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Speciation analysis of mercury in natural water and fish samples by using capillary electrophoresis–inductively coupled plasma mass spectrometry

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ABSTRACT

A environment-friendly microwave-assisted extraction used to extract trace mercury compounds from fish samples, and a ultra-sensitive method for the analysis of Hg(II), methylmercury (MeHg) and ethylmercury (EtHg) by using capillary electrophoresis–inductively coupled plasma mass spectrometry (CE–ICP–MS) were described in this study. The extraction method is environment-friendly, simple, effective, and can be used to extract trace mercury compounds in fish samples with a satisfied recovery within several minutes. The CE–ICP–MS analytical method has a detection limit as lower as 0.021–0.032 ng Hg/mL for MeHg, EtHg and Hg(II), and can be used to determined ultratrace MeHg, EtHg and Hg(II) in natural water and fish samples directly without any preconcentration. With the help of the above methods, we have successfully determined MeHg, EtHg and Hg(II) in dried fish (*Tapertail anchovy*) muscle and natural water within 25 min with a RSD (relative standard deviation, $n=6$) <5% and a recovery of 94–103%. Our results showed that dried muscle of *T. anchovy* contained only one species of mercury, MeHg, indicating that MeHg is easier to be accumulated by aquatic organisms.

1. Introduction

Mercury is one of the most toxic elements impacting on human and ecosystem health, the United State Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) have listed mercury and its compounds in the third place on the "Priority List of Hazardous Substances". So far, a lot of researches have shown that any mercury released into the environment undergoes biogeochemical transformation processes and can be converted into the more toxic organic mercury form [1,2]. Therefore, there is a wide range of mercury species exists within natural water, and the chemical form of mercury not only controls its bioavailability and toxicity but also controls its transport and persistence. It was well known that the concentrations of mercury escalate up the food chain because of its high bioaccumulation. For example, predatory fish can have up to 10^6 times higher mercury concentrations than the ambient water and up to 95% of this mercury can be in the form of organic mercury [2]. For above reasons, the World Health Organization (WHO) recommends a maximum intake of methylmercury of 1.6 μg/kg per week and the organomercury compounds were banned from agricultural use in the 1970s in the world [3]. In order to control the effectiveness of these legal provisions and to ensure the safety of aquatic organisms for consumption, it is very important to develop a sensitive and accurate analytical method for the quantification of each species of mercury in natural water and aquatic organisms.

So far, the main techniques used for the speciation analysis of mercury are based on the combination of separation technology and sensitive element-selective detectors. For example, liquid chromatography (LC) and gas chromatography (GC) coupled with atomic fluorescence spectrometry (AFS) [4–8], atomic emission spectrometry (AES) [9,10], and inductively coupled plasma mass spectrometry (ICP–MS) [8,11–14]. However, for ionic Hg species, GC-based techniques require a previous derivatization step due to its low volatility and LC-based techniques require a previous complexation step in order to form non-polar [2]. The derivatization is one of the most critical steps in the speciation analysis of mercury, low yield as well as degradation phenomena in derivatization can heavily affect the quality of the results. LC-based techniques also suffer from the inadequate stability (combined with ICP–MS) and second environmental pollution due to the usage of much organic solvent. In addition, chromatographic separations provide interactions of species between stationary and mobile phase, probably resulting in the destruction of complexes [15,16].

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In comparison with chromatographic techniques, capillary electrophoresis (CE) has several advantages such as higher separation efficiency for all ionic and neutral species, much smaller sample and reagent consumption, no interaction between the sample and the stationary phase, various separation modes and low operating cost etc. [17–19]. Combined with different detectors such as UV-spectrometry [20–22], atomic fluorescence spectrometry (AFS) and ICP–MS [23–27], CE has been tried to use in the analysis of various mercury compounds. However, the combination of CE and UV or AFS has obvious limitations such as lower sensitivity, poorer stability and so on, and CE–ICP–MS methods reported previously can not separate methylmercury (MeHg) and ethylmercury (EtHg) completely and have a lower sensitivity and poorer stability [25–27]. In addition, most chromatography-based methods previously reported are focused on natural water samples whose matrix is relatively simple, the speciation analysis of mercury in aquatic organisms has been few reported [11,12].

The main aim of the present study is to develop a novel method for the simultaneous determination of ultratrace level of MeHg, EtHg and Hg(II) by using CE–ICP–MS and establish a environment-friendly extraction method for the extracting of all species of mercury in aquatic organisms, in hope of providing a realistic approach for the evaluation of safe consumption of seafood.

2. Experimental

2.1. Chemicals and reagents

The analytical grade of three species of mercury compounds, namely mercuric chloride, methylmercury-chloride and ethylmercury-chloride, were purchased from Best Chengdu Reagent Co., Ltd. (Chengdu, China). The 1000 μg/mL stock standard solution of mercuric chloride was prepared by dissolving above standard matter in Milli-Q water. The 1000 μg/mL stock standard solution of methylmercury-chloride and ethylmercury-chloride were prepared by dissolving above standard matters in methanol solution. Mercaptoacetic acid (MAA) was obtained from Sigma Co., Ltd. (China). All the stock standard solutions were stored at 4 °C. Working standard solutions were prepared by diluting the stock solutions to the desired concentration with Milli-Q water. The analytical grade sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$) and sodium dihydrogenphosphate ($NaH_2PO_4{\cdot}2H_2O$) were purchased from Shanghai Reagents Co. Ltd. (Shanghai, China). The running buffer solution of 50 mmol/L H_3BO_3–12.5 mmol/L $Na_2B_4O_7$ (pH 9.20) was prepared by dissolving above reagents in Milli-Q water. All solutions were treated by ultrasonic agitation and filtered through a 0.22 μm membrane filter before use.

All experiments were performed at room in which the temperature was regulated in 25–27 °C by an air conditioner, and water used in this experiment is Milli-Q water (18.2 MΩ/cm) prepared by a Milli-Q equipment (Millipore, Bedford, USA).

2.2. CE–ICP–MS system

The CE–ICP–MS system consists of a CEi-SP20 CE–Interface system (Reeko Instrument Co. Ltd., Xiamen, China) and an Agilent 7500ce ICP–MS (Agilent Technologies, USA). The CE–Interface was made according to the principle reported in our previous paper [28]. The CE capillary was conditioned daily by purging with Milli-Q water for 10 min, 0.1 mol/L NaOH solution for 10 min, Milli-Q water for 10 min and running buffer solution for 10 min, respectively. Between each run, the CE capillary was flushed with Milli-Q water and running buffer solution for 2 min respectively in order to clean any analyte or matrix adsorbed on the surface of capillary.

2.3. Determination of MeHg, EtHg and Hg(II) in natural water and dried fish muscle

Natural water sample was collected from Minjiang river in Fuzhou of China. The water was immediately filtered through a 0.22 μm membrane filter after sampling, and then was used for CE–ICP–MS analysis directly. Mercury in dried fish (*Tapertail anchovy*) muscle was extracted with dilute hydrochloric acid. Firstly, about 0.5 g sample was accurately weighed and put into a 100 mL Teflon beaker, which coupled with a screwed cover, and 20.0 mL of 1 mol/L HCl solution was added into it. Then, the beaker, which closed with screwed cover, was put into a microwave digester (Sineo Microwave Chemical Technology Co. Ltd., Shanghai, China). The microwave system was programmed to heat the whole at 70 °C for 5 min under 400 W power. After the whole was cooled to room temperature, the extract was separated by filtering it through a 0.22 μm membrane filter, and the residue was repeatedly extracted once again with the same manner. Then, the total extract was evaporated to near dryness by using a pressured nitrogen blowing concentrator with a moderate stream and the residue was diluted to the appropriate volume with buffer solution again (according to the mercury content in the sample). The final solution was used for the CE–ICP–MS analysis with continuous sample-introduction mode.

For the determination of MeHg, EtHg and Hg(II) with CE–ICP–MS, above 10 μL of above sample solution or mixed standard solution of MeHg, EtHg and Hg(II) was firstly put into a microtube, and 40 μL of 0.1% MAA solution was added. Then, the whole was plenty agitated for 10 min in order to complex mercury compounds with MAA. Finally, the whole solution was diluted to 100 μL with running buffer solution, and the final solution was injected into CE–ICP–MS for determination with electro-migration injection.

3. Results and discussion

3.1. Optimization of CE–ICP–MS conditions for the analysis of MeHg, EtHg and Hg(II)

The CE separation of MeHg, EtHg and Hg(II) is still a very difficult problem because these mercury compounds in solution are present as undissociated molecules [29]. It has been reported that an effective way to solve this problem is to complex mercury compounds with an ionic agent having thiol group [21]. In this study, the effect of complexing agents on the separation of MeHg, EtHg and Hg(II) was studied by using cysteine and MAA as complexing agent, and the results showed that MAA is more suitable. After complexing with MAA, MeHg, EtHg and Hg(II) can be completely separated within 25 min by CZE (capillary zone electrophoresis).

In CE-based analysis, the buffer solution including its chemical components, pH and concentration greatly affected the separation of analytes by affecting the electroosmosis flow (EOF). In the experiment, several different buffer solutions including H_3PO_4–NaH_2PO_4, NaH_2PO_4–$Na_2B_4O_7$, H_3BO_3–$Na_2B_4O_7$ were used to separate MeHg, EtHg and Hg(II). The result showed that MeHg, EtHg and Hg(II) can be more completely separated when the H_3BO_3–$Na_2B_4O_7$ solution ($H_3BO_3/Na_2B_4O_7$ = 4/1, mole concentration) was used as buffer solution.

The pH of buffer solution greatly affects the migration times/resolution. The relationship between migration time/resolution and pH was studied in detail in the range of 9.00–9.40 with 50 mmol/L H_3BO_3–12.5 mmol/L $Na_2B_4O_7$ as buffer solution. From the results shown in Fig. 1A, we found that higher pH is favorable to prolong the migration time and improve the separation of MeHg, EtHg and Hg(II), three mercury compounds