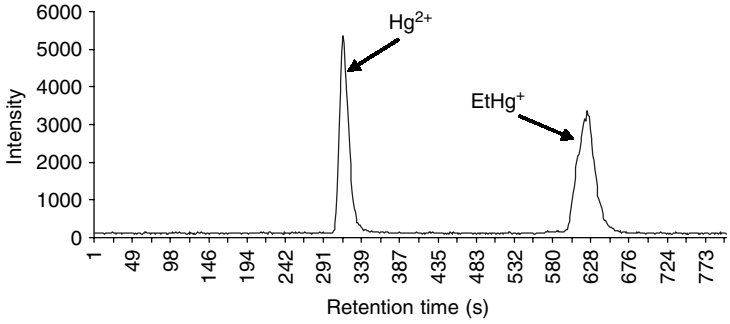

Figure 1. Typical chromatogram for separation of inorganic mercury and ethylmercury. Flow rate: 1 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 μm Supelcosil LC-18 HPLC column.

Technologies, Palo Alto, CA, USA) containing Li, Y, Ce and Tl in 30% methanol. Time-resolved analysis (TRA) mode was engaged. The operating conditions for the HPLC-ICP-MS set-up are reported elsewhere.[23]

A direct mercury analyzer (DMA-80, Milestone, Monroe, CT, USA) was used in this study to determine the total mercury content in each of the extraction and purification steps. The operation conditions for DMA-80 used throughout this work were based on the guidelines provided in the EPA Method 7473 protocol.[24,25]

Reagents and standards

Double-deionized (DDI) water (18 MΩ cm^{-1}), prepared from a Barnstead NANOpure Ultrapure Water System (Dubuque, IA, USA), was used in the preparation of all solutions throughout this study. Reagent-grade HCl, $Na_2S_2O_3$, toluene, ammonium acetate, 2-mercaptoethanol (98%) and optima-grade methanol were obtained from Fisher Scientific (Pittsburgh, PA, USA). The reagent-grade tetraethyltin $(C_2H_5)_4Sn$ (98%) was obtained from Alfa Aesar (Ward Hill, MA, USA).

Standard solutions containing 1 mg ml^{-1} of $HgCl_2$ in 5% HNO_3 and C_2H_5HgCl were commercially available from Alfa Aesar (Ward Hill, MA, USA). ^{201}HgO, lot no. VX3060, was obtained from Isotech Inc. (Miamisburg, OH, USA). HPLC speciation mobile phase [30% (v/v) methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate], was prepared by diluting 300 ml of methanol, 50 μl of 2-mercaptoethanol and 4.8 g of ammonium acetate in 700 ml of DDI water.

Synthesis procedure

Naturally abundant mercury (II) oxide was used for optimization studies. Mercury oxide was first converted to $HgCl_2$. For this purpose, 307.5–501.9 μmol of Hg^{2+} (Alfa Aesar, Ward Hill, MA, USA) were dissolved in 5 ml 6.0 mol l^{-1} HCl in a 30 ml amber glass bottle and stirred for 5 min. A 1.29 mol l^{-1} methanolic solution of $(C_2H_5)_4Sn$ was prepared by dissolving approximately 0.60 g of $(C_2H_5)_4Sn$ in 2.0 ml of methanol and then the mixture was transferred quantitatively into the $HgCl_2$ solution and the glass vial cap was replaced. The resulting reaction mixture was then stirred for 1 h at the different temperature (60, 75 or 90 °C) in a water bath. The reaction mixture was cooled to room temperature and extracted three times with toluene (3 × 7.5 ml). The monoethylmercury chloride extracted into toluene was then washed three times with DDI water (3 × 20 ml). The monoethylmercury chloride was then back-extracted three times into 1% $Na_2S_2O_3$, (3 × 25 ml). The synthesis method produces only a single mercury product of C_2H_5HgCl. All of the extracts were stored in amber glass vials in a cold room at ~4 °C until analysis.

RESULTS AND DISCUSSION

Optimization of synthesis conditions

A total of four monoethylmercury chloride syntheses were performed during this study. Rahman *et al.*'s[23] procedure for synthesis and purification of monomethylmercury was followed step-by-step at the beginning of this study. Mercury present in the reaction mixture (left after toluene extraction), in the DDI water wash, in the toluene phase (left after 1% $Na_2S_2O_3$ extraction) and in the 1% $Na_2S_2O_3$ extract was analyzed as total mercury using a direct mercury analyzer-80 (DMA-80, Milestone, GmbH, Germany), whereas high-performance liquid chromatography connected with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used to analyze for both mercury species (inorganic mercury and ethylmercury) in the reaction mixture, in the water wash, in the toluene phase and in the 1% $Na_2S_2O_3$. A typical HPLC-ICP-MS chromatogram for inorganic mercury and ethylmercury is shown in Fig. 1. The total mercury results obtained from the DMA-80 and HPLC-ICP-MS analyses agreed with each other at the 95% confidence interval. Therefore, the final speciation results from the HPLC-ICP-MS analyses and the respective synthesis conditions are reported in Table 1. The results are presented as percentage recovery in parentheses and mercury content in each fraction in μmol unit.

From Table 1, it is found that only 43.71 ± 2.79% of the HgO was converted to ethylmercury during trial 1 at 60 °C.

Table 1. HPLC-ICP-MS analyses results for the preliminary and final synthesis of isotopically enriched ethylmercury chloride[a]

	Mercury content (μmol) [recovery (%)]							
	Trial 1		Trial 2		Trial 3		Trial 4[b]	
Synthesis step	Hg^{2+}	$C_2H_5Hg^+$	Hg^{2+}	$C_2H_5Hg^+$	Hg^{2+}	$C_2H_5Hg^+$	Hg^{2+}	$C_2H_5Hg^+$
Reaction mixture	240.6 ± 5.8 [47.94 ± 1.16]	<DL	6.2 ± 0.8 [2.03 ± 0.25]	1.5 ± 0.1 [0.49 ± 0.04]	0.3 ± 0.1 [0.08 ± 0.03]	0.5 ± 0.1 [0.15 ± 0.04]	19.2 ± 2.6 [1.99 ± 0.27]	3.1 ± 1.1 [0.32 ± 0.11]
DDI water wash	NW	NW	<DL	9.7 ± 1.3 [3.15 ± 0.42]	<DL	16.6 ± 2.7 [4.98 ± 0.80]	<DL	26.2 ± 6.3 [2.71 ± 0.65]
Toluene phase	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
$Na_2S_2O_3$ extract	48.4 ± 5.8 [9.64 ± 1.16]	219.4 ± 14.0 [43.71 ± 2.79]	1.7 ± 0.6 [0.54 ± 0.21]	287.9 ± 13.7 [93.64 ± 4.45]	<DL	316.0 ± 19.8 [94.94 ± 5.96]	<DL	914.4 ± 88.1 [94.55 ± 9.11]
Total	508.3 ± 16.2 [100.35 ± 3.40]		307.0 ± 13.8 [99.44 ± 4.49]		333.4 ± 20.0 [100.15 ± 6.02]		963.0 ± 88.4 [99.57 ± 9.14]	

[a] Synthesis conditions: trial 1, 501.9 μmol Hg^{2+}, 5 ml 6.0 mol l^{-1} HCl, 5 min, 0.591 g $(C_2H_5)_4Sn$, 1 h, **60** °C; trial 2, 307.5 μmol Hg^{2+}, 5 ml 6.0 mol l^{-1} HCl, 5 min, 0.591 g $(C_2H_5)_4Sn$, 1 h, 75 °C; trial 3, 332.9 μmol Hg^{2+}, 5 ml 6.0 mol l^{-1} HCl, 5 min, 0.593 g $(C_2H_5)_4Sn$, 1 h, 90 °C.
[b] Trial 4, 967.1 nmol $^{201}Hg^{2+}$, 5 ml 6.0 mol l^{-1} HCl, 5 min, 0.345 g $(C_2H_5)_4Sn$, 1 h, 90 °C.
NW, not washed with DDI water. DL, detection limit (0.5 ng ml^{-1}).
Uncertainties are at 95% confidence interval with $n = 4$.

As was observed by Rahman *et al.*,[23] the reaction time and excess amount of tetramethyltin compound had little effect on the final product; therefore, it was decided to optimize only the reaction temperature. All other synthesis conditions were maintained as constant throughout the study. It is observed that the percentage yield increased from 43.71 ± 2.79% (trial 1) to 94.94 ± 5.96% (trial 3) at 90 °C. A similar higher yield was also obtained in trial 2 at 75 °C, but the product was not pure in ethylmercury. A small amount of inorganic mercury (0.54 ± 0.21%) was present along with the ethylmercury (in 1% $Na_2S_2O_3$ extract) in trial 2. Therefore, the reaction temperature was increased to 90 °C in trial 3 for complete conversion of the reactant into ethylmercury. From trial 3, it is observed that almost 100% of HgO was converted into ethylmercury. During HPLC-ICP-MS analysis of the product (in DDI water wash and in 1% $Na_2S_2O_3$), only ethylmercury was detected; no unreacted inorganic mercury or diethylmercury was found. Therefore, the conditions applied in trial 3 were considered as optimized and applied for the synthesis of isotopically labeled monoethylmercury (trial 4 in Table 1).

Characterization of the synthesized isotopically enriched monoethylmercury

After optimization of the synthesis procedure, an isotope-enriched monoethylmercury ($C_2H_5{}^{201}Hg^+$) was synthesized using ^{201}HgO and $(C_2H_5)_4Sn$ and all the synthetic fractions were analyzed using both DMA-80 and HPLC-ICP-MS (Table 1 and Fig. 2). From the final result it was observed that a highly pure and higher yield (94.55 ± 9.11% in 1% $Na_2S_2O_3$ fraction) of isotopically enriched monoethylmercury was synthesized. The HPLC chromatogram contained neither inorganic mercury nor any other mercury peak, but only an ethylmercury peak. In order to compare the peak position of the isotopically enriched monoethylmercury synthesized during this study with the naturally abundant

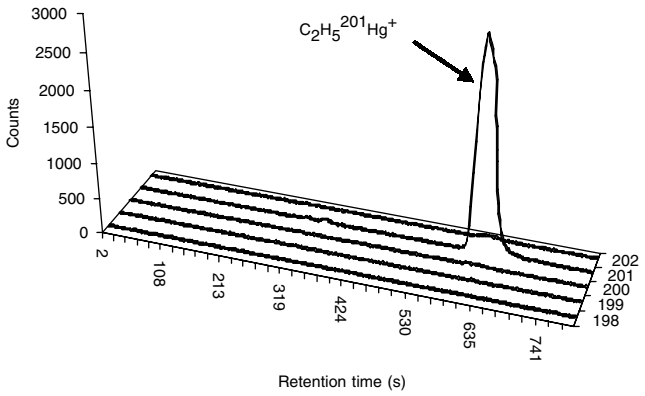

Figure 2. Chromatogram for synthesized isotope-enriched monoethylmercury ($C_2H_5{}^{201}Hg^+$). Flow rate: 1 ml min^{-1}; eluent: 30% methanol + 0.005% 2-mercaptoethanol + 0.06 mol l^{-1} ammonium acetate; column: 5 μm Supelcosil LC-18 HPLC column.

Table 2. Results for characterization of synthesized isotopically enriched monoethylmercury with ICP-MS

Isotope	Enriched ^{201}HgO (certified)	Enriched $C_2H_5{}^{201}Hg^+$ (determined)
Hg-196	<0.05	0.001 ± 0.001
Hg-198	0.08	0.07 ± 0.03
Hg-199	0.10	0.10 ± 0.04
Hg-200	0.45	0.44 ± 0.06
Hg-201	98.11	98.19 ± 0.22
Hg-202	1.18	1.13 ± 0.08
Hg-204	0.08	0.07 ± 0.02
Total	100.00	100.00 ± 0.25

monoethylmercury standard, these two standards were mixed at a 25 : 1 ratio and analyzed by HPLC-ICP-MS. The chromatogram demonstrated that both monoethylmercury standards overlapped with each other and appeared as a single peak at the same elution time, confirming that the product synthesized was the isotope-enriched monoethylmercury.

The isotopic abundances of the synthesized isotopically enriched monoethylmercury were determined and compared with the isotopic material supplier's certified values. This study was performed using an Agilent HP 4500 ICP-MS. The standard solution was aspirated in direct mode and isotope abundances for each isotope were calculated. The results are reported in Table 2 with 95% confidence intervals. The values measured correspond well with the certified values in most cases.

The concentration of the isotopically enriched synthesized monoethylmercury (in 1% $Na_2S_2O_3$ extract) was also determined by reverse IDMS on 23 March 2005 and found to be 3.35 ± 0.09 μg g^{-1}. The concentration indicated a yield of 94.93 ± 2.55%. In order to determine the stability and shelf-life of the synthesized product, the concentration was determined again with RIDMS on 30 June 2005 as 3.31 ± 0.06 μg g^{-1}. From both RIDMS analyses, it was observed that the product was stable at least for 90 days. The concentration of the product will be evaluated periodically for stability over time. The product will be used for the application of SSIDMS and SIDMS to the analyses of ethylmercury species in different environmental and biological matrices.

CONCLUSIONS

Isotopically labeled monoethylmercury chloride ($C_2H_5{}^{201}HgCl$) has been synthesized using commercially available ^{201}HgO and tetraethyltin with a yield of more than 95%. The synthesis procedure requires 1 h at 90 °C and does not produce any toxic diethylmercury. The product is highly pure and stays stable in cold conditions (~4 °C). The product is now commercially available from AIT for IDMS, SSIDMS and SIDMS analysis of ethylmercury from environmental or biological matrices.‡

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‡ To assist in the use of SSIDMS and SIDMS in speciated analysis, some of this species is now available as a commercial product (AIT part no. 30510) from Applied Isotope Technologies Inc. (e-mail info@sidms.com).