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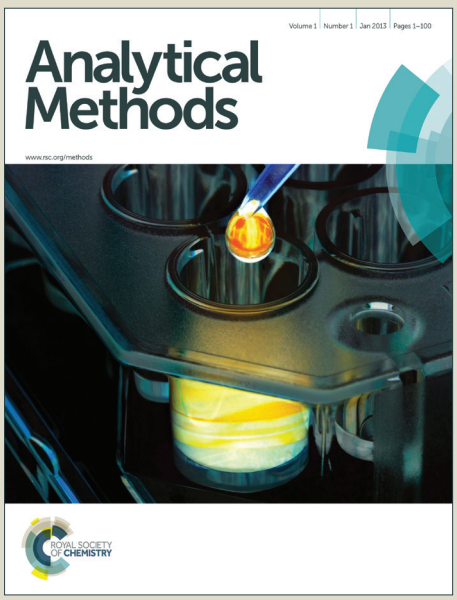

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Preparation of hollow molecular imprinting polymer for determination of ofloxacin in milk

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Abstract

A porous hollow molecular imprinting polymer (MIPs) with ofloxacin (OFL) as template and SiO_2 nanoparticle (250 nm) as sacrifice core was synthesized. Dibutyl isophthalate was used as plasticizer to strengthen the polymer shell in MIP preparation firstly, which was necessary to avoid the shell broken. Infrared spectra (IR) and transmission electron microscope (TEM) were used to verify the successful synthesis of the hollow MIPs. The adsorption behavior of MIPs was evaluated by adsorption capacity, imprint factor and adsorption model. The MIPs could obtain the adsorption capacity of 147 mg g^{-1} to OFL in theory and imprint factor of 2.6 when the initial OFL concentration was 900 μg mL^{-1}. The MIPs could adsorb not only OFL but other fluoroquinolone antibiotics (FQs), which was useful to analyze FQs together. At the same time, it hardly adsorbed other compounds with dissimilar structure. The MIPs used as adsorbent to enrich the FQs from milk and the good selectivity and enrichment ratios were obtained. Coupling with high performance liquid chromatography (HPLC), the FQs in milk were determined with no more than 30 ng mL^{-1} of the limit of quantitation (LOQ).

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Keywords fluoroquinolone antibiotics hollow molecular imprinting polymer milk plasticizer

Introduction

Fluoroquinolone antibiotics (FQs) are widely used as not only human but veterinary medicine in recent years. The residual FQs in edible animal's meat and milk will be adsorbed again by human and further cause pathogen resistance [1]. FQs are prohibited for use in food producing animals worldwide and their maximum residue limits in food must be less than 100 μg kg^{-1} at least [2]. Milk is the common food especially for young children and it is very important to be sure its safety because the trace amount of antibiotics residue would be dangerous for the babies.

Nowadays, the residues of FQs in environment have aroused a concern. A lot of sensitive HPLC analytical methods to determine FQs are set up coupled with mass spectrometry detector [1,3], UV detector or diode array detector [4-5], fluorescence detector [6] or chemiluminescence detector [2]. However, the analysis of trace FQs in complicate matrix such as food still faces great challenge and the sample pretreatment is necessary.

For getting lower detection limit in the samples with complicate matrix, usually solid phase extraction (SPE), solid phase microextraction (SPME) and matrix solid-phase dispersion (SPD) [7-8] are preferred for enrichment and cleaning of samples. The molecular imprinting polymers (MIPs) are becoming the main sorbents for these techniques. For improving the selectivity or adsorption capacity, new types of MIPs are prepared continually with various methods. Besides the traditional precipitation polymerization [9], the electropolymerization [10], water compatible MIPs used to aqueous samples [11-13], metal ion mediated MIPs [14-15], surface MIPs on basic adsorbents such as silica [16] and mesoporous carbon [5], hollow porous MIPs [17,19] and MIPs with more than one functional monomers [20] are present recently. The sites left by template molecules are distributed homogeneously in the MIPs, it is difficult to use the innermost efficiently, which reduce the adsorption efficiency of MIPs and prolong the adsorption time. Hollow porous MIPs with thin shell make it possible to finish the adsorption in short time easily owing to the lower mass transfer resistance and the larger adsorption interface. Liu etc [17] prepared the hollow porous MIPs of bisphenol A using mesoporous MCM-48 nanospheres (about

500 nm) [18] as the sacrificial support. The hollow MIPs of fenpropathrin were prepared [19] with the size of 100 μm. However, hollow MIPs were easy to be broken during the usage and thick shell had to be made to avoid the breach, which was conflicted with its advantages. Smaller particles used as sacrificial support could make the MIPs own larger special surface to increase adsorption capacity and resist pressure to avoid breach in the operation.

In the paper, we synthesized a new hollow porous MIPs using ofloxacin (OFL) as template molecules and further used the MIPs as SPE sorbent to determine the trace FQs in milk coupling with HPLC method. The hollow MIPs had smaller core compared with the literatures and achieved a nonbreakable thin shell owing to a plasticizer used to enhance the mechanical strength of MIPs.

Experimental

Materials and reagents

Tetraethoxysilicane (TEOS), Methacrylic acid (MAA), 3-Methylacryloxypropyl-trimethoxysilane (MATMS) and Ethylene glycol dimethacrylate (EGDMA) were purchased from Alfa Aesar (Beijing, China). EGDMA was distilled before used. azo-bis-isobutyronitrile (AIBN) was obtained from yuefeng chemical company(Tianjin, China). Dibutyl isophthalate (DBP) was obtained from Beijing chemical factory (Beijing, China). Ofloxacin (OFL), enrofloxacin (ENR), norfloxacin (NOR) and sulfamerazine (SMZ) were from Shanghai Yuanye biology technique company (Shanghai, China). Ibuprofen (IBU) was obtained from Hubei Baike Gelai Pharmaceutical Company (Wuhan, China). Sudan I was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). The solvents used including methanol, ethanol and acetonitrile (ACN) were distilled before used. Acetic acid, triethylamine and hydrofluoric acid (HF) were used directly. All the reagents were of analytical grade. Ultra pure water used throughout the experiments was obtained from the MILLI-Q (Millipore, Bedford, MA, USA) purification system. HPLC-grade methanol was from Dima Technology (RichmondHill, USA).

Instrumental analysis

The chromatographic analytical system consisted of two Model 210 HPLC pumps and a UV detector (Varian Prostar, USA). All separations were carried out on a C18 column (Dikma Technologies, 5 μm, 250×4.6 mm). Mobile phase was consisted of Methanol/phosphate buffer (0.01mol/L, pH 2.80, v/v=25/75) and filtered by 0.45 μm membrane. The rate was 1.0 mL min^{-1} and column temperature was 40 ℃. UV detection wavelength was changed as following: 0-14.8 min 293 nm; 14.8-25.0 min 277 nm. Injection volume was 10 μL.

UV measurement was accomplished at Beijing Puxi TU-1810-UV spectrophotometer (Beijing, China). The Fourier transform infrared (FTIR) spectra were acquired with an FTIR spectrometer (Thermo Mattson, Madison, WI, USA). Transmission electron microscopy (TEM) was carried out by a JEM1200EX instrument (JEOL Tokyo, Japan).

Preparation of MIPs

First, SiO_2 nanoparticles were synthesized and followed by surface modification of MIPs polymers with OFL as template molecules. Later, SiO_2 was etched by HF.

SiO_2 nanoparticles were synthesized according to the reference [21]. 860 mg of TEOS was resolved in 50 mL of ethanol and 1.0 mL of aqueous ammonia (25% concentrated ammonia in water) was added dropwise. The system was kept stirring at 25 ℃ for 4 h. After the deposit was centrifuged at 15000 rpm and washed with ethanol, it was dispersed in ethanol again and centrifuged at 4000 rpm to remove the larger particles. Finally, the SiO_2 nanoparticles were dried.

One gram of SiO_2 nanoparticles were dispersed in ethanol and 0.5 mL of MATMS was added. The reaction system was kept stirring for 24 h to get SiO_2@MATMS at 25 ℃. The product was dried under vacuum.

0.5 g of SiO_2@MATMS, 0.1 mmol of OFL and 0.4 mmol of MAA were dispersed in 20 mL of ACN with stirring. The mixture was stirred for 8 h in ice bath for prepolymerization and then 2.0 mmol of EGDMA as crosslinker, 0.14 mmol of DBP as plasticizer and 0.12 mmol of AIBN as initiator were added. N_2 gas was passed into the mixture to drive O_2 away and then under N_2 protection the reaction was lasted

for 24 h at 60 ℃ with stirring. The product was named as MIP1. The procedure was repeated except adding DBP and the corresponding product was named as MIP1a.

40% (v/v) HF aqueous solution was used to soak 100 mg of the above materials completely. The mixture was with vortex for 15 min and kept static for another 2 h. After centrifuged, the materials were washed with methanol and dried under vacuum.

Finally, the hollow MIP particles were put into a column and washed with methanol–acetic acid (4:1, v/v) to remove the template molecules, then dried under vacuum at 40 ℃ and stored at ambient temperature before use.

For comparison, several polymers including non molecular imprint polymers (NIPs) were synthesized on the surface of SiO_2. The reactant ratios were summarized in table 1. The synthesis of hollow MIP was shown in Fig 1.

(Table 1)

(Fig 1)

Adsorption experiments

Static adsorption experiments

Each 20 mg of MIPs or NIPs was added to 10 mL of ACN containing 100 or 500 μg mL^{-1} of OFL. The mixture was shaken for 24 h at 25 ℃ followed by 9000 rpm of centrifugation. The supernatant was filtered to measure the concentration of OFL with UV spectrophotometry method at 300 nm. According to the results, the MIP2, which possessed the largest adsorption capacity, was further studied.

20 mg of MIP2 or NIP2 was added to 10 mL of ACN solution containing 20-900 μg mL^{-1} of OFL. The adsorption experiments were accomplished as the above procedures. The data of static absorption experiment were further processed according to the Scatchard equation (1) [22] to estimate the binding parameters of MIP2.

(1)

Here Q is the amount of OFL bound to MIP2 at equilibrium, Q_{max} is the maximum binding capacity, C_{free} is the equilibrium concentration of OFL and K_d is the dissociation constant, respectively.

Adsorption kinetic studies

Adsorption kinetic studies were carried out as following: 20 mg of MIP2 or NIP2 was suspended in 10 mL of ACN containing 20, 200 or 1000 μg mL^{-1} of OFL. The mixture was incubated at 25 ℃ with shaking. Eight samples were taken at defined time intervals (at 1, 2, 3, 5, 7, 9, 12 and 24 h, respectively). The residual concentrations of OFL were measured with UV spectrophotometry method.

Selectivity evaluation and competitive adsorption

First, 20 mg of MIP2 was equilibrated with 10 mL of ACN containing 100 μg mL^{-1} of OFL, SMZ, sudan I or IBU respectively to evaluate the selectivity of MIP2. Second, 20 mg of MIP2 was put into the mixture containing each 100 μg mL^{-1} of OFL, NOR, ENR and SMZ to evaluate the selectivity of MIPs and the competitive capacity of OFL. The samples were shaken for 2 h at 25 ℃ to facilitate the adsorption. The concentrations of free analytes were determined by UV spectrophotometry (for the solution containing single analyte) or HPLC-UV methods (for the mixture).

Determination of real samples

Milk samples were purchased from retail markets in Lanzhou, China. These samples were kept at 4 ℃ until analysis.

Extraction of the FQs from 10 mL of spiked or original milk was carried out by adding 1.0 mL of ACN and 0.1 mL of HCl (5 mol L^{-1}). The mixture was oscillated and centrifuged at 12,000 rpm for 10 min. The supernatant was collected and transferred to another 15 mL tube, and the residues were extracted again with 1.0 mL of ACN. The pooled extract was adjusted pH with NaOH and up to 10.0 mL with H_2O. 20.0 mg of MIP2 was added and shaken for the further dispersive solid phase extraction for 15 min. After centrifugation, the analytes were eluted from the adsorbent and dried with N_2. The residue was resolved in 0.5 mL of mobile phase for HPLC analysis. For comparison, the milk was protein-deposited and concentrated to 0.5 mL for direct analysis.

Results and discussion

Characterization

The materials were observed with TEM (Fig 2). We got the regular SiO_2 particles with size of about 250 nm (a). The polymer layer could be found to cause the conglutination of particles (b). The hollow polymers particles with DBP adding were holonomic with the layer thickness of about 70 nm (c) but the particles broken without adding DBP (d). DBP was one of the most used plasticizer, which could increase flexibility and extensibility of the polymer. Because keeping the particle shape was necessary for the reproducibility and stability of extraction, all the MIPs without adding DBP would not be used further.

(Fig 2)

The materials with DBP and SiO_2 particles were certified with IR spectra, shown in fig 3. The IR spectrum of SiO_2 particles was shown in (a). 468 and 800 cm^{-1} were the symmetric stretching peaks and 1100 cm^{-1} was the anti-symmetric stretching peak of Si-O-Si. 957 cm^{-1} was the bending vibration peak of Si-OH. (b) belonged to SiO_2 –MIP2 in which 1734 cm^{-1} was the special peak of C=O from DBP, MATMS and EGDMA; 758 ,879 and 957 cm^{-1} belonged to substituted benzene ring in DBP; 2960 and 2991 cm^{-1} were from $–CH_2$ group. The reduced strength of 1100 cm^{-1} verified the successful modification of MIP2 on SiO_2. (c) and (d) were from hollow MIP2 and NIP2, respectively. It was found the special peaks of SiO_2 disappeared after SiO_2 was etched and the in-plane bending vibration peak of –OH group (1258 cm^{-1}) and the stretching vibration peak of C-O group (1159 cm^{-1}) became distinct without the disturbance of SiO_2.

(Fig 3)

Adsorption evaluation

The adsorption capacity Q (the adsorption amount (mg) of OFL on 1.0 g materials) and imprint factors α of the hollow MIPs (the ratio of OFL adsorption amount on MIP and NIP under same conditions) were compared each other at initial concentrations of 100 and 500 μg mL^{-1} of OFL. The results were shown in table 2.

(Table 2)

Q_{MIP2} and Q_{MIP3} was similar each other under the same conditions and always higher than Q_{MIP1}. The imprint factors α from different MIPs were higher than 1

under any condition studied with the similar values, which meant MIPs had higher adsorption capacities than corresponding NIPs. Increasing the polymer thickness was helpful to increase adsorption capacity. But when the layer thickness was increased so much, the adsorption capacity was not continue increase, owing to lower mass transfer and adsorption efficiency resulting from hidden adsorption sites. In addition, the site of template molecules was not increased in direct proportion with polymer thickness. So MIP3 could not display better properties than MIP2. At last MIP2 was chosen for further experiments.

The adsorption capacities of MIP2 and NIP2 were further compared in samples with different OFL concentrations (Fig 4). When the initial concentration of OFL was higher than 300 μg mL^{-1}, the adsorption capacity of MIP2 was becoming much higher than of NIP2. NIP2 got the adsorption capacity of 27.0 mg g^{-1} and the adsorption capacity of MIP2 could not get the constant yet in the experimental scope. The samples with higher concentrations of OFL did not be studied because of the limit of solubility of OFL. When the initial concentration of OFL was 900 μg mL^{-1}, the imprint factor α was about 2.6. Scatchard curve was set up according to the experimental results of MIP2. The two distinct linear portions were obtained, which meant two kinds of adsorption sites existing in MIP2. One was with higher adsorption selectivity, the corresponding Kd_1 and Q max_1 was 2.342 mmol L^{-1} and 0.133 mmol g^{-1}. Another one was with low adsorption selectivity, the Kd_2 and Q max_2 was 0.459 mmol L^{-1} and 0.274 mmol g^{-1}, respectively. The maximal adsorption capacity would be 147 mg g^{-1} in theory, which was larger than the values obtained from the other hollow MIPs reported in literatures [17,19]. Langmuir and Freundlich adsorption isotherm equations were used to analyze the sorption equilibrium and the correlation coefficient R^2 for Langmuir equation was 0.9739, which was smaller than that from Freundlich equation (R^2=0.9910). It could be deduced that OFL molecules were adsorbed on heterogeneous sites of MIP2 with a non-uniform distribution of energy levels [23]. The results from Scatchard curve and Freundlich equation were concordant with each other.

(Fig 4)

The adsorption time up to the equilibrium depended on the initial OFL concentration seriously in the dynamic experiments (Fig 5). The larger initial concentration of OFL, the longer equilibrium time was. When OFL concentration was 20 μg mL^{-1}, the equilibrium was gotten within 30 min. Under the same conditions, it needed 60 min for the MIPs prepared with precipitation polymerization (the data were not shown, which was accepted by Journal of Lanzhou University (natural sciences)). But the equilibrium needed 9 h to get in 1000 μg mL^{-1} of OFL sample. In addition, MIP2 needed longer time than NIP2 to get equilibrium because it contained the deep caves left by template molecules. The pseudo-first-order kinetic model and the pseudo-second-order kinetic model [23] were used to study the adsorption data too. It was found the kinetic data fit pseudo-first-order format better than pseudo-second-order format with the R^2 larger than 0.97. The calculated Q_{max} of MIP2 was 109.3 mg g^{-1}, which was smaller than the value from Freundlich equation (147 mg g^{-1}). Even though the shell layer of MIP2 was thin enough, the resistance of mass transfer was still existed to decrease the adsorption capacity.

(Fig 5)

Selectivity and competitive adsorption

The selectivity of MIP2 was evaluated by two experiments. The results were shown in fig 6. MIP2 showed very low adsorption capacity to SMZ, IBU and sudan I as expected because of their dissimilar structures with OFL. The adsorption capacity of MIP2 to OFL was about 16 times more than to SMZ and IBU, and eight times more than to Sudan I. MIP2 displayed higher adsorption capacity to OFL and NOR because of their similar structures and a little higher imprint factor for OFL. MIP2 did not show the satisfied adsorption capacity for another FQ molecule - ENR. The possible reason was that the three-membered ring of ENR was rigid body and it was difficult to enter the sites left by OFL in MIP2. The adsorption capacity of MIP2 to OFL decreased in the mixture containing the similar molecules. The total adsorption amount of MIP2 for OFL and NOR in the mixture was nearly equal to the adsorption amount of OFL in the pure OFL solution.

NIP2 displayed higher adsorption capacity to OFL and NOR too. The possible

reason was hydrogen bonding existed as the main interaction force between analytes and the materials because of the FQs containing the element F. Owing to the rigid ring existed in ENR molecule, it might be difficult to get the suitable angle to form hydrogen bonding.

(Fig 6)

Sample analysis

Optimal conditions of adsorption and desorption

First, pH (2.0-7.0) of milk sample was adjusted. Satisfied recovery ratio was obtained when sample pH was 3.0 to 4.0, the possible reason may be that interaction between the residual casein (the main protein in milk, pI 4.6) and OFL (pKa 5.49) was relatively weak in such a pH scope. Lower pH would change the surface property of MIP and the charge distribution of OFL, which made the recovery lower.

Second, the ion strength of milk was adjusted by adding NaCl to the sample. It was found that adding NaCl could decrease the recovery.

Third, the mixture of acetic acid and methanol or ACN (v/v, 10/90) was used to desorb OFL from the MIP2. It was found that methanol mixture offered higher recovery. Then the volume of acetic acid-methanol mixture was optimized (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL). 2.0 mL was chosen to elute OFL from 20 mg of MIP2.

Analysis method

HPLC–UV method was set up for determination of FQs in milk. Under the optimal conditions, the method performance was evaluated in the linear range of 0.03-2.5 μg mL^{-1} ($R^2 > 0.9994$) in milk and 0.03 μg mL^{-1} was affirmed as the limit of quantitation (LOQ) for the ENR and 0.02 μg mL^{-1} for OFL and NOR (signal/noise=6). The accuracy and repeatability of this method were also evaluated by recoveries of spiked milk at 0.0625, 0.5 and 1.0 μg mL^{-1}. The results were shown in table 3.

(Table 3)

Fig 7 was the chromatograms of blank milk (a) and spiked milk with 62.5 ng mL^{-1} FQs (b) after treatment with MIP2. No FQs were found in non-spiked milk samples. Fig 8 was the chromatograms of the spiked milk with FQs (125 ng mL^{-1}), without (a) or with (b) MIP2 treatment. The effect of MIP2 treatment was distinct for cleaning the sample. Without MIP2 treatment, the peaks of OFL and NOR could not

be identified and lower than the LOQs, and all the peaks of FQs were disturbed with interferences, especially ENR (a). After treated with MIP2, the peaks OFL and NOR increased distinctly (b). The interferences, which disturbed the ENR peak, were eliminated because they were not adsorbed on MIP2 and the pure peak was obtained.

(Fig 7)

(Fig 8)

Conclusion

A new kind of hollow MIPs nanoparticles of OFL was prepared. DBP was added in the MIP synthesis procedure for the first time to avoid the particles broken. The new material offered faster adsorption rate than prepared by precipitation polymerization and the higher adsorption capacity than the other hollow MIPs reported with larger sizes and thicker shells. The MIPs could enrich OFL and reduce the interference efficiently in milk samples.

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Table 1 Various materials synthesized in the work

SiO_2@MATMS (g)	OFL (mmol)	MAA (mmol)	EGDMA (mmol)	DBP (mmol)	AIBN (mmol)	Name of polymer
0.5	0.1	0.4	2.0	0.14	0.12	MIP1
0.5	0	0.4	2.0	0.14	0.12	NIP1
0.5	0.1	0.4	2.0	0	0.12	MIP1a
0.5	0.1	0.8	4.0	0.28	0.12	MIP2
0.5	0	0.8	4.0	0.28	0.12	NIP2
0.5	0.1	0.8	4.0	0	0.12	MIP2a
0.5	0.1	1.2	6.0	0.42	0.12	MIP3
0.5	0	1.2	6.0	0.42	0.12	NIP3
0.5	0.1	1.2	6.0	0	0.12	MIP3a

Table 2 Adsorption capacities and imprint factors of materials

	100 μg/mL OFL			500 μg/mL OFL		
	Q_{MIP} (mg g^{-1})	Q_{NIP} (mg g^{-1})	α	Q_{MIP} (mg g^{-1})	Q_{NIP} (mg g^{-1})	α
1*	11.73	7.56	1.55	27.44	18.97	1.44
2	16.31	10.6	1.54	36.82	26.95	1.36
3	15.22	9.97	1.53	32.60	24.57	1.32

*1, 2 or 3 meant the number in the name of MIP or NIP. n=3

Table 3 Recoveries and RSDs of SPE-HPLC method for milk samples

added[a]		OFL	NOR	ENR
0.0625	Recoveries (%)	97.6	97.7	98.4
	RSD%[1]	4.2	4.9	4.7
	RSD%[2]	3.5	4.1	1.4
0.5	Recoveries (%)	102.6	93.7	90.9
	RSD%[1]	2.9	3.0	3.7
	RSD%[2]	3.6	4.8	4.3
1.0	Recoveries (%)	101.8	98.9	102.1
	RSD%[1]	3.9	1.4	2.6
	RSD%[2]	2.5	2.8	4.8

[a] added concentration: μg/mL ; [1] intraday RSD%; [2] interday RSD%. n=3

Captions

Fig1 Synthesis route of hollow MIPs

Fig2 TEM of materials

(a) SiO_2 nanoparticles; (b) SiO_2 coated with MIP2; (c) hollow MIP2; (d) hollow MIP2a. All the bars in the pictures mean 200 nm.

Fig3 IR spectra of materials

(a) SiO_2; (b) SiO_2 coated with MIP2; (c) hollow MIP2; (d) hollow NIP2

Fig4 Adsorption isotherm of MIP2 and NIP2 (a) and Scatchard curves of MIP 2 (b)

Conditions: 20 mg of MIP2 or NIP2; 10 mL of ACN solution with different concentration of OFL; adsorption time: 24 h. temperature: 25 ℃

Fig5 Adsorption kinetic curves of MIP2 and NIP2

Conditions: 20 mg of MIP2 or NIP2; 10 mL of ACN solution with 20, 200 or 1000 μg mL^{-1} of OFL; temperature: 25 ℃.

Fig6 Adsorption amounts of different compounds on MIP2

Conditions: 20 mg MIP2; 10 mL of ACN containing 100 μg mL^{-1} OFL or SMZ, sudan I and IBU, respectively (a); 10 mL of ACN containing OFL, NOR, ENR and SMZ with each 100 μg mL^{-1} (b).

Fig7 Chromatograms of blank and spiked milk samples

Blank milk sample (a); Milk sample spiked with 62.5 ng mL^{-1} of OFL, NOR and ENR (b).

Conditions: 10 mL of milk, treated with 20 mg of MIP2; final volume after treatment: 0.5 mL; Chromatographic conditions: C18 column, Methanol/phosphate buffer (0.01mol/L, pH 2.80, v/v=25/75) , flow rate:1.0 mL min^{-1} , column temperature :40 ℃. UV detection wavelengths: 0-14.8 min, 293 nm;14.8-25.0 min, 277 nm; Injection volume:10 μL. Peak 1, OFL; Peak 2, NOR; Peak 3, ENR.

Fig8 Chromatograms of milk samples spiked with 125 ng mL^{-1} of three FQs

Conditions: milk sample was protein-deposited and concentrated directly to 0.5 mL(a); milk sample was further treated with MIP2 after protein-deposited (b).

The treatment conditions and the chromatographic conditions: same as Fig 7.

Fig 1

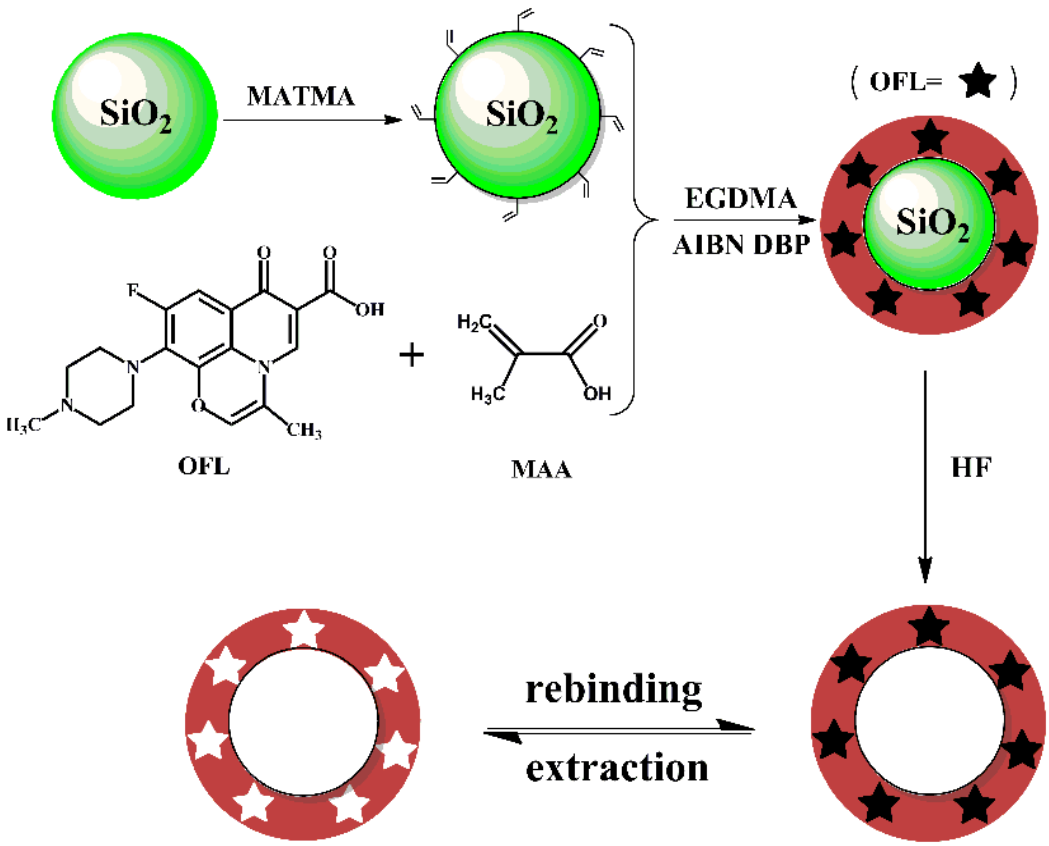


Fig 1 Synthesis route of hollow MIPs

Fig 2

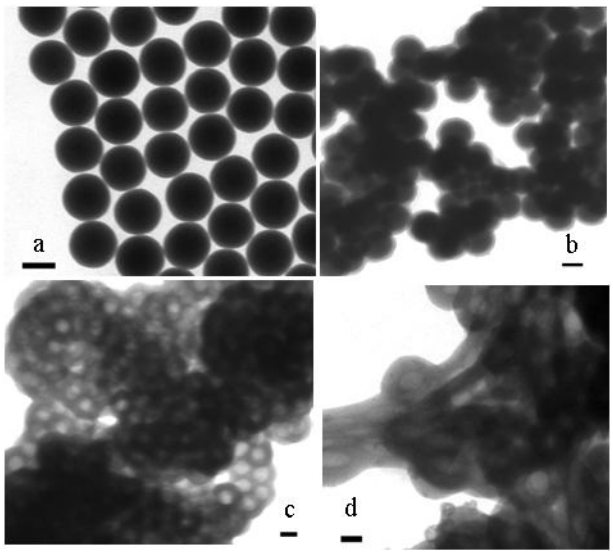


Fig 2 TEM of materials

(b) SiO_2 nanoparticles; (b) SiO_2 coated with MIP2; (c) hollow MIP2; (d) hollow MIP2a. All the bars in the pictures mean 200 nm.

Fig 3

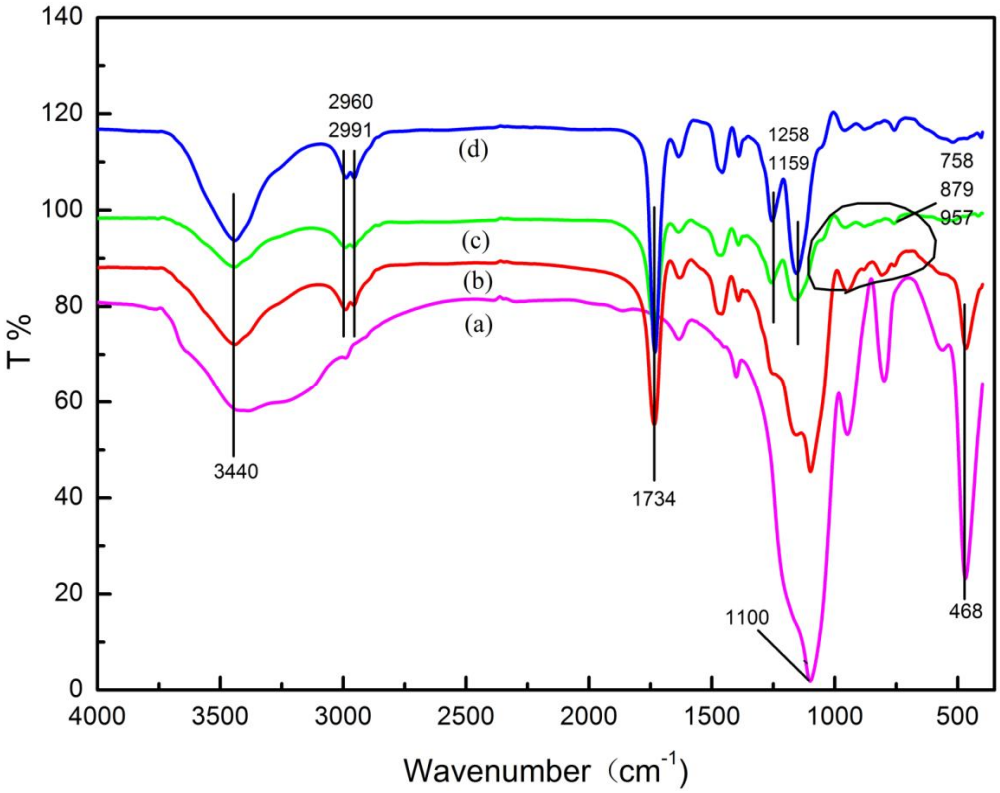


Fig 3 IR spectra of materials

(b) SiO_2; (b) SiO_2 coated with MIP2; (c) hollow MIP2; (d) hollow NIP2

Fig 4

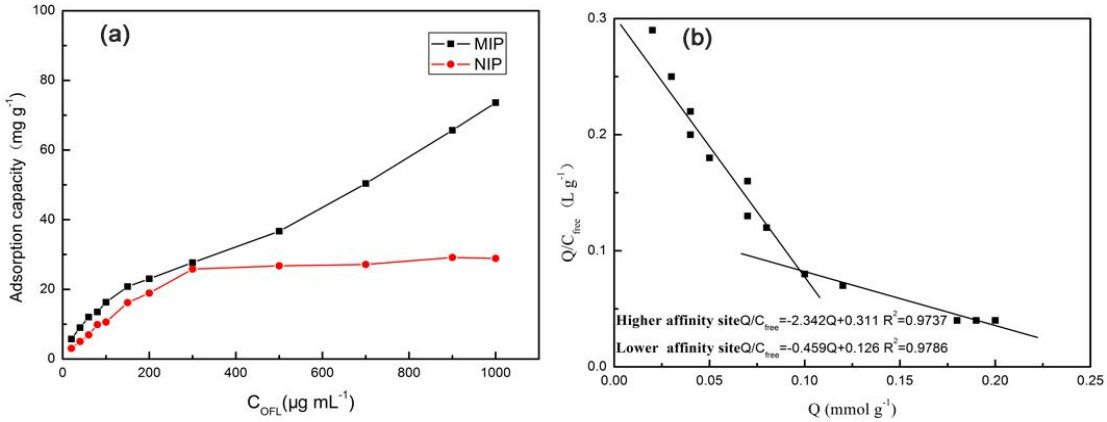


Fig4 Adsorption isotherm of MIP2 and NIP2 (a) and Scatchard curves of MIP 2 (b)

Conditions: 20 mg of MIP2 or NIP2; 10 mL of ACN solution with different concentration of OFL; adsorption time: 24 h. temperature: 25 ℃

Fig 5

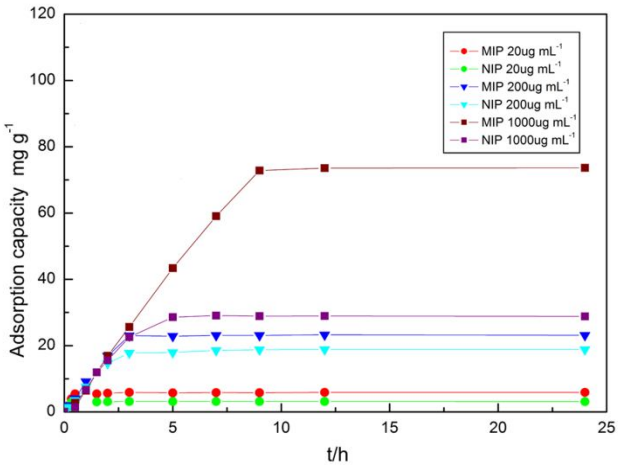


Fig5 Adsorption kinetic curves of MIP2 and NIP2

Conditions: 20 mg of MIP2 or NIP2; 10 mL of ACN solution with 20, 200 or 1000 μg mL^{-1} of OFL; temperature: 25 ℃.

Fig 6

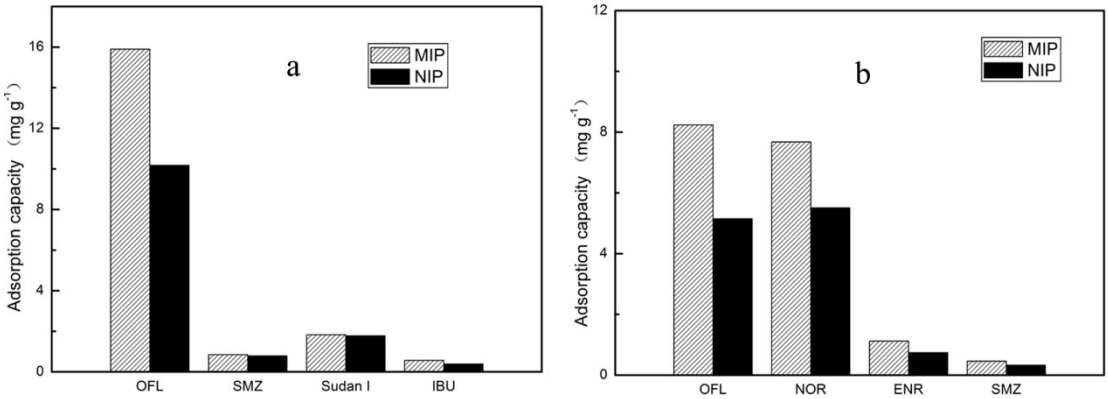


Fig6 Adsorption amounts of different compounds on MIP2

Conditions: 20 mg MIP2; 10 mL of ACN containing 100 μg mL^{-1} OFL or SMZ, sudan I and IBU, respectively (a); 10 mL of ACN containing OFL, NOR, ENR and SMZ with each 100 μg mL^{-1} (b).

Fig 7

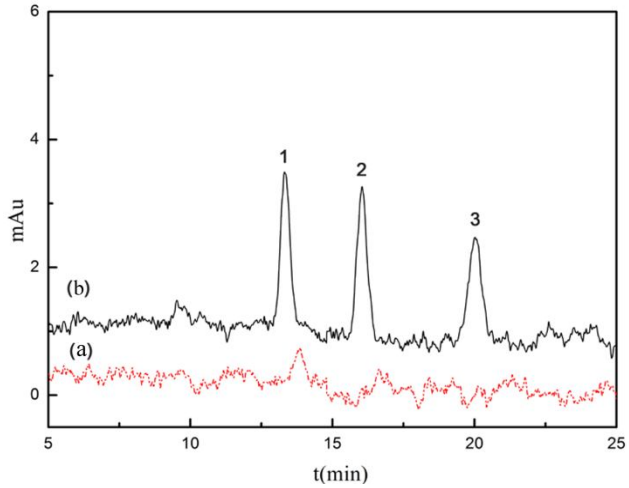
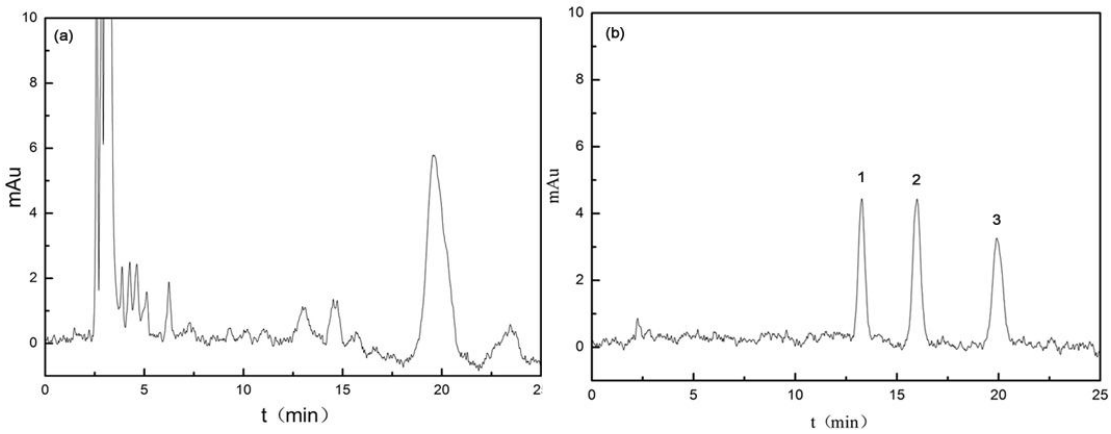


Fig7 Chromatograms of blank and spiked milk samples

Blank milk sample (a); Milk sample spiked with 62.5 ng mL^{-1} of OFL, NOR and ENR (b). Conditions: 10 mL of milk, treated with 20 mg of MIP2; final volume after treatment: 0.5 mL; Chromatographic conditions: C18 column, Methanol/phosphate buffer (0.01mol/L, pH 2.80, v/v=25/75) , flow rate:1.0 mL min^{-1} , column temperature :40 ℃. UV detection wavelengths: 0-14.8 min, 293 nm;14.8-25.0 min, 277 nm; Injection volume:10 μL. Peak 1, OFL; Peak 2, NOR; Peak 3, ENR.

Fig 8



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