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Metal ion mediated molecularly imprinted polymer for selective capturing antibiotics containing beta-diketone structure

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ABSTRACT

A new molecularly imprinted polymer (MIP) targeting to quinolones (Qs) and tetracyclines (TCs) was synthesized using itaconic acid (ITA) and ciprofloxacin (CIP) as a functional monomer and template molecule, respectively. Factors affecting the overall performance of MIP were investigated, and the results showed that Fe^{3+} ion play a vital role in the formation of MIP with high molecular imprinting effect. Meanwhile, the chelating ability of monomer, species of template molecule, as well as the molar ratio of monomer and template also contribute to the performance of the obtained MIP. Cyclic voltammetry verified that, with the participation of Fe^{3+} ions, a ternary complex of ITA–Fe^{3+}–CIP could be formed before polymerization. Compared with conventional MIP prepared from commonly used monomer, methacrylic acid (MAA), the new MIP show significantly enhanced molecular imprinting effect and higher capacity for specific adsorption of target compounds as revealed by static and dynamic binding experiments. The MIP was successfully used as solid-phase extraction (SPE) adsorbent for enriching a broad spectrum of antibiotics containing beta-diketone structure from surface water sample. HPLC detection showed that high recovery rate (78.6–113.6%) was found in these spiked antibiotics, whereas recovery rate for the non structurally related drugs, epinephrine (EP) and dopamine (DOPA), was very low (4.7–7.6%) on the MIP cartridges. The results demonstrate that the MIP prepared by the strategy proposed in this work, could specifically target to a series of structurally related antibiotics containing beta-diketone structure.

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1. Introduction

In recent years, drug contaminations in aquatic systems released from municipal sewage have becoming a serious environmental problem [1–3]. Quinolones (Qs) and tetracyclines (TCs) are two representative families of antibiotics, which are widely used to prevent and treat a large variety of infectious diseases in human and veterinary medicine with broad-spectrum against bacteria [10]. The majority of quinolones in clinical use belong to the members of fluoroquinolones [4]. In comparison with other antibiotic classes, fluoroquinolones have the highest risk of causing colonization with MRSA and *Clostridium difficile* [5]. A general avoidance of fluoroquinolones is recommended based on the available evidence and clinical guidelines, since increased toxicity to certain organs have been reported [6]. Tetracyclines are a class of protein synthesis inhibitors, mainly including tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC). Side effects caused by tetracyclines were also found in human. Trace residues of such antibiotics released from municipal and industrial wastewaters may persist in natural water system, imposing potential hazard to human health and ecological system [7,8]. Thus, it is important to develop analytical methods for determining these drugs existing in water samples at trace levels. However, most of the analytical methods established thus far for Qs and TCs detection are not sensitive enough to directly detect the trace amount of drugs existing in water samples without pre-concentration step. Over the last few years, solid-phase extraction (SPE) has become the most commonly used sample preparation technique. It not only can clean the sample matrix but also enrich the objective analytes prior to analysis. Nevertheless, due to the lack of selectivity of the commercial SPE sorbents, other materials with high selectivity, such as immunosorbents (ISs) and molecularly imprinted polymers (MIPs), have been developed and applied to sample extraction procedures. The expensive and time-consuming procedure for the production and isolation of antibodies as well as lack of availability have limited the IS application, and led to MIP being exploited widely as SPE materials [9].

The selectivity of MIP is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, is dissolved in a solvent together with one or more functional monomers. The complex formation between functional monomers and template occurs before polymerization, the strength of which will be

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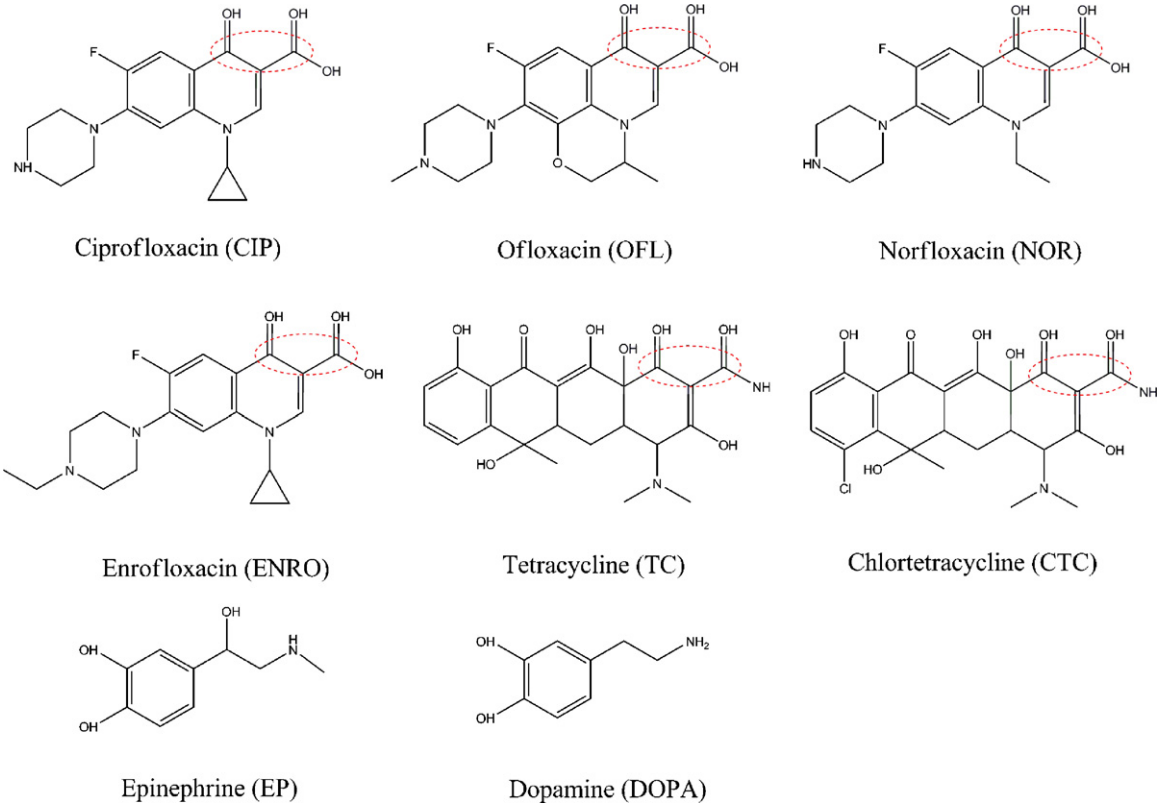

Fig. 1. The molecular structures of six antibiotics containing beta-diketone structure and two kinds of non-structurally related drugs for control experiment.

reflected in the selectivity of the MIP polymer. Accordingly, the design of the appropriate template–monomer composition is crucial. Part of the process can be carried out by molecular modeling, experimental design or by screening methods [10,11], all of which was based on the knowledge of molecular interaction between monomer and template. The interaction between monomer and template of MIP may include hydrogen bond, hydrophobic, charge transfer, or other forms in non-covalent MIP and reversible covalent or coordination interaction in covalent MIP. At present, a few MIPs targeting to fluoroquinolones had been successfully obtained via bulk polymerization in water–methanol systems [12–14], since hydrogen bonding and electrostatic forces existed between methacrylic acid (MAA) monomer and fluoroquinolones template. However, the formation of template–monomer complex under aqueous conditions cannot be efficient enough, owing to the hydrogen bonding interaction between drugs and monomers can be interrupted by polar solvent. In order to avoid the disturbance caused by water during the polymerization, metal ions as mediator had been introduced during the pre-polymerization to form the complex of template–metal ion–monomer [15,16]. This might be appropriate for the synthesis of water-compatible MIPs targeting to fluoroquinolones and tetracyclines, both of which have common β-diketone structure with high metal chelating capability especially for Fe(III) [17]. In fact, coordination interaction is a strong interaction, and metal ion-mediated MIP has been demonstrated to show high selectivity and has been exploited in several types of selective recognition systems [18–21] and catalysis systems [22]. The use of metal ion mediated recognition has a great potential for the synthesis of MIP systems, the effective recognition of metal ions with monomer and template are critical for us to develop MIP with novel and enhanced selective characteristics. It has been reported that Fe^{2+} could bridge between TC and MAA to form the complex of TC–Fe^{2+}–MAA, and the Fe^{2+} mediated MIP for TC was prepared [15]. However, coordinating capability of MAA with metal ion is far weak compared with TC, which has strong chelating ability with multivalent metal ions [23–25], leading to the instability of the ternary complex. In this work, commonly used MAA was replaced by itaconic acid (ITA), which is a kind of functional monomer with stronger chelating ability. We found that the molecular imprinting effect of MIP could be greatly improved by using ITA–Fe^{3+}–ciprofloxacin (CIP) system. This effect might owe to that a more stable ternary complex of monomer–metal ions–template can be formed in aqueous phase before polymerization, in which ITA and CIP could strongly chelate with Fe^{3+} ions through double carboxyl groups and β-diketone groups, respectively. After polymerization the CIP is removed leaving behind imprinted sites that are sterically and chemically complementary to the structurally similar molecules. In this case, the imprinted sites are capable of binding a class of quinolones and tetracyclines antibiotics with beta-diketone structure. This novel strategy proposed in this work provides an example for preparation of desirable water-compatible MIPs, which can selectively extract target drugs from aqueous samples. As far as we know, this is first report on using itaconic acid as a functional monomer to prepared metal ion mediated MIP targeting to structurally related antibiotics.

2. Experimental

2.1. Materials and reagents

Ciprofloxacin (CIP), enrofloxacin (ENRO), ofloxacin (OFL), norfloxacin (NOR), tetracycline (TC), chlortetracycline hydrochloride (CTC), epinephrine (EP) and dopamine (DOPA) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Their molecular structures were shown in Fig. 1. Itaconic acid (ITA) and ethyleneglycol dimethacrylate (EDMA) were supplied from Acros (New Jersey, USA). Methacrylic acid (MAA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2,2′-

Azobisisobutyronitrile (AIBN), phosphoric acid, and triethylamine were purchased from Wynca Chemical Group Co. (Zhejiang, China). Ferric trichloride ($FeCl_3$) was obtained from Jinshan Chemical Co. (Shanghai, China). HPLC-grade acetonitrile and methanol were purchased from TEDIA (Fairfield, USA) and Shield Company (Tianjin, China), respectively. All the other reagents used in the experiment were analytically pure. Water used in the experiment was doubly distilled.

ITA–methanol solution was prepared by dissolving 2.60 g ITA in 50 mL of methanol to a final concentration of 0.40 mol L^{-1}. 0.5 mol L^{-1} $FeCl_3$ solution was prepared by dissolving 6.75 g $FeCl_3{\cdot}6H_2O$ in 50 mL pure water. Standard stock solutions of CIP, ENR, OFL, NOR, TC and CTC were prepared by diluting appropriate weight of the drug powder in pure water to a final concentration of 1.00 mmol L^{-1}. These solutions were stored in refrigeration (4 °C) before usage.

2.2. Synthesis of molecularly imprinted polymers (MIPs) and non-imprinted polymers (NIPs)

The MIPs were prepared using antibiotics and ITA as the template and functional monomer, respectively. Fe^{3+} was chosen as the mediator to bridge them for its high affinity to both monomer and template molecules through coordinating force. Particularly, CIP and CTC were selected respectively as the template molecules to synthesize two kinds of MIPs, The procedure could be described as follows: 0.8 mL $FeCl_3$ solution and 4 mL ITA–methanol solution were mixed in a 50 mL centrifuge tube. The pH value of the solution was adjusted to 2.8 with 1.0 mol L^{-1} NaOH solution. Oxygen in the solution was removed by purging it with nitrogen gas for 10 min. Then, CIP or CTC (0.40 mmol) dissolved in methanol was added into the mixture mentioned above. The solution was diluted to 15 mL with methanol:water solvent (9:1, v/v), and mixed thoroughly by sonicating it for 15 min.

EDMA (3.7 mmol) and AIBN (0.4 mmol) were added into the above solution in order. After purged with nitrogen gas once again for 15 min, the tube was sealed and kept in 70 °C water bath for 20 h. The resulting bulk rigid polymer was ground into fine powders, Fe^{3+} ions coordinated in the polymers were eluted with 1 M HCl until the eluted solution was free of Fe^{3+} ions, which could be detected by KSCN solution. Then the template molecules were eluted with mixed solution of methanol and acetic acid (4:1, v/v) until the template could no longer be detected by UV spectrometer. Finally, the polymer was washed with distilled water to neutral and dried at 60 °C. The non-imprinted polymers (NIPs) were prepared in the same procedure, except that the template molecules were not added into the mixed solution before polymerization.

To compare the performance of MIP sorbents prepared in different conditions, several kinds of MIPs were synthesized using ITA and MAA as functional monomer, with or without Fe^{3+} mediating, respectively. The name of each kind of MIP sorbents and their preparative conditions were listed in Table 1.

2.3. Cyclic voltammetry

Cyclic voltametric analysis was performed with a CHI810B electrochemical analyser(Shanghai Chenhua Instrument Company, China) to verify whether Fe^{3+} bridged between template and monomer. Solution containing 2-(N-morpholino)ethanesulfonic acid (MES) buffer (25 mmol L^{-1}, pH 5.0), CIP (1.00 mmol L^{-1}), Fe^{3+} (1.00 mmol L^{-1}) and ITA (4.00 mmol L^{-1}) was prepared. Mixed solutions of Fe^{3+}–CIP, Fe^{3+}–ITA and Fe^{3+}–ITA–CIP were stirred for 1 h and purged with nitrogen for 3–5 min respectively before the solutions were analyzed by a CHI810B electrochemical analyser. Golden disk electrode (1 mm I.D.), Ag/AgCl and platinum wire were chosen as working electrode, reference electrode and auxiliary electrode, respectively. Experimental condition for cyclic voltammetry was set as follows: scanning potential range: −1.5 to 1.5 V; scanning velocity = 50 mV/s; sensitivity = 10^{-5} A/V.

Table 1
Conditions for preparation of MIP sorbents used in the experiment.

Name of MIP sorbent	Functional monomers	Template molecules	Fe^{3+} ion mediation
ITA–CIP	ITA	CIP	No
ITA–CTC	ITA	CTC	No
ITA–Fe^{3+}–CIP	ITA	CIP	Yes
ITA–Fe^{3+}–CTC	ITA	CTC	Yes
MAA–CIP	MAA	CIP	No
MAA–CTC	MAA	CTC	No
MAA–Fe^{3+}–CIP	MAA	CIP	Yes
MAA–Fe^{3+}–CTC	MAA	CTC	Yes

ITA, itaconic acid; MAA, methacrylic acid; CIP, ciprofloxacin; CTC, chlortetracycline hydrochloride.

2.4. Dynamic binding and adsorptive capacity measurement

Dynamic binding experiment was conducted on a SPE cartridge, which was prepared by pumping the slurry of 100 mg MIP or NIP into an empty plastic cartridge (Supelco, USA) with volume of 3 mL at a flow rate of 3.0 mL min^{-1} with a D100-B digital peristaltic pump (Shanghai Qingpu Instrumental Corp., China). Before the dynamic binding experiment, the SPE cartridge was first conditioned with methanol and then with distilled water. Distilled water spiked with target antibiotics was pumped through the cartridge at a flow rate of 1 mL min^{-1}. On-line detection was realized by connecting the outlet of cartridge with an UV-100 detector (Dalian Elite, China). The breakthrough curve was recorded at a wavelength of 275 nm until the UV absorptive signal reached a maximal platform. Solution flew through the cartridge was collected and analyzed with an UV-2800 spectrometer (Hitachi, Japan). The absorptive capacity of the SPE cartridge could be calculated by subtracting the amount of antibiotics molecules in the percolate solution from the total amount of antibiotics in original water sample.

2.5. Equilibrium binding

In order to investigate the equilibrium binding capacity of the MIPs in water environment, 20 mg MIP or NIP particles, and 2.0 mL solution with different concentrations of target compounds (CIP and CTC) ranging from 0.25 to 2.0 mol L^{-1} were respectively placed into 10 mL plastic centrifugation tubes. After being shaked for 24 h at room temperature, each solution was centrifuged for 3 min at 4000 rpm. The concentrations of CIP or CTC in supernatant were analyzed by Agilent 1200 HPLC system (Agilent, USA), and the static adsorptive capacity of MIP or NIP could be calculated based on HPLC results. The data of the static absorption experiment were further processed with the Scatchard equation to estimate the binding parameters of the MIPs [13]. The equation could be expressed as:

$$\frac{Q}{C_{free}} = \frac{Q_{max} - Q}{K_D}$$

where Q is the amount of antibiotic bound to MIPs at equilibrium, Q_{max} is the maximum binding capacity, C_{free} is the equilibrium concentration of antibiotic, and K_D is the dissociation constant.

2.6. Solid-phase extraction of antibiotics from water samples using MIPs

SPE cartridge packed with 100 mg MIP or NIP was prepared according to the procedure described in Section 2.4. Before the SPE experiment, the cartridge was first conditioned with methanol and

then with distilled water. Surface water samples were collected from *Tiesha* river in Hangzhou city. 0.2 g L^{-1} of Na_2EDTA solution was added into the water sample to prevent the chelating of metal ion with antibiotics. All the water samples were filtered through Whatman filter membrane with pore size of 0.22 μm before usage. The samples were spiked with six kinds of antibiotics, and the concentration of spiked antibiotics in water sample was 0.05 μmol L^{-1} (OFL), 0.10 μmol L^{-1} (TC), 0.025 μmol L^{-1} (NOR), 0.025 μmol L^{-1} (CIP), 0.05 μmol L^{-1} (ENR) and 0.20 μmol L^{-1} (CTC), respectively. 50 mL of water sample was loaded onto the cartridge at a flow rate of 1 mL min^{-1}. After all the water samples were pumped through the MIP sorbent, the cartridge was washed with 3 mL of 10% methanol water solution to remove the weakly adsorbed species. The adsorbed components were eluted with 3 mL mixed solution of methanol and acetic acid (6:1, v/v), and the eluted solution was concentrated by evaporating the solvent at 85 °C water bath to near dryness. Finally, the concentrated samples were re-dissolved in 1.0 mL of distilled water for HPLC analysis.

The recoveries of EP and DOPA on the MIP and NIP cartridges could be also determined by the same methods described above.

2.7. Conditions of HPLC separation and detection

All HPLC measurements were performed using an Agilent 1200 LC system equipped with a UV detector. Chromatographic separation was carried out on a ZORBAX SB-C18 HPLC column (4.6 mm × 150 mm, 5 μm) from Agilent Technologies (Wilmington, DE, USA). The column was kept in a constant temperature of 30 °C. The mobile phase consisted of mixture of phosphoric acid–triethylamine solution (pH 3.0) and methanol (79:21, v/v). The flow rate of the mobile phase was of 1.0 mL min^{-1}. 20 μL of sample was injected, and the separated components were detected by a UV detector at the wavelength of 275 nm.

3. Results and discussion

3.1. Factors affecting molecular imprinting effect of MIP materials

In this paper, TE (template effect) factor was used for evaluating the molecular imprinting effect of MIP. We defined TE factor as the ratio of adsorptive capacity of MIP to NIP. A larger TE factor is an indicative of higher molecular imprinting effect or specific binding capability of MIP, whereas TE factor close to 1 means that there is no obvious difference in binding capability between MIP and NIP. Experimental data concerning the effect of Fe^{3+} ions, species of monomers, as well as the template molecules on the adsorption capability and TE factor of MIP were listed in Table 2. For comparison, MAA and ITA were respectively chosen as functional monomers in MIP preparation; while the CIP and CTC were selected as template molecules, respectively. Table 2 showed that Fe^{3+} ion played a vital role in the formation of MIP materials with higher TE factor. Adsorption capacity of MIP without metal ions involved was almost the same as that of NIP. In other words, the TE factor of all these MIP is closed to 1, showing a low molecular imprinting effect. However, not all the MIP materials with Fe^{3+} ion participation have a remarkable larger TE factor. It can be inferred from the results of Table 2 that other factors such as the species of functional monomers and template molecules also exert impact on molecular imprinting effect of MIP. By using ITA as monomer and CIP as template respectively, the TE factor significantly increased compared with MAA and CTC as a counterpart. Among all the MIPs prepared in this work, MIP prepared from ITA–Fe^{3+}–CIP had the highest TE factor, which could reach 3.84 and 7.68 for CIP and CTC, respectively. According to the above mentioned descriptions, metal ions such as Fe^{3+}, could substantially improve the adsorption capacity of MIPs targeting to the template and structurally related molecules and thus dominate the imprinting effects. In addition, using functional monomer with stronger chelating capability and suitable template molecules could also contribute to the specific binding capability of MIP.

Table 2
TE factor of several MIPs prepared under varies condition.

	CIP*			CTC*		
	AC_{MIP}	AC_{NIP}	TE	AC_{MIP}	AC_{NIP}	TE
ITA–CIP	6.06	6.16	0.98	2.06	2.45	0.84
ITA–CTC	6.95	6.16	1.13	3.21	2.45	1.31
ITA–Fe^{3+}–CIP	24.52	6.38	3.84	8.37	1.09	7.68
ITA–Fe^{3+}–CTC	6.99	6.38	1.10	1.57	1.09	1.44
MAA–CIP	5.84	5.12	1.14	1.35	1.21	1.12
MAA–CTC	4.37	5.12	0.85	0.97	1.21	0.80
MAA–Fe^{3+}–CIP	8.32	6.67	1.24	3.16	1.17	2.70
MAA–Fe^{3+}–CTC	4.94	6.67	0.74	2.52	1.17	2.15

CIP* means choosing CIP solution (0.01 mmol L^{-1}) as the sample in dynamic adsorption experiment.
CTC* means choosing CTC (0.01 mmol L^{-1}) solution as the sample in dynamic adsorption experiment.
AC_{MIP} means adsorption capacity of MIPs.
AC_{NIP} means adsorption capacity of NIPs.
Template effect (TE) factor = AC_{MIP}/AC_{NIP}.

Data shown in Table 3 manifested that the molar ratio of ITA to CIP/Fe^{3+}, as well as the molar percentage of crosslinker EDMA also significantly affected the molecular imprinting effect of the MIP (CIP–Fe^{3+}–ITA). The value of TE factor could reach highest when the molar ratio of ITA to CIP/Fe^{3+}(1:1) was controlled at 4. Whereas little or excess amount of ITA would cause the decrease of TE factor. The molar ratio dependent phenomena indicated that the ratio of chelating ligands in the CIP–Fe^{3+}–ITA ternary complex would also remarkably affect the molecular imprinting effect of the MIP materials.

3.2. The Fe^{3+} mediated interaction between CIP and ITA

Theoretically, the complex of template–functional monomer should be formed before polymerization for MIPs production, which is usually verified by spectrometric analysis [26]. In this work, cyclic voltammetric (CV) analysis was employed to confirm the formation of monomer–metal ion–template complex. Since the Fe^{3+} ion is an electro-active species, any ligands coordinating with the ion will cause the negative shift in reductive peak potential, and a larger shift in peak potential is indicative of higher stability of the complex. To investigate whether or not Fe^{3+} ion acts as the mediator for CIP and ITA to form the ideal complex, solutions of Fe^{3+} ion, CIP–Fe^{3+}, ITA–Fe^{3+}, CIP–Fe^{3+}–ITA were sequentially scanned by cyclic voltametric analysis. As shown in Fig. 2, neg-

Table 3
TE factors of ITA–Fe^{3+}–CIP MIPs synthesized under varies condition.

	CIP*			CTC*		
	AC_{MIP}	AC_{NIP}	TE	AC_{MIP}	AC_{NIP}	TE
Template:Fe^{3+}:monomer						
1:1:1	7.24	6.40	1.13	3.37	2.75	1.23
1:1:2	9.88	6.71	1.47	4.37	2.76	1.58
1:1:4	24.52	6.38	3.84	8.37	1.09	7.68
1:1:8	13.67	6.48	2.11	6.60	4.49	1.47
1:1:16	7.36	6.94	1.06	6.08	4.61	1.32
EDMA (mol%)						
60	12.74	8.68	1.47	1.25	1.21	1.03
70	24.52	6.38	3.84	8.37	1.09	7.68
80	14.72	6.71	2.19	3.52	2.59	1.36

EDMA (mol%) means the molar proportion of EDMA account for the sum of EDMA and monomer.

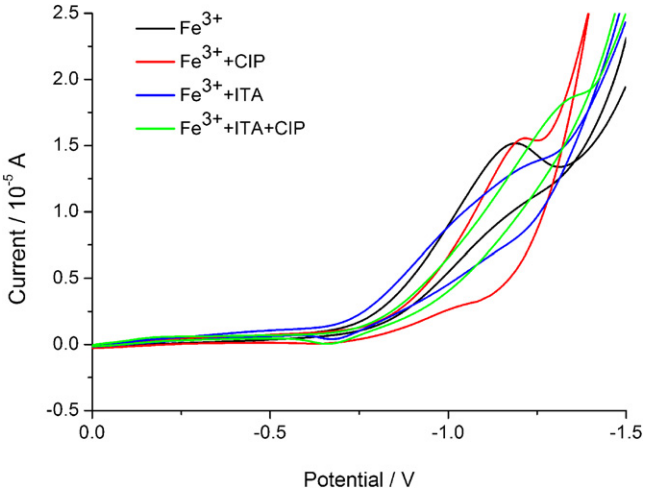

Fig. 2. Cyclic voltammetry of Fe^{3+} ions with or without the presence of monomer (ITA) and template molecules (CIPs). Range of scanning potential: −1.5 to 1.5 V; scanning velocity = 50 mV/s; sensitivity = 10^{-5} A/V.

ative shift in reductive peak potential of CIP–Fe^{3+} and ITA–Fe^{3+} were observed compared with free Fe^{3+} ions. Compared with these binary components, larger negative shift in the reductive peak potential could be found in CIP–Fe^{3+}–ITA, suggesting that a ternary complex of CIP–Fe^{3+}–ITA was formed before polymerization. To elucidate whether the complex of CIP–Fe^{3+}–ITA is more stable than that of CIP–Fe^{3+}–MAA, cyclic voltammetry of MAA–Fe^{3+}, and CIP–Fe^{3+}–MAA was also conducted, and the reductive peak potential was compared in Table 4. It could be deduced that the stability of ITA–Fe^{3+} complex was higher than that of MAA–Fe^{3+}, because the reductive peak potential of ITA–Fe^{3+} shifted to a more negative value. Also, the ternary complex of CIP–Fe^{3+}–ITA should be more stable than CIP–Fe^{3+}–MAA for the same reason. Based on all the facts presented above, it could be inferred that the stability of the ternary complex played an important role in the synthesis of metal mediated MIP with higher TE factor. This work demonstrated that using itaconic acid, a monomer with higher Fe^{3+} ion chelating capability, could significantly improve the molecular imprinting effect of metal ion mediated MIP.

Table 4
The results of cyclic voltammetry analysis.

	Reductive peak potential (V)	ΔV[a]
Fe^{3+}	−1.2736	
MAA–Fe^{3+}	−1.3282	−0.0546
CIP–Fe^{3+}–MAA	−1.4579	−0.1843
ITA–Fe^{3+}	−1.4494	−0.1758
CIP–Fe^{3+}–ITA	−1.4746	−0.2010

[a] $\Delta V = V - V(Fe^{3+})$

3.3. Static binding capacity of MIP materials

Adsorption isothermal curves (Fig. 3a and c) indicated that the MIP prepared from CIP–Fe^{3+}–ITA had higher binding capacity to target analytes than the MIP prepared from CIP–Fe^{3}–MAA. The two distinct linear portions in Scatchard analysis (Fig. 3b and d) indicated two classes of binding sites existed in the imprinted polymer: one is high selectivity or affinity with high binding energy and the

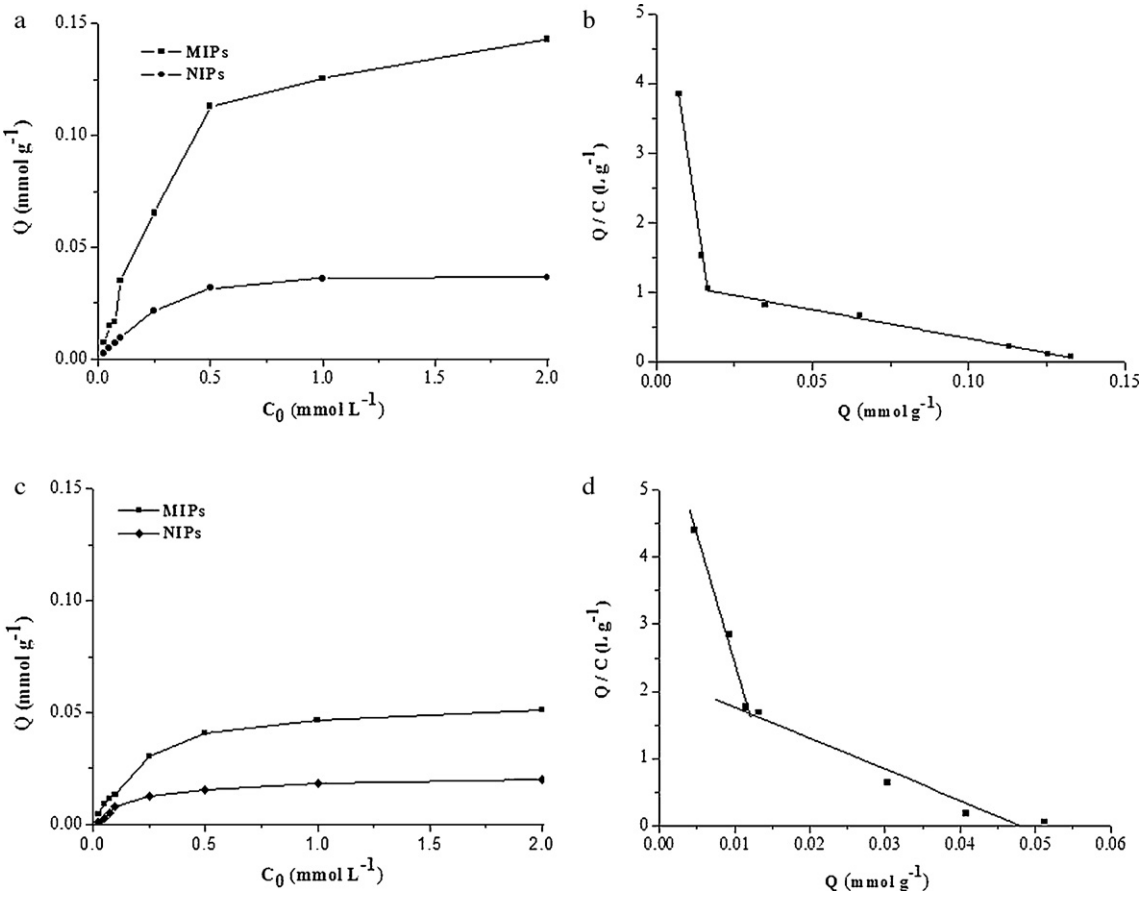

Fig. 3. Adsorption isotherms of CIP on the MIP (ITA–Fe^{3+}–CIP) and corresponding NIP; (b) Scatchard analysis curve of CIP on the MIP (ITA–Fe^{3+}–CIP); (c) adsorption isotherms of CIP on the MIP (MAA–Fe^{3+}–CIP) and corresponding NIP; (d) Scatchard analysis curve of CIP on the MIP (MAA–Fe^{3+}–CIP).

Table 5
The results of Scatchard analysis.

	Binding sites	Linearity	K_D (μmol L^{-1})	Q_{max} (mmol g^{-1})
ITA–Fe^{3+}–CIP	Higher affinity site	$Q/C = 1.17 - 8.29Q$	120.63	141.13
	Lower affinity site	$Q/C = 6.04 - 302.44Q$	3.31	19.97
MAA–Fe^{3+}–CIP	Higher affinity site	$Q/C = 2.15 - 44.41Q$	22.52	48.41
	Lower affinity site	$Q/C = 5.713 - 565.9Q$	2.63	16.41

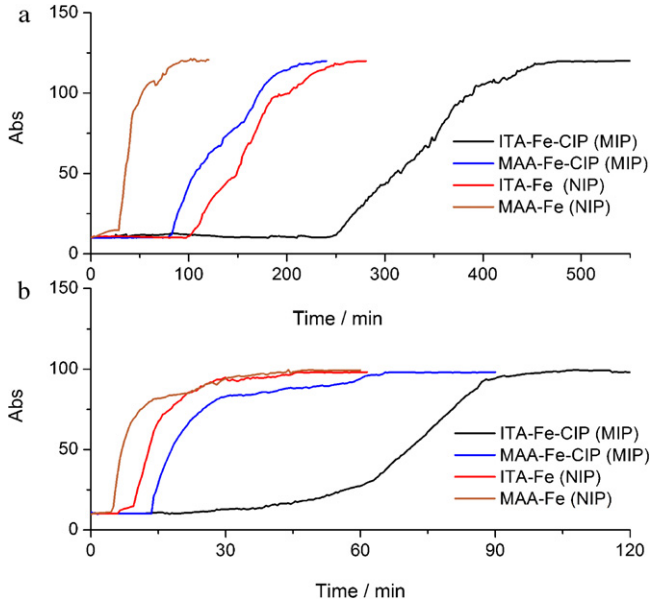

Fig. 4. Breakthrough curve of (a) CIP and (b) CTC on SPE cartridges filled with MIP and NIP prepared from different functional monomers.

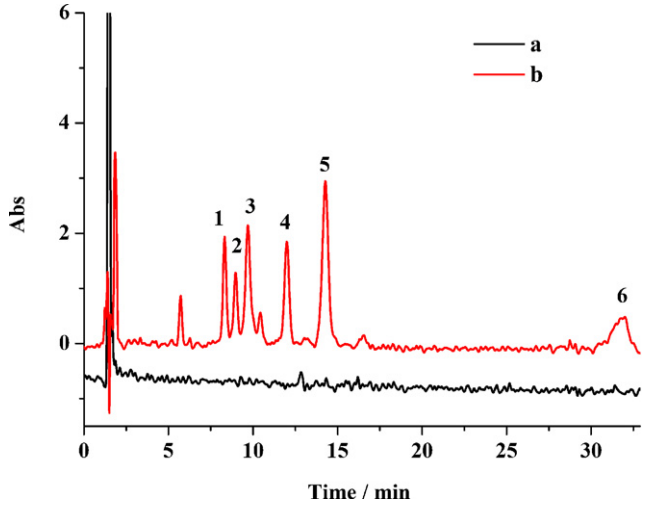

Fig. 5. Chromatogram of antibiotics spiked in water samples (a) without SPE enrichment (b) and with SPE enrichment. Peak 1~6 represent OFL, TC, NOR, CIP, ENR, CTC, respectively. HPLC column: ZORBAX SB-C18 (4.6 mm × 150 mm, 5 μm); column temperature: 30 °C; mobile phase: phosphoric acid–triethylamine solution (pH 3.0) and methanol (79:21, v/v); flow rate: 1.0 mL min^{-1}; sample volume: 20 μL; wavelength of UV detector: 275 nm.

other is low affinity with low binding energy. The respective K_D and Q_{max} values are calculated from the slopes and intercepts of the two linear portions of Scatchard analysis. The results listed in Table 5 clearly demonstrated that the chemical structure of functional monomer could significantly affect the absorption capacity. MIP produced from ITA monomers possesses more high affinity sites than MIP using MAA monomers.

3.4. Breakthrough curve of target molecules on metal ion mediated MIP and NIP cartridges

In order to evaluate the dynamic adsorption capacity of the obtained MIPs, binding experiments on SPE cartridges containing 100 mg of MIPs prepared from ITA–Fe^{3+}–CIP and MAA–Fe^{3+}–CIP were carried out. For contrast, each experiment was done in parallel with the corresponding NIPs. Solutions of CIP and CTC with the concentration of 1×10^{-5} mol L^{-1} were pumped through the cartridges continuously, and the breakthrough of drug molecules from MIP or NIP cartridges was monitored by a UV-detector. Breakthrough curves of CIP and CTC from ITA–Fe^{3+}–CIP and MAA-Fe^{3+}–CIP were illustrated in Fig. 4. The adsorption capacity and molecular imprint effect in dynamic flow condition could be observed by comparing the initial and full breakthrough time of target molecules on the MIP and the corresponding NIP cartridge. The results further confirmed that a more successful imprinting effect was observed on the MIP prepared from ITA–Fe^{3+}–CIP. Moreover, the strong imprinting effect of the MIP to both CIP and CTC was found, although CIP and CTC belong to different kinds of drug with remarkable different structures. We think that the metal ion mediated process might a major factor deciding the selectivity of the MIPs. A common beta-diketone structure with metal ion chelating capability exist in both quinolones and tetracyclines drugs. In the MIP preparation, the beta-diketone groups in template molecules (CIP) take part in the formation of ternary complex of ITA–Fe^{3+}–CIP before monomer polymerization. As a result, the extraction of template molecules will leave an imprinting site with a similar orientation, which can be fitted by molecules containing beta-diketone structure. In the environmental analysis, MIP targeting to a category of structurally related contaminants are needed, because a wide variety of pollutant residues should be screened in most of analytical tasks.

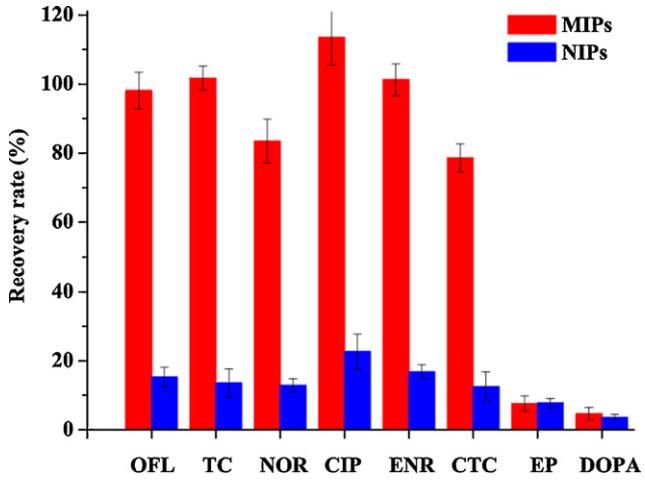

Fig. 6. Mean recovery of different kinds of drugs on MIP and NIP cartridges ($n = 5$).

3.5. Enrichment of antibiotics in water samples with the MIPs prepared from ITA–Fe^{3+}–CIP

To evaluate whether the MIPs have wide selectivity to antibiotics containing beta-diketone structure, surface water spiked with trace amount of OFL, TC, NOR, CIP, ENRO and CTC was loaded onto a SPE cartridge filled with the MIP prepared from ITA–Fe^{3+}–CIP. Meanwhile, control experiment on corresponding NIP cartridge was also conducted. After 50 mL of water sample was pumped through the cartridges, and the weakly adsorbed species was washed away, the antibiotics captured on the MIP or NIP cartridge were eluted with 3.0 mL mixture of methanol:acetic acid (6:1, v/v). The eluted solutions were blew to near dryness with nitrogen gas, and re-dissolved in 1.0 mL of distilled water before it was analyzed by HPLC as described in Section 2.7. For demonstrating the enrichment effect of the MIP, water sample before enrichment step was also subjected to HPLC analysis. As shown in Fig. 5a the chromatographic peak of each antibiotic in water sample without enrichment step is negligible, for the concentration of each spiked drug in water sample was below the detection limit of UV detector in the current HPLC system. After the water sample was enriched by the MIP cartridge, chromatographic peaks of each spiked antibiotics could be well detected as shown in Fig. 5b, demonstrating a significant enrichment effect of the MIP material. The recovery of each antibiotic enriched by MIP and NIP cartridge was calculated based on HPLC detection, As illustrated in Fig. 6, all the antibiotics enriched by MIP cartridge had remarkably high recoveries compared with the results obtained on NIP cartridge. The results implied that MIP prepared in this work could target to a category of structurally related antibiotics, which containing beta-diketone groups as shown in Fig. 1.

To further elucidate whether the prepared MIP is only specific to the structurally related compounds, two types of drugs without beta-diketone groups, the epinephrine (EP) and dopamine (DOPA), were also spiked in the water sample. The procedure for sample enrichment and detection was the same as described before. As shown in Fig. 6, the mean recovery for EP on MIP and NIP cartridge is about $7.6 \pm 2.0\%$ and $7.8 \pm 1.3\%$, respectively, while the mean recovery for DOPA on MIP and NIP cartridge is $4.7 \pm 1.8\%$ and $3.1 \pm 0.9\%$, respectively. Noting that the recovery for the two drugs on MIP was significantly low, and recovery differences between the MIP and NIP cartridges are not significant, we can conclude that drugs without beta-diketone structure cannot be selectively captured by the MIP. These results further confirm that the MIP prepared in this work is selective only towards to structrually related compounds with beta-diketone structure.

4. Conclusions

A new kind of water-compatible MIPs targeting to quinolones (Qs) and tetracyclines (TCs) was developed based on the metal ion mediated imprinting methods. Both Qs and TCs with beta-diketone structure, have an ability to coordinate with metal ion. Using functional monomer and template with the metal chelating ability, a complex of monomer–metal ion–template could be formed before polymerization. In this work, itaconic acid (ITA) and ciprofloxacin (CIP) were chosen as functional monomer and template, respectively. With the participation of Fe^{3+}, a novel MIP with strong molecular imprinting effect could be obtained. This kind of MIP is superior to that synthesized from MAA, which is a commonly used functional monomer in MIP preparation. The obtained water-compatible MIPs were successfully used as SPE adsorbent for selective adsorption and enrichment of four Qs and two TCs from surface water samples with high recovery. The strategy proposed in this work might find pervasive application in preparation of MIPs targeting to a category of structurally related compounds.

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References

[1] S. Perez, D. Barceló, Trends Anal. Chem. 26 (2007) 494.
[2] J. Fick, H. Soderstrom, R.H. Lindberg, C. Phan, M. Tysklind, D.G.J. Larsson, Environ. Toxicol. Chem. 28 (2009) 2522.
[3] M. Yasojima, N. Nakada, K. Komori, Y. Suzuki, H. Tanaka, Water Sci. Technol. 53 (2006) 227.
[4] C.L. Tong, G.H. Xiang, J. Fluoresc. 16 (2006) 831.
[5] E. Tacconelli, G. De Angelis, M. Cataldo, E. Pozzi, R. Cauda, J. Antimicrob. Chemother. 61 (2008) 26.
[6] M. Falagas, P. Rafailidis, E. Rosmarakis, Int. J. Antimicrob. Agents 29 (2007) 374.
[7] E. Caro, R.M. Marc, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 562 (2006) 145.
[8] M.E. Lindsey, M. Meyer, E.M. Thurman, Anal. Chem. 73 (2001) 4640.
[9] C. Cacho, E. Turiel, A. Martin-Esteban, C. Perez-Conde, C. Camara, Bioanal. Chem. 376 (2003) 491.
[10] F. Lanza, B. Sellergren, Anal. Chem. 71 (1999) 2092.
[11] S.A. Piletsky, K. Karim, E.V. Piletska, C.J. Day, K.W. Freebairn, C. Legge, A.P.F. Turner, Analyst 126 (2001) 1826.
[12] H.W. Sun, F.X. Qiao, J. Chromatogr. A 1212 (2008) 1.
[13] H.Y. Yan, F.X. Qiao, K.H. Row, Anal. Chem. 79 (2007) 8242.
[14] H.Y. Yan, K.H. Row, G.L. Yang, Talanta 75 (2008) 227.
[15] G. Qu, S. Zheng, Y. Liu, W. Xie, A. Wu, D. Zhang, J. Chromatogr. B 877 (2009) 3187.
[16] L. Wu, Y. Li, Anal. Chim. Acta 517 (2004) 145.
[17] R. Benassi, E. Ferrari, R. Grandi, S. Lazzari, M. Saladini, J. Inorg. Biochem. 101 (2007) 203.
[18] J. Matsui, I.A. Nicholls, T. Takeachi, K. Mosbach, I. Karube, Anal. Chim. Acta 335 (1996) 71.
[19] P.K. Dhal, F.H. Arnold, Macromolecules 25 (1992) 7051.
[20] T. Rosatzin, L.I. Andersson, W. Simon, K. Mosbach, J. Chem. Soc. Perkin Trans. 2 (1991) 1261.
[21] S. Vidyasankar, M. Ru, F.H. Arnold, J. Chromatogr. A 775 (1997) 51.
[22] T. Yamazaki, E.E. Yilmaz, K. Mosbach, K. Sode, Anal. Chim. Acta 435 (2001) 209.
[23] C.R. Anderson, H.S. Rupp, W.H. Wu, J. Chromatogr. A 1075 (2005) 23.
[24] J.J. Werner, W.A. Arnold, K. Mcneill, Environ. Sci. Technol. 40 (2006) 7236.
[25] L. Jin, X. Amaya-Mazo, M.E. Apel, S.S. Sankisa, E. Johnson, M.A. Zbyszynska, A. Han, Biophys. Chem. 128 (2007) 185.
[26] X.Z. Shi, A.B. Wu, G.R. Qu, R.X. Li, D.B. Zhang, Biomaterials 28 (2007) 374.