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Poly(acrylamide-vinylpyridine-*N*,*N*′-methylene bisacrylamide) monolithic capillary for in-tube solid-phase microextraction coupled to high performance liquid chromatography

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Abstract

In-tube solid-phase microextraction (SPME) based on a poly(acrylamide-vinylpyridine-*N*,*N*′-methylene bisacrylamide) monolithic capillary was investigated and on-line coupled to HPLC for the determination of trace analytes in aqueous samples. The polymer monolith was conveniently synthesized in a fused silica capillary by in situ polymerization method. Several groups of analytes including non-steroidal anti-inflammatory drugs, phenols, non-peptide angiotensin II receptor antagonists and endocrine disrupting chemicals were extracted by the monolithic capillary. High extraction efficiency was achieved for the analytes investigated and great improvement of the limits of detection were obtained in comparison to that of direct chromatographic analysis and strong hydrophobic and ion-exchange interactions between the analytes and the polymer were confirmed. The newly developed monolithic capillary showed excellent reusability and high stability under extreme pH conditions during extraction. The possibility of applying the established method to water sample analysis was also demonstrated.

Keywords: Poly(acrylamide-vinylpyridine-*N*,*N*′-methylene bisacrylamide); Monolithic capillary; In-tube SPME; Liquid chromatography

1. Introduction

Solid-phase microextraction (SPME) was introduced as a sample pretreatment method in the early of 1990s [1] and has gained wide acceptance for the determination of various kinds of analytes, including drugs, pesticides and pollutants in recent years [2–4]. Compared to traditional extraction methods such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE), SPME bears the advantages that it is solvent-free and miniaturizable, and has been combined to GC [3], HPLC in off-line [5] or on-line mode [6] and CE [7] for many application areas in the past several years. In-tube SPME-HPLC is the on-line mode of SPME coupling to liquid chromatography, where an inner-wall coated capillary is generally used as the extraction medium. Better precision compared to the off-line mode of SPME-HPLC is achieved and analysis time can also be shortened remarkably [6].

Initially, inner-wall coated capillaries for GC were directly used as the extraction medium for in-tube SPME-HPLC. However, determination of trace amount of analytes from complex sample matrices such as biological and environmental samples requires further development of the extraction capillaries so that they are able to provide high extraction efficiency. Therefore, novel coatings for extraction capillary have been introduced into in-tube SPME-HPLC, including polypyrrole coating [8–10], TiO_2-derived PDMS coating [11] and so on. Another effective way to achieve high extraction efficiency is to utilize “packed” capillaries (or tubes), including molecular imprinted polymer (MIP) particles-filled PEEK tube [12], silica monolithic column [13] and fiber-inserted PEEK tubes [14]. Due to the increase in the volume of the extraction sorbent, obvious improvement of the extraction efficiency was demonstrated.

Organic polymer monolithic materials obtained by in situ polymerization in a “mold” have generated intense research interests in the past few years [15–17]. The synthesis of such materials requires only one-step polymerization and a simple post-treatment procedure, and the porous structure and surface properties of the polymer are usually tunable [17]. Monolithic

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materials have been widely employed for separation purposes such as CEC [17] and SPE [18]. Recently, we introduced poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillary into in-tube SPME-HPLC and performed successful extraction of abuse drugs [19,20] and xanthines [21] from biological samples.

Vinylpyridine has been selected as the relatively hydrophilic monomer to modulate the surface properties of hydrophobic polymers such as poly(styrene-divinylbenzene) and thus improve their extraction capacity towards polar analytes [22–26]. Polymer materials containing vinylpyridine have also been investigated as sorbent for acidic and metallic analytes [27–29], where ion exchange and complexation interactions are involved in the extraction process. Besides, vinylpyridine is also a crucial functional monomer in the field of molecular imprinting. The distinct selectivity of the resulting imprinted polymer was achieved in many reported works and the existence of strong electrostatic interactions between the pyridyl group and the acidic analytes was demonstrated [30–32]. Therefore, extraction media containing vinylpyridine are expected to be the ideal sorbent for enrichment of acidic analytes from aqueous samples.

In the present work, we developed a poly(acrylamide-vinylpyridine-*N*,*N*′-methylene bisacrylamide) (AA-VP-Bis) monolithic capillary as the extraction medium for in-tube SPME on-line coupled to HPLC. The extraction of several groups of analytes including acidic drugs, environmental priority pollutant phenols and endocrine-disrupting chemicals was investigated.

2. Experimental

2.1. Chemicals and materials

Acrylamide (AA) was purchased from Tianjin Chemical Plant (Tianjin, China). 4-Vinylpyridine (4-VP) was obtained from Acros (Sweden). *N*,*N*′-Methylene bisacrylamide (Bis), 2,2′-azobis(2-methylpropionitrile) (AIBN), dodecanol and DMSO were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China) and were of analytical reagent grade.

Several groups of analytes selected for investigation are displayed in Fig. 1. The non-steroidal anti-inflammatory drugs (NSAIDs), ketoprofen (2-(3-benzoylphenyl) propionic acid, KEP), fenbufen (3-(4-biphenylylcarbonyl) propionic acid, FEP), and ibuprofen (2-(4-isobutylphenyl) propionic acid, IBP) were obtained from Pharmacy Administration of Hubei Province (Wuhan, China).

The phenolic compounds including phenol (Ph), 2-nitrophenol (2-NP), 3-nitrophenol (3-NP), 4-nitrophenol (4-NP) and 2,4-dinitrophenol (2,4-DNP), were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China).

The non-peptide angiotensin II receptor antagonists (ARAs) for therapy of hypertension, losartan (2-*n*-butyl-4-chloro-1-[*p*-(*o*-1H-tetrazol-5-ylphenyl) benzyl]-imidazole-5methanol monopotassium salt, LOR), valsartan ((*S*)-*N*-valeryl-*N*-[29-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl)-valine, VAL), olmesartan medoxomil (5-methyl-2-oxo-1,3-dioxolen-4-yl)

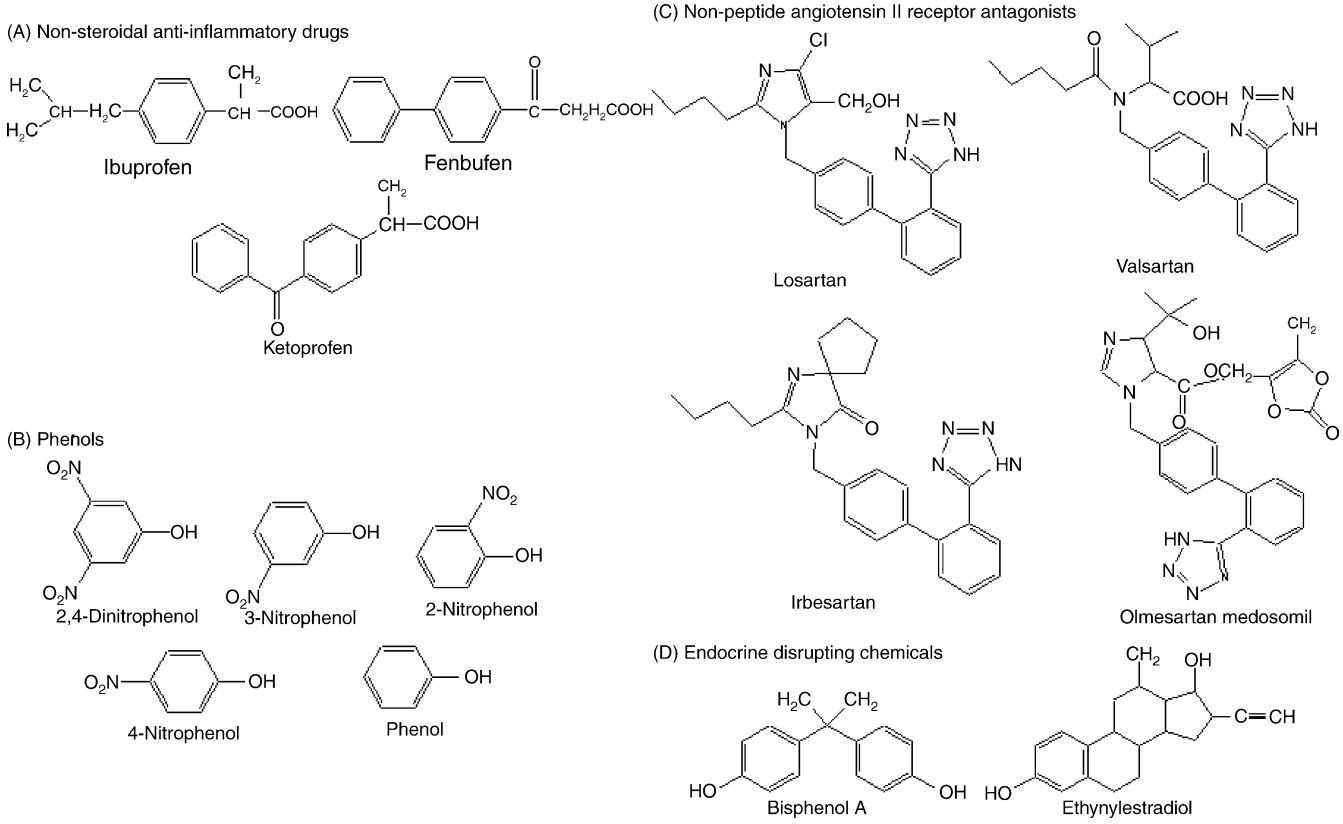

Fig. 1. Molecular structures of the analytes studied.

methoxy-4(1-hydroxy-1-methylethyl)-2-propyl{4-[2-(tetrazol-5-yl)-phenyl]phenyl} methylimidazol-5-carboxylase, OLM), and irbesartan ((2-butyl-3-[(29-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]1,3-diazaspiro[4,4]non-1-en-4-one), IRB) were obtained from Pharmacy Administration of Jiangsu Province (Nanjing, China).

The endocrine-disrupting chemicals (EDCs), bisphenol A (BPA) and ethynylestradiol (ED), were obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China) and Drug and Pharmacy Administration of China (Beijing, China), respectively.

A standard solution of 1 mg/mL for each analyte was prepared in methanol. The stock mixture standard solution of each group of analytes was prepared by diluting the standard solution to 50 μg/mL with double distilled water. Then the sample solution for extraction was obtained by diluting the mixture standard solution to the desired concentration.

2.2. Preparation of poly(AA-VP-Bis) monolithic capillary

The poly(AA-VP-Bis) monolithic capillary was synthesized inside a fused silica capillary (20 cm × 0.25 mm, i.d., Yongnian Fiber Plant, Hebei, China) by a heat-initiated polymerization method. Firstly, the capillary was derivatized with 3-(triethoxysilyl) propyl methacrylate using a procedure described previously [21]. Then, the pre-polymerization mixture consisting of monomer AA 56 mg (7.9 wt%), 4-VP 57 mg (8.1 wt%,), crosslinker Bis 63 mg (8.9 wt%), porogenic solvent DMSO 370 mg (52.3 wt%) and dodecanol 161 mg (22.8 wt%), initiator AIBN 2 mg (1 wt% of monomer and crosslinker) was filled into the capillary. The capillary was immediately sealed with silicon rubber and the reaction was initiated at 60 °C for 18 h, followed by washing with methanol to remove the unreacted component and porogenic solvent. The total length of the polymer monolith in the capillary was 15 cm, which was able to give both satisfactory extraction efficiency and adequate permeability.

2.3. Instrumental and analytical conditions

The configuration of the in-tube SPME-HPLC system is shown in Fig. 2 [21]. The whole system consisted of an extraction segment, which included a Shimadzu SIL-1A six-port valve (valve 1), a Jasco PU-1580 pump (pump A) (Jasco, Tokyo, Japan) and a PEEK tube (0.03 in. i.d., 700 μL total volume), and an analytical segment, which included a Jasco PU-1580 pump (pump B) (Jasco, Tokyo, Japan), Rheodyne 7725i six-port valve (valve 2) with a 20 μL loop (Cotati, CA, US) and a Unimicro UV-detector (Unimicro Technologies, CA, USA). The analytical column for separation of NSAIDs, phenols and EDCs was 150 mm × 4.6 mm, i.d. packed with Kromasil ODS (5 μm), which was purchased from Eka Chemicals (Bohus, Sweden). For NSAIDs, the mobile phase consisted of 25% 0.025 mol/L of Na_2HPO_4 solution at pH 4.5 and 75% methanol in volume ratio; the detection was performed at 223 nm. For phenols, the mobile phase consisted of 50% 0.02 mol/L of NaAc buffer solution at pH 4.5 and 50% methanol in volume ratio; the detection was performed at 280 nm. EDCs were separated with a mobile phase consisting of 30% 0.025 mol/L of Na_2HPO_4 solution at pH 4.5 and 70% methanol in volume ratio; the detection was performed at 220 nm. As to the chromatographic separation of ARAs, the analytical column was a Betasil C18 column (200 mm × 4.6 mm i.d.; 5 μm) (Elite, Dalian, China). The mobile phase consisted of 55% of HAc (0.2%, v/v) and 45% acetonitrile in volume ratio; the detection was performed at 250 nm. The flow rate for all the separation was set to 1 mL/min.

The extraction procedure has been described previously [21]. In brief, valve 2 was initially set to LOAD position, and the mobile phase was driven by pump B to directly flow through the analytical column to obtain a stable baseline in preparation for chromatographic separation. Before extraction, valve 1 was switched to LOAD position and the carrier solution, water for instance, was driven by pump A to flow through the capillary for conditioning at 0.04 mL/min. At the same time, the PEEK tube was filled with the sample solution with a syringe. When

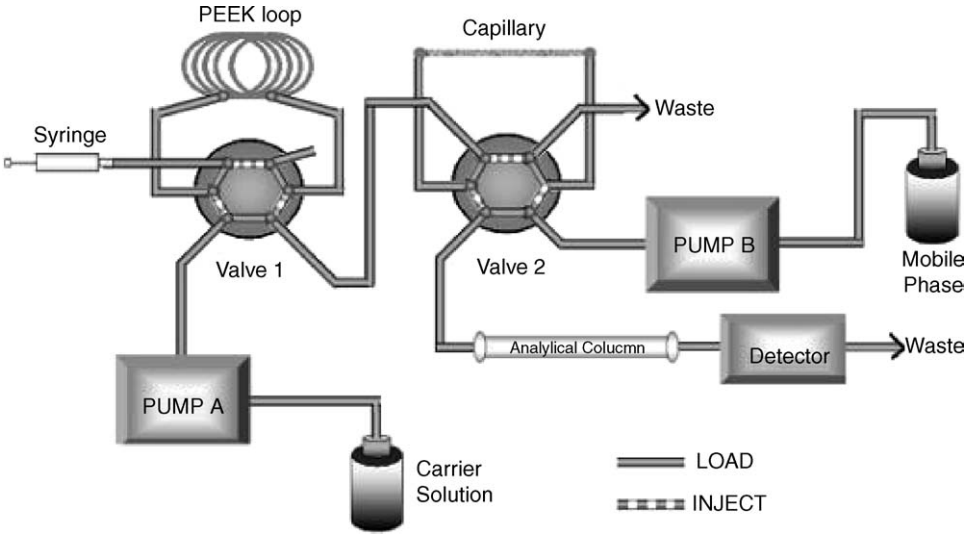

Fig. 2. Construction of in-tube SPME-HPLC.

extraction began, valve 1 was directed to INJECT position to perform extraction for a given time interval and returned to LOAD position immediately thereafter. Thus, the volume of the sample solution through the monolithic capillary could be calculated accurately from the flow rate and the valve 1 switching time interval. Then the extracted analytes were desorbed from the monolithic capillary to the analytical column with the mobile phase at a flow rate of 0.02 mL/min for 5 min by simply switching valve 2 to INJECT position, followed by adjustment of the flow rate of mobile phase to 1 mL/min for separation.

Scanning electron microscopy (SEM) was performed with a KYKY-EM3200 instrument (KYKY Technology Development Ltd., Beijing, China). The elemental analysis was performed with MOD-1106 elemental analyzer (Carlo Erba, Italy). A FTIR 8000 Series instrument (Shimadzu, Japan) was used for Fourier transform infrared spectroscopy. The measurement of pore size of the monolithic capillary was carried out with a Coulter SA 3100 plus surface area and pore size analyzer (Beckman, USA).

3. Results and discussion

3.1. Characterization of poly(AA-VP-Bis) monolithic capillary

The poly(AA-VP-Bis) monolithic capillary could be obtained conveniently by in situ radical polymerization method. The resulting polymer monolith was taken for FT-IR characterization with the spectra shown in Fig. 3. The band at 3200–3600 cm^{-1} and 1668 cm^{-1} were characteristic of the N–H and C=O stretching frequency of the acrylamide, respectively, and the band at 1590 cm^{-1} was indicative of the presence of pyridyl groups. The C%, N%, and H% (wt) of the polymer monolith were determined by elemental analysis as 56.4, 13.2 and 6.0%, respectively. Comparing to the theoretical nitrogen percentage of the polymer monolith based on the feed composition, the pyridyl content in the polymer monolith was somewhat higher than the theoretical value. Fig. 4 shows the morphology of the resulting monolithic capillary observed by SEM. From Fig. 4, the interconnected skeletons and interconnected textural pores of the monolith can be easily observed; and it is also

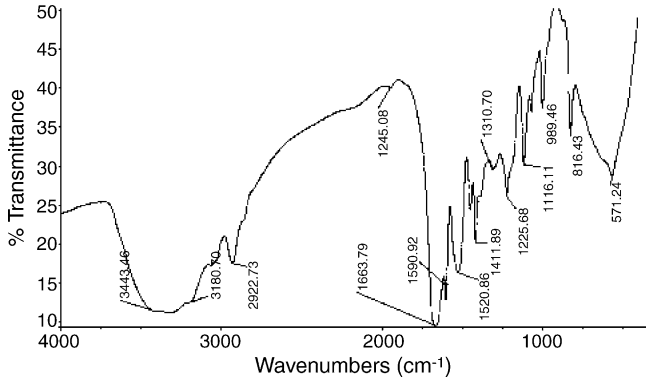

Fig. 3. FT-IR spectra of poly(AA-VP-Bis) monolithic capillary.

obvious that the monolith was attached tightly to the inner-wall of the capillary. The average skeleton pore size of the monolith determined by N_2 sorption method was 3.3 nm.

Compared to the preparation of the inner-wall coated capillaries, the synthesis of monolithic capillary was simpler and convenient. The column-to-column reproducibility was also evaluated by calculating the relative standard deviation (RSD) of the extracted amount while performing the extraction of BPA from aqueous sample. The results showed that RSD of 1.6% ($N = 5$) could be achieved when performing extraction with independently prepared monolithic capillaries, which demonstrated the robustness of the preparation method of the monolithic capillaries.

3.2. In-tube SPME with poly(AA-VP-Bis) monolithic capillary

In order to assess the extraction capacity of the poly(AA-VP-Bis) monolithic capillary, several groups of analytes, mostly bearing acidic functional groups, were investigated. After extraction, the desorption of the extracted analytes into the analytical column could be achieved by simply directing the mobile phase to flow through the capillary. A blank sample was taken for extraction after performing SPME of the spiked sample and no analyte peaks were found. This result indicated that the mobile phase could completely elute the extracted analytes from the

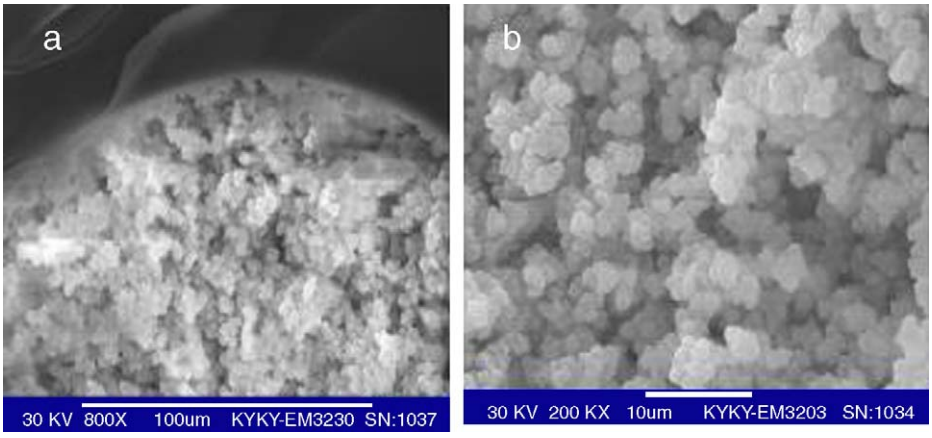

Fig. 4. Scanning electron microscopy images of poly(AA-VP-Bis) monolithic capillary. (a) Wide-view and (b) close-up-view.

Table 1
In-tube SPME of analytes from aqueous samples with poly(AA-VP-Bis) monolithic capillary

Compound		Extraction amount (ng)	Extraction yield[a] (%)	Enrichment factor[b]	RSD[c] (%)
NSAIDs	KEP	284	71	64.2	2.3
	FEP	274	69	62.0	1.7
	IBP	263	66	59.6	4.2
Phenols	Ph	26	6	5.7	23
	2-NP	215	54	48.7	5.9
	3-NP	108	27	24.5	2.4
	4-NP	326	82	73.8	1.2
	2,4-DNP	347	87	78.6	5.6
ARAs	LOR	370	93	83.9	2.8
	VAL	344	86	77.9	1.7
	OLM	264	66	59.8	0.6
	IRB	381	95	86.2	1.9
EDCs	BPA	255	64	57.7	0.4
	ED	161	40	36.6	1.6

[a] The extraction yields (%) are the percentages of extracted amount of the analytes per initial amounts of the analytes in the sample solution passing through the monolithic capillary.
[b] Enrichment factor is calculated from the ratio of the peak area obtained with in-tube SPME to that without preconcentration, i.e. the direct injection of the same sample volume as equal to the void volume of the extraction capillary.
[c] RSDs are calculated for the extracted amount of the analytes. The analytes were spiked in water at a concentration of 1 μg/mL.

capillary. Therefore, the amount of the analytes extracted by the monolithic capillary and the extraction yields, which represented the extraction efficiency, could be calculated directly from the chromatographic results [33], as listed in Table 1.

The preconcentration capacity of the poly(AA-VP-Bis) monolithic capillary toward the selected analytes could be evaluated by the enrichment factor [34], which was calculated from the ratio of the peak area obtained with in-tube SPME to that by direct injecting the sample solution (the injecting volume is equal to the void volume of the extraction capillary). The results are listed in Table 1. It is obvious that most of the selected analytes can be extracted by poly(AA-VP-Bis) monolithic capillary with high extraction efficiency. Fig. 5 presents chromatograms illustrating in-tube SPME and direct injection (20 μL) of the sample solution with NSAIDs spiked at 1 μg/mL. Great enhancement in the peak height is achieved, indicating that much lower limits of detection can be achieved for these analytes by the proposed in-tube SPME method.

The extraction time profiles of phenols were constructed in order to further evaluate the extraction ability of the poly(AA-VP-Bis) monolithic capillary for these analytes. By extracting 1 μg/mL of sample solutions for progressively longer periods of the extraction time from 2 to 23 min, i.e. valve 1 switching time interval, the profiles were obtained and shown in Fig. 6: similar trends of the profiles were found for 2,4-DNP, 2-NP and 4-NP and the extraction equilibrium was not achieved within 23 min of extraction time, indicating that the poly(AA-VP-Bis) monolithic capillary exhibited strong extraction capacity for these analytes. However, the extraction equilibrium for 3-NP was reached at 10 min of extraction time, suggesting that the retention of 3-NP in the monolithic capillary was weaker than that of the other nitrophenols. Taking the results here and the enrichment factor data listed in Table 1 into account, we can find out that the most acidic analyte 2,4-DNP exhibited the greatest enrichment; while and the weakest acidic analyte 3-NP exhibited smallest extraction yield. It is also noticeable that a slight decrease in extraction efficiency for 3-NP was found when prolonging the extraction time to over 10 min, which was indicative of the occurrence of the competitive adsorption.

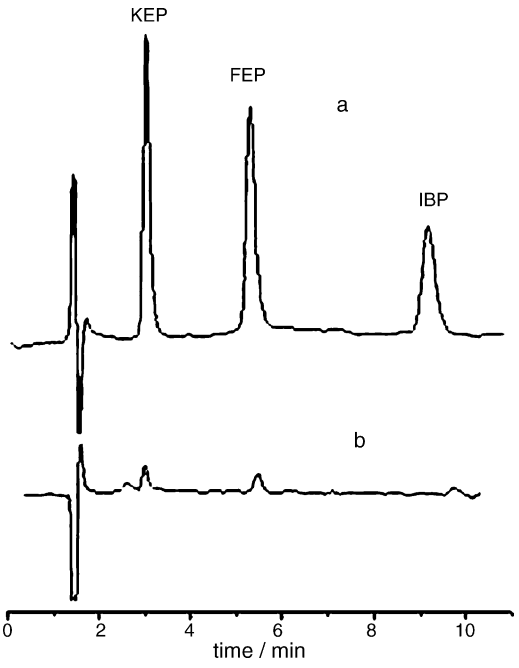

Fig. 5. Chromatograms of NSAIDs obtained by (a) in-tube SPME with monolithic capillary and (b) direct injection of standard sample solution (20 μL). The analytes were spiked at 1 μg/mL. In-tube SPME conditions: extraction time, 10 min; flow rate, 0.04 mL/min. The mobile phase consisted of 25% 0.025 mol/L of Na_2HPO_4 solution at pH 4.5 and 75% methanol in volume ratio. The flow rate for separation was 1 mL/min. The detection was performed with a UV detector at 223 nm.

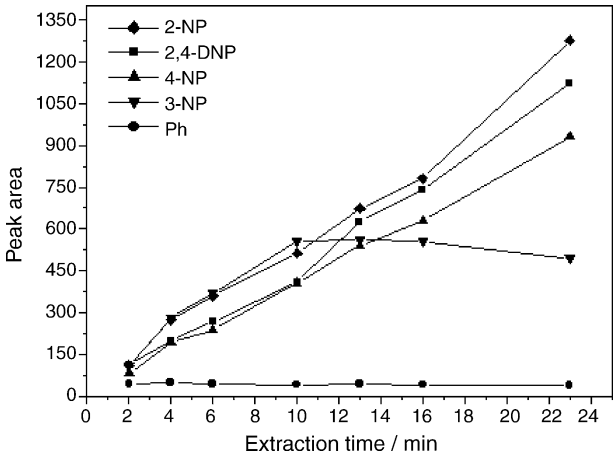

Fig. 6. Extraction time profiles for phenols. The analytes were spiked at 1 μg/mL. The extraction flow rate was 0.04 mL/min. The mobile phase consisted of 50% 0.02 mol/L of NaAc buffer solution at pH 4.5 and 50% methanol in volume ratio. The flow rate for separation was 1 mL/min. The detection was performed with a UV detector at 280 nm.

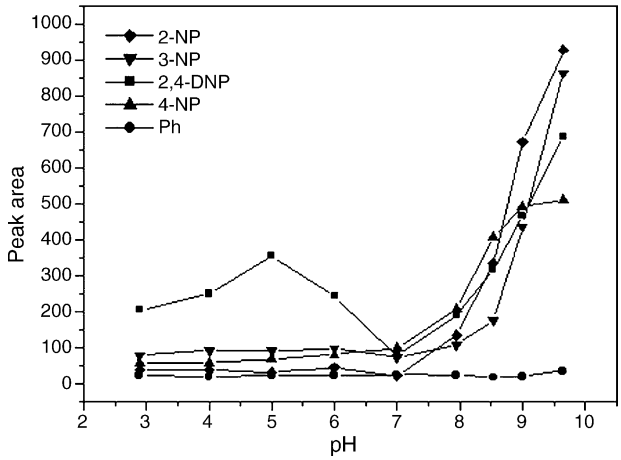

Fig. 7. The relationship between sample pH and the extraction efficiency (represented by peak area) of phenols. The analytes were spiked at 1 μg/mL, and the sample matrix consisted of 0.01 mol/L PBS for pH 2–7 and Tris for pH above 7. In-tube SPME was performed for 10 min at a flow rate at 0.04 mL/min; the HPLC conditions were the same as depicted in Fig. 6.

Besides, the monolithic capillary almost showed no extraction capacity for phenol, which could also be attributed to its weak acidic character and low hydrophobicity.

ARAs could also be extracted with high extraction efficiency by the poly(AA-VP-Bis) monolithic capillary, as shown in Table 1. The tetrazol-5-yl-phenyl benzyl functional group they had in common provided the acidic center to interact with the monolithic capillary. However, the enrichment factor order of the four analytes, IRB > LOS > VAL > OLM, was not in accordance with that of their p*K*a values, LOR ≈ VAL < OLM < IRB. This result implied that the hydrophobic interaction also contributed remarkably to the retention of the ARAs because IRB bearing a hydrophobic group, diazaspiro[4,4]non-1-en-4-one, exhibited the greatest extraction yield. High extraction efficiency of the neutral analytes, BPA and ED, as shown in Table 1, confirmed that hydrophobic interaction did play an important role in the SPME process.

The precision for performing in-tube SPME with the poly(AA-VP-Bis) monolithic capillary was also evaluated by calculating the RSD values for the extracted amount of the analytes. As can be seen from Table 1, the precision is acceptable for quantification analysis, suggesting that the presented method is promising in practical analysis.

3.3. In-tube SPME of analytes from different sample matrix

In order to further understand the extraction process and improve the extraction performance of the monolithic capillary for the selected analytes, the influence of the sample matrices on the extraction efficiency was investigated.

When performing the extraction of NSAIDs, phenols and ARAs from the sample matrices with the pH ranging from 2 to 7, the variations of the extraction efficiency for these analytes were found to be similar, i.e. the highest extraction efficiency was obtained at about pH 5 while lower or higher pH values led to the decrease in the extraction efficiency, suggesting that ion-exchange interaction was involved in the extraction process [20,35] due to the pyridyl groups as the ion-exchange sites [36,37].

When the pH of the sample matrix increased to above 7 (buffered with Tris), unexpected increase in the extraction efficiency for NSAIDs and nitrophenols were observed. For nitrophenols, as depicted in Fig. 7, the extraction efficiency obtained at pH 9–10 was even higher than that at pH 5, suggesting that Tris might act as a cooperative agent to help in enhancing the hydrophobic interaction between the analytes and the monolithic capillary. This was proved by the fact that the extraction efficiency for nitrophenols decreased when substituting the organic buffer by borate buffer solution. For ARAs, only a slight increase in the extraction efficiency with the increase of the pH was found for LOS, VAL and IRB, as shown in Fig. 8, suggesting

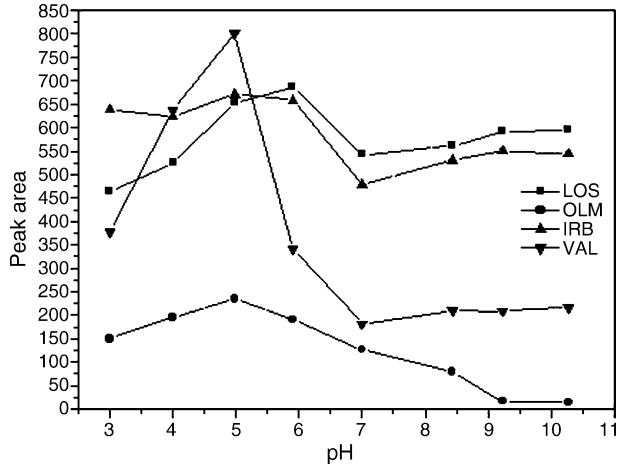

Fig. 8. The relationship between sample pH and the extraction efficiency (represented by peak area) of ARAs. The analytes were spiked at 1 μg/mL, and the sample matrix consisted of 0.01 mol/L PBS for pH 2–7 and Tris for pH above 7. In-tube SPME was performed for 10 min at a flow rate at 0.04 mL/min. The mobile phase consisted of 55% of HAc (0.2%, v/v) and 45% acetonitrile in volume ratio. The flow rate for separation was 1 mL/min. The detection was performed with a UV detector at 250 nm.

that the hydrophobic interaction was dominant in this pH range. The extraction efficiency of OLM was found to decrease with increase of the pH value due to hydrolysis. The results clearly indicated that the extraction efficiency for acidic analytes could be greatly increased by selecting appropriate buffering system.

When the neutral analytes BPA and ED were extracted from the sample matrices with different pH, as reported in our other recent work [38], no fluctuation of the extraction efficiency was found in the pH range of 2–8, while a steep decrease was found when pH increased to above 9. This could be attributed to the deprotonation of the molecules, which led to electrostatic repulsion between the analytes and the monolithic capillary and thus weakened the interactions between the analytes and the monolithic capillary.

The development of high pH-resistant sorbent is thought to be necessary since high pH of sample matrix might satisfy the extraction or desorption requirements [11]. In our experiment, in order to evaluate the stability of the poly(AA-VP-Bis) monolithic capillary, it was rinsed with 0.01 mol/L of NaOH solution and 0.01 mol/L PBS at pH 12–13, respectively, up to 1000 times of the void volume. The damage of the capillary was not found and the RSDs of the extracted amount of phenols and EDCs before and after rinsing were smaller than 5.4 and 4.6%, respectively, which demonstrated that the capillary was stable under extreme high pH conditions.

3.4. Application to water sample analysis

The in-tube SPME-HPLC system based on the poly(AA-VP-Bis) monolithic capillary was applied to the analysis of phenols from real water samples. Before SPME, only a centrifugation step is needed in order to remove the particle components.

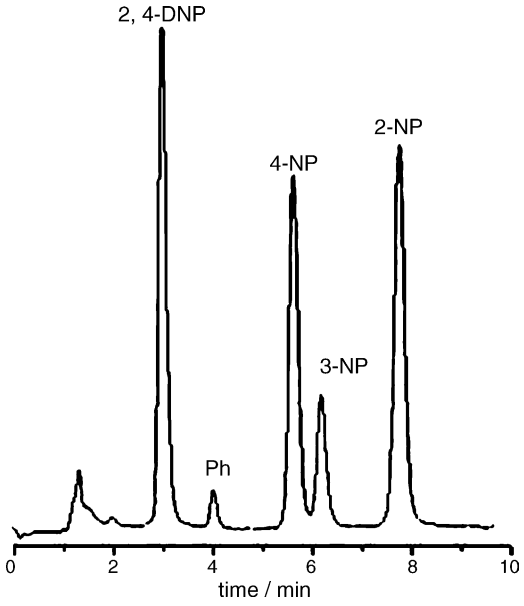

Fig. 9. In-tube SPME of phenols from spiked lake water sample. 2,4-DNP, 2-NP, 3-NP, and 4-NP were spiked at 1 μg/mL. Phenol was spiked at 4 μg/mL. Extraction was performed for 10 min at a flow rate at 0.04 mL/min; HPLC conditions were the same as depicted in Fig. 6.

Phenols were extracted from spiked lake water sample (Donghu Lake, Wuhan) and the chromatogram is depicted in Fig. 9. No decrease in extraction efficiency was found on extracting 2,4-DNP, 2-NP, and 4-NP from the spiked lake sample and the peak area reproducibility achieved was found to be 1.4, 2.7, and 1.0%, respectively. However, the extraction recovery of 3-NP decreased to 40% of that obtained by extracting standard sample and the RSD of the peak area was 10%, which could be attributed to the strong competition effect originated from the sample matrix. In the spiked concentration range of 50–2500 ng/mL, good linearity was obtained for in-tube SPME of the phenols with the correlation coefficient values better than 0.998 and the limits of detection (S/N = 3) for 2,4-DNP, 4-NP, 3-NP and 2-NP were found to be 4, 6, 16 and 5 ng/mL, respectively.

The poly(AA-VP-Bis) monolithic capillary was demonstrated to be robust since its extraction performance did not appear to deteriorate and abnormal backpressure fluctuation was not found after hundreds of real water sample extractions. It clearly indicates that the poly(AA-VP-Bis) monolithic capillary method is promising for application to real water sample analysis.

4. Conclusions

A poly(acrylamide-vinylpyridine-*N*,*N*′-methylene bisacrylamide) monolithic capillary was synthesized and used as an extraction medium for in-tube SPME-HPLC system. Great improvement in the limits of detection for several groups of acidic and neutral analytes were obtained, which could be attributed to the greater sorbent loading amount than that used in open-tubular capillaries and convective mass transfer provided by the monolithic capillary. Remarkable enrichment factors were achieved for acidic analytes and the ion exchange and hydrophobic interactions were found dominant in the extraction process.

Since in-tube SPME is a non-exhaustive extraction method based on equilibrium sampling, it is able to make use of more kinds of extraction media in comparison to other micro extraction methods such as micro SPE and thus provides us with more versatile sample pretreatment tools. With polymer monolithic capillary as the extraction medium, the established in-tube SPME method will be very attractive because it provides higher extraction efficiency and more convenient manipulation. Although the extraction of a limited number of analytes were reported in the present work, the poly(AA-VP-Bis) monolithic capillary is able to efficiently extract more kinds of acidic and neutral analytes from environmental and biological samples. The excellent stability and highly reproducible extraction efficiency of the monolithic capillary are expected to make it a promising sorbent for in-tube SPME, which might also encourage people to introduce more kinds of organic polymer monolithic materials into SPME.

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