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Determination of ultra-trace amount methyl-, phenyl- and inorganic mercury in environmental and biological samples by liquid chromatography with inductively coupled plasma mass spectrometry after cloud point extraction preconcentration

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ABSTRACT

The cloud point extraction (CPE) preconcentration of ultra-trace amount of mercury species prior to reverse-phase high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) detection was studied. Mercury species including methyl-, ethyl-, phenyl- and inorganic mercury were transformed into hydrophobic chelates by reaction with sodium diethyldithiocarbamate, and the hydrophobic chelates were extracted into a surfactant-rich phase of Triton X-114 upon heating in a water bath at 40 °C. Ethylmercury was found partially decomposed during the CPE process, and was not included in the developed method. Various experimental conditions affecting the CPE preconcentration, HPLC separation, and ICP-MS determination were optimized. Under the optimized conditions, detection limits of 13, 8 and 6 ng l^{-1} (as Hg) were achieved for $MeHg^+$, $PhHg^+$ and Hg^{2+}, respectively. Seven determinations of a standard solution containing the three mercury species each at 0.5 ng ml^{-1} level produced relative standard deviations of 5.3, 2.3 and 4.4% for $MeHg^+$, $PhHg^+$ and Hg^{2+}, respectively. The developed method was successfully applied for the determination of the three mercury species in environmental water samples and biological samples of human hair and ocean fish.

1. Introduction

It is well known that the toxicity of mercury is highly dependent on its chemical forms, and alkylmercury compounds are deemed to be the most toxic substances among the various mercury species. The determination of mercury species in environmental and biological samples has attracted much attention of chemical analysts [1–3].

The common analytical techniques used for mercury speciation are based on coupling chromatographic separation to mercury specific detection [4–7]. Compared with gas chromatography, high performance liquid chromatography (HPLC) is the preferred separation technique used for mercury speciation, because the mercury species need not to be derived to volatile compounds before HPLC separation. Thus, HPLC has been coupled to various atomic spectrometric techniques for mercury speciation in the past several decades [2–4]. Since the concentration of mercury species in non-polluted environmental and biological samples is usually at tens ng l^{-1} or pg g^{-1} levels, inductively coupled plasma mass spectrometry (ICP-MS) is, among various elemental specific detection techniques, the most preferable detection technique for mercury speciation due to its excellent detectability for mercury [8–10]. Thus, various coupled HPLC–ICP–MS systems [11–21] have in recent years been reported for the determination of mercury species in environmental or biological samples since the first work reported by Bushee [18]. Even so, determination of mercury species in water samples with HPLC–ICP–MS is still challenging, because the detection limits of mercury species obtained by the HPLC–ICP–MS are usually in the range of 0.x–0.0x μg l^{-1}. Therefore, preconcentration of mercury species prior to HPLC–ICP–MS determination is sometimes required. It has been reported that solid-phase extraction preconcentration of mercury species with diethyldithiocarbamate-immobilized C18 [12] or dithiocarbamate resin [19], and of insoluble diethyldithiocarbamate chelates of mercury species [11] with C18 before HPLC–ICP–MS determination lowered the detection limits down to several ppt levels.

Cloud point extraction (CPE) [22–23], which is based on the temperature induced phase-transfer of non-ionic surfactants in

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aqueous solution, is an efficient and analyst/environment-friendly alternative for sample pretreatment. Compared with conventional solvent extraction, it greatly reduces the extraction time, solvent consumption and waste-disposal costs. Thus, CPE has in recent years been used for preconcentration of various trace metal ions with posterior quantification by an atomic spectrometric technique [23]. However, only a few works have been reported on CPE preconcentration of mercury species. de Wuilloud et al. [24] reported a CPE approach for preconcentration of trace inorganic mercury in tap water samples with chelating reagent 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol and non-ionic surfactant polyethyleneglycolmono-*p*-nonylphenylether prior to flow injection cold vapor generation inductively coupled plasma optical emission spectrometry, and achieved a detection limit of 4 ng l^{-1} inorganic mercury. Li and Hu [25] developed a non-chromatographic technique for the speciation of methylmercury and inorganic mercury in seafood by sequential CPE combined with inductively coupled plasma optical emission spectrometry, obtaining the detection limits of 56.3 and 94.6 ng l^{-1} for Hg^{2+} and $MeHg^{+}$ (as Hg), respectively. Yin [26] reported a dual CPE technique for capillary electrophoresis (CE) speciation of mercury. In their method, mercury species were first extracted into Triton X-114 surfactant-rich phase as hydrophobic 1-(2-pyridylazo)-2-naphthol (PAN) chelates, which were then back extracted to aqueous phase by forming hydrophilic mercury complexes of cysteine. Using a CE system with UV detector for posterior separation and determination, the author reported detection limits of 47.5, 45.2, 4.1, and 10.0 μg l^{-1} (as Hg) for methyl-, ethyl-, phenyl-, and inorganic mercury, respectively. Yu [27] developed a CPE approach for preconcentration of methyl-, ethyl-, phenyl-, and inorganic mercury species as their pyrrolidinedithiocarbamate (APDC) chelates prior to HPLC–cold vapor generation-atomic fluorescence spectrometry (CV-AFS) for speciation of mercury in fish. The author claimed detection limits ranging from 2 to 9 ng l^{-1} (as Hg) for the methyl-, ethyl-, phenyl-, and inorganic mercury. To the best of our knowledge, however, no report about coupling of CPE preconcentration to HPLC–ICP–MS for speciation of ultra-trace mercury has been published.

The purpose of the present work is to develop a sensitive, analyst/environment-friendly and cost-effective method for the determination of ultra-trace mercury species in environmental waters and biological samples by coupling CPE preconcentration to HPLC–ICP–MS system.

2. Experimental

2.1. Instrumentation

A model 7500a ICP-MS (Agilent, USA) was used in this work. An Agilent 1100 HPLC system (Agilent, Germany) consisted of a degassing system, a triple-gradient pumping system and a Rheodyne Model 7725 injection valve was employed. Separation was performed on a Discovery C18 column (4.6 mm i.d. × 15 mm, 5 μm) (Supelco, PA, USA). The outlet of the LC column was directly connected to the sample introduction system of ICP-MS via a 30 cm of 0.18 mm i.d. Teflon tubing. The optimized chromatographic and ICP-MS operating conditions are summarized in Table 1. The HPLC–ICP–MS system worked at time resolved analysis mode, and Software B 03.03 ICP-MS chemstation was used to control the system, and the collected data were processed with the Version C. 01.00 Chromatographic Data Analysis software provided by the manufacturer.

A Biofuge Primo R7500 centrifuge (Kendro laboratory, GmbH, Hanau, Germany) was used to accelerate the phase separation during CPE. All the vessels used for trace analysis were soaked in 10% nitric acid for 24 h and subsequently rinsed at least three times with pure water.

Table 1
Operational parameters of the HPLC–ICP–MS system.

HPLC part	
Column	Discovery C18, 4.6 mm i.d. × 15 mm, 5 μm
Mobile phase	35% methanol–40% acetonitrile–25% water containing 1.0×10^{-4} mol l^{-1} DDTC
Flow rate of the mobile phase	0.8 l min^{-1}
Sampling volume	20 μl
ICP-MS part	
Forward power	1500 W
Reflect power	<5 W
Spray chamber temperature	−5 °C
Plasma gas and its flow rate	Ar, 15 l min^{-1}
Auxiliary gas and its flow rate	Ar, 0.2 l min^{-1}
Carrier gas and its flow rate	Ar, 0.5 l min^{-1}
Make up gas and its flow rate	Ar, 0.4 l min^{-1}
Optional gas and its flow rate	Ar/O_2 = 4:1 in volume, 0.3 l min^{-1}
Sampling depth	11 mm
Monitoring masses	Hg, m/z = 202
Acquisition mode	Time resolved analysis
Integration time	1 s

2.2. Reagents

All reagents were of analytical or better grade, and pure water (18.2 MΩ cm) obtained from MILLI-Q elemental-grade water purification system (Millipore Corp., Molsheim, France) was used throughout the work. Triton X-114 (>93%) was obtained from Acros Organics (New Jersey, USA) and used without further purification. A 0.05 mol l^{-1} sodium diethyldithiocarbamate (DDTC) was prepared by dissolving the reagent (Shanghai No. 3 Reagent Factory, China) in methanol–water (1:1, v/v). Methanol and acetonitrile (HPLC grade) were obtained from Tedia (Tedia Inc., OH, USA).

A stock standard solution of 1000 mg l^{-1} Hg^{2+} prepared in 5% nitric acid was obtained from National Standard Material Center (GSB G 62069-90, Beijing, China). Stock standard solutions of $MeHg^{+}$, $EtHg^{+}$, and $PhHg^{+}$ at 1000 mg l^{-1} (as Hg) were prepared by individually dissolving appropriate amounts of methylmercury chloride (≧95%), ethylmercury chloride(≧95%), both from Alfa Aesar (A Johnson Matthey Company, MA, USA), and phenylmercuric acetate (98%, Acros Organics, New Jersey, USA) in methanol, respectively. All these stock solutions were stored in plastic bottles and kept at 4 °C. Working standard solutions were prepared from the corresponding stock solutions by stepwise dilution with water just before use. Standard reference material of human hair was obtained from National Standard Material Center (GBW 07601, Beijing, China), and the ocean bonito fish was purchased from local ocean fish market.

2.3. Procedure for cloud point extraction

Aliquots of 25 ml water samples or standard solutions were pipetted to 50 ml plastic centrifuge tubes. Then, 1 ml of 0.05 mol l^{-1} borate buffer (pH 9.0), 0.4 ml of 5% Triton X-114 and 0.4 ml of 0.05 mol l^{-1} DDTC were sequentially added and completely mixed with the sample or standard solutions. The centrifuge tubes containing the mixed solutions were heated in a thermostatic water bath at 40 °C for 10 min. Separation of the aqueous and surfactant-rich phases was accomplished by centrifuging at 4000 rpm for 10 min. The supernatant aqueous waste in the tubes was removed with a pipette. Then, 0.5 ml of methanol was added to dilute the