Talanta 99 (2012) 270–276

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Molecularly imprinted solid phase microextraction fiber for trace analysis of catecholamines in urine and serum samples by capillary electrophoresis

Xinfeng Zhang [a,b], Shuxia Xu [a,b], Jae-Min Lim [b], Yong-Ill Lee [b,*]

[a] Mineral Resources Chemistry Key Laboratory of Sichuan Higher Education Institutions, College of Material and Chemistry & Chemical Engineering, Chengdu University of Technology, Chengdu, Sichuan 610059, China

[b] Anastro Laboratory, Department of Chemistry, Changwon National University, Changwon 641-773, Republic of Korea

ARTICLE INFO

Article history:
Received 14 March 2012
Received in revised form
22 May 2012
Accepted 23 May 2012
Available online 7 June 2012

Keywords:
Molecularly imprinted polymer fiber
Capillary electrophoresis
Solid phase miroextraction
Dapamine
Epinephrine
Norepinephrine

ABSTRACT

A selective and flexible monolithic moleculary imprinted polymer (MIP) fiber was developed in batch for solid phase microextraction (SPME) of catecholamines (CAs), i.e., dopamine (DA), epinephrine (E) and norepinephrine (NE), and coupled with capillary electrophoresis (CE) for trace analysis of urine and serum samples. The polymer fiber was synthesized *in-situ* simply using a flexible capillary as a mold and the polymerization protocols and SPME experimental conditions were examined in detail. The reproducibility of fiber to fiber fabrication ($n=5$) was in range of 5.9–9.8% for three CAs. The fiber also shows high stability without any deterioration of extraction performance after 30 times use. Under the established optimum conditions, the limits of detection for DA, E, and NE were 7.4, 4.8, and 7.1 nmol L^{-1}, respectively, with the enhancement factor over 100 after MIP-SPME. The specific selectivity to three CAs was discovered with the developed MIP fibers compared with non-imprinted polymer (NIP) fiber. Finally, the MIP fibers were successfully applied for selective extraction of CAs in urine and serum samples with the relative recoveries ranging from 85% to 103%. The fabricated MIP-fibers were promising in preparation of biological samples in batch followed by CE–UV detection.

1. Introduction

Catecholamines (CAs), such as epinephrine (E), norepinephrine (NE) and dopamine (DA), are a group of small molecules generated in nerve tissue and adrenal glands. They are known to be correlated with stress, heart disease, neurological disorders, pheochromacytoma and cancerous tumors [1,2]. For example, the CAs level in urine may provide useful information for diagnoses of Parkinson disease, schizophrenia and so on. Therefore, the precise determination of CAs level in urine, serum and other body fluids is very essential for the clinical diagnostics.

In recent years, capillary electrophoresis (CE) has become a very important technique for simultaneous analysis of CAs because of its remarkably high separation efficiencies, short analysis time and relatively simple instrumentation [3–6]. The major drawback of CE technique, in case of UV detection, is relatively low sensitivity in concentration of target analytes, compared to high-performance liquid chromatography (HPLC) due to the short optical length. Meanwhile, the complex biological samples usually need a cleanup step prior to CE detection. Thus, a preconcentration as well as cleanup technique are always important essentially for CE-UV analysis.

Sample preparation procedures such as solid-phase extraction (SPE) [7–11] and liquid–liquid extraction (LLE) [12,13] have been most frequently used for the determination of CAs. However, these methods require large volumes of organic solvents or time-consuming operation. Recently, newly developed sample pretreatment methods including single drop microextraction [14], microchip-based extraction [15], and solid-phase microextraction (SPME) [16–18] have been reported for extraction of CAs in complicated matrices. Among these methods, SPME is considered to be advantageous because the integration of sampling and sample preparation steps, in conjunction with extraction, preconcentration, and matrix removal in SPME, provides the merits of simplicity, convenience, and high preconcentration capacity. Several coating materials such as boronate [16], slica-C_8 [17], and carbowax-templated resin [18] have been employed to achieve optimal performance for extraction of CAs. However, the main problem associated with these materials is lack of selectivity, preventing the determination of CAs at trace levels especially in complex biological samples.

Molecularly imprinted polymers (MIP) acclaimed as artificial antibody has been known for the high selectivity to the template molecules, and characterized with excellent chemical stabilities, easy preparation and long lifetime [19]. The

* Corresponding author. Tel.: +82 55 213 3436; fax: +82 55 213 3439.
E-mail address: yilee@changwon.ac.kr (Y.-I. Lee).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.05.050

combination application of MIP with SPME was considered to be very promising for sample preparation [20–24]. Although MIP has been shown as important materials for sensing and separation of CAs [25–27], to the best of our knowledge, no research has been carried out for the extraction of CAs by using the MIP fibers.

The aim of this work is to develop a novel monothilic MIP fiber for extracting CAs and then to couple it with CE for the quantitative analysis of urine and serum samples. The MIP fiber was fabricated by thermally induced polymerization within a 25 cm flexible capillary mold. After completing the reaction, the capillary was cut into 3 cm and the polymer was entirely pushed out for complete removal of template. Then, the fiber was inserted back to the capillary mold with remaining 1 cm of the fiber outside the capillary for extraction. Such a strategy makes the preparation of MIP fiber in batch mode very easy. The remarkable features of the prepared fibers are monolithic and flexible enough for a long life-span use. More importantly, the capillary mold makes the prepared fibers uniform in diameter which can ensure the reproducibility during batch processing of samples.

2. Experimental

2.1. Materials

All the chemical reagents were at least of analytical grade. DA, E and NE standard were purchased from Sigma–Aldrich (St. Louis, MO, USA). The stock solutions of analytes, 10 mmol L^{-1} DA, E, and NE were prepared in methanol and kept at 4 °C. The 25 mmol L^{-1} borate solution in pH 9.00 was used as running buffer. NaOH and HCl solutions (both 1 mol/L) were used to adjust pH to the desired value. Ethylene dimethacrylate (EDMA), methacrylic acid (MAA) and azo(bis)-isobutyronitrile (AIBN) were also obtained from Sigma–Aldrich. Acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF), methanol, ethanol, acetone, chloroform, diethyl ether and benzene were purchased from Daejung Chemical & Metals Co. (Gyeonggi-do, South Korea). Purified water prepared by Milli-Q water purification system of Millipore (Daihan Labtech Co., Gyeonggi-do, South Korea) was used during the experiments.

2.2. Instrumental

All the experiments were carried out using a self-assembled CE instrument, which consists of a high power supply system (CZEPN10, High voltage Electronic Co., USA), an UV detector (Sappire 800 CE, Ecom Ltd., USA), and a Labview data collecting system (Labview 7.1, National instruments, Korea). An uncoated 75 μm (i.d.) silica capillary (Hebei Reafine Chromatography LTD., Hebei, China) with a total length of 55 cm and effective length of 47 cm was used for separation of analytes. The detection wavelength was set at 210 nm.

2.3. Preparation of MIP fiber

The MIP was prepared through the thermally induced radical coplymerization of MAA and EDMA using E as a template in a capillary mold. First, 0.12 mmol E was dissolved in 0.4 mL mixture of DMF and ACN (1:1, V/V) in a 1.5 mL tube, and then 0.3 mmol MAA, 1.8 mmol EDMA, and 3.0 mg AIBN were added to prepare for a prepolymer solution. After being sonicated and degassed by a stream of nitrogen gas for 5 min, the solution was filled in a 25 cm long capillary i.d., (530 μm, Hebei Reafine Chromatography LTD., Hebei, China) with the help of a syringe. Then, both ends of the capillary were closed with two small pieces of rubber, and allowed the polymerization reaction of the filled solution in a 60 °C water bath for 12 h. After accomplishing polymerization, the capillary was cut into 3 cm. The produced polymer fiber was entirely pushed out from the capillary mold using an o.d. 360 nm silica fiber after being dried in 40 °C oven for 30 min. Sequentially, the polymer fibers were immersed in the mixture of methanol: acetic acid (1:1, V/V) to remove the templates by ultrasonication. This procedure was repeated for several times until no CE signal was observed for blank solution. Then, the polymer fiber was inserted back to the capillary mold and left 1 cm outside the capillary for extraction. Only 1 cm part of the polymer fiber was immersed in sample solutions during SPME process. The non-imprinted polymer (NIP) fibers were also prepared as described above without the addition of a template. The morphological evaluation of the obtained fibers was performed by a field emission scanning electron microscope (FESEM, Tescan Mira, USA).

2.4. SPME procedures

Three milliliter of standard or sample solutions in ACN was added into a 5 mL glass vial, and the MIP fiber was fixed by inserting it into a rubber cap which can cover the mouth of glass vial. When the extraction process was preceded, only 1 cm part of the MIP polymer fiber was immersed into the solutions for 60 min to extract analytes at the stirring rate of 300 rpm of magneton. Then, the fiber was pulled out and washed with 1.5 mL of ACN for 5 min under low stirring to decrease nonspecific bindings and clean up the sample matrix. Subsequently, the target analytes in polymer fiber were desorbed by immersing the fiber in 180 μL of the mixture of acetone: acetic acid (7:3, V/V) for 15 min. The desorption process was accomplished by pulling out the MIP fiber. The solutions were dried with a gentle nitrogen gas and the residuals were redissolved in 5 μL running buffer solution for CE analysis. Before extraction, fibers were reconditioned under the mixture of acetone: acetic acid (7:3, V/V) and ACN for 10 min in sequence.

2.5. CE procedures

Before the first use, the capillary was rinsed with 0.1 mol L^{-1} NaOH for 30 min. Subsequently, 0.1 mol L^{-1} HCl, double distilled water, and running buffer solution for 10 min were used for further clean up and reconditioning, respectively. Prior to each run, the capillary was flushed with 0.1 mol L^{-1} NaOH for 2 min. and with a running buffer solution for 2 min. The CE conditions in this work were as follows: (1) the running buffer was 20 mM borate buffer with pH 9.0; (2) The separation voltage was set at 14 kV; and (3) The sample solution was injected by raising the inlet end of the capillary 15 cm higher than the outlet end for 10 s.

2.6. Sample preparation

Human urine samples were donated by two drug-free and healthy volunteers and the human serum samples were purchased from Sigma–Aldrich. A 3-mL volume of human urine or serum sample was adjusted to pH 7 with 1 mol L^{-1} NaOH. Then, 3 mL of ACN was added to the samples to precipitate proteins. The sample solution was centrifuged at 6000 rpm for 10 min to remove the precipitates. The supernatant of urine and spiked serum sample was evaporated to dryness on a rotary evaporator. The analytes in the residuals were extracted with 3 mL of ACN under ultrasonication for 10 min. After being filtered through 0.45 μm membrane filter, the sample solution was used for SPME extraction.

3. Result and discussion

3.1. Preparation of MIP fiber

After completing the polymerization in capillary mold, the MIP fiber was fabricated by two methods: (I) pushed out 1 cm of the polymer fiber and then removed the template directly; and (II) withdrew the entire polymer (Fig. 1A) out to remove all templates in fiber by ultrasonication and subsequently inserted the fiber back to the mold with 1 cm of the fiber outside the capillary for extraction, as shown in Fig. 1B. Because of the simplicity of method (I), it was adapted first, but the results showed that the template leakage was serious during extraction process probably due to the impediment for removing all templates in fiber of the capillary mold. In contrast, no template leakage was found using fibers prepared by method (II) and high extraction efficiency was observed. The prepared fiber was flexible and not fragile. With a 25 cm capillary mold, eight MIP fibers can be fabricated at a time and uniform diameters in fibers were perceived. Thus method (II) was chosen to prepare reproducible MIP fiber in batch.

3.2. Optimization of MIP preparation conditions

The properties of MIP fiber depend on the composition of polymer (i.e., polymerization solvent, template, function monomer, cross-linker) and polymerization time. The CE detection signals were utilized to investigate the effects of these factors on SPME extraction efficiency. The choice of solvents for polymerization which has influenced on solubility of template, polymer morphology, and extraction reproducibility, is an important factor for molecular recognition. Using ACN as a solvent, it was recognized that the template cannot be dissolved at all. The subsequent optimization was processed using methanol, DMF, and the mixture of DMF and ACN (5:5, V/V) as solvents. As shown in Fig. 2, the extraction ability was proved to be the best when the fiber was prepared in the mixture of DMF and ACN.

The selection of template molecules is very important to molecular imprinting. 0.12 mmol DA, E, and NE were used separately as templates to prepare MIP fibers with the mixture of DMF and ACN as a solvent. The results showed that DA and NE could not be dissolved easily, whereas E was dissolved completely in the present condition. Hence, E was selected as the imprinting template. The molar quantity of template molecule was investigated from 0.03 mmol to 0.24 mmol by keeping the molar ratio of function monomer and cross linker at 1:6 and polymerization time of 24 h. The higher concentration of template molecule always led to better extraction efficiency because of more active imprinted sites. However, the 0.24 mmol template was found to be insoluble in the used solvent. The effects of molar ratio of MAA and EDMA were also studied from 1:3 to1:12 by holding constant the concentration of the template at 0.12 mmol and polymerization time of 24 h, and the fiber prepared with the molar ratio of 1:6 (MAA: EDMA) exhibited the best extraction ability. It was mainly due to the enhancement of non-homogenous polymerization and the decrease of binding sites at high molar ratio, which has been proved by the increase of the non-specific adsorption.

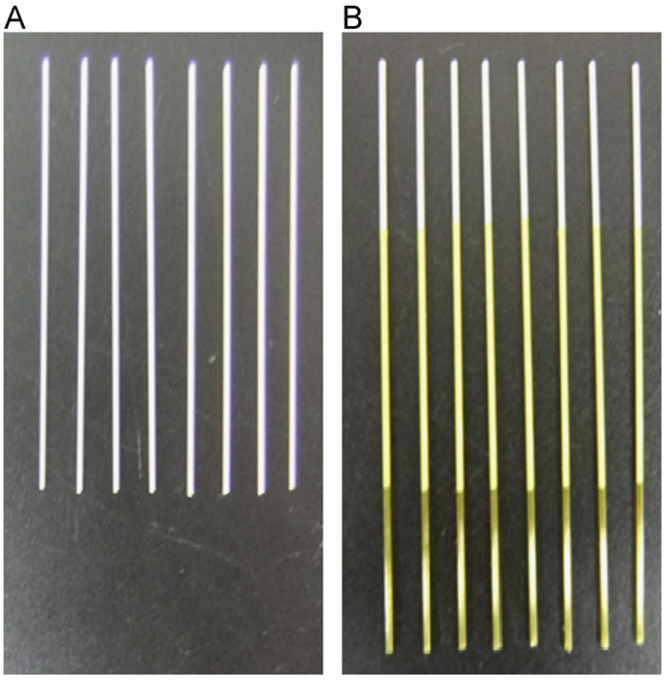

Fig. 1. Photographs of MP fibers without capillary mold (A) and after being inserted back to capillary mold (B).

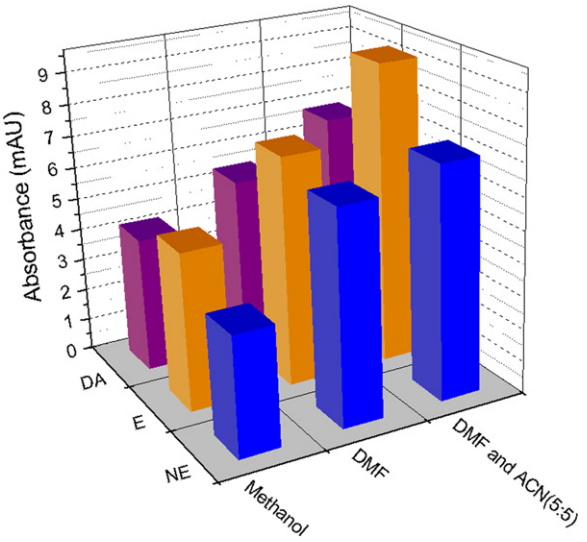

Fig. 2. Effect of polymerization solvent on extraction efficiency. Extraction condition: analyte concentration, 300 nmol L^{-1}; extraction solvent, ACN; extraction time, 60 min; desorption solvent, mixture of acetone and acetic acid (7:3, V/V); desorption time, 20 min. CE conditions: effective capillary length, 47 cm; running voltage, 14 kV; running buffer, 25 mmol L^{-1} of pH 9.00 borate solution.

Polymerization time could affect the porosity of MIP fibers by controlling the degree of polymerization reaction. The optimization experiments were performed under the optimum quantity of template at 0.12 mmol and the molar ratio of function monomer and cross at 1:6, to ensure not only the good reproducibility but also the fiber quality to achieve the best extraction capacity by regulating the polymerization time from 3 h to 24 h. Generally, the binding sites could not be fully created at short polymerization time, while the over long polymerization time might result in high degree of cross-linking and a less porous structure. A polymerization time of 12 h was chosen as an optimum and stringently controlled for both MIP- and NIP-fiber preparation (Table 1).

3.3. Characterization of MIP fiber

3.3.1. SEM graphs of MIP fiber

The scanning electron micrographs of the MIP fibers formed were illustrated in Fig. 3. The MIP fiber was monothlic without any support materials, as can be seen clearly in Fig. 3A. As exhibited in Fig. 3B, C and D, the polymers have a homogenous and high cross-linked structure, and the diameter of polymer

particles was decreased using DMF as a solvent compared with those using methanol, leading to less porous structure of polymers. The best morphological structure of the polymer particles (Fig. 3D) with very tiny homogeneous particles was accomplished using the mixture of DMF and ACN (5:5, V/V) as a solvent. It can provide more compact and porous network which allows more recognition sites than other two polymer structures (Fig. 3B and C).

3.3.2. Infrared spectrum of MIP fiber

The infrared spectrum of MIP fiber was shown in Fig. 4. It indicated that the main functional groups of the MIP fiber could be found with corresponding infrared absorption peaks as follows: 3565 or 3442 cm^{-1}, stretching vibration of O–H bonds from MAA; 3000 cm^{-1}, stretching vibration of H–C=; 2955 cm^{-1}, stretching vibration of H–C–; 1732 cm^{-1}, stretching vibration of C=O;1632 cm^{-1}, stretching vibration of C=C;1477, 1455 cm^{-1}, bending vibration of CH_2; 1387 cm^{-1}, bending vibration of CH_2; 1293 cm^{-1}, in plane bending vibration of C–H and 1161 cm^{-1}, stretching vibration of C–O.

Table 1
Optimization of molar quantity of template, the molar ratio between function monomer and cross linker, and polymerization time.

Template[a]		Function monomer and cross linker[b]		Polymerization time[c]	
E (mmol)	Signal (mAU)	Ratio	Signal (mAU)	Time (h)	Signal (mAU)
0.03	6.023	1:3	7.834	3	5.385
0.06	7.342	1:6	9.025	6	7.231
0.12	8.563	1:9	7.269	12	8.913
0.24*	–	1:12	5.873	24	8.123

* Not dissolved.

3.3.3. Stability of MIP fiber

The thermal stability of the fiber was evaluated by thermogravimetric analysis (TGA). The results indicated that the obvious mass loss occurred at around 210 °C for MIP fiber. When the temperature was less than 210 °C, the MIP fiber has good thermal stability. The chemical stability of MIP fiber was tested by immersing it in various solvents such as methanol, ethanol, water, benzene, diethyl ether, acetone, hexane, and 50% acetic acid in acetone. After keeping it in those solvents for 1 h, no obvious change was observed for the surface structure of MIP fiber including deformation, cracking, swelling, etc. Thus, we could conclude that the MIP fiber has high chemical stability.

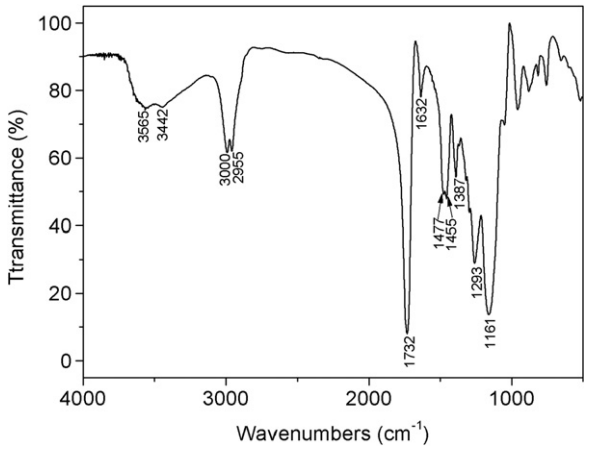

Fig. 4. Infrared spectrum of MIP fiber prepared in the mixture of DMF and ACN.

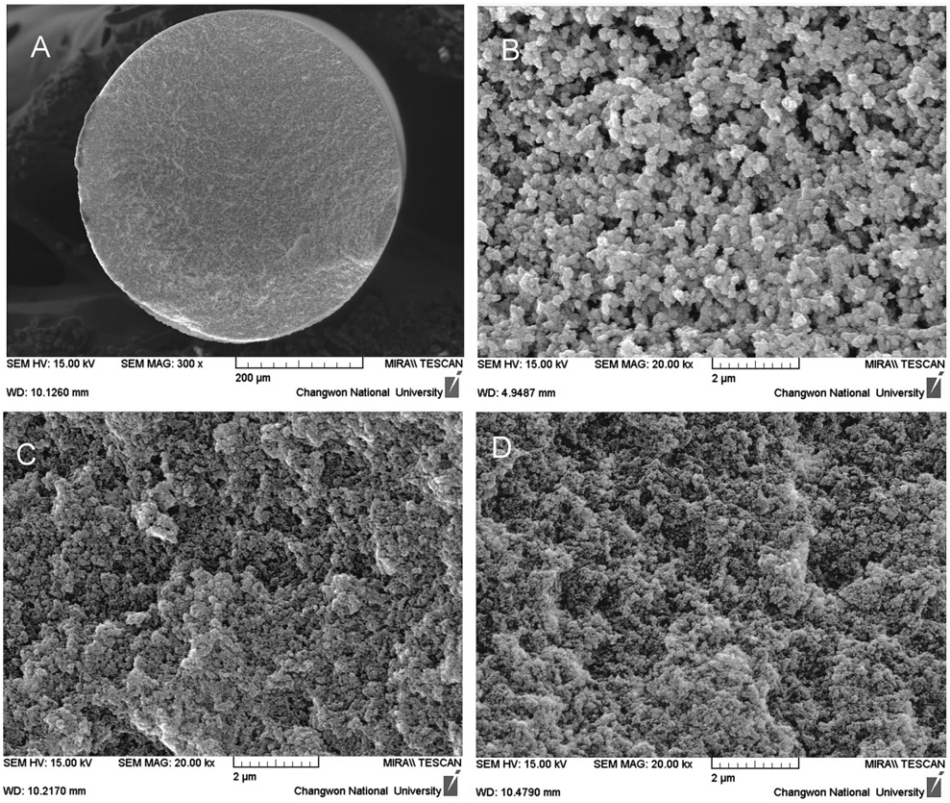

Fig. 3. Scanning electron micrographs of MIP fibers fabricated with different polymerization solvents. (A) methanol, ×300; (B) methanol, ×20 k; (C)DMF, ×20 k and (D) mixture of DMF and ACN, ×20 k.

3.4. Optimization of CE conditions

The chemical composition, pH, and the concentration of the buffer solution can affect the baseline stability, peak shape, and separation efficiency. Three kinds of buffer systems, i.e., phosphate, bicarbonate, and borate solutions, were used to separate the target analytes, DA, E, and NE. The influence of buffer pH on separation was examined in the range of 8.5–10.0, and it was demonstrated that the optimum pH value of running buffer for separation was 9.0. The best resolution and peak shapes for the target analytes were obtained using borate buffer system. The effect of buffer concentration on separation was also studied in the range from 5 mM to 40 mM. As the buffer concentration increased, the resolutions of analytes were gradually improved. However, the baseline became unstable when buffer concentrations were higher than 25 mM, because high buffer concentrations resulted in considerable Joule heating. Thus, 25 mM of pH 9.0 borate buffer solution was chosen as the best running buffer for further investigation.

3.5. Optimization of SPME conditions

3.5.1. Effect of extraction solvent and time

Several common organic solvents including benzene, chloroform, acetone, ACN, methanol, and water were examined as extraction solvents using a standard solution prepared by mixing 300 nmol L^{-1} DA, E, and NE. Highest extraction efficiency of three CAs was found when ACN was used as an extraction solvent. This is probably due to that strong solvophobic interaction between target analytes and solvent, which enhances the binding ability of analytes in MIP cavity [28]. In addition, ACN which is the most similar to polymerization solvent (mixture of ACN and DMF) can reproduce the environment established during synthesis and improve the recognition ability [29]. Thus, ACN was considered as the optimum extraction solvent. The effect of adsorption time on the extraction efficiency was also explored from 5 min to 100 min, and the results were shown in Fig. 5. The adsorption of three CAs to MIP fiber could reach equilibrium after 60 min, so the optimum extraction time was considered to be 60 min.

3.5.2. Effect of desorption solvent and desorption time

The desorption experiments of analytes from MIP fibers were carried out using six solvents (methanol, ethanol, acetone, chloroform, benzene, and hexane). We found that chloroform, benzene, and hexane could hardly desorb CAs. Although methanol, ethanol, and acetone could elute the analytes from the MIP fiber, the electropherogram showed all of them have some impurities interfering the detection at 210 nm. The migration times of impurities in methanol and ethanol were the same with that of DA, while the impurities in acetone were eluted before the three analytes. Therefore, acetone was considered as a proper solvent for analyte desorption. It is noteworthy that the addition of acetic acid can improve desorption efficiency, especially for DA and NE. The 30% acetic acid in acetone was used for desorption of analytes in this work. Fig. 6 shows the desorption kinetics of three CAs with the MIP fiber. It was revealed that the desorption process is much faster than that of adsorption. The desorption amount of CAs was increased remarkably as the desorption time increased, and about 80% of the adsorbed CAs could be quickly desorbed within 10 min. The desorption process seems to reach the equilibrium within 15 min. Thus, 15 min was selected as an optimum desorption time.

3.5.3. Extraction capacity

Under the established optimum condition, a series of experiments was conducted to investigate the extraction capacity of these monothlic MIP fibers for three CAs in the concentrations of 10–1800 nmol L^{-1}. Fig. 7 illustrates the dependence of the amount of the analytes extracted by the MIP fibers vs. the initial concentration of the analytes in the mixed standard solutions. It was noticed that the adsorption amounts of CAs increased linearly

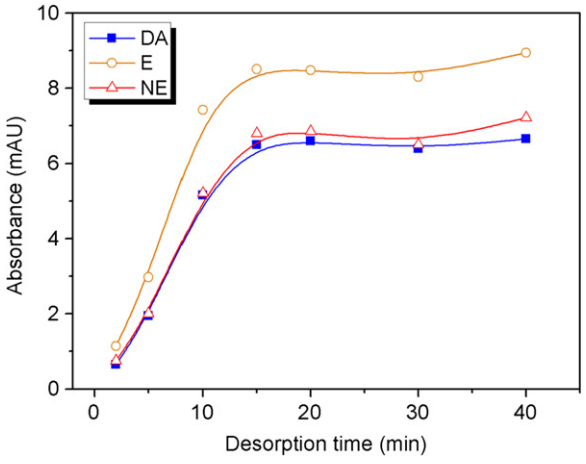

Fig. 6. Effect of desorption time on extraction efficiency. Extraction condition: desorption times were varied from 2 min to 40 min; other conditions were the same as Fig. 2.

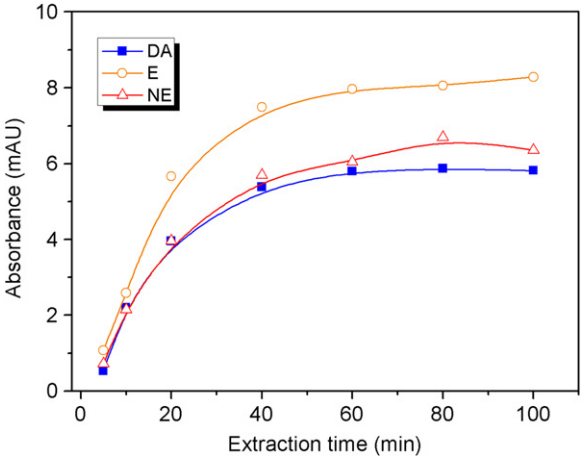

Fig. 5. Variation of extraction efficiencies of MIP-fibers depending on extraction time. Extraction condition: extraction time was varied from 5 min to 100 min; other conditions were the same as Fig. 2.

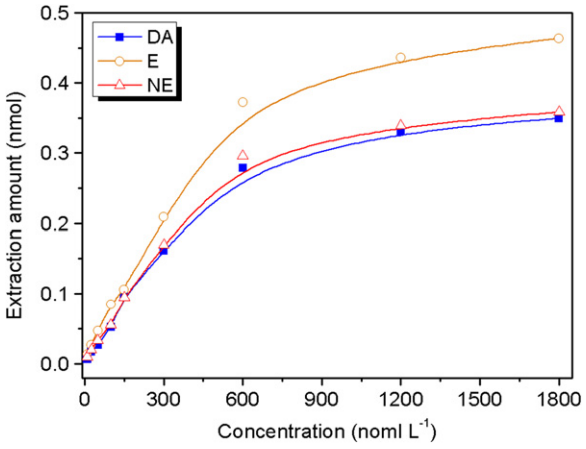

Fig. 7. Extraction amounts of DA, E, NE, and Phe at an initial concentration of 300 nmol L^{-1}. Desorption time, 15 min; other conditions were the same as Fig. 3.

with increasing the concentration within 10–600 nmol L^{-1}, so this concentration range was suitable for quantification. Further increase the analyte concentration caused the fiber to saturate. The extraction capacities of DA, E and NE per fiber were about 0.34 nmol (51 ng), 0.46 nmol (84 ng) and 0.35 nmol (59 ng), respectively. The adsorption capacity of this monothilic MIP fiber was higher than those of MIP fibers with only a coating film (less than 5 ng) [30].

3.6. Selectivity

The selectivity of the MIP fiber was studied with a mixed solution of 300 nmol L^{-1} target analytes and phenylalanine (Phe) as a reference compound. The result revealed that the MIP fiber possessed the best extraction ability for E with the extraction yields of 23%, while those for the reference compound was only 4.2%. It was mainly attributed to the specific geometric shapes of cavities in MIP for the target analytes, and correspondingly, the different special structures of reference compound from the template led to a low extraction efficiency. Owing to the similar chemical structure of DA and NE with E, much higher extraction yields (16.7% and 17.8% for DA and E, respectively) than that of reference compound was also found. Thus, the MIP fiber showed good selectivity for the template molecule and its analogues.

Table 2
The LODs comparison between MIP-SPME-CE-UV and other methods.

Method	MIP-SPME-CE-UV	CE-LIF	CE-MS	HPLC-UV	HPLC-MS
DA (μmol mL^{-1})	0.0071	0.0012	0.30	–	0.13
E (μmol mL^{-1})	0.0048	0.00064	0.10	0.16	0.082
NE (μmol mL^{-1})	0.0074	0.0014	0.30	0.12	0.089
Ref.	This work	[31]	[32]	[33]	[34]

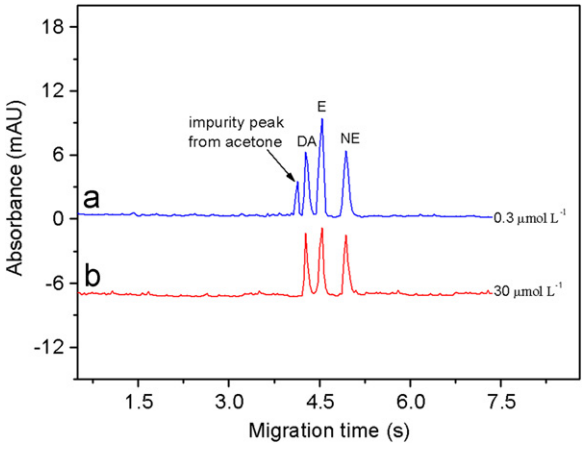

Fig. 8. Typical electropherograms of (a) 0.3 μmol L^{-1} mixed standard solution of DA, E, and NE with MIP extraction, and (b) 30 μmol L^{-1} mixed standard solution of DA, E, and NE with direct injection. Desorption time, 15 min; other conditions were the same as Fig. 3.

3.7. Evaluation of analytical performance

3.7.1. Fiber reproducibility, linearity, and limit of detection

The fiber reproducibility was examined with a mixed standard solution containing 300 nmol L^{-1} each for DA, E, and NE. The relative standard deviations (RSDs) for five consecutive measurements with the same MIP fiber were 5.9% for DA, 6.1% for E, and 4.3% for NE. The extraction reproducibility with different fibers ($n=5$) were 9.8%, 6.6% and 5.9% for DA, E and NE, respectively. In addition, the prepared fiber could remain in extraction performance after the use of more than 30 times.

The linearity and limits of detection (LOD) of the MIP fiber-SPME-CE method has been evaluated with a series of DA, E and NE mixed standard solutions under optimized conditions. The good linearities were achieved in range of 10–600 nmol L^{-1} for all CAs with correlation coefficient, $(r) > 0.9950$. The LOD calculated based on signal-to-noise of 3 were 4.8 nmol L^{-1}, 7.1 nmol L^{-1}, 7.4 nmol L^{-1} for E, DA and NE, respectively, which were over 100-fold lower than those obtained with direct CE-UV detection. Without an expensive instrument or labeling procedure, the LODs for CAs obtained by present method are only slightly higher than those obtained by CE-LIF but much lower than those with CE-MS, HPLC-UV and HPLC-MS detection (Table 2). The electropherograms in Fig. 8 proved the remarkable enhancement of the sensitivity by using the developed MIP extraction system.

3.7.2. Accuracy and repeatability for urine and serum sample analysis

The MIP fiber-SPME-CE was employed to quantify CAs in human urine and serum samples. Fig. 9 shows the typical electropherograms produced by direct CE detection and NIP/MIP fiber-SPME-CE system with urine and CAs spiked serum samples. With direct injection, CAs in urine and spiked serum samples could not be detected, and the electropherograms were quite complicated (Fig. 9a). Although the NIP-fiber possessed a little

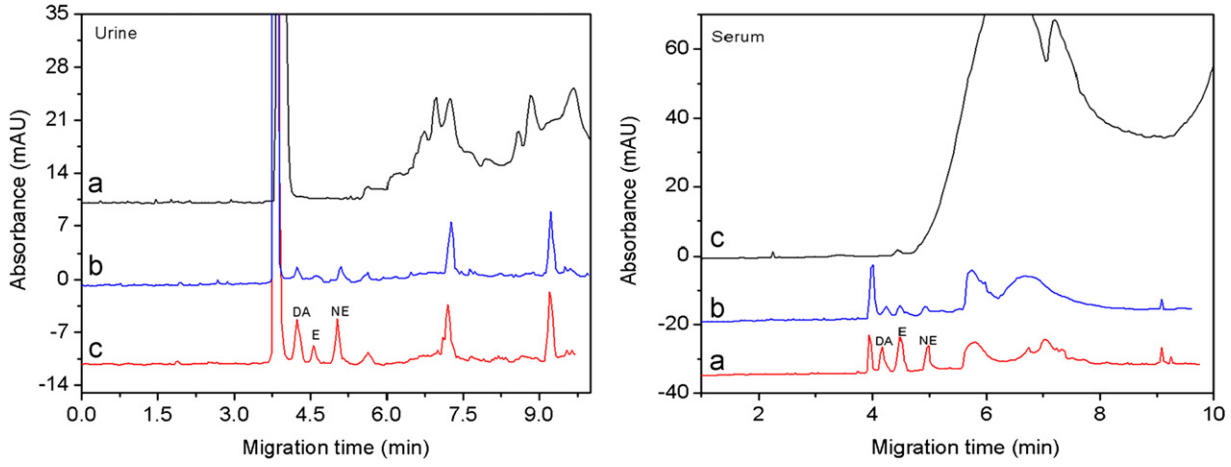

Fig. 9. Elelectropherograms of urine and spiked serum samples. (a) direct injection after filtered through 0.45 μm membrane filter; (b) sample solution extracted with NIP fiber and (c) sample solution extracted with MIP fiber. Desorption time, 15 min; other conditions were the same as Fig. 3.

Table 3
Accuracies of three CAs in urine sample.

Sample no.	Analyte	Found (nM)*	Added (nM)	Total found (nM)*	Recovery (%, RSD, $n=3$)
1	DA	247.6 ± 19.6	200	416.1 ± 33.1	85 (4.7)
	E	67.1 ± 10.2	100	152.8 ± 15.4	86 (6.0)
	NE	218.8 ± 19.5	200	397.9 ± 22.3	90 (3.3)
2	DA	278.5 ± 22.3	300	560.3 ± 45.7	94 (4.9)
	E	45.3 ± 8.7	50	89.1 ± 15.6	88 (10.4)
	NE	127.2 ± 14.6	100	219.5 ± 32.2	92 (4.2)

* Average ± 1.68 × standard deviation with the confidence level at 90% ($n=3$).

Table 4
Accuracies of three CAs in serum sample.

Sample no.	Analyte	Found (nM)	Added (nM)	Total found (nM)*	Recovery (%, RSD)
1	DA	nd	150	138.6 ± 25.7	92 (11.0)
	E	nd	150	132.2 ± 24.7	88 (11.1)
	NE	nd	150	128.0 ± 31.2	85 (14.4)
2	DA	nd	300	308.8 ± 30.5	103 (5.9)
	E	nd	300	289.5 ± 29.6	96 (6.1)
	NE	nd	300	283.5 ± 19.9	94 (4.2)

nd: not detectable.

* Average ± 1.68 × standard deviation with the confidence level at 90% ($n=3$).

extraction capacity, low analytes signals were observed (Fig. 9b). This implies that the extraction with non-specific adsorption could be seriously interfered by the matrix effect. After MIP fiber extraction, obvious higher signals for CAs were appeared and the matrix interferences were also diminished markedly in the eletropherogram (Fig. 9c).

The analytical results of urine and serum samples were summarized in Tables 3 and 4. Two pristine urine samples contained the CAs in the concentration level ranging from 45.3 nM to 278.5 nM; While, the CAs in serum itself were not found by the present system. The recoveries of CAs for the spiked urine and serum samples were 85–94% (RSD: 3.3–10.4%) and 85–103% (RSD: 4.2–14.4%), respectively, by using different fibers. These recoveries were calculated by comparing the peak areas of the spiked sample with those of standard solutions following the same procedure. The intraday repeatability was investigated by five consecutive extractions of urine and serum samples spiked with 150 nM. The RSD values were 8.3–14.2% for urine samples and 5.4–13.5% for spiked serum samples.

4. Conclusions

This work demonstrated that the combination of SPME and the simply fabricated MIP fiber was a powerful tool for quantitative analysis of CAs in urine and serum samples. A novel E imprinted SPME fiber was developed by using a capillary as polymerization mold and the fabricated MIP fibers were flexible, homogeneous, highly cross-linked and porous. It was also found to be useful to overcome two main problems generally encountered in CE-UV analysis of complex samples, i.e., low sensitivity and matrix interference. It should be noticed that the fabrication procedure is very simple and reproducible which makes the preparation of MIP fiber in a batch mode easy. The developed MIP-fiber possessed the specific selectivity and enhanced sensitivity to three CAs and therefore, the E-imprinted polymer fiber was suitable and promising for extraction of trace CAs before CE detection.

Acknowledgements

The authors gratefully acknowledge the financial support for the Basic Research Program (NRF 2011-0010155), the Priority Research Centers Program (NRF 2010-0029634) through the National Research Foundation (NRF) of Korea, the National Natural Science Foundation of China (No. 21005010), and the cultivating program of middle-aged backbone teachers of Chengdu university of Technology.

References

[1] D. Durstewitz, M. Kelc, O. Gunturkun, J. Neurosci. 19 (1999) 2807–2822.
[2] B. Kagedal, D.S. Goldstein, J. Chromatogr. 429 (1988) 177–233.
[3] W.L. Tseng, S.M. Chen, C.Y. Hsu, M.M. Hsieh, Anal. Chim. Acta 613 (2008) 108–115.
[4] Y. Hu, X. Li, Z. Pang, J. Chromatogr. A 1091 (2005) 194–198.
[5] M.E. Yue, T.F. Jiang, Y.P. Shi, J. Sep. Sci. 28 (2005) 360–364.
[6] J. Kang, X.B. Yin, X. Yang, E. Wang, Electrophoresis 26 (2005) 1732–1736.
[7] B. Claude, R. Nehme, P. Morin, Anal. Chim. Acta 699 (2011) 242–248.
[8] W.H. de Jong, E.G. de Vries, B.H. Wolffenbuttel, I.P. Kema, J. Chromatogr. B 878 (2010) 1506–1512.
[9] M. Lasakova, D. Thiebaut, P. Jandera, V. Pichon, J. Sep. Sci. 32 (2009) 1036.
[10] D. Talwar, C. Williamson, A. McLaughlin, A. Gill, D.S. O'Reilly, J. Chromatogr. B 769 (2002) 341.
[11] K. Vuorensola, H. Siren, J. Chromatogr. A 895 (2000) 317–327.
[12] F.A. van der Hoorn, F. Boomsma, A.J. Man in 't Veld, M.A. Schalekamp, J. Chromatogr. 563 (1991) 348–355.
[13] H. Tsuchiya, T. Hayashi, J. Pharmacol. Method 23 (1990) 21–30.
[14] H.F. Wu, C.H. Lin, Rapid Commun. Mass Spectrom. 20 (2006) 2511–2515.
[15] C. Cakal, J.P. Ferrance, J.P. Landers, P. Caglar, Anal. Chim. Acta 690 (2011) 94–100.
[16] J. He, Z. Liu, L. Ren, Y. Liu, P. Dou, K. Qian, H.Y. Chen, Talanta 82 (2010) 270–276.
[17] A. El-Beqqali, A. Kussak, M. Abdel-Rehim, J. Sep. Sci. 30 (2007) 421–424.
[18] J. Auger, R. Boulay, B. Jaillais, S. Delion-Vancassel, J. Chromatogr. A 870 (2000) 395–403.
[19] F.G. Tamayo, E. Turiel, A. Martin-Esteban, J. Chromatogr. A 1152 (2007) 32–40.
[20] E. Turiel, J.L. Tadeo, A. Martin-Esteban, Anal. Chem. 79 (2007) 3099–3104.
[21] L. Qiu, W. Liu, M. Huang, L. Zhang, J. Chromatogr. A 1217 (2010) 7461–7470.
[22] Y. Hu, Y. Wang, X. Chen, G. Li, Talanta 80 (2010) 2099–2105.
[23] X. Hu, J. Pan, Y. Hu, G. Li, J. Chromatogr. A 1216 (2009) 190–197.
[24] M. Liu, M. Li, B. Qiu, X. Chen, G. Chen, Anal. Chim. Acta 663 (2010) 33–38.
[25] D. Lakshmi, A. Bossi, M.J. Whitcombe, I. Chianella, S.A. Fowler, S. Subrahmanyam, E.V. Piletska, S.A. Piletsky, Anal. Chem. 81 (2009) 3576–3584.
[26] P.Y. Chen, P.C. Nien, C.T. Wu, T.H. Wu, C.W. Lin, K.C. Ho, Anal. Chim. Acta 643 (2009) 38–44.
[27] B.Y. Huang, Y.C. Chen, G.R. Wang, C.Y. Liu, J. Chromatogr. A 1218 (2011) 849–855.
[28] S. Boonpangrak, V. Prachayasittikul, L. Bülow, L. Ye, J. Appl. Polym. Sci. 99 (2006) 1390–1398.
[29] E. Caro, R.M. Marcé, F. Borrull, P.A.G. Cormack, D.C. Sherrington, Trend Anal. Chem. 25 (2006) 143–154.
[30] X.G. Hu, G.M. Dai, J.J. Huang, T.T. Ye, H.J. Fan, T. Youwen, Y. Yu, Y. Liang, J. Chromatogr. A 1217 (2010) 5875–5882.
[31] S. Xiong, H. Han, R. Zhao, Y. Chen, G. Liu, Biomed. Chromatogr. 15 (2001) 83–88.
[32] Z.D. Peterson, D.C. Collins, C.R. Bowerbank, M.L. Lee, S.W. Graves, J. Chromatogr. A 776 (2002) 221–229.
[33] A. Mishra, A. Upadhyay, A. Patra, S. Chaudhury, P. Chattopadhyay, Sci. Pharm. 77 (2009) 367–374.
[34] Q. Gu, X. Shi, P. Yin, P. Gao, X. Lu, G. Xu, Anal. Chim. Acta 609 (2008) 192–200.