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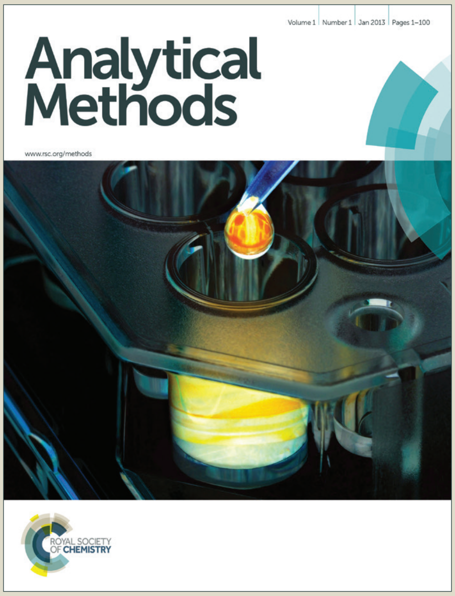

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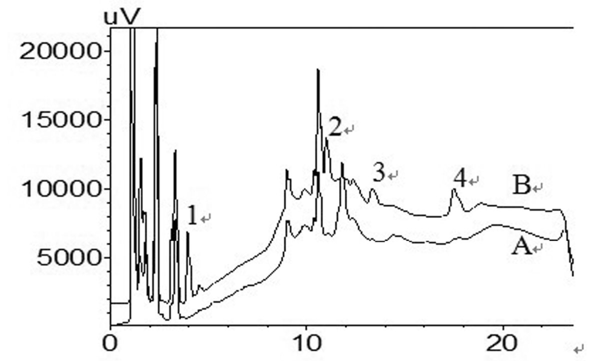

The PVDF membrane was used as the extraction phase in TFME for the first time, which developed for the extraction of some endocrine disrupting chemicals in aqueous samples prior to their determination by HPLC.

Thin-film microextraction for the preconcentration of some endocrine disrupting chemicals in aqueous samples before chromatographic analysis

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Abstract: Poly (vinylidene fluoride) (PVDF) has attracted enormous attention as a membrane material with regard to its excellent properties in recent years. In this paper, a new sample preparation technique, the PVDF membrane as the extraction phase has been applied to the extraction of four endocrine-disrupting compounds: Bisphenol A (BPA), 4-*t*-octylphenol (4-*t*-OP), nonylphenol (NP), and 4-nonylphenol (4-NP) from water samples for the first time, prior to their determination by high performance liquid chromatography–ultraviolet detection. Some important experimental parameters affecting the extraction efficiency were optimized. Good linearity was observed over the concentration range of 1.0–100.0 ng mL^{-1} for BPA, and 2.0–100 ng mL^{-1} for 4-*t*-OP, NP and 4-NP with the correlation coefficients (r) greater than 0.9934. The limit of detection ($S/N = 3$) of the method was 0.2 ng mL^{-1} for BPA, and 0.4 ng mL^{-1} for 4-*t*-OP, NP and 4-NP. The method is convenient, cost effective and can be a useful alternative approach for the extraction and determiantion of the endocrine-disrupting compounds in water samples.

Keywords: Thin-film microextraction; Poly (vinylidene fluoride); Endocrine-disrupting compounds; High performance liquid chromatography

Introduction

Bisphenol A (BPA) is mostly applied to manufacture polycarbonate plastics and epoxy resins. It can be found in a number of products and materials that consumers can easily encounter everyday.[1] 4-*t*-octylphenol (4-*t*-OP), nonylphenol (NP), and 4-nonylphenol (4-NP) are widely used as surfactant materials. They may leak into the environment through urban and industrial wastewaters.[2] BPA, 4-*t*-OP, NP, and 4-NP are considered to be endocrine disrupting compounds (EDCs).[3] The United States Environmental Protection Agency (EPA) classifies these contaminants as priority pollutants.[4] Monitoring the widely exsited EDCs in environment is of great interest.

Several different sample preparation methods, such as solid phase extraction (SPE),[5, 6] liquid–liquid extraction (LLE),[7] dispersive liquid liquid microextraction (DLLME),[8-11] solid-phase microextraction (SPME),[12, 13] liquid-phase microextraction (LPME),[3] and emulsion liquid membrane (ELM) extraction,[14-16] have been developed for the extraction of environmental contaminants from different samples. However, SPE and LLE are time-consuming, laborious, and need large volumes of samples and toxic organic solvents. For SPME, the extraction fiber is fragile, relative expensive, the small volume of the extraction phase and a limited life-time.[17] In the ELM process, three steps, i.e. emulsification, extraction, and demulsification, are included. And ELM was limited by the emulsion instability. Recently, thin film microex-traction (TFME), a new geometry for solid-phase microextraction, was introduced by Pawliszyn.[18] In TFME, a thin and wide membrane is employed as a sorbent for microextraction procedure. TFME has higher extraction efficiency and load ability due to the larger volume of its extraction phase and its higher surface-to-volume ratio. The thin film of polydimethylsiloxane (PDMS) membrane,[18, 19] PDMS tape,[20] polyaniline-nylon-6 nanofiber film,[21] thin sheet of C18,[22] ethylene/vinyl acetate film,[23] chemically modified cellulose paper,[24] polyacrylonitrile–polystyrene–divinylbenzene, polyacrylonitrile–phenylboronic acid,[25] and octadecyl-silica glass[26] have been used as the extracting phase in TFME.

Poly (vinylidene fluoride) (PVDF) is one of the more popular microporous membrane materials. PVDF has attracted enormous attention as a membrane material with regard to its excellent properties such as high mechanical strength, thermal stability, chemical resistance, and high hydrophobicity, compared to other commercialised polymeric materials.[27] PVDF membranes have been extensively applied in ultrafiltration and microfiltration for ordinary separation purposes, and are currently being explored as potential candidates in the applications of wastewater treatment.[28, 29]

In this paper, the PVDF membrane was used as the extraction phase in TFME for the first time. The extraction performance of PVDF membrane for BPA, 4-*t*-OP, NP, and 4-NP in water samples was investigated. Some important experimental parameters affecting the extraction efficiency were also optimized.

Theoretical considerations

The specific surface area of the extraction phase determines the equilibration time and extraction rate, and the fluid contacting the surface of the extraction phase is always stationary. This static layer of defined thickness is the Prandtl boundary layer.[30] Theoretically, the initial rate of TFME extraction is directly proportional to the surface area of the extraction phase.[31] According to the aspect of SPME extraction kinetics, extraction kinetics can be improved when the traditional cylindrical fiber geometry is changed to a thin film configuration which will provide a faster extraction rate for the analytes of interest.[32, 33]

Experimental

Chemicals and materials

BPA, 4-*t*-OP, NP, and 4-NP were purchased from Aladdin-reagent (Shanghai, China). The chemical structures of the endocrine-disrupting compounds are shown in Fig. 1. Chromatography-grade acetonitrile, methanol, and other chemicals (acetone, hydrochloric acid, and sodium hydroxide) were purchased from Huaxin Chemical Reagent Company (Baoding, China). Sodium chloride (NaCl) was from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). The water used throughout the work was purified by a SZ-93 automatic double-distiller purchased from Yarong Biochemistry Instrumental Factory (Shanghai, China). A 85-2B temperature-controlled magnetic stirrer was obtained from Jintan (Jiangsu, China).

PVDF membrane (50 mm diameter and 0.45 μm average pore size) was bought from Xingya (Shanghai, China). Polypropylene, nylon and polytetrafluoroethylene membrane were purchased from Taoyuan (Haining, China).

Reservoir water was collected from Angezhuang reservoir (Baoding, China), and lake water was collected from Wumazhuang (Baoding, China).

A mixture stock solution containing each of BPA, 4-*t*-OP, NP, and 4-NP at 10.0 mg mL^{-1} were prepared in methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with methanol in a 10 mL volumetric flask. All the standard solutions were stored at 4 ^{o}C and protected from light.

Fig. 1

Instruments

The high performance liquid chromatography (HPLC) system (Shimadzu, Japan) was equipped with two LC-20AT VP pumps, a SPD-20A ultraviolet detection (UV) /vis detector and a LC-solution software. Chromatographic separations were performed on a Promosil C_{18} column (150 mm × 4.6 mm I.D., 5.0 μm) from Bonna-Agela technologies (Tianjin, China). Acetonitrile–water was used as mobile phase. The system was run in the following gradient elution: 0-5 min, from 50% acetonitrile to 80% acetonitrile, held for 15 min. After 20 min, 80% acetonitrile to 50% acetonitrile, stopped at 25 min. The flow rate of the mobile phase was 1 mL min^{-1}. The UV monitoring wavelengths were chosen at 225 nm. The sample injection volume was 15.0 μL.

Preparation of membrane and water samples

A square piece of the membrane (2.5 cm^2) were washed with acetone in an ultrasonic bath for 5 min to remove the possible contaminants in the membrane and dried. Then it was hanged vertically in a glass vial.

Before use, reservoir water and lake water samples were filtered through 0.45 μm micro-pore membranes and stored in brown glass containers at the temperature of 4 ^{o}C.

PVDF based TFME procedure

Extraction was performed as follows: the prepared membrane was immersed in 20.0 mL of sample solution in a 25 mL glass vial, and stirred at 600 rpm for 30 min. After extraction, the membrane was taken out from the sample solution, and dried gently with filter paper. It was then transferred into a 0.5-mL centrifuge tube and 100.0 μL acetonitrile was added for desorption of the adsorbed analytes by vortexing for 1 min. Finally, 15.0 μL desorption solution was injected into HPLC for analysis.

Results and discussion

In order to obtain a high extraction efficiency, several important parameters, including extraction time, the pH of the sample solution, salt addition, stirring rate as well as the desorption condition were investigated. 20.0 mL sample solution spiked with 50.0 ng mL^{-1} each of the EDCs was employed to optimize the experimental conditions. All the experiments were performed in triplicate and the means of the results were used for evaluation.

Effect of the type of membrane materials

We have also investigated other membrane materials as film absorbers, such as polypropylene, nylon and polytetrafluoroethylene. According to the results, PVDF membrane achieved better extraction efficiency towards the target compounds than other membranes, so we select PVDF as membrane material.

Effect of the sample solution pH

The pH of the solution plays an important role for the extraction efficiency in membrane extraction technique. pH changes the formation of the analytes and thereby the adsorption of analytes into the membrane can be enhanced. So, the influence of the sample pH on the extraction efficiency was investigated by adjusting the pH in the range of 2–10 via adding 1.0 mol L^{-1} sodium hydroxide solution or 1.0 mol L^{-1} hydrochloric acid. Fig. 2 (A) shows that the peak areas of BPA, 4-*t*-OP, NP, and 4-NP had no significant change in the range of pH 2-7, but obviously decreased in the range of pH 8-10. When pH is above 7, the solubility of these compounds in aqueous solution increased owing to their phenolic hydroxyl group. The pH of the water samples were below 7, so the pH of the sample solution was not adjusted in the subsequent experiments.

Fig. 2 (A)

Effect of the ionic strength

Addition of salt to the aqueous sample can decrease the solubility of analytes in the aqueous phase and enhance the availability of analytes for extraction. Nevertheless, it could increase the viscosity and density of the aqueous phase. The effect of the concentrations of NaCl on the extraction efficiency was investigated in the range from 0 to 12% (w/v) at an interval of 3% in the sample solution. Fig. 2 (B) shows that salt addition had no significant effect on peak areas of these analytes. Consequently, no NaCl was added for further analysis.

Fig. 2 (B)

Effect of the extraction time

Membrane extraction is an equilibrium-based extraction procedure and therefore a time-dependent process. In this study, the extraction time was tested from 5 to 60min, and the results were shown in Fig. 2 (C). It was found that the peak areas of the analytes increased with increasing extraction time to 30 min. And longer extraction times did not have much effect on the analytes. The extraction process time of 30 min was therefore chosen.

Fig. 2 (C)

Effect of the stirring rate

Magnetic stirring accelerates extraction and shortens the time needed to reach thermodynamic equilibrium between the aqueous and the membrane. In general, the larger the stirring rate, the faster the mass transfer between the aqueous phase and the membrane. However, when stirring rate was too fast, air bubbles are tending to form outside the membrane. These could affect the extraction accuracy and reproducibility. In this work, the stirring rate range studied was 200–1000 rpm. As shown in Fig. 2 (D), the peak areas of the analytes increased with increasing the stirring speed from 200 to 600 rpm and when the stirring rate was greater than 600 rpm, the peak areas kept almost constant. Therefore, 600 rpm was selected.

Fig. 2 (D)

Effect of the desorption condition

After extraction, the membrane was transferred to a centrifuge tube for desorption. In this experiment, three different organic solvents, i.e., acetonitrile, methanol and acetone, were tested as the desorption solvent. The results showed that the desorption power of acetonitrile was mainly similar to either acetone or methanol. Considering to the mobile phase and the shape of the chromatographic peak, acetonitrile was selected as the desorption solvent. The volume of acetonitrile was optimized in the range from 30 to 100 μL and as a result, 100 μL yielded the best desorption result. Desorption time was investigated by vortexing the tube for the time in the range from 1 to 5 min. As a result, there was no obvious difference between the tested desorption times. 1 min was chosen.

Validation of the method

A series of experiments with regard to the linear range (LR), limits of detection (LOD), and repeatability were performed to validate the proposed method under the optimized extraction conditions. The results are listed in Table 1. Calibration curves were established for all the analytes in the concentration range of 1.0–100.0 ng mL^{-1} using six spiked concentrations in double-distiller water. For each concentration five replicate extractions and determinations were performed. Good linearity was observed over the concentration range of 1.0–100.0 ng mL^{-1} for BPA, and 2.0–100 ng mL^{-1} for 4-*t*-OP, NP, and 4-NP with the correlation coefficients (r) greater than 0.9934. Based on the signal-to-noise ratio of 3 ($S/N = 3$), the LOD was 0.2 ng mL^{-1} for BPA and 0.4 ng mL^{-1} for 4-*t*-OP, NP, and 4-NP. The relative standard deviations (RSDs, $n = 5$) at the concentration of 10.0 μg L^{-1} for each of the analytes were from 6.5% to 7.3%. These results showed that the method had a high sensitivity and good repeatability.

Table 1

The enrichment factor (*EF*) was defined as the ratio between the analyte concentration in the desorption solution (100 μL acetonitrile) and the initial analyte concentration in the sample solution. Under the above optimized experimental conditions, the enrichment factors of this method for BPA, 4-*t*-OP, NP, and 4-NP were 34, 43, 54, and 41, respectively. And the absolute extraction recoveries of BPA, 4-*t*-OP, NP, and 4-NP were 17%, 22%, 27% and 21%, respectively.

Analysis in the real sample

In order to test the applicability of the developed method, the method was applied to analyze the analytes in different environmental water samples, including reservoir, and lake water samples. The results are shown in Table 2. No residues of the analytes were detected in reservoir water sample and only low concentration of 4-NP (2.32 ng mL^{-1}) was found in lake water sample. In order to validate the accuracy of the method, the water samples were spiked with the four analytes standards at the concentrations of 10.0 and 50.0 ng mL^{-1}, respectively. For each concentration level, five replicate experiments were operated. The recoveries for the four analytes fell in the range from 86.0% to 108.7%, indicating a good accuracy of the method. Fig. 4 shows the typical chromatograms of the lake water sample before and after being spiked at 10.0 ng mL^{-1} each of the four analytes.

Table 2

Fig. 4

Comparison of PVDF-TFME method with other sample preparation methods

To evaluate the performance of PVDF-TFME method, a comparison between the current method and other reported methods coupled with HPLC was conducted from the viewpoint of linearity, LOD and RSD.[12, 34-37] The comparative results are listed in Table 3. It can be observed that the current method has comparable or even lower LODs with those of the other reported methods except for MSPE. Moreover, the PVDF membrane used in the current method is disposable because of its cheapness, which can prevent the possible cross-contaminations and carry-over effects. In addition, the current method is easy to handle and more environmental friendly. Therefore, the PVDF-TFME method is indeed simple, rapid, and sensitive for the determination of the analytes in environmental water samples.

Table 3

Conclusions

In the present research, a simple and sensitive PVDF membrane extraction combined with HPLC-UV detection was developed for the determination of BPA, 4-*t*-OP, NP, and 4-NP in environment water samples. The membrane in the method is disposable, the carry-over problems can be overcame. Moreover, the method does not need special apparatus and other additional clean-up processes. The method can be a useful alternative approach for the analysis of the contaminants in complex samples.

Acknowledgements

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Table Captions

Table 1 The linear ranges (LRs), correlation coefficients (*r*), limits of detection (LODs) and relative standard deviations (RSDs) in reservoir, and lake water samples.

Table 2 Recoveries obtained for the determination of BPA, 4-*t*-OP, NP, and 4-NP in water samples.

Table 3 Comparison of PVDF-TFME method with other sample preparation methods.

Figure Captions

Fig. 1 Chemical structures of BPA, 4-*t*-OP, NP, and 4-NP.

Fig. 2 Effect of extraction conditions on PVDF membrane microextraction efficiency. (A) Effect of the sample solutions pH. (B) Effect of salt addition. (C) Effect of extraction time. (D) Effect of stirring rate.

Fig. 3 The typical chromatograms for lake water (A) and the lake water sample spiked with BPA, 4-*t*-OP, NP, and 4-NP at 10.0 ng mL^{-1} (B). Peak identification: (1) BPA, 4-*t*-OP, NP, and 4-NP. Detection wavelength: 225 nm.

Table 1 The linear ranges (LRs), correlation coefficients (*r*), limits of detection (LODs) and relative standard deviations (RSDs) in reservoir, and lake water samples.

Analytes	LRs (ng mL^{-1})	*r*	LODs (ng mL^{-1})	RSDs (%) (*n* = 5)
BPA	1.0-100.0	0.9988	0.2	6.5
4-*t*-OP	2.0-100.0	0.9964	0.4	6.8
NP	2.0-100.0	0.9934	0.4	7.0
4-NP	2.0-100.0	0.9975	0.4	7.3

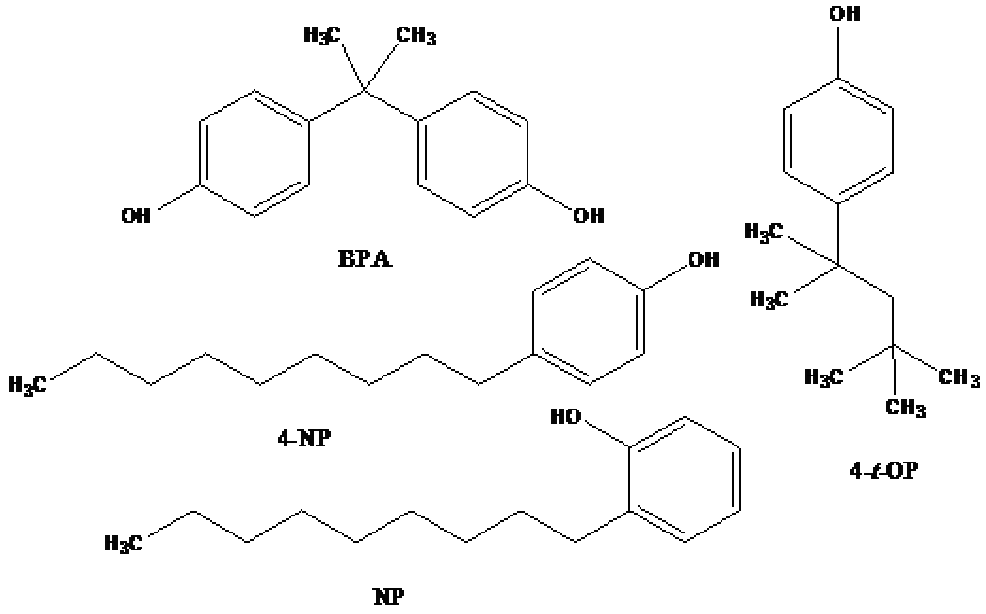
Table 2 Recoveries obtained for the determination of BPA, 4-*t*-OP, NP, and 4-NP in water samples.

Analytes	Spiked (ng mL^{-1})	Reservoir water (n = 5)			Lake water (n = 5)		
		Found (ng mL^{-1})	R^{a} (%)	RSDs (%)	Found (ng mL^{-1})	R^{a} (%)	RSDs (%)
BPA	0.0	nd^{b}			nd^{b}		
	10.0	9.53	95.3	6.6	10.06	100.6	6.5
	50.0	49.1	98.2	6.8	52.26	104.5	6.9
4-*t*-OP	0.0	nd^{b}			nd^{b}		
	10.0	8.93	89.3	7.0	8.60	86.0	6.7
	50.0	48.3	96.6	6.7	47.34	94.7	7.0
NP	0.0	nd^{b}			nd^{b}		
	10.0	9.76	97.6	7.4	10.87	108.7	6.9
	50.0	50.4	100.8	6.8	48.66	97.3	6.8
4-NP	0.0	nd^{b}			2.32		
	10.0	9.90	99.0	6.9	12.0	96.8	7.2
	50.0	49.63	99.3	7.2	53.57	102.5	6.9

R^{a}: recovery of the method; nd^{b}: not detected.

Table 3 Comparison of PVDF-TFME method with other sample preparation methods.

Methods	LRs (ng mL^{-1})	LOD (ng mL^{-1})	RSD (%)	Samples	References
DLLME	1.0–300.0	0.23-0.48	4.6-5.5	water	34
SPME	1.0-90.0	0.32–0.52	1.3-8.4	water	12
MSPE	--	<0.036	<34	water	35
HF-LPME	--	0.54	<4.8	water	36
SPE	10-150000	2.8-5.6	<11.4	serum	37
PVDF-TFME	1.0-200.0	0.2-0.4	<7.3	water	This method



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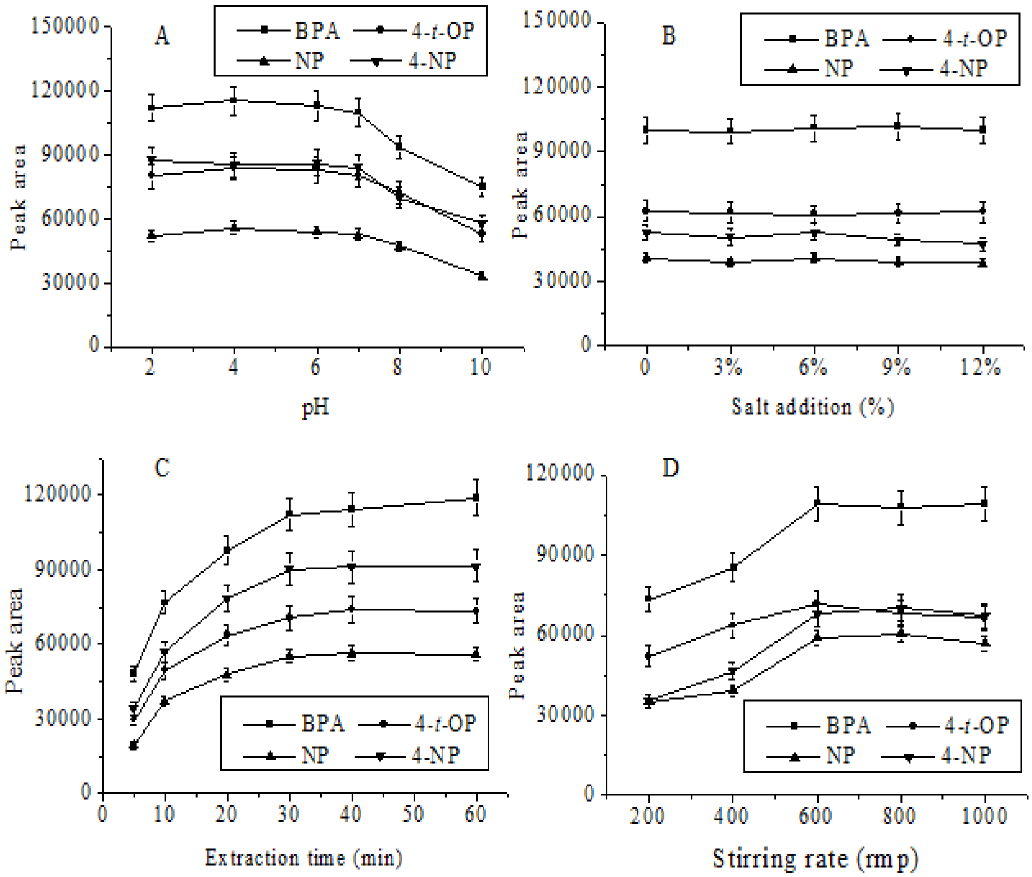
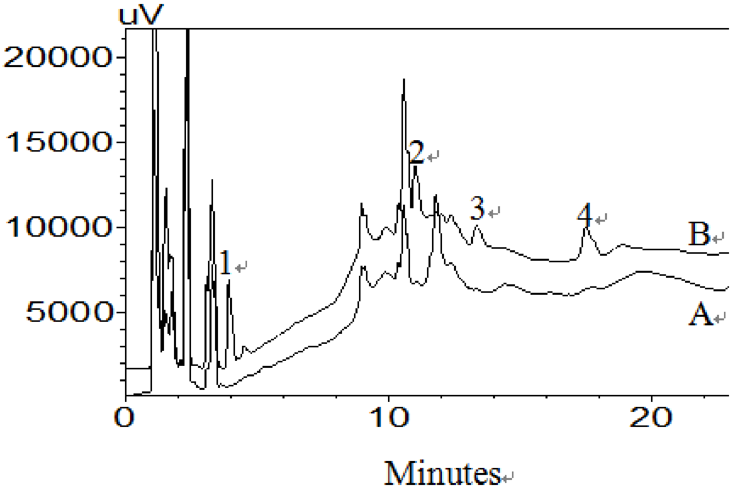


Fig. 2 Effect of extraction conditions on PVDF membrane microextraction efficiency (*n* = 3). (A) Effect of the sample solutions pH. (B) Effect of salt addition. (C) Effect of extraction time. (D) Effect of stirring rate.



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