

Molecularly imprinted polymer microspheres derived from pickering emulsions polymerization in determination of di(2-ethylhexyl) phthalate in bottled water samples

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ABSTRACT: The mono-dispersed macroporous Molecularly Imprinted microspheres (MIPMs) selective for Diethylhexyl Phthalate (DEHP) were synthesized by Pickering emulsion polymerization. Silica nanoparticles were stabilizers in forming a stable oil-in-water emulsion, while the polymeric system was prepared by radical polymerization using methacrylic acid as functional monomer and ethylene glycol dimethacrylate as cross-linker. The results of scanning electron microscopy and nitrogen adsorption desorption measurement indicated that the obtained polymer microspheres had regularly porous structure and narrowly diameter distribution (100 nm), besides the specific surface area (S_{BET}) was $452 \text{ m}^2 \text{ g}^{-1}$, pore volume was $9.685 \text{ cm}^3 \text{ g}^{-1}$, and pore diameter was 5.089 nm. The equilibrium adsorption capacity of MIPs was 1.75 mg g^{-1} at 298 K. Good selectivity for DEHP in another two kinds of analogies (DBP and DAP) was demonstrated with high selectivity coefficients, respectively 17.753 and 19.450. In the end, DEHP-MIP was used as packing of solid-phase extraction to form an sensitive analytical method in extraction and enrichment DEHP in bottled water samples with the limits of detection of $1.7\text{--}2.5 \mu\text{g L}^{-1}$. The recoveries at three spiking level (0.05, 0.1, and 1 mg L^{-1}) were varied between at 97.5 and 103.1% with RSD values below 3.5. © 2016 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 43484.

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INTRODUCTION

Phthalate esters (PAEs) are extensively utilized in different manufacturing plastics industry for improving the flexibility and mold characteristics.^{1,2} They penetrate into the ecosystem,³ food chain,⁴ and effluent,⁵ leading to ubiquitous contamination in various matrices during their usage or disposal. In China, phthalate compounds,⁶ such as diethylhexyl phthalate (DEHP), diethyl phthalate (DEP), and di-n-butylphthalate (DBP) have been classified as the occurring PAEs priority pollutants⁷ and endocrine-disrupting compounds.⁸ Amongst PAEs, DEHP is an increasing widely used plasticizer.² They do not form a covalent bond with plastic substrates, rather they are linked together by the hydrogen bond and Van der Waal force. Although phthalates have low solubility in pure water,⁹ studies have indicated that they show a strong adsorption and affinity with solid particles as well as living body.¹⁰ Hence, PAEs were hard to migrate to atmosphere in water environment, mainly depending on a

series of reactions of adsorption,¹¹ hydrolysis,¹² photochemical reaction,¹³ and microbial degradation.¹⁴ Recent evidences have shown that PAEs have become a major public health concern, which cause cancer and deformities affecting the endocrine system and reproductive capacity.^{15,16}

In the PAEs, DEHP has been a widely used plasticizer, gradually distributes in all kinds of aquatic environment.¹⁷ As DEHP belongs to the long side chain of PAEs, it is much difficult to degrade in the natural environment. The half-life is 105 years for photolysis and as long as 2000 years for hydrolysis.¹⁸ Up to now, the main analysis methods to identification and determination DEHP in environmental samples rely on the prior enrichment technology, such as liquid-liquid extraction¹⁹ and solid phase microextraction (SPME),²⁰ followed by gas chromatography (GC),²¹ high performance liquid chromatography (HPLC),²² or gas chromatography and mass spectrometry (GC-MS).²³ Commercial SPE sorbents retain low selectivity, which

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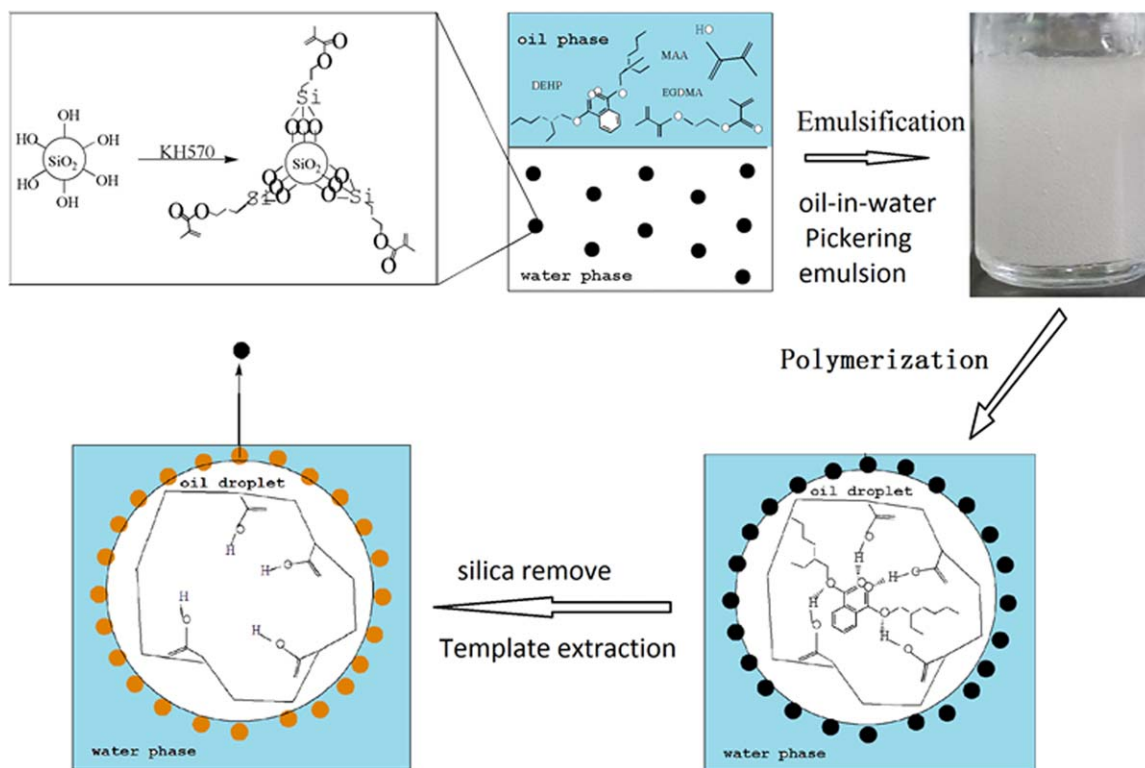


Figure 1. Schematic illustration of the preparation of molecularly imprinted polymer microspheres (MIPMs) and recognition process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

impose restrictions on the trace enrichment of DEHP and its application.

Molecular imprinting is, an emerging technology, was firstly put forward by Wulff^{24–26} aimed at preparing molecular recognition materials with specific selectivity and affinity. The prepared molecularly imprinted polymers (MIPs) are microporous matrices owning microcavities complementary in size, shape, and functional groups after the template is removed from the resulting polymer matrix.²⁷ Thus, they exhibit the characteristics of structure-activity reservation, specific identification and long-term stability, possessing great advantages in chromatographic separations,²⁸ enzyme-like catalysis,²⁹ solid-phase extraction, and bio-sensors.³⁰ However, many inherent challenges of molecular imprinting polymers hinder their advancement, which most imprinted recognition sites are embedded in the interior of highly cross-linked polymer matrix. In surface molecular imprinting technique (SMIT),³¹ imprinted sites are mostly situated at or closer to surface of imprinted material. This improves binding capacity, kinetics, site accessibility, and enhances surface to volume ratio.

Pickering emulsion is a kind of milky emulsion³² stabilized by solid particles adsorbed to oil–water interface, such as colloidal silicon particles. The phenomenon was described by S.U. Pickering in 1907.³³ The small oil droplets can disperse in water and eventually coalesce in oil–water interface. Once solid particles are added to the mixture, they will be combined into the surface of the interface and prevent oil droplets agglomeration so that to make the emulsion stable. Pickering emulsion has not only high stability that ordinary emulsion cannot achieve, but

also good environmental compatibility.³⁴ More recently, we synthesized poly-porous polymer microspheres by Pickering emulsion polymerization, which can solve the defects of traditional MIP synthesized in organic solvent. Then, the synthetic MIP adsorbents have shown a significant priority and become increasingly common in extracting DEHP from the complicated waters, compared with the weak adsorption of natural adsorbents such as silica gel, activated carbon, and alumina.

In this study, we aimed to synthesize the mono-disperse polymer microspheres with a series of closely packed mesoporous on their surface by Pickering emulsion polymerization. Figure 1 demonstrated the schematic diagram of imprinted microspheres in detail with functional monomer (MAA), initiator (AIBN), and porogen (toluene) in oil phase formed by cross-linker (EGDMA). The water phase was Triton X-100 water solution and NaOH solution to insure a PH of 7 during the whole reaction system. The MIP microspheres were fabricated by radical polymerization in oil–water interface and served as solid phase extraction (SPE) adsorbents to detect the adsorption performance towards DEHP in bottled water samples with the help of HPLC. In addition, the synthetic mechanism of the polymer beads was studied through a variety of characterization methods as well, such as SEM, FT-IR, and nitrogen adsorption test.

EXPERIMENTAL

Materials and Instruments

Diethylhexyl Phthalate (DEHP, 99%), Dibutyl phthalate (DBP, 99.5%), Diallyl phthalate (DAP, 98%), 3-Methacryloxypropyltrimethoxy-silane (KH-570), MPS, tetraethyl

orthosilicate (TEOS), $\text{NH}_3 \cdot \text{H}_2\text{O}$ (28 wt %), Methacrylic acid (MAA, 99%), and ethylene glycol dimethacrylate (EGDMA) were supplied by Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Triton X-100, Tetrahydrofuran (THF), toluene, methanol, ethanol, acetic acid and azodiisobutyronitrile (AIBN) were supplied from Sinopharm Chemical Reagent (Shanghai, China). Methanol (for HPLC) was obtained from Tedia (Ohio, USA). Ultrapure water (18.2 M Ω) was obtained from Millipore Milli-Q purification system. NaOH solution (1 M) was prepared. All reagents belong to analytic grade and with no further purification processing.

The surface morphology of polymer microspheres and their dispersion performance was examined by field emission scanning electron microscopy (S-4800, Hitachi, Japan). KRÜSS DSA25 contact angle measuring device supplied by KRÜSS (Germany) was used to reflect the hydrophilic–hydrophobic property of SiO_2 . Nexus 470 Fourier Transform Infrared Spectrometer (FT-IR, U.S.A.) were showed by JEM-2100 (HR) transmission electron microscopy to show various vibration mode of the MIP atomic group intensively. Potassium bromide squash technique was used, samples and background scanning frequency were scanned 32 times at instrument resolution of 4 cm^{-1} and wave number of 4000–400 cm^{-1} . Surface area, pore volume, and size of polymers were figured out by the surface area analyzer (Nova 2000e) with Brunauer–Emmett–Teller (BET) nitrogen adsorption and desorption method. The nitrogen adsorption isotherm was firstly obtained at 77.4 K and then polymers were degassed at 384.3 K.

Synthesis of Silica Nanoparticles

Silica and SiO_2 modified with KH570, was used as stabilizer to form a stable oil-in-water Pickering emulsion, the layer of molecularly imprinted was formed on the surface of silicon in oil–water interface. Micrometer-sized and mono-dispersed silica nanoparticles were synthesized by the modified stöber process,³⁵ with TEOS acting as silicon source and ammonia as basic catalyst. To begin with, 25 mL absolute ethanol/methanol/isopropanol and 2.1 mL ultrapure water were mixed in a three-necked flask, subsequently moderate ammonia (0.61 mL) was added to adjust the pH of solution at 10. Then, mix 4.2 mL TEOS with 5 mL ethanol and drip into the above solution slowly. Finally, the flask was moved into 25 °C water bath and mechanically stirred for 24 h. Thereafter the colloidal solution was separated by a brief centrifugation (9000 r/min), and the gel silica was ultrasonically cleaned with ethanol for more than four times and dried in vacuum at 40 °C overnight.

Synthesis of Modified Nanosilica by KH-570

With the grafting by KH-570, SiO_2 can be more hydrophilic and conductive to form stable Pickering emulsion in the later experiments. In this process, the hydrolysis of siloxane base for silane coupling agent KH-570 can produce silicon hydroxyl and combine with silica surface in acrylic system. Concretely, 5 g silica was added into a 250 mL mouth flask containing 100 mL methanol and ultrasonic dispersion for 15 min. As followed, certain ammonia was added in the silica dispersion, and the flask was shifted into 45 °C water bath under mechanical stir, adding KH-570 methanol solution (KH-570: SiO_2 = 5%, mass ratio) slowly. After 24 h of reaction, the outcome was

centrifuged (10,000 r/min) and ultrasonic rinsed with ethanol for three times when it was cooled to room temperature. At last, the gel shaped modification of silica was obtained by drying in 30 °C under vacuum for 8 h and light gently lapping.

Preparation of MIP by Pickering Emulsion Polymerization

About 35 mg silica nanoparticles and 0.17 mL MAA (2 mmol) was dissolved in a vial of 6 mL 0.3% Triton X-100, sonicated for 10 min. Then, the pH value of the mixed solution was adjusted to 7 by adding 1M NaOH solution. Subsequently, adding quantitative EGDMA (2 mL, 10 mmol), template DEHP (0.195 g, 0.5 mmol), porogen toluene (0.5 mL), and initiator AIBN (20 mg), the mixed solution was sonicated for 10 min again and tempestuously shaken for 2 min by hand. Afterward, the vial was let stand for 36 h until there was no oil droplets coagulated, which a stable Pickering emulsion was formed. Finally, the Pickering emulsion polymerization was proceed at 70 °C in nitrogen atmosphere for 16 h until composite imprinted microspheres were obtained. After the vial was cooled, moderate THF was added to remove the soluble polymers for 2–3 times. Followed by, the reaction products were firstly washed with methanol and water while the products dried under vacuum condition. Generally, in order to remove SiO_2 on the surface of MIP, plastic centrifuge tubes containing 30% HF aqueous solution were placed in a thermostatic oscillator, vibrated for 12 h at least. Later on, the template of MIP microspheres was wiped off by soxhlet extraction with methanol including 10% acetic acid until no template could be examined by ultraviolet spectrophotometer. Following, the polymeric particles were washed with methanol and placed in vacuum drying. Correspondingly, nonimprinted polymers (NIP) were prepared with the same steps without adding the template.

Chromatographic Conditions

DEHP was tested using high performance liquid chromatography in the wavelength of 245 nm. The mobile phase ratio of determination was methanol: water = 95:5 (V/V), the flow rate was 1 mL min^{-1} . The chromatographic column is Waters Symmetry-C18 (5 μm , 4.6 \times 150 nm).

Adsorption Performance Evaluation of Polymer Microspheres

In this article, the capacity of adsorbent for DEHP was determined in batch mode by means of static adsorption experiment and selectivity. The prepared MIP microspheres were carried out to evaluate the kinetics, isothermal and selective adsorption performance at 298 K. It was preliminarily discussed on the specific recognition, binding performance and their recognition mechanism of DEHP. First of all, it was needed to use ultraviolet spectrophotometer to detect the characteristic wavelength of DEHP, DAP, and DBP. To determine the chromatographic conditions should ensure that the three structural analogues could be completely separated under the same criteria. Secondly, their standard curves were established with concentration in 2–100 mg L^{-1} and the corresponding linear equations as well as correlation coefficient were acquired.

Taking an example of MIP, the adsorption kinetics experiments were conducted as follows: a series of certain amount samples (20 mg) were put into some centrifuge tubes containing 6 mL (V) DEHP solution in methanol with a fixed concentration of 10 mg L^{-1} (C_0). Then put them in thermostatic oscillator and

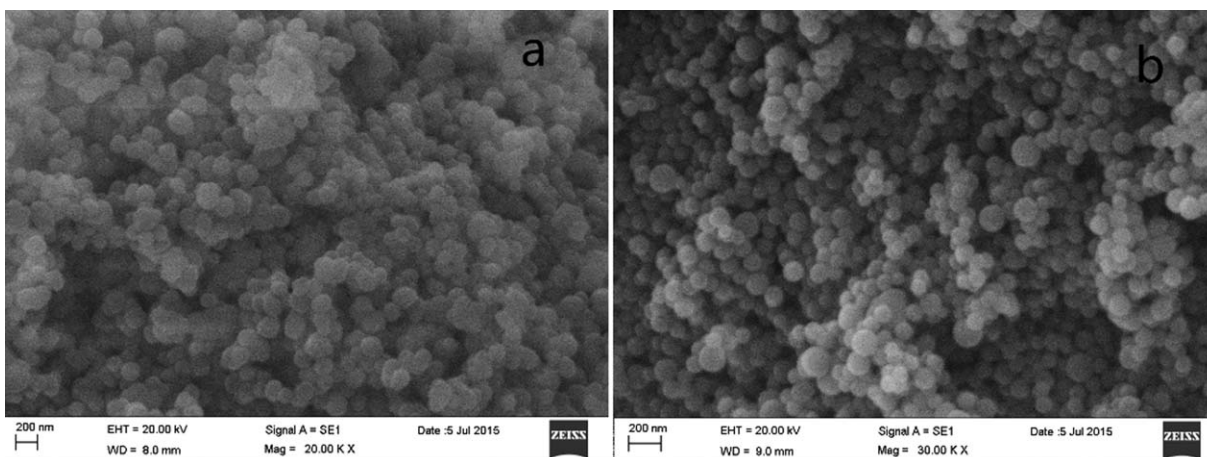


Figure 2. SEM images of silica nanoparticles synthesized by using (a) ethanol and (b) methanol as solvent.

take out the corresponding ones according to the predetermined time intervals in turn, subsequently the supernatant solutions were collected with a syringe having a 0.22 microns porous membrane at the needle to filter solid particles. Finally, with the help of HPLC, DEHP concentration in the solution could be tested at the wavelength of 245 nm. According to the opposite standard curve equation, the concentration of supernatant fluid (C_t) at the counter time was received. Thus, we could calculate the adsorption quantity (Q_t) of polymers for DEHP at different time using the following equation:

$$Q_t = \frac{(C_0 - C_t)V}{m} \quad (2)$$

where Q_t (mg g^{-1}) represents the adsorption capacity of DEHP at the specified time, C_0 (mg L^{-1}) is the initial concentration of DEHP solution, C_t (mg L^{-1}) is the concentration of solution that does not adsorb by MIP/NIP, V (mL) is the volume of DEHP solution, and m (mg) is the quality of the adsorbent.

Similarly, the experiments of adsorption isotherm were as follows: 10 copies of MIP/NIP with the same quality (20 mg) were added to the centrifuge tubes containing 6 mL (V) DEHP

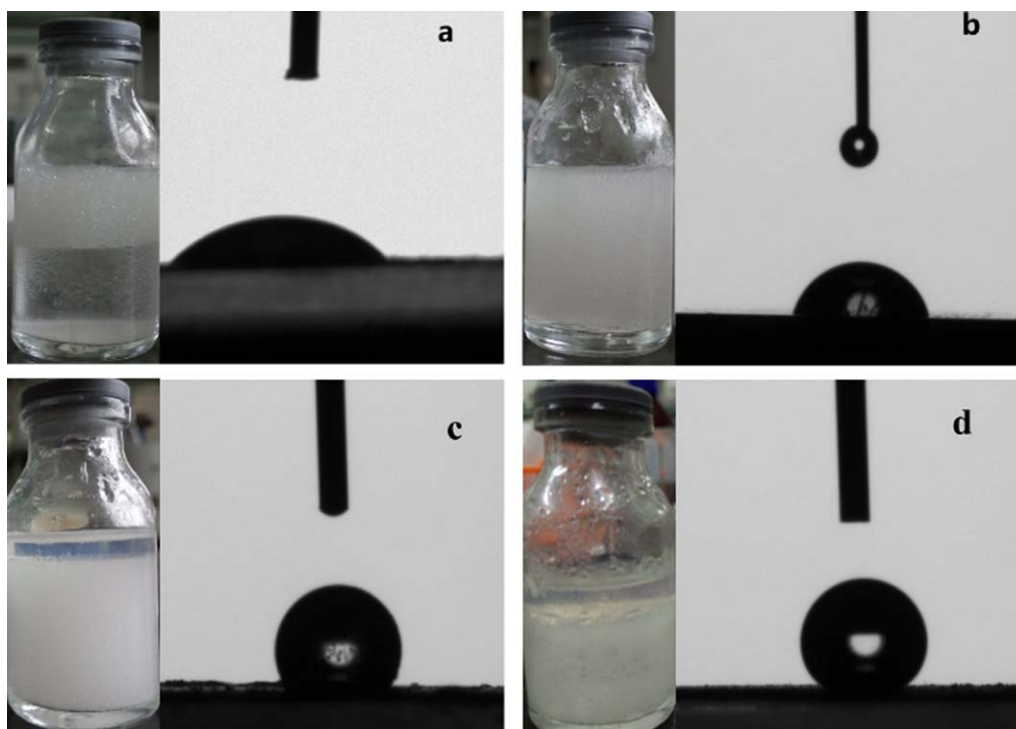


Figure 3. The photograph of contact angle (θ) for silica nanoparticles modified by different amount of KH-570 and their corresponding Pickering emulsions after placing for 36 h. (a) S_{K0} , (b) S_{K1} , (c) S_{K2} and (d) S_{K3} . [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



Figure 4. Pictures of DEHP Pickering emulsion stabilized by SiO_2 at different concentration after placing for 36 h (0, 0.02, 0.035, 0.05, 0.08 g, from left to right). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

solution of different concentration (C_0), varying from 10 to 100 mg L^{-1} . Then put them in thermostatic oscillator shocking for 24 h until the adsorption equilibrium were reached. In the same way, take the supernatant with a syringe with a 0.22 microns at the needle to filter solid particles and using HPLC to measure the adsorption quality (C_e) of supernatant resolution for DEHP that did not adsorb. the adsorption capacity (Q_e) of substrate at different concentration was calculated by eq. (2):

$$Q_e = \frac{(C_0 - C_e)V}{m} \quad (3)$$

where Q_e (mg g^{-1}) is the adsorption of MIP/NIP for DEHP when the adsorption reached equilibrium, C_e is on behalf of the concentration of supernatant solution adsorbed for DEHP under the condition of adsorption equilibrium.

To examine the selective recognition of MIP microspheres toward DEHP, DBP as well as DAP were selected as comparison. The adsorb-ability of template or the competition substances was experiments, 10 mg MIP/NIP were put into 10 mL which is 9 mL of methanol and 1 mL of acetic acid, mixed solution (DEHP/DBP, DEHP/DAP), which the concentration was 40 mg L^{-1} . Placing in a thermostatic oscillator for 24 h till the equilibrium was reached. As well as adsorption kinetics and isotherm, the concentrations (C_e) in the supernatant were tested. Respectively using eqs. (4–6), distribution coefficient (K), selectivity coefficient (k), and relative selectivity coefficient (k') were calculated, which acted as selective evaluation parameters and indexes for polymers.

$$K_d = \frac{Q_e}{C_e} \quad (4)$$

$$k = \frac{K_d(\text{DEHP})}{K_d(R)} \quad (5)$$

$$k' = \frac{k_{\text{SiO}_2 @ \text{MIPs}}}{k_{\text{SiO}_2 @ \text{NIPs}}} \quad (6)$$

where K_d is the distribution coefficient, C_e is the equilibrium concentration of template and its analogues, k is the selectivity coefficient of MIP for the combining of DEHP with respect to the competition substances DAP and DBP, K_d (DEHP) is the distribution coefficient of template, K_d (R) is the distribution coefficient of analogues, k' is relative selectivity coefficient.

Analysis of Adsorption and Regeneration in Bottled Water Samples

The Pickering imprinted microspheres were acted as SPE adsorbents to evaluate the specific adsorption and regeneration for DEHP in bottled water (as real water) that bought in supermarket of Jiangsu University. The water was spiked with DEHP and DBP with concentration of $0.05/0.1/1 \text{ mg L}^{-1}$ and sonicated more than half an hour. During the entire process of solid phase extraction, 300 mg of MIP/NIP was placed into the polypropylene columns and fixed with two pieces of polyethylene sieve plates, which MIP-SPE column and NIP column were prepared. Each of the SPE columns was activated firstly with 10 mL of methanol then 10 mL of pure water as a preprocessing. Following, the spiked water sample was got through the cartridge. Repeating many times and collecting each filtrate until adsorption equilibrium was reached. The column packing was then washed by 10 mL of methanol–water solution (1:9 v/v), and target template was eluted with 10 mL of methanol/acetic mixed solution, besides the leachate and eluent were collected and filtered with 0.22 microns porous membrane in order to use HPLC with content analysis. As for the investigation regeneration performance of polymers, five times adsorption and experiment were carried out in mixed water solution above-mentioned. Finally, the recovery efficiency, referring to the percentage of the adsorption amount with initial adsorption quantity was compared.

RESULTS AND DISCUSSION

Influence Factors on Stability of Pickering Emulsion

Comparatively authority theory believes that the stability of Pickering emulsion is mainly by means of solid particles (silica nanoparticles) adsorbed on the oil–water interface, forming a single or multilayer solid membrane to prevent oil droplets coalescence and stabilize emulsions. Figure 2, respectively, compared the morphology and particle sizes of the synthesized silica by using methanol and ethanol as solvent, the corresponding particle sizes were 100 and 200 nm. The silica prepared by



Figure 5. Pictures of DEHP Pickering emulsion stabilized by SiO_2 nanoparticles after placing for 36 h at $\text{pH} \sim 4, 7, 10$ (from left to right), respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

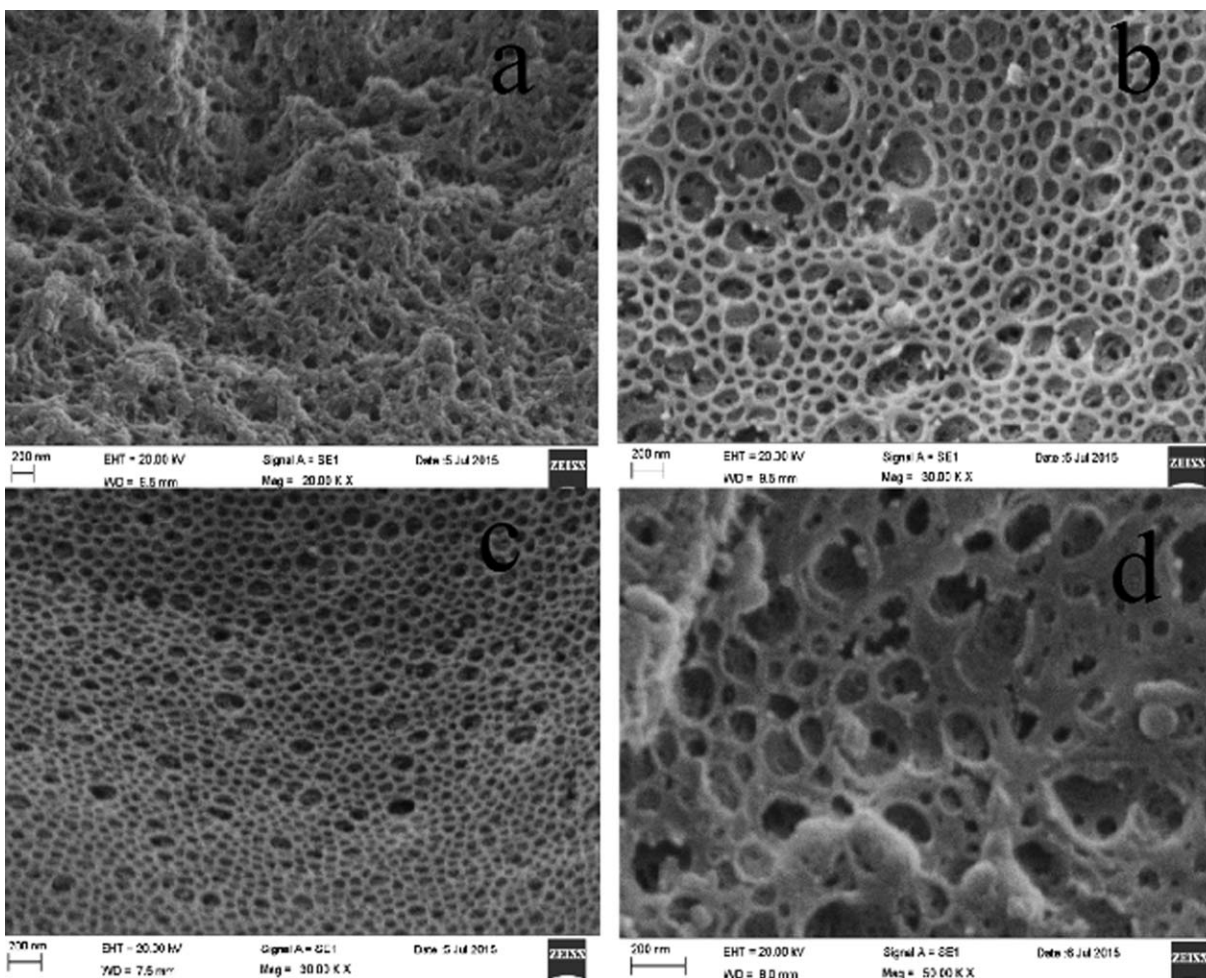


Figure 6. Photographs of SEM images of prepared imprinted polymers with different volume of toluene after placing for 36 h: (a) 0 mL, (b) 0.3 mL, (c) 0.5 mL, and (d) 0.8 mL.

isopropanol was of much larger particle size. Therefore, we chose methanol as subsequent solvent. Besides, the properties of Pickering emulsion were mainly influenced by the surface wettability of solid particles, particle mass, pH and porogen.

Surface Wettability of Silica Nanoparticles. The surface contact angle (θ) is an important measurement for surface wettability of silica, which determines the type of Pickering emulsion. Here, the θ of silica modified by different amount of KH-570 and their corresponding Pickering emulsions after placing for 36 h were shown in Figure 3 by means of compression method to reflect the hydrophilic–hydrophobic property. Hence, the stability of Pickering emulsion was compared with the silica having different wettability on their surface. θ was about 30° in Figure 3(a), and the unmodified silica (S_{K0}) was hydrophilic with a lot of hydroxyl on the surface, therefore the prepared emulsion was layered with silica dissolving in the water phase and oil phase suspending in the upper. Figure 3(b–d) were θ and the emulsions with silica modified by different amounts of KH-570, respectively S_{K1} of 85° , S_{K2} of 120° and S_{K3} of 135° . In Figure 3(b), droplets of Pickering emulsions could be the highest coalescence stability and minimum size, whereas there were oil phase on the top in Figure 3(c,d). As a result, we could observe

that emulsions made by S_{K1} were the most stable at the θ of nearly 90° .

Particle Mass. Since the premised formation condition of Pickering emulsions is that silica should form a strong and stable interface membrane in the oil/water boundary, the particle concentration should reach the minimum threshold on the surface to prevent emulsion droplets from coalescing. It had studied that the particle size for emulsion decreased along with the increase of the mass of silica. As shown in Figure 4, the effect of SiO_2 mass (m_p) on Pickering emulsions with five different levels was discussed, respectively 0, 0.02, 0.035, 0.05, and 0.08 g. When m_p was inferior to 0.02 g, there was a large number of oil phase separated from the upper emulsions, which could be explained the inadequate for silica to coat oil droplets. With the increase of silica, the volume of emulsion phase firstly increased when m_p reached to 0.035 g then decreased with excessive amount of silica particles dispersing in the water phase at the bottom of vial and produced apparently flocculation and increasing water overflowing when m_p was in excess of 0.05 g. Therefore, we set the amount of silica to 0.035 g in the following experiment.

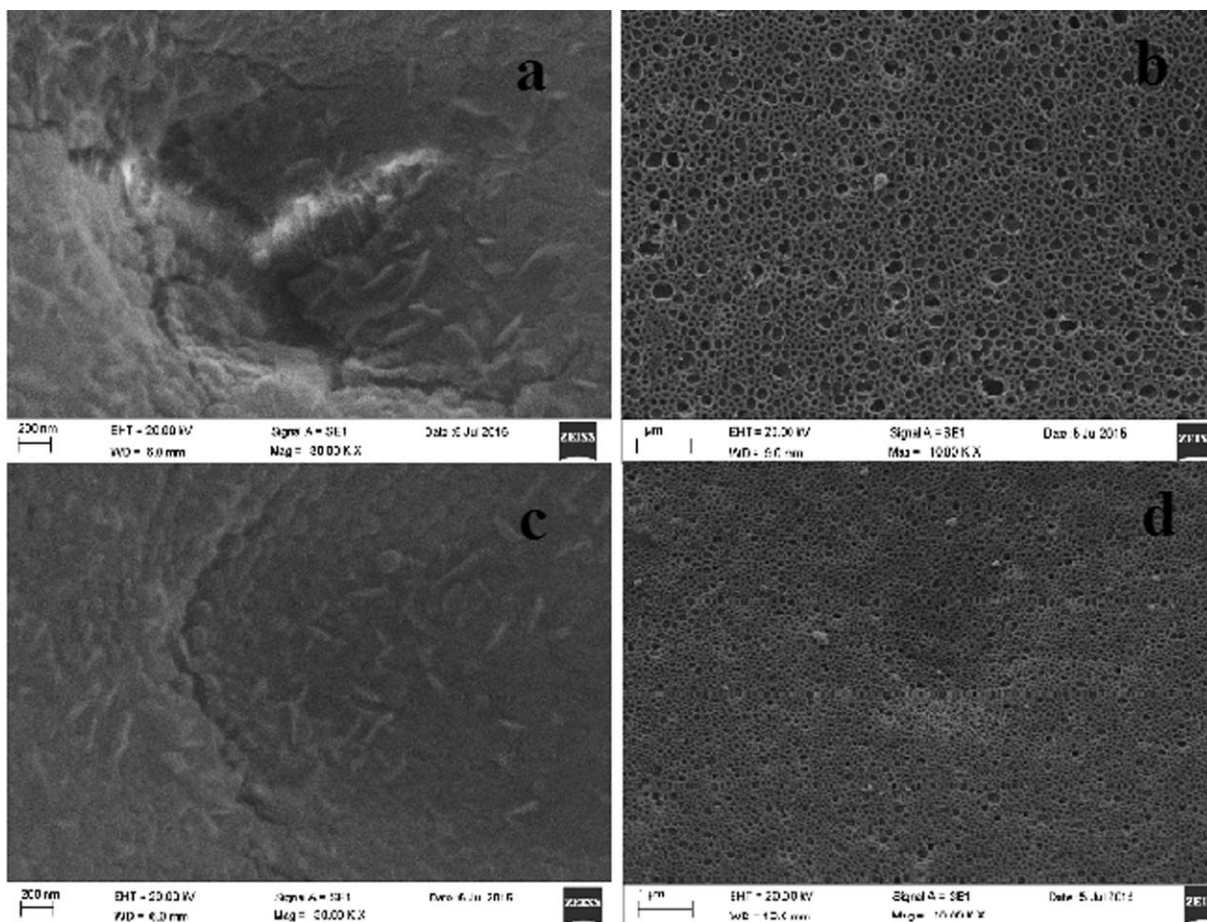


Figure 7. SEM images of (a) SiO₂@NIP, (b) SiO₂@NIP after eluting silica, (c) SiO₂@MIP, and (d) SiO₂@MIP after eluting silica.

pH Value in Water Phase. pH value affects the stability of DEHP Pickering emulsions by interfacial tension and the diameter distribution of emulsion droplets. Emulsions stabilized by SiO₂ nanoparticles were simply discussed at pH~4,7,10 (from left to right) in Figure 5. In this study, we found that with the increase of the pH value, the phase volume fraction of Pickering emulsions increased at first and then decreased. It showed up worse stability and more hydrophobic under alkaline environments (pH = 10) than in acidic condition (pH = 4), and aqueous dispersion separated from emulsion phase. The phenomenon could be explained that the electrostatic repulsion between modified silica was less under the condition of acid, which could be conducive to their adsorption on the interface. Uniform and stable Pickering emulsion was prepared at the pH of 7. Hence, pH was set to neutral in the later experiment.

Porogen. In the process of molecular imprinting, toluene, a kind of porogen with weak hydrogen bonding interaction, has good solubility property of polymer and then generates abundant pore structure at the surface of polymeric microspheres. The different volume of toluene was added into the Pickering emulsions, ulteriorly, the photographs of SEM images of prepared imprinted polymers were observed in Figure 6. In detail, the pictures of DEHP Pickering emulsions above after placing for 36 h were observed in Supporting Information Figure S1. When there was no or little toluene, only a small amount of oil

droplets were distributed between water phase in the top layer and oil phase below. Whereas the volume of toluene was 0.8 mL, the position of oil–water phase was upside down and oil phase in the top. Figure 6(a) made clear that the polymers

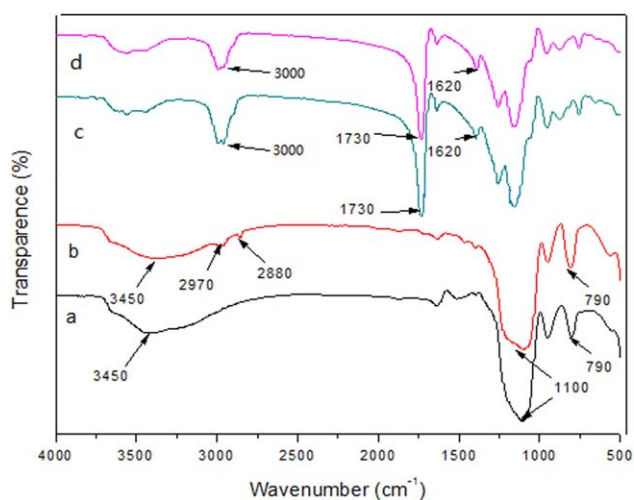


Figure 8. FT-IR spectra of (a) SiO₂, (b) SiO₂ modified with KH-570, (c) SiO₂@MIP, (d) SiO₂@MIP after template removal, and (e) SiO₂@NIP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Textural Features of Silica, MIP, and NIP

	Surface area ($\text{m}^2 \text{g}^{-1}$)			Pore volume ($\text{cm}^3 \text{g}^{-1}$)		Pore diameter (nm)		
	S_{BET}	S_{BJH}^1	S_{BJH}^2	V_{BJH}^1	V_{BJH}^2	D_{ave}	D_{BJH}^1	D_{BJH}^2
Silica	32.89	10.21	10.21	50.48	37.10	8.472	50.48	37.10
MIP	452.0	1295.4	1305.6	9.685	9.727	5.089	8.553	8.177
NIP	559.7	1369.7	1388.2	10.790	10.793	6.360	9.269	9.512

S_{BET} represents BET surface area. S_{BJH}^1 , S_{BJH}^2 stands for BJH adsorption and desorption cumulative surface area (1.7–300 nm). V_{BJH}^1 , V_{BJH}^2 stands for BJH adsorption and desorption cumulative volume of pores (1.7–300 nm). D_{ave} represents adsorption average pore width (4V/A by BET). D_{BJH}^1 , D_{BJH}^2 stands for BJH adsorption and desorption average pore diameter (4V/A).

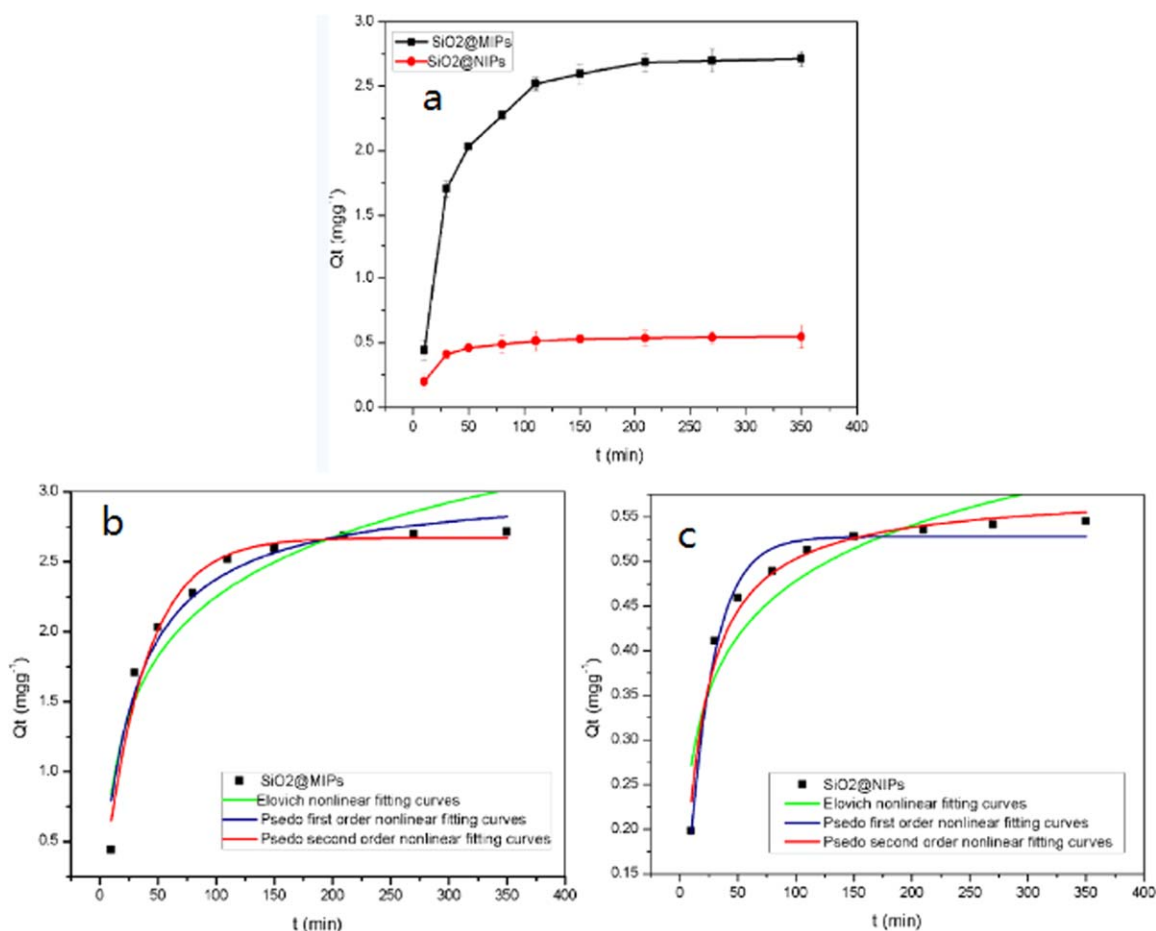


Figure 9. Nitrogen adsorption isotherms and pore size distributions on silica, MIP, and NIP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Kinetic Parameters of the Pseudo Order and Elovich Equations for Adsorption of MIP/NIP

	Pseudo-first-order			Pseudo-second-order			Elovich			
	$Q_{e,e}$ mg g^{-1}	$Q_{e,c}$ mg g^{-1}	k_1 min^{-1}	R^2 nonlinear	$Q_{e,c}$ mg g^{-1}	k_2 $\text{mg g}^{-1} \text{min}^{-1}$	R^2 nonlinear	a mg g^{-1} min^{-1}	b g mg^{-1}	R^2 nonlinear
MIP	2.7	3.051	0.011	0.953	2.672	0.028	0.974	0.240	1.631	0.856
NIP	0.47	0.528	0.046	.0979	0.579	0.115	0.965	0.184	11.13	0.842

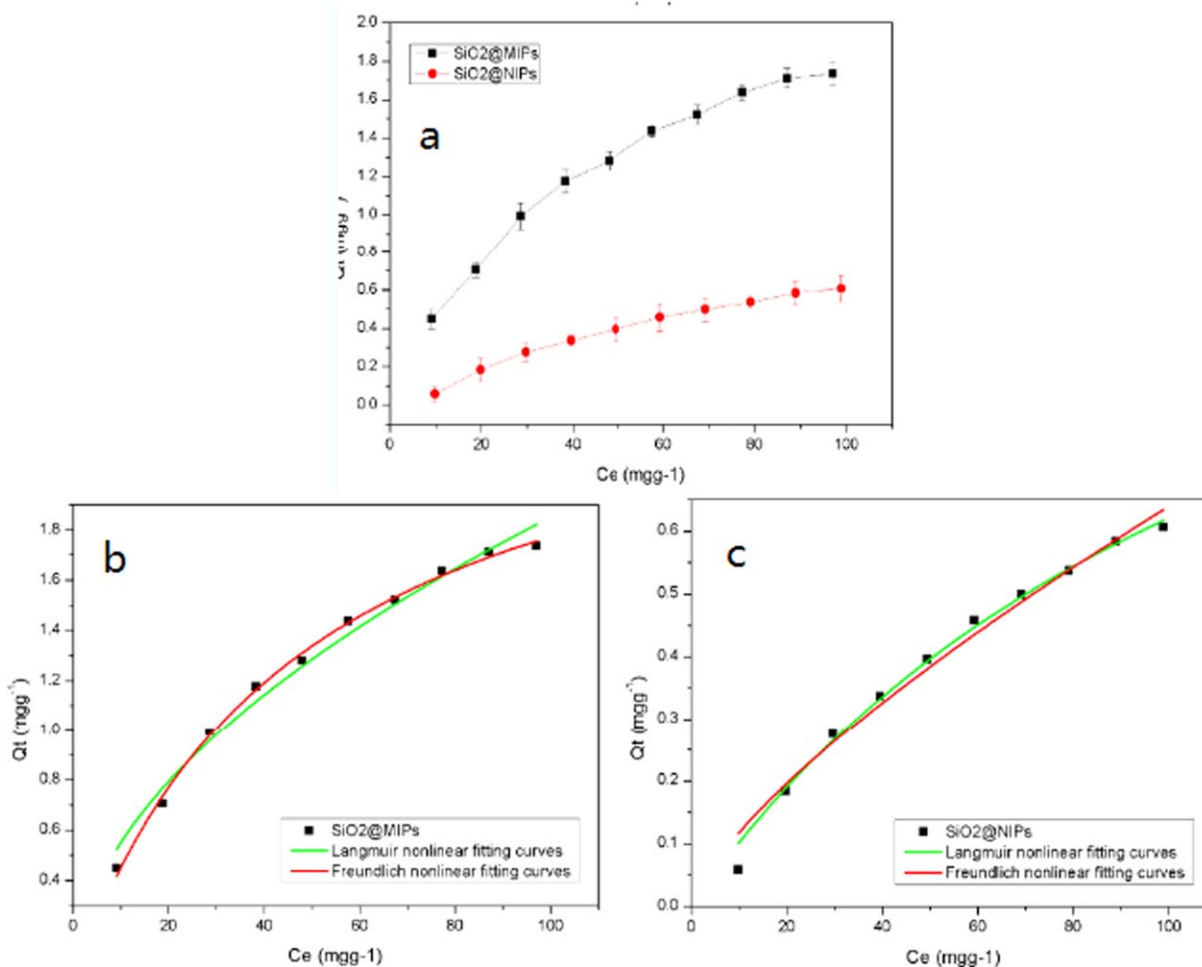


Figure 10. Adsorption kinetic and curves of SiO₂@MIP (a) and SiO₂@NIP (b) as well as their pseudo-first- and second-order nonlinear fitting curves. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

without toluene showed the chaotic mesoporous structure. Polymer microspheres in Figure 6(b–d) were all orderly countless network structure in the presence of the toluene. The only difference was that relatively uniform pore diameter at the volume of 0.5 mL of toluene. When it was 0.3 or 0.8 mL, the diameter was heterogeneity and the structure became deteriorate. In consequence, 0.5 mL was selected as the optimal volume.

Characterization of the MIP/NIP

SEM. According to the upper experimental optimization conditions, corresponding imprinted/nonimprinted polymer microspheres with uniform diameters were prepared under neutral

condition, and the volume of porogen was 0.5 mL. The SiO₂@MIP with modified and no-modified were compared in Supporting Information Figure S2, the former had much smaller and more uniform particle size, nearly 100 nm, which the later was up to 200 μm in size. SiO₂@NIP/MIP before and after eluted DEHP were analyzed with their particle sizes and surface morphology in Figure 7. A large number of spherical bulge was seen in Figure 7(a, c), however the photo of MIP was more smoother on the surface. In addition, the diameter of MIP (100 nm) was smaller and more uniform than NIP (200 nm). This may be explained by the specific adsorption of imprinted polymers to make silica more compact and orderly arrangement.

Table III. Isothermal Parameters of the Langmuir and Freundlich Equations for Adsorption of MIP/NIP

Isotherm modes	Langmuir			Freundlich		
	k_L L mg ⁻¹	Q_m mg g ⁻¹	R^2 nonlinear	k_F	n_F	R^2 nonlinear
DEHP-MIP	0.001	2	0.983	0.1	1.3	0.997
DEHP-NIP	0.007	1	0.991	0.4	2.4	0.979

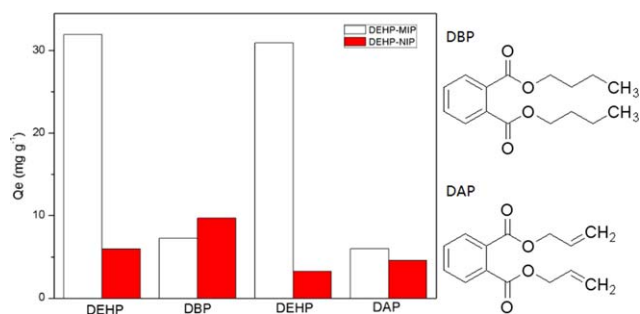


Figure 11. Effect of adsorption capacity towards DEHP, DBP and DAP of MIP/NIP, and the structure of Dibutyl phthalate (DBP) as well as Diallyl phthalate (DAP). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FT-IR Spectra. In order to prove the successfully modified of nanosilica and the formation for layer of imprinted polymer, FT-IR spectra of SiO₂ (a), SiO₂ modified with KH-570 (b), SiO₂@MIP after DEHP was eluted (c), and SiO₂@NIP after eluting (d) were demonstrated in Figure 8. In both Figure 8(a,b), we could see that the mutual absorption bands of the asymmetric, symmetric stretching vibration peak of Si-O-Si and wide absorption peak of -OH, respectively, at 1100, 790, and 3450 cm⁻¹. However, Figure 8(b) had the characteristic absorption bands of C-H on methyl and methylene at 2970 and 2880 cm⁻¹, which confirmed the introduction of organic groups on the modified silica. In Figure 8(c, d), the absorbances at 3000, 1620, and 1730 cm⁻¹ were because of vibration of -OH on MAA, C=C and C=O on EGDMA, which proved the existence of polymerization of functional monomer and cross-linker. Besides, Figure 8(c, d) were nearly same but did not have the feature peak, which illustrated DEHP in MIP has been completely washed out.

Nitrogen Adsorption and Desorption. Both silica and MIP/NIP complied with “type IV” adsorption isotherm, characteristic of mesoporous materials as there existed hysteresis because of the capillary condensation in Supporting Information Figure S3. The width of hysteresis was small, suggesting the distribution of pore size was narrow. Besides, the tendency of saturated adsorption was shown at the relative pressure of 1, which conformed the neat mesoporous structure. The detailed textural features of silica, MIP, and NIP were shown in Table I. When silica and template DEHP was eluted after Pickering emulsion polymerization, the MIP microspheres had smaller diameter size (5.089 nm), pore volume (9.685 cm³ g⁻¹) and consequently small specific surface area (452 m² g⁻¹) than NIP, accordingly

6.36 nm, 10.79 and 559.7 m² g⁻¹, which indicated the formation of an imprinted layer on the surface and internal cavities of silica.

Adsorption Kinetics

Adsorption kinetics were mainly served to study the adsorption mechanism type by using DEHP-MIP/NIP as adsorbent for solid-phase extraction, then the kinetic data were further fitted by pseudo first and second order as well as Elovich nonlinear dynamic equations eqs. (7–9) to figure up the equilibrium adsorption in Figure 9:

$$q_t = q_e(1 - e^{-k_1 t}) \quad (7)$$

$$q_t = q_e^2 k_2 t / (1 + q_e k_2 t) \quad (8)$$

$$q_t = \ln(a * b) / b + \ln(t) / b \quad (9)$$

where k_1 and k_2 were rate constant of first- and second-order models, a was adsorption rate constant, b was surface coverage related constant, t was adsorption time.

Figure 9(a) denoted the kinetic curves for DEHP on MIP and NIP at the fixed concentration (10 mg L⁻¹) at 298 K. Both of them appeared at two stages during the whole adsorption process, firstly the external binding sites quickly adsorbed DEHP with a small steric hindrance. Secondly, DEHP began to combine with interior binding sites, hence the adsorption rate was slowed down on account of the increasing mass transfer resistance. Because of the specific adsorption with DEHP and high surface area, the saturated adsorption capacity of MIP (2.7 mg g⁻¹) was significantly higher than NIP (0.47 mg g⁻¹), and the adsorption equilibrium (200 min) was four times longer than NIP.

The dynamic data of MIP/NIP was nonlinear fitted in Figure 9(b,c), and their rate constants and linear regression values of three kind of kinetic equations were listed in Table II. For MIP, R^2 for pseudo-second-order equation (0.9745) was the best, and the calculated value ($Q_{e,c}$, 2.6715 mg g⁻¹) was nearly the same with experimental result ($Q_{e,e}$, 2.7 mg g⁻¹). Wherever, R^2 of NIP had a significant association with pseudo-first-order equation. It was proved that pseudo-second-order model could be well fitted the experimental data of MIP as the specific binding bites.

Adsorption Isotherm

The adsorption isotherm reflects the relations between the adsorption capacity for DEHP-MIP/NIP with their concentration of adsorption equilibrium. Simultaneously, the experimental data were fitted by Langmuir and Freundlich nonlinear equations:

Table IV. Selective Recognition Parameter of DEHP-MIP and NIP

Mixed solution	Compounds	DEHP-MIP		DEHP-NIP		k'
		K_d	k	K_d	k	
DEHP/DBP	DEHP	3.959	-	0.175	-	
	DBP	0.223	17.753	0.321	0.545	32.574
DEHP/DAP	DEHP	3.419	-	0.088	-	
	DAP	0.176	19.450	0.128	0.688	28.271

$$q_e = (q_m * k_L * C_e) / (1 + k_L * C_e) \quad (10)$$

$$q_e = k_F * C_e^{1/n} \quad (11)$$

where q_m and k_L were the maximum adsorption capacity for mono-layer and Langmuir constant, C_e was equilibrium concentration, k_F and n were isothermal parameters and adsorption capacity, respectively.

Figure 10(a) was the isotherm of MIP/NIP, and the adsorption quantity increased with the increase of C_0 until the adsorption equilibrium was reached, but MIP was 1.75 mg g^{-1} , much higher than NIP (0.62 mg g^{-1}). Table III and Figure 10(b,c) compared the fitting results of two kinds of isotherm models. It was Langmuir model that had more relevant with the experimental data of MIP ($R^2 = 0.9969$), whereas Freundlich model identified with NIP ($R^2 = 0.9914$).

Selective Adsorption Analysis

Two other phthalates, DBP and DAP, were chosen as structural analogues. As shown in Figure 11, mixed DEHP with DBP/DAP into the adsorption system at the concentration of 40 mg L^{-1} to determine the specific adsorption performance for MIP/NIP toward DEHP by static selective adsorption experiments. We could find that DEHP-MIP had huge adsorption capacity in both of the two mixed solution. The correspondingly selective recognition parameters (K_d , k , K') were listed in Table IV. The distribution coefficients were the highest for DEHP relatively to DAP and DBP. Besides, the selectivity coefficients for MIP (17.753, 19.450) were nearly 30 times for NIP (0.545, 0.688), indicating a higher selective recognition ability.

Analysis of DEHP in Bottled Water

DEHP-MIP was suitable for the packing of solid-phase extraction in separation and enrichment of trace materials in bottled water samples. The optimal elution condition was confirmed with 9 : 1 for methanol/acetic in volume. Linearity and the correlation coefficient (0.995) were obtained by different concentrations of DEHP solution between 0.02 and 2 mg L^{-1} , besides the limits of detection was $1.7\text{--}2.5 \mu\text{g L}^{-1}$. The recoveries and RSDs for three kinds of added aqueous solutions (0.05/0.1/1.0 mg L^{-1}) were, respectively, 101.5/103.1/97.5 (%) and 3.0/2.8/3.5. It proved that the prepared MIP by means of SPE-HPLC method was practical in determining DEHP from bottled water samples.

CONCLUSIONS

Macroporous microspheres ($\text{SiO}_2\text{@MIP}$) were successfully synthesized by Pickering emulsion polymerization with silica as stabilizing agent in conforming a stable O/W emulsion. DEHP-MIP had mono-disperse (100 nm) and porous structure, as a result higher specific surface area and better adsorption capacity for extraction of DEHP in bottled water samples in SPE-HPLC procedures at low concentration. It provided a feasibility for future research towards separation and enrichment hydrophobic organic pollutants.

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