Determination of Chlorpyrifos in Rice Based on Magnetic Molecularly Imprinted Polymers Coupled with High-Performance Liquid Chromatography

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Abstract The magnetic molecularly imprinted polymers (MMIPs) were prepared and used for the selective separation of chlorpyrifos from rice samples. The MMIPs were synthesized by surface-imprinted polymerization, using functionalized Fe_3O_4 particles as magnetic cores, chlorpyrifos as template, methacrylic acid as functional monomer, and trimethylolpropane trimethacrylate as cross-linker. Magnetic particles were characterized by scanning electron microscopy, Fourier-transform infrared spectrometry, physical property measurement system, and thermogravimetric analyzer. Adsorption studies were carried out to investigate the specific binding capacity, kinetics, and recognition specificity. The kinetic property of MMIPs was well fitted to the pseudo-second-order equation. The selective recognition experiment demonstrated that MMIPs had high selectivity towards chlorpyrifos over reference compound. Chlorpyrifos extracted from the rice samples was purified with MMIPs and detected by high-performance liquid chromatography. Under the optimal conditions, the detection limit of chlorpyrifos was 0.0072 $\mu g\ g^{-1}$. The relative standard deviations of intra- and interday ranging from 2.4 to 4.6 % and from 3.5 % to 7.3 % were obtained, respectively. The recoveries of chlorpyrifos at three different fortified concentrations (0.025, 2.5, and 5 $\mu g\ g^{-1}$) were in the range of 81.2–92.1 %.

Introduction

Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] (CPF) is an effective broad spectrum organophosphorous agricultural insecticide displaying activity against a broad range of insects and household pests (Cho et al. 2002). Agricultural, industrial, and residential uses of CPF resulted in its intentional and accidental introduction into terrestrial and aquatic ecosystem, and brought a potential health hazard to mammals. As one of the most common pesticides in food, CPF is associated with chromosomal damage and bladder cancer (Lee et al. 2004; McKellar et al. 1976; Vidal et al. 1998), many works about detection of CPF were carried out (Cherta et al. 2012; Deme et al. 2012; Omeroglu et al. 2013).

Owing to the complexity of the matrix, the analysis of food often requires efficient sample pretreatment techniques. Typically, CPF was extracted from food under shaking, and then the extracts obtained were cleaned up by liquid–liquid extraction (LLE) or solid phase extraction (SPE). However, the LLE and classic SPE may retain not only the target analytes but also other chemicals, which could interfere with the detection of the compounds of interest. The QuEChERS (quick, easy, cheap, rugged, and safe) method also had used to determine the organophosphorous (Marchis et al. 2012). It was a sample preparation technique for multiresidue analysis of pesticides in food (Anastassiades et al. 2003). The advantages of the method included high sample throughput and low amounts of

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solvent, glassware, and bench space required (Payá et al. 2007). But sometimes, the selectivity of this method was not satisfying, and the impurities could not be cleaned completely.

Molecularly imprinted polymers (MIPs) are stable synthetic cross-linked polymers possessing selective molecular cognition sites, which have highly specific recognition ability for target molecules or similar compounds (Feás et al. 2009; He et al. 2007, 2008; Li et al. 2008; Puoci et al. 2009; Tamayo et al. 2007; Zhang et al. 2009a). MIPs are produced by growing a polymer around the template molecules (Ai et al. 2009; Lisa and Evangelyn 2005; Schirmer and Meisel 2009; Xie et al. 2003). After polymerization, templates are removed from the polymers, leaving specific cavities that can selectively rebind template molecules in size, shape and functional groups (Alexander et al. 2006). Their most significant advantages like high stability, long life, and easy preparation have led to the development of various MIPs applications in chromatographic separation (Ou et al. 2007), chemical sensors (Kandimalla and Ju 2004; Kirsch et al. 2001; Malitesta et al. 2012), chiral separation (Davies et al. 2004), SPE (Mohamed et al. 2007; Urraca et al. 2007), and catalysis (Pasetto et al. 2005; Wulff 2002). During the past few years, the increasing interest and attention toward MIPs have been attributed to its predetermination, recognition, and practicability (Katz and Davis 1999).

The MIP-SPE was applied to determine organophosphate residues in strawberries (Xu et al. 2010), vegetables (Baldim et al. 2012), and water samples (Liu et al. 2010). In the studies above, disulfoton, dichlorvos, and chlorpyrifos were used as templates, respectively. Good recovery and low quantification limits were obtained in these methods. However, the drawbacks like a tedious column packing procedure, high backpressure, and a low flow rate owing to cartridge mode (Ji et al. 2009) have also restricted its widespread application.

Combining magnetism with MIPs can build a controllable rebinding process and enable magnetic separation to replace the centrifugation and filtration step in a more convenient and economical way (Zhang et al. 2009b), and the new method is easier, quicker, simpler, and more effective to perform than MIP-SPE with cartridge mode. Recently, magnetic molecularly imprinted polymers (MMIPs) have been prepared and used for the extraction and determination of pyrethroids pesticide (Guan et al. 2013), triazines pesticide (Zhang et al. 2009b), bisphenol A (Ji et al. 2009), and β-agonists (Hu et al. 2011). In our previous studies, MMIPs were used to analyze tetracyclines (Chen et al. 2009a), sulfonamides (Chen et al. 2009b), and fluoroquinolones (Chen et al. 2010) in different matrix. The results proved that target analytes can be selectively recognized. However, there were still the problems of incomplete template removal and slow binding kinetics due to limitations of synthesis methods of MMIPs.

The aim of this study is to develop a new method to improve the analysis of CPF in rice, which is the main food in China. In the work, MMIPs were synthesized by surface-imprinted polymerization using CPF as template molecules, methacrylic acid (MAA) as functional monomer, and trimethylolpropane trimethacrylate (TRIM) as cross-linker. Surface MIPs show many advantages, including high selectivity, more accessible sites, fast mass transfer, and binding kinetics, due to the imprinted sites mostly situated on the surface of MMIPs. The characteristics of the MMIPs and binding experiment were investigated. The polymers were used as sorbents for the separation of CPF in the extract of rice sample, followed by high-performance liquid chromatography (HPLC) analysis. The CPF was selectively isolated, and the matrix interferences were eliminated in a short time using MMIPs, which simplified the sample pretreatment procedure.

Material and Methods

Materials

Chromatographic grade methanol was obtained from Fisher (Pittsburgh, PA, USA). CPF, MAA, iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 3-methacryloxypropyltrimethoxysilane (MPS), potassium persulfate ($K_2S_2O_8$), polyethylene glycol (PEG 6000), and TRIM were purchased from Aladdin (Shanghai, China). Methanol, ethanol, and ethyl acetate were obtained from Kermel (Tianjin, China). Ammonia solution (25 %) and acetic acid were purchased from Guangfu (Tianjin, China). Tetraethyl orthosilicate (TEOS) was obtained from Sinopharm (Shanghai, China). The purity of analytical standard CPF was 99 %. All other chemicals employed in the study were of analytical grade. High-purity water was obtained from a Milli-Q water system (Millipore, Billerica, MA, USA).

The standard stock solution of CPF was prepared by dissolving CPF in methanol, and the concentration was 1 mg mL^{-1}. It was stored in a refrigerator at 4 °C. Work standard solutions with concentrations from 0.1 to 200.00 μg mL^{-1} were daily prepared by diluting the stock solution.

Rice samples were randomly obtained from the local markets in Harbin (China). The samples were shattered and stored at room temperature. Sample processing procedure should result in analytical portions that are representative of the analytical sample (Omeroglu et al. 2013). In this study, the shattered rice samples were mixed and reduced by coning and quartering to get a homogeneous mass in sample processing procedure (Wagner et al. 2001). The principle of

this method was that the sample was mixed and subsequently arranged into a flattened pile, subsequently divided into four "equal" quadrants, and repeated on two opposing quadrants until the required reduced sample mass is reached. As rice was easy to shatter and mix uniformly, 2.0 g rice was representative and used for the following sample preparation.

One sample was checked to be free of CPF by National standard method used in China (Ministry of Health of the People's Republic of China 2008), and it was used as blank sample for calibration and validation purposes. The spiked rice samples were prepared by adding CPF standard solution into rice samples. Then, the mixture was incubated uniformly and evaporated to dryness with nitrogen gas continuously.

Instrumentation

The feature of MMIPs was observed with a scanning electron microscopy (SEM; JEM-6700F, JEOL, Tokyo, Japan). Fourier-transform infrared spectrometry (FT-IR360, Nicolet, Madison, WI, USA) was used to determine the functional groups of products. Magnetic properties were measured by a physical property measurement system (PPMS; Quantum Design Instrument, San Diego, CA, USA) at 298 K. Themogravimetric analyzer (TGA; Pyris 1, Perkin Elmer, Waltham, MA, USA) was employed to determine the thermal stability of MMIPs.

Chromatographic analysis was performed on a LC-15C liquid chromatograph (Shimadzu, Kyoto, Japan). A Zorbax SB-C18 column (250×4.6 mm I.D., 5 μm) was used as an analytical column (Palo Alto, CA, USA). A KQ5200E ultrasonic apparatus (Kunshan Instrument, Kunshan, China) was used for assisting extraction of CPF from rice samples. A DZKW-C thermostatic bath (Shanghai, China) was used to keep a constant temperature during the experiment of synthetic reaction. A shaking table (Shengtang, Jintan, China) was used for adsorption process.

Preparation of Polymers

The first step was the preparation of Fe_3O_4 by co-precipitation method according to our previous work (Chen et al. 2009a). The Fe_3O_4 (0.5 g) obtained was dispersed in 100 mL of alcohol and 20 mL of water by ultrasound for 15 min, followed by the addition of 3.0 mL of ammonia solution and 2.0 mL of TEOS. The system was stirred for 24 h at 300 rpm, and Fe_3O_4 was encapsulated by SiO_2 ($Fe_3O_4@SiO_2$). The magnetic precipitates were isolated from the solvent by a permanent magnet and washed several times with water. Surface modification of $Fe_3O_4@SiO_2$ was

carried out by the reaction of $Fe_3O_4@SiO_2$ and 0.15 mL of MPS dissolved in 100 mL of acetic acid/water (1:9, v/v). The system was stirred for 5 h at 60 °C. The magnetic particles were separated from the solvent by a permanent magnet and washed several times with water.

The CPF (0.5 mmol) and MAA (2.0 mmol) was dissolved in 10 mL ethanol and stored for 24 h. Then the mixture was added into a three-necked flask containing 150 mL 5 % PEG solution, the modified $Fe_3O_4@SiO_2$ and 5.0 mmol TRIM. After being subjected to ultrasound for 10 min, the initiator $K_2S_2O_8$ (0.1 g) was added into the system. The reaction was allowed to proceed at 60 °C and stirred at 300 rpm for 24 h. After polymerization, the product was collected by an external magnetic field. Then, the template was eluted from polymers with the mixture of methanol/acetic acid (8:2, v/v) by Soxhlet extraction until the CPF could not be detected by HPLC. Finally, the MMIPs were dried at 60 °C in vacuum. The process of preparing MMIPs is shown in Fig. 1.

The magnetic nonimprinted polymers (MNIPs) were prepared and processed similarly as above, except that the template molecules were not added. MMIPs and MNIPs could be kept and used for a long time.

Binding Experiment

The recognition property of MMIPs for CPF was studied by the static equilibrium adsorption. Each binding experiment was carried out by adding 10.0 mg of MMIPs or MNIPs in a glass tube containing 10.0 mL of CPF standard solution and shaking for some time. Then, the precipitates were collected with a magnet, and the target analyte in residual solution was detected. The amount of CPF bounded on the polymers was obtained by subtracting the free concentration from initial concentration of CPF added to the mixture.

The selectivity of the MMIPs was investigated using reference compound acephate, which was also belonging to organophosphorous.
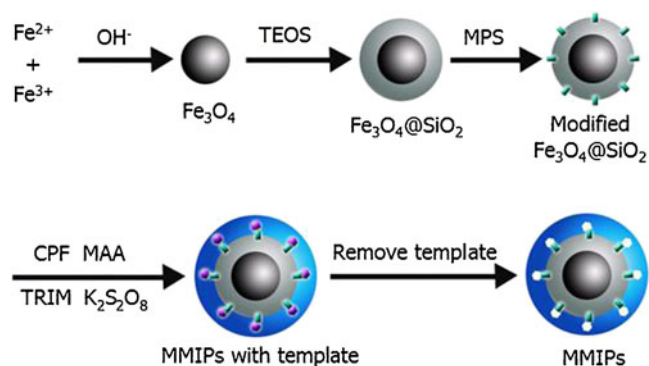

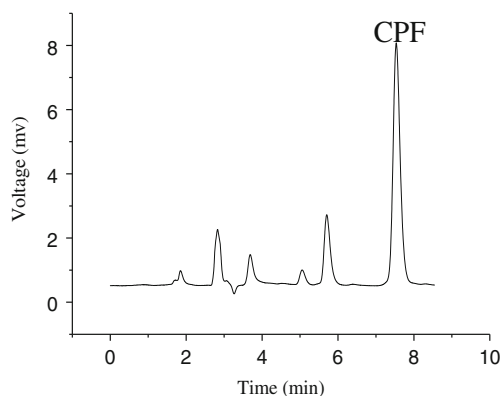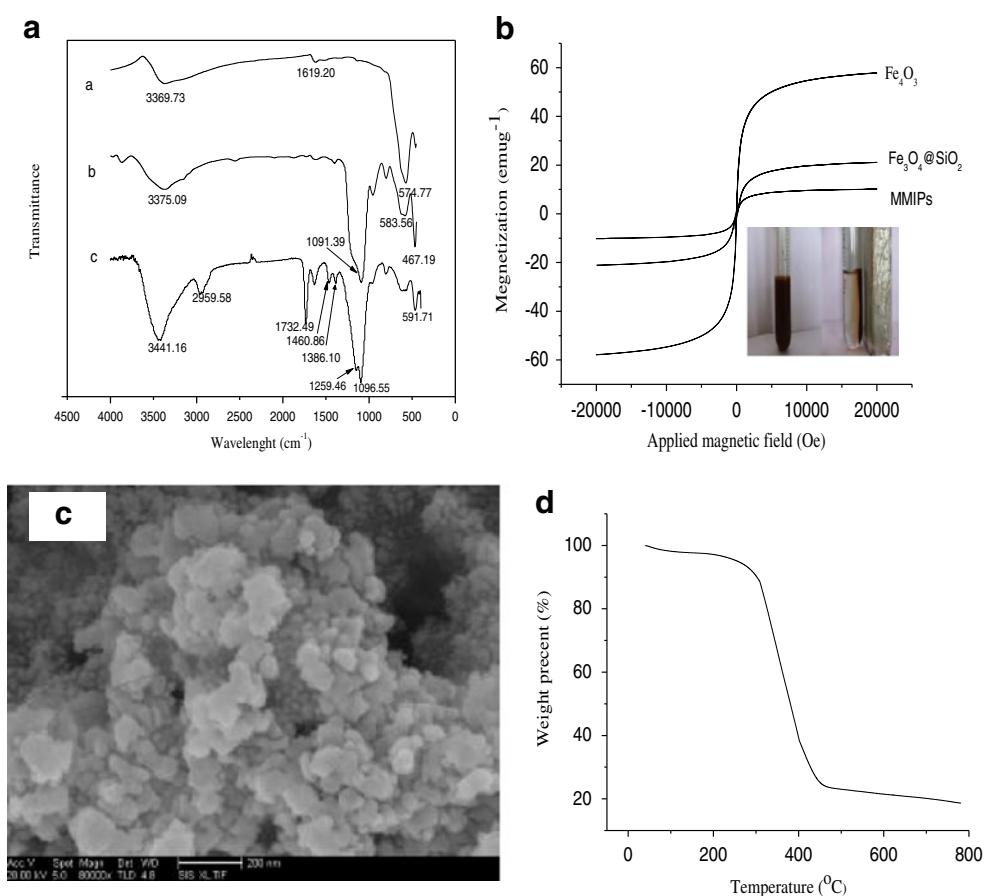**Fig. 1** Schematic representation of preparation for MMIPs

Fig. 2 HPLC chromatogram of CPF in rice sample

Sample Preparation

The rice sample (2.0 g) was extracted with 10 mL ethyl acetate under ultrasound assisted for 10 min and centrifuged for 5 min at 5,000 rpm. Supernatant was collected, dried under nitrogen gas, and redissolved with 3.0 mL of 10 % aqueous methanol. Finally, the mixture was subjected to the extraction procedure by MMIPs.

Extraction Procedure

The MMIPs (5 mg) were added to above sample extract and shaken for 10 min. After that, the polymers were separated with a magnet and washed with 3.0 mL 20 % aqueous methanol. The CPF was eluted from the MMIPs with 3×1.0 mL (1.0 mL every time and eluted three times) of methanol/acetic acid (97:3, v/v) under ultrasound, dried with nitrogen gas, and redissolved in 0.5 mL of 85 % aqueous methanol for HPLC analysis.

The extraction conditions including amount of MMIPs, shaking time, washing solvent, elution solvent, and elution times affecting the performance of the extraction were investigated. The optimization was carried out one variable at a time. Every sample was analyzed three times.

HPLC Analysis

The analysis of CPF was performed by HPLC. The HPLC mobile phase was the 85 % aqueous methanol. The flow rate was 1.0 mL min^{-1}. UV wavelength was set at 234 nm. The column temperature was at room temperature, and the injection volume was 20 μL.

Fig. 3 FTIR spectra of Fe_3O_4, $Fe_3O_4@SiO_2$, and MMIPs (**a**), hysteresis loops of Fe_3O_4, $Fe_3O_4@SiO_2$, and MMIPs (**b**), SEM micrographs of MMIPs (**c**), and thermogravimetric curve of MMIPs (**d**)

Fig. 4 Binding isotherms (**a**), Scatchard plot analysis of the binding of CPF onto the MMIPs (**b**), and MNIPs (**c**)
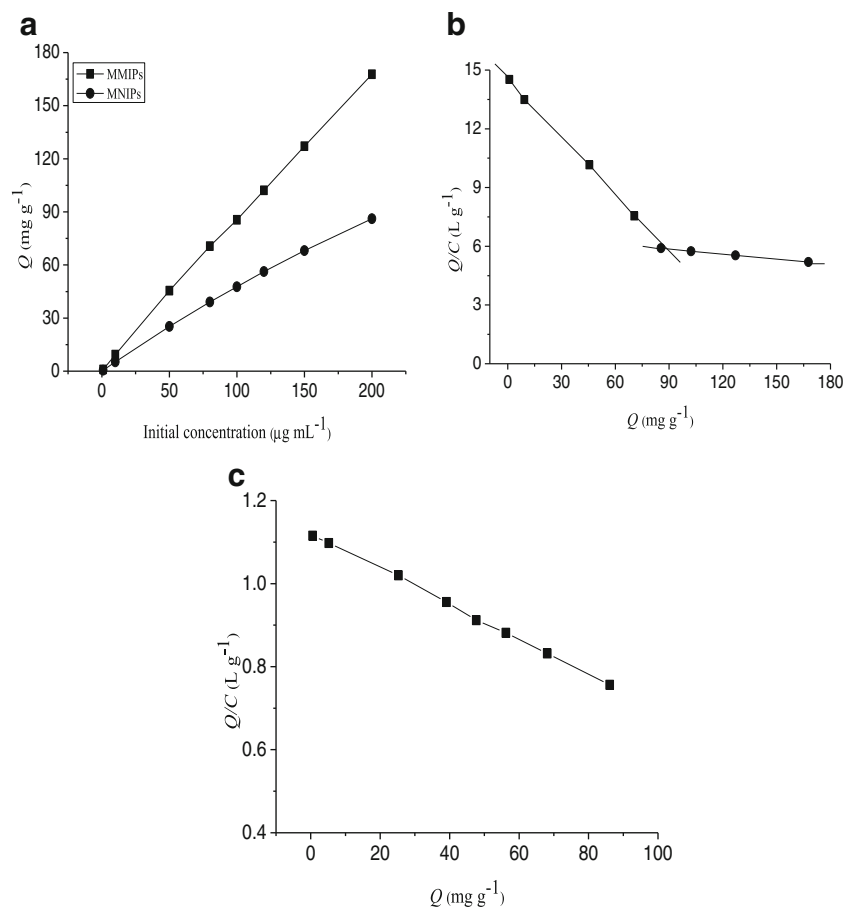
Typical chromatogram of CPF in rice samples is shown in Fig. 2.

Validation Study

Several basic analytical parameters in the method validation were evaluated. The chromatographic identification of the CPF was realized by its relative retention time. Calibration curve was obtained by plotting the peak area of the studied analyte versus the theoretical concentration of the analyte added to rice samples. The data were subjected to least squares regression analysis to provide information on linearity of the method. Limit of detection (LOD) and limit of quantification (LOQ) are considered as the minimum concentrations of analyte that can be confidently identified and quantified by the method, respectively. LOD and LOQ were estimated as the concentration of analyte producing a signal/noise ratio of 3 and 10. For the intraday assay precision and accuracy (Baldim et al. 2012), six replicates of control samples were analyzed for the same day at three different fortified concentrations of 0.025, 2.5, and 5 μg g^{-1}. For the interday assay precision and accuracy, control samples were analyzed once a day for six sequence

days at three different fortified concentrations of 0.025, 2.5, and 5 μg g^{-1}.

Results and Discussion

Characterizations of the MMIPs

FTIR spectra of Fe_3O_4, $Fe_3O_4@SiO_2$, and MMIPs were obtained. As can be seen from Fig. 3a, the absorption band of Fe−O bond at 574 cm^{-1} attributed to the Fe_3O_4 nanoparticles, the peaks at 583 and 591 cm^{-1} were also found in the spectra of $Fe_3O_4@SiO_2$ and MMIPs, respectively, which further proved that Fe_3O_4 was embedded in MMIPs. In comparison with the infrared data of pure Fe_3O_4, the characteristic peak of Si−O−Si group was at 1,091 cm^{-1} in $Fe_3O_4@SiO_2$. In MMIPs, the peak at 1,096 cm^{-1} was found, which indicated that SiO_2 was embedded in MMIPs. In the spectra of MMIPs, the adsorption band around 3,441 cm^{-1}, revealed the stretching vibration of O–H group in MAA, and the adsorption band around 2,959 cm^{-1}, unveiled the telescopic vibration of C–H group in CH_3. The band around 1,732 cm^{-1} was attributed to the C=O stretching

Fig. 5 Kinetics adsorption (**a**), pseudo-first-order kinetic for adsorption of MMIPs and MNIPs (**b**), and pseudo-second-order kinetic for adsorption of MMIPs and MNIPs (**c**)
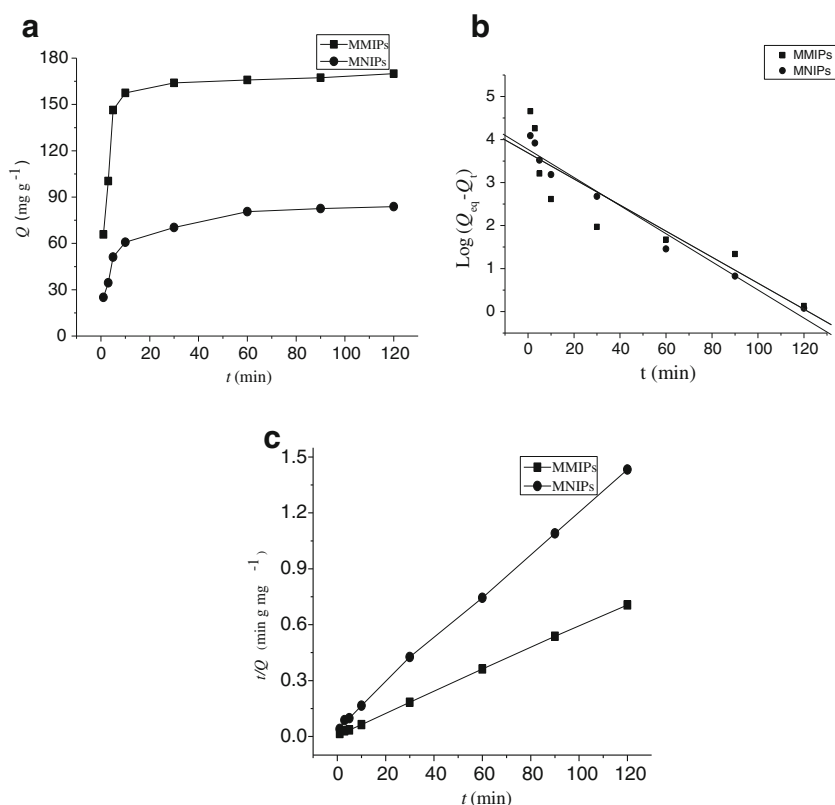
vibration. The peak at 1,259 cm^{-1} indicated the existence of C-O stretching vibration in MMIPs.

The magnetic properties of Fe$_3$O$_4$, Fe$_3$O$_4$@SiO$_2$, and MMIPs were studied by PPMS. Figure 3b showed the magnetic hysteresis loops of Fe$_3$O$_4$, Fe$_3$O$_4$@SiO$_2$, and MMIPs. There was a similar general shape of these three curves, being symmetrical about the origin. The figure illustrated that the materials could respond magnetically to an external magnetic field, and this response vanished upon the removal of the magnetic field. The saturation magnetization of Fe$_3$O$_4$, Fe$_3$O$_4$@SiO$_2$, and MMIPs were 57.86, 21.13, and 10.15 emu g^{-1}, respectively. As shown in the inserted photograph of Fig. 3b, without the external magnetic field, a brown homogeneous dispersion existed; with the external magnetic field, the brown particles were attracted to the wall of vial.

The SEM image of the MMIPs is shown in Fig. 3c. The amount of Fe$_3$O$_4$ encapsulated in MMIPs was measured

through TGA. As shown in Fig. 3d, when the temperature was slowly increased to 200 °C, the flat curve might be water loss of 5 %. From 200 to 600 °C, the polymer particles accounted for the significant mass loss 78 %, which might be due to decomposition of organic matter in MMIPs. The remaining mass was attributed to the thermal resistance of Fe$_3$O$_4$ particles, and their content in the MMIPs was 17 wt%.

Isothermal Absorption Experiment

The isothermal absorption experiment was carried out at the concentration of 1–200 μg mL^{-1}. The amount of CPF adsorbed by the polymers was calculated as:

$$Q = (C_i - C_f)V / m \tag{1}$$

where Q (mg g^{-1}) is the amount of CPF adsorbed on per gram of polymers, C_i (μg mL^{-1}) is the initial concentration

Table 1 Kinetic parameters for adsorption of CPF onto MMIPs and MNIPs

Adsorption material	Q_{eq} (mg g^{-1})	Pseudo-first-order			Pseudo-second-order		
		K_1 (min^{-1})	Q_e (mg g^{-1})	R^2	K_2 (g mg^{-1} min^{-1})	Q_e (mg g^{-1})	R^2
MMIPs	171.12	0.035	39.57	0.7343	0.0040	172.42	0.9998
MNIPs	84.85	0.0421	46.82	0.9805	0.0028	86.21	0.9992

Table 2 Selective properties of MMIPs and MNIPs

Adsorption material	Recognized molecule	δ (mL g^{-1})	α	β
MMIPs	CPF	4.895	6.282	6.179
	Acephate	0.779	–	–
MNIPs	CPF	0.698	1.017	–
	Acephate	0.686	–	–

of CPF, C_f (µg mL^{-1}) is the final concentration of CPF, V (mL) is the total volume of the adsorption mixture, and m (mg) is the mass of the polymers. As shown in Fig. 4a, CPF adsorbed on the polymers increased with increase in the initial concentration of CPF. Furthermore, the MMIPs showed significantly higher CPF loading than the MNIPs. In order to further study the binding properties of MMIPs, Scatchard analysis was performed. The equation of Scatchard analysis can be expressed as follows (Chen et al. 2009a):

$$\frac{Q}{C} = \frac{Q_{max} - Q}{K_d} \tag{2}$$

where K_d (µg mL^{-1}) is the dissociation constant of binding sites, Q_{max} (mg g^{-1}) is the maximum amount of apparent binding, C (µg mL^{-1}) is the equilibrium concentration of CPF in solution, Q is the same as Eq. (1). Scatchard graph was plotted based on Eq. (2). As can be seen from Fig. 4b, the relationship between Q/C and Q could be expressed using two straight lines, which illustrated that two kinds of different binding sites existed in MMIPs. For the two kinds of binding sites, the linear regression equation for the left part of the cure was $Q/C = -0.0983Q + 1.4534$ when the initial concentration was in the range of 1–100 µg mL^{-1}; K_d and Q_{max} were 10.17 mg L^{-1} and 14.79 mg g^{-1}, respectively. The linear regression equation for the right part of the cure was $Q/C = -0.0085Q + 0.6623$ when the initial concentration was in the range of 100–200 µg mL^{-1}, K_d and Q_{max} were 117.65 mg L^{-1} and 77.92 mg g^{-1}, respectively.

While for MNIPs, as shown in Fig. 4c, the relationship between Q/C and Q could be expressed using a liner equation. The equation was $Q/C = -0.0042Q + 0.1120$ when the initial concentration was in the range of 1–200 µg mL^{-1}. K_d and Q_{max} were 238.10 mg L^{-1} and 26.67 mg g^{-1}, respectively.

Kinetics Adsorption Experiment

Kinetics adsorption experiment shown in Fig. 5a was carried out. In this study, two of the most widely used kinetic models, i.e., pseudo-first-order equation and pseudo-second-order equation were used to research the adsorption kinetic behaviors of CPF onto MMIPs and MNIPs. The pseudo-first-order kinetic model is expressed by the following equation (Chen and Wang 2006):

$$\ln\left(Q_{eq} - Q_t\right) = \ln Q_e - K_1 t \tag{3}$$

where Q_{eq} and Q_t are the amount of CPF adsorbed (mg g^{-1}) at equilibrium and time t (min), respectively. K_1 is the equilibrium rate constant of pseudo-first-order sorption (min^{-1}). Q_e is the theoretic adsorption capacity of the pseudo-first-order kinetic model. The values of K_1 and Q_e can be calculated from the slope and intercept of the linear line plotted in versus t. The binding of CPF to the MMIPs and MNIPs were analyzed by pseudo-first-order kinetic model and described in Fig. 5b. The linear regression equations of the curves were as follows:

$$\ln\left(Q_{eq} - Q_t\right) = -0.0350t + 3.6780, \tag{4}$$

$$\ln\left(Q_{eq} - Q_t\right) = -0.0421t + 3.8463 \tag{5}$$

Equations (4) and (5) referred to MMIPs and MNIPs, respectively.

Another kinetic model is pseudo-second-order model, which is expressed as follow (Sheng et al. 2009):

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} + \frac{t}{Q_e} \tag{6}$$

where K_2 is the equilibrium rate constant of pseudo-second-order adsorption (g mg^{-1} min^{-1}). Q_e is the theoretic adsorption capacity of the pseudo-second-order kinetic model. The slope and intercept of the plot of t/Q_t versus t are used to calculate K_2 and Q_e. The binding of CPF to the MMIPs and MNIPs were analyzed by pseudo-second-order kinetic model and described in Fig. 5c. By

calculating, the equations were as follows:

$$\frac{t}{Q_t} = 0.0058t + 0.0085 \tag{7}$$

$$\frac{t}{Q_t} = 0.0116t + 0.0482 \tag{8}$$

Equations (7) and Eq. (8) referred to MMIPs and MNIPs, respectively.

Different kinetic parameters were shown in Table 1. The best-fit model was selected based on the linear regression correlation coefficient (R^2) values. All the experimental data of CPF adsorption onto MMIPs and MNIPs showed better compliance with pseudo-second-order kinetic model in terms of higher R^2 values and closer values between Q_e and Q_{eq}.

Selective Adsorption for CPF

The static distribution coefficient (δ), separation factor (α), and relative separation factor (β) were used to evaluate the selectivity of MMIPs (Tan et al. 2008).

$$\delta = C_p \big/ C_s \tag{9}$$

where C_p and C_s are the adsorbed and unadsorbed concentration, respectively. δ reflects the adsorption capacity. The bigger is δ, the stronger is the adsorption capacity.

$$\alpha = \delta_1 \big/ \delta_2 \tag{10}$$

where δ_1 and δ_2 are the static distribution coefficients of CPF and acephate. The parameter α embodies the selectivity.

$$\beta = a_1 \big/ a_2 \tag{11}$$

where α_1 and α_2 are the separation factors of MMIPs and MNIPs, respectively. The parameter β characterized the selective difference between MMIPs and MNIPs. The bigger is β, the stronger is the selectivity.

The data of the selectivity are listed in Table 2. According to the values of δ, a marked preference of CPF was observed for MMIPs while there was a little difference in rebinding acephate and CPF for MNIPs. The value of β was 6.179, confirming again that MMIPs had a greater selectivity than the MNIPs. This might have resulted from the imprinting effect. During the preparation of MMIPs, the template of CPF was incorporated into organic networks. After the removal of CPF, specific binding sites in a predetermined orientation were formed, whereas the MNIPs had no such imprinted cavities and specific binding sites.

Extraction Conditions

The extraction conditions were optimized by analyzing the rice samples. According to the National standard method used in China, ethyl acetate was chosen as extraction solvent for the extraction of CPF from rice samples (Ministry of Health of the People's Republic of China 2008). The extraction solvent volume and extraction time were optimized, and the results showed that CPF can be extracted from the rice by 10 mL ethyl acetate assisted with ultrasound for 10 min. After the extraction of CPF from rice, 10 % aqueous methanol was used for rebinding CPF. This is because the result of experiment indicated that complete rebinding of CPF could be obtained with this solvent.

The Amount of MMIPs

During the absorption procedure, the MMIPs were used to rebind the CPF. The optimal amount of sorbents required getting efficient recovery was investigated. The amounts of MMIPs ranging from 1 to 6 mg were applied. Figure 6a showed that the adsorption capacity of MMIPs increased with increase in dosage in <5 mg. Five milligrams MMIPs almost enabled to get complete recovery of CPF.

The recycling of the sorbent was then studied, and the results showed that the sorbent could be used at least five times with the satisfied recoveries in the range of 83.6–90.5 %.

Shaking Time

The experimental results indicated that the shaking time had an effect on the adsorption of target analyte. The adsorption process of CPF must have enough time to approach equilibrium to obtain satisfactory recovery. In this study, the shaking time ranging from 1 to 15 min were investigated. The result is shown in Fig. 6b, which showed that 10 min was sufficient to achieve satisfactory adsorption, and the increase in the shaking time beyond 10 min could not improve the recoveries.

Washing Solvent

Due to the rice samples contained complex matrix components, a washing step was required to remove the interfering compounds from sample matrixes without desorbing the target analyte. Different percentages of aqueous methanol were used as the washing solution. As shown in Fig. 6c, the recoveries of CPF decreased when methanol was more than 20 % in the washing solution. Finally, 3 mL 20 % aqueous methanol was used as washing solution.
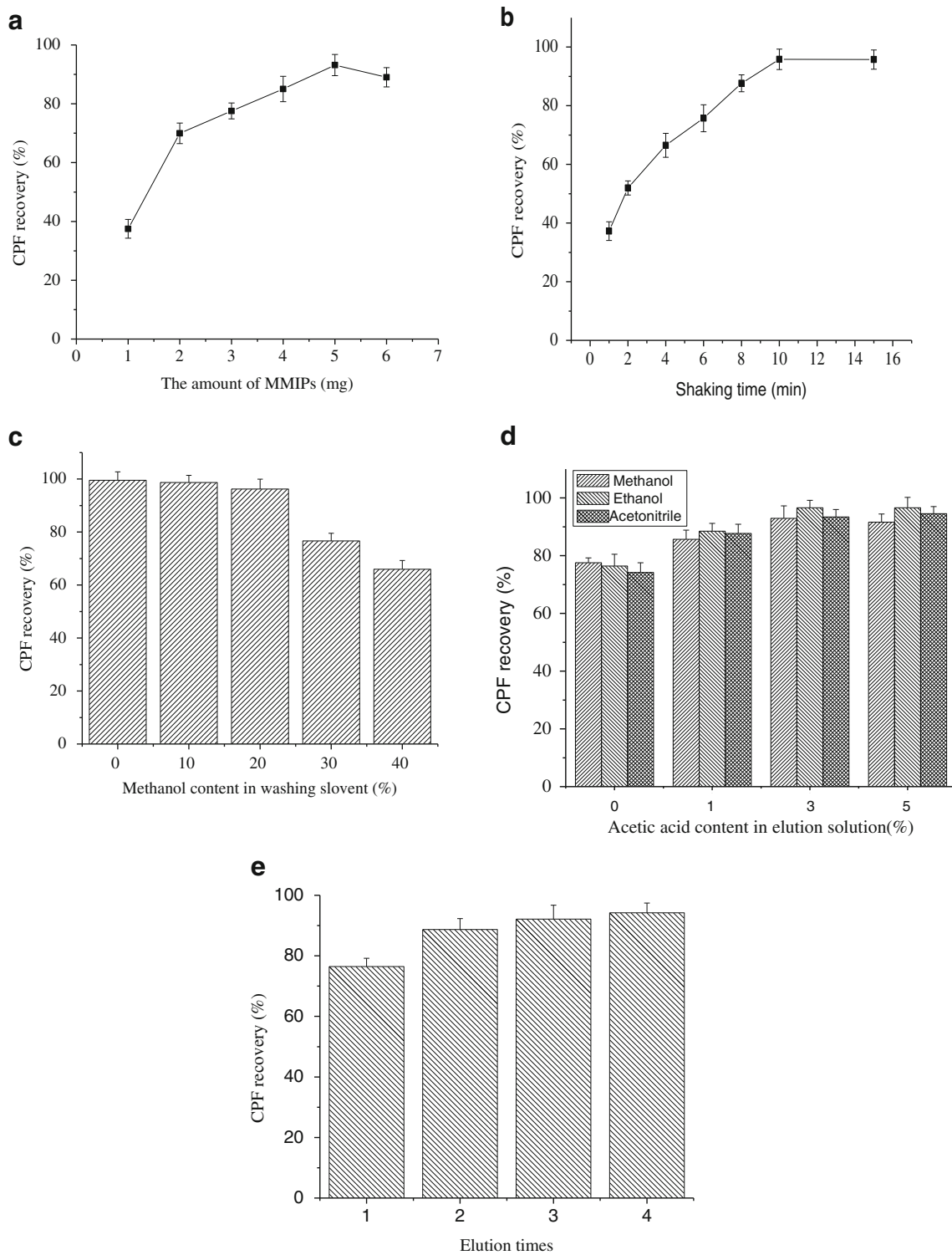
Fig. 6 Effects of MMIPs amount (**a**), shaking time (**b**), washing solvent (**c**), elution solvent (**d**), and elution times (**e**) on the recovery of CPF ($n=3$)

Elution Solvent

Three different organic solvents (ethanol, methanol, and acetonitrile), acetic acid/methanol (1:99, 3:97 and 5:95, v/v), acetic acid/ethanol (1:99, 3:97, and 5:95, v/v), acetic acid/acetonitrile (1:99, 3:97, and 5:95, v/v) were used as elution solvents to investigate their impacts on the process of extraction. As can be seen from Fig. 6d,

Table 3 Comparison of the proposed method with other methods used in the literatures

Sample	Sample preparation	Detection	LOD ($\mu g\ g^{-1}$)	Recovery (%)	Precision (RSD, %)	Reference
Rice plant	Shaking extraction with 30 mL acetone for 1 h and clean-up by Florisil cartridge	GC-ECD	0.006	88–105	1.6–5.4	Zhang et al. 2012
Rice	Ultrasonic extraction with 60 mL acetone for 15 min and clean-up by LLE with 60 mL dichloromethane	GC-FPD	0.005 (LO-Q)	90–102	<4.5	Zhang et al. 2010
Rice	Swelling the sample with 10 mL water and extracting with 10 mL acetonitrile, anhydration with 4 g anhydrous MgSO$_4$ and clean-up using dispersive SPE with 375 mg primary secondary amine	GC-MS/MS	6.7	97.0–101.2	2.6–4.6	Hou et al. 2013
Crop	Ultrasonic extraction with 200 mL acetone for 30 min followed by dichloromethane partitioning, and clean-up by gel permeation chromatography equipped with a column packed with 8.5 g Envirosep SX3 styrene-divinylbenzene copolymer	GC-ECD	0.01	87.7–88.2	–	Gelsomino et al. 1997
Vegetables	Matrix solid-phase dispersion of sample with 10 g of Florisil and 8 g of acid washed sand and clean-up by 0.5 g C$_8$	GC-MS	–	93	8	Viana et al. 1996
Vegetables	A 2.5 g sample, 10 g sodium sulfate anhydrous and 12 g graphitized carbon were packed in the pressurized liquid extraction vessel, and then extracted with 6.6 mL ethyl acetate at 100 °C for 10 min	GC-MS	3	71–82	5.6–21	Tanaka et al. 2007
Rice	Ultrasonic extraction with 10 mL ethyl acetate for 10 min and clean-up by 5 mg MMIPs for 10 min	HPLC	0.0072	81.2–92.1	2.4–4.6	The proposed method

different solvents showed different elution efficiency. The lower recovery of CPF was obtained using organic solvents without acetic acid. In the study, acetic acid/methanol (3:97, *v/v*) was the choice of elution solvent.

Elution Times

In order to obtain the best elution efficiency, elution times were investigated. One milliliter elution solvent was used every time for eluting CPF. The result, shown in Fig. 6e, indicated that CPF was almost eluted from the MMIPs when the polymers were eluted three times.

Method Validation

The linear range of CPF was 0.025–10 $\mu g\ g^{-1}$ and the correlation coefficient was 0.999. The LOD and LOQ of CPF obtained in this study were 0.0072 and 0.024 $\mu g\ g^{-1}$, respectively. The maximum residue limit (MRL) of CPF established by China (Ministry of Health of the People's Republic of China 2005) and Codex (Codex Alimentarious Commission 2010) were 0.1 and 0.05 mg kg^{-1}, respectively. Compared with them, the value of LOD proved that the method was sensitive.

The relative standard deviations (RSDs) of intra- and interday ranging from 2.4 to 4.6 % and 3.5 to 7.3 % were obtained, respectively. In all three fortified levels,

recovery of CPF was in the range of 81.2–92.1 %. SANCO provided that RSD must be below 20 % and the recovery should be in the range of 70–120 % (SANCO 2011). RSD and recovery in the work satisfied the requirements of SANCO, which illustrated that the method was feasible.

The analytical results obtained by the proposed method were compared with those obtained by the methods used in the literatures for analyzing CPF (Gelsomino et al. 1997; Hou et al. 2013; Tanaka et al. 2007; Viana et al. 1996; Zhang et al. 2010, 2012). The results presented in Table 3 indicated that the proposed method provided similar sensitivity, recovery, and precision. However, the sample preparation time and consumption of solvent were decreased in our method. Moreover, MMIPs prepared in this work have selective molecular cognition sites, which can adsorb CPF selectively. The magnetic polymers adsorbing analytes were easily collected by an external magnetic field without any additional centrifugation or filtration, which makes separation easier and faster. The dosage of sorbent in this study (5 mg per 2.0 g sample) was lower than that of other studies. It indicates that the MMIPs have high adsorption ability for CPF.

Application to Real Samples

To demonstrate the application of the method, three rice samples were detected. CPF residues at detectable levels were not found in these samples. The recovery of CPF

was studied by analyzing the spiked samples under the optimized conditions. The level of CPF added into the samples was 2.5 $\mu g\ g^{-1}$. The following steps were according to the procedure described in experiment. The recoveries of CPF obtained were 87.7 ± 4.2 %, which indicated good accuracy of the method.

Conclusion

In the study, MMIPs were synthesized using CPF as template. The obtained MMIPs were characterized by SEM, FTIR, PPMS, and TGA. The high adsorption capacity and good selectivity were obtained for CPF with MMIPs. The equilibrium data of MMIPs was well described by Scatchard analysis. The adsorption kinetics was fitted to the mechanism of the pseudo-second-order equation. The method based on coupling of MMIPs extraction with HPLC detection was successfully applied in analyzing CPF in rice samples. The MMIPs not only provided a convenient separation but also overcame problems of low selectivity and high operating cost.

Conflict of Interest   Guifu Ma and Ligang Chen declare that they have no conflicts of interest. This article does not contain any studies with human or animal subjects.

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