Chunhe Yu
Bin Hu

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan, P. R. China

Received February 8, 2010
Revised April 16, 2010
Accepted April 16, 2010

Research Article

Automated stir plate (bar) sorptive extraction coupled to high-performance liquid chromatography for the determination of polycyclic aromatic hydrocarbons

Automated methods of PDMS/β-CD/divinylbenzene-coated stir plate sorptive extraction (SPSE) coupled to HPLC-fluorescence detector were reported for the first time. Three automation modes, static SPSE, circular flow SPSE and continuous flow SPSE, were evaluated and critically compared with stir bar sorptive extraction by using six polycyclic aromatic hydrocarbons as model analytes. It was found that the operable sample volume for circular flow SPSE and continuous flow SPSE was larger than that for static SPSE. Under the same extraction conditions, continuous flow SPSE exhibited the highest extraction efficiencies in all automated modes and manual stir bar sorptive extraction for the target compounds. Compared with the manual operation (approximately 5–10 min), automated SPSE required a relatively short time (117–180 s) to finish sampling, washing and sample loading. Besides being labor-saving and time-saving, automated SPSE has other advantages, such as no time limit and non-attended operation. The proposed continuous flow PDMS/β-CD/divinylbenzene-coated SPSE-HPLC-fluorescence detector was successfully applied to environmental water analysis.

Keywords: Automation / HPLC-fluorescence detector / PDMS/β-CD/divinylbenzene coating / Polycyclic aromatic hydrocarbons / Stir plate (bar) sorptive extraction
DOI 10.1002/jssc.201000091

1 Introduction

As a crucial step in qualitative and quantitative analysis, sample pretreatment is commonly used to isolate, concentrate or convert the target analytes into the forms tailored to the instrumental analysis [1], and it would solve those problems generally encountered in direct instrumental analysis of real-world samples, such as matrix effect and the concentration level not up to the quantitative limit. With the development and application of diverse sample pretreatment techniques in recent years, the tendency of miniaturization, environmentally friendly, convenient operation, time-saving, low cost and automation for sample pretreatment techniques is becoming more and more manifest [2, 3]. Automation of an analytical method would provide a number of advantages over manual method, such as high sample throughput, improved reproducibility, no time limit and non-attended operation. Automation of a certain sample pretreatment technique or analytical method would definitely broaden its acceptance and application field [4, 5].

At present, many sample pretreatment techniques including liquid–liquid extraction [6], liquid-phase microextraction [7], SPE [8] and solid-phase microextraction (SPME) [5] have been automated and online coupled to subsequent analytical instrumentation. Stir bar sorptive extraction (SBSE) is another form of sorptive extraction and it is based on the same principle as SPME. In SBSE, the sorbent is coated on a magnetic stirrer, and the analytes are extracted when the stir bar stirs the aqueous solution. After extraction, the stir bar is desorbed thermally (for GC analysis) or by organic solvents (mainly for LC analysis) [9]. Compared with other microextraction techniques, including SPME and liquid-phase microextraction, SBSE would provide higher extraction efficiency and better reproducibility due to much more amount of extraction phase coated on the stir bar and no more special skills required, but the main disadvantage is the difficulty in full automation [4, 10,

Correspondence: Professor Bin Hu, Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, P. R. China
E-mail: binhu@whu.edu.cn
Fax: +86-27-68754067

Abbreviations: ANA, acenaphthene; **DVB,** divinylbenzene; **FLD,** fluorescence detector; **FLT,** fluoranthene; **NAP,** naphthalene; **PAH,** polycyclic aromatic hydrocarbon; **PHE,** phenanthrene; **PYR,** pyrene; **SBSE,** stir bar sorptive extraction; **SPME,** solid-phase microextraction; **SPSE,** stir plate sorptive extraction

11]. Although the commercially available thermal desorption systems allow automatic control of all desorption, trapping and injection conditions, the user needs to manually transfer the stir bar into the sample vial and from the sample vial to the liner tray [5, 12]. Once the fully automated SBSE is achieved, a greater widespread application of this technique can be foreseen.

The purpose of this work is to develop a fully automated SBSE-HPLC system for the determination of six polycyclic aromatic hydrocarbons (PAHs) in environmental waters. To facilitate the automation, a concept of ''stir plate'' instead of stir bar was proposed, and the stir plate was coated with PDMS/β-CD/divinylbenzene (DVB) in this work. The procedure of stir plate sorptive extraction (SPSE), including automatic extraction, desorption and sample loading, was controlled by a programmable flow injection system. Automated SPSE in three modes (static SPSE, circular flow SPSE and continuous flow SPSE) were optimized and investigated critically. For comparison, manual SBSE using PDMS/β-CD/DVB-coated stir bars and automatic SBSE using a commercial PDMS-coated stir bar were also studied. The continuous flow SPSE was successfully applied to the analysis of six PAHs in tap water and Yangzi River water.

2 Materials and methods

2.1 Chemicals and materials

Hydroxyl-terminated PDMS (OH-PDMS) and β-CD were purchased from Aldrich (Milwaukee, WI, USA). Methyltrimethoxysilane, DVB and TFA were purchased from China Medicine (group) Shanghai Chemical Reagent Corporation (Shanghai, China). Poly(methylhydrosiloxane) was obtained from the Chemical Plant of Wuhan University (Wuhan, China). The quartz glass capillary (0.53 μm id) was obtained from Ruipu Chromatography Equipment (Yongnian, Hebei, China); the capillary glass bars were obtained from Apparatus Factory of West China University of Medical Sciences (Chengdu, Sichuan, China) and the glass slides (25.4 wide × 76.2 mm long × 1 mm thick, Cat. No. 7101) were purchased from Huida Medical Instruments (Yancheng, Jiangsu, China). The stir bars (Twister^{TM}) coated with 10 mm in length and 1 mm film thickness PDMS were purchased from Gerstel GmbH (Mülheim a/d Ruhr, Germany). Sodium chloride and all solvents used in this study were of analytical grade. High purity water obtained by a Milli-Q water purification system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout the whole experiments.

PAHs including naphthalene (NAP, 99%), acenaphthene (ANA, 95%), phenanthrene (PHE, 95%), anthracene (ANT, 98%), fluoranthene (FLT, 95%) and pyrene (PYR, 98%) were purchased from Tianchang Chemical (Anshan, Liaoning, China). Each standard solution of PAHs was prepared in methanol at a concentration of 0.1 mg/mL and stored at 4°C in the refrigerator.

2.2 Instrumental

The chromatographic system consisted of an Agilent 1100 series HPLC-fluorescence detector (FLD) with 100-μL sample loop and a reversed phase C_{18} HPLC column (Lichrospher ODS, 5 μm, 4.6 mm × 200 mm id, Hanbon, Jiangsu, China). Methanol–water (80:20, v/v) was used as an isocratic eluent at a flow rate of 1 mL/min. The retention time (t), excitation wavelength (Ex) and emission wavelength (Em) for the studied compounds are as follows: t_{NAP} = 7.45 min, λEx = 220 nm, λEm = 330 nm; t_{ANA} = 13.237 min, λEx = 226 nm, λEm = 359 nm; t_{PHE} = 14.679 min, λEx = 246 nm, λEm = 370 nm; t_{ANT} = 16.793 min, λEx = 250 nm, λEm = 406 nm; t_{FLT} = 21.442 min, λEx = 234 nm, λEm = 440 nm; t_{PYR} = 24.334 min, λEx = 270 nm, λEm = 390 nm. The images of the prepared SPSE coatings were observed by Nikon 76 TE2000-U Microscope (Nikon, Japan).

2.3 Preparation of stir plate

The microscope slide was cut into small pieces (8 mm × 8 mm) and was activated by 1 mol/L NaOH for 3 h. Then, these glass plates were cleaned by water and dried at the room temperature. A hybrid material PDMS/β-CD/DVB was chosen as the coating of stir plate according to our previous work [13], which indicates that PDMS/β-CD/DVB have better extraction ability for PAHs than pure PDMS. The prepared sol solution was made up of 150 μL PDMS, 50 mg β-CD, 50 μL DVB, 100 μL methyltrimethoxysilane, 10 μL poly(methylhydrosiloxane), 150 μL CH_2Cl_2 and 40 μL 95% TFA. After ultrasonication, 20 μL of the homogeneous sol solution was coated on one side of each glass plate and the coated plate was then placed into a constant temperature drier for 24 h at 60°C for gelation. Subsequently, the sol solution consisting of the above components was prepared again and coated onto the other side of the glass plate. After aging for 24 h at 60°C, the coated glass plate was mounted by a fit iron ring for stirring (Fig. 1). Prior to use, the

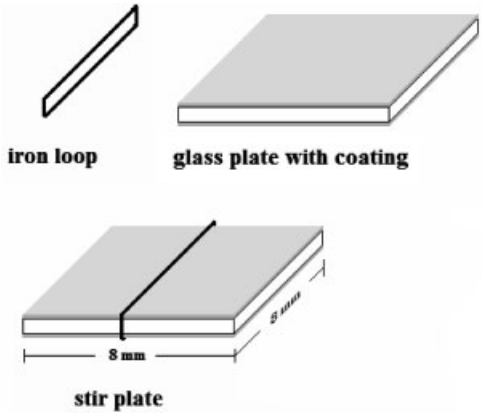

Figure 1. Setup of stir plate.

PDMS/β-CD/DVB-coated ''stir plate'' should be cleaned in methanol by ultrasonication for 10 min to get rid of the organic contaminants in the coating.

PDMS/β-CD/DVB-coated stir bars (1 mm id and 20 mm length) were prepared according to Ref. [13], using the same sol solution as stir plate.

2.4 Automation of SPSE-HPLC-FLD

The automation of SPSE-HPLC-FLD was controlled by an FIA-3110 Flow Injection Analyser (Beijing Titan Instruments, China) consisting of two pumps (each pump has two triple channels, one on the top and the other on the bottom), an eight-way valve, polyfluortetraethylene tubes, organic solvent-tolerant tubes and some connectors. One 15-cm-long quartz glass capillary (0.53 μm id) connected with the pump was placed vertically to the bottom of the extraction vial for pumping out the waste (valve position 0) or the eluent (valve position 1), and another 15-cm-long quartz glass capillary was inserted into the six-port valve of HPLC for the sample loading. As can be seen from Fig. 2, the flow design and tube connections for circular flow SPSE and continuous flow SPSE are similar, and the only difference is the place where the waste tube was put into. For circular flow SPSE, the waste tube was put into the vial of sample solution; hence, the sample solution could be pumped in and out for many times; for continuous flow SPSE, the waste tube was put into the waste vial. The flow design for static SPSE is the same as that of continuous flow SPSE.

The designed programs included eight steps for all the three modes of automated SPSE, and the magnetic stirrer was kept running during the whole procedure. The process of static SPSE was described as follows (Table 1). Step 1: the eight-way valve was in position 0, and pump A started clockwise at the rate of 120 rpm for 55 s to pump 5 mL sample solution into the extraction vial (13 mm id); step 2: pump A was stopped and the stir plate was stirring for 1200 s (time of this step was set as 600 s and repeated once because the time range is 0–999 s in one run); step 3: pump

Table 1. Program of static SPSE, circular flow SPSE and continuous flow SPSE

Step	Time (s)	Valve	Pump A (rpm)	Pump B (rpm)	Repeat
Static SPSE					
1	055	Fill	+[a] 120	+ 000	1
2	600	Fill	+ 000	+ 000	2
3	060	Fill	+ 000	+ 120	1
4	007	Fill	−[b] 120	+ 000	1
5	030	Fill	+ 000	+ 120	1
6	003	Inject	− 120	+ 000	1
7	600	Inject	+ 000	+ 000	1
8	025	Inject	+ 000	+ 030	1
Circular flow SPSE					
1	022	Fill	+ 120	+ 000	1
2	600	Fill	+ 100	+ 100	2
3	030	Fill	+ 000	+ 120	1
4	007	Fill	− 120	+ 000	1
5	030	Fill	+ 000	+ 120	1
6	003	Inject	− 120	+ 000	1
7	600	Inject	+ 000	+ 000	1
8	025	Inject	+ 000	+ 030	1
Continuous flow SPSE					
1	022	Fill	+120	+000	1
2	600	Fill	+009	+009	2
3	030	Fill	+000	+120	1
4	007	Fill	−120	+000	1
5	030	Fill	+000	+120	1
6	003	Inject	−120	+000	1
7	600	Inject	+000	+000	1
8	025	Inject	+000	+030	1

a) +, clockwise rotate.
b) −, counter-clockwise rotate.

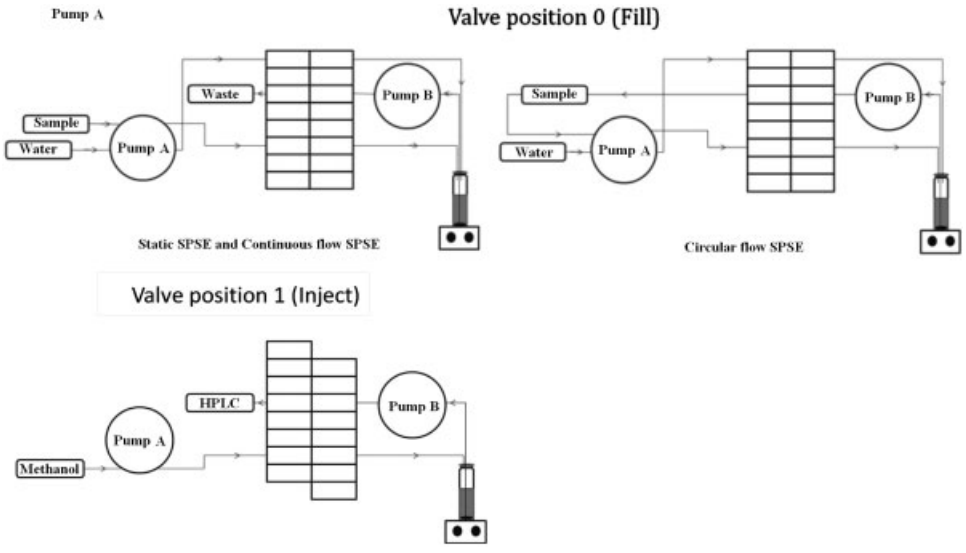

Figure 2. Schematic representation of static SPSE, circular flow SPSE and continuous flow SPSE.

B was started clockwise at the rate of 120 rpm for 60 s to drain the sample solution; step 4: pump A was started counter-clockwise at the rate of 120 rpm for 7 s to pump high purity water into the vial for clean-up; step 5: pump B was started clockwise at the rate of 120 rpm for 30 s to drain the waste; step 6: the eight-way valve was moved to position 1 automatically and pump A was started counter-clockwise simultaneously at the rate of 120 rpm for 3 s to pump in 150 μL methanol for desorption; step 7: both pumps were stopped and the stir plate was stirring in the methanol for 600 s to desorb the analytes; step 8: pump B was started clockwise at the rate of 30 rpm for 25 s to pump the eluent into six-port valve (load position), then 100 μL of the eluent in the sample loop of HPLC was manually injected into the HPLC for subsequent analysis.

The programs of circular flow and continuous flow SPSE are quite similar except for the procedure of pumping the sample solution into the extraction vial. In the mode of circular flow SPSE, 1.5 mL of sample solution was pumped into the extraction vial firstly in step 1 to keep the stir plate immersed in sample solution, and then the rest of the sample solution was pumped circularly to flow through the stir plate at the rate of 100 rpm in step 2 until the finish of extraction. In the mode of continuous flow SPSE, 1.5 mL of sample solution was also pumped into the extraction vial firstly in step 1 and then the rest of the sample solution was pumped at the speed of 0.425 mL/min to flow through the stir plate.

It is noteworthy that automated SPSE costs only 117–180 s to finish sampling, washing and sample-loading, whereas a skilled operator usually needs 5–10 min to finish these processes for the conventional SBSE, which means automated SPSE reduces not only labor intensity but also operation time.

2.5 Automation of commercial SBSE-HPLC-FLD

Prior to use, the Twister was preconditioned by methanol. Since the total length of the stir bar is about 15 mm, larger vials (20 mm id) were used for extraction, and the volume of desorption solvent was increased to 300 μL to immerse the stir bar. The sample volume is 10 mL and the automated mode is continuous flow. The program of automated SBSE is the same as that of continuous flow SPSE (Table 1), except that the time of pumping desorption solvent into the extraction vial was changed to 5 s in step 6.

2.6 Manual operation of SBSE

Referring to our previous work [13], the PDMS/β-CD/DVB-coated stir bar was stirred in a 5 or 10 mL aqueous solution at 700 rpm for 20 min, and then transferred into a small test tube containing 90 μL methanol. After desorption by ultrasonication for 10 min, the stir bar was taken out to dry its surface carefully and was placed into 2 mL methanol for cleaning. Seventy microliter of the elution was injected into HPLC-FLD for analysis.

2.7 Sample preparation

Tap water, Yangzi River water and the spiked water samples were treated by continuous flow SPSE. For recovery assays, the tap water sample was spiked at 25 ng/L and the Yangzi River water sample was spiked at 50 ng/L for each target PAH.

3 Results and discussion

3.1 Preparation of stir plates

In the proposed automation system, both extraction and desorption were processed in the same vial. Taking into account the volume of HPLC sample loop, the volume of desorption solvent should be around 100 μL. Thus, smaller extraction vials (13 mm id was used) than the commonly used ones (approximately 20 mm id) should be employed and consequently the available stir bars should be less than 10 mm in length. To increase the surface area of coating as much as possible in the designed setup, an 8 mm × 8 mm glass slide was used as the support for PDMS/β-CD/DVB coating, which was named as ''stir plate''. The surface area of the prepared stir plate was calculated as 128 mm^2, which is twice as much as that of a stir bar in 20 mm length and 1 mm id (62.8 mm^2).

The preparation reproducibility of stir plate (8 mm × 8 mm) and stir bar (20 mm length × 1 mm id) was evaluated under the similar conditions in manual mode. The stir plate/bar was stirred at 700 rpm for 20 min, using 5 mL aqueous solution at 50 ng/L of each PAH, and then desorbed in 150 μL methanol for 10 min. The results in Table 2 show that the preparation reproducibility of stir plates is better than that of stir bars because PDMS/β-CD/DVB sol could be coated quantitatively on the surface of the glass plates, whereas quantitative coating is difficult to achieve for the cylindrical surface of stir bars.

Figure 3 shows microscope graphs of a stir plate. It shows that the coating on the surface of the stir plate is not very uniform (Fig. 3A). By measuring ten random selected

Table 2. Preparation reproducibility data for PDMS/β-CD/DVB-coated stir plates and stir bars

Compounds	Plate to plate ($n = 6$)	Bar to bar ($n = 6$)
NAP	7.1	10.1
ANA	4.2	12.6
PHE	5.0	16.6
ANT	7.5	18.6
FLT	12.5	14.5
PYR	13.2	13.4

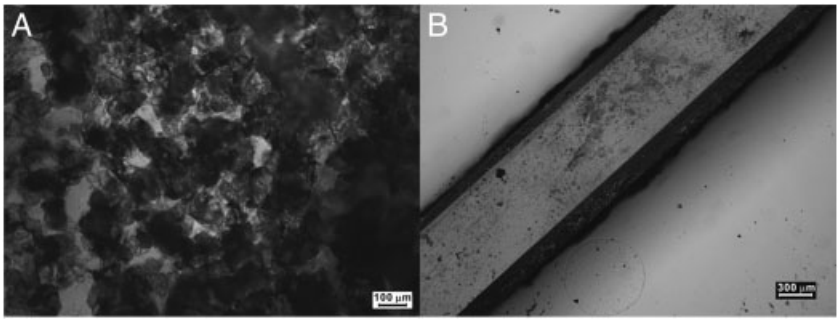

Figure 3. Microscope graphs of (A) the surface and (B) the cross section of PDMS/β-CD/DVB-coated stir plate.

points, the average thickness of the coating is found to be about 150.9 μm (Fig. 3B).

3.2 Optimization of extraction conditions for automated SPSE

3.2.1 Static SPSE and circular flow SPSE

In static SPSE, the whole sample solution was pumped into the extraction vial in one step for the stir plate extraction, and the volume of the sample solution was limited to 5 mL because the force moment of the stir plate is not large enough to make the upper solution be stirred in a relatively large volume of sample solution. To make much larger volume of sample solution operable in this system, circular flow SPSE was designed, in which the sample solution was circulated through the pumps and extraction vial at a high speed. In brief, the only difference between static SPSE and circular flow SPSE is the operable sample volume: smaller than or equal to 5 mL for static SPSE and larger than 5 mL for circular flow SPSE.

The optimal extraction conditions for circular flow SPSE were investigated by using the standard aqueous solution at 200 ng/L for each analyte. The effect of extraction time on the extraction was studied with extraction time varying from 5 to 30 min, and the results showed that extraction equilibrium could be reached in 20 min. The desorption time in the range of 5–20 min was evaluated and the best desorption efficiency was obtained at 10 min. By keeping the extraction time of 20 min, the effect of sample volume on the extraction was investigated and it was found that the extraction efficiency of circular flow SPSE was increased with the increase of sample volume from 5 to 10 mL and reached a plateau when the sample volume is above 10 mL. Therefore, circular flow SPSE was processed with 10 mL sample solution at 700 rpm stirring rate for 20 min and 150 μL methanol desorption for 10 min. The optimized conditions for static SPSE were the same as that for circular flow SPSE except for the sample volume of 5 mL.

3.2.2 Continuous flow SPSE

The desorption conditions of continuous flow SPSE were kept the same as that of static and circular flow SPSE because of the same desorption process. Continuous flow SPSE is a dynamic non-equilibrium extraction, in which the sample solution was flowed continuously through the stir plate. Since desorption time was 10 min, 20 min was selected as extraction time to match with the HPLC separation (30 min). Under these conditions, the effect of sample flow rate on the extraction was evaluated at 0.225, 0.425, 0.675, 0.925 and 1.425 mL/min (the corresponding rotation speeds of the pumps are 5, 9, 15, 20 and 30 rpm, respectively) and the results demonstrated that the extraction efficiency of continuous flow SPSE was increased with the increase of sample flow rate to 0.425 mL/min and then leveled off with further increase of sample flow rate to 1.425 mL/min. Consequently, 0.425 mL/min was selected as the sample flow rate, which corresponds to 10 mL sample for continuous flow SPSE. The other operation conditions were similar to static SPSE and circular flow SPSE.

3.2.3 Continuous flow SBSE

Commercial stir bar coating is PDMS, its thickness is 1 mm and its extraction mechanism is based on the absorption. As reported in some literatures [14–17], the equilibrium time of commercial PDMS stir bars for the extraction of PAHs is in the range of 1.5–14 h. As the chromatographic separation takes about 30 min, while stirring bar desorption needs 10 min, in order to match with the separation, the extraction time was also selected as 20 min for continuous flow SBSE. The effect of desorption time ranging from 5 to 20 min on the extraction was evaluated and the experimental results demonstrated that the equilibrium of desorption was reached in 10 min. Subsequently, the desorption volume of methanol (150–400 μL) was also optimized because continuous flow SBSE has to employ a larger extraction vial to contain the longer stir bar over the stir plate. It was found that 300 μL of methanol gave the best desorption efficiency, and 300 μL is also the smallest volume of methanol required to immerse the stir bar completely. Therefore, continuous flow SBSE was processed with 10 mL sample solution at 700 rpm stirring rate for 20 min and 300 μL methanol desorption for 10 min, by using the commercial stir bar coated with 10 mm length and 1 mm film thickness PDMS.

3.3 Analytical performance

The analytical performance of automated circular flow/continuous flow PDMS/β-CD/DVB-coated SPSE-HPLC-FLD with sample volume of 10 mL and automated static PDMS/β-CD/DVB-coated SPSE-HPLC-FLD with sample volume of 5 mL was evaluated, respectively, and the results are shown in Fig. 4. The LODs were calculated as the concentration of the target analyte that produced a S/N of 3, and the enrichment factors were calculated by the ratio of the slope of the linear curves obtained with and without extraction. For comparison, the analytical performance of manual PDMS/β-CD/DVB-coated SBSE-HPLC-FLD using the self-made stir bar (20 mm length, 1 mm id) for the extraction of 5- and 10-mL samples, and continuous flow SBSE using commercial stir bar in 10 mm length for the extraction of 10-mL sample were also investigated. For the extraction of six PAHs in 5-mL sample solution, static SPSE showed a little better reproducibility (except for PHE) because of programmable control and non-attended operation, although not as much as expected probably due to multi-step program. The LODs and enrichment factor obtained by static SPSE were worse than that obtained by manual SBSE, mainly because the utilization rate of desorption solvent in manual SBSE was higher than that in static SPSE. For manual SBSE, the desorption solvent volume was 90 μL in which 70 μL was injected for subsequent HPLC analysis; while in static SPSE, the desorption solvent volume was 150 μL in which 100 μL was injected for subsequent HPLC analysis. When the sample volume was increased to 10 mL, the automated SPSEs (circular flow SPSE and continuous flow SPSE) provided lower LODs than manual SBSE for the target PAHs (except for PHE) because the extraction efficiency of stir plate with larger surface area over the stir bar would be improved greatly with the increase of the sample volume, although the utilization rate of desorption solvent was a little lower. The LODs of PAHs obtained by circular flow SPSE and continuous flow SPSE were at the same level but the LODs of PAHs obtained by circular flow SPSE were a little higher than those obtained by continuous flow SPSE, which resembled the results of extraction efficiency (calculated as the ratio of practical enrichment factor and theoretical enrichment factor) of these two methods. It is interesting that the extraction efficiency of continuous flow SPSE for all the six PAHs is a little higher than that of circular flow SPSE in the same extraction conditions. Since in the coating of PDMS/β-CD/DVB, PDMS contributes the most to the extraction [13], this can be explained by the extraction mechanism of partition equilibrium of the analytes between sample solution and coating for PDMS/β-CD/DVB-coated SPSE. For circular flow SPSE, the concentration of the analytes in the sample solution was continuously decreasing, while the concentration of the analytes in coatings was continuously increasing until the extraction equilibrium was reached. For continuous flow SPSE, fresh sample solution was kept pumping through the stir plate and the concentration of the analytes in the sample solution are almost invariable; much more analytes were extracted into the coating to achieve the extraction equilibrium, resulting in an improved extraction efficiency.

From Fig. 4, it could be seen that the enrichment factor obtained by manual SBSE is higher than those of static SPSE and automated SBSE for the target PAHs except for NAP because the utilization rate of desorption solvent in manual SBSE is higher than that in automated SPSE and SBSE (commercial stir bar). However, it was found that automated SPSEs exhibited much higher extraction efficiency than manual SBSE for NAP, ANA and FLT, and continuous flow SPSE showed the highest extraction efficiency for NAP, ANA, FLT and PYR in manual SBSE,

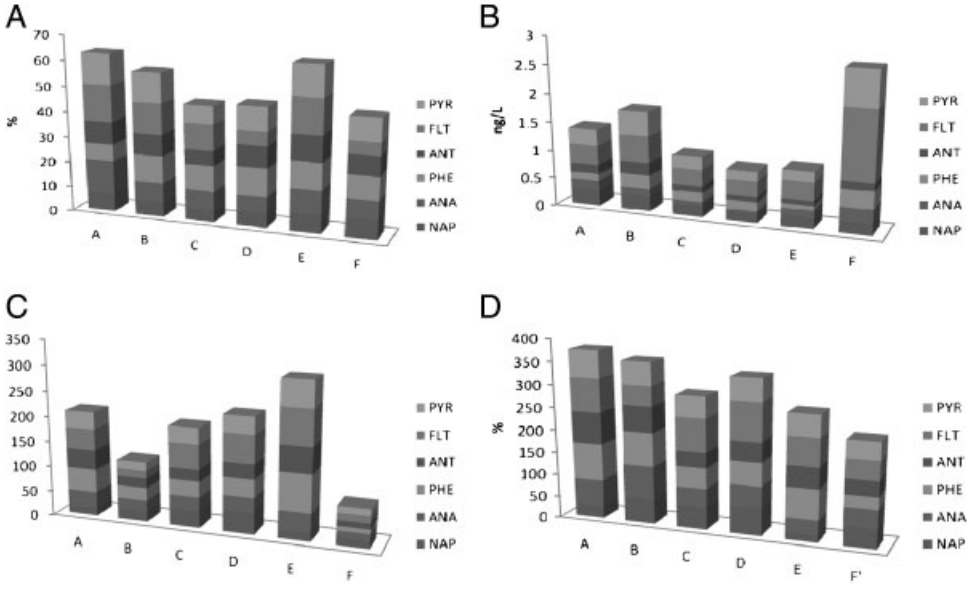

Figure 4. Comparison of (A) RSDs, (B) LODs, (C) enrichment factors and (D) extraction efficiencies of five extractions.

Table 3. Linear ranges and correlation coefficients of manual SBSE (5 and 10 mL), static SPSE, circular flow SPSE, continuous flow SPSE and continuous flow–commercial SBSE[a)]

Compounds	Linear range (ng/L)						Correlation coefficient (*r*)					
	A	B	C	D	E	F	A	B	C	D	E	F
NAP	1–1000	1–1000	0.5–1000	0.5–1000	1–1000	1–1000	0.9986	0.9988	0.9997	0.9993	0.9940	0.9908
ANA	0.5–1000	0.5–1000	0.5–1000	0.5–1000	0.5–1000	1–1000	0.9997	0.9960	0.9934	0.9999	0.9991	0.9934
PHE	0.5–1000	1–1000	0.5–1000	0.5–1000	0.5–1000	1–1000	0.9974	0.9944	0.9962	0.9958	0.9998	0.9941
ANT	1–1000	1–1000	0.5–1000	0.5–1000	0.5–1000	1–1000	0.9982	0.9960	0.9985	0.9967	0.9996	0.9986
FLT	1–1000	2–1000	1–1000	1–1000	1–1000	1–1000	0.9995	0.9965	0.9976	0.9982	0.9902	0.9826
PYR	1–1000	2–1000	1–1000	0.5–1000	1–1000	1–1000	0.9973	0.9982	0.9986	0.9988	0.9967	0.9869

a) A, manual SBSE (5 mL); B, static SPSE (5 mL); C, circular flow SPSE (10 mL); D, continuous flow SPSE (10 mL); E, manual SBSE (10 mL); F, continuous flow–commercial SBSE (10 mL).

Table 4. PAHs concentrations in tap water and Yangzi River water

	Tap water			Yangzi River		
	Added (ng/L)	Found (ng/L)	Recovery (%)	Added (ng/L)	Found (ng/L)	Recovery (%)
NAP	0	–	–	0	–	–
	25	24 ± 2	96	50	45 ± 3	89
ANA	0	–	–	0	60 ± 4	–
	25	26 ± 2	104	50	113 ± 7	106
PHE	0	–	–	0	–	–
	25	23 ± 3	92	50	53 ± 6	106
ANT	0	–	–	0	–	–
	25	26 ± 3	104	50	44 ± 3	88
FLT	0	–	–	0	–	–
	25	27 ± 4	108	50	55 ± 5	110
PYR	0	–	–	0	–	–
	25	26 ± 2	104	50	51 ± 3	102

continuous flow SPSE and circular flow SPSE by using 10 mL sample solution, as shown in Fig. 4d. This indicated that not only the increase of coatings but also the continuous sample introduction would improve the extraction efficiency of SPSE/SBSE for the most of target PAHs.

The LODs and extraction efficiency of PAHs obtained by continuous flow SBSE using the commercial stir bar are not as good as expected. The main reason for this is that the extraction time of 20 min was too short to achieve the extraction equilibrium and the utilization rate of desorption solvent (100 of 300 μL eluent for injection) was very low. The LODs obtained by continuous flow SBSE could be improved remarkably with the extraction time extended to 4 h, as shown in Fig. 4. Table 3 listed the linear ranges and correlation coefficients obtained by all these six extraction methods.

It should be pointed out that a carry-over effect was found in the automated system. If the concentration of target analyte was lower than 10 ng/L, the system should be cleaned by about 2 mL methanol and 2 mL high purity water sequentially for two times before switching on the program for next SPSE or SBSE.

3.4 Sample analysis

Generally, the concentration level of PAHs is very low in environmental water samples but the available amount of environmental water is abundant; hence, tap water and Yangzi River water samples were selected for the determination of the six target PAHs by the continuous flow SPSE-HPLC-FLD system. As can be seen from the results summarized in Table 4, no target analyte was found in tap water but 60 ± 4 ng/L of ANA was found in Yangzi River water and the recoveries of the target analytes for the spiking water samples ranged from 92 to 108% for tap water and from 88 to 110% for Yangzi River water. Figures 5 and 6 are the chromatograms of sample analysis obtained by continuous flow SPSE-HPLC-FLD.

4 Concluding remarks

An automated SBSE-HPLC-FLD system was developed for the first time. With self-made PDMS/β-CD/DVB-coated SPSE instead of conventional SBSE, three different automated SPSE

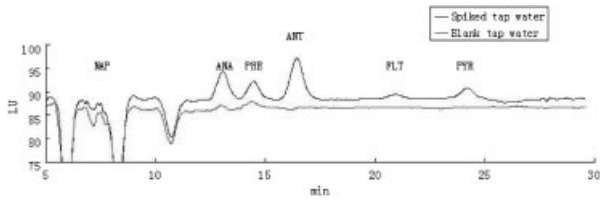

Figure 5. HPLC-FLD chromatograms of tap water sample obtained by continuous flow PDMS/β-CD/DVB-coated SPSE. Spiking concentration for all target compounds is 25 ng/L.

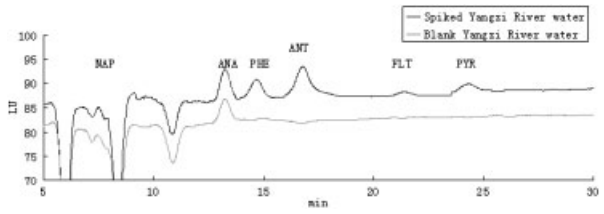

Figure 6. HPLC-FLD chromatograms of Yangzi River water sample obtained by continuous flow PDMS/β-CD/DVB-coated SPSE. Spiking concentration for all target compounds is 50 ng/L.

operation modes, including static SPSE, circular flow SPSE and continuous flow SPSE, were designed and optimized for online HPLC-FLD determination of six target PAHs, and SBSE in manual and automated continuous flow mode was also investigated for comparison. Continuous flow SPSE exhibited slightly better enrichment factors and extraction efficiencies than circular flow SPSE and manual SBSE, probably due to continuous introduction of fresh sample solution into the sample vial. The improved reproducibility by automated SPSE or SBSE was not as much as expected, maybe due to the designed multi-step program. The automated SPSE provided a relatively short time (117–180 s) to finish sampling, washing and sample loading, whereas it would take 5–10 min to fulfill these processes for the conventional manual SBSE. Besides being labor-saving and time-saving, automated SPSE is reliable, effective, has no time limit and is a non-attended operation. However, although automatic extraction, desorption and sample loading could be achieved in this work, automation of sample injection has not been realized. To solve this problem, further research is under way.

Financial supports from the Science Fund for Creative Research Groups of NSFC (No. 20621502, No. 20921062), National Nature Science Foundation of China (20775057) and MOE of China (NCET-04-0658) are gratefully acknowledged.

The authors have declared no conflict of interest.

5 References

[1] Rosenfeld, J. M., *Sample Preparation for Hyphenated Analytical Techniques*, Blackwell Publishing, UK 2004, p. 1.

[2] Pavlovic, D. M., Babic, S., Horvat, A. J. M., Kastelan-Macan, M., *Trends Anal. Chem.* 2007, *26*, 1062–1075.

[3] Beyer, A., Biziuk, M., *Food Chem.* 2008, *108*, 669–680.

[4] Ridgway, K., Lalljie, S. P. D., Smith, R. M., *J. Chromatogr. A* 2007, *1153*, 36–53.

[5] O'Reilly, J., Wang, Q., Setkova, L., Hutchinson, J. P., Chen, Y., Lord, H. L., Linton, C. M., Pawliszyn, J., *J. Sep. Sci.* 2005, *28*, 2010–2022.

[6] Vreulsa, J. J., Louterb, A. J. H., Brinkmana, U. A. T., *J. Chromatogr. A* 1999, *856*, 279–314.

[7] Ouyang, G., Zhao, W., Pawliszyn, J., *J. Chromatogr. A* 2007, *1138*, 47–54.

[8] Hennion, M.-C., *J. Chromatogr. A* 1999, *856*, 3–54.

[9] Hyotylainen, T., Riekkola, M.-L., *Trends Anal. Chem.* 2007, *26*, 788–808.

[10] Lambropoulou, D. A., Albanis, T. A., *Anal. Bioanal. Chem.* 2007, *389*, 1663–1683.

[11] Buchberger, W., Zaborsky, P., *Acta Chim. Slov.* 2007, *54*, 1–13.

[12] David, D., Sandra, P., *J. Chromatogr. A* 2007, *1152*, 54–69.

[13] Yu, C., Yao, Z., Hu, B., *Anal. Chim. Acta* 2009, *641*, 75–82.

[14] Popp, P., Bauer, C., Wennrich, L., *Anal. Chim. Acta* 2001, *436*, 1–9.

[15] Kolahgar, B., Hoffmann, A., Heiden, A. C., *J. Chromatogr. A* 2002, *963*, 225–230.

[16] Zuin, V. G., Montero, L., Bauer, C., Popp, P., *J. Chromatogr. A* 2005, *1091*, 2–10.

[17] Perez-Carrera, E., Leon, V. M. L., Parra, A. G., González-Mazo, E., *J. Chromatogr. A* 2007, *1170*, 82–90.

