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Automated dispersive liquid-liquid microextraction coupled to high performance liquid chromatography - cold vapour atomic fluorescence spectroscopy for the determination of mercury species in natural water samples

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ABSTRACT

An automated, home-constructed, and low cost dispersive liquid-liquid microextraction (DLLME) device that directly coupled to a high performance liquid chromatography (HPLC) – cold vapour atomic fluorescence spectroscopy (CVAFS) system was designed and developed for the determination of trace concentrations of methylmercury ($MeHg^+$), ethylmercury ($EtHg^+$) and inorganic mercury (Hg^{2+}) in natural waters. With a simple, miniaturized and efficient automated DLLME system, nanogram amounts of these mercury species were extracted from natural water samples and injected into a hyphenated HPLC-CVAFS for quantification. The complete analytical procedure, including chelation, extraction, phase separation, collection and injection of the extracts, as well as HPLC-CVAFS quantification, was automated. Key parameters, such as the type and volume of the chelation, extraction and dispersive solvent, aspiration speed, sample pH, salt effect and matrix effect, were thoroughly investigated. Under the optimum conditions, linear range was 10–1200 ng L^{-1} for $EtHg^+$ and 5–450 ng L^{-1} for $MeHg^+$ and Hg^{2+}. Limits of detection were 3.0 ng L^{-1} for $EtHg^+$ and 1.5 ng L^{-1} for $MeHg^+$ and Hg^{2+}. Reproducibility and recoveries were assessed by spiking three natural water samples with different Hg concentrations, giving recoveries from 88.4–96.1%, and relative standard deviations <5.1%.

1. Introduction

Mercury (Hg) is considered as one of the most toxic elements for its accumulative and persistent character in the environment and biota. However, the toxicity, biogeochemical behavior, and the bioavailability of Hg are highly dependent on its chemical form. Consequently, the development of simple, sensitive, selective, and environmental friendly techniques for the determination of mercury species at trace levels in environmental samples is of particular significance.

The most commonly used methods for mercury speciation analysis in environmental samples are gas chromatography (GC) [1,2], high-performance liquid chromatography (HPLC) [3,4], ion chromatography (IC) [5] and capillary electrophoresis (CE) [6] coupled with atomic absorption spectrometry (AAS) [2,7], atomic fluorescence spectrometry (AFS) [3,4,8], or inductively coupled plasma-mass spectrometry (ICP-MS) [5]. However, due to the extremely low concentrations of mercury species in freshwater samples and the complexity of the matrix, sample preparation steps prior to the analytical method are generally required.

Dispersive liquid-liquid microextraction (DLLME), which was introduced by Rezaee and his co-workers, represents an important development in the field of sample preparation for its simplicity, miniaturization and speed [4,9] and has been successfully applied for the determination of mercury species [10–13]. One of the most interesting and challenging aspects of DLLME is its capacity for automation and its direct coupling to a detector, which offers several advantages, such as minimizing the errors associated with manual handling, reducing sample and reagent consumption, increasing sample throughput and improving sensitivity and precision [14]. A common approach to the automation of DLLME is the use of continuous flow techniques such as flow injection analysis (FIA), sequential injection analysis (SIA), and multisyringe flow injection analysis (MSFIA). Anthemidis et al. [15] reported an on-

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line SIA-DLLME system coupled with a flame atomic absorption spectrometric detector for the determination of copper and lead in water samples. The analytes were retained in a micro-column after extraction and then eluted to the detector. However, the necessity to use a micro-column for retention and the requirement for several hundred microliters of solvent for elution were limitations. Recently, automated in-syringe magnetic stirring-assisted DLLME coupled to a spectrophotometric detector was developed [16–18]. In this approach, an alternating magnetic field was generated in the syringe using a specially designed driving device placed around the syringe barrel. Thus, the magnetic stir bar in the syringe could rotate to facilitate the extraction process. The extraction was carried out within the syringe pump and the extracts were then injected into the detector. However, the specially designed driving device is not very easy to obtain. Recently, Lee et al. [19–22] pioneered the development of a fully automated DLLME that seamlessly coupled to gas chromatography-mass spectrometry (GC–MS) using a commercially available multipurpose autosampler equipped with microsyringes of different capacities, thus opening up a new horizon for the automation of DLLME.

In the present work, a low cost, home-assembled, automated DLLME device has been designed and integrated with a high performance liquid chromatography (HPLC) – cold vapour atomic fluorescence spectroscopy (CVAFS) system, and the performance characteristics of the device have been demonstrated by application to the determination of mercury species in natural waters. Using a simple, miniaturized and efficient automated DLLME system that we recently developed [23], trace concentrations of methylmercury ($MeHg^+$), ethylmercury ($EtHg^+$) and inorganic mercury (Hg^{2+}) were extracted and injected into the hyphenated HPLC-CVAFS system for quantification. Key parameters influencing the performance of the automated DLLME-HPLC-CVAFS method were investigated and its applicability was validated against a number of natural water samples collected from Chengdu, China.

2. Experimental

2.1. Reagents and standards

10 mg L^{-1} Hg^{2+} stock standard solution was obtained from Merck (Darmstadt, Germany). 10 mg L^{-1} stock standard solutions of $MeHg^+$ and $EtHg^+$ were prepared by dissolving of methylmercury chloride and ethylmercury chloride that purchased from Dr. Ehrenstorfer (Augsburg, Germany) in methanol. Calibration and working solutions were daily prepared by sequentially diluting of the stock standard solutions. All solutions were stored in the dark at 4 °C.

Ionic liquids including 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$), 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$) and 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$) were purchased from Shanghai Cheng Jie Chemical Co. Ltd. (Shanghai, China). L-Cysteine (L-Cys), and sodium diethyldithiocarbamate (DDTC) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2-Mercaptoethanol was purchased from Beyotime (Haimen, China). HPLC grade acetonitrile, methanol, ammonium acetate and acetic acid were purchased from Tedia (Ohio, USA). Hydrochloric acid HCl (GR), potassium borohydride KBH_4 (AR), sodium hydroxide NaOH (GR), sodium chloride NaCl (AR), potassium persulfate $K_2S_2O_8$ (AR), and HPLC grade acetone were purchased from Kelong (Chengdu, China).

All solutions were prepared in ultra-pure water obtained from a Milli-Q Integral 3 system from Millipore (MA, USA).

To avoid Hg contamination, all the glass and plastic vessels used for the analysis were lightly dusted with sulfur powder, eliminating the volatilization of Hg, then cleaned with deionized water and soaked in a 35% (v/v) nitric acid bath for at least 24 h. They were thoroughly rinsed with Milli-Q water before use.

2.2. Instrumentation

A schematic diagram of the automated DLLME-HPLC-CVAFS system is shown in Fig. 1. It comprised a 9600-step syringe pump (SP) with a highest speed of 2400 steps per second (Ristron PVS-100, Zhejiang, China), and a rotary 10-port multi-position valve (MPV; VICI, Schenkon, Switzerland).

A three-way solenoid valve (SV1; Ristron), and a 10 mL syringe (Hamilton, Bonaduz, Switzerland) were connected to the SP. SV1 was either connected to the centre port of the MPV or to the HPLC injection valve (Rheodyne model 7725i) with a 50 μL sample loop (California, USA). The MPV was used for handling sample/blank, standard, extractant, dispersive solvent, chelating agent, air, Milli-Q water, methanol, waste, and was connected to the mixing chambers (C1, C2, and C3) with a 10, 2 and 1 mL cylinder syringes respectively. Polytetrafluoroethylene (PTFE) tubing (0.79 mm i.d.) was used to connect all system components.

A HPLC-CVAFS that we previously developed [3,13,24] was seamlessly integrated with the automated DLLME system for the quantification of the extracted Hg species.

2.3. Samples and automated DLLME-HPLC-CVAFS procedures

Natural water sample collected at a depth of 5 cm from the surface of the Pi River (Chengdu, China) were used for this study. A 5 L bulk sample was collected in a PVC container and filtered through a 0.22 μm membrane immediately after collection. The bulk sample was divided into ten 500 mL sub-samples which were stored in brown bottles at 4 °C until used.

After adjusting the sample pH to 3.5 using 0.05 M HNO_3 and NaOH solutions, Hg^{2+}, $MeHg^+$ and $EtHg^+$ were extracted and analyzed using the automated DLLME-HPLC-CVAFS system as follows. Initially, the syringe and the connecting tubing were cleaned three times by aspiration of 10 mL of Milli-Q water (at MPV in position 9 and SV1 in position a) into the syringe followed by discharge to waste (at MPV in position 6). Afterwards, 5 mL of sample (MPV in position 4 and SV2 in position c), 0.1 mL chelating agent (SV2 in position d) and 1 mL of air (at MPV in position 8) were sequentially aspirated into the syringe and subsequently expelled to C1 through a mixing coil (MC1) at MPV position 1. In order to improve the chelating efficiency for Hg species, the mixtures in C1 were again aspirated into the syringe and then expelled to C1 three times. After that, 800 μL of acetone as the dispersive solvent (at MPV in position 5), 30 μL of $[C_6MIM][PF_6]$ as the extractant (at MPV in position 10) and 1 mL of air (at MPV in position 8) were subsequently aspirated into the syringe and expelled into C2 through MC2 at MPV position 3. In order to improve the mixing of dispersive solvent and extractant, the mixtures in C2 were again aspirated into the syringe and then expelled to C2, and again discharged into the syringe. Hereafter, the sample that was held in C1 was rapidly aspirated into the syringe (MPV in position 1) at an aspiration speed of 2400 step s^{-1}. At this time, fine droplets were formed, facilitating mass transfer of the target analytes into the ionic liquid phase. Phase separation was achieved in 2 min and the extractant phase was sedimented at the bottom of the syringe.

For the collection and injection of the extracts, SV3 was switched to position f, all the sample expelled with the MPV at position 2, and the extracts were finally held in MC3. After that, 90 μL of methanol (at MPV in position 7) and the extracts held in MC3 (at MPV in position 2) were aspirated into the syringe. In order to enhance the mixing of extracts and methanol, the mixture was propelled to C3 (with MPV in position 2 and SV3 in position e) and then back-aspired to the syringe three times. Afterwards, SV1 was switched