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Titania immobilized polypropylene hollow fiber as a disposable coating for stir bar sorptive extraction–high performance liquid chromatography–inductively coupled plasma mass spectrometry speciation of arsenic in chicken tissues

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ABSTRACT

The bottleneck of applying stir bar sorptive extraction (SBSE) to elemental speciation analysis is lack of suitable extraction phases with good affinities to different elemental species. In this paper, a newly high polar extraction phase of titania immobilized polypropylene hollow fiber (TiO_2-PPHF) was prepared by sol–gel immersion and low temperature hydrothermal process and the obtained TiO_2-PPHF inherits the adsorption properties of TiO_2 and the toughness of PPHF. With a suitable size of stainless steel magnetic bar inserted into the prepared TiO_2-PPHF, a disposable TiO_2-PPHF coating stir bar was obtained. The prepared TiO_2-PPHF was characterized by X-ray diffraction spectrometry and scanning electron microscopy and the significant parameters affecting the extraction efficiency of different arsenic species were studied. Based on the above facts, a new method of SBSE combined with high performance liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry (ICP-MS) was developed for the speciation of phenyl arsenic compounds and their possible transformation products in chicken tissues. Under the optimal conditions, limits of detection (LODs) of the developed method for eight target arsenic species were in the range of 11.4–64.6 ng L^{-1} with enrichment factors of 8.5–22.3 (theory enrichment factor was 50), and the relative standard deviations (RSDs) were varying from 6.3 to 12.6% ($c_{AsIII/V}$ = 5 μg L^{-1}, $c_{MMA,DMA,p\text{-}ASA,4\text{-}OH,3\text{-}NHPAA,PA,4\text{-}NPAA}$ = 10 μg L^{-1}, n = 7). The proposed method was successfully applied to the speciation of arsenic in chicken meat/liver samples and the recoveries for the spiked samples were in the range of 78.5–120.4%. In order to validate the accuracy of the proposed method, a certified reference material of BCR-627 tuna fish tissue was analyzed and the determined values were in good agreement with the certified values. The TiO_2-PPHF was demonstrated to be a highly selective coating for the target arsenic species, and could be easily prepared in batches with low cost. In addition, with the disposable coating, the carry-over effect commonly encountered in conventional SBSE was avoided.

1. Introduction

Arsenic, as a ubiquitous element, exists as many different chemical species in the environment and organisms. The chemical form and oxidation state of arsenic play a very important role in its toxicity and bioavailability. Inorganic arsenic compounds (arsenite As(III) and arsenate As(V)) are strong carcinogenic substances. Simple methylated arsenic species such as monomethylarsonic acid (MMA) and dimethyl arsinic acid (DMA) are identified as possible cancer promoters. Arsenobetaine (AsB), arsenocholine (AsC), arsenic sugars and arsenolipids are considered to be non-toxic species [1]. Some phenylarsonic acid compounds, for example, p-amino phenyl arsenic acid (p-ASA), 3-nitro-4-hydroxyphenylarsonic acid (3-NHPAA, Roxarsone), and 4-nitrophenylarsonic acid (4-NPAA), are capable of controlling intestinal parasites, improving feed efficiency, and promoting rapid growth of the poultry. Thus, phenylarsonic acid compounds are extensively used in the poultry industry [2]. However, the abuse of those feed additives containing arsenic will bring potential hazards to human health and environment. Most of the phenylarsonic acid compounds are excreted virtually unchanged in the manure [3], but could be eventually converted to toxic arsenic compounds (such as As(V)) to contaminate water and soil in the role of sunlight and micro-organisms [4,5]. Therefore, it is of great significance to develop rapid, sensitive and simple arsenic speciation methods for further investigation and better understanding of arsenic toxicity and bioavailability in biological and environmental systems.

At the end of 1970s in the 20th century, hyphenation technique by coupling chromatographic separation with element-specific detection was proposed by Van Loon [6] and Suzuki for trace ele-

Abbreviations: As(III), arsenite; As(V), arsenate; MMA, monomethylarsonic acid; DMA, dimethyl arsinic acid; 4-OH, 4-hydroxyphenylarsonic acid; 3-NHPAA, 3-nitro-4-hydroxyphenylarsonic acid; PA, phenylarsonic acid; p-ASA, 4-aminophenylarsonic acid; 4-NPAA, 4-nitrophenylarsonic acid.

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ment speciation analysis. Afterwards, the hyphenation technique has been rapidly developed and become one of the most commonly used approaches for elemental speciation. This is especially the case for arsenic speciation. Nowadays, different separation techniques (gas chromatography (GC), capillary electrophoresis (CE), high performance liquid chromatography (HPLC), etc.) coupled with highly sensitive and element specific atomic spectrometry or atomic mass spectrometry (such as inductively coupled plasma mass spectrometry (ICP-MS)) has been proven to be one of the most effective means for the speciation of arsenic in different samples with complex matrix [7]. GC provides excellent separation capacity for volatile arsenicals, but most naturally occurring arsenic species are non-volatile which should be derivatized into volatile species prior to GC analysis. Derivatization complicates the analytical process and results in possible analyte loss or species transformation, which greatly limits the application of GC in the field of arsenic speciation [8]. CE is a high efficiency separation method but lacks of sensitivity and selectivity for real samples with complex matrix. CE–ICP-MS provides higher selectivity and improved sensitivity compared with CE–UV detection for arsenic species, but is usually limited by complicated interface and poor relative detection limits [9]. HPLC–ICP-MS has been demonstrated to be the most effective method in arsenic speciation due to the advantages of no derivatization required, simple interface and the flow rate of HPLC effluent matching with the sample uptake rate of the ICP pneumatic nebulizer. Different HPLC separation modes including ion exchange (IE) [2], reversed phase (RP) [10], reversed phase ion pair (RP-IP) [11] and micellar HPLC [12] have been employed for arsenic speciation, but IE HPLC is one of the most used separation techniques for arsenic speciation.

For elemental speciation in real-world samples, appropriate sample pretreatment techniques are often required because of the extremely low concentration of single species for a given element and very complicated matrix in the samples. In recent years, different sample pretreatment techniques have been employed for arsenic speciation, such as solid phase extraction (SPE) [13] and solid phase microextraction (SPME) [8]. SPME offers numerous advantages in sample preparation for speciation analysis, such as easy to combine with virtually any detection system, quick achievement of sample-matrix separation, solvent free and miniaturization of manipulation in sample pretreatment techniques. The combination of in-tube SPME with HPLC for arsenic speciation analysis can obviously exhibit the advantages of simple operation and good sensitivity [14]. Stir bar sorptive extraction (SBSE) which was first developed in 1999 [15] is a sample preparation technique derived from SPME, and its extraction mechanism and advantages are similar to those of SPME. Generally, the volume of extraction phase of SBSE is 50–250 times larger than that of SPME fiber, thus, better reproducibility and higher sensitivity than SPME are expected when SBSE is used. Therefore, SBSE is especially suitable for trace/ultra-trace analysis. The SBSE extraction process and its analytical applications have been thoroughly described in recent reviews [16–18]. Like SPME, the further development and application of SBSE are highly dependent on the exploration of new extraction coatings. However, to the best of our knowledge, polydimethylsiloxane (PDMS), as the most commonly used coating for extraction of non-polar and weak polar organic compounds by SBSE [19], is the only commercially available coating. To improve the extraction performance of SBSE for polar compounds, some novel extraction coatings with good capability have been explored extensively, including dual-phase stir bar (PDMS/activated carbons) [20], restricted access material (RAM) alkyl-diol-silica (ADS) [21], polyurethane foams (PU) [22], PDMS/β-cyclodextrin (β-CD) [23], polyphthalazine ether sulfone ketone (PPESK) [24], nylon-6 polymer imprinted with L-glutamine [25], vinylpyrrolidone (VPL)/divinylbenzene (DVB) monolithic material [26], and PDMS/polyvinylalcohol (PVA) [27], etc. However, very few reports on the application of SBSE to the trace elements and their species analysis have been published until present, mainly due to lacking of suitable coatings and coating techniques. Pu et al. [28] proposed a zirconia coated graphite bar sorptive extraction for electrothermal vaporization (ETV)–ICP-MS determination of trace Cd, Hg and Pb with limits of detection at 0.05, 0.42 and 0.06 pg mL^{-1}, respectively. Vercauteren et al. [29] have determined ppq-level traces of organotin compounds in environmental samples using SBSE combined with thermal desorption-capillary GC–ICP-MS. Similarly, Prieto et al. [30] has utilized headspace stir bar sorptive extraction (HS-SBSE)–thermal desorption–GC–MS to the speciation of methylmercury and butyltin species in environmental samples. Duan et al. [31] employed HS-SBSE–GC–ICP-MS for the speciation of dimethylselenide and dimethyldiselenide in biological samples.

A few high polar inorganic metallic oxides, such as titania, alumina, and zirconia, have been extensively used in SPE for trace elements and their speciation analysis [32,33], indicating an application possibility of inorganic metallic oxides as the coating for stir bar sorptive extraction of high polar target analytes. Unfortunately, it is difficult to coat or bond the inorganic metallic oxides powder on the surface of glass stirring rod and even through sol–gel coating technique, the coating will easily peel off during the process of preparation and stirring extraction. To obtain an inorganic metallic oxide coated stir bar with good stability, an appropriate preparation technique is required. Employing polypropylene hollow fiber (PPHF) as template and combined with sol–gel process, Xu and Lee et al. [34] synthesized a zirconia hollow fiber by repeatedly impregnating PPHF in zirconia sol precursor and calcinating it to burn off the template. The prepared zirconia hollow fiber had been demonstrated to be lack of mechanical strength, and therefore, a shaker instead of a stirrer was used to facilitate the extraction process to prevent its possible damage. As the template PPHF itself has good toughness, it is assumed that the obtained material would inherit the toughness advantage of PPHF if the template is not burned off.

In this work, using PPHF as the support and template, a novel high polar SBSE extraction phase of titania (TiO_2) immobilized PPHF was prepared by sol–gel immersion and low temperature hydrothermal process. With a suitable size of stainless steel magnetic bar inserted into the prepared TiO_2-PPHF, a disposable TiO_2-PPHF coating stir bar was obtained and a new method of TiO_2-PPHF SBSE coupled with HPLC–ICP-MS was developed for simultaneous speciation of phenylarsonic acids and their possible transformation products in biological samples.

2. Experimental

2.1. Instrument

A quadrupole (Q) ICP-MS (Model Agilent 7500a, Hewlett-Packard, Yokogawa Analytical Systems, Tokyo, Japan) with a Babington nebulizer was used for the determination of arsenic species with a single-ion-monitoring mode (m/z 75). An HPLC system consisting of a LC-10AD high-pressure pump, CTO-10A column oven, SPD-10AV UV–vis spectrometry detector, C-R6A chromatopac (Shimadzu, Japan) and CAPCELL PAK C18 MG column (5 μm, 250 mm × 4.6 mm i.d., Shiseido, Japan) was used for the separation of arsenic species. ICP-MS was used as an on-line detector for HPLC by connecting HPLC column outlet (1.0 mL min^{-1}) to the Babington nebulizer situated in the spray chamber via a minimum length piece of Teflon tubing (i.d. 0.5 mm). Optimization of the ICP-MS instrument (i.e., lens settings, sampling depth, and carrier gas flow rate) was performed with conventional pneumatic nebulization (PN)-ICP-MS prior to being connected with HPLC. Typical

Table 1
Operating conditions for the analytical instrument.

HPLC	
Stationary phase	CAPCELL PAK C18 MG
Mobile phase	Methanol:water (2:98, v/v), water containing 2.5 mmol L^{-1} of sodium butanesulfonate and 4.0 mmol L^{-1} of malonic acid (pH 3.0), isocratic elution
Flow rate	1.0 mL min^{-1}
Column temperature	40 °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

operating conditions of the HPLC and ICP-MS are summarized in Table 1.

Lab X-3000 X-ray powder diffractometer (Shimadzu, Japan), ASAP 2020 accelerated surface area and porosimetry analyzer (Micromeritics, USA) and Hitachi modal X-650 scanning electron microscopy (Tokyo, Japan) were used for the characterization of the prepared TiO_2-PPHF. The pH values were measured by a Mettler Toledo 320-S pH meter (Mettler Toledo Instruments Co., Ltd., China) supplied with a combined electrode.

2.2. Standard solutions and reagents

Sodium arsenite (As(III), 90%) and sodium arsenate (As(V), 99%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 4-Hydroxyphenylarsonic acid (4-OH, 98%) and 3-nitro-4-hydroxyphenylarsonic acid (3-NHPAA, 98%) were bought from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Monomethylarsonic acid (MMA, 99%) and dimethyl arsinic acid (DMA, ≥98.5%) were purchased from J&K Chemical Ltd. (Beijing, China), and phenylarsonic acid (PA, 97%), 4-aminophenylarsonic acid (p-ASA, ≥98%) and 4-nitrophenylarsonic acid (4-NPAA) were purchased from Alfa Aesar, China, Fluka, USA and Shanghai Chemical reagent Co., Ltd., China, respectively. The standard stock solutions (1.00 g L^{-1} as As) of each arsenic species were prepared by dissolving certain amount of respective species in high purity deionized water and stored at 4 °C in the dark. Certified reference material BCR-627 tuna fish tissue was purchased from European Commission Joint Research Centre Institute for Reference Materials and Measurements (IRMM, Belgium).

Tetrabutyl titanate, acetyl acetone, acetic acid, anhydrous ethanol, sodium butanesulfonate, malonic acid, KH_2PO_4, acetone and other reagents used were of analytical reagent grade. High purity water obtained by a Milli-Q water purification system (18.2 MΩ cm, Millipore, Bedford, MA, USA) was used throughout the whole experiments.

2.3. The preparation of TiO_2-PPHF disposable coating stir bar

The titania sol was prepared as follows. 10 mL of tetrabutyl titanate was dissolved in 30 mL of anhydrous ethanol. The rapid hydrolysis of tetrabutyl titanate could be controlled effectively by using ethanol as the solvent. After stirring for 10 min, 0.5 mL acetyl acetone and 0.5 mL glacial acetic acid were added. The resulting suspension was stirred for 3 h at room temperature, followed by addition of 0.5 mL high purity water under vigorous stirring. The solution was stirred continuously for 2 h and the obtained sol solution was aged overnight for further use.

The preparation procedure of stir bar with disposable TiO_2-PPHF coating was described as follows. Firstly, a polypropylene hollow fiber (i.d. 600 μm, 200 μm wall thickness, 0.2 μm pore size, Q3/2 Accurel, Wuppertal, Germany) was cut into 2.7 cm small segments which were ultrasonically cleaned in acetone and air-dried before use. The cleaned fibers were immerged into the synthesized titania sol solution for 2 h and taken out for air drying. The immersion and airing drying procedure was repeated for three times. Then, the obtained fibers were placed in a constant-temperature water bath for 6 h at 80 °C and then dried in air. Finally, a 0.5 mm × 22 mm stainless steel magnetic bar was inserted into the TiO_2-PPHF and the excess membrane was cut off to keep 2.2 cm valid length (shown in Fig. 1).

2.4. SBSE procedures

SBSE was carried out in a 20 mL vial containing 5 mL sample solution with the TiO_2-PPHF coating stir bar. The vial was placed on the 85-2A constant temperature magnetic stirrer (Ronghua Instrument Factory, China). After stirring for 25 min, the stir bar was taken out and dried by a filter paper. A 200 μL pipette tip was sealed on the base end by an alcohol burner and the stir bar was put into it for ultrasonic desorption (Shengyuan Instrument Factory, China) with 100 μL desorption solution. The desorption solution was filtered by a 0.22 μm filter membrane and then injected into the sample loop for HPLC–ICP-MS analysis. To avoid the carry-over effect, the TiO_2-PPHF stir bar coating was discarded after use.

2.5. Preparation of real samples

Chicken leg meat and liver were purchased from a local market. Samples were cut into small pieces and freeze-dried in Alpha1-2 lyophilizer (Christ, Germany). The freeze-dried sample was homogenized by grinding and stored frozen at −20 °C under refrigeration until analysis.

Total arsenic determinations. 1.0 g freeze-dried chicken tissue sample was placed in a Teflon digestion vessel and 6.0 mL of HNO_3 and H_2O_2 (5:1, v/v) were added overnight for pre-digestion. A WX-4000 microwave digestion system (Shanghai EU Microwave Chemistry Technology Co., Ltd., China) with the following microwave program was used for digestion: 250 W, 2 min; 0 W, 2 min; 250 W, 6 min; 400 W, 5 min; 550 W, 8 min; ventilation 8 min. After microwave digestion, the samples were placed on heating-board (80 °C) and evaporated to near dryness. Then the residuals were dissolved by 10 mL dilute HNO_3 (1%). High purity water without analyte addition was employed as the blank and subjected to the same procedure described above. Total arsenic concentration in freeze-dried chicken tissue samples was determined by PN–ICP-MS.

Extraction of arsenic species. Similar to the procedure reported by Palacios and co-workers [35], methanol–water (1:1, v/v) was used as the extractant to extract different arsenic species from the biological samples. Briefly, 5 mL methanol–water (1:1, v/v) was added into 0.1 g sample and the mixture was maintained in a con-

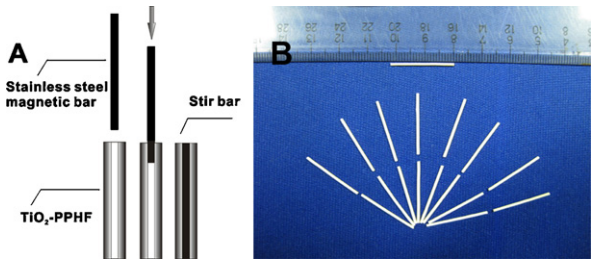


Fig. 1. Scheme of TiO_2-PPHF stir bar. (A) Preparation procedure and (B) finished stir bar.

stant temperature bath at 55 °C for 10 h and then ultrasonicated for 30 min. The samples were centrifuged for 15 min at 3000 rpm, the extract was then removed using a pipette and the residue was re-extracted following the same procedure. The two combined extracts were mixed, evaporated to dryness using a centrivap evaporator and cold trap system, diluted by deionized water. After filtered through a 0.45 μm nylon syringe filter, the extracts were subjected to SBSE–HPLC–ICP-MS analysis. Chicken leg meat, liver and BCR 627 Tuna fish and blank samples were all subjected to this procedure.

3. Results and discussion

3.1. Characterization of TiO_2-PPHF disposable coating

3.1.1. XRD

TiO_2 can exist in different crystal forms including anatase, rutile and boorkite. Among them, anatase TiO_2 is the most attractive due to its excellent performance in photocatalytic reactions and better chemical stability, and it has been used as photocatalysis, photoelectric chemical conversion materials and SPE sorbents. To prevent the destruction of PPHF template and inactivation of TiO_2, low temperature hydrothermal method [36] was employed to the synthesis of anatase TiO_2 in this work instead of the traditional high temperature sintering process. As can be seen from Fig. 2, a characteristic peak of anatase TiO_2 was obviously observed after 80 °C hydrothermal treatments.

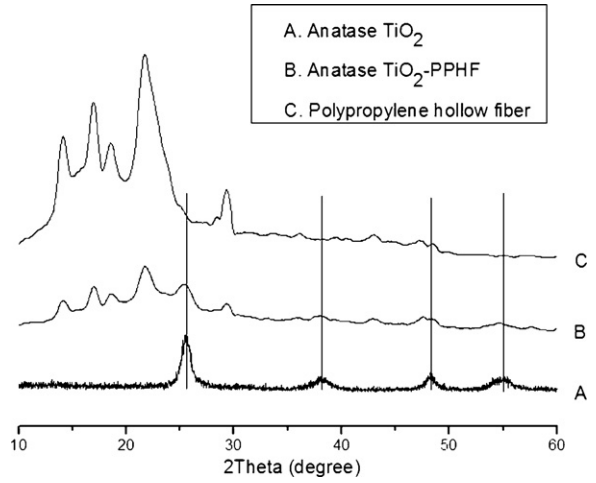


Fig. 2. XRD spectra of TiO_2-PPHF. (A) Sintering at 550 °C and (B) hydrothermally treated at 80 °C.

3.1.2. SEM, specific surface area and porosimetry analysis

The PPHF support and as-synthesized TiO_2-PPHF were characterized by scanning electron microscopy (SEM) and their SEM patterns are shown in Fig. 3. Fig. 3(A) and (B) exhibited the cross section and side wall of TiO_2-PPHF, indicating a 500 μm inner diameter for the as-synthesized TiO_2-PPHF. Fig. 3(C) and (D) illustrated the surface morphology of TiO_2-PPHF and PPHF, respectively. It can be seen that the morphology of TiO_2-PPHF is similar to its template PPHF but has more dense micropores. Surface area and pore structure analysis of TiO_2-PPHF were carried out, and the results showed that after immobilized with TiO_2, the surface area of PPHF was increased from 26 to 36 $m^2\,g^{-1}$ and the average pore volume was increased from 0.09 to 0.26 $mL\,g^{-1}$.

By employing PPHF as the support and template of TiO_2, the toughness of TiO_2 was improved and the obtained TiO_2-PPHF could be easily used as the stir bar coating through a suitable size of stainless steel magnetic bar inserted into it. Furthermore, the porous structure of PPHF results in the increase of surface area of TiO_2, which is benefit to improve the extraction efficiency.

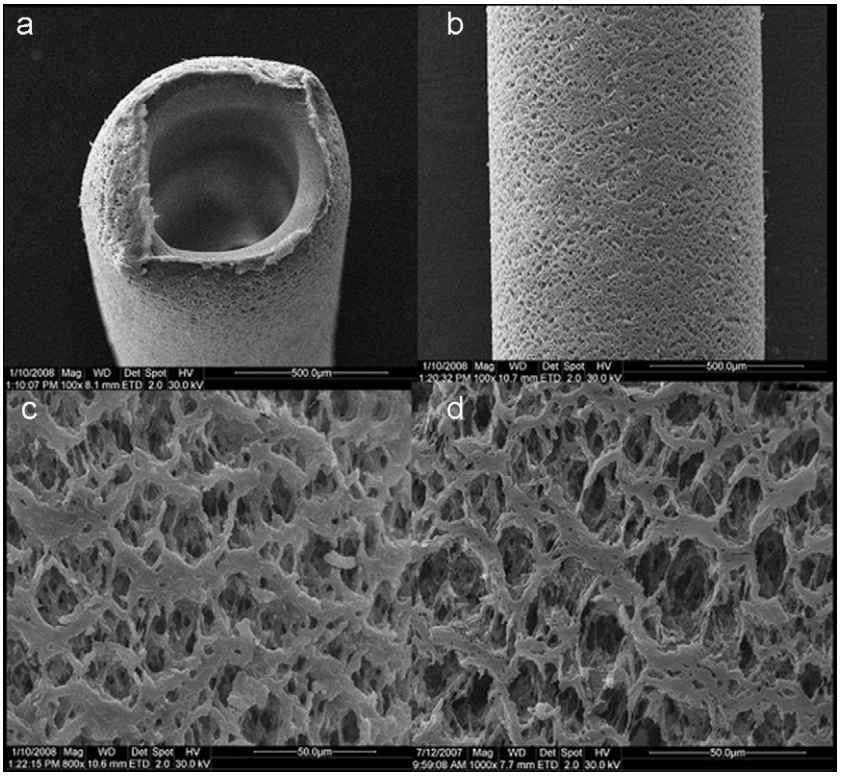


Fig. 3. SEM spectra of TiO_2-PPHF. (A) and (B) were the cross section and side wall of TiO_2-PPHF, (C) and (D) illustrate the surface morphology of TiO_2-PPHF and PPHF, respectively.

3.1.3. Reproducibility of stir bar with disposable TiO_2-PPHF coating

Reproducibility of the prepared TiO_2-PPHF disposable coating stir bar was also evaluated in this work. Seven bars prepared in the same batch and five bars from different batches were tested for the extraction of arsenic species (inorganic As 5 ng mL^{-1}, others 10 ng mL^{-1}) from aqueous solution. The relative standard deviations of 6.3–12.6% ($n = 7$) within one batch and 8.3–20.0% ($n = 5$) among different batches for the preparation of TiO_2-PPHF disposable coating stir bars were obtained for target arsenic species, indicating a good reproducibility of the proposed stir bar coating preparation method.

3.2. The optimization of HPLC mobile phase

With sodium butanesulfonate (2.5 mmol L^{-1}) as ion pair reagent and using malonic acid to adjust the pH of mobile phase (4.0 mmol L^{-1}, pH 3.0), HPLC separation of target arsenic species was studied. It was found that the retention time of phenylarsonic acid compounds was obviously affected by methanol proportion and column temperature. Either increasing the proportion of methanol in mobile phase or enhancing the column temperature could shorten the retention time of target arsenic species.

Finally, the optimized HPLC mobile phase and separation conditions were as follows: methanol:water (2:98, v/v), water containing 2.5 mmol L^{-1} of sodium butanesulfonate and 4.0 mmol L^{-1} of malonic acid (pH 3.0), isocratic elution, and column temperature of 40 °C.

3.3. The optimization of SBSE procedure

3.3.1. Effect of sample pH

The pH value of sample solution plays a great role on the extraction of target arsenic species by SBSE and an appropriate pH value can improve their extraction efficiency. As(III/V), MMA, DMA, p-ASA, 4-OH, 3-NHPAA, PA and 4-NPAA were weak acids with different pK_a values (as shown in Table 2) and they could be ionized and adsorbed on the TiO_2 surface at the appropriate pH by the electrostatic effect and surface complexation [37]. The effect of pH on the extraction efficiency of disposable TiO_2-PPHF coating SBSE for the target arsenic species was studied from pH 2 to 10, and the results indicated that the maximum signal intensity were obtained for all of the arsenic species within a pH range of 4–7. When pH value was greater than the isoelectric point of TiO_2 (around 5.8) [37], the TiO_2 surface was negatively charged and the extraction efficiency for negative charged arsenic species would be decreased rapidly. Thus, pH 6 was used for the simultaneous extraction of eight target arsenic species by TiO_2-PPHF coating SBSE in further experiments.

3.3.2. Effect of desorption solution

Ultrasonic desorption was employed and 100 μL desorption volume which was the minimal volume for entirely immersing the stir bar was used. The effects of four kinds of desorption solutions were studied, including acid, base, organic solvent and inorganic salt solutions and the results are shown in Fig. 4. As could be seen, the target arsenic species could not be desorbed by HNO_3 (A), methanol (D) or $NaNO_3$ (E). Though the target arsenic species could be desorbed by NaOH (B), the serious peak variation and trailing were observed. Only three arsenic species could be desorbed by ammonia (C). 1 mol L^{-1} KH_2PO_4 (pH 4.5) buffer (F) could provide the best desorption for the target arsenic species, but it would influence the subsequent chromatographic separation. Therefore, the effect of pH values of KH_2PO_4 solution on the desorption efficiency and the subsequent chromatographic separation were studied. It was found that the pH values of KH_2PO_4 solution had no obvious effect on the desorption efficiency of target arsenic species, but had influence on the chromatographic separation of the target arsenic species. Fig. 5 shows the effect of pH values of KH_2PO_4 solution on the separation of different arsenic species. As could be seen, when pH value of desorption solution was 1 and 2.5, the chromatographic separation was better than pH 4.5. To prevent the damage of the ordinary ODS column under low pH value, 1 mol L^{-1} KH_2PO_4 (pH 2.5) was used as the desorption solution. The possible desorption mechanism was competitive adsorption. It is assumed that $H_2PO_4^-$ has the similar structure to $H_2AsO_4^-$, which could be easily adsorbed on the TiO_2 surface and release arsenic species simultaneously.

3.3.3. Effect of extraction time, desorption time and stirring rate

SBSE is based on the extraction equilibrium between the stir bar coating and the sample solution. Usually, extended extraction time could improve the extraction efficiency. The effect of extraction time on the extraction efficiency of target arsenic species was evaluated from 5 to 30 min, and the results showed that the extraction efficiency was increased with the increase of extraction time from 5 to 25 min, and kept constant or decreased a little with further increasing the extraction time. Therefore, a 25 min extraction time was chosen for subsequent experiments.

Then the effect of desorption time on the desorption of arsenic species was investigated. The desorption efficiency was increased along with the increase of desorption time from 5 to 10 min and remained almost constant after 10 min. Then, 15 min was selected as desorption time.

The effect of stirring rate on the extraction efficiency was also studied. The experimental results showed that extraction efficiency was increased with the increase of stirring rate from 400 to 800 rpm and descended with further increasing the stirring rate. Therefore, a stirring rate of 600 rpm was selected for the further work.

3.4. The tolerance of coexisting ions

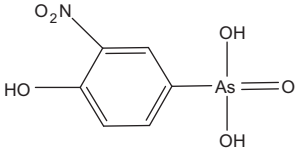
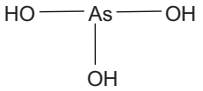
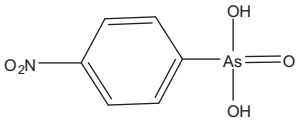
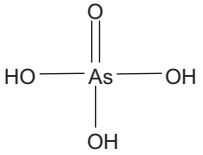
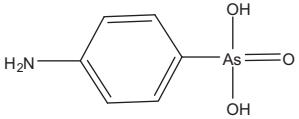
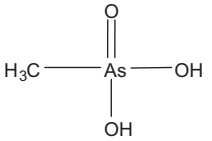
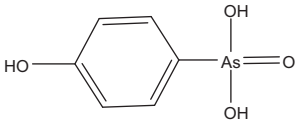
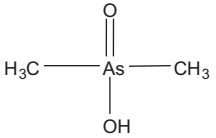
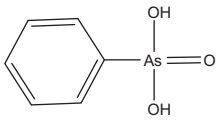
Fixing the concentration of inorganic As at 5 ng mL^{-1} and other arsenic species at 10 ng mL^{-1}, the interference of common coexisting ions found in biological samples, such as K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Cl^-, SO_4^{2-}, and NO_3^- on the preconcentration and determination of target arsenic species were investigated. The results indicated that in the presence of 1000 mg L^{-1} K^+, Na^+, Ca^{2+}, Mg^{2+}, 1000 mg L^{-1} Cl^-, SO_4^{2-}, NO_3^-, 200 mg L^{-1} Cu^{2+}, Zn^{2+}, and 100 mg L^{-1} Fe^{3+}, no significant interferences were observed under the optimum conditions described above. It shows that the proposed method has good selectivity for arsenic species and is suitable for the analysis of samples with complicated matrix.

3.5. Analytical performance

Based on the above studies, the optimal SBSE conditions for arsenic species were as follows: sample solution (pH 6) was extracted for 25 min with a stirring rate of 600 rpm, and desorbed ultrasonicately by 100 μL of 1 mol L^{-1} KH_2PO_4 (pH 2.5) for 15 min.

The analytical performance of the proposed TiO_2-PPHF coating SBSE–HPLC–ICP-MS method has been validated and the linear range, limits of detection (LODs), enrichment factors and relative standard deviations (RSDs) are listed in Table 3. The linear range was found to cover 3 orders of magnitude (50–100,000 ng L^{-1}) with correlation coefficient of 0.9996–0.9999. Based on $3\sigma_{blank}$ approach as recommended by IUPAC for spectrochemical measurements, the LODs of the proposed method for the eight target arsenic species were found to be in the range of 11.4–64.6 ng L^{-1}. The RSDs of method was varied from 6.3 to 12.6% ($n = 7$, inorganic As 5 ng mL^{-1}, others 10 ng mL^{-1}). The enrichment factor, defined as the slope ratio of the calibration curve obtained after SBSE to the original one without SBSE, was calculated as 8.5–22.3 (theory enrichment fac-

Table 2
Structures, pK_a and log P values of arsenic compounds used in this work.

Analyte	Molecular structure	pK_a	log P	Analyte	Molecular structure	pK_a	log P
3-Nitro-4-hydroxy phenylarsonic acid (Roxarsone), 3-NHPAA		3.5	−0.510	Arsenite, As(III)		9.2 12.1	–
4-Nitrophenyl arsonic acid, 4-NPAA		3.0	−0.271	Arsenate, As(V)		2.3 6.7 11.6	−1.882
4-Aminophenyl arsonic acid, p-ASA		2.0 4.0 8.9	−1.283	Monomethylarsonic acid, MMA		4.6 7.8	−1.753
4-Hydroxyphenyl arsonic acid, 4-OH		3.9	−0.737	Dimethyl arsinic acid, DMA		6.2	−1.624
Phenylarsonic acid, PA		3.6 8.8	−0.001				

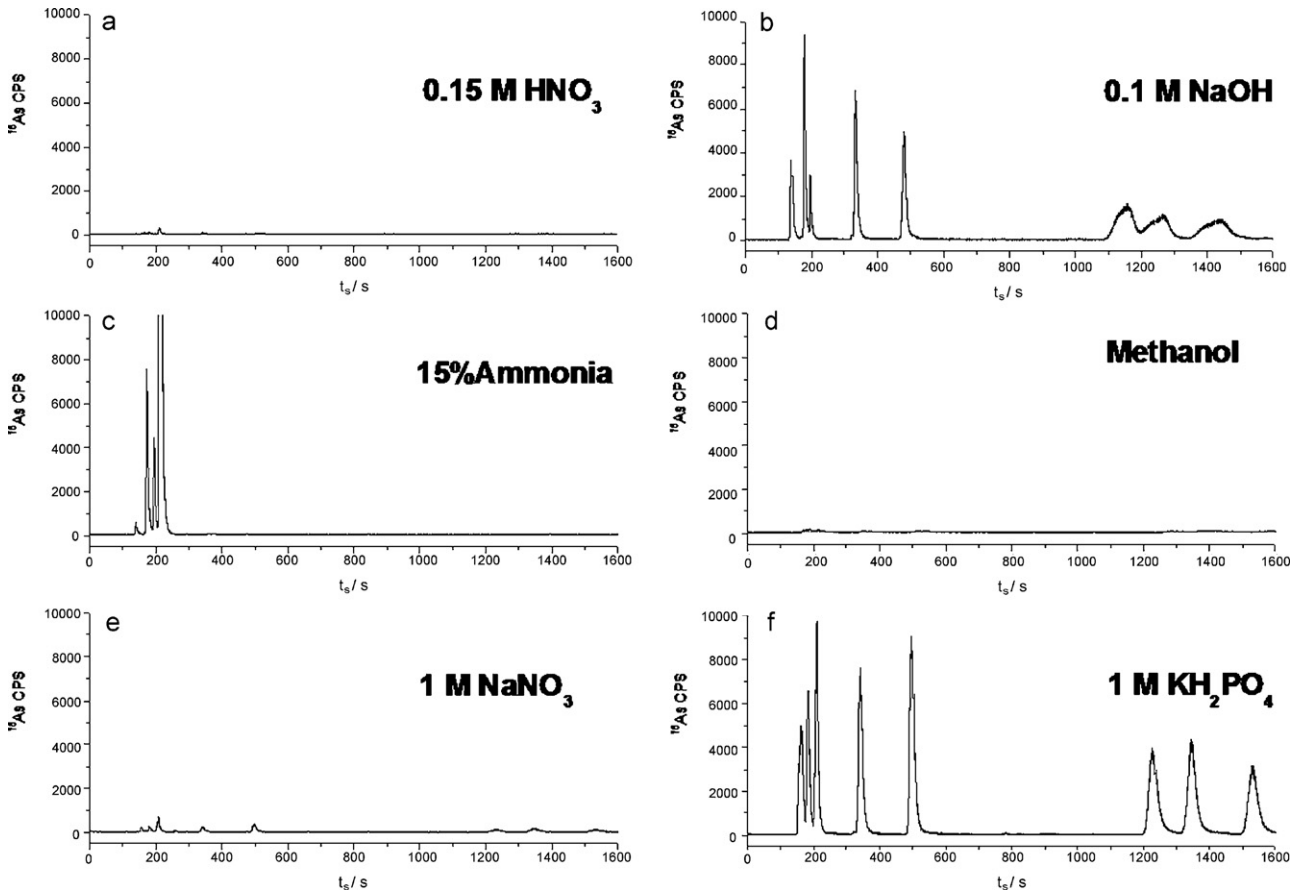


Fig. 4. Effect of different kinds of desorption solutions. *Conditions*: inorganic As 5 ng mL^{-1}, others 10 ng mL^{-1}, extraction 30 min, desorption 30 min, 4 mL sample solution, 100 μL different kinds of desorption solutions.

tor was 50). A comparison of LODs for the arsenic species obtained by this method and other different approaches is also shown in Table 4. As could be seen, the proposed method was one of the most sensitive methods for arsenic speciation.

3.6. Real sample analysis

The developed method was validated by the analysis of certified reference material of BCR-627 Tuna fish tissue. The determined values for total As (4.3 ± 0.2 μg g^{-1}) and DMA (0.17 ± 0.02 μg g^{-1}) were in good agreement with the certified values (4.8 ± 0.3 μg g^{-1} for total As and 0.15 ± 0.01 μg g^{-1} for DMA, respectively). The method was also used to analyze real chicken meat/liver samples and the analytical results along with the recovery for the spiked samples are listed in Table 5. Fig. 6 shows the chromatograms of unspiked and spiked chicken meat samples after SBSE pretreatment. Inorganic arsenic (35–57 ng g^{-1}) and DMA (65–108 ng g^{-1}) were found in chicken meat and liver samples, 4-NPAA (36 ng g^{-1})

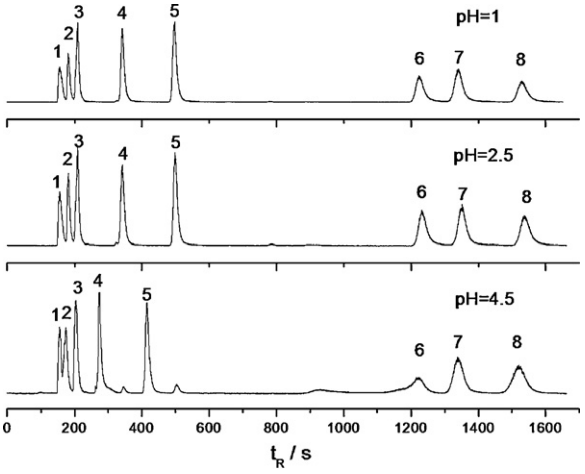


Fig. 5. Effect of the pH of KH_2PO_4 desorption solution on chromatogram figures of analytes. Peaks of 1–8 represent inorganic arsenic (As^{III}/As^{V}), MMA, DMA, p-ASA, 4-OH, 3-NHPAA, PA and 4-NPAA, respectively.

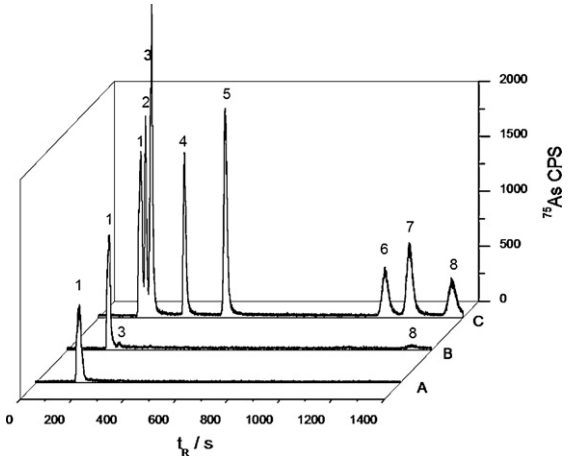


Fig. 6. SBSE–HPLC–ICP-MS analysis of (A) blank, (B) chicken leg extract, (C) chicken leg extract spiked with 8 arsenic species. Peaks of 1–8 represent inorganic arsenic (As^{III}/As^{V}), MMA, DMA, p-ASA, 4-OH, 3-NHPAA, PA and 4-NPAA, respectively.

Table 3
Analytical performance data by SBSE with HPLC–ICP-MS.

Analytes	Linear range (ng L^{-1})	Linear equation	R^2	Enrichment factor (EF)[a]	LOD[b] (ng L^{-1})	RSD[c] % (n = 7)
Inorganic As	200–100,000	$y = 124074 + 80687x$	0.9999	15.3	64.6	6.7
MMA	50–100,000	$y = 16405 + 60563x$	0.9999	8.5	19.8	6.3
DMA	50–100,000	$y = 71752 + 121190x$	0.9998	10.4	12.9	7.5
p-ASA	50–100,000	$y = 55041 + 107561x$	0.9998	17.3	14.1	8.5
4-OH	50–100,000	$y = 50415 + 118872x$	0.9999	22.3	12.9	8.7
3-NHPAA	50–100,000	$y = 76629 + 85783x$	0.9996	20.6	11.4	9.4
PA	50–100,000	$y = 57012 + 114524x$	0.9999	19.1	14.8	11.7
4-NPAA	50–100,000	$y = 62202 + 90030x$	0.9998	15.2	13.7	12.6

[a] EF = slope ratio of the calibration curve obtained after SBSE versus the one that obtained without SBSE.
[b] LOD = 3 × standard deviation of background/slope of calibration curve obtained after SBSE.
[c] RSD (sample concentration: inorganic As 5 ng mL^{-1}, others 10 ng mL^{-1}).

Table 4
Comparison of detection limits found in the literatures for determination of arsenic species.

Analytical technique	LOD for As (ng mL^{-1})									Ref.
	As(III)	As(V)	MMA	DMA	p-ASA	4-OH	3-NHPAA	PA	4-NPAA	
RP-μ HPLC–ICP-MS	0.10	0.10	–	–	0.10	0.10	0.12	–	0.26	[10]
RP-IP-μ HPLC–ICP-MS	0.6	0.4	–	–	0.9	0.8	n.d		n.d	
μ HPLC–ES-MS–MS	–	–	0.305	0.072	0.107	0.25	0.15	–	0.145	[38]
CE–HEN–ICP-MS	1.4	0.97	2.2	1.3	3.2	1.1	1.1			[9]
SPME–GC–QITMS	–	–	0.6	0.22	–	–	2.69	–	–	[8]
IC–ICP-MS										
As7 anion exchange column	0.024	0.008	0.006	0.019	0.053	–	0.027	–	–	
As14 anion exchange column	0.112	0.079	0.061	0.044	0.076	–	0.254	–	–	[2]
As16 anion exchange column	0.015	0.029	0.014	0.011	0.018	–	0.061	–	–	
SBSE–HPLC–ICP-MS	0.065		0.020	0.013	0.014	0.013	0.011	0.015	0.014	This work

Table 5
Analytical results for arsenic species in spiked real sample.

Samples	Analytes	Added (μg g^{-1})	Determined (μg g^{-1})	Added (μg g^{-1})	Determined (μg g^{-1})	Recovery (%)
Chicken liver	Inorganic As	0	0.035 ± 0.014	1.350	1.540 ± 0.381	111.5
	MMA	0	nd	1.350	1.098 ± 0.019	81.3
	DMA	0	0.108 ± 0.030	1.350	1.532 ± 0.157	105.5
	P-ASA	0	nd	0.675	0.718 ± 0.071	106.4
	4-OH	0	nd	0.675	0.757 ± 0.001	112.1
	3-NHPAA	0	nd	0.675	0.635 ± 0.058	94.1
	PA	0	nd	0.675	0.657 ± 0.002	97.3
	4-NPAA	0	nd	0.675	0.550 ± 0.054	81.5
Chicken leg	Inorganic As	0	0.057 ± 0.001	1.350	1.549 ± 0.167	110.5
	MMA	0	nd	1.350	1.070 ± 0.158	79.3
	DMA	0	0.065 ± 0.015	1.350	1.690 ± 0.140	120.4
	P-ASA	0	nd	0.675	0.780 ± 0.037	115.6
	4-OH	0	nd	0.675	0.736 ± 0.077	109.0
	3-NHPAA	0	nd	0.675	0.548 ± 0.053	81.2
	PA	0	nd	0.675	0.590 ± 0.028	87.4
	4-NPAA	0	0.036 ± 0.012	0.675	0.566 ± 0.022	78.5

was found in chicken meat. The amounts of arsenic in real samples were lower than the food hygiene standards of China (500 ng g^{-1}). The recoveries for eight target arsenic species in spiked samples were in the range of 78.5–120.4%.

4. Conclusion and outlook

A novel high polar TiO_2-PPHF was synthesized by sol–gel and low temperature hydrothermal process and was employed as disposable coating for stir bar sorptive extraction of different arsenic species. This disposable TiO_2-PPHF stir bar coating inherits both the adsorption properties of TiO_2 and the toughness of PPHF, and exhibits good extraction efficiency and selectivity for different arsenic species. Compared with the commercially available SBSE coatings, the disposable TiO_2-PPHF stir bar coating is low cost, free of carry-over effect and easy to prepare in large batch, indicating a good commercialization potential. From the view of application, it is suitable for the analysis of high polar species or compounds. Besides, similar composite materials such as Al_2O_3-, ZrO_2-PPHF fiber could also be synthesized and used as extraction coatings for SBSE. The developed method of SBSE–HPLC–ICP-MS is one of the most sensitive methods for arsenic speciation and could be applied to the speciation of arsenic in various samples with complicated matrix.

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References

[1] B. Hu, Z.C. Jiang, Chromatography Hyphenated with Atomic Spectometry/Mass Spectrometry for Elemental Speciation, Science Press, Beijing, 2005, p. 6.
[2] B.P. Jackson, P.M. Bertsch, Environ. Sci. Technol. 35 (2001) 4868.

[3] J.R. Garbarino, A.J. Bednar, D.W. Rutherford, R.S. Beyer, R.L. Wershaw, Environ. Sci. Technol. 37 (2003) 1509.
[4] D.W. Rutherford, A.J. Bednar, J.R. Garbarino, R. Needham, K.W. Staver, R.L. Wershaw, Environ. Sci. Technol. 37 (2003) 1515.
[5] J.F. Stolz, E. Perera, B. Kilonzo, B. Kail, B. Crable, E. Fisher, M. Ranganathan, L. Wormer, P. Basu, Environ. Sci. Technol. 41 (2007) 818.
[6] J.C. Van Loon, Anal. Chem. 51 (1979) 1139A.
[7] C. B'Hymer, J.A. Caruso, J. Chromatogr. A 1045 (2004) 1.
[8] A.R. Roerdink, J.H. Aldstadt, J. Chromatogr. A 1057 (2004) 177.
[9] C.G. Rosal, G.M. Momplaisir, E.M. Heithmar, Electrophoresis 26 (2005) 1606.
[10] S.A. Pergantis, E.M. Heithmar, T.A. Hinners, Analyst 122 (1997) 1063.
[11] G.M. Momplaisir, C.G. Rosal, E.M. Heithmar, U.S. EPA, NERL-Las Vegas (2001) (TIM No. 01-11) available at: http://www.epa.gov/nerlesd1/chemistry/labmonitor/labresearch.htm.
[12] J.W. Hong Ding, J.G. Dorsey, J.A. Caruso, J. Chromatogr. A 694 (1995) 425.
[13] C.Z. Huang, B. Hu, Z.C. Jiang, Spectrochim. Acta Part B 62 (2007) 454.
[14] J.C. Wu, Z. Mester, J. Pawliszyn, Anal. Chim. Acta 424 (2000) 211.
[15] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcolumn Sep. 11 (1999) 737.
[16] F. Sanchez-Rojas, C. Bosch-Ojeda, J.M. Cano-Pavon, Chromatographia 69 (2009) S79.
[17] F.M. Lancas, M.E.C. Queiroz, P. Grossi, I.R.B. Olivares, J. Sep. Sci. 32 (2009) 813.
[18] A. Prieto, O. Basauri, R. Rodil, A. Usobiaga, L.A. Fernandez, N. Etxebarria, O. Zuloaga, J. Chromatogr. A 1217 (2010) 2642.
[19] L. Montero, P. Popp, A. Paschke, J. Pawliszyn, J. Chromatogr. A 1025 (2004) 17.
[20] C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, F. David, P. Sandra, J. Chromatogr. A 1094 (2005) 9.
[21] J.P. Lambert, W.M. Mullett, E. Kwong, D. Lubda, J. Chromatogr. A 1075 (2005) 43.
[22] N.R. Neng, M.L. Pinto, J. Pires, P.M. Marcos, J.M.F. Nogueira, J. Chromatogr. A 1171 (2007) 8.
[23] Y.L. Hu, Y.J. Zheng, F. Zhu, G.K. Li, J. Chromatogr. A 1148 (2007) 16.
[24] W. Guan, Y.J. Wang, F. Xu, Y.F. Guan, J. Chromatogr. A 1177 (2008) 28.
[25] X.L. Zhu, Q.S. Zhu, J. Appl. Polym. Sci. 109 (2008) 2665.
[26] X.J. Huang, N.N. Qiu, D.X. Yuan, B.L. Huang, Talanta 78 (2009) 101.
[27] C.H. Yu, B. Hu, J. Sep. Sci. 32 (2009) 147.
[28] X.L. Pu, Z.C. Jiang, B. Hu, J. Mass Spectrom. 41 (2006) 887.
[29] J. Vercauteren, C. Peres, C. Devos, P. Sandra, F. Vanhaecke, L. Moens, Anal. Chem. 73 (2001) 1509.
[30] A. Prieto, O. Zuloaga, A. Usobiaga, N. Etxebarria, L.A. Fernandez, C. Marcic, A. de Diego, J. Chromatogr. A 1185 (2008) 130.
[31] J.K. Duan, X. Li, C.H. Yu, B. Hu, J. Anal. At. Spectrom. 24 (2009) 297.
[32] C.Z. Huang, Z.C. Jiang, B. Hu, Talanta 73 (2007) 274.
[33] C.Z. Huang, B. Hu, M. He, J. Duan, J. Mass Spectrom. 43 (2008) 336.
[34] L. Xu, H.K. Lee, Anal. Chem. 79 (2007) 5241.
[35] I. Pizarro, M. Gomez, C. Camara, M.A. Palacios, Anal. Chim. Acta 495 (2003) 85.
[36] Y.H. Yue, Z. Gao, Chem. Commun. (2000) 1755.
[37] C.Y. Jing, X.G. Meng, S.Q. Liu, S. Baidas, R. Patraju, C. Christodoulatos, G.P. Korfiatis, J. Colloid Interf. Sci. 290 (2005) 14.
[38] S.A. Pergantis, W. Winnik, D. Betowski, J. Anal. At. Spectrom. 12 (1997) 531.