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Automated dynamic hollow fiber liquid–liquid–liquid microextraction combined with capillary electrophoresis for speciation of mercury in biological and environmental samples

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ABSTRACT

A simple home-made automatic dynamic hollow fiber based liquid–liquid–liquid microextraction (AD-HF-LLLME) device was designed and constructed for the simultaneous extraction of organomercury and inorganic mercury species with the assistant of a programmable flow injection analyzer. With 18-crown-6 as the complexing reagent, mercury species including methyl-, ethyl-, phenyl- and inorganic mercury were extracted into the organic phase (chlorobenzene), and then back-extracted into the acceptor phase of 0.1% (*m*/*v*) 3-mercapto-1-propanesulfonic acid (MPS) aqueous solution. Compared with automatic static (AS)-HF-LLLME system, the extraction equilibrium of target mercury species was obtained in shorter time with higher extraction efficiency in AD-HF-LLLME system. Based on it, a new method of AD-HF-LLLME coupled with large volume sample stacking (LVSS)-capillary electrophoresis (CE)/UV detection was developed for the simultaneous analysis of methyl-, phenyl- and inorganic mercury species in biological samples and environmental water. Under the optimized conditions, AD-HF-LLLME provided high enrichment factors (EFs) of 149–253-fold within relatively short extraction equilibrium time (25 min) and good precision with RSD between 3.8 and 8.1%. By combining AD-HF-LLLME with LVSS-CE/UV, EFs were magnified up to 2195-fold and the limits of detection (at $S/N = 3$) for target mercury species were improved to be sub ppb level.

1. Introduction

Mercury is one of the most toxic elements because of its accumulative and persistent character in the environment and biota. Furthermore, the toxicity, bioavailability and bioaccumulation of mercury are highly dependent on its chemical species [1]. Organomercury compounds with alkyl-/aryl-substituent bonded to the mercury atom are generally more toxic than inorganic mercury salts. Nowadays, particular concern has been devoted to monitoring the level of mercury in environment and biological samples and investigation of its existing species, which is of great importance to evaluate the risk of mercury exposure to human being. Due to the high separation efficiency, species-friendly separation conditions and extremely low sample/reagent consumption, capillary electrophoresis (CE) is deemed to be one of the most suitable analytical tools for elemental speciation [2–8]. However, some problems are commonly encountered in CE analysis of mercury species, mainly including insufficient sensitivity and poor anti-interference ability for real sample analysis, as target mercury species in real environmental and biological samples usually presents at a very low concentration with complicated matrix. To overcome such limitations, usually an extraction and preconcentration step is required prior to their analysis by CE.

Microextraction techniques, such as solid-phase microextraction (SPME) [9,10], liquid phase microextration (LPME) [5,8] and stir bar microextration (SBSE) [11,12] have been used for the enrichment of mercury species and clean-up of sample matrix. Among these emerging techniques, LPME with the advantages of simplicity, ease of implementation, and insignificant startup cost is almost accessible to all laboratories and thus recognized as one of most powerful sample preparation techniques [1,13].

So far, the overwhelming majority of LPME are focused on organic compounds, while the analysis of metals and organometallic compounds involving LPME are still relatively few [14]. Especially, when LPME was coupled with high performance liquid chromatography (HPLC) or CE, three-phase LPME mode (LLLME) is usually preferred because the aqueous extraction phase (acceptor solution) is directly compatible with the mobile phase or the

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electrolyte buffer used in HPLC or CE [5,15]. LLLME is only suitable to extract those metal and metallic compounds, which could be converted by reactions (protonation or complexation) into species that have a high affinity for the acceptor phase. To the best of our knowledge, only limited researches have been reported on the application of LLLME for mercury speciation [4,5,7,8,16].

An ideal LPME system should provide: (1) high extraction efficiency for target analytes with good selectivity; (2) fast extraction kinetics; and (3) minimum of manual handling. Therefore, besides the exploration of selective and sensitive LPME system, the research efforts have been oriented toward improving and innovating extraction mode or device to enhance the extraction kinetic or simplify operation. Compared with the static LPME method, the dynamic LPME methods were reported to lead to a higher enrichment factor and a lower detection limit in relatively shorter time. Automation and optimization of the extraction process benefits the simplification of the overall analysis by integration of sampling loading, extraction and washing, resulting in improved reproducibility. So far, many research works have been focused on the development of dynamic or automatic dynamic methods to extract the organic compounds both in two phase and three phase LPME [17–28]. Lee and Lee [24] developed an automated dynamic in-syringe two phase LPME with on-column derivatization approach for the analysis of carbamate pesticides from water samples by gas chromatography/mass spectrometry (GC/MS). Valcarcel et al. [25] reported a supported liquid membrane (SLM) device combined with commercially CE equipment for the direct determination of chlorophenols in surface water samples. A planar supported liquid membrane (SLM) was developed by Kuban and Bocek [26] and coupled on-line with CE for direct analysis of amino acids in human body fluids. Nitiyanontakit et al. [28] designed an in-line hollow-fiber-assisted three-phase LPME to extract Cr (VI) using a hybrid flow analyzer, and the automatic handling of the donor and acceptor aqueous solutions was realized via programmable flow. However, low extraction efficiency (13.2%) and low enrichment (10.9) were obtained, probably because the designed device limited the volume of sample contacted with organic solvent. In our previous work, we designed a dynamic extraction unit named membrane supported (MS)-LLLME and proposed a new sample pretreatment mode termed phase transfer based liquid–liquid–liquid microextraction (PT-LLLME) to facilitate the extraction of inorganic and organic mercury species [5]. The developed PT-LLLME-CE/UV method for mercury speciation was demonstrated to be high sensitive and selective, with short equilibrium time and fast extraction kinetics. However, full manual operation and careful attentions during extraction procedures are still required.

In the present work, to improve the sensitivity, reproducibility and ease of operation of dynamic LPME for mercury speciation, a new automated dynamic hollow fiber (AD-HF)-LLLME device was designed and constructed. The AD-HF-LLLME procedure, including automatic sample loading, washing and extraction, was controlled by a programmable flow injection system. The factors affecting the extraction efficiency of AD-HF-LLLME were studied. A new method of AD-HF-LLLME coupled with large volume sample stacking (LVSS)-CE/UV detection was established for simultaneous quantification of inorganic and organomercury species in both environmental and biological samples.

2. Experimental

2.1. Reagents and materials

The stock standard solution of inorganic mercury (Hg^{2+}) (1000 mg/L) was prepared from mercury chloride (AR, Shanghai Chemicals Reagent Co. Ltd., Shanghai, China) in diluted nitric acid. The stock solutions of methylmercury ($MeHg^{+}$), ethylmercury ($EtHg^{+}$), and phenylmercury ($PhHg^{+}$) of 1000 mg/L (as Hg) were prepared, by dissolving appropriate amounts of methylmercury chloride, ethlymercury chloride, and phenylmercury chloride (AlfarAesar, Ward Hill, MA, USA) in methanol. All stock solutions were stored at 4 °C in the dark. Working standard solutions were prepared by diluting their stock standard solution with high purity water to the required concentration. 3-Mercapto-1-propanesulfonic acid, sodium salt (MPS) (>98%) was purchased from Westingarea (Shanghai, China). The aqueous MPS solution was prepared daily. 18-crown-6 (99%) was obtained from Shanghai Jingchun Chemical Reagent Company (Shanghai, China). Other reagents used were of analytical reagent grade. High-purity water obtained by a Milli-Q system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout the whole experiment. Accurel polypropylene (PP 50/280) hollow fiber was purchased from Membrana GmbH (Wuppertal, Germany). The thickness of the wall was 200 μm, and the pore size was 0.2 μm. Toluene, cyclohexane, octanol, nitric acid, hydrochloric acid, sodium hydroxide and other reagents used were of analytical reagent grade.

2.2. CE system

All separation experiments were performed by Agilent 3D CE system (California, CA, USA) equipped with programmable, multiwavelength UV/vis detector. Fused-silica capillary of dimensions 48.5 cm (40 cm to the detector) × 50 μm i.d. × 360 μm o.d. (Yongnian Optical Fiber, Hebei, China) was used, and the capillary temperature was maintained at 25 °C. The detection wavelength used was 200 nm. A modified CE automatic sample vial as described in Ref. [15] was used for CE sample injection. 35 mmol/L borate buffer (pH 9.10) was used as back ground electrolyte (BGE). Prior to use, BGE was filtered through a 0.45 μm syringe filter and then ultrasonic degassed. In standard injection mode, the sample solution was injected hydrodynamically at 50 mbar for 5 s. Prior to use, the capillary was pretreated by flushing it sequentially, for 10 min each time, with 1 mol/L NaOH, high purity water, and the BGE. Between runs, the capillary was rinsed with BGE for 4 min.

2.3. AD-HF-LLLME unit and extraction procedure

The AD-HF-LLLME system is mainly comprised of a magnetic stirrer, a hollow fiber, a homemade extraction vial and a flow injection analyzer. An 85-2A constant temperature magnetic stirrer (Ronghua, Jiangsu, China) was used for stirring the sample solution. Accurel polypropylene (PP 50/280) hollow fiber (i.d. 280 μm; wall thickness, 200 μm; pore size, 0.2 μm) was purchased from Membrane GmbH (Wuppertal, Germany). The automation of AD-HF-LLLME was controlled by an FIA-3110 Flow Injection Analyzer (Beijing Titan Instruments, China) consisting of two peristaltic pumps and a standard rotary injection valve (8-channel, 16-port, multifunctional injector). A 10 mL flask (25 mm i.d., 25 mm height) with two connectors (3 mm length, 1 mm i.d. each) was home-made and used as the extraction vial. The extraction device (see magnified view in Fig. 1A) can be easily assembled daily. Briefly, two pieces of 5-cm long quartz glass capillary (530 μm i.d., 680 μm o.d.) were inserted into the two ends of hollow fiber separately and tightly, and the joint were 3 mm each. Then one end of capillary together with hollow fiber was horizontally inserted through the connectors of extraction vial and the hollow fiber was in the middle of the extraction vial with a slight U-shape. The joint between the connectors of extraction vial and capillaries was sealed using Parafilm to prevent the leak of samples solution. After that, the capillaries were connected with tubes and other parts of extraction system.

The designed programs included eight steps for AD-HF-LLLME, and the magnetic stirrer was kept running during the whole