Carbohydrate Polymers 101 (2014) 517–523

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Synthesis of chitosan molecularly imprinted polymers for solid-phase extraction of methandrostenolone

Yun Wang[a,*], Enlan Wang[a], Ziming Wu[a], Huan Li[a], Zhi Zhu[a], Xinsheng Zhu[b], Ying Dong[a]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Zhenjiang Products Quality Supervision and Inspection Center, Zhenjiang 212132, China

ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 13 June 2013
Accepted 24 September 2013
Available online 29 September 2013

Keywords:
Chitosan
Methandrostenolone
Molecularly imprinted polymers
Solid-phase extraction
HPLC

ABSTRACT

Chitosan molecularly imprinted polymers (CHI-MIPs) for selective extraction of methandrostenolone (MA) was synthesized by cross-linking of chitosan with epichlorohydrin in the presence of MA as the template molecule. Systematic investigations of the influences of template, functional polymer, cross-linker as well as porogen concentrations on the rebinding capacity of CHI-MIPs were carried out. Adsorption and kinetic binding experiments indicated that the synthesized CHI-MIPs had high adsorption and excellent affinity to MA. Solid-phase extraction (SPE) using the prepared CHI-MIPs as adsorbent was then investigated, and the optimum loading and eluting conditions for SPE of the MA were established. The optimized SPE procedure was used to extract the MA from several spiked samples and a good sample clean-up was obtained with the average recoveries ranged from 95.97 to 101.79%.

1. Introduction

The trace level quantification of organic contaminants in complex matrices usually requires a step of sample pretreatment before instrumental analysis. Solid-phase extraction (SPE) is one of the most popular cleanup techniques due to factors such as convenience, time saving and simplicity (Picó, Fernández, Ruiz, & Font, 2007; Pragst, 2007). Nevertheless, the main drawback of conventional SPE sorbent is lack of selectivity, leading to co-extraction of matrix interference components with the target analyte. The best way to overcome this drawback is by performing selective extraction targeted for the compound of interest. The first sorbent used for selective extraction was immuno-sorbent, which relies upon reversible and highly selective interactions between analyte and antibody (Hennion & Pichon, 2003; Senyuva & Gilbert, 2010). However, the obtainment of antibody is difficult, time-consuming and expensive, their application is to some extent limited.

In recent years, molecularly imprinted polymers (MIPs) have attracted much attention due to their outstanding advantages, such as predetermined recognition ability, stability, relative ease and low cost of preparation, and potential application to a wide range of target molecules (Alexander et al., 2006; Chen, Xu, & Li, 2011; Turiel & Martín-Esteban, 2010). MIPs are synthetic polymers possessing specific cavities designed for a target molecule. In the most common preparation process, monomers form a complex with a template molecule through covalent or non-covalent interactions and are then joined by using a cross-linking agent. After removing of the imprinting molecules by chemical reaction or extraction, binding sites are exposed which are complementary to the template molecule in size, shape, and position of the functional groups, and consequently allow their selective uptake. MIPs have been successfully used as artificial receptors in separations (López Mdel et al., 2012; Santos, Vitor, Andrade, Martins, & Figueiredo, 2012), sensors (Pardieu et al., 2009), catalysis (Volkmann & Brüggemann, 2006), and drug development and screening (Shi, Wu, Qu, Li, & Zhang, 2007).

Chitosan is produced by the deacetylation of chitin, which is known to be the most abundant natural amino-polysaccharide presenting non-toxicity, bio-degradability and bio-compatibility (Rinaudo, 2006). The presence of multiple functional groups such as amino and hydroxyl groups on its polysaccharide chain provides the flexibility for structural modifications and for preparing molecularly imprinted polymers, and it is, therefore, interesting not only as an abundant resource but also as a novel type of functional materials. The range of its applications has been enormously expanded in various fields including biotechnology, water-treatment, membranes, cosmetics, food industry, and medicine (Rinaudo, 2006; Wan Ngah, Teong, & Hanafiah, 2011). However, few works deal with the combination of chitosan in molecular imprinting (Guo, Xia, Wang, Song, & Zhang, 2005; Kyzas, Lazaridis, & Bikiaris, 2013;

* Corresponding author. Tel.: +86 511 88797059; fax: +86 511 88780201.
E-mail address: wangy1974@ujs.edu.cn (Y. Wang).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.078

Monier & El-Sokkary, 2010; Nishad, Bhaskarapillai, Velmurugan, & Narasimhan, 2012; Tong, Guan, Wang, Xu, & He, 2011).

Methandrostenolone (MA) is a kind of synthetic anabolic androgenic steroids and has been strictly regulated in most countries over the world due to its adverse effects on human health. In the present study, a novel chitosan molecularly imprinted polymer (named hereafter as CHI-MIP) intended to clean-up and pre-concentration of MA from natural samples was prepared. The surface structure and the physicochemical properties of the polymers were characterized; the adsorption capacity and the selectivity toward the template in the presence of structurally related compounds were determined. The performance of the developed MIPs for the clean up and preconcentration of MA from several actual samples was also evaluated.

2. Materials and methods

2.1. Chemicals

Chitosan with an average molecular weight of 100 kD and a deacetylation degree of 93% was purchased from Golden-Shell Biochemical Co., Ltd. (Yuhuan, China). Methandrostenolone, trenbolone (TB), testosterone propionate (TP), epichlorohydrin and sodium borohydride ($NaBH_4$) were supplied by Sigma–Aldrich (St. Louis, MO, USA). High-performance liquid chromatography-grade methanol was obtained from Fisher Scientific, Inc. (Pittsburgh, PA, USA). All other chemicals were of analytical grade and supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Milli-Q water was obtained from ultrapure water system supplied by Chengdu Ultrapure Technology Co., Ltd. (Chengdu, China).

2.2. Preparation of CHI-MIPs

Chitosan was cross-linked with epichlorohydrin and molecularly imprinted with MA as the template molecule following a single-step procedure as previously described (Aburto & Le Borgne, 2004; Wang, Xu, Zhang, Wang, & Dong, 2011a), with modifications. Firstly, 0.6 g of chitosan was dissolved in 30 mL of 2% acetic acid aqueous solution (v/v). The resulted chitosan solution was mixed with 15 mL of methanol containing 100 mg of MA and the mixture was stirred for 30 min. 50 mL of liquid paraffin containing 0.3 mL of span-80 and 2 g of sucrose were added into the mixture solution and stirred at 50 °C for 10 min. Then, 1 mL of formaldehyde aqueous solution (37%, w/w) performed as amino protective solute was added into the mixture and stirred for 30 min. After adjusted pH to 9.0 with 2 M sodium hydroxide aqueous solution, epichlorohydrin was added dropwise into the mixture to the final concentration raged from 0.5% to 2.5%. The cross-linking reaction was performed by agitation at 70 °C for 3 h with the pH maintained at 9.0 throughout. To remove the template molecules and the residuals of the polymers, the prepared CHI-MIPs were extracted in a Soxhlet apparatus using 80% methanol for 24 h and then digested with 0.1% hydrochloric acid to remove the amino protective solute. Finally, the CHI-MIPs were thoroughly washed with deionized water and dried in a vacuum drier. Chitosan-based non-imprinted polymers (CHI-NIPs) were prepared with the same procedure in the absence of template molecule.

2.3. Optimization of CHI-MIPs preparation

The optimum porogen content, molar ratios of cross-linker (epichlorohydrin) and template (MA) to polymer (chitosan) were determined through the rebinding capacity of the prepared CHI-MIPs.

2.3.1. Methanol:water ratio

The volume ratios of methanol to water tested for the CHI-MIPs preparation were 1:3, 1:2, 1:1, 2:1 and 3:1. Sucrose content, molar ratios of epichlorohydrin to chitosan and MA to chitosan were 1%, 1:6 and 1:10 respectively, whereas all the other conditions were as formerly described.

2.3.2. Sucrose content

The contents of sucrose tested for the optimal preparation of CHI-MIPs were 0.5%, 1%, 1.5%, 2% and 2.5%. Volume ratio of methanol to water, molar ratios of epichlorohydrin to chitosan and MA to chitosan were 1:2, 1:6 and 1:10 respectively, whereas all the other conditions were as previously described.

2.3.3. Molar ratio of epichlorohydrin to chitosan

The molar ratios of epichlorohydrin versus chitosan (glucosamin) tested for each type of CHI-MIPs were 1:10, 1:8, 1:6, 1:4 and 1:2. Volume ratio of methanol to water, sucrose content and molar ratio of MA to chitosan were 1:2, 1% and 1:10 respectively, whereas all the other conditions were as previously described.

2.3.4. Molar ratio of MA to chitosan

Similarly, molar ratios of MA to chitosan (glucosamin) ranged from 1:20, 1:10, 1.5:10 and 1:5 were tested for CHI-MIPs preparation. Volume ratio of methanol to water, sucrose content and molar ratio of epichlorohydrin to chitosan were 1:2, 1% and 1:6 respectively, whereas all the other conditions were as formerly described.

2.4. Characterization of CHI-MIPs and CHI-NIPs

The synthesized CHI-MIPs and CHI-NIPs were characterized with scanning electron microscope (S-4800, Hitachi Ltd., Japan) and Fourier transform infrared spectroscopy (Nicolet Nexus 670, Thermo Fisher Scientific, USA). The pore size in polymers surface was measured by nitrogen adsorption porosimetry (NOVA e2000, Quantachrome, USA). The average pore diameter was calculated using the BJH analysis of the desorption branch.

2.5. Kinetic adsorption

In the kinetic experiments, 0.2 g of CHI-MIPs were placed in a conical flask and mixed with 10 mL of 800 μg/mL MA dissolved in 10% methanol. The suspensions were incubated for 5 hours at room temperature. Samples were collected at fixed intervals (30 min) and analyzed by HPLC method (Wang, Xu, Zhang, Wang, & Dong, 2011b). The same experiments were performed for the respective CHI-NIPs.

2.6. Static adsorption

The effect of initial MA concentration on the adsorption was determined by mixing of CHI-MIPs with 10 mL of MA solutions at definite concentrations (0.1–1 mg/mL). Immediately after mixing, the mixture was incubated at room temperature for 5 h. After adsorption, the residual concentration of the MA was determined by HPLC. The static adsorption capacity was calculated based on the following formula (Shaikh, Memon, Khan, Bhanger, & Nizamani, 2012):

$$Q = \frac{(C_i - C_f) \times V}{m}$$

where Q (mg/g) was the mass of MA adsorbed per gram of polymers, C_i (mg/L) was the initial concentration of MA, C_f (mg/L) was its final concentration after adsorption, V (L) was the total volume of adsorption mixture, and m (g) was the mass of polymers. The same experiments were performed for the respective CHI-NIPs.

The saturation binding data were further processed to generate a Scatchard equation to estimate the binding properties of CHI-MIPs and CHI-NIPs. The Scatchard equation was as follows:

$$\frac{Q}{Q_s} = \frac{Q_{max} - Q}{K_d}$$

where Q was the amount of MA bound to polymers at equilibrium, Q_{max} was the apparent maximum adsorption capacity, Q_s was the free MA concentration at equilibrium and K_d was the dissociation constant. The values of K_d and Q_{max} could be calculated from the slope and intercept of the linear curve plotted at Q/Q_s versus Q.

2.7. Selectivity

To verify whether the prepared CHI-MIPs are selective to the imprinting molecule, the rebinding of two structurally related compounds on the polymers was investigated. A mixture solution of MA, TB and TP with an initial individual concentration of 100 μg/mL was mixed with the MIPs. The binding amounts of tested analytes were determined using HPLC method.

The specificity of the CHI-MIPs and CHI-NIPs was estimated by the partition coefficient (K) of selected phthalates between polymers and the solution. Parameter of K is defined as follows:

$$K = \frac{Q}{Q_s}$$

where Q was the amount of tested analyte bound to polymers at equilibrium, and Q_s was the concentration of tested analyte remaining in the solution at equilibrium.

In addition, the imprinting factor (IF) and selectivity coefficient (SC) were used to evaluate the selectivity properties of MIPs and NIPs toward MA and its close structural analogs (TP and TB). The imprinting factor and selectivity coefficient were calculated by the following formula:

$$IF = \frac{K_i}{K_c}$$

$$SC = \frac{IF_{MA}}{IF_i}$$

where K_i and K_c represent the partition coefficients of analytes for MIPs and NIPs, IF_{MA} and IF_i were the imprinting factors for MA and the other two analogs, respectively.

2.8. Binding evaluation parameters by solid-phase extraction

About 0.15 g of CHI-MIPs was introduced into a solid-phase extraction cartridge. 200 μg of MA dissolved in different solutions including 2% methanol, 10% methanol or PBS buffer was loaded onto the cartridge. After thoroughly washing with distilled water, the bound MA was released from the polymers using methanol with different concentration (10%, 50% and 100%). The MA content in flow-through and eluants were determined by HPLC, and the binding efficiency and releasing efficiency were used to evaluate the SPE conditions.

2.9. Solid-phase extraction of MA from spiked real samples

The animal tissue (pork and pork liver), feed (maize powder) and egg samples purchased from local market were spiked with MA at the final concentration of 1.5 and 6.0 ng/g. About 5 g of spiked animal tissue and feed samples were homogenized and agitated for 5 min in 20 mL of tert-butyl methyl ether (Wang et al., 2011a). After 10 min of ultrasonic extraction, the mixtures were centrifuged at 8000 rpm for 10 min. The supernatants were collected and the pellets were repeatedly extracted using tert-butyl methyl ether for 2 times followed by ultrasonic extraction and centrifugation as before. All the supernatant fractions were pooled and evaporated to dryness. The residues were dissolved in 10% methanol and loaded on the SPE column. For egg sample treatment, the same procedure was applied with tert-butyl methyl ether replaced by 10% Na_2CO_3 in ethanol (w/v). Under optimal SPE conditions, the eluate was collected and MA content was determined to estimate the recovery rate during SPE cleanup.

3. Results and discussion

3.1. Synthesis of CHI-MIPs and CHI-NIPs

In this study, natural polymer chitosan instead of the monomer was used to prepare the MA-imprinted polymers through a single-step procedure. In preparation of CHI-MIPs, the influences of cross-linker, template as well as the porogen concentrations on the properties of the CHI-MIPs were assessed.

3.1.1. Porogen content

The porogenic solvent should not only provide the environment for the cross-linking reaction, but also produce a porous structure in the resulting polymers and influence the polymers morphology and the strength of non-covalent interactions (Cormack & Elorza, 2004; Lasáková & Jandera, 2009). In order to obtain MIPs which demonstrate specific recognition ability in aqueous environment, MIPs synthesized in water-containing system were investigated using different proportions of methanol–water as porogenic solvents. As shown in Fig. 1a, the rebinding ability of the prepared MIPs was critically influenced by the proportion of methanol. When the ratio of methanol–water was 1:2 (v/v), the obtained polymers exhibited the highest rebinding ability (46.58 mg/g).

In addition to organic solvents, sucrose is also proved to be a good porogenic agent for molecular imprinting (Wu et al., 2010). Fig. 1b depicts the effect of sucrose content on MA rebinding capacity of the prepared MIPs. Increasing the percentage of sucrose in the reaction mixture, the rebinding capacity of the MIPs was shown to increase and reached the highest level (45.40 mg/g) when the sucrose content was 2.0%, which was selected as the optimum porogen. When excessive amount of sucrose was used, the rebinding capacities of the MIPs declined possibly due to the rigidity framework (Chang et al., 2010).

3.1.2. The molar ratio of cross-linker to chitosan

The molar ratio of cross-linker to chitosan (glucosamine) is a key parameter which greatly affected the rebinding capacities and imprinting effects of the CHI-MIPs. As shown in Fig. 1c, the rebinding capacities of MIPs were shown to increase with the increasing of cross-linker/glucosamine ratio in reaction mixture until a value of 1:4, exporting the rebinding capacity of 37.48 mg/g. This is not surprising because it is well known that a high degree of cross-linking resulted in a certain rigidity of the polymer matrix, which is important in preserving the imprinted memory and maintaining specific recognition sites in the resulted polymers (Chang et al., 2010). When the molar ratio of cross-linker to chitosan is greater than 1:4, the amino groups besides the hydroxyl groups in the chitosan molecules will be participated in the cross-linking reaction (Yu, Deng, & Yu, 2008). Therefore, the rebinding capacity of the MIPs decreased along with the increase of cross-linker content due to the number of specific recognition sites were reduced for imprinting molecule.

3.1.3. The molar ratio of template molecule to chitosan

The ratio of template molecule to functional polymer could affect both imprinting results and adsorption properties. The less imprinting molecules, the less imprinted sites are available for the

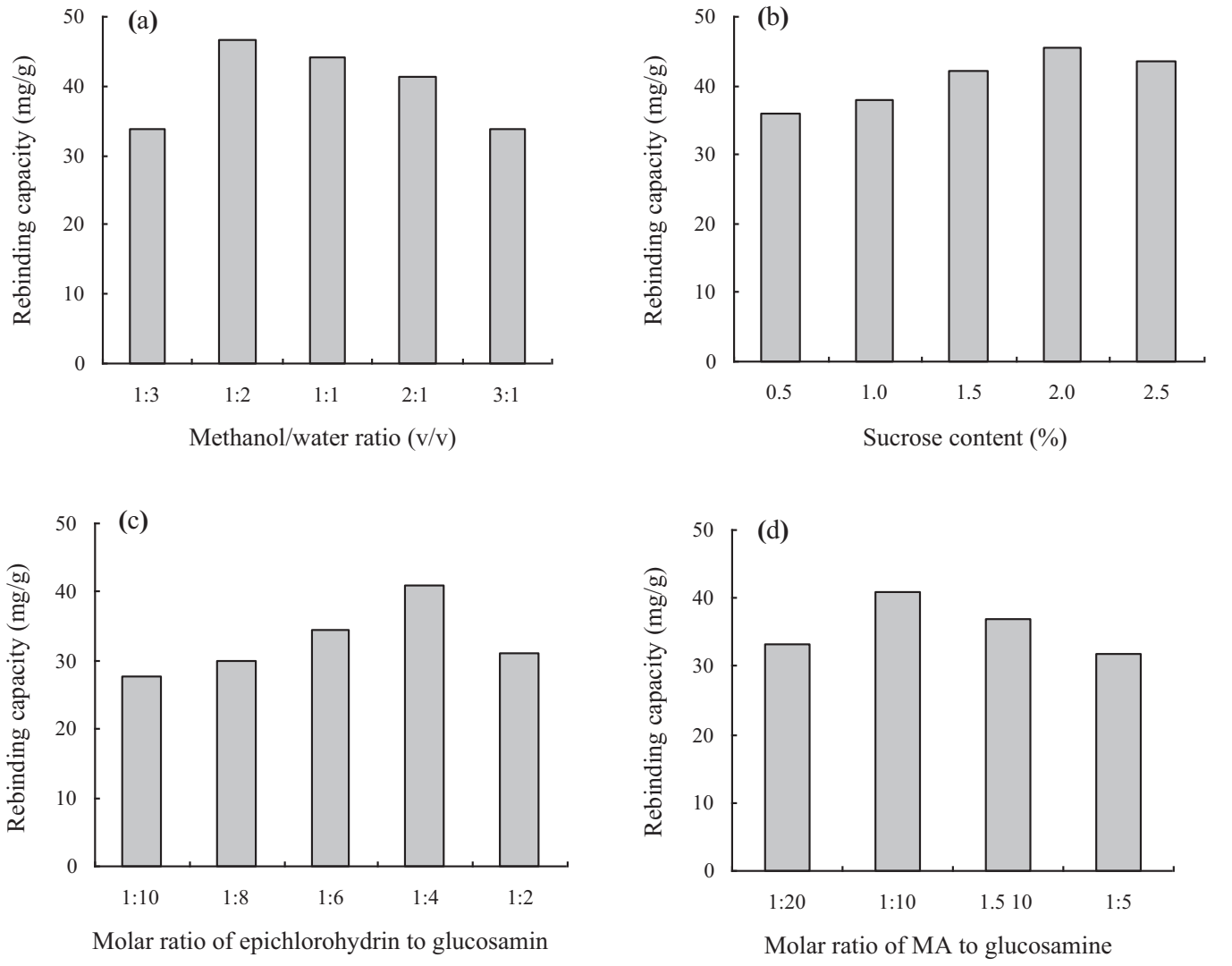

Fig. 1. Effect of reaction conditions on the rebinding property of CHI-MIPs. Volume ratio of methanol to water (a), sucrose content (b), molar ratio of epichlorohydrin to chitosan (c) and molar ratio of MA to chitosan (d).

recognition of MA. However, too much imprinting molecules may lead to the formation of MIPs defects due to the relative lack of the polymer functional groups. Fig. 1d summarizes the relation between rebinding capacity and the amount of template molecule used in cross-linking reaction. It can be seen that the suitable molar ratio of MA/glucosamine was 10 and the highest rebinding capacity of the imprinted polymers was 38.94 mg/g. However, when the amount of template molecule was too large, the rebinding capacity of the prepared polymers declined.

3.2. Characterization of CHI-MIPs

Scanning electron micrographs of the prepared CHI-MIPs and CHI-NIPs are presented in Fig. 2. It can be seen that the CHI-NIPs present a relatively smooth surface compared to the rough porous surface presented by CHI-MIPs with an average pore diameter of about 32 nm, which could be attributed to the cavities left after the leaching of the imprinting molecules out of the cross-linked network.

The FT-IR spectra of CHI-NIPs, MA and CHI-MIPs (before MA was removed) were presented in Fig. 3. The spectrum of MA exhibits the characteristic peaks of O—H at 3452 cm^{-1} and C=O at 1664 cm^{-1}. The spectrum of CHI-NIPs shows the absorption peaks at about 3442 cm^{-1} for the stretching vibration of O—H and N—H, at about 1637 cm^{-1} attributed to the NH_2CO in chitosan. In Fig. 3c, the broaden absorption peak at about 3500–3000 cm^{-1} is corresponding to the hydrogen bond strength. In compare with that of CHI-MIPs, the O—H and N—H adsorption peak at 3442 cm^{-1} in the spectrum of CHI-NIPs was shifted to 3421 cm^{-1}, and the C=O absorption peak was moved from 1637 cm^{-1} to 1654 cm^{-1}. Altogether, these changes can be considered as providing evidence that MA has been successfully imprinted in chitosan possibly via hydrogen bonds between $-NH_2$ and —OH in the functional polymer chitosan and —OH, C=O in the template molecule MA (Espinosa-García et al., 2007; Ma, Chen, Zheng, Youn, & Chen, 2011).

3.3. Binding properties of the CHI-MIPs and CHI-NIPs

3.3.1. Kinetic adsorption

It can be seen that the amount of MA binding to the MIPs is much higher than that of the NIPs (Fig. 4). A monotonous increasing trend with high rates at the beginning of adsorption for both CHI-MIPs and CHI-NIPs was observed in the first 1 h, and then the adsorption curve became relatively flat and almost reached the binding equilibrium within 3 h. The adsorption rate of MA to CHI-MIPs is relatively fast, which could be probably due to the groups of the MIPs structure with high complexation potency (Spivak, 2005).

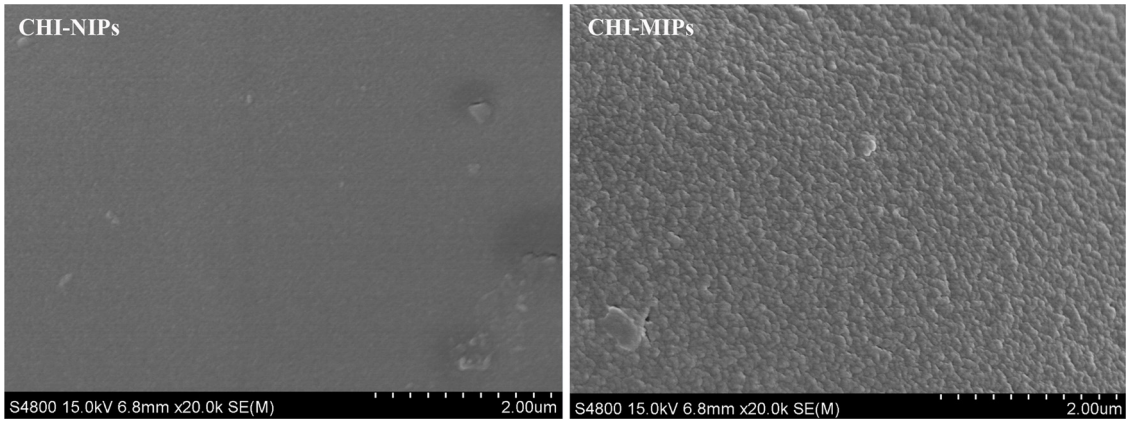

Fig. 2. Scanning electron micrographs of CHI-MIPs and CHI-NIPs.

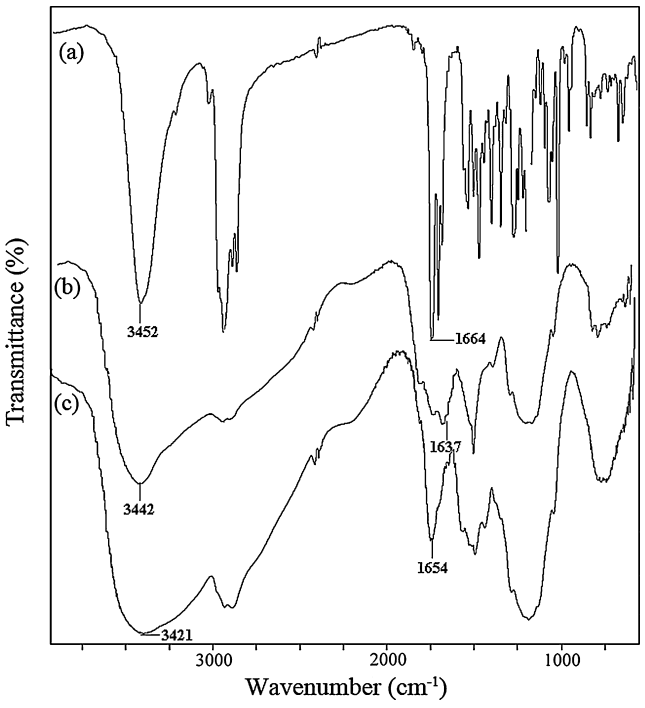

Fig. 3. FT-IR spectra of MA (a), CHI-NIPs (b) and CHI-MIPs before template removed (c).

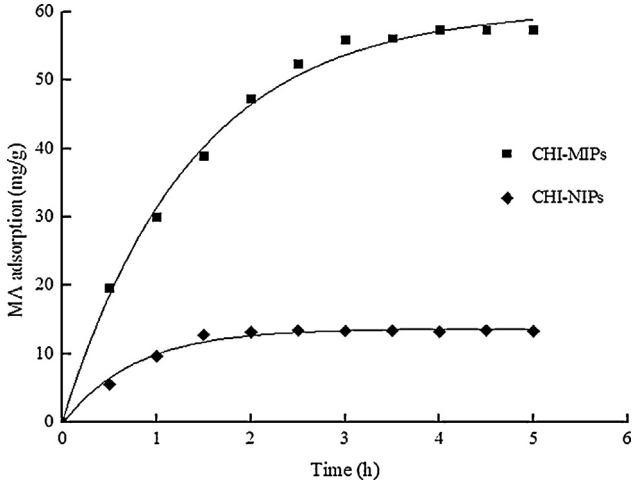

Fig. 4. Adsorption kinetics of MA on CHI-MIPs and CHI-NIPs.

3.3.2. Static adsorption

In order to investigate the affinity of CHI-MIPs and CHI-NIPs, a steady-state binding experiment and subsequent Sactchard analysis were carried out by varying the initial concentration of MA in the range of 0.1–1 mg/mL. As shown in Fig. 5a, the adsorption of polymers increased with increasing the initial MA concentration. The results also showed that amount of MA bound to CHI-MIPs is much higher in comparison with the CHI-NIPs, indicating large population of MA specific binding sites were produced during imprinting reaction.

The saturation binding data were further processed to generate a Scatchard equation to estimate the binding properties of CHI-MIPs and CHI-NIPs. It can be seen in Fig. 5b, two straight lines were obtained in the case of CHI-MIPs in the plot region, which indicated that there were two different binding sites in CHI-MIPs. The linear regression equations for the slope of biphasic curve were $Q/Q_s = 245.95 - 5.85Q$ and $Q/Q_s = 154.85 - 2.24Q$, respectively. The dissociation constant (K_d) of 0.17 and 0.45 mg/mL, and the apparent maximum binding capacities (Q_{max}) of 42.04 and 69.13 mg/g could be calculated from the slopes and the intercept of the linear equilibrations. For CHI-NIPs, biphasic curve was not observed which revealed that only one binding site existed. K_d (0.19 mg/mL) and Q_{max} (10.16 mg/g) can be similarly calculated from the fitting linear equation ($Q/Q_s = 125.92 - 16.53Q$). The specific affinity and binding capacity of CHI-NIPs for MA were much lower than that of CHI-MIPs.

3.3.3. Selectivity

The binding specificity of the prepared CHI-MIPs was evaluated by the experiment of simultaneous adsorption of imprinting molecules in the presence of its structurally related compounds (TP and TB). It can be seen in Table 1, the bound amount of MA for CHI-MIPs was much higher than that of the other two competitive analogs. The adsorption of small amount of other competitive analogs might be attributed to the non-specific interaction. Moreover, the calculation of selectivity coefficients indicated that the imprinted polymers were specific and about 10 times the non-imprinted ones. All these data suggested that the prepared

Table 1
Distribution coefficient, imprinting factor and selectivity coefficient of MA, TP and TB onto CHI-MIPs and CHI-NIPs.

Analytes	K_{MIPs} (mL/g)	K_{NIPs} (mL/g)	Imprinting factor	Selectivity coefficient
MA	82.79	6.62	12.51	–
TP	5.64	4.34	1.30	9.62
TB	5.47	4.16	1.31	9.55

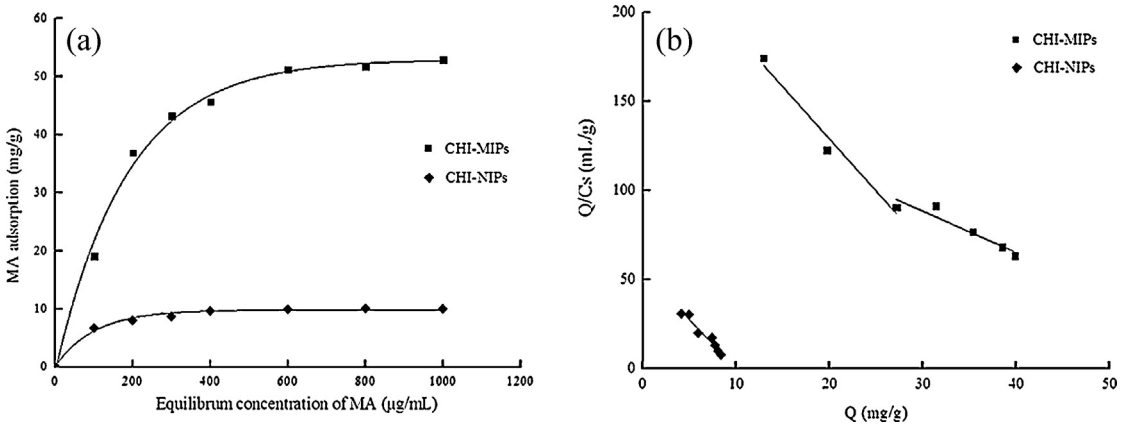

Fig. 5. Adsorption isotherms (a) and Scatchard plot analysis of the binding of MA to CHI-MIPs and CHI-NIPs (b).

CHI-MIPs had a relatively higher affinity and selectivity to its template molecule.

3.4. Molecularly imprinted solid-phase extraction of MA from spiked samples

The aim of this study is to investigate the feasibility of the prepared CHI-MIPs for selective extraction of template molecule from complex matrices. The prepared CHI-MIPs were packed into a solid-phase extraction cartridge to evaluate their characteristics. In order to optimize the chromatographic extraction conditions, different loading buffer and eluting buffer were tested. The highest extraction efficiency was achieved by using 10% methanol and pure methanol as loading and eluting buffer, respectively.

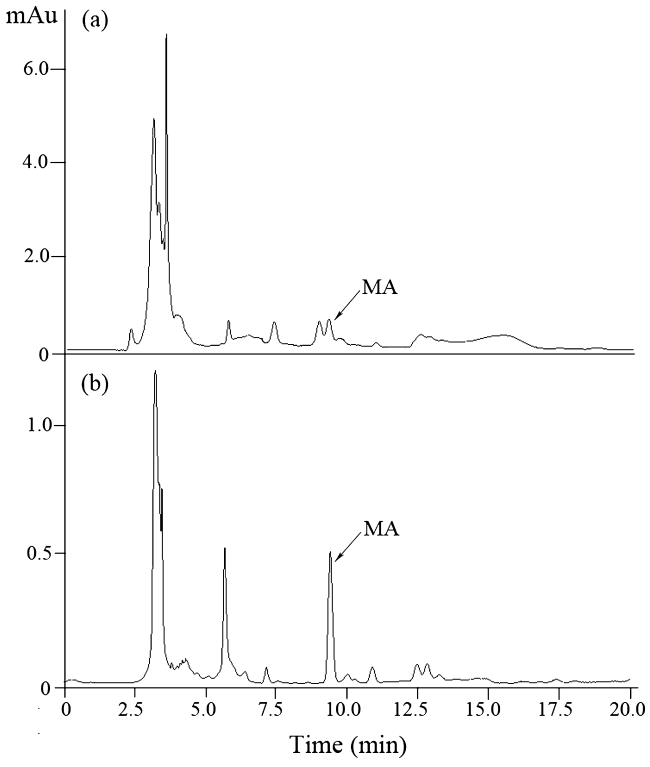

Fig. 6. Representative chromatogram of spiked maize powder (a) and chromatogram of spiked maize powder after MIP-SPE (b). Detection wavelength: 250 nm; mobile phase: acetonitrile/water (80:20, v/v); flow rate: 0.7 mL/min; column temperature: 25 °C. Sample spiked level: 6 ng/g.

Table 2
Recoveries of MA from spiked samples by SPE using prepared CHI-MIPs ($n = 3$).

Sample	Spiked level (ng/g)	Recovery rate (%)	RSD (%)
Pork	1.5	95.97	2.08
	6.0	96.04	1.94
Egg	1.5	97.87	3.42
	6.0	101.46	1.80
Maize powder	1.5	99.06	1.99
	6.0	96.62	1.23
Pork liver	1.5	98.21	2.56
	6.0	101.79	4.68

Solid-phase extraction prior to chromatographic quantitative determination was used in order to achieve high sample cleanup and pre-concentration. To demonstrate the potential application to selective cleanup of complex matrices, the prepared CHI-MIPs were challenged with four kinds of natural samples including pork, pork liver, egg and maize powder fortified with MA at the levels of 1.5 and 6.0 ng/g. Fig. 6 shows a representative chromatogram obtained from a spiked maize powder with or without a step of MIP-SPE cleanup. As it can be found, a number of interfering peaks were removed after MIP-SPE pretreatment. Quantitative analysis indicated that the average recoveries of MA from the fortified samples were in the range of 95.97–101.79% with relative standard deviation (RSD) less than 4.68% (Table 2). These results suggested that the CHI-MIPs prepared in the present work could be effectively applied for the determination of MA in real samples.

4. Conclusions

In the present study, molecularly imprinted polymers selective for MA were prepared using chitosan as functional polymer and epichlorohydrin as cross-linker, and the conditions in imprinting stage were optimized. Through evaluated in a series of adsorption experiments, the synthesized CHI-MIPs exhibited good recognition and selectivity to imprinting molecule. Furthermore, a procedure of extraction of MA from real samples using the MIPs as solid-phase extraction adsorbent was developed. The proposed MIP-SPE method provided adequate recoveries of MA from different samples, suggesting that it could be a useful tool for analytical purposes.

Acknowledgements

Gratitude is expressed to Dr. Afoakwah Akowuah Newlove for critical reading of the manuscript. This work was co-supported by Natural Science Foundation of Jiangsu Province,

China (BK2008242), China Postdoctoral Science Foundation (20070420974) and PAPD of Jiangsu Higher Education Institutions.

References

Aburto, J., & Le Borgne, S. (2004). Selective adsorption of dibenzothiophene sulfone by an imprinted and stimuli-responsive chitosan hydrogel. *Macromolecules*, *37*, 2938–2943.

Alexander, C., Andersson, H. S., Andersson, L. I., Ansell, R. J., Kirsch, N., Nicholls, I. A., et al. (2006). Molecular imprinting science and technology: A survey of the literature for the years up to and including 2003. *Journal of Molecular Recognition*, *19*, 106–180.

Chang, Y., Zhang, L., Ying, H., Li, Z., Lv, H., & Ouyang, P. (2010). Desulfurization of gasoline using molecularly imprinted chitosan as selective adsorbents. *Applied Biochemistry and Biotechnology*, *160*, 593–603.

Chen, L., Xu, S., & Li, J. (2011). Recent advances in molecular imprinting technology: Current status, challenges and highlighted applications. *Chemical Society Reviews*, *40*, 2922–2942.

Cormack, P. A. G., & Elorza, A. Z. (2004). Molecularly imprinted polymers: Synthesis and characterization. *Journal of Chromatography B*, *804*, 173–182.

Espinosa-García, B. M., Argüelles-Monal, W. M., Hernandez, J., Félix-Valenzuela, L., Acosta, N., & Goycoolea, F. M. (2007). Molecularly imprinted chitosan-genipin hydrogels with recognition capacity toward o-xylene. *Biomacromolecules*, *8*, 3355–3364.

Guo, T. Y., Xia, Y. Q., Wang, J., Song, M. D., & Zhang, B. H. (2005). Chitosan beads as molecularly imprinted polymer matrix for selective separation of proteins. *Biomaterials*, *26*, 5737–5745.

Hennion, M. C., & Pichon, V. (2003). Immuno-based sample preparation for trace analysis. *Journal of Chromatography A*, *1000*, 29–52.

Kyzas, G. Z., Lazaridis, N. K., & Bikiaris, D. N. (2013). Optimization of chitosan and β-cyclodextrin molecularly imprinted polymer synthesis for dye adsorption. *Carbohydrate Polymers*, *91*, 198–208.

Lasáková, M., & Jandera, P. (2009). Molecularly imprinted polymers and their application in solid phase extraction. *Journal of Separation Science*, *32*, 799–812.

López Mdel, M., Pérez, M. C., García, M. S., Vilariño, J. M., Rodríguez, M. V., & Losada, L. F. (2012). Preparation, evaluation and characterization of quercetin-molecularly imprinted polymer for preconcentration and clean-up of catechins. *Analytica Chimica Acta*, *721*, 68–78.

Ma, X., Chen, R., Zheng, X., Youn, H., & Chen, Z. (2011). Preparation of molecularly imprinted CS membrane for recognizing naringin in aqueous media. *Polymer Bulletin*, *66*, 853–863.

Monier, M., & El-Sokkary, A. M. A. (2010). Preparation of molecularly imprinted cross-linked chitosan/glutaraldehyde resin for enantioselective separation of L-glutamic acid. *International Journal of Biological Macromolecules*, *47*, 207–213.

Nishad, P. A., Bhaskarapillai, A., Velmurugan, S., & Narasimhan, S. V. (2012). Cobalt (II) imprinted chitosan for selective removal of cobalt during nuclear reactor decontamination. *Carbohydrate Polymers*, *87*, 2690–2696.

Pardieu, E., Cheap, H., Vedrine, C., Lazerges, M., Lattach, Y., Garnier, F., et al. (2009). Molecularly imprinted conducting polymer based electrochemical sensor for detection of atrazine. *Analytica Chimica Acta*, *649*, 236–245.

Picó, Y., Fernández, M., Ruiz, M. J., & Font, G. (2007). Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *Journal of Biochemical and Biophysical Methods*, *70*, 117–131.

Pragst, F. (2007). Application of solid-phase microextraction in analytical toxicology. *Analytical and Bioanalytical Chemistry*, *388*, 1393–1414.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, *31*, 603–632.

Santos, M. G., Vitor, R. V., Andrade, F. L., Martins, I., & Figueiredo, E. C. (2012). Molecularly imprinted solid phase extraction of urinary diethyl thiophosphate and diethyl dithiophosphate and their analysis by gas chromatography–mass spectrometry. *Journal of Chromatography B*, *909*, 70–76.

Senyuva, H. Z., & Gilbert, J. (2010). Immunoaffinity column clean-up techniques in food analysis: A review. *Journal of Chromatography B*, *878*, 115–132.

Shaikh, H., Memon, N., Khan, H., Bhanger, M. I., & Nizamani, S. M. (2012). Preparation and characterization of molecularly imprinted polymer for di(2-ethylhexyl) phthalate: Application to sample clean-up prior to gas chromatographic determination. *Journal of Chromatography A*, *1247*, 125–133.

Shi, X., Wu, A., Qu, G., Li, R., & Zhang, D. (2007). Development and characterisation of molecularly imprinted polymers based on methacrylic acid for selective recognition of drugs. *Biomaterials*, *28*, 3741–3749.

Spivak, D. A. (2005). Optimization, evaluation, and characterization of molecularly imprinted polymers. *Advanced Drug Delivery Reviews*, *57*, 1779–1794.

Tong, Y., Guan, H., Wang, S., Xu, J., & He, C. (2011). Syntheses of chitin-based imprinting polymers and their binding properties for cholesterol. *Carbohydrate Research*, *346*, 495–500.

Turiel, E., & Martín-Esteban, A. (2010). Molecularly imprinted polymers for sample preparation: A review. *Analytica Chimica Acta*, *668*, 87–99.

Volkmann, A., & Brüggemann, O. (2006). Catalysis of an ester hydrolysis applying molecularly imprinted polymer shells based on an immobilised chiral template. *Reactive and Functional Polymers*, *66*, 1725–1733.

Wan Ngah, W. S., Teong, L. C., & Hanafiah, M. A. K. M. (2011). Adsorption of dyes and heavy metal ions by chitosan composites: A review. *Carbohydrate Polymers*, *83*, 1446–1456.

Wang, Y., Xu, Y., Zhang, X., Wang, E., & Dong, Y. (2011a). Development and characterization of a chitosan-supported immunoaffinity chromatography column for the selective extraction of methandrostenolone from food and feed samples. *International Journal of Biological Macromolecules*, *49*, 428–432.

Wang, Y., Xu, Y., Zhang, X., Wang, E., & Dong, Y. (2011b). Preparation of an immunoaffinity column and its application in sample cleanup for methandrostenolone residues detection. *Journal of Chromatography B*, *879*, 2149–2154.

Wu, L., Wu, W., Li, Q., Zhang, Q., Wang, Y., Lu, W., et al. (2010). The preparation of functionalized crosslinked macroporous chitosan microspheres and their adsorption properties for bilirubin. *Macromolecular Symposia*, *297*, 179–187.

Yu, Q., Deng, S., & Yu, G. (2008). Selective removal of perfluorooctane sulfonate from aqueous solution using chitosan-based molecularly imprinted polymer adsorbents. *Water Research*, *42*, 3089–3097.