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Magnetic molecularly imprinted nanoparticles based on dendritic-grafting modification for determination of estrogens in plasma samples

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ABSTRACT

In order to resolve the low imprinting efficiency of surface molecularly imprinted polymers (MIPs), a dendritic-grafting method introducing more functional groups was proposed to modify the SiO_2-coated magnetic nanoparticles (SiO_2-coated MNPs). And then magnetic MIPs (MMIPs) were obtained using 17-ethyl estradiol (EE2) as a pseudo template with dendronized SiO_2-coated MNPs as the supporter, aiming to avoid residual template leakage and to increase the imprinting efficiency. The resulting MMIPs showed high adsorption capacity, quick binding kinetics and good selectivity for trace estrogens. Meanwhile, MMIPs were used as magnetic dispersive solid-phase extraction (MDSPE) materials coupled with HPLC-UV for the detection of trace estrogens. The amounts of three estrogens which were detected from the plasma samples of pregnant women were 5.28, 5.31 and 4.17 ng mL^{-1}, and the average recoveries were 87.8%, 93.1% and 90.6% for the spiked samples with RSDs in the range of 1.4–6.3%, respectively. All these results reveal that MMIPs as MDSPE materials has good applicability to selective extraction and separation of trace estrogens from complex samples.

1. Introduction

As we all know, estrogens play important roles at different stages of mammalian development including prenatal development, growth, reproduction and sexual behaviors. The most potent naturally occurring estrogen is estradiol (E2), which is converted to the less potent estrone (E1) in liver, and then metabolized to estriol (E3) that has limited estrogenic activity. A deficiency of estradiol is often related to such diseases as menopausal symptoms or heart diseases and osteoporosis [1]. In addition, estrogens have strong physical activity, and the concentration of estrogens has direct influence on human life (especially pregnant women and fetuses) [2–5]. Therefore, the determination of estrogen in human plasma, especially maternal plasma is vitally important. However, because of the low concentration of estrogens in blood plasma and the interference of complex matrix components, improving detecting sensitivity of estrogens is a major analytical challenge. In order to effectively solve this problem, several pretreatment methods using molecular imprinting technology for extraction of estrogens have been established [6–8].

Due to high selectivity, chemical stability and easy preparation, molecularly imprinted polymers (MIPs) were used as sorbents in separation and enrichment trace target molecules or its analogues from the complex systems. Currently, the research about surface molecular imprinting technique (SMIT) has become a mainstream. And linear-grafting modification on the surface of nanoparticles to introduce additional functional groups was widely used in surface MIPs preparations [9–11]. But the low graft density and a small amount of imprinted sites resulted in a low rebinding capacity of the surface-imprinting materials. Therefore, how to improve the imprinting efficiency of the MIPs shell was concerned [12,13].

At present, dendrimers as the novel supramolecular nanomaterials have attracted more and more attentions in different research fields because of their huge potential applications [14–16]. Dendrimers have some characteristics including regular structures, rich functional groups, so the molecularly imprinted nanoparticles based on dendritic-grafting modification were designed to resolve the low imprinting efficiency of surface MIPs. Recently, the inorganic nanoparticles and electrodes which modified by dendrimers have been reported [17–20]. Whereas, the correlative researches about the combination with dendrimers and MIPs were mainly restricted in bulk polymerization [21–23].

In this paper, dendrimers and SiO_2-coated magnetic nanoparticles (SiO_2-coated MNPs) were effectively introduced to the study of MIPs. Firstly, SiO_2-coated MNPs could provide a relatively rapid and

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convenient way for withdrawal of magnetic polymers from sample matrices by a magnet without additional centrifugation or filtration [24–27]. Subsequently, a large number of functional groups were introduced to SiO_2-coated MNPs through the dendritic-grafting modification, and the novel magnetic MIPs (MMIPs) were designed by hydrogen bonding interactions between templates and functional groups. The resulting MMIPs have high adsorption capacity, quick binding kinetics and good selectivity for estrogens by using 17-ethyl estradiol (EE2) as the pseudo template. Lastly, MMIPs were successfully used as magnetic dispersive solid-phase extraction (MDSPE) sorbents coupled with HPLC-UV for the detection of trace estrogens in the plasma samples of pregnant women.

2. Experimental

2.1. Chemicals and regents

Estrone (E1), 17β-estradiol (E2), estriol (E3), ethinylestradiol (EE) and diethylstilbestrol (DES) were all purchased from Xianju Pharmaceutical Co., Ltd. (Zhejiang, China). Aminopropyltriethoxysilane (APTES), trimethylolpropane trimethacrylate (TRIM) and glycidyl methacrylate (GMA) were all obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Bisphenol A (BPA), 2-amino-1, 3-propanediol, tetraethoxysilane (TEOS), methyl acrylate (MA), dimethylformamide (DMF), triethylamine, O-methylhydroquinone (MEHQ) and ammonium hydroxide (25%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

All other chemicals were of analytical grade and obtained commercially. Ultra pure water used throughout the experiments was obtained from laboratory purification system (PALL, Germany).

2.2. Apparatus

HPLC was performed with a Shimadzu (Japan) system comprising LC-10ATVP pump, SPD-10AVP UV-detector, CTO-10ASVP column oven, and HW-2000 chromatographic work station. An Agilent (USA) 1200SL Series liquid chromatographic system interfaced to an Agilent 6410B Triple Quad LC–MS/MS. FT-IR spectra were recorded on a TENSOR27 infrared scanner (Bruker, Germany). Other instruments used included M27407 vibrating sample magnetometry (VSM) (Lake Shore Ltd.), Rigaku D/max22500 X-ray diffraction (Rigaku Ltd., Japan), JSM-5900 scanning electron microscope (SEM) (JEOL Ltd., Japan) and JEM1010 transmission electron microscope (TEM) (JEOL Ltd., Japan)

2.3. Dendritic-grafting modification of SiO_2-coated MNPs

The SiO_2-coated MNPs were obtained by the preparation method according to the literatures [6,28]. And then SiO_2-coated MNPs were modified as the follows:

(a) 100 mg SiO_2-coated MNPs were dispersed in 100 mL of toluene by ultrasonic vibration and then 10 mL of APTES was added. The mixture was stirred vigorously and purged with nitrogen gas while the temperature increased to 120 °C. After 24 h, the SiO_2-coated MNPs with amino groups (MNPs-NH_2) on the surface were obtained by the magnetic separation with magnet.
(b) The MNPs-NH_2 obtained by step (a) was dispersed in 80 mL of methanol by ultrasonic vibration and 15 mL of MA was added. The reaction mixture was purged with nitrogen and sealed, then vigorously stirred for 30 h at 60 °C. The SiO_2-coated MNPs with the terminal ester groups (MNPs-OCH_3) were obtained by the magnetic separation.
(c) 15 g 2-amino-1,3-propanediol was dissolved in 100 mL of dimethylformamide (DMF) and the MNPs-OCH_3 obtained by step (b) was dispersed by ultrasonic vibration. 24 g K_2CO_3 was added as catalyst. With the protection of nitrogen, the mixture was first carried out at 25 °C for 12 h and then completed at 40 °C for 18 h. So, the SiO_2-coated MNPs with the terminal hydroxyl groups (MNPs-OH) were obtained.
(d) The MNPs-OH was dispersed in 50 mL of acetonitrile and 30 mL of GMA was added. An appropriate amount of triethylamine and MEHQ were added as catalyst and polymerization inhibitor, respectively. The reaction mixture was stirred vigorously and purged with nitrogen gas at 60 °C for 24 h. And then the modified SiO_2-coated MNPs with the terminal methacrylate groups (MNPs-MA) were obtained.

2.4. Synthesis of MMIPs

In this study, EE2 as pseudo template was synthesized to prepare the MMIPs. The synthesis method was published in our previous report [29].

EE2 (0.4 mM) and the MNPs-MA (50 mg) were dispersed in 50 mL of toluene/acetonitrile (9:1, v/v) by ultrasonic vibration and stirred for 1 h at room temperature. Then, the functional monomer MAA (0.8 mM) was added to the suspension under stirring for another 1 h. Subsequently, the cross-linking agent TRIM (4.8 mM) and AIBN (40 mg) were then dissolved into the above solution. The mixture was stirred under nitrogen for 10 min and sealed. The prepolymerization was first carried out at 50 °C for 8 h, and the final polymerization was completed at 60 °C for 20 h. The products were further aged at 85 °C for 8 h to obtain a high cross-linking density.

Finally, the synthesized MMIPs were obtained by the magnetic separation, ultrasonically cleaned by methanol/acetic acid (9:1, v/v) as the eluted reagent and washed with deionizer water until no template was detected in the rinses. As a reference, magnetic non-imprinted polymers (MNIPs) were synthesized simultaneously under the same procedure in the absence of template molecule. Fig. 1 illustrated the possible preparation protocol of MMIPs.

2.5. Adsorption experiment of MMIPs

To evaluate the static adsorption, 20 mg of MMIPs and MNIPs microspheres were separately dispersed into 5 mL of toluene/acetonitrile (9:1, v/v) with various EE2 concentrations (0.02–15.0 mM) by ultrasonic treatment. Following this, the mixture was shaken for 1 h at room temperature, and then the supernatant was separated and evaluated by HPLC-UV analysis. Besides these, we also investigated the adsorption kinetics of MMIPs at the same concentration with different volumes.

In order to evaluate the selectivity of MMIPs and MNIPs, the structural analogues (E2, EE, E3 and E1) and the reference compounds (DES) were selected to investigate.

2.6. Chromatographic conditions

The chromatographic separation was performed on a Diamonsil® C18 column (5 μm, 150 mm × 4.6 mm). The mobile phase was composed of acetonitrile, methanol and water (40:10:50, v/v/v). The gradient of flow-rate was carried out starting from 0.8 to 1.0 mL min^{-1} in 5 min, held for 10 min, then to 1.2 mL min^{-1} in 3 min, held another 2 min, and then to 0.8 mL min^{-1} in 3 min, held for 2 min. UV wavelength was set at 280 nm. The injection volume was 20 μL.

2.7. Sample preparation

Stock solutions of estrogens were prepared at concentration of 1 mg mL^{-1} in acetonitrile. Diluting the stock solution serially with

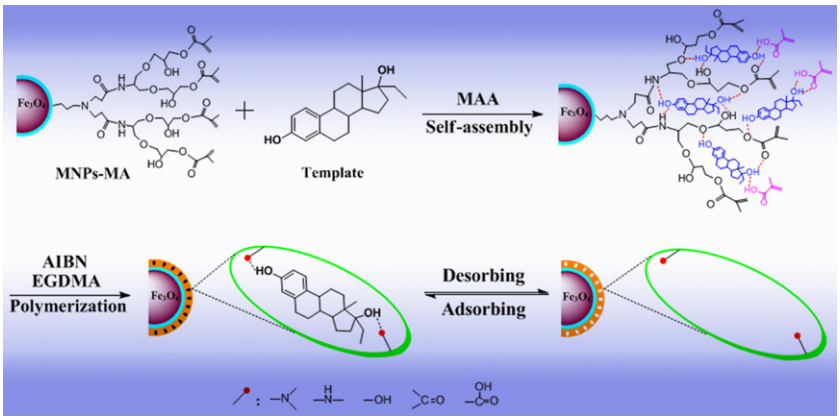

Fig. 1. Schematic representation of the possible preparation process of MMIPs.

acetonitrile yielded standard solutions. All solutions were sealed and refrigerated at 4 °C until use.

0.05 mL of estrogen standard solutions with different concentrations were placed in the centrifugal tubes and dried under a nitrogen stream and then 1 mL of plasma of pregnant women were added and vortex-mixed for 1 min, respectively. Subsequently, 2 mL of acetonitrile were sequentially added, the mixtures were vortexed another 5 min and then centrifuged at 10,000 rpm for 10 min. The supernatant was transferred into an empty tube and dried. Then 20 mg MMIPs as MDSPE materials were added to the resulting residue, reconstituted with 1 mL of toluene/acetonitrile (9:1, v/v), shook at room temperature for 20 min. The MMIPs were obtained by the magnetic separation and washed with 1 mL eluent by ultrasonic treatment for 20 min, and then supernatant was collected and dried under a nitrogen stream. In the end, the resulting residues were dissolved in 0.1 mL of the mobile phase for further

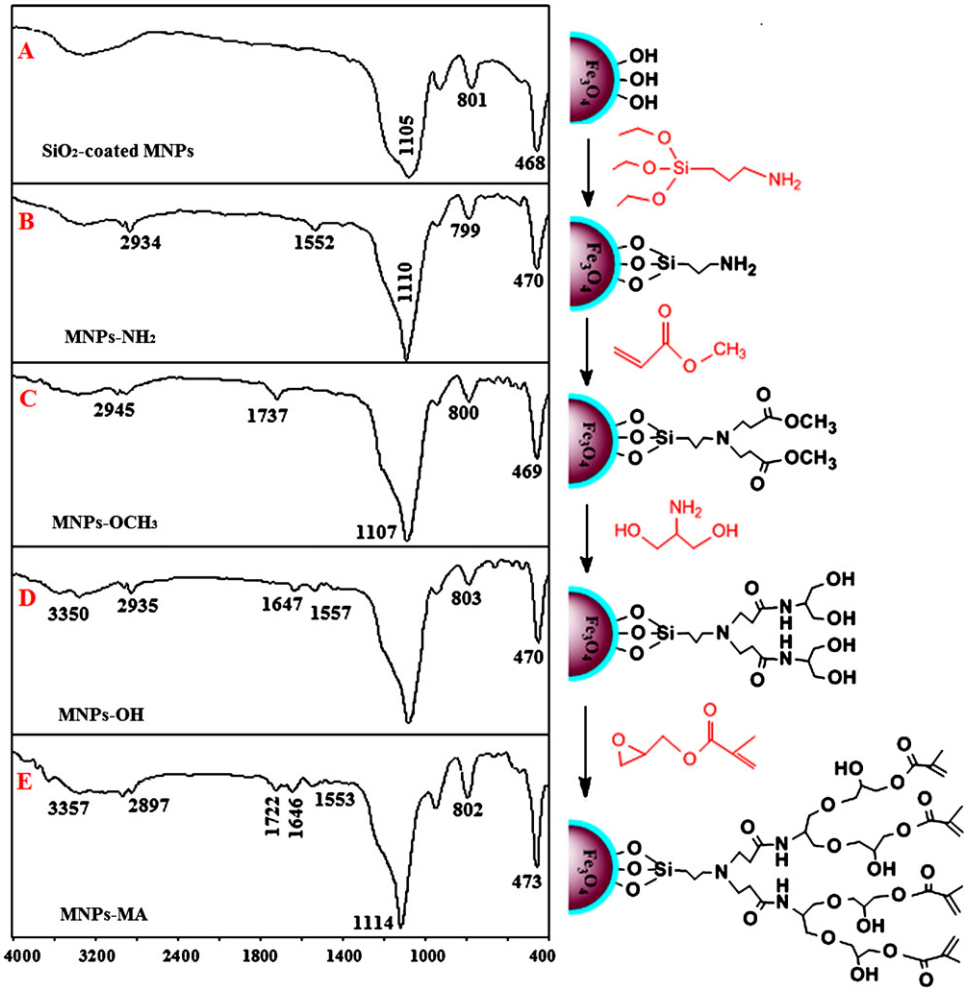

Fig. 2. FT-IR spectra of (A) SiO_2-coated MNPs, (B) MNPs-NH_2, (C) MNPs-OCH_3, (D) MNPs-OH and (E) MNPs-MA.

HPLC-UV analysis and ascertained the unequivocal identification of target compounds in the plasma samples of pregnant women by HPLC–MS/MS.

3. Results and discussion

3.1. Characterizations

3.1.1. Modification of SiO_2-coated MNPs and FT-IR spectrum

In this study, SiO_2-coated MNPs was firstly modified by APTES to produce the amino groups. And then the dendritic MNPs-OH were successfully prepared by divergent synthesis using the MNPs-NH_2 as raw materials by means of Michael addition reaction and amidation reaction with MA and 2-amino-1, 3-propanediol. Subsequently, the ring-opening reaction of epoxy groups in GMA and the terminal hydroxyl groups was occurred, and the methacrylate groups were obtained. Dendrimers were synthesized on the surface of SiO_2-coated MNPs, so the separation and purification of the product were very convenient by an external magnetic field.

To ascertain the presence of functional groups on the surface of SiO_2-coated MNPs, each modification stage was tracked by FT-IR spectra. As shown in Fig. 2, compared with the infrared characteristic peaks of SiO_2-coated MNPs (A), the MNPs-NH_2 (B) displayed clearly the characteristic peaks including the —NH_2 peak at 1552 cm^{-1}, and the relatively strong peak at 2934 cm^{-1} corresponded to the stretching vibration of C—H bonds to the methyl (or methylene) groups for APTES. After the Michael addition reaction, the stretching vibration absorption of C=O bonds in MNPs-OCH_3 (C) was appeared at 1737 cm^{-1}, and the previous —NH_2 absorption peaks were disappeared. This suggested that MA was successfully modified on the surface of MNPs-NH_2 and the terminal ester groups were obtained. Because of the amidation reaction with 2-amino-1,3-propanediol, some new characteristic peaks of the functional groups in MNPs-OH (D) including the —OH absorption peaks at 3350 cm^{-1}, the C=O and N—H bonds of secondary amine at 1647 cm^{-1} and 1550 cm^{-1} were observed, respectively. Meanwhile, the previous peak of ester carbonyl group at 1737 cm^{-1} disappeared. At last, compared with the infrared characteristic peaks of the MNPs-OH, the MNPs-MA (E) displayed clearly a new characteristic peak about the C=O bonds in GMA at 1722 cm^{-1}. These data confirmed the success of chemical modification on the surface of SiO_2-coated MNPs.

3.1.2. Magnetic properties of nanoparticles

Meanwhile, the magnetic properties of several magnetic materials were measured by VSM. As shown in Fig. 3A, the Fe_3O_4, MNPs-MA and MMIPs achieved a saturation magnetization value of 74.92, 23.64 and 7.78 emu g^{-1}, respectively. These data illustrated that dendrimers and MIPs layers on the surface of SiO_2-coated MNPs most likely contribute to the decrease of the saturation magnetization. However, MMIPs with less magnetite encapsulation also possess enough magnetic response to meet the need of magnetic separation within a short time.

3.1.3. X-ray diffraction (XRD) analysis

The XRD patterns of Fe_3O_4 and MMIPs are compared in Fig. 3B, indicating the existence of iron oxide particles (Fe_3O_4) in MMIPs, which has magnetic properties and can be used for the magnetic separation. The results of XRD analysis for Fe_3O_4 were mostly coincident with MMIPs. Six characteristic peaks for Fe_3O_4 were observed in the 2θ range of 30–80°, and the diffraction peak 10–30° is the amorphous diffraction of SiO_2.

3.1.4. SEM and TEM characterization

Representative SEM and TEM images of SiO_2-coated MNPs and MMIPs were provided in Fig. 4. From SEM images, it was obvious

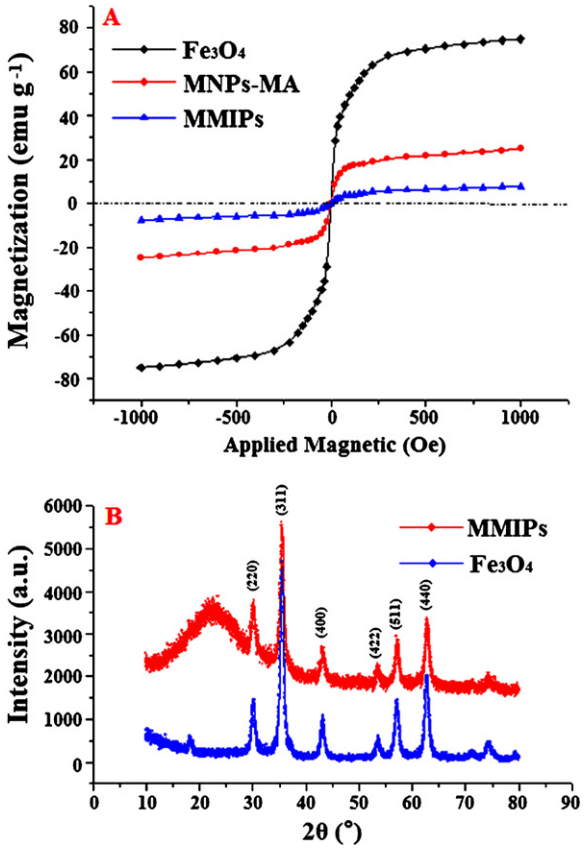

Fig. 3. (A) VSM analysis of Fe_3O_4, MNPs-MA and MMIPs. (B) XRD patterns of Fe_3O_4 and MMIPs.

that MMIPs were regular spheres with a size of 450–550 nm and the surface of synthesized MMIPs was rough. TEM images revealed that the thickness of MMIPs was estimated to be about 20–30 nm, suggesting that most recognition sites were well distributed on the surface of SiO_2-coated MNPs.

3.2. Evaluation of the adsorption characteristic of MMIPs

3.2.1. Adsorption isotherm investigation

The adsorption isotherm for MMIPs and MNIPs was plotted by the batch rebinding experiments which were conducted in toluene/acetonitrile (9:1, v/v) using a range of EE2 concentrations from 0.02 to 15.0 mM. In Fig. 5A, it is observed that the magnetic polymers showed increased binding amounts as the initial concentration increased. However, the extraction capacity of MMIPs was 330.6 μmol g^{-1} for EE2, and MNIPs' was only 107.3 μmol g^{-1}. This suggested that a good molecular imprinting effect of MMIPs was observed, and indicated that MNIPs had the non-specific adsorption capacity due to the abundant functional groups on the surface of the modified SiO_2-coated MNPs.

Meanwhile, to further thoroughly investigate the imprinting effects, the binding properties of isotherms could be estimated by application of Freundlich isotherm model (Eq. (1)). Where Q was the bound amount of adsorbed analyte per unit of polymer mass, and C was the concentration of the analyte in solution. The constant α was a measure of the capacity and the average affinity. The parameter m was the heterogeneity index, which varies from 0 to 1. In addition, the number of binding sites (N) and association constant (K) can be estimated for the subset of binding sites that are accessed within the concentration limits of the binding isotherm. This range is set by the experimental binding isotherm (C_{min} to C_{max}) as defined by Eq. (2). The weighted average affinity constant

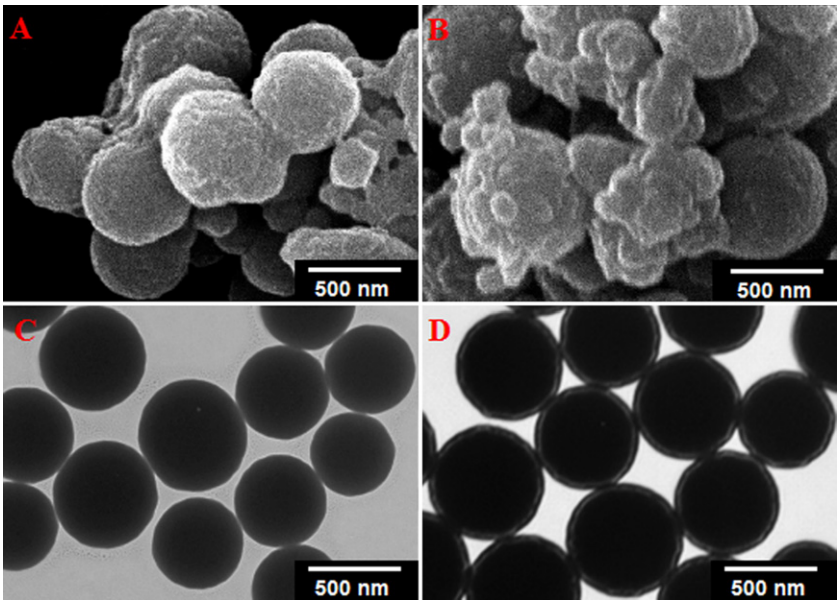

Fig. 4. SEM and TEM images of SiO_2-coated MNPs (A and C) and MMIPs (B and D).

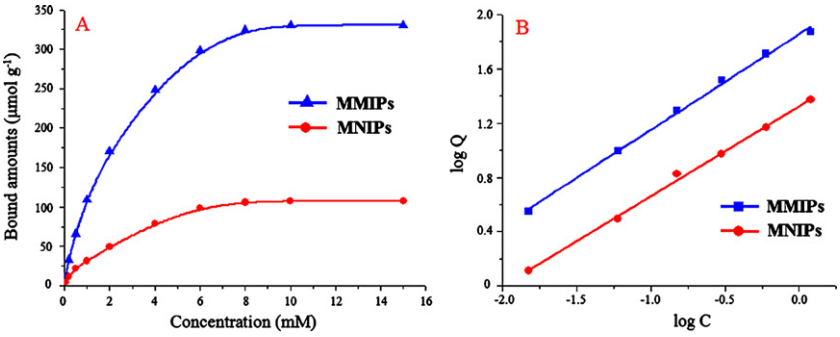

Fig. 5. Adsorption isotherm of EE2 on MMIPs and MNIPs (A) and the fitting plots with Freundlich isotherm model (B).

($\bar{K}_{K_{min}-K_{max}}$) and number of binding sites ($N_{K_{min}-K_{max}}$) for the polymers was calculated using Eqs. (3) and (4) [29–31]. The calculated results were listed in Table 1.

$$\log\ Q = m \log C + \log \alpha \quad (1)$$

$$K_{max} = \frac{1}{C_{min}} \qquad K_{min} = \frac{1}{C_{max}} \quad (2)$$

$$N_{K_{min}-K_{max}} = \alpha(1 - m^2)\left(K_{min}^{-m} - K_{max}^{-m}\right) \quad (3)$$

$$\bar{K}_{K_{min}-K_{max}} = \left(\frac{m}{m-1}\right)\left(\frac{K_{min}^{1-m} - K_{max}^{1-m}}{K_{min}^{-m} - K_{max}^{-m}}\right) \quad (4)$$

The number of binding sites measured by $N_{K_{min}-K_{max}}$ were 39.31 mg g^{-1} and 12.67 mg g^{-1} for MMIPs and MNIPs, respectively. These results indicated the successful imprinting of SiO_2-coated MNPs. And both of the magnetic materials contained a heterogeneous distribution of binding sites with heterogeneity indexes (m) of 0.7047 for MMIPs and 0.6639 for MNIPs.

3.2.2. Adsorption kinetics investigation

Binding kinetics of the template EE2 with MMIPs were evaluated at the different volumes. As shown in Fig. 6A, the adsorption capacities of MMIPs reached gradually equilibrium over time, and the adsorption equilibrium times were different at the different volumes. This was expected because MMIPs could recognize the template molecular effectively, correctly and quickly in a small volume. Meanwhile, the data of desorption kinetics experiments showed that desorption could reach equilibrium in 20 min (Fig. 6B). The short equilibrium time was mainly ascribed to sequential self-assembly process, regular dendritic nanostructures and most of recognition sites of MMIPs existing at surface or in the proximity of the surface, so the diffusional resistance could be decreased.

3.2.3. Selectivity investigation

Successful imprinting cannot solely be evaluated on the ability of MMIPs to rebind the template but also on its discrimination between analogue molecules. Therefore, the cross-selectivity of MMIPs was investigated with E2, EE, E3 and E1, the four estrogens

Table 1
Isotherm parameters for EE2 on MMIPs and MNIPs estimated by fitting data to the Freundlich isotherm models.

	Equation	R^2	$N_{K_{min}-K_{max}}$ (mg g^{-1})	$\bar{K}_{K_{min}-K_{max}}$ (L g^{-1})
MMIPs	$\log Q = 0.7047 \log C + 1.8555$	0.9965	39.31	5.51
MNIPs	$\log Q = 0.6639 \log C + 1.3262$	0.9967	12.67	5.84

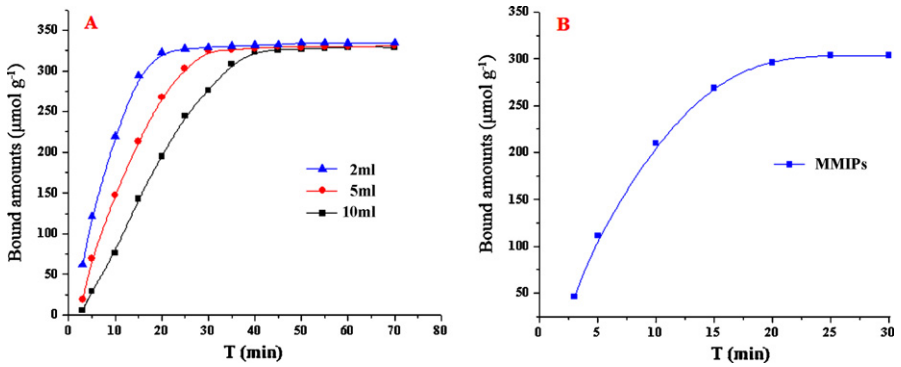

Fig. 6. (A) Adsorption kinetic curves of MMIPs for EE2 in three different volumes and (B) desorption kinetic curve of MMIPs.

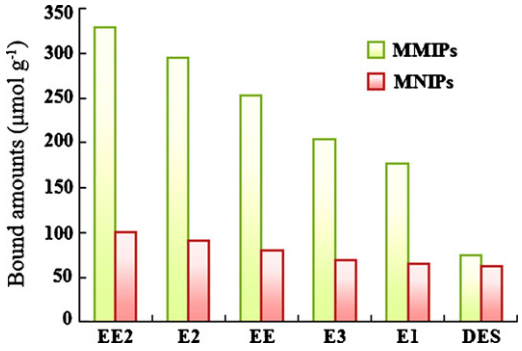

Fig. 7. The selective recognition property of each compound with MMIPs and MNIPs at the concentration of 15 mM, respectively.

with similar molecular structure, as well as the reference compound (DES). The adsorption amounts of different compounds on the polymers were calculated and illustrated in Fig. 7. It could been seen that MMIPs had high selectivity and good extraction capacities for the analogues of EE2 and poor affinity for the reference compound owning to the large difference to EE2 in molecular and size.

As shown in Table 2, the discrimination ability of the polymers was also demonstrated by the distribution coefficient (K_d), the selectivity coefficient (k) and the relative selectivity coefficient (k'). These results were calculated using Eqs. (5)–(7). The selectivity parameters were measured at the initial concentration of 8 mM of each compound. From the parameters, it could be implied that created recognition sites of MMIPs can distinguish between EE2 and other compounds based on their molecular size and chemical structure.

$$K_d = \frac{Q_e}{C_e} \tag{5}$$

$$k = \frac{K_{d(\mathrm{EE2})}}{K_{d(\mathrm{Other\ Compounds})}} \tag{6}$$

Table 2
Data of distribution coefficients (K_d), selectivity coefficients (k) and relative selectivity coefficients (k') of the selective adsorption.

	MMIPs		MNIPs		k'
	K_d	k	K_d	k	
EE2	37.94		10.45		
E2	33.47	1.13	9.40	1.11	1.02
EE	28.10	1.35	8.34	1.25	1.08
E3	19.79	1.92	7.14	1.46	1.31
E1	17.19	2.21	6.74	1.55	1.42
DES	7.77	4.88	6.42	1.63	3.00

$$k' = \frac{k_{\mathrm{MMIPs}}}{k_{\mathrm{MNIPs}}} \tag{7}$$

where K_d represents the distribution coefficient (mL g^{-1}); Q_e (μmol g^{-1}) is the equilibrium adsorption capacity; C_e (mM) is the equilibrium concentration; k is the selectivity coefficient; k' is the relative selectivity coefficient.

3.3. Analytical performance

Under optimal conditions, a series of experimental parameters, including linear range, correlation coefficients (R^2), precision, limits of detection (LODs) and limits of quantification (LOQs), were tested to validate the proposed method (Table 3). The results indicated good linearity was achieved in the range 1.0–25.0 ng mL^{-1} for three estrogens (E1, E2, E3 and EE2) with R^2 of 0.9992, 0.9993, 0.9992 and 0.9995, respectively. The LODs and LOQs of this proposed method ranged from 0.3 ng mL^{-1} to 0.4 ng mL^{-1} and from 0.8 ng mL^{-1} to 1.0 ng mL^{-1}, respectively. The precision of the proposed method was evaluated by measuring intra- and inter-day relative standard deviations (RSDs) at concentrations 10.0 ng mL^{-1} for E1, E2, E3 and EE2, which were within the range of 3.2–3.8% and 4.0–5.3%, respectively. Some methods have been reported to determine estrogens [11,32–34]. Compared with the other sample pretreatment methods (Table 4), this developed method is simple, timesaving, reliable, sensitive and good selectivity.

3.4. Applications to plasma samples of pregnant women

The proposed method was applied successfully to the analysis of estrogens in the plasma samples of pregnant women. Fig. 8A shows the samples' chromatogram obtained by HPLC-UV analysis with MMIPs-MDSPE protocol (curves b and d). In contrast to the chromatogram of direct injection without enrichment (curve a), the obvious target peaks and the obviation of matrix interference are observed, indicating that the remarkable preconcentration ability of the novel MDSPE sorbent to estrogens. The curve c shows that there was no such selectivity with MNIPs as MDSPE materials. The target compounds in the obtained solution as mentioned above have been identified by HPLC–MS/MS (Fig. 8B).

In addition, the amounts of E1, E2 and E3 that were detected from the plasma samples of pregnant women were 5.28, 5.31 and 4.17 ng mL^{-1}, respectively. And the accuracy of the MDSPE method was estimated by determining the plasma samples of pregnant women spiked with E1, E2 and E3 at three different concentration levels. As listed in Table 5, the average recoveries of three estrogens (E1, E2, E3 and EE2) were 87.8%, 93.1%, 90.6% and 95.9% for the spiked samples with RSDs in the range

Table 3
The performance characteristics of the proposed method ($n = 5$).

Compounds	Linearity range (ng mL^{-1})	R^2	RSD (%)		LODs (ng mL^{-1})	LOQs (ng mL^{-1})
			Intra-day	Inter-day		
E1	1.0–25.0	0.9992	3.8	4.6	0.4	1.0
E2	1.0–25.0	0.9993	3.2	4.0	0.3	0.8
E3	1.0–25.0	0.9992	3.4	5.3	0.3	0.9
EE2	1.0–25.0	0.9995	3.6	4.7	0.4	1.0

Table 4
Comparison with other published methods for the determination of estrogens by HPLC.

Extraction method	Analytical method	Sample type	LODs (ng mL^{-1})	Compounds	Reference
C18-SPE[a]	HPLC-FL[b]	Human urine	2.70–8.30	E2, E3, EE	[30]
E3-MIPs-DSPE[c]	HPLC-UV	Milk tablets	1.49–1.83	E2, E3	[11]
EE2-PMIPs-DSPE	HPLC-UV	Chicken tissue	5.40–8.10	E1, E2, E3	[29]
MIPs-SPME[d]	HPLC-UV	Fish and Shrimp	0.98–2.39	E1, E2, E3, EE	[31]
MMIPs-MDSPE	HPLC-UV	Plasma	0.30–0.40	E1, E2, E3	This article

[a] SPE: solid-phase extraction.
[b] FL: fluorescence.
[c] DSPE: dispersive solid-phase extraction.
[d] SPME: solid-phase microextraction.

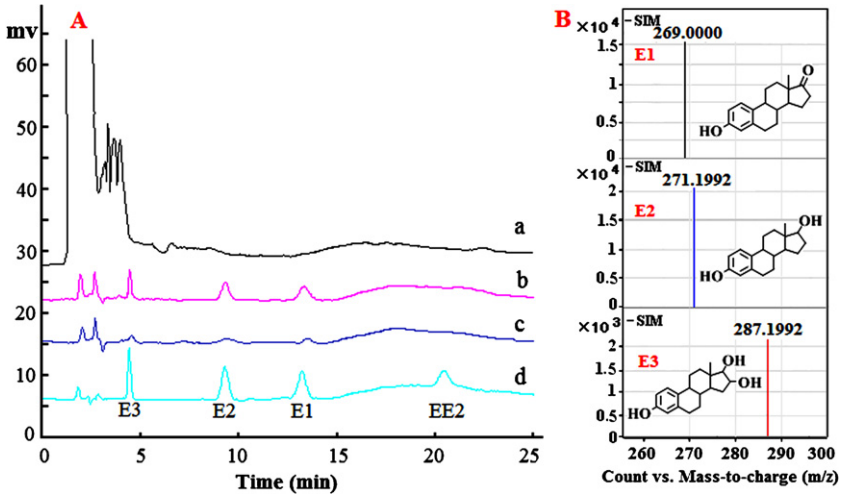

Fig. 8. (A) Chromatograms of estrogens in the plasma samples of pregnant women (a) by direct injection without enrichment, (b) with MMIPs-MDSPE protocol, (c) with MNIPs-MDSPE protocol and (d) the spiked sample with MMIPs-MDSPE protocol. (B) Mass spectrum of E1, E2 and E3 in the plasma samples of pregnant women by HPLC–MS/MS.

of 1.4–6.3%, respectively. From these results, it can be concluded that MMIPs as MDSPE materials have good applicability to selective extraction of the mentioned compounds from complex samples.

Table 5
Determination of estrogens in the plasma samples of pregnant women ($n = 5$).

Compounds	Concentration (ng mL^{-1})	Spiked amounts (ng mL^{-1})	Recovery (%)	RSD (%)
E1	5.28	3.00	87.2	1.4
		5.00	89.6	3.2
		7.00	86.6	2.8
E2	5.31	3.00	92.7	3.8
		5.00	94.1	4.1
		7.00	92.5	2.6
E3	4.17	3.00	90.6	5.6
		5.00	91.2	6.3
		7.00	89.9	5.2
EE2	N.D.	3.00	95.7	3.4
		5.00	95.8	4.6
		7.00	96.2	5.7

N.D.: not detected.

4. Conclusions

In this study, the leakage of residual template was successfully avoided because EE2 as pseudo template is very similar with most of estrogens in shape, size and functionality, and it can be well separated from other estrogens by HPLC as displayed in Fig. 7A. Dendrimers and SiO_2-coated MNPs were effectively introduced to the MIPs which have a number of effective imprinted sites, high adsorption capacity, good selectivity and fast adsorption kinetics. MMIPs as MDSPE materials were used to selectively enrich and determine estrogens in the plasma samples of pregnant women. The results demonstrated that MDSPE protocol was simple, rapid and effective method for sample preparation. In view of these advantages of MMIPs, this proposed method also provides an effective tool for the monitoring of estrogens in food or environment.

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