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Novel molecularly imprinted polymer prepared by nanoattapulgite as matrix for selective solid-phase extraction of diethylstilbestrol

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ABSTRACT

Using nanoattapulgite as matrix, both diethylstilbestrol surface molecularly imprinted polymer and non-imprinted polymer were synthesized in this work. Compared with each other, the diethylstilbestrol surface molecularly imprinted polymer is superior to non-imprinted polymer in adsorption capacity, selectivity and mass transfer property. The maximum static adsorption capacities of diethylstilbestrol surface molecularly imprinted polymer, non-imprinted polymer and nanoattapulgite for diethylstilbestrol was 105.14, 78.54 and 28.50 mg g^{-1}, respectively. As the packing material of solid-phase extraction, the diethylstilbestrol surface molecularly imprinted polymer has been applied to concentrating diethylstilbestrol in pond water and fish samples. A corresponding analytical method to determine diethylstilbestrol has been developed. The limit of detection for diethylstilbestrol in pond water sample and fish samples were 3 μg L^{-1} and 15 μg kg^{-1}.

1. Introduction

Diethylstilbestrol (DES) is a synthetic estrogen used to prevent spontaneous abortion clinically. However, the long-term intake of DES can lead to the increased risk of breast cancer [1]. Compared with natural estrogen, estrogenic drugs such as DES are more stable and remain in body longer [2]. Although use of growth-promoting drugs for fattening livestock has been banned in the European Union since 1986 [3], DES is still used sometimes for increasing the flesh of cattle, sheep, chook and fish in pursuit of illegal benefit. Thus, it is important to determine the residual DES in meat. With growing concerns over human health and the need to increase sample-throughput in analytical testing laboratories, there is a constant requirement for more accurate, simpler and faster analytical methods for the determination of DES residue in food-safety area.

DES is analyzed by various analytical methods, including the pressurized capillary electrochromatography (pCEC) [4], the combination of gas chromatography–electron ionization mass spectrometry (GC–EI-MS) and solid-phase microextraction (SPME) GC–MS [5] or enzyme-linked immunosorbent assay (ELISA) [6]. Although the methods cited above have the advantage of high sensitivity, they suffered from low specificity except ELISA method. When the sample matrix is complicated, sample pretreatment is needed. In one our previously work [7], it has been reported that DES in the fish sample can be determined by the molecularly imprinted polymer solid-phase extraction–high performance liquid chromatography (MIP-SPE–HPLC) method with the detection limit of 60 ng mL^{-1}. The aim of the present study was to develop a new molecularly imprinting polymer to improve the specificity of the method.

MIP gives an effective way for molecular recognition via the template-directed synthesis of highly crosslinked polymeric matrices [8], which can extract selectively the small molecules from complex mixtures [9]. The various templates have been reported in literature and their use is quite simple and timesaving. MIP has been extensively used in SPE [10,11], catalysis [12,13], sensors [14,15], and as artificial antibodies in binding assays [16,17]. There are many kinds of matrixes for synthesis of MIP such as silica nanoparticles [18], silica nanotubes [19], Fe_3O_4 magnetic nanoparticles [20], and quantum dot [21]. Nanosized materials have the higher binding capacity and faster binding kinetics than normalones.

Nanoattapulgite (ATP) is a kind of phyllosilicate charactered by microfibrous morphology, high surface charge and large specific surface area [22,23]. Because of its specific structure, it has been widely used as an environmental absorbent, catalyst, carrier for pesticides and fertilizers, rheological control agent and so on [24–26]. Recently ATP modified by organic group has been used to adsorb organic contaminant [27,28] too. The adsorption was based on the hydrophobic interaction between the organic layer and the organic molecules. In our lab, a MIP of DES was firstly synthesized

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using ATP as matrix. The material was applied as SPE packing to extract DES in pond water and fish samples, followed by HPLC determination, which provided high selectivity for DES.

2. Experimental

2.1. Materials and chemicals

Attapulgite nanofibrillar clay (ATP) with an average diameter of 325 mesh was provided by Gansu ATP, (Gansu, China). It was dried in vacuum at 110 °C for 48 h before use.

Methacrylic acid (MAA), ammonium persulfate (APS), *p*-toluenesulfonic acid (*p*-TSA), ethanol, acetonitrile and acetic acid were from Tianjin Guangfu Fine Chemical Research institute (Tianjin, China). γ-Aminopropyltriethoxysilane (APTES) and 2,2-bis(hydroxymethyl)-propionic acid (bis-MPA) were purchased from Alfa Aesar (Beijing, China). Acrylamide (AA), *N*,*N*-methylene-biacrylamide (MBAA) and *N*,*N*,*N*′,*N*′-tetramethylethlenediamine (TEMED) were obtained from Chemistry Reagent Factory of Chinese QianJin (Tianjin, China). Diethylstilbestrol (DES) was purchased from Ying Fa Peng Na (Ningbo, China).

Toluene and dimethylformamide (DMF) (Tianjin Chemical Reagent Co., Tianjin, China) were of analytical reagent grade and used after being stirred overnight over CaH_2 and distilled under reduced pressure. These chemicals above were of analytical grade.

Ultra pure water used throughout the experiments was obtained from a purification system MILLI-Q (Millipore, Bedford, MA, USA). HPLC-grade acetonitrile was from Dima Technology (Richmond Hill, USA). The mobile phase used for HPLC experiments was a mixture of acetonitrile (ACN), and ultra pure water (40:60, v/v), and was filtered through 0.45 μm filter and ultrasonic treatment to degas before use.

2.2. Instrumentation

The chromatographic system consisted of a Model 210 HPLC pump and a UV detector (Varian Prostar). All separations were carried out on a C_{18} column (Hanbon Science & Technology, 150 mm × 4.6 mm) with a flow rate of 0.8 mL min^{-1} at room temperature (about 21 °C). The UV detector was operated at 254 nm.

The MIP-SPE study was done using a solid-phase extraction cartridge supplied by Dalian institute of Chemical Physics, Chinese Academy of Sciences. One hundred milligrams of MIP sorbent was packed into SPE cartridge, respectively.

2.3. Preparation of MIP sorbent

2.3.1. Hyperbranched aliphatic polyester grafted attapulgite (HAPE-ATP).

The composites of HAPE-ATP were performed by the procedure as described in the literature [29]. γ-Aminopropyltriethoxysilane (APTES) was first assembled onto the surface of the attapulgite nanofibrillar clay by the following procedure: 3.0 g ATP and 5.0 mL APTES were dispersed into 100 mL dried toluene with ultrasonic agitation for 30 min, the mixture was refluxed for 8 h with electromagnetic stirring. After cooling to room temperature, the product, aminopropyl modified attapulgite (A-ATP), was filtered and thereafter thoroughly washed with ethanol and dried in a vacuum at 40 °C overnight.

Then 2.0 g A-ATP, 5.0 g bis-MPA, and 0.50 g *p*-TSA were added into 40 mL DMF. The mixture was irradiated ultrasonically for 30 min and refluxed for 8 h with N_2 bubbling throughout. The hyperbranched aliphatic polyester grafted attapulgite (HAPE-ATP) was separated from the nongrafted hyperbranched aliphatic polyester by several cycles of dispersion in DMF with ultrasonic vibrations for 30 min and precipitated by centrifugation at 5600 × *g* for 30 min. It was then washed throughout with ethanol and dried in a vacuum at 40 °C.

2.3.2. MAA grafted HAPE-ATP (MAA-ATP)

A 300 mL round-bottomed flask was equipped with a reflux condenser, a three-way stopcock, and a magnetic stirring bar and flushed with dry nitrogen. 30 mL benzene, 0.2 g hydroquinone, 0.4 g *p*-TAS, 0.1 g boric acid, 1.29 g methacrylic acid and 0.5 g HAPE-ATP were placed in the flask, and the mixture was refluxed with stirring and flushing with dry nitrogen for 72 h. The product MAA-ATP was filtered and thereafter thoroughly washed with water and diethyl ether and dried in a vacuum at 40 °C.

2.3.3. Synthesis of MIP

DES (17 mg) was dissolved in a mixture of ethanol, and ultra pure water (50:50, v/v), and then MAA-ATP (500 mg) was dispersed in the solution. Under stirring, appropriate AA (36 mg) and MBAA (175 mg) were added. The polymerization was started by adding APS and TEMED under N_2 protection at 37 °C, and then it was stirred for 8 h. The polymer was collected and washed with ultra pure water and methanol subsequently in order to sufficiently remove the template. All process was showed in Fig. 1.

For comparison, molecularly non-imprinted polymer (NIP) was immobilized on the surface of ATP in the similar manner described as above, except for the absence of DES.

The obtained products were characterized with Nicolet Nexus 670 Fourier transform infrared (FTIR) (MN, USA) spectrometer and JEM1200EX transmission electron microscope (TEM) (Tokyo, Japan).

2.4. Static adsorption test

The capacity of the MIP to recognize and bind DES was evaluated in ethanol.

Static adsorption test was done as following: 10 mL of various concentrations of DES were dissolved in ethanol, and then 20 mg of MIP were added. The mixture was shaken for 24 h at room temperature to facilitate adsorption of DES onto the DES-imprinted sorbent. After the solution was filtrated, the concentrations of the DES were determined by HPLC.

Adsorption kinetic studies were carried out as following: in a centrifuge tube, 20 mg of MIP, NIP or ATP was suspended in 10 mL of DES with an initial concentration of 500, 200, 50 or 10 mg L^{-1}. The tube was incubated at room temperature (about 21 °C) with shaking. Ten samples were taken at defined time intervals (at 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 h, respectively). The residual concentrations of DES were measured by HPLC.

Competitive recognition studies were performed with DES and the structurally similar compound trans-resveratrol (3,5,4′-trihydroxy-stilbene, RES) and bisphnol A (4-(2-(4-hydroxyphenyl) propan-2-yl) phenol, BPA) at 500 mg L^{-1} level.

2.5. Selectivity of the imprinted sorbent in dynamic adsorption

A sample of 100 mg of MIP or NIP was placed into an empty SPE cartridge. After pretreated with 10 mL methanol and 10 mL pure water, 20 mL of 1 μg mL^{-1} RES, BPA and DES mixture (pH 4.0) was loaded onto the SPE cartridge. After washing with 2.0 mL methanol–water mixture (80:20, v/v), the cartridge was eluted with 2.0 mL of the MeOH:HAc (99:1, v/v) mixture. The eluent was measured by HPLC.

2.6. Comparison of retention behavior of DES between C_{18} SPE and DES-MIP-SPE

For comparison, 100 mg of C_{18} and 100 mg of DES-imprinted sorbent were placed into two empty SPE cartridges, respectively. After

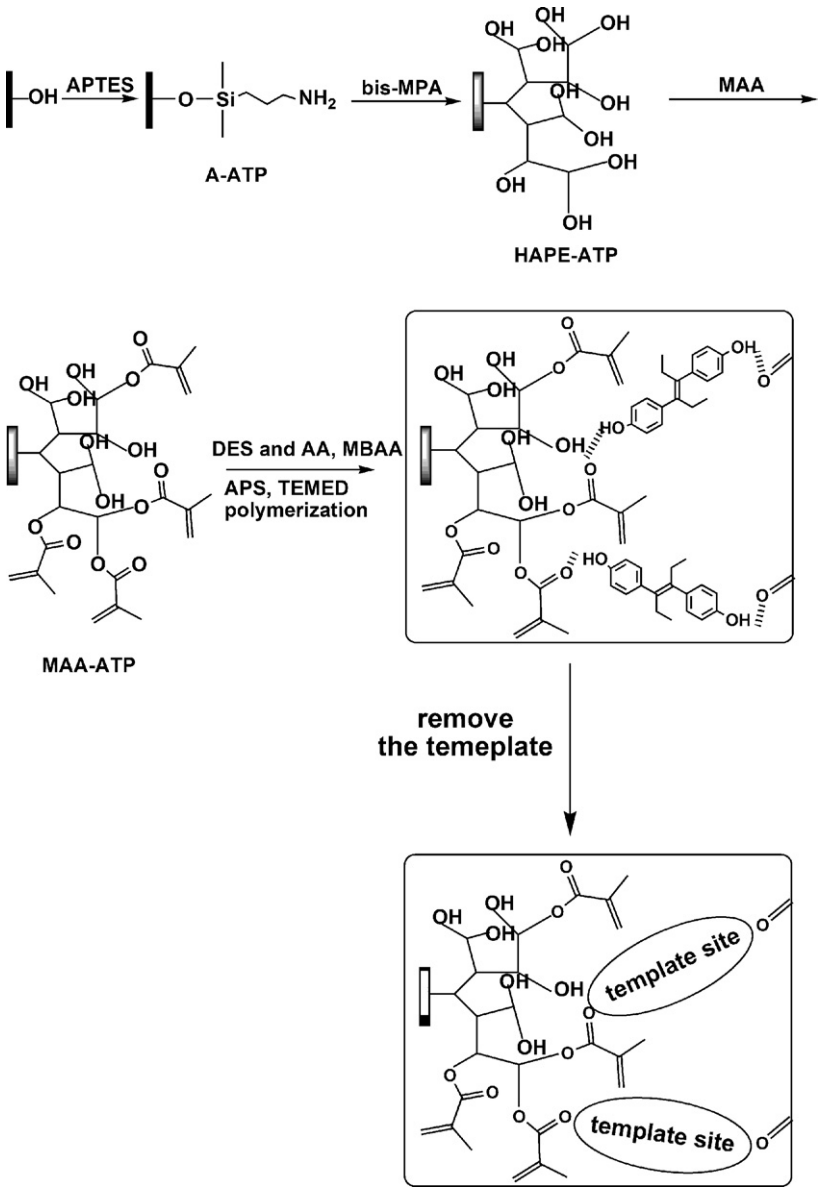

Fig. 1. Preparation procedure of molecularly imprinted polymer. Columns in filled represent ATP and the shaded columns represent A-ATP.

they were pretreated with 10 mL methanol and 10 mL pure water, 10 mL of 1 μg mL^{-1} RES, BPA and DES mixture was treated on the C_{18} SPE cartridges according to the conditions in the literatures [7]. The same solution was treated on the MIP-SPE cartridges at the optimal condition.

2.7. Determination of DES in pond water and fish samples

2.7.1. Determination of DES in pond water sample

Pond water was filtrated through a 0.22 μm filter before use. SPE cartridges were prepared by packing 100 mg MIP and washing with 10 mL MeOH and 10 mL H_2O. Pond water sample was processed in the SPE at 4 mL min^{-1} flow rate. The retained DES was then eluted with adequate volumes of MeOH:HAc (99:1, v/v) solution. The eluent was collected directly for HPLC analysis.

2.7.2. Determination of DES in fish samples

According to reference [30], the eatable parts of the fish were stripped away and a meat chopper was used to grind them and then stored at 4 °C. For each analysis, 2.00 g fish were quantified in a 15 mL centrifuge tube. 0.2 mL of ultrapure water or standard solutions, 0.5 mL of 10% Na_2SO_4 and 4.5 mL of acetonitrile were added to the tube and mixed in the shaker (QL-861 vortex shaker, Jiangsu Haimen Kylin-Bell Lab Instruments, Haimen, China) to extract DES. The tube was then centrifuged for 3 min at a speed of 960 × *g*. The supernatants were collected in a flask and then vaporized at 40 °C, and then dissolved with 1 mL 10% methanol solution (pH 4) and centrifuged for 3 min at 1860 × *g*. The supernatants were then transferred to SPE treatment.

3. Result and discussion

3.1. Preparation of MIP

In order to form more hydroxyl groups on the surface of ATP, the hyperbranched aliphatic polyester grafting onto the ATP was conducted by the polycondensation of the bis-MPA, in the presence of the amino group modified A-ATP and *p*-TSA as catalyst. The reaction was probably facilitated because the interaction between the carboxyl groups of bis-MPA and the amino groups of A-ATP has the

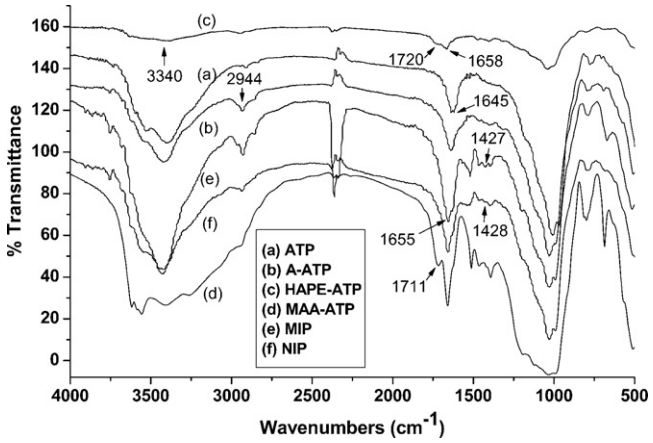

Fig. 2. FTIR spectra of ATP (a), A-ATP (b), HAPE-ATP (c), MAA-ATP (d), MIP (e) and NIP (f).

higher reactive activity than the interactions between the carboxyl and hydroxyl groups of AB_2-type monomer.

To synthesize the MIP, the matrix should have vinyl groups and carbonyl groups. Vinyl groups could react with the crosslinker to strengthen mechanical strength of MIP. The carbonyl groups could form hydrogen bond with template, Therefore MAA was chosen to graft onto HAPE-ATP.

AA was chosen as the functional monomer and MBAA was used as the crosslinker to get DES imprinted polymers. MBAA could form hydrophilic surface. It was favorable to contact sufficiently between aqueous solution and the surface of MIP.

3.2. Characteristics of molecular imprinting polymers

FTIR spectra of ATP, A-ATP, HAPE-ATP, MAA-ATP, MIP and NIP were shown in Fig. 2.

After the assembly of γ-aminopropyltriethoxysilane onto the surfaces of attapulgite, the band of methyl and methylene groups in A-ATP were found at 2941 cm^{-1}.

The absorbance band at 1734 cm^{-1} of ester groups and the obvious broaden of O–H peak about 3350 cm^{-1} showed the hyperbranched aliphatic polyester had been grafted onto the surfaces of the ATP nanofibrillar clay already. The presence of the characteristic band at about 1655 cm^{-1} of amide groups also showed that the hyperbranched aliphatic polyester was grafted onto the surfaces of the ATP nanofibrillar clay via the amidation reaction between the carboxyl groups of the monomer and the amino groups on the A-ATP.

The obvious increase of C=O peak intensity indicated that the carboxylic groups of MAA had grafted to the HAPE-ATP.

The presence of the bands at about 1427 cm^{-1} of C–N and the disappearance of band at 1734 cm^{-1} of ester groups showed that the MBAA had polymerized to the surface of MAA-ATP.

The TEM images of ATP, NIP and MIP were shown in Fig. 3. It could be seen that the crosslinker had markedly grafted onto the surface of the ATP.

3.3. Static adsorption test

3.3.1. Adsorption capacity of DES-imprinted sorbent for DES

The adsorption capacity was an important factor to evaluate MIP. A series of DES solutions with concentrations of 100–2000 mg L^{-1} were studied. As can be seen in Fig. 4, the adsorbed amount increased with the increasing initial concentration of DES solution. The static adsorption capacities of ATP, NIP and MIP for DES were 28.50, 78.54 and 105.14 mg g^{-1}, respectively. Obviously, the static adsorption capacity of the MIP was larger than that of the NIP and ATP. The results showed that the MIP had a higher adsorption capacity for DES and it would be better for enriching trace DES in samples. The S_{BET} of ATP, NIP and MIP were 163, 64.4 and 71.2 m^2/g, respectively. Although ATP had the higher specific surface area, its adsorption capacity was lower than the NIP and the MIP. Because the pores of ATP were covered organic layer, the MIP and NIP had low surface areas. Obviously, the carbonyl groups or amino groups of NIP and MIP make the adsorption of DES favorably. The MIP had the highest adsorption capacity owing to its more effective sites.

3.3.2. Adsorption kinetics

Fig. 5(a) showed the kinetic adsorption processes of DES at 500 mg L^{-1} onto MIP, NIP and ATP. As can be seen, the adsorption reached equilibrium on the MIP in 12 h. The higher adsorption capacity indicated that molecularly imprinting process had resulted

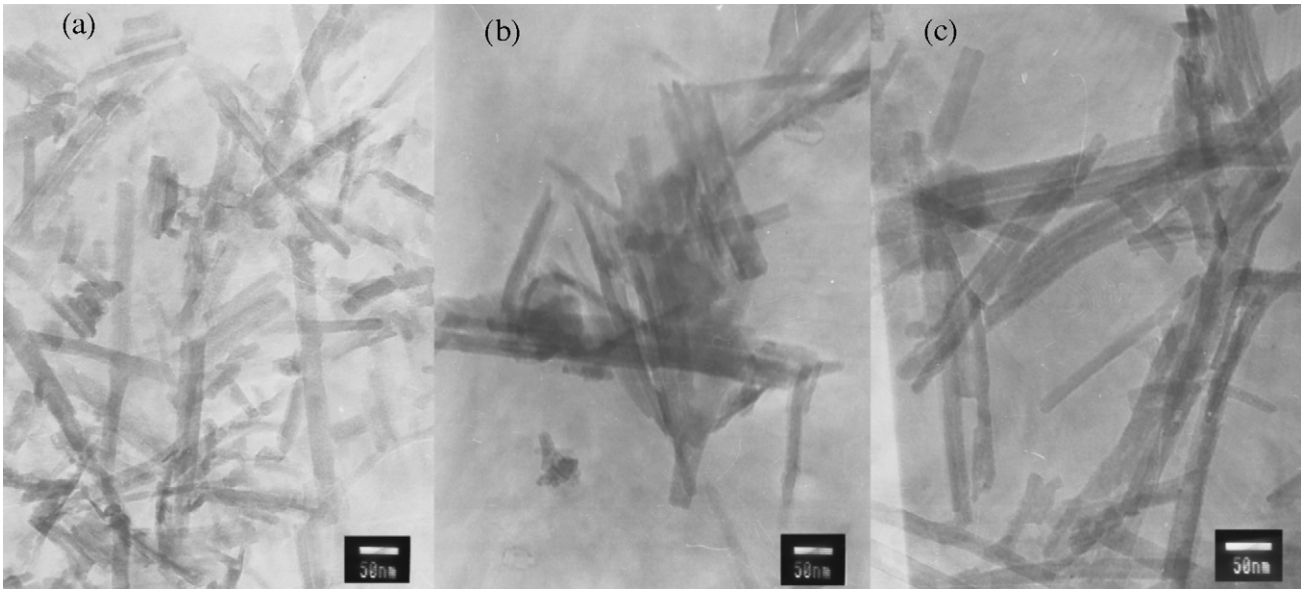

Fig. 3. TEM of ATP (a), NIP (b) and MIP (c).

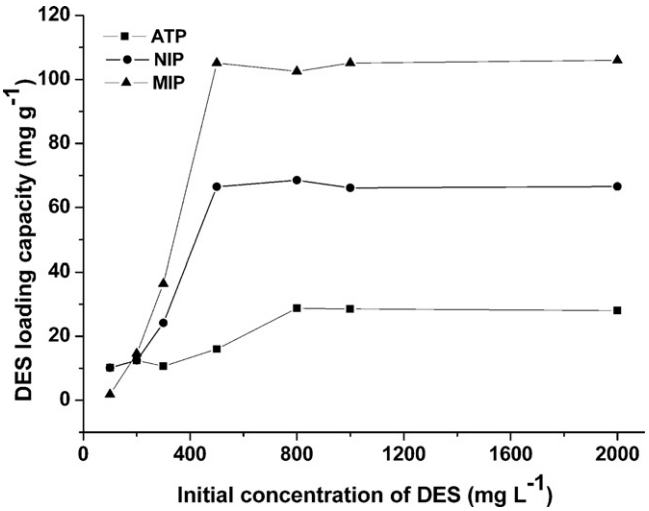

Fig. 4. Loading isotherm of DES onto ATP, NIP and MIP. 20 mg of each material was suspended in 10 mL DES solution with different concentrations and the adsorption was kept for 24 h.

in the formation and preservation of specific recognition cavities on the surface of MIP which benefited for DES to diffuse into the inner cavities of the polymer. As for the NIP, the non-specific adsorption played a dominant role. There were no suitable cavities on the surface for DES to diffuse into the inner of polymer. In the case of the ATP, the adsorption of DES did not reach equilibrium in 24 h and the adsorption procedure was non-specific and slow.

Adsorption equilibrium time is depended on the sample concentration and the media. When DES in ethanol solution was 500, 200, 50, 10 mg L^{-1}, the adsorption reached equilibrium on the MIP in 12 h, 3 h, 10 min, 5 min, respectively. With DES solution in water, all adsorption reached equilibrium within 5 min. The results were showed in Fig. 5(b).

3.3.3. Selectivity of the imprinted sorbent

Recognition coefficient (α) calculated from formulae below Table 1 [31] was used to evaluate the recognition ability. It was noticed that α was higher for DES, indicating the MIP had high selectivity for DES over the structurally similar compound RES and BPA.

RES and BPA were absorbed to some extent in the active centers of MIP. However, the ability of specific recognition is restricted and affected by the competitive molecule. More similar the competitive molecule to the template molecule was, lower coefficient α was obtained. That is the reason why the analogue of template synthesis method give the little different α [32,33]. The adsorption of RES, DES and BPA was due to the formation of hydrogen bonds between the carbonyl groups of MIP and the hydroxyl groups in RES, BPA and DES. Besides, the adsorption was attributed to the interaction between specific recognition sites and the DES in terms of size and shape.

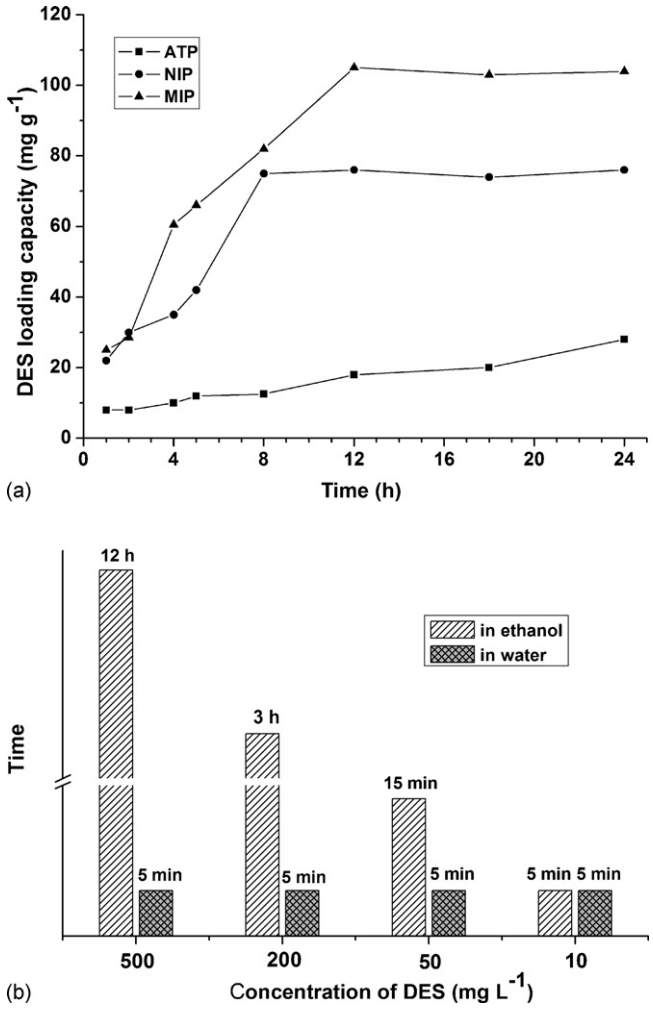

Fig. 5. (a) Adsorption kinetic curves of ATP, NIP and MIP and (b) adsorption equilibrium time with the sample media. 20 mg of each material was suspended in 10 mL of solution with 500 mg L^{-1} DES

3.4. Selectivity of the imprinted sorbent in dynamic adsorption

According to the formula below Table 2, the α values for DES, RES, BPA were calculated as 6.93, 1.00 and 0.91, respectively. The higher $\alpha_{(DES)}$ value than that from static adsorption was owing to much specific adsorption retained and non-specific adsorption washed away.

3.5. Application of the MIP to SPE

3.5.1. Optimization of pH

The pH value is essential not only for achieving high capacity, but also for bringing forward the selectivity of the polymer in aqueous media. The adsorption behavior of DES on the MIP was studied over a wide pH range of 2–10. Fig. 6 shows that pH significantly

Table 1
Competitive loading of DES, RES and BPA on MIP and NIP from static adsorption test.

Samples	C_o (mg L^{-1})			C_{final} (mg L^{-1})			S (mg g^{-1})			α		
	DES	RES	BPA	DES	RES	BPA	DES	RES	BPA	DES	RES	BPA
MIP	500	500	500	362	429	443	69	36	29	1.94	1.01	0.74
NIP	500	500	500	429	430	423	36	35	39			

Note: S, bonded amount of analytes with sorbent, $S = (C_o - C_{final}) \times$ solution volume (L)/sorbent mass (g), where C_o and C_f represent the initial and final concentrations of the analytes, respectively; α, recognition coefficient, $\alpha = S_{MIP}/S_{NIP}$, where S_{MIP} and S_{NIP} are the analytes binded amount to MIP and NIP, respectively.

Table 2
Competitive loading of DES, RES and BPA on MIP and NIP from dynamic adsorption test.

Samples	C_o (mg L^{-1})			C_{eluent} (mg L^{-1})			S (mg g^{-1})			α		
	DES	RES	BPA	DES	RES	BPA	DES	RES	BPA	DES	RES	BPA
MIP	1	1	1	9.84	0.88	1	0.20	0.02	0.02	6.93	1.00	0.91
NIP	1	1	1	1.42	0.88	1.1	0.03	0.02	0.02			

Note: C_o represents the initial concentrations; S, bonded amount of analytes with sorbent, $S = C_{eluent} \times$ eluent volume (L)/sorbent mass (g), where C_{eluent} represented the eluent concentration of the analytes; α, recognition coefficient, $\alpha = S_{MIP}/S_{NIP}$, where S_{MIP} and S_{NIP} were the analytes binded amount to MIP and NIP SPE cartridge, respectively.

affects the recovery of DES on the MIP and the adsorbed amount decreased with increasing pH. The recovery of the adsorbed DES remains above 98.0% at pH 2–4, and then began to decrease with pH increasing. Thus, the further studies were carried out at pH 4.0.

The adsorption of DES varying with pH can be elucidated by considering the dissociation of analyte and the surface charge of the MIP. In general, only the undissociated form of the analyte that will be easily extracted by an absorptive-type of material [34]. At lower pH more protons will be available to protonate the organo-surface of MIP [27], and the electrostatic attractions between DES molecules and MIP sites led to the high recovery of DES. At pH 5–10, the surface of the MIP is deprotonated and DES existed in the dissociated form so that the adsorption is restricted.

3.5.2. Optimization of loading volume

Different volumes of 0.5 μg mL^{-1} DES aqueous solutions (10, 25, 30, 40, 50, 60 and 80 mL (pH 4.0)) were loaded to MIP-SPE column to find the largest loading volume. The elute was collected and analyzed by HPLC. The results showed that when the loading volume was more than 20 mL, the sample could not be adsorbed completely. So the loading volume for SPE will be 20 mL.

3.5.3. Optimization of washing solution

The 20 mL sample spiked DES was loaded onto the cartridge as described above. Wash the cartridge with 2.0 mL methanol:water solution (20:80, 40:60, 50:50, 60:40 and 80:20, v/v). Different concentrations of methanol can wash away different polar impurities, but it may wash away DES to a certain extent. Based on the recoveries obtained, 20% methanol solution was chosen as the washing solution.

3.5.4. Optimization of eluent volume

The mixture MeOH:HAc (99:1, v/v) was used to elute DES from the MIP-SPE cartridges. Using the same DES sample was loaded, different volumes of the eluent between 0.50 and 4.00 mL were tested. Eluent volumes of 2.00 mL or larger permitted the extraction efficiencies of around 100% to be achieved. 2.00 mL was then employed as the eluent volume. To get the greatest enrichment factor, the eluent was evaporated and redissolved in 0.50 mL MeOH for HPLC analysis.

3.6. Determination of the repeatability with one single MIP cartridge

The repeatability of the extraction efficiency with one single MIP cartridge was evaluated. The rebinding/elution process was repeated ($n = 10$) using the same MIP cartridge. Under the conditions optimized above, a RSD value of 2.9% was obtained, indicating good repeatability using a single cartridge and demonstrating the inherent reusability of these MIP-packed cartridges.

3.7. Comparison of retention behavior of DES between C_{18} SPE and DES-MIP-SPE

Recoveries of the DES, RES and BPA mixtures from the MIP-SPE and C_{18} SPE were shown in Fig. 7. The recovery of DES was 98.7% on the C_{18} SPE cartridge and 99.5% on the MIP-SPE cartridge. That means the imprinted sorbent can be the substitute of the traditional C_{18} sorbent and DES had the similar adsorption behavior on the two sorbents. However, RES and BPA were retained strongly on the C_{18} SPE cartridge and retained poorly on the MIP-SPE cartridge. The DES-imprinted sorbent exhibited high selectivity and molecular recognition function.

3.8. Determination of DES in pond water and fish samples

3.8.1. Determination of DES in spiked pond water

The samples were extracted as described in the Section 2.7.2. The recoveries and reproducibility of the method were calculated and summarized in Table 3. As can be seen, the average recoveries of the MIP-SPE method was 93.4% in pond water at the studied levels

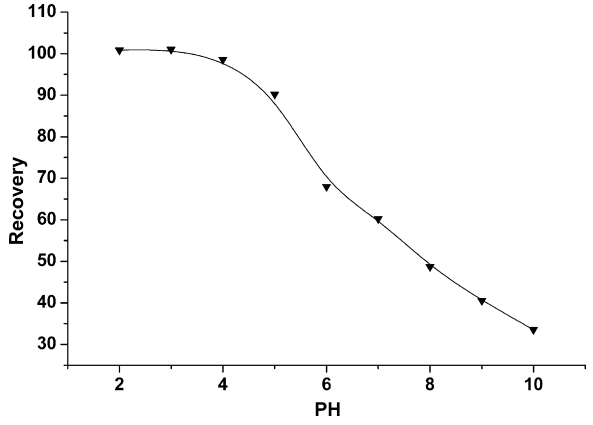

Fig. 6. Effect of pH on DES recovery from MIP. 10 mL of spiked solutions with 1 μg mL^{-1} DES over the pH range of 2–10 were loaded onto the MIP-SPE cartridges with 100 mg of sorbent, respectively. 2 mL methanol–acetic acid (v/v, 90:10) solution was used as eluent.

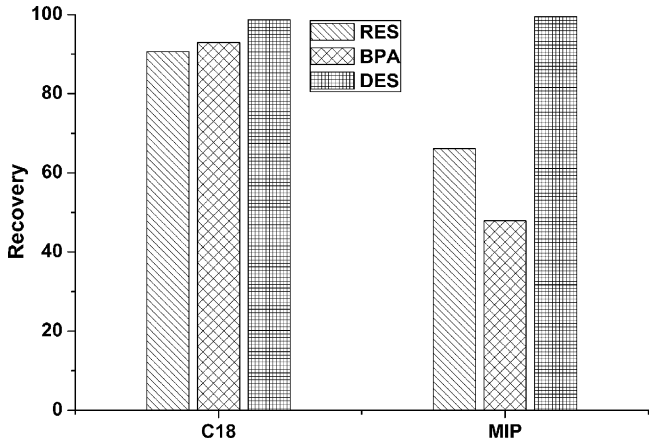

Fig. 7. Recoveries of RES, BPA and DES from C_{18} cartridge and MIP-SPE cartridge. Concentration of RES, BPA and DES was each 1 μg mL^{-1}, 100 mg of C_{18} or MIP sorbent used.

Table 3
Recoveries of DES after MIP-SPE of spiked pond water sample and fish samples.

Sample content	Added	Found ± SD (n = 6)	%Recovery ± %RSD (n = 6)
In	0	0	
pond	15.00	13.0 ± 0.6	84.0 ± 5.1
water	100.0	97.0 ± 3.6	97.0 ± 3.7
	1000	1000.2 ± 19.0	100.2 ± 1.9
Wuchang	0	0	
fish	50.00	43.6 ± 2.7	87.2 ± 6.2
	500.0	476.0 ± 19.0	95.2 ± 4.0
	1000	1064.0 ± 23.1	100.5 ± 2.3
Herbivorous	0	0	
fish	50.00	43.7 ± 2.5	87.4 ± 5.8
	500.0	483.5 ± 16.4	96.7 ± 3.4
	1000	966.3 ± 53.9	96.6 ± 5.6
Cyprinoid	0	87.0 ± 5.9	
	50.00	46.6 ± 2.2	93.2 ± 4.8
	500.0	509.5 ± 10.1	84.5 ± 2.4
	1000	1085.2 ± 19.9	99.8 ± 2.0

The unit to pond water sample and fish sample are $\mu g\,L^{-1}$ and $\mu g\,kg^{-1}$, respectively.

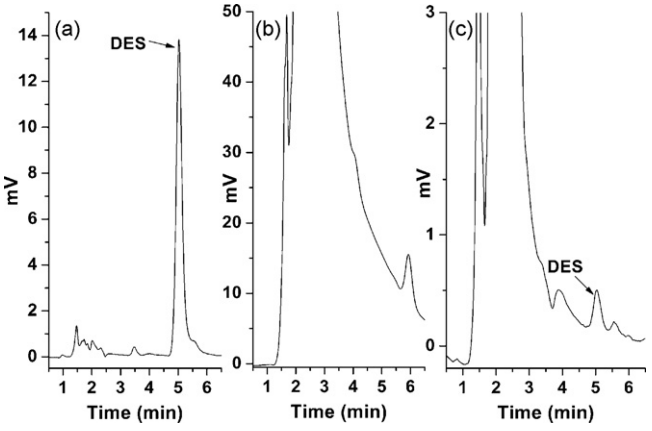

Fig. 8. Chromatograms of DES standard solution (3 $\mu g\,mL^{-1}$) (a), cyprinoid sample before SPE (b) and cyprinoid sample after SPE (c).

and good linearity was obtained over the calibration curve covered the range of 9–1000 $\mu g\,L^{-1}$.

The detection limit (LOD) was obtained from the signal to noise ratio (S/N). A signal value of three times the noise (S/N = 3) was used to calculate the LOD of the calibration curve. The LOD obtained was 3 $\mu g\,L^{-1}$ and the LOQ was 9 $\mu g\,L^{-1}$.

3.8.2. Determination of DES in fish samples

The proposed method was applied to the analysis of DES in three kinds of fish samples. DES standard was added at levels of 45–3000 $\mu g\,kg^{-1}$ to the herbivorous fish to create a calibration curve.

LOD was obtained from the signal to noise ratio (S/N). In this work, the baseline noise was measured from the chromatogram of fish samples. A signal value of three times the noise (S/N = 3) was used to calculate the LOD. The LOD obtained was 15 $\mu g\,kg^{-1}$ and the LOQ was 45 $\mu g\,kg^{-1}$. The results for the DES concentrations showed in Table 3.

Chromatograms of the DES standard, cyprinoid sample before and after SPE are shown in Fig. 8, which showed excellent selectivity of MIP for DES.

4. Conclusion

MIP of DES using ATP as matrix was synthesized. It showed the good adsorption capacity and mass transfer property in static adsorption and kinetic studies. By selectivity study and the comparison of MIP with the commercial C_{18}, the MIP material was proved to be with the good selectivity for template molecule of DES. This MIP used as an SPE sorbent and coupled to sensitive HPLC detection could quantitatively detect DES at low concentration levels in complex samples. The methodology developed in this work was shown be useful for the selective determination of DES in pond water and fishes.

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