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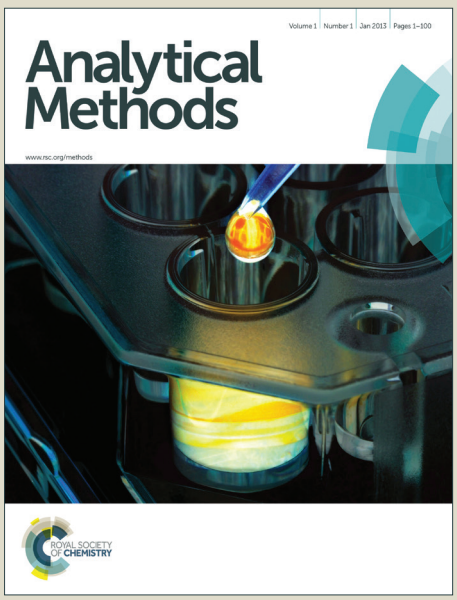

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Novel Sample Preparation Technique based on Functional Nanofibers Mat for sensitive and precise determination of Phenolic Environmental Estrogens in Environmental Water

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Abstract

Present study described a novel sample preparation technique for phenolic environmental estrogens (PEEs) including bisphenol A (BPA), nonylphenol (NP) and octylphenol (OP) in environmental water samples. Hydrazine-modified polyacrylonitrile (HM-PAN) nanofibers mat followed by amino group functionalization was prepared with electrospining technique. A disks solid-phase extraction (SPE) was developed utilizing the functional nanofibers mat as sorbent for extraction of PEEs prior to high performance liquid chromatography- tandem mass spectrometry (HPLC-MS/MS). Critical factors affecting the extraction efficiency of sorbent to target analytes were tested and optimized. Under optimized condition, home-made disks SPE device for simple and fast sample preparation procedure was achieved with noticeable reduced sorbent bed mass (4.0 mg) and eluent volume (600 μL). The detection limits for the targeted PEEs were in the range of 0.01- 0.03 ng mL^{-1} (S/N=3). We validated the applicability of the proposed method using four real environmental water samples and satisfactory spiked recoveries in the range of 84.6 - 101.9% with low RSD ≤ 8.6% (n=6) were achieved. A comparison of present method with the existing methods for the determination of PEEs was made and the results exhibited that proposed method had better recoveries, sensitivity and reproducibility. The results demonstrated that HM-PAN nanofibers mat based SPE

technique was a viable alternative for sensitive and precise analysis of PEEs in environmental water.

Keywords

Nanofibers mat; Sample preparation; Phenolic environmental estrogen; Disks solid-phase extraction; Water sample

1. Introduction

Biphenyl A (BPA), a primary raw material, is widely used in the industry as an important intermediate in the production of epoxy resins and polycarbonates plastic[1]. Nonylphenol (NP) and octylphenol (OP) are the main degradation products of alkylphenols polyethoxylates, which are extensively utilized in the production of elasticizers, abstergents and pesticide emulsifiers [2]. These phenolic compounds can immigrate into the aquatic environment from household or industry products and may end up in ground water or surface waters[3, 4]. As representative and ubiquitous phenolic environmental estrogens (PEEs) in environmental water, BPA, NP and OP are endocrine disrupters [5] and show toxic effect to reproductive system, nervous system and immune system of aquatic organism and humans even at very low concentration (ng L-1 or μg L-1) [6-8]. Alkylphenols (APs) have been sent onto preferred controlled pollutant black list by the Japan's environment ministry, European Commission and Canada Environmental Protection Agency [9]. Therefore, the pollution from PEEs has drawn worldwide increasing attention, and monitoring the residuals of PEEs in environmental water is essential.

Due to low PEEs concentration and complex matrix, sample pretreatment is usually required for the analysis of PEEs in environmental water. Nowadays, some sample preparation techniques like liquid-liquid extraction (LLE) [10], ultrasonic extraction (USE) [11], microwave-assisted extraction (MAE) [12], pressurized liquid extraction (PLE) [13], solid-phase micro-extraction (SPME) [14], and solid-phase extraction (SPE) have been proposed for the extraction of PEEs. SPE is the most frequently employed method because it is environment-friendly, easy to operate, and can provide high enrichment factors. It is believed that that the heart of SPE technique is

sorbent material [15], and the effective and selective SPE sorbent is a key point to perform SPE excellently. So, the search for new optimum sorbent seems to be a never-ending task in the environmental analytical chemistry and pollutants monitoring.

Compared with traditional SPE sorbents, nanomaterials can offer higher extraction capacity and extraction efficiencies due to their very large surface areas. Electrospining is the most frequently used method to prepare nanofibers. Some researchers[16-19], and our group[20-24], have explored the application of electrospun nanofibers as SPE sorbent. As shown in our previous studies, electrospun nanofibers own a high surface-to-volume ratio and length-to-diameter ration, leading to can achieve a larger specific surface and more active situs for adsorption. Accordingly, the attachment of target molecules become stronger, thus, a few sorbent bed mass(about milligrams) and organic solvent ($<$ 1 milliliter) would be sufficient for effective extraction. In addition, electrospun nanofibers are easily obtained as a form of membrane or mat, which makes it convenient for disks SPE. So it is much easier to deal with larger volume samples to obtain a much better enrichment coefficient than the nano-sized sorbent packed column, which always suffers from the high backpressure [25]. Moreover, the most compelling characteristic of electrospun nanofibers is that they are easy to be functionalized via choosing different polymer or modification with diverse functional group [15, 26]. The ability to incorporate various chemical groups prior or post-electrospinning may enable functionalized electrospun nanofibers designable for effective and selective sample preparation applications.

Polyacrylonitrile (PAN), a common and inexpensive commercial product, has been studied for electrospun nanofibers [27]. The electrospun PAN fibers have desirable chemical resistance, thermal stability, low flammability, and good mechanical properties [28]. Furthermore, polymers based on the acrylonitrile group present reactive pendant nitrile groups (–C≡N), which can readily be modified by different kinds of reagents, such as amindoxime [29], hydrazine [30], diethylenetriamine-modified [31] and so on, through nucleophilic addition. Polyaniline-based polymer with amino groups ($–NH_2$) and imino groups (–NH–), has been found to be suitable for the extraction of polar compounds, such as phenols and chlorophenols from environmental samples [32]. Aminopropyl cartridges are used at clean up step for sample pretreatment of BPA and 4-NP in human milk and polyphenols in tobacco [33]. The results indicated that the retaining behavior for

these materials to targets is probably due to the interactions of polar functional groups (–NH_2). Inspired by above work, PAN nanofibers, prepared by electrospinning and modified to introduce –NH_2 on their surface, could be applicable for the extraction of PEEs. However, to the best of our knowledge, there has been no report using modified PAN nanofiber mat on the enrichment and measurement of organic pollutant.

The goal of present study was to develop and optimize a novel sample preparation technique based on disks SPE method with functional nanofibers mat for analyzing the PEEs in environmental water samples. Hydrazine-modified Polyacrylonitrile (HM-PAN) nanofibers mat was prepared and chosen as SPE adsorption medium for the extraction of target analytes prior to a high performance liquid chromatography-mass spectrometry (HPLC-MS). The method was validated and tested by analyzing real water samples.

2. Experimental

2.1 Reagents and materials

BPA, NP and OP were selected as model analytes. For the purpose of internal standard calibration deuterated compounds NP-d_8 and BPA-d_{16} were used. All the high purity standards (>99.0 %) were supplied by Sigma-Aldrich (St. Louis, MO, USA). HPLC grade dichloromethane (DCM), methanol (MeOH), ethanol and ammonium acetate were purchased from Tedia Company, Inc. (Ohio, USA) and Kemiou Chemical Reagent Co., Ltd (Tianjing, China). Analytical grade solvents acetic acid, sodium acetate, hydrazine hydrate 85% and dimethylformamide were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). PAN made by copolymer (91.4% acrylonitrile and 8.6% methlacrylate with Mw=150000) was supplied by J&K Scientific Ltd., (Beijing, China). Milli-Q water (Millipore, Bedford, USA) was used throughout.

A stock solution (0.1 mg mL^{-1}) of PEEs (BPA, NP, OP) was prepared in ultrapure water. All the standard solution was stored at 277 K in the refrigerator. The working solutions at a concentration of 10 μg mL^{-1} were prepared daily by diluting the stock solutions with

MeOH. The internal standard solutions of NP-d_8 and BPA-d_{16} were prepared at 0.1 mg mL^{-1} daily by serially diluting the stock solution with MeOH.

2.2 Preparation of raw PAN nanofibers and HM-PAN nanofibers

Firstly, PAN nanofibers were prepared using the electrospinning method according to the following procedures [34]. Briefly, PAN was dissolved in DMF and stirred at room temperature for 24 h to obtain 15% (w/v) homogeneous solution. The PAN nanofibers mat was collected on the aluminum foil at a high voltage of 10 KV. The solution feed rate was 1.0 mL h^{-1} and the spinneret diameter was 0.5 mm. The distance between the collector and the spinneret was 15 cm.

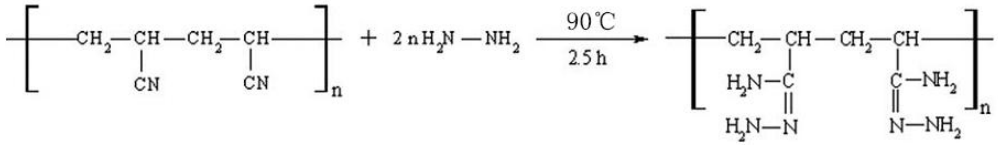
Subsequently, the raw PAN nanofibers were functionalized with amino group by reacting with hydrazine hydrate, transforming nitrile into amidrazone as presented in Scheme 1 [30]. Briefly, hydrazine (100 mL) and the PAN nanofibers mat (0.2 g) were mixed in a reaction vessel and then heated at 363 K for 2.5 h. HM-PAN nanofibers mat was prepared after conversion. The nanofibers mat was washed several times with distilled water and ethanol, and then dried in an oven at 333 K. The conversion of nitrile group in the PAN was calculated as follows[35]:

$$C(\%) = \frac{W_1 - W_0}{W_0} \times \frac{53}{32} \times 100$$

Where C (%) is the conversion percent of nitrile group into amidrazone, W_0 and W_1 represent the weight of the original and modified PAN nanofibers in grams, 32 and 53 denote the molecular weight of hydrazine hydrate and acrylonitrile monomer, respectively.

The surface morphologies of raw PAN and HM-PAN nanofibers were examined under a scanning electron microscopy (SEM, Hitachi S-3000N，Hitachi, Japan). A Fourier transform infrared spectrometer（FT-IR, Nicolet IS10, Thermo Fisher Scientific, USA）was used to analyze the chemical and/or physical interaction over the wave number range of 400 - 4000 cm^{-1}. The surface chemical compositions were analyzed using X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Scientific, USA).

Scheme 1 Reaction of hydrazine with PAN nitrile groups.



2.3 SPE procedure

The HM-PAN nanofibers mat was cut in circular format with a diameter of about 2.0 cm (4 mg), and fixed in a home-made disks SPE device tightly [24]. The nanofibers mat was preconditioned successively with 300 μL MeOH, 300 μL DCM and 500 μL water. A 20 μL of 1 μg mL^{-1} TBBPA and 1 μg mL^{-1} NP-D8 were added to 10 mL lake water sample as internal standard substance. Then lake water samples were pushed through the pre-conditioned filter at the optimum flow rate. The PEEs retained on the mat were eluted with an optimal volume of eluant. The eluent was concentrated to dryness under nitrogen at 310 K, and then reconstituted with mobile phase to a final volume of 0.2 mL for HPLC analysis.

2.4 HPLC–MS/MS analysis

HPLC analysis was performed using a Thermo system, consisting of a Thermo UltiMate 3000 series LC system connected to a Thermo scientific TSQ Vantage series spectrometer system. Chromatography was performed on a Unitary C18 (100 mm × 2.1 mm, 2.8 μm) column at room temperature with an injection volume of 10 μL. The mobile phase consisting of a solvent A (water) and solvent B (MeOH) was delivered at a flow rate of 0.3 mL min^{-1}. The gradient program was as follows: 0-2 min: 90% B, 2-4 min: 98% B, 4-6min: 90% B.

Mass spectrometry was carried out using a triple quadrupole tandem mass spectrometer (TSQ vantage, Thermo scientific, USA) equipped with an electrospray interface (ESI), and with SRM scan mode. Ions were created in the negative ion mode setting the sprayer voltage at 6.0 kV. The capillary temperature and the vaporizer temperature were at 623 K and 573 K respectively. The Sheath Gas Pressure, Aux Gas pressure and Ion sweep Gas pressure were set 40 arb, 15 arb and 5 arb, respectively. The optimized collision energy, precursor, product ions and S-Lens for each analytes are listed in Table 1.

Table 1 MS/MS Parameters of the Target Compounds

Compounds	Qualitative Transition	S-Lens	Collision Energy (eV)
BPA	227.0>212.0	95	21
	227.0>133.0	95	31
NP	219.3>106.0	91	23
	219.3>119.0	91	43
OP	205.2>106.0	103	22
	205.2>119.0	103	43

2.5 Water samples

Four environmental water samples from the Daming Lake (Jinan City, Shandong Province, China) were analyzed to evaluate the feasibility of the developed method. Sample S1-S2 and S3-S4 were respectively taken from the inlet and outlet of Daming Lake (Fig. 1). All the collected water samples were stored in brown glass bottles at 277 K. In order to remove organic impurities, all the collection and storage containers and the laboratory glassware were orderly washed with detergents, water, MeOH and double-distilled water, and dried for subsequent using.

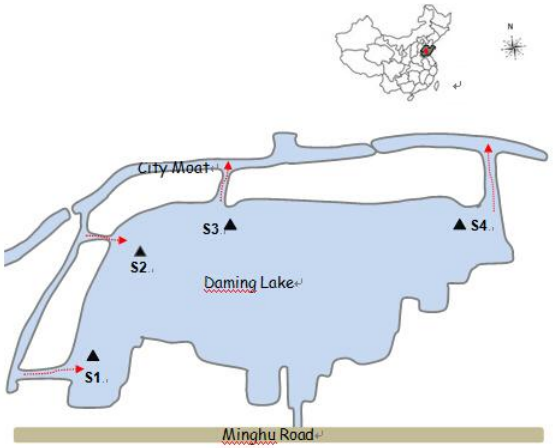


Fig. 1 Sample sites in Daming Lake (Jinan-China).

3. Results and discussion

3.1 Characterization of HM-PAN nanofibers

SEM images in Fig. 2 exhibit the surface morphological characteristic of raw PAN nanofibers and HM-PAN nanofibers. Electrospun PAN nanofibers [Fig. 2(a)] have uniform surface with large length-diameter ratio and high porosity, indicating the large surface area. By contrast, the surface morphology of HM-PAN nanofibers [Fig. 2(b)] showed groove like cracks and more roughness, suggesting that functional groups have combined on the surface of PAN nanofibers. The different average diameters of PAN nanofibers (500 nm) and HM-PAN nanofibers (510 nm) calculated by software Image J also gave the same hint.

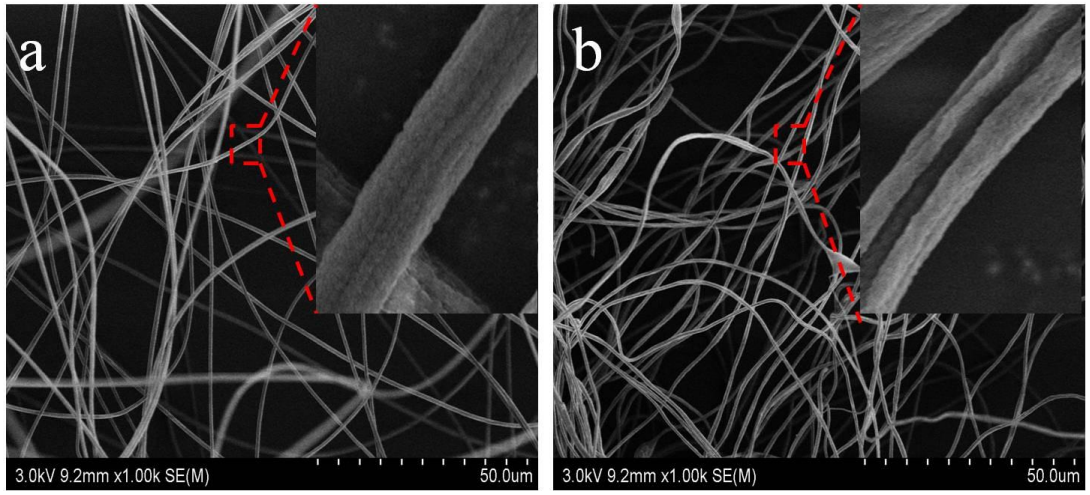


Fig. 2 SEM images of the (a) PAN and (b) HM-PAN nanofibers, which were electrospun from a 15% (w/v) solution.

The surface composition of functionalized and raw PAN nanofibers was examined and contrasted using FT-IR. In the spectrum of raw PAN nanofibers [Fig. 3(a)], the characteristic absorption peak of a stretching vibration at 2241 cm^{-1} (nitrile –C≡N) 1730 cm^{-1} (carbonyl –C=O) 1250 and 1150-1060 cm^{-1} (ether –C–O) indicated that the PAN was a copolymer of acrylonitrile and methylacrylate. A new peak at 3100 - 3400 cm-1 in the spectrum of HM-PAN nanofibers [Fig. 3(b)] was assigned to the N–H stretching vibration of the secondary amino group. Meanwhile, the vibration adsorption of nitrile groups (–C≡N) at 2241 cm-1 decreased significantly in intensity after hydrazine treatment. These results revealed that hydrazine was chemically attached onto the PAN nanofibers to generate amidrazone by partially converting nitrile groups (–C≡N) to amino groups ($–NH_2$) (conversion was measured as 24.8%). Additionally, the peak found at about 1700 - 1630 cm-1 was probably related to the coupling of carbonyl (–C=O) and nitrile groups (–C≡N), whereas the weak band at about 1570 - 1560 cm^{-1} was resulted from the overlapping of the C–N stretching vibration with the N–H bending vibration, also implying

that was embedded in PAN molecular structure.

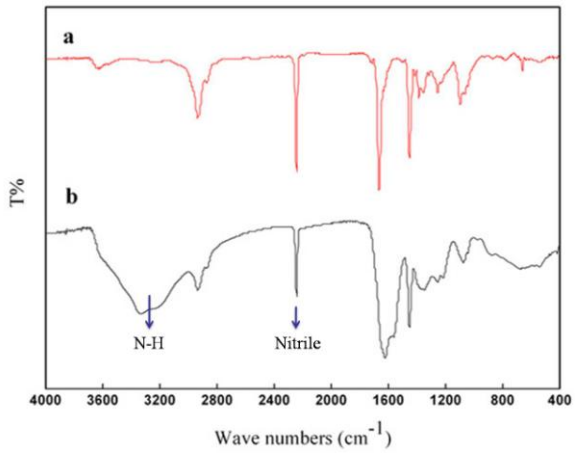


Fig. 3 FTIR spectra of the (a) PAN and (b) HM-PAN nanofibers.

In order to further examine the modification behaviors of hydrazine onto PAN nanofibers surface, the XPS analysis was performed. The N1s peaks for raw PAN and HM-PAN nanofibers mat are shown in Fig. 4. For raw PAN [Fig. 4(a)], the N1s signal has one single component centered at about 399.1 eV due to the –C≡N in PAN chains. When the nitrile groups (–C≡N) is conversed to amino groups ($–NH_2$), the N1s core-level spectrum of the HM-PAN nanofibers mat [Fig. 4(b)] can be fitted into two peak components. The main component of N1s peak at 401.6 eV is attributable to the –NH– species. The minor components at low (399.7 eV) binding energies are assigned to imine nitrogen (=N–) and nitrile groups (–C≡N).

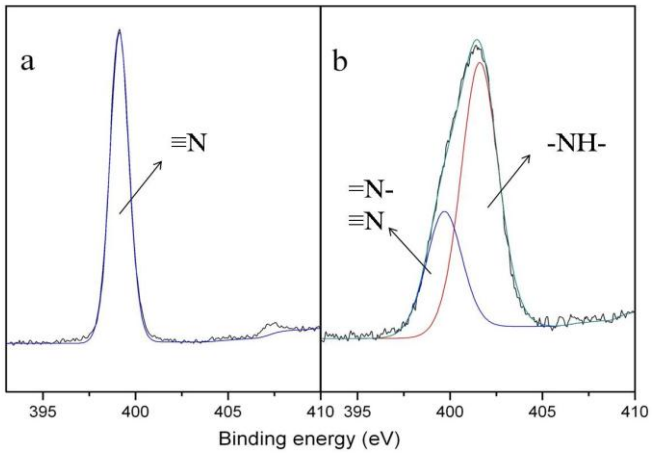


Fig. 4 XPS spectra of the (a) PAN and (b) HM-PAN nanofibers.

All the results above suggested that amino groups ($–NH_2$) were successfully immobilized on the surface of PAN nanofibers via hydrazine functionalization with chemical adsorption.

3.2. Evaluation of the novel sample preparation technique

3.2.1 Comparison of raw and functional nanofibers mat as SPE sorbent

To evaluate the suitability of the functional nanofibers mat, the extraction efficiency of raw PAN nanofibers mat and HM-PAN

nanofibers mat for PEEs in environmental water samples was compared. Three PEEs mentioned above were selected as model analytes. The results demonstrated that under the same experimental conditions, the recoveries for the analytes raised from 70.4 - 92.6% to 84.6 - 101.9% using HM-PAN nanofibers mat as the adsorbent, especially for BPA (loading volume: 10mL).

As we know, the functional groups on the surface have a vital influence on the adsorption efficiency of sorbents. Polar groups were introduced into sorbent material for the extraction of polar analytes because it could increase the wettability of sorbent and provide dipole-dipole and hydrogen bonding interaction sites, enhancing the rention of polar analytes[36]. Dipole-dipole interaction refers to one kind of non-covalent weak interaction among polar molecules. The nitrile groups (–C≡N) in PAN molecular chain, and hydroxyl groups (-OH) in three analytes both belong to polar groups, probably leading to dipole-dipole interaction between raw PAN nanofibers mat and target analytes. However, after hydrazine modification, newly generated amino groups ($–NH_2$) on the surface of HM-PAN nanofibers mat may provide a much stronger interaction force (hydrogen bonding) toward target analytes, except for dipole-dipole interactions. This combined interaction forces from HM-PAN nanofibers mat showed an enhanced extraction efficiency to target analytes, which resulted in significant improvement of extraction capacity. It is also worth noting that compared to NP and OP, the recoveries of BPA were higher using HM-PAN nanofibers mat, which probably attributed to that BPA had two phenolic hydroxyls in molecular structure and NP and OP only had one, so BPA had stronger hydrogen bonding forces with HM-PAN nanofibers mat, thus obtained better extraction efficiencies.

3.2.2 Evaluation of HM-PAN nanofibers mat as SPE sorbent

To evaluate the performance of HM-PAN nanofibers mat as SPE sorbent, important parameters affecting the extraction efficiency, including eluant consumption, adsorbent dosage, pH, sample volume, flow rate of sample were tested and optimized. All the experiments were performed using spiked Milli-Q water (2 ng mL^{-1}) under 3 duplicate samples.

Eluant volume is directly related to efficient elution of retained analytes, but excessive eluant consumption would fail in obtaining high enrichment factor, and is also unfriendly to environment. Therefore, we investigated elution volumes of MeOH and DCM in the

range of 2 × 0.1 to 2 × 0.6 mL. The results (see Fig. S1 in supplementary material) showed that the extraction recoveries of three analytes increased along with increasing elution volumes from 0.1 to 0.3 mL. When elution volumes were above 0.3 mL, variations of recoveries for three analytes were not remarkable, which suggested that 0.3 mL MeOH and 0.3 mL DCM would be enough for eluting target compounds from HM-PAN nanofibers mat.

A significant benefit of promising adsorbents was capable of using as few adsorbent as possible to extract target compounds efficiently. Therefore, the amount of HM-PAN nanofibers mat was examined in the range of 1.0 - 5.0 mg. The results (see Fig. S2 in supplementary material) showed that the recoveries of three analytes increased quantitatively with the amount of HM-PAN nanofibers mat from 1.0 to 4 .0 mg, and the recoveries of three analytes did not change obviously thereafter. The increase of recoveries was due to more available sorption sites. In this paper, only 4.0 mg HM-PAN nanofibers mat was sufficient for the extraction of three analytes under experiment conditions.

The pH of working solutions or samples for SPE is a fatal factor because it determines the surface charge of the adsorbent and the chemical speciation of analytes [37]. The amino groups ($–NH_2$) on HM-PAN can be protonated to form $–NH_3^+$ depending on the pH value of the solution [38]. Our results (see Fig. S3 in supplementary material) indicated that the highest recovery efficiency of three PEEs could obtain at pH 7.0 of working solutions or samples. At low pH solution, a relatively high concentration of protons would be available to protonate amino groups ($–NH_2$) of HM-PAN chain, which suppress the formation of hydrogen bonding between amino groups ($–NH_2$) and phenol hydroxy groups (–OH) from phenol estrogens, resulting in the decrease of adsoption capacity of the PEEs at low pH values. However, at high pH solution, phenols can be hydrolyzed to phenoxides negative ions, also bad for the interaction of hydrogen bonds. These results are in agreement with those obtained by Zhao *et al*, who indicated that neutral initial solution condition was favorable for amino functionalized adsorbents adsorbing phenol estrogens in water samples (Zhao et al., 2012). Therefore, in the following experiments the pH of samples was adjusted to 7.0.

Given that the concentrations of target analytes are very low in environmental water samples, the effect of sample volume on the

extraction efficiency was evaluated to ensure reliable analytical results and high enrichment efficiency. Meanwhile, if the sample volume exceeds breakthrough volume, target compounds could be flushed away. Therfore, nonoccurrence of sample breakthrough should be ensured. In this section, sample volume was investigated in the range of 10 - 200 mL. The results (see Fig. S4 in supplementary material) showed that when the volume was in the range of 10 - 80 mL, it could obtain satisfactory enrichment performance and recovery barely changed. When the volume was increased from 80 to 150 mL, recovery got slightly lower but still can obtain satisfactory enrichment performance（>85%）, and the decrement rate of BPA, OP and NP was 6.3%, 4.2% and 2.06%, respectively. However, when the sample volume was added up to 200 mL, three targets decline rate were 20.6%, 17.8% and 5.5%, respectively, despite the recovery rate of NP didn't change obviously, the recoveries of BPA and OP declined visibly. So breakthrough volume was defined as 150 mL.

Considering the trace exists of target compounds, with the view of achieving superior LOD and less analysis time simultaneously, the least possible loading volume was tested, *e.g.* 10 mL, 84.6 - 101.9% of the recoveries could be achieved for the PEEs tested, indicating good sensitivity and precision of the developed method. In the further experiment, 10 mL of loading sample volume was used.

Sample flow rate can affect the analysis time and the recoveries of analytes by means of controlling the contact time of target compounds and adsorbents. So it was investigated in the range of 1.0 - 5.0 mL min^{-1}. According to results, when the flow rate reached 3.0 mL min^{-1}, not only the recoveries for the target compounds were still above 90%, but also the time of the aqueous sample (10 mL) passing through the HM-PAN nanofibers mat was reduced. But when the flow rate was above 3.0 mL min^{-1}, the recoveries of analytes began to reduce. Therefore, in the subsequent analysis, 3.0 mL min^{-1} was adopted.

3.2.3 Comparison of HM-PAN nanofibers mat and other SPE sorbents

The results of the comparison between HM-PAN nanofibers mat and other SPE sorbents reported in literatures for the same target analytes were presented in Table 2. It was confirmed that the proposed method based on HM-PAN nanofibers mat showed advantages in lower consumption of organic solvent, less sorbents, better repeatability and precision compared to the existing methods. When

increasing sample volume to breakthrough volume (150 mL), the method LOD was from 0.001 to 0.003 ng mL^{-1}, lower than that in the literature method.

The good extraction performance of HM-PAN nanofibers mat as SPE adsorbent for BPA, NP and OP was attributed to large specific surface area and functional amino groups ($–NH_2$). As a result, sorbent bed mass and eluent volumes decreased remarkably, which enabled HM-PAN nanofibers a suitable candidate to environment friendly sorbent to miniaturize SPE. Meanwhile, disks SPE technique based on HM-PAN nanofibers mat could improve mass transfer process and allow sample processing at higher flow velocity. Hence, it was much easier to deal with large volume samples with better enrichment coefficient and lower LOD obtained.

In addition, as an advantage of HM-PAN nanofibers mat compared with other SPE sorbents, the reusability of HM-PAN nanofibers mat was also evaluated, which could improve the availability of HM-PAN nanofibers mat. The mat was used to deal with samples multiple times. MeOH, DCM and water were employed to pass through HM-PAN nanofibers mat respectively before next SPE extraction to make sure the possible residue of PEEs was removed. The result indicated that a piece of membrane could be used for seven times at least before discarded since no significant statistical differences ($P > 0.05$) in recoveries of the three estrogens were observed. The satisfactory recoveries above 90% for all three compounds can be achieved at the seventh time use.

Table 2 Comparison with other kinds of SPE sorbents in literature

Sorbents	Analytes	Amount (mg)	Organic solvent comsumption (mL)	Enrichment multiple	LOD (ng mL^{-1})	RSD (%)	Absolute Recovery (%)	Ref
HM-PAN (n=3)	BPA,NP,OP	4.0	1.2	750	0.001-0.003	≤8.6	≥84.6	this work
C18	NP,OP,BPA,	500	15.0	500	0.02-0.1	≤9.0	≥72.0	[39]
HLB	BPA,NP,OP	500	21.0	500	0.001-0.008	≤11.1	≥60.5	[40]
silica-magnetite nanoparticle	BPA,NP,OP	100	–	1600	0.007-0.02	≤10.0	≥68.0	[41]
Cetyltrimethylammonium Bromide-Coated Magnetic Nanoparticles	BPA,NP,OP	150	–	800	0.0012-0.0034	≤9.6	≥82.0	[42]
Aminopropyl(NH_2) catridges	BPA, NP	≈300	34	–	0.09-0.5	≤5.7	≥82.0	[33]

–: Not given
Enrichment multiple: volume of sample/volume of eluent

3.3. Analytical performance of developed method

Under the above optimum conditions, the HM-PAN nanofibers mat-based SPE method was first validated with the spiked water samples from Daming Lake as matrix. All the three compounds of the lake sample were detected. The amounts of three compounds in the blank sample were subtracted from those obtained by spiking. The analytical performance was evaluated in terms of linear range, correlation coefficients (r2), limits of detection (LODs, S/N=3) and recovery for target analytes.

The linear ranges are 0.05-20.0 μg L-1 for BPA and 0.1-20.0 μg L-1 for NP and OP, which possess good linearity (r2>0.999 for all analytes). The LODs of BPA, NP and OP were 0.01, 0.03 and 0.03 ng mL^{-1}, respectively. At the same time, the method precisions were evaluated by 10 mL spiked lake water sample containing standard PEEs at 2 ng mL^{-1}, the RSD(n=3) were found to be below 8.6% and recoveries were above 84.6% for all three compounds. Satisfactory spiked recoveries at low, medium and high concentration (0.05, 1.0 and 10.0 ng mL^{-1}) for BPA, (0.1, 1.0 and 10.0 ng mL^{-1}) for NP and OP were obtained in the range of 87.4-120.5% (n=6).

The proposed method was then applied to the determination of PEEs in Daming Lake water to evaluate its analytical applicability. The representative selective reaction monitoring (SRM) chromatograms of calibration standard (0.1 ng mL^{-1}) and real water samples were shown in Fig.5.

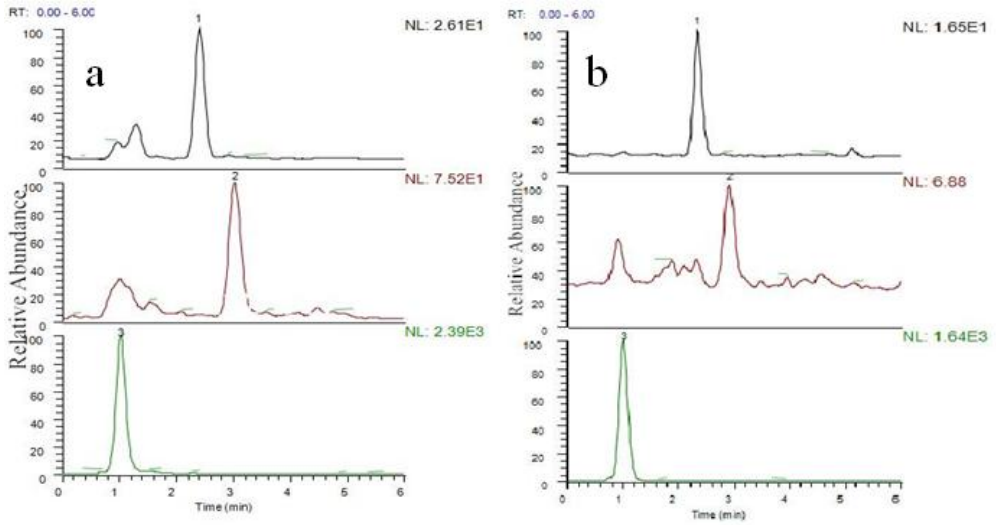


Fig. 5 The SRM mode chromatograms of low calibration standard (0.1ng mL^{-1}) (a) and real water samples (b) Peak identification: (1) OP; (2) NP; (3) BPA.

The results indicated that all the three compounds had been detected in Daming Lake water. Among three analytes, BPA and OP can be quantitated precisely, and their average concentrations (n=3) in lake water samples were 0.195 and 0.106 ng mL^{-1} (S1), 0.235 and 0.120 ng mL^{-1} (S2), 0.230 and 0.101 ng mL^{-1} (S3), 0.190 and 0.106 ng mL^{-1} (S4), respectively. The concentration of three compounds in four lake water samples didn't show obvious difference. This phenomenon may be due to that flow velocity at water inlets and outlets are fast, and the water in Daming Lake is moving.

According to National standard GB 5749-2006 of China, the limit for BPA in drinking water is 0.01mg L^{-1}, which is higher than we detected. It is reported that industrial effluents were the main source of BPA, NP and OP contamination in the aquatic environment. Daming lake is located in the center of Jinan city without factories nearby, whose major pollution source was sewage runoff, so the

concentration of three target compounds were low in Daming Lake [43, 44]. However, even at such low concentration, these compounds may present activity as endocrine disrupters, which can be potential risk for the environmental and in the future may be for humans, especially because synergistic and long-term effects are not fully understood.

4. Conclusions

This study revealed that HM-PAN nanofibers mat with amino groups ($–NH_2$) introduced by hydrazine functionalization, can be used as a superior disks-SPE adsorbent for extraction of PEEs from environmental water. The target PEEs can be quantitatively adsorbed on 4.0 mg HM-PAN nanofibers and consumed only 1.2 mL organic solvent during SPE process with satisfactory recovery and repeatability. It further confirmed the new method offered better analytical performance in terms of higher recovery, lower detection limits and better precision comparing to the existing ones used in literatures. The applicability of the method was further validated by successful application to determine target compounds in real environmental water.

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