Journal of Chromatography B, 877 (2009) 3187–3193

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Metal ion mediated synthesis of molecularly imprinted polymers targeting tetracyclines in aqueous samples

Guorun Qu[a,b], Sulian Zheng[b], Yumin Liu[c], Wei Xie[a], Aibo Wu[b,*], Dabing Zhang[a,b,*]

[a] Medical School, Southeast University, 87 Dingjiaqiao Road, Nanjing 210009, Jiangsu Province, PR China
[b] School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China
[c] The Instrumental Analysis Center, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China

ARTICLE INFO

Article history:
Received 20 June 2009
Accepted 11 August 2009
Available online 18 August 2009

Keywords:
Molecular imprinting
Water-containing system
Metal ion
Tetracyclines
Extraction

ABSTRACT

Molecularly imprinted polymers (MIPs) prepared in water-containing systems are more appropriate as adsorption materials in analyte extraction from biological samples. However, water as a polar solvent involved in the synthesis of MIPs frequently disrupts non-covalent interactions, and causes non-specific binding. In this study Fe^{2+} was used as mediator to prepare MIPs, targeting tetracyclines (TCs) of tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC), with TC as template molecule and methacrylic acid (MAA) as functional monomer. The subsequent binding assay indicated that Fe^{2+} was responsible for substantially improved specific binding in recognition of TCs by decreasing the non-specific binding. Spectrophotometric analysis suggested the existence of the strong interactions among TC, metal ions and MAA in the mixture of methanol and water. Moreover, mass spectrometric measurements verified that Fe^{2+} could bridge between TC and MAA to form a ternary complex of one TC, one Fe^{2+} and four MAAs with a mass of 844.857. Furthermore, combined with molecularly imprinted solid-phase extraction (MISPE) for sample pretreatment, HPLC–UV analysis data revealed good performance of the obtained MIPs as adsorbents. The recoveries of TC, OTC and CTC in urine samples were 80.1–91.6%, 78.4–89.3% and 78.2–86.2%, respectively. This research strategy provides an example for preparation of desirable water-compatible MIPs extracting target drugs from aqueous samples by introducing metal ion as mediator into conventional polymerization system.

1. Introduction

For the determination of target chemicals in routine analytical tasks, a thorough pretreatment procedure is generally required, and solid-phase extraction (SPE) is widely used for sample preparation [1]. Nowadays, molecularly imprinted solid-phase extraction (MISPE) is ubiquitously utilized for sample pretreatment, using molecularly imprinted polymers (MIPs) as adsorption materials [2–5], due to its high selectivity and stability. Unfortunately, most polymers obtained in aprotic and low polar organic solvents usually have poor recognition of the target compound within aqueous conditions, which lead to a large degree limit of the MIPs application in bioseparation. To overcome it, the MIPs prepared in water-containing system have good performance on improving the extraction efficiency from aqueous samples [6,7], provided that the non-covalent interaction between the template and functional monomer is not disrupted by the polarity of water.

Currently, a few MIPs targeting fluoroquinolones had been successfully obtained via bulk polymerization in water–methanol systems [7–9], because strong hydrogen bonding and electrostatic forces existed between methacrylic acid (MAA) and fluoroquinolones. However, other groups of target drugs cannot efficiently form the complex of template-monomer under aqueous conditions, thus the developed system of fluoroquinolones mentioned above is not suitable for generic preparation of the water-compatible MIPs for other drugs. Therefore, it is in great need of developing feasible approaches of molecular imprinting in water-containing systems for production of water-compatible MIPs as specific adsorption materials carrying higher specificity and selectivity against target analytes.

Tetracyclines (TCs) are a large representative family of widely used antibiotics with broad-spectrum against bacteria and have been used extensively in animal husbandry [10], mainly including tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC). As reported, TCs have the ability to bind with multivalent ions [11–14]. The A ring (tricarbonyl) or BCD ring (phenolic β-diketone) of TCs can form chelating complexes with multivalent cations in aqueous conditions [14]. As far as we know, most MIPs toward TCs were prepared in an organic solvent, either methanol or acetonitrile [15–19], owing to the hydrogen bonding interaction between TCs

* Corresponding authors at: School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, PR China.
Tel.: +86 21 34205073; fax: +86 21 34204869.
E-mail addresses: aibowu@sjtu.edu.cn (A. Wu), zhangdb@sjtu.edu.cn (D. Zhang).

1570-0232/$ – see front matter.
doi:10.1016/j.jchromb.2009.08.012

and MIPs was interrupted by polar solvent, the extraction amounts of TCs were low in the polar solvent of methanol [19].

In order to avoid the disturbance caused by water during the polymerization, we try to introduce metal ions as mediator during the prepolymerization to form the complex consisting of template-metal ion-monomer, probably to create stronger ionic interactions, and replace hydrogen bonding interactions with the template and functional monomer. This might be appropriate for synthesis of water-compatible MIPs targeting TCs. In this study, we originally prepared Fe^{2+} mediated MIPs for TCs in water-containing system and found that Fe^{2+} could bridge between TC and MAA to form the complex of one TC, one Fe^{2+} and four MAA. The applicability of MISPE with the obtained water-compatible MIPs as inner adsorption materials was confirmed by HPLC–UV analysis of various spiked urine samples. This novel strategy may provide an example for preparation of desirable water-compatible MIPs extracting target drugs from aqueous samples.

2. Experimental

2.1. Materials and chemicals

TC as the template, MAA as functional monomer, enrofloxacin (ENRO), propranolol, furacillin (FURA), oxytetracycline (OTC) and chlortetracycline (CTC) for selectivity evaluation were purchased from Sigma–Aldrich (Steinheim, Germany). Chloramphenicol (CAP) and ethylene glycol dimethacrylate (EGDMA) as cross-linker were from Fluka (Steinheim, Germany). 2,2′-Azobis (2-isobutyronitrile) (AIBN) was from the China National Medicines Co. Ltd. (Shanghai, China). HPLC-grade acetonitrile (ACN) for mobile phases was purchased from Fisher (Fair Lawn, NJ, USA). All other chemicals were of analytical grade from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Double distilled and MilliQ water were obtained from a MilliQ purification system (Millipore, USA).

2.2. UV–vis spectrophotometric analysis

Solutions consisting of 0.04 mmol L^{-1} TC and a series of various concentrations of metal ions and MAA were prepared in 20 mmol L^{-1} Tris–HCl buffer at different pH values. Corresponding solutions without TC were set as references for the determination of their specific absorption spectra. An UV–vis spectrophotometer (UV mini1240, Shimadzu, Tokyo, Japan) was used for these analyses.

2.3. Preparation of imprinted polymers

The polymers were synthesized as follows: TC, one kind of metal ion (Fe^{2+}, Mg^{2+} or Cu^{2+}) and MAA were dissolved in 5 mL of methanol: water (9:1, v/v), presented in Table 1. Then, 990 mg (5 mmol) of EGDMA and 12 mg (0.07 mmol) of AIBN were added and dissolved by sonicating, and before the flask was sealed, the oxygen inside was removed by bubbling nitrogen for 10 min. The polymerization was performed in a 60 °C water bath for 24 h in darkness. The polymers were packed and washed with methanol–acetic acid (9:1, v/v) in a soxhlet apparatus until no TC was detected as confirmed by HPLC–UV analysis. After removing the residual acetic acid with methanol, the polymers were dried at 50 °C until constant weight. The reference polymers were prepared and treated identically except for the exclusion of the metal ion {P(TC)}, TC {P(Fe) and P(Mg)} or both {P(Blank)}.

2.4. Binding assay

Polymers (40 mg) were added to a 5 mL volume glass bottle with 2 mL of solution, consisting of 0.4 mmol L^{-1} TC in methanol–water (9:1, v/v), and incubated at 25 °C for 24 h with stirring at 300 rpm. The filtered solutions were dried with a stream of nitrogen, and then re-dissolved with acetonitrile, and then the concentrations of free TC in the supernatant solutions [TC] were assayed. The amount of TC bound to MIPs or their reference polymers, Q, was calculated by subtracting the [TC] from the initial concentration, *C*. Each test was measured in triplicate.

Table 1
The compositions of TC/Metal ion/MAA for synthesis of molecularly imprinted polymers (MIPs) and non-imprinted polymers (NIPs).

Polymer	TC[a] (mmol)	Metal ions[b] (mmol)			MAA[c] (mmol)
		Mg^{2+}	Fe^{2+}	Cu^{2+}	
P(TC/Mg)	0.1	0.5			0.2
	0.1	0.5			0.4
	0.1	0.5			0.8
P(TC/Fe)	0.1		0.1		0.2
	0.1		0.1		0.4
	0.1		0.1		0.8
	0.1		0.2		0.4
	0.1		0.2		0.6
	0.1		0.2		0.8
P(TC/Cu)	0.1			0.2	0.4
	0.1			0.2	0.8
P(Mg)		0.5			0.2
		0.5			0.4
		0.5			0.8
P(Fe)			0.1		0.2
			0.1		0.4
			0.1		0.8
			0.2		0.4
			0.2		0.6
			0.2		0.8
P(Cu)				0.2	0.4
				0.2	0.8
P(TC)	0.1				0.2
	0.1				0.4
	0.1				0.6
	0.1				0.8
P(Blank)					0.2
					0.4
					0.6
					0.8

[a] TC: 44.4 mg (0.1 mmol).
[b] Mg^{2+}: 101.5 mg $MgCl_2{\cdot}6H_2O$ (0.5 mmol); Fe^{2+}: 27.8 mg $FeSO_4{\cdot}7H_2O$ (0.1 mmol); Cu^{2+}: 49.9 mg $CuSO_4{\cdot}5H_2O$ (0.2 mmol).
[c] MAA: 68.8 mg (0.8 mmol).

HPLC analysis was performed at room temperature using a HPLC system (Beckman, Berkeley, CA, USA) equipped with a UV detector, and a 7725I sample injection valve with a 20 μL sample loop, and a Beckman ODS C18 column ODS C18 cartridge (4.6 mm id × 250 mm, 5 μm). The mobile phase consisted of 0.1% TFA in water–ACN (70:30, v/v). The flow rate was set at 1.0 mL min^{-1}.

2.5. Mass spectrometric analysis

Mass spectrometric measurements, for investigating the interaction of TC, Fe^{2+} and MAA, were performed on a Q-TOF Premier (ESI-Q-TOF) instrument (Waters, Milford, MA, USA). A solution consisting of 0.04 mmol L^{-1} TC, 0.08 mmol L^{-1} $FeSO_4$ and 0.32 mmol L^{-1} MAA was prepared in methanol–water (9:1, v/v). During mass spectra scanning, data were acquired from 50 to 1000 *m/z* and analyzed by Masslynx software. The TOF mass analyzer operated in the negative mode was set as follows: the desolvation gas flow rate of 600 L h^{-1} at 350 °C with the cone gas flow at 50.0 L h^{-1} and the source temperature of 120 °C. The capillary voltage and the sampling cone volt-

age were 3.0 kV and 35 V, respectively. The collision energy was 4.0 V.

2.6. Solid-phase extraction

For MISPE, the SPE cartridges of 3.0 mL (Supelco, Bellefonte, PA, USA) were used and 100 mg of dry imprinted polymers suspended in 2 mL of isopropanol–methanol (2:1, v/v) were packed between two glass–wool frits. Then the cartridges were washed with acetonitrile–pure acetic acid–1.0 mol L^{-1} HCl in water (8:1:1, v/v/v), and preconditioned with 5 mL of double distilled water and 1.0 mL of 0.25 mmol L^{-1} $FeSO_4$. The drug dissolved in water was loaded individually with 2.0 mL of the working solutions at 0.04 mmol L^{-1}. Water–methanol (1:4, v/v) was used as washing solution. The elution solution was 2.0 mL of a mixture of acetonitrile–pure acetic acid–1.0 mol L^{-1} HCl in water (8:1:1, v/v/v). These experiments were carried out on a Supelco VisiprepTM DL SPE vacuum manifold.

2.6.1. Sample extraction

Human urine samples without TCs were collected from healthy volunteers and confirmed by HPLC analysis, then treated with KH_2PO_4. An aliquot of 0.2 mol L^{-1} KH_2PO_4 (1 mL) was added to 9.0 mL of urine which was mixed with such standard solutions as TC, OTC and CTC. The spiking concentrations for each drug were set with five levels of 10, 20, 50, 100 and 200 μg L^{-1}, respectively. The samples were whirled for 2 min and loaded onto MISPE which were preconditioned with $FeSO_4$. 1.5 mL of PBST (0.1% phosphate-buffered saline, 0.1% Tween-20, pH 7.4) was used as washing solution. After MISPE, the eluted solution was adjusted to pH 2.0, dried with a stream of nitrogen, and then re-dissolved with 200 μL of the mobile phase for HPLC analysis.

3. Results and discussion

3.1. Binding capacity of MIPs

When the molar ratio of MAA to TC was 8:1, the binding capacities were mainly determined by the nature of metal ions in the order of adsorption capabilities: P(TC/Mg) > P(TC/Fe) > P(TC/Cu) (Fig. 1a). By contrast, without metal ions involved in the process of polymerization, the adsorption capacity of P(TC) was almost the same as that of P(Blank). Since Cu^{2+} was precipitated with MAA during the preparation of P(Cu), the non-specific binding of P(TC/Cu) could not be evaluated. As for Mg^{2+} mediated MIPs, the adsorption amount was 11.13 μmol g^{-1} on P(TC/Mg) and 6.73 μmol g^{-1} on P(Mg). Thus, the non-specific binding of P(TC/Mg) was very high. For P(TC/Fe), the adsorption amount was 9.16 μmol g^{-1} whilst that of P(Fe) was 2.38 μmol g^{-1}, indicating that P(TC/Fe) had better imprinting effects. Therefore, P(TC/Fe) was selected in the following experiments.

TC can bind metal ions and form soluble complexes of metal ion-TC with the molar ratio of 2:1 or 1:1 [20]. For this purpose, the molar ratio of Fe^{2+} to TC was optimized. As shown in Fig. 1b, when the molar ratio of Fe^{2+} to TC decreased from 2:1 to 1:1, the adsorption capacity of P(TC/Fe) was slightly changed from 9.20 to 9.16 μmol g^{-1}. However, that of P(Fe) was significantly decreased from 2.38 to 1.68 μmol g^{-1}. Furthermore, when the ratio of MAA to TC was decreased from 8:1 to 4:1, the adsorption capacities of P(TC/Fe) and P(TC) did not vary much, whilst the adsorption capacity

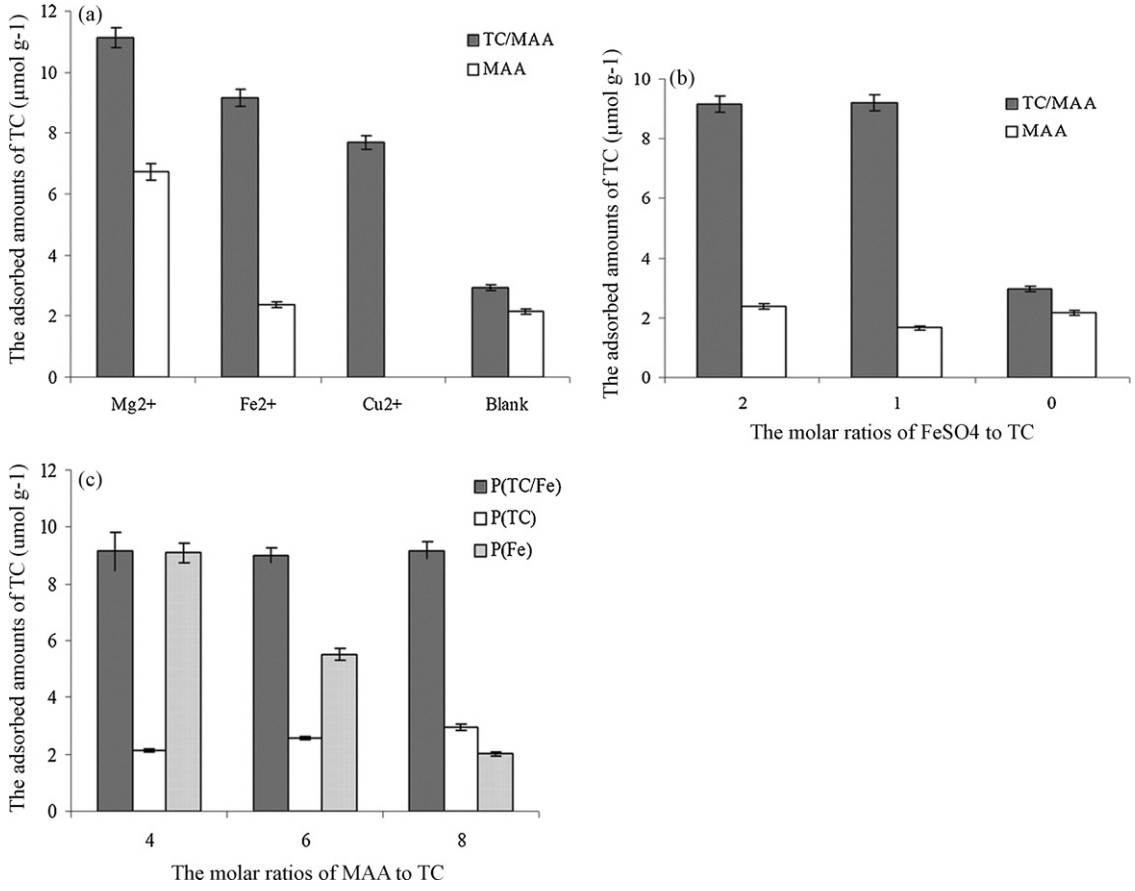

Fig. 1. The adsorption of tetracycline (TC) on metal ions mediated MIPs and the control polymers. (a) The different kinds of metal ions used in the preparation of MIPs and NIPs with the molar ratio of MAA/TC of 8; (b) the different molar ratios of Fe^{2+}/TC used in the preparation of MIPs and NIPs with the molar ratio of MAA/TC of 8; (c) the different molar ratios of MAA/TC used in the preparation of P(TC/Fe), P(TC) and P(Fe).

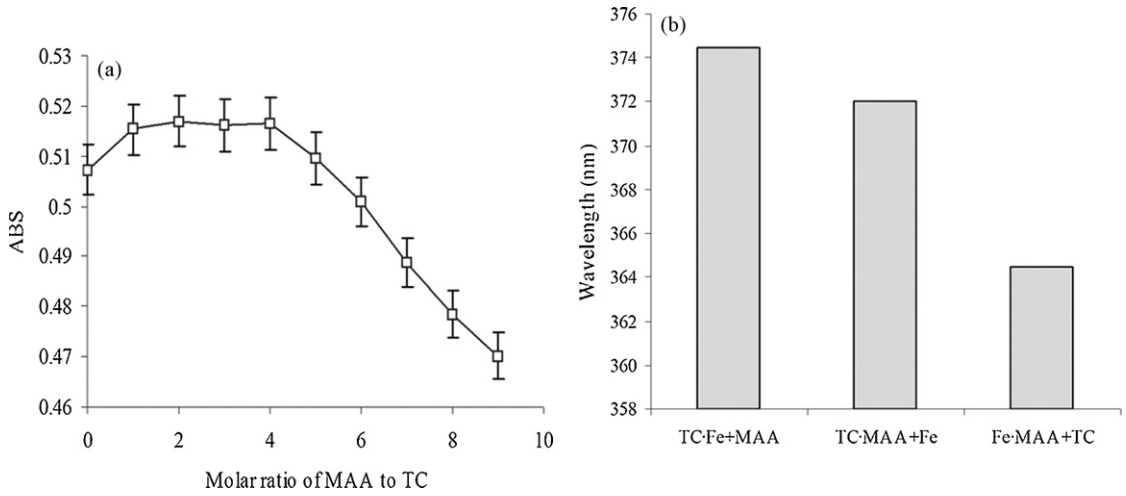

Fig. 2. Absorption spectra of TC for addition of Fe^{2+} and MAA. (a) The absorption value at 365 nm changed with the improved molar ratio of MAA to tetracycline; (b) UV spectra wavelength peak of TC affected by the mode of mixing.

of P(Fe) increased from 2.03 μmol g^{-1} to 9.09 μmol g^{-1} (Fig. 1c), demonstrating that P(TC/Fe) synthesized under the ratio of MAA to TC at 8:1 had the best performance in terms of the specific binding capacity.

According to the above mentioned descriptions, metal ions, exemplified by Fe^{2+}, can substantially improve the adsorption capacity of MIPs targeting the template molecules and thus dominate the imprinting effects. In addition, the less amount of MAA as functional monomer in polymerization contributed to the non-specific binding of P(TC/Fe).

3.2. The Fe^{2+} mediated interaction between TC and MAA

Theoretically, the complex of template-functional monomer should be generated before polymerization for MIPs production, which is usually revealed by UV spectrophotometry or NMR analysis [21]. To investigate whether or not Fe^{2+} could be the mediator for TC and MAA to form the ideal complex, single TC, TC–metal ion and TC–metal ion–MAA complexes were sequentially scanned by UV–vis analysis to reflect the changes caused by the amount of MAA and the order of addition of TC, Fe^{2+} and MAA.

As shown in Fig. 2a, the absorption peak was observed to be about 0.51 when the molar ratio of MAA to TC was 1 and almost kept at the value of 0.51–0.52 until the ratio reached 4. Then, the absorption value was obviously decreased when the ratio was more than 4 (Fig. 2a). As described before, Fe^{2+} has six ligands for binding and two ligands bridge the –OH and =O groups of TCs [22,23]. Four positions were possibly remained for the interaction of MAA [14,24], suggesting that a ternary complex of TC/Fe^{2+}/MAA was formed during prepolymerization (Fig. 3). When the molar ratio of MAA to TC/Fe^{2+} was over 4, TC/Fe^{2+} had no capability to adsorb more MAA.

Moreover, as shown in Fig. 2b, the absorption wavelengths for different orders of mixing of TC/Fe^{2+}/MAA were 364.5, 372.0 and 374.5, respectively. This indicated that the sequence of mixing TC, Fe^{2+} and MAA could change the position of the absorption peaks of TC.

In order to elucidate the composition of the formed ternary complex, time-of-flight mass spectra (TOF-MS) was used to provide some structural evidence, referring to previous studies on TC–metal ion complexes [25]. As shown in Table 2, the m/z and its assigned structure indicated that TC–Fe^{2+}–MAA_4 is the only composition of the ternary complex, corresponding to the peak at 844.857. As a result, this MS data confirmed the above speculation based on the results of UV–vis analysis (Fig. 2a).

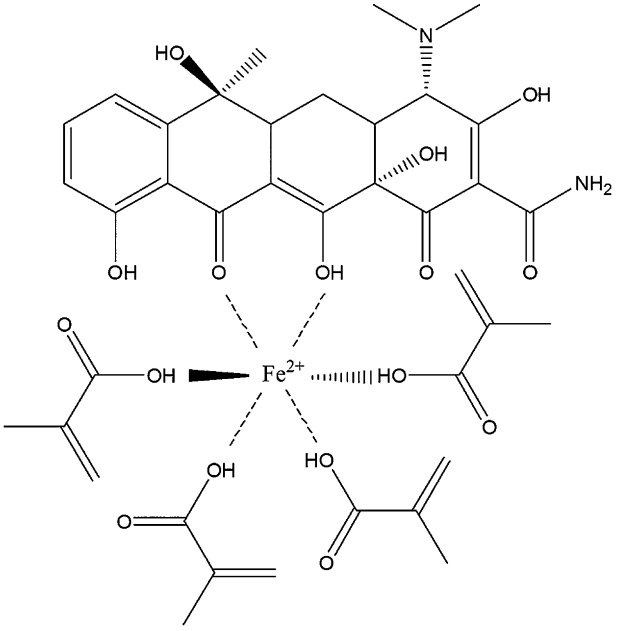

Fig. 3. The ternary complex of TC/Fe^{2+}/MAA formed during prepolymerization in water-containing system.

3.3. Optimization of MISPE conditions

The optimum recognition for MIPs usually occurs in the solution with similar composition to that used in prepolymerization [5]. Thus, Fe^{2+} is required for rebinding of the template of TC, according to most cases [26].

In this case, the MISPE conditions for loading and eluting were easily obtained, which was described in Section 2. As for washing solution, the ratio of methanol to water was firstly adjusted,

Table 2
Assignment of TC and the ternary complex of TC/Fe^{2+}/MAA detected in the time-of-flight mass spectra (TOF-MS).

Peak	Mass	Formula
1	443.132	TC^-
2	444.135	TC
3	445.1622	TC^+
4	844.857	TC–Fe–MAA_4

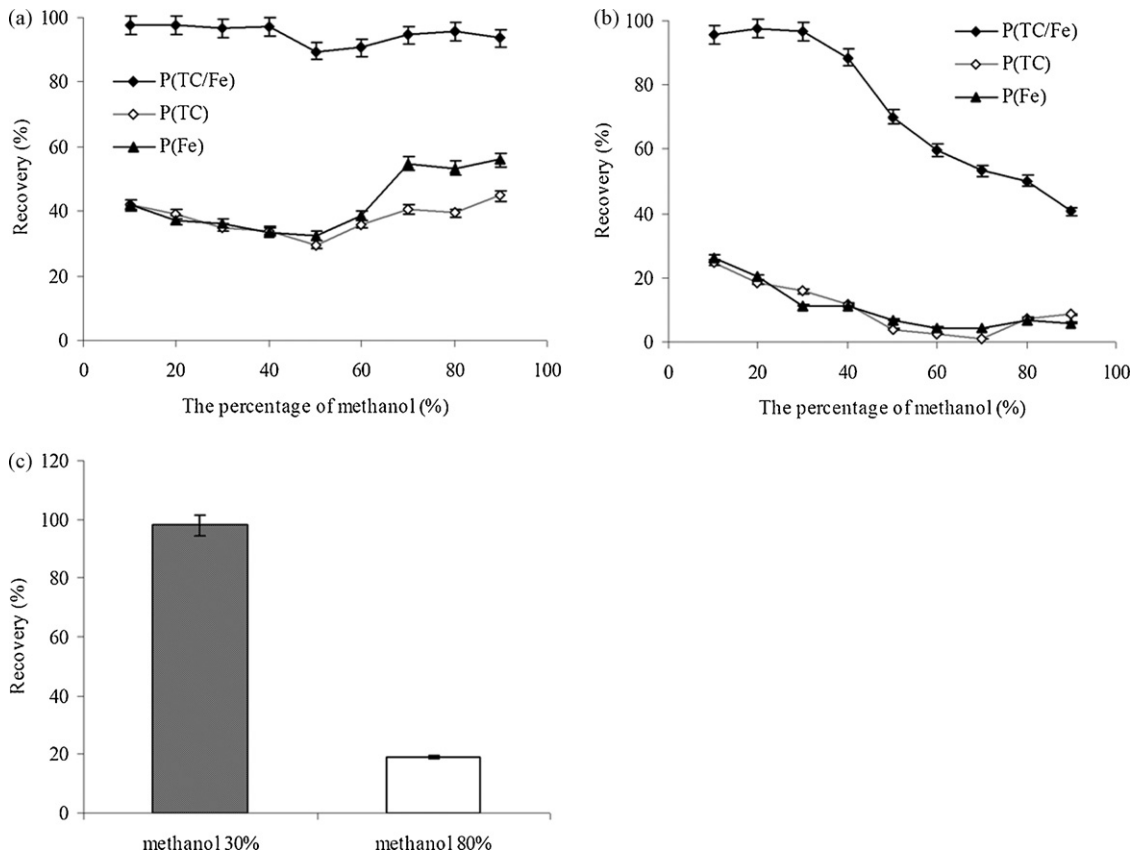

Fig. 4. Recoveries of TC on P(TC/Fe), P(TC) and P(Fe) cartridges and furacillin (FURA) on P(TC/Fe) cartridges using different washing and preconditioning solutions. (a) The different percentages of methanol in water as the washing solutions and 0.25 mmol L^{-1} $FeSO_4$ as the preconditioning solution; (b) the different percentages of methanol in water as the washing solutions and water as the preconditioning solution; (c) the volume of loading was 2 mL. The concentration of FURA was 0.04 mmol L^{-1}.

with the goal of improvement on the specific adsorption and minimization of the non-specific binding of TCs on MISPE cartridges. With the addition of Fe^{2+} on MISPE cartridge, the recoveries of P(TC/Fe) were almost about 95% (Fig. 4a), using different percentages of methanol in water as washing solution. In contrast, without Fe^{2+}, the recovery of P(TC/Fe) in 80% methanol in water was only 50.2% (Fig. 4b). Although the recovery of P(TC/Fe) in 30% methanol was not affected (Fig. 4b), the recoveries of P(TC) and P(Fe) were simultaneously decreased to 15.8% and 11.4%, respectively. Moreover, 80% methanol was able to wash 80.9% of FURA retained on MISPE cartridge whilst 30% methanol could only remove 1.3% of FURA (Fig. 4c). Overall, the specificity of water-compatible MIPs was affected by the mediation of metal ions.

3.4. Selectivity evaluation of the MIPs

To evaluate the selectivity of the MIPs, OTC, CTC, CAP, FURA, ENRO and propranolol were selected. The standard solution (2 mL)

Table 3
Recoveries of three TCs of TC, OTC, and CTC for the spiked urine samples at five levels on MISPE cartridge and NISPE cartridge ($n = 3$).

Compound	Spiked level (μg L^{-1})	MISPE			NISPE		
		Found level (μg L^{-1})	Recovery[a] (%)	RSD[b] (%)	Found level (μg L^{-1})	Recovery[a] (%)	RSD[b] (%)
TC	200	183.2	91.6	5.7	39.2	19.6	5.1
	100	87.9	87.9	4.3	10.6	10.6	4.9
	50	42.6	85.3	4.4	6.6	13.2	5.5
	20	16.8	83.8	3.1	/	/	/
	10	8.0	80.1	3.2	/	/	/
OTC	200	198.6	89.3	4.6	37.4	18.7	5.2
	100	87.8	87.8	4.3	8.2	8.2	4.6
	50	42.3	84.6	3.9	5.4	10.8	5.8
	20	16.3	81.4	3.6	/	/	/
	10	7.8	78.4	3.5	/	/	/
CTC	200	172	86.2	5.9	72.2	36.1	4.5
	100	85.1	85.1	4.8	33.3	33.3	4.7
	50	41.9	83.8	4.1	19.9	38.8	6.1
	20	15.8	79.5	3.4	/	/	/
	10	7.8	78.2	3.3	/	/	/

"/" could not be determined quantitatively.
[a] Mean of three replicates.
[b] Relative standard deviation.

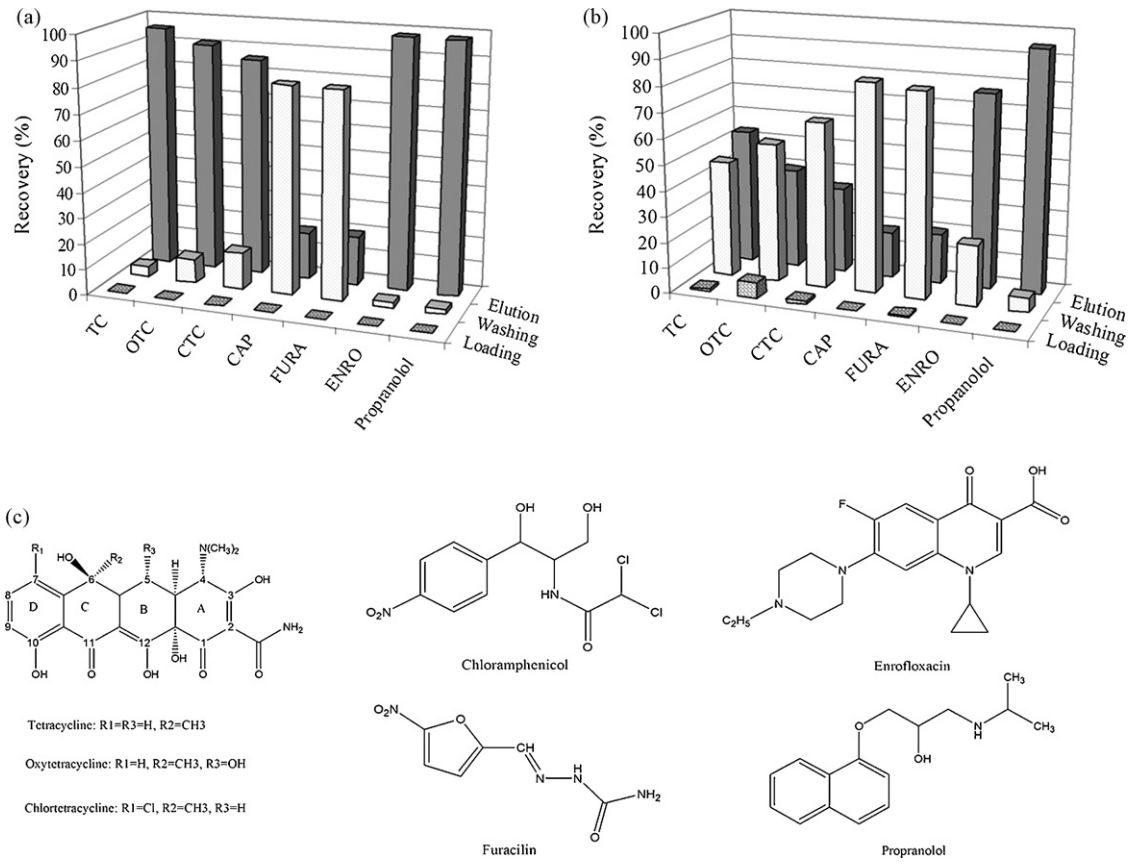

Fig. 5. Selectivity evaluation of the obtained Fe^{2+} mediated MIPs. (a) The recoveries on the MIPs; (b) the recoveries on the NIPs. The volume of loading was 2 mL. OTC oxytetracycline, CTC chlortetracycline, FURA furacillin, CAP chloramphenicol, ENRO enrofloxacin.

of each drug at 0.04 mmol L^{-1} was used as loading solution, then washed with 1.2 mL of water–methanol (1:4, v/v), eluted with 2.0 mL of the mixture consisting of acetonitrile, pure acetic acid and 1.0 mol L^{-1} HCl in water (8:1:1, v/v/v). As shown in Fig. 5, the recovery was 98 ± 3% for ENRO and 98 ± 2% for propranolol on MISPE cartridges and 77 ± 5% recovery for ENRO and 94 ± 4% for propranolol on NISPE cartridges. This indicates that the high non-specificity of MISPE cartridges existed toward ENRO and propranolol. In practice, the detection of TCs would not be affected even under the presence of ENRO and propranolol in real samples. This is due to the fact that TCs were measured at 360 nm whilst ENRO and propranolol were detectable at 282 nm and 290 nm, respectively. As shown in Fig. 5a, most CAP and FURA were removed during the washing step, and the recoveries were 19 ± 1% for CAP and 19 ± 2%, respectively. Meanwhile, the recoveries for TC, OTC and CTC were 96 ± 4%, 90 ± 4% and 86 ± 5%, respectively.

These analytical data may indicate that the obtained P(TC/Fe) can be used for simultaneous extraction of three TCs, including TC, OTC and CTC. The molecular size and shape of the template of TC, together with hydrophobic effects and ionic interactions, play a pivotal role in the specific binding between the water-compatible MIPs and the analytes.

3.5. Urine sample analysis

The urine sample analysis was performed not only with the MIPs but also with the NIPs to evaluate the selectivity of MISPE in loading extracts (Table 3). The TCs of TC, OTC and CTC spiked urine samples were selected for validation of the obtained MIPs and optimized MISPE procedure. The identity of TC, OTC and CTC peaks was firstly confirmed (Fig. 6c) by HPLC–UV analysis.

The spiking concentration for each drug was at 50 μg L^{-1} level, which was hard to be detected by conventional HPLC–UV method (Fig. 6a). As described in Section 2.6.1, the concentrations of TC, OTC and CTC could be enriched 50 times by the MISPE procedure. As shown in Fig. 6, compared to direct HPLC–UV analysis (Fig. 6a), the sensitivities of three TCs in spiked urine sample were substantially improved within the MISPE-HPLC (Fig. 6b). Interestingly, after the MISPE treatment, the interferences were almost removed whilst the spiked TC, OTC and CTC were selectively extracted and

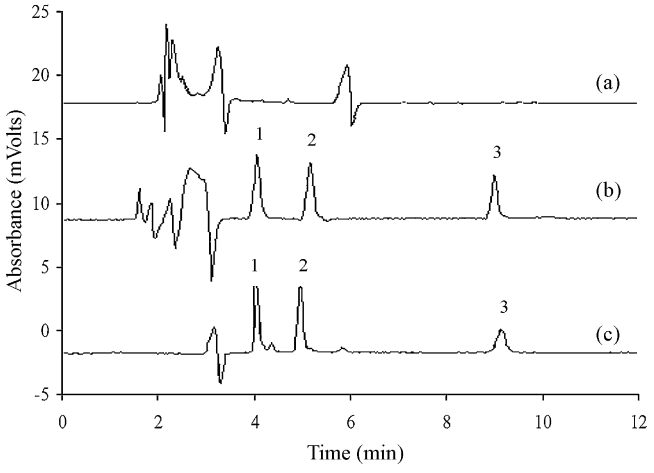

Fig. 6. Chromatograms of HPLC–UV analysis of the spiked urine samples of three TCs of CTC, TC and OTC at 50 μg L^{-1}, without and with MISPE. (a) TCs spiked sample solution; (b) Spiked sample solution extracted with MISPE; (c) TCs mixed standard solution. 1: OTC; 2: TC; 3: CTC. Injection volume, 20 μL. TCs tetracyclines.

detected, then the peaks (Fig. 6b) could be clearly observed, consistent with those of the mixed standard solution (Fig. 6c). The recoveries of TC, OTC and CTC in the various spiked urine samples were 80.1–91.6%, 78.4–89.3% and 78.2–86.2%, respectively. The relative standard deviation (RSD) values varied from 3.1% to 5.9% (Table 3). When 1.0 mL of the eluting solvent was used, the concentration of TC, OTC and CTC in this solution was increased by approximately 10 times and the limit of quantification (LOQ) reached 50 $\mu g\,L^{-1}$ in HPLC–UV analysis, which was lower than the maximum residue limits (MRL) established by the Commission of the European Union at 100 $\mu g\,L^{-1}$.

In summary, the above experimental results demonstrated that Fe^{2+}-mediated water-compatible MIPs could be applied as adsorption materials in MISPE cartridges to simultaneously extract three target analytes of TC, OTC and CTC from urine samples, with desirable specificity and high extraction efficiency.

4. Conclusions

A new scheme of preparing water-compatible MIPs was developed in that most drugs could not be directly imprinted in water-containing systems. With the described metal ion mediated imprinting, the interference of water was substantially reduced due to the formation of the ternary complex of TC/Fe^{2+}/MAA during prepolymerization. Thus, the obtained water-compatible MIPs with the template of TC were successfully used for specific adsorption materials in the MISPE cartridges for clean-up and enrichment of three TCs of TC, OTC and CTC from aqueous urine samples. In conclusion, the research method proposed in this work may provide a novel strategy or model for preparing valuable MIPs in water-containing systems and the development of water-compatible adsorption materials targeting drugs in the field of sample preparation.

Acknowledgments

This research was supported by the National “863”High-Tech Project (2006AA10Z438, 2008AA100804), the National Key Project of Scientific and Technical Supporting Programs of China (2006BAK02A09), Shanghai Commission of Science and Technology (07dz19508) and Shanghai Leading Academic Discipline Project (B205).

References

[1] E. Blahova, E. Brandsteterova, Chem. Pap. 58 (2004) 362.
[2] G. Theodoridis, P. Manesiotis, J. Chromatogr. A 948 (2002) 163.
[3] A. Blomgren, C. Berggren, A. Holmberg, F. Larsson, B. Sellergren, K. Ensing, J. Chromatogr. A 975 (2002) 157.
[4] E. Caro, R.M. Marcé, F. Borrull, R.A.G. Cormack, D.C. Sherrington, Trends Anal. Chem. 25 (2006) 143.
[5] C. Alexander, H.S. Andersson, L.I. Andeersson, R.J. Ansell, N. Kirsch, I.A. Nicholls, J.O. Mahony, M.J. Whitcombe, J. Mol. Recogn. 19 (2006) 106.
[6] K. Haupt, A. Dzgoev, K. Mosbach, Anal. Chem. 70 (1998) 628.
[7] H.Y. Yan, F.X. Qiao, K.H. Row, Anal. Chem. 79 (2007) 8242.
[8] H.Y. Yan, K.H. Row, G.L. Yang, Talanta 75 (2008) 227.
[9] H.W. Sun, F.X. Qiao, J. Chromatogr. A 1212 (2008) 1.
[10] O.C. Seamus, S.A. Diana, Trends Anal. Chem. 26 (2007) 456.
[11] H.F. Dos Santos, E.S. Xavier, M.C. Zerner, W.B. De Almeida, J. Mol. Struct. 527 (2000) 193.
[12] L. Jin, X. Amaya-Mazo, M.E. Apel, S.S. Sankisa, E. Johnson, M.A. Zbyszynska, A. Han, Biophys. Chem. 128 (2007) 185.
[13] J.J. Werner, W.A. Arnold, K. Mcneill, Environ. Sci. Technol. 40 (2006) 7236.
[14] C.R. Anderson, H.S. Rupp, W.H. Wu, J. Chromatogr. A 1075 (2005) 23.
[15] S. Roongnapa, S. Teerapol, C. Thitima, K. Urairut, J. Chromatogr. B 811 (2004) 191.
[16] C. Wensheng, B.G. Ram, Sep. Purif. Technol. 35 (2004) 215.
[17] E. Caro, R.M. Marce, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 552 (2005) 81.
[18] Y. Xiong, H.J. Zhou, Z.J. Zhang, D.Y. He, C. He, Analyst 131 (2006) 829.
[19] X.G. Hu, J.L. Pan, Y.L. Hu, Y. Huo, G.K. Li, J. Chromatogr. A 1188 (2008) 97.
[20] C. Gu, K.G. Karthikeyan, Environ. Sci. Technol. 39 (2005) 2660.
[21] X.Z. Shi, A.B. Wu, G.R. Qu, R.X. Li, D.B. Zhang, Biomaterials 28 (2007) 3741.
[22] M. Arias, M.S. Garcia-Falcon, L. Garcia-Rio, J.C. Mejuto, R. Rial-Otero, J. Simal-Gandara, J. Food Eng. 27 (2007) 69.
[23] A. Aleksandrov, J. Proft, W. Hinrichs, T. Simonson, ChemBioChem 8 (2007) 675.
[24] L.Q. Wu, Y.Z. Li, Anal. Chim. Acta 482 (2003) 175.
[25] J.M. Wessels, W.E. Ford, W. Szymczak, S. Schneider, J. Phys. Chem. 102 (1998) 9323.
[26] A.G. Mayes, M.J. Whitcombe, Adv. Drug Deliv. Rev. 57 (2005) 1742.