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Determination of methylmercury and inorganic mercury by coupling short-column ion chromatographic separation, on-line photocatalyst-assisted vapor generation, and inductively coupled plasma mass spectrometry

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ABSTRACT

We have combined short-column ion chromatographic separation and on-line photocatalyst-assisted vapor generation (VG) techniques with inductively coupled plasma mass spectrometry to develop a simple and sensitive hyphenated method for the determination of aqueous Hg^{2+} and $MeHg^{+}$ species. The separation of Hg^{2+} and $MeHg^{+}$ was accomplished on a cation-exchange guard column using a glutathione (GSH)-containing eluent. To achieve optimal chromatographic separation and signal intensities, we investigated the influence of several of the operating parameters of the chromatographic and photocatalyst-assisted VG systems. Under the optimized conditions of VG process, the shortcomings of conventional $SnCl_2$-based VG techniques for the vaporization of $MeHg^{+}$ was overcome; comparing to the concentric nebulizer-ICP-MS system, the analytical sensitivity of ICP-MS toward the detection of Hg^{2+} and $MeHg^{+}$ were also improved to 25- and 7-fold, respectively. With the use of our established HPLC–UV/nano-TiO_2–ICP-MS system, the precision for each analyte, based on three replicate injections of 2 ng/mL samples of each species, was better than 15% RSD. This hyphenated method also provided excellent detection limits—0.1 and 0.03 ng/mL for Hg^{2+} and $MeHg^{+}$, respectively. A series of validation experiments—analysis of the NIST 2672a Standard Urine Reference Material and other urine samples—confirmed further that our proposed method could be applied satisfactorily to the determination of inorganic Hg^{2+} and $MeHg^{+}$ species in real samples.

1. Introduction

Although mercury is used widely in industrial, medical, and agricultural fields, it has many harmful biological effects; for example, it is believed to be a factor in neurotoxicity, neurological problems, immunosuppression, myocardial infarction, autism, Alzheimer's disease, and renal damage [1–5]. In particular, methylated mercury derivatives are hundreds of times more toxic than inorganic mercury compounds [6,7]. Because inorganic and methylated mercury are both excreted from the human body in the urine and stool and because a positive correlation exists between the mercury concentrations in urine and serum [8–10], urinary mercury concentrations are used widely as an exposure biomarker for mercury poisoning. Notably, however, the concentrations of mercury in urine are generally at or below the level of micrograms per liter [11]; thus, it remains a challenge to develop sensitive and reliable analytical techniques that are capable of measuring nanogram levels of mercury species.

Sensitive speciation techniques are indispensable in the quest to reveal the biological behavior of mercury. Cold vapor (CV) atomic absorption spectrometry, CV atomic fluorescence spectrometry, and inductively coupled plasma mass spectrometry (ICP-MS) can all be used to determine mercury at subnanogram-per-milliliter detection limits. To date, only a few mercury speciation techniques have been reported that do not incorporate chromatographic separation procedures into these atomic spectrometry techniques [12–14]. Among the reported hyphenated techniques, gas chromatography (GC) is used most widely to separate Hg^{2+} and $MeHg^{+}$ species [15]. The chemical derivatization of mercurials into nonpolar, volatile, and thermally stable derivatives prior to GC separation is, however, laborious and time-consuming. In addition, the interconversion of mercury species, resulting from the high column temperature, may also provide erroneous results [16]. Although capillary electrophoresis (CE) combines rapid separation with high efficiency and very small sample volumes (usually a few nanoliters), the poor representativity of the sample and the

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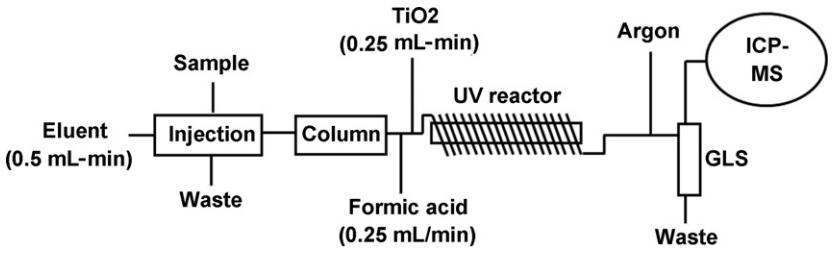

Fig. 1. Schematic representation of the on-line HPLC–UV/nano-TiO_2–ICP-MS system.

inferior concentration detection limits, approximately two orders of magnitude worse than those of liquid chromatographic (LC) separations, render it unsuitable for analyzing most real-world samples [17]. In view of minimizing species interconversion while providing sufficiently sensitive determination of mercury species in biological samples, LC separations using anion-exchange [18], cation-exchange [15,19], and reverse-phase [16,20–23] columns have been developed in both isocratic and gradient modes. For cation-exchange separation, severe memory effects may occur as a result of Hg^{2+} ions adsorbing onto the column. To avoid this problem during chromatographic separation, the addition of L-cysteine as a complexing reagent allows Hg^{2+}– and $MeHg^{+}$–cysteine complexes to be separated successfully at the selected pH [19].

The determination of ultra-trace levels of mercury using atomic optical or mass spectrometry requires the assistance of vapor generation (VG) techniques to (i) eliminate the matrix effect resulting from the concomitant matrix and (ii) improve the detection limit. Among the reported chemical VG methods [24,25], the chemical reagents $SnCl_2$ and $NaBH_4$ are used most frequently as reductants for the determination of mercury. According to Bramanti et al. [26,27], the vaporization efficiency of Hg^{2+} ion could be severely suppressed due to the formation of thiol complexes and a molar excess of $NaBH_4$ of up to several orders of magnitude became required for the complete reduction. To minimize the adverse effects resulting from a thiol-containing effluent, Bramanti et al. [28] developed an on-line oxidation system to convert various mercurial thiol complexes to Hg^{2+} ions prior to $NaBH_4$ reduction.

Because several metal ions can be converted into their metallic forms via TiO_2 photocatalytic reduction process [29,30], photocatalyst-assisted reduction has been applied successfully in a direct VG device for the speciation of selenium and mercury by coupling high-performance liquid chromatography (HPLC) separation with ICP-MS or atomic fluorescence spectrometric (AFS) detection [31,32]. Our aim in this study was to develop a simple and sensitive hyphenated technique for the determination of mercury species in biological samples; we chose to use a short cation-exchange column to separate Hg^{2+} ions from $MeHg^{+}$ ions with a GSH-containing eluent and then to develop an on-line photocatalyst-assisted reduction device to directly derivatize both the Hg^{2+}– and $MeHg^{+}$–thiol complexes into volatile species after their chromatographic separation, without requiring pre-oxidation. Based on the results of chromatographic separation, we were able to separate the mercury species of interest and prevent their adsorption onto the column by the use of GSH-containing NaCl solution as eluent. To develop an effective hyphenated method, we positioned a gas–liquid separator in series as the interface between the developed photocatalyst-assisted reduction device and the ICP-MS system; we found that the resulting on-line HPLC/photocatalyst-assisted VG-ICP-MS hyphenated system facilitated the analytical differentiation of Hg^{2+} ions from $MeHg^{+}$ ions in urine samples, without the problems—found in the case of conventional chemical VG processes—associated with vaporization of mercurial thiol complexes in the column effluent.

Table 1
Operating conditions for the HPLC–UV/nano-TiO_2–ICP-MS system.

Parameter	Setting
ICP-MS	Agilent 7500a
Plasma gas	15 L/min
Auxiliary gas	0.8 L/min
Carrier gas	1.35 L/min
Plasma forward power	1350 W
Chromatographic separation	
Mobile phase	250 mM NaCl/100 μM GSH
pH	3.0
Flow rate	0.5 mL/min
UV/nano-TiO_2 reduction	
TiO_2 concentration	3 g/L (mixing flow rate: 0.25 mL/min)
Formic acid	100 mM (mixing flow rate: 0.25 mL/min)
Reaction coil length	2 m

2. Experimental

2.1. Instrumentation

The HPLC–UV/nano-TiO_2–ICP-MS hyphenation system is depicted in Fig. 1. The HPLC pump (Alltech Associates, Deerfield, IL, USA) was equipped with a metal-free on-line injector (Rheodyne 9010, Cotati. CA, USA) and a 50-μL poly(ether-ether-ketone) (PEEK) sample loop. The column was connected to the UV/nano-TiO_2 device through polytetrafluoroethylene (PTFE) tubing (0.030 in. I.D. × 5 cm long). The filtrate of the urine samples was separated using a guard column packed with poly(hydroxy methacrylate) gel (Shodex, MSpak SP-80 4B, 8 μm, 50 mm × 4.60 mm I.D., functional group: sulfopropyl, Japan). A peristaltic pump (Watson-Marlow Bredel, Wilmington, MA, USA) was used to mix the TiO_2 suspension and a mixture of HCOOH and HNO_3 (flow rates: 0.25 mL/min) with the column effluent and to adjust the pH of the mixture. After HPLC separation and mixing with the TiO_2 suspension and the mixture of HCOOH and HNO_3, the mercury species in the effluent were delivered directly into the UV/nano-TiO_2 device and then reduced in the presence of TiO_2 (3 g/L), GSH (100 μM), and HCOOH (250 mM) under UV irradiation. After vaporization, the gaseous mercury products generated were carried by a stream of argon into the ICP-MS system (Agilent 7500a, Tokyo, Japan) for measurement. Adjustment of the sampling position and ion lenses for the optimal signal for mercury at m/z 202 was performed using a mercury standard solution (5 μg/L). Detailed descriptions of the instrumental system and conditions are provided in Table 1.

2.2. Chromatographic separation

The chromatographic separation was conducted at ambient temperature. Aliquots (50 μL) of the analytical samples or the corresponding standard solutions were injected. The mobile phase was 250 mM NaCl (pH 3.0); the flow rate was 0.5 mL/min. The NaCl solution was filtered through a Millipore 0.22-μm filter and degassed prior to use.