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Synthesis of mixed coating with multi-functional groups for in-tube hollow fiber solid phase microextraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in human urine

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ABSTRACT

A novel method based on in-tube hollow fiber-solid phase microextraction (in-tube HF-SPME) on-line coupled with ion pair reversed phase high performance liquid chromatography (IP-RP-HPLC)–inductively coupled plasma mass spectrometry (ICP-MS) was developed for arsenic speciation. Partially sulfonated poly(styrene) (PSP) and mixed-sol of 3-mercapto propyltrimethoxysilane (MPTS) and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) were prepared and immobilized in the pores and the inner surface of polypropylene hollow fiber (HF). The prepared MPTS–AAPTS/PSP immobilized HF was characterized by FT-IR spectroscopy and scanning electron microscope (SEM). With arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AsB) and arsenocholine (AsC) as model arsenic species, a series of factors that influence the extraction of target arsenic species by in-tube HF-SPME, including pH value, sample volume and flow rate, elution conditions and interference of co-existing ions were investigated in details, and the conditions for subsequent HPLC–ICP-MS determination were also optimized. Under the optimal conditions, the sampling frequency was 6.5 h^{-1}, the detection limits for six target arsenic species were in the range of 0.017–0.053 $\mu g\,L^{-1}$ with the relative standard deviations ($c_{As(V),MMA} = 0.1\ \mu g\,L^{-1}$, $c_{As(III),DMA,AsB,AsC} = 0.5\ \mu g\,L^{-1}$, $n = 5$) ranging in 3.1–8.7%, and the enrichment factors were varied from 4 to 19-fold. To validate the accuracy of this method, certified reference materials DORM-2 (dogfish) and CRM No. 18 (human urine) were analyzed, and the determined values were in good agreement with the certified values. The proposed method was also successfully applied for arsenic speciation in human urine samples, and the recoveries for the spiked samples were in the range of 92.6–107%. The self-designed in-tube HF-SPME–HPLC–ICP-MS system shows high efficiency and good stability, and the proposed method is sensitive and suitable for simultaneous speciation of organic and inorganic arsenic species (including anions and cations) in biological samples.

1. Introduction

Non-metallic element arsenic is widely distributed in nature as various species [1] which can enter and accumulate in human body to cause the toxic effect. The toxicity of arsenic highly depends on its chemical forms and oxidation state [2]. Inorganic arsenic species such as arsenite (As(III)) and arsenate (As(V)) are considered as strong toxic and carcinogenic compounds. The toxicity of monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA) is much lower than that of inorganic arsenic, while arsenobetaine (AsB) and arsenocholine (AsC) are considered as non-toxic arsenic compounds. Recent researches indicated that trivalent methylated arsenic is highly toxic, and may be more toxic than some inorganic arsenic compounds [3]. Therefore, total arsenic determination cannot effectively reveal the toxicity of arsenic and its chemical behavior in organism, the speciation analysis of arsenic has become one of the hot research topics in analytical community [4,5].

At present, hyphenated techniques by combining high efficient separation techniques with sensitive element-specific detectors are most widely used for arsenic speciation [6–8]. Among the element-specific detection techniques [6,9–11], ICP-MS [6,12–14] is widely applied due to its merits of high sensitivity, wide linear range, and easy to couple with various separation techniques. Separation techniques for arsenic speciation could be classified into chromatography methods [15–20] and non-chromatography methods [21,22]. With a variety of separation modes, good precision, and easy to interface with the subsequent detectors such as

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ICP-MS, high performance liquid chromatography (HPLC) is widely applied for arsenic speciation, especially ion-pair reversed phase (IP-RP)–HPLC [12,13,17] and ion exchange chromatography (IEC) [18–20] which are suitable for polar compounds separation.

For elemental speciation in real world, in most cases the concentrations of some species are at or below the detection limits of HPLC–ICP-MS, and the sample matrix is often complex which will affect the accuracy of the analytical results. Therefore, it is of great significance to apply a sample pretreatment method to effectively separate the matrix and preconcentrate the target arsenic species prior to HPLC–ICP-MS analysis [23]. However, it is a challenge for analytical chemists to develop a method that can simultaneously enrich various arsenic species with different physical and chemical properties, and has the merits of simplicity, fast speed, easy automation, and compatibility to subsequent separation/detection techniques. Up to now, the used sample pretreatment methods for arsenic speciation include solvent extraction [24], solid phase extraction (SPE) [25], microwave assisted extraction [26], in-tube solid phase microextraction (SPME) [27], matrix solid-phase dispersion (MSPD) [28], stir bar sorptive extraction (SBSE) [29] and so on, but most of them involve multi-steps process and are difficult to automate.

In-tube SPME (also called capillary microextraction (CME)), which can be used as an on-line preconcentration technique coupling to HPLC–ICP-MS, has demonstrated great potential in the arsenic speciation due to its merits of multiple-coating options, low-cost, simple operation, rapidness, good reproducibility, low sample/reagents consumption, and easy atomization. Pawliszyn et al. [27] had successfully employed in-tube SPME coupled to HPLC/electrospray ionization mass spectrometry (ESI-MS) for the speciation of four organic arsenic species (MMA, DMA, AsB, and AsC). The extraction efficiency and enrichment factor for four target arsenic species were in range of 3.42–22.01% and 2.28–14.67-fold, respectively. However, no inorganic arsenic species were included in this work probably due to the limitation of the employed coatings. On the other hand, the amount of the coatings on the inner surface of the fused-silica capillary is limited, which will lead to a low adsorption capacity and restrict the application of in-tube SPME. Compared with in-tube SPME based on the support of fused-silica capillary, porous membrane supported SPME shows relatively large specific surface area, high extraction rates and extraction efficiency, but this mode cannot be easily automated or on-line coupled to HPLC [30–33].

The purpose of this approach is to develop a novel in-tube hollow fiber (HF)-SPME and on-line couple it to HPLC–ICP-MS for both inorganic and organic arsenic speciation in human urine samples. For extraction of different arsenic species including As(III), As(V), MMA, DMA, AsB and AsC, partially sulfonated poly(styrene) (PSP)/3-mercapto propyltrimethoxysilane (MPTS)-N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) immobilized polypropylene hollow fiber (HF) was prepared, and a series of factors that influence the extraction of target arsenic species by in-tube HF-SPME were investigated in details. The developed method was validated by the speciation of arsenic in biological samples.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a LC-10AD high-pressure pump, a CTO-10A column oven, and a DGU-12A degasser (Shimadzu, Japan). CAPCELL PAK C18 column (250 mm × 4.6 mm, 5 μm) was used for the separation of six target arsenic species. As an on-line detector, ICP-MS (Agilent 7500a, Japan) with a Babington nebulizer was interfaced to HPLC via a minimum length piece of Telfon tubing (i.d. 0.5 mm, length 30 cm) with a finger-tight PEEK fitting. The operating conditions for HPLC–ICP-MS are given in Table 1.

Table 1
Operating conditions for the analytical instrument.

HPLC	
Stationary phase	CAPCELL PAK C18
Sample loop volume	200 μL
Mobile phase	2.5 mmol L^{-1} sodium butanesulfonate, 4 mmol L^{-1} malonic acid, 0.5% (v/v) methanol
Flow rate	1.0 mL min^{-1}
Column temperature	25 °C
ICP-MS plasma	
Rf power	1150 W
Rf matching	1.5 V
Sampling depth	6.8 mm
Carrier gas	1.1 L min^{-1}
Time-resolved data acquisition	
Scanning mode	Peak-hopping
Dwell time	100 ms
Integration mode	Peak area
Detected isotope	^{75}As

An IFIS-C flow injection system (Ruimai Tech. Co. Ltd., Xi'an, China) was used for on-line coupling in-tube HF-SPME to HPLC–ICP-MS. The Accurel Q3/2 polypropylene hollow fiber membrane (i.d. 600 μm, 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). A Mettler Toledo 320-s pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) with a combined electrode was used to control the pH. TS2-60 Multi-Syringe pump (Baoding Longer Precision Pump Co. Ltd., Baoding, China) was used to pump the coating materials into the hollow fiber. The WX-3000 microwave accelerated system was obtained from EU Chemical Instruments Co. Ltd. (Shanghai, China), and ECH-1 temperature control heating panel was purchased from Sineo Microwave Chemistry Technology Co. Ltd. (Shanghai, China).

The structure of MPTS–AAPTS/PSP coating was characterized by 170SX FI-IR (NICOL ET, USA). And the scanning electron micrograph (SEM) of the MPTS–AAPTS/PSP immobilized HF was obtained using an X-650 scanning electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 25 kV.

2.2. Standard solutions and reagents

Stock solutions (1.000 g L^{-1} as As) of As(III), As(V), MMA, DMA, AsB and AsC were prepared by dissolving a certain amount of $NaAsO_2$ (>90%, Wako, Japan), $Na_2AsO_7 \cdot H_2O$ (>99%, Wako, Japan), $CH_3AsO_3Na_2$ (98.5%, J&K Chemical Ltd., China), $C_2H_6AsO_2Na \cdot H_2O$ (>98.5%, Genebase Bioscience Co., Ltd., China), $C_5H_{11}AsO_2$ (95%, Wako, Japan) and $C_5H_{14}AsBrO$ (95%, Wako, Japan) in high purity deionized water, respectively. Tetramethoxysilane (TMOS), 3-mercapto propyltrimethoxysilane (MPTS) and N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AAPTS) were purchased from Organic Silicon Material Company of Wuhan University (Wuhan, China). And poly(styrene) (PS) (Alfa Aesar, MA, USA) was used to synthesize partially sulfonated poly(styrene) (PSP). Acetic anhydride, 1,2-dichloroethane (DCE) and malonic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), butanesulfonate was obtained from Tianjin Aoran Fine Chemical Research Institute (Tianjin, China).

All reagents used were of analytical reagent grade. High purity deionized water obtained by a Milli-Q Element system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout this work. All laboratory ware was cleaned by soaking in 10% (v/v) nitric acid for at least 24 h.