Analytical Methods

A novel molecularly imprinted polymer of the specific ionic liquid monomer for selective separation of synephrine from methanol–water media

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A B S T R A C T

A novel molecularly imprinted polymer (MIP) using the specific ionic liquid (i.e. 1-vinyl-3-carboxymethylimidazolium bromide, 1-vinyl-3-carboxethylimidazolium bromide, 1-vinyl-3-carboxybutoxylimidazolium bromide, or 1-vinyl-3-carboxypentylimidazolium bromide) as functional monomer was prepared via precipitation polymerization, which can be used to selectively separate synephrine (SYN) from methanol–water media. Ionic liquids are facile to be designed with varying the cation or anion, which enables the specific ionic liquid to be effectively designed to be a functional monomer for the preparation of MIP. The MIP showed a good selectivity and high adsorption capacity for SYN in methanol–water media. The adsorption process could be described by the pseudo-first-order model, which meant that the adsorption kinetics described a diffusion-controlled process. The equilibrium data fitted well to the Freundlich model, indicating multilayer adsorption. Finally, the MIP were successfully applied as sorbent to selectively enrich and separate SYN from the extracts of Aurantii Fructus Immaturus with a relatively high recovery (80–90%).

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1. Introduction

Aurantii Fructus Immaturus (Chinese name: Zhishi), is the immature dried fruits of Citrus aurantium L. (bitter oranges) or Citrus sinensis Osbeck (sweet oranges), which are widely used in herbal medicine, herbal weight loss products, or dietary supplements (Santana, Sharpless, & Nelson, 2008). Octopamine (OCT), synephrine (SYN) and tyramine (TYR) are main alkaloids in the herbs (Scheme 1). The adverse effects and safety of CITRUS are still hot debates (Rossato, Costa, Limberger, de Lourdes Bastos, & Remião, 2011; Stohs, Preuss, & Shara, 2011); therefore, selective enrichment or clean-up of SYN in the extracts of Citrus fruit is very significant. In our previous work (Fan et al., 2012), a molecularly imprinted polymer (MIP) was prepared via bulk polymerization using methacrylic acid as a functional monomer and used to selectively extract SYN from Aurantii Fructus Immaturus in acetonitrile media. In that work, we think, there are still at least two shortcomings to be resolved. Firstly, bulk polymerization often exhibits poor site accessibility to the target analyte, time-consuming in using a crash-and-sieve process and has low mass transfer efficiency in the sorption–desorption process. Secondly, the performance of MIP is poor in polar protic solvents, especially in aqueous media. Furthermore, the usual extraction solvent of SYN is not acetonitrile but a methanol/water mixture because of higher cost and toxicity of acetonitrile (Fan et al., 2012; Santana et al., 2008). Therefore, based on these considerations, a novel MIP should be prepared for selective separation of SYN in the extracts of Aurantii Fructus Immaturus.

MIPs have been called “antibody mimics” because they attempt to mimic the interactions of their natural counterparts. With their rapid development and wide use (Baggiani et al., 2012; Chen, Xu, & Li, 2011; Qu, Zhang, Gao, & Yang, 2012; Quesada-Molina, Claude, García-Campaña, del Olmo-Iruela, & Morin, 2012), MIPs still face a number of challenges, one of which is that MIP is incompatible with aqueous media, which restricts its applications in various aspects (Chen et al., 2011). Now, some approaches have been developed to deal with this challenge. (Ambrosini, Shinde, De Lorenzi, & SELLERGEN, 2012; Ma, Zhang, Guo, & Zhang, 2012; Pan, Zhang, Ma, Li, & Zhang, 2011; Shen, Xu, & Ye, 2012; Tarannum & Singh, 2011; Stohs, 2001; Stohs, Preuss, & Shara, 2011).
media based on the monomer for preparation of MIP which can be used in aqueous should be noted that ionic liquids have been used as the functional (Ambrosini et al., 2012; Bi, Tian, & Row, 2012; Guo, Deng, Fang, Gao, & Shuo, 2011; Luo et al., 2011). Ionic liquids have many fascinating properties, among which designability makes it easy to synthesize specific ionic liquid as a functional monomer to prepare MIP. Moreover, ionic liquids have also been used as solvent or porogen in the preparation of MIPs, which can accelerate the synthesis process and improve the selectivity and adsorption of MIP (Xu, Fang, & Wang, 2010; Xu, Zhou, Zhao, Qiao, & Yang, 2010). Therefore, the use of the specific ionic liquid as the unique functional monomer for preparation of selective MIPs is very meaningful.

In this work, several specific polymerizable Brønsted acidic imidazolium-type ionic liquids were prepared [Scheme 1], i.e., 1-vinyl-3-carboxymethylimidazolium bromide ([COOHevim]Br), 1-vinyl-3-carboxylethylimidazolium bromide ([COOHpvim]Br), 1-vinyl-3-carboxybutylimidazolium bromide ([COOHavim]Br), and 1-vinyl-3-carboxyethylimidazolium bromide ([COOHvim]Br), and firstly used as a functional monomer for synthesis of the novel SYN MIPs via precipitation polymerization. After optimization and due to the limited solubility of SYN in pure water, the binding and separation experiment was operated in methanol/water (4:1, v/v) media.

2. Materials and methods

2.1. Instruments and materials

The surface morphology of the MILPs was observed by LEO-1503 field emission scanning electron microscope (LEO Electron Microscopy Ltd., Wiesbaden, Germany), under high vacuum condition at an accelerating voltage of 10.0 kV. The stir-baked Aurantii Fructus Immaturus with bran was purchased from Zhangshu Tianqitang Traditional Chinese Medicine Yinpian Co., LTD, Zhangshu, China. SYN hydrochloride (> 98% purity) and OCT hydrochloride (> 98% purity) were purchased from Shangai Sciphar Biotechnology Co. Ltd., Xi’an, China, and Shaanxi Dongke Medicine Science and Technology Incorporate Company, Yanglin, China, respectively. TYR (> 98% purity), Ethylene glycol dimethacrylate (EGDMA) and Amberlite IR-120 (Na+ form) cation exchange resin were purchased from Shanghai Aladdin Reagent Company, Shanghai, China. Reverse-phase silica gel (CEC18) was supplied by Sepax Technologies, Inc., Suzhou, China. Macroporous resin (D101) was supplied by Anhui Wandong Resin Technology Co., Ltd., Bengbu, China. Silica gel was purchased from the Qingdao Haiyang Chemical Co., Ltd., Qingdao, China. 2- azoisobutyronitrile (AIBN) was purchased from the Damao Chemical Reagents Co., Tianjin, China. HPLC grade methanol was purchased from Tianjin Shield Company, Tianjin, China. These specific ionic liquids (i.e. [COOHevim]Br), [COO-Hpvim]Br, [COOHavim]Br, and [COOHvim]Br were synthesized according to our previous work (Tong, Fan, Xiao, & Tian, 2011). All the other chemicals were analytical grade reagents; all solutions were prepared from deionized water. SYN hydrochloride and OCT hydrochloride were converted to the free base by passing a solution of their hydrochloride salt in water into an ethanol/water prewashed column of Amberlite IR-120 (H+ form) and subsequent elution with a concentrated ammonium hydroxide/ethanol (65:35) solution. The eluate was concentrated to dryness to afford the free base as a white powder.

2.2. HPLC conditions

The HPLC separation was performed on an Agilent Technologies 1100 LC system consisting of a vacuum degasser (type G1311A), a quaternary pump (type G1311A), an autosampler (type G1313A), and a diode-array detector (type G1315A). Samples were analyzed on a Hypersil BDS-C18 column (4.6 × 200 mm, 5 μm particle size, Sepax Technologies, Inc) at 20 °C with a mobile phase composed of methanol and an aqueous solution containing 0.02% phosphoric acid, 0.02% triethylamine and 0.1% sodium dodecyl sulfate in a volume ratio of 60:40 at a flow rate of 1.0 mL min⁻¹. All analytes were detected at 224 nm and identified by retention time and comparison with the UV–visible spectrum of the standard.

2.3. Preparation of Aurantii Fructus Immaturus extracts

A 0.5 g of pulverized stir-baked Aurantii Fructus Immaturus with bran in 65% ethanol aqueous solution (6 mL) was sonicated for 16 min with an ultrasonic power of 420 W, after which the extract was centrifuged for 20 min at 5000 rpm. An aliquot (1 mL) of the supernatant was diluted to 10 mL with methanol/water (4:1, v/v).

2.4. MIP preparation using the specific ionic liquids as the functional monomer

MILPs were prepared according to the literature methods (Funaya & Haginaka, 2012; Luo et al. 2011). In a typical synthesis procedure, an amount of 0.2 mmol SYN (template) and 0.8 mmol specific ionic liquid (functional monomer, their structures were shown in Scheme 1) were dissolved in 25 mL of methanol/water by Anhui Wandong Resin Technology Co., Ltd., Bengbu, China. Silica gel was purchased from the Qingdao Haiyang Chemical Co., Ltd., Qingdao, China. 2- azoisobutyronitrile (AIBN) was purchased from the Damao Chemical Reagents Co., Tianjin, China. HPLC grade methanol was purchased from Tianjin Shield Company, Tianjin, China. These specific ionic liquids (i.e. [COOHevim]Br), [COO-Hpvim]Br, [COOHavim]Br, and [COOHvim]Br were synthesized according to our previous work (Tong, Fan, Xiao, & Tian, 2011). All the other chemicals were analytical grade reagents; all solutions were prepared from deionized water. SYN hydrochloride and OCT hydrochloride were converted to the free base by passing a solution of their hydrochloride salt in water into an ethanol/water prewashed column of Amberlite IR-120 (H+ form) and subsequent elution with a concentrated ammonium hydroxide/ethanol (65:35) solution. The eluate was concentrated to dryness to afford the free base as a white powder.

![Scheme 1. Chemical structures of SYN, OCT, TYR, specific ionic liquids (i.e. [COOHevim]Br), [COOHpvim]Br, [COOHavim]Br, and [COOHvim]Br) and EGDMA.](image-url)
(4:1, v/v) (porogenic solvent). The resulting mixture was sonicated for 10 min and then stood overnight. Then 4 mmol EGDMA (cross-linker) and 0.02 mmol AIBN (initiator) were added to the solution. The pre-polymerization solution was shaken and sonicated for 10 min. The mixture was sealed and deoxygenated with a stream of nitrogen and then the polymerization was carried out at 60 °C for 24 h, in a thermostat-controlled water bath, until the polymerization was completed. The resulting particles were washed by methanol/acidic acid solution (9:1, v/v) until no template molecule could be detected by HPLC. Subsequently, the products were washed with methanol to remove residual acidic acid solution and dried at 50 °C under vacuum and stored at the room temperature. NIPs were prepared with the same procedures as above described and the only difference was that no SYN was added into the reaction mixture.

2.5. Static binding selectivity studies

Selectivity studies, carried out under static binding conditions, were conducted for MIP, NIP or conventional sorbent with a constant sorbent amount (5 mg). The NIP was used to determine the extent of random, nonspecific binding resulting from interactions with the cross-linked, dispersed functional monomer. The sorbent was incubated in an aliquot of the analyte solution (5 mL). The resulting mixture was oscillated by a wrist action shake for 12 h and then centrifuged at 8000 rpm for 20 min. An aliquot of the supernatant was then analyzed by HPLC, and the concentration of free analyte determined. The same rebinding experiments were performed using solutions of the template analogue OCT and TYR, which allows to verify the selectivity of the process. The adsorption capacity (Q) of analyte on all sorbents were determined by the following equations: (Dai et al., 2012; Gu et al., 2010; Yang et al., 2012).

\[ Q_e = \frac{(C_0 - C_t)V}{m} \]  \hspace{1cm} (1)

\[ Q_l = \frac{(C_0 - C_t)V}{m} \]  \hspace{1cm} (2)

where \( Q_e \) and \( Q_l \) (\( \mu \text{mol g}^{-1} \)) are amounts of the analyte bound on the sorbent at equilibrium and time \( t \), respectively; \( C_0 \) (\( \mu \text{mol mL}^{-1} \)), \( C_t \) (\( \mu \text{mol mL}^{-1} \)) and \( C_t \) (\( \mu \text{mol mL}^{-1} \)) are the concentrations of analyte initially, at equilibrium, and at time \( t \), respectively; \( V \) (mL) is the volume of the sample solvent; and \( m \) (g) is the mass of the sorbent used.

2.6. Selective separation of SYN from the mixture solution containing SYN, OCT and TYR standard, and from the extract of Aurantii Fructus Immaturus in methanol–water media

To validate the selectivity of MIPs, OCT and TYR were chosen as the competitors of SYN in competitive recognition studies. SYN was selectively separated from the mixture solution containing SYN, OCT and TYR standard by a modified solid phase extraction (MSPE) process using the synthesized MIP as the sorbent according to the literature methods. (Puoci et al., 2012). The loading step was represented by the rebinding experiments in which 50 mg MIP was added to a 3 mL mixture solution containing 0.1 mmol L\(^{-1}\) SYN, OCT and TYR standard in methanol/water (4:1, v/v) media, shaken at room temperature for 4 h and then separated centrifugally 20 min (8000 rpm). Thereafter, rinsing and eluting steps were performed by an acetonitrile/water mixture (1:9, v/v) and methanol/acidic acid mixture (9:1, v/v). After each step, MIP was centrifuged for 20 min (8000 rpm) and the liquid phase analyzed by HPLC to detect the three analyte amounts. The same rebinding experiments were performed by using NIP as the sorbent, with the aim to verify the selectivity of the process.

The MSPE protocol of Aurantii Fructus Immaturus sample extracts was similar to that of the SYN standard solution.

3. Results and discussion

3.1. Optimization of polymerization conditions and the MIP binding solvent

In this work, the MIPs of SYN were prepared via precipitation polymerization using the specific ionic liquid as the functional monomer, EGDMA as the cross-linker, and AIBN as initiator. The main factors affecting the properties of MIPs (Table 1) were evaluated by variation of the types of the functional monomer, the amount of EGDMA, porogenic solvents and binding solvent.

It is well-known that the functional monomer affects the selectivity towards adsorption capacity (Q) of target compounds. Therefore, the influence of functional monomer types on the MSPE performance was considered. For this purpose, four specific ionic liquids (i.e. [COOHevim]Br, [COOHpvim]Br, [COOHavim]Br, or [COOHvim]Br) were used as the functional monomers for the preparation of the MIPs. In these cases, there are mainly two kinds of interactions between analyte and functional groups of the ionic liquids. One is p–p interactions between the phenyl group of the analyte and imidazolyl of the specific ionic liquids; another is the hydrogen bond interactions between the carboxylic acid group of the specific ionic liquids and the amine or the hydroxyl group of the analyte (Supplementary Scheme S1A). To improve the interaction between the specific ionic liquids and SYN and enhance the MIP selectivity, SYN hydrochloride was converted to the free base before it was used as the template. Hence, MIP1, MIP2, MIP3 and MIP4 were evaluated with respect to their alkyl chain length of the carboxylic acid group. The morphology of the evaluated by scanning electron microscope was shown in Supplementary Fig. S1. It could be seen that the surface of polymers was rough and irregular, which could create a large surface and space volume for SYN to embed into the cavity of the MIPs, resulting in its easier adsorption and elution. In Table 1, among the several specific ionic liquids (MIP1, MIP2, MIP3 and MIP4), [COOHavim]Br (MIP3) was the most efficient functional monomer for MIP due to its appropriate alkyl chain length of the carboxylic acid group, which improves the interactions between the functional monomer and the analyte. Therefore, the specific ionic liquid, [COOHavim]Br, was selected as the functional monomer for further optimization.

The polymerization conditions and the MIP binding solvents were optimized by binding experiments; the summarized results as to the adsorption capacity (Q) are listed in Table 1. Acetonitrile, methanol, acetonitrile/water (9:1, v/v), acetonitrile/water (4:1, v/v), methanol/water (4:1, v/v), and methanol/water (7:3, v/v) were selected as porogenic solvent for preparation of MIP3, MIP5, MIP6, MIP7, MIP8, and MIP9, respectively. The results showed that MIP8 using methanol/water (4:1, v/v) as the porogenic solvent had a higher adsorption capacity than the others. Moreover, lower concentrations of methanol aqueous were not investigated due to the limited solubility of SYN in pure water. Therefore, Methanol/water (4:1, v/v), was selected as the porogenic solvent for further optimization.

The effect of the MIP binding solvent (MIP8, MIP10, MIP11 and MIP12) on the adsorption capacity was obvious. When methanol/water (4:1, v/v) (MIP11) was used as the MIP binding solvent, a higher adsorption capacity than that of the others was observed, since the degrees of shrink or swelling were least when the binding solvent was the same as porogenic solvent (Xu, Fang, et al., 2010;
and $C_0$ are the amounts of SYN adsorbed on the sorbent at equilibrium and time $t$, respectively. $k_1$ is the pseudo-first-order rate constant. The pseudo-first-order model can be expressed as follows:

$$Q_t = Q_e - Q_e e^{-k_1 t}$$

where $Q_t$ is the amount of SYN adsorbed at time $t$, $Q_e$ is the adsorption capacity at equilibrium, and $t$ is the time.

To investigate the adsorption capacity, the amount of cross-linker (EGDMA) in the monomer mixture would influence the degree of polymerization, degree of cross-linking and the polymer’s properties, such as rigidity of the cavity structure, flexibility of the polymer and so on (Sergeyeva et al., 2001; Zhang et al., 2011). Hence, the adsorption capability of the MIP depending on the amount of cross-linker in the monomer mixture was investigated (MIP11, MIP13, MIP14, MIP15 and MIP16 in Table 1). These data indicate that the MIPs with too low and too high amount of EGDMA have lower adsorption capability in comparison with better performing MIP (MIP11, 4 mmol EGDMA). For the too low amount of cross-linker (MIP13 and MIP14), the rigidity of the resultant MIP was not enough. After template molecules were removed, it is difficult to maintain its spatial structure and cavity to match the binding sites. However, too high cross-linker amount (MIP15 and MIP16) will generate a higher percentage of non-specific binding sites. Therefore, methanol/water (4:1, v/v) was used as the MIP binding solvent for the subsequent experiments.

As widely recognized, the amount of cross-linker (EGDMA) in the monomer mixture would influence the degree of polymerization, degree of cross-linking and the polymer’s properties, such as rigidity of the cavity structure, flexibility of the polymer and so on (Sergeyeva et al., 2001; Zhang et al., 2011). Hence, the adsorption capability of the MIP depending on the amount of cross-linker in the monomer mixture was investigated (MIP11, MIP13, MIP14, MIP15 and MIP16 in Table 1). These data indicate that the MIPs with too low and too high amount of EGDMA have lower adsorption capability in comparison with better performing MIP (MIP11, 4 mmol EGDMA). For the too low amount of cross-linker (MIP13 and MIP14), the rigidity of the resultant MIP was not enough. After template molecules were removed, it is difficult to maintain its spatial structure and cavity to match the binding sites. Increasing cross-linker amount reduces flexibility and thus improves the contribution of specific binding to imprinted receptor sites. However, too high cross-linker amount (MIP15 and MIP16) will generate a higher percentage of non-specific binding sites, and will also make it difficult for the SYN to enter the binding cavities in MIP. Consequently, at cross-linker (EGDMA) amount of 4 mmol an optimum of MIP affinity (MIP11) was obtained, with a high adsorption capability of the MIP.

Finally, after investigation of the effects of the types of functional monomer, the amount of cross-linker, porogenic solvents and MIP binding solvents, the optimal polymerization conditions were achieved. Briefly, the MIP is prepared using 0.8 mmol [COOHavim]Br, 0.2 mmol SYN (template), 4 mmol EGDMA (cross-linker), 0.02 mmol AIBN (initiator) in 25 mL porogenic solvent under a nitrogen atmosphere for 24 h at 60 °C.

### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Functional monomer</th>
<th>Amount of EGDMA (mmol)</th>
<th>Porogenic solvent</th>
<th>MIP binding solvent$^b$</th>
<th>$Q$ (µmol/g)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP1</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Acetonitrile</td>
<td>Acetonitrile</td>
<td>23.6</td>
</tr>
<tr>
<td>MIP2</td>
<td>[COOHpvim]Br</td>
<td>4</td>
<td>Acetonitrile</td>
<td>Acetonitrile</td>
<td>29.5</td>
</tr>
<tr>
<td>MIP3</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Acetonitrile</td>
<td>Acetonitrile</td>
<td>38.7</td>
</tr>
<tr>
<td>MIP4</td>
<td>[COOHhvim]Br</td>
<td>4</td>
<td>Acetonitrile</td>
<td>Acetonitrile</td>
<td>32.2</td>
</tr>
<tr>
<td>MIP5</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Methanol/water (9:1, v/v)</td>
<td>Acetonitrile</td>
<td>35.62</td>
</tr>
<tr>
<td>MIP6</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Acetonitrile/water (4:1, v/v)</td>
<td>Acetonitrile</td>
<td>30.26</td>
</tr>
<tr>
<td>MIP7</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Acetonitrile/water (4:1, v/v)</td>
<td>Acetonitrile</td>
<td>22.25</td>
</tr>
<tr>
<td>MIP8</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Methanol/water (4:1, v/v)</td>
<td>Acetonitrile</td>
<td>48.7</td>
</tr>
<tr>
<td>MIP9</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Methanol/water (7:3, v/v)</td>
<td>Acetonitrile</td>
<td>39.56</td>
</tr>
<tr>
<td>MIP12</td>
<td>[COOHavim]Br</td>
<td>4</td>
<td>Methanol/water (4:1, v/v)</td>
<td>Methanol/water (7:3, v/v)</td>
<td>63.5</td>
</tr>
<tr>
<td>MIP13</td>
<td>[COOHavim]Br</td>
<td>2</td>
<td>Methanol/water (4:1, v/v)</td>
<td>Methanol/water (4:1, v/v)</td>
<td>60.49</td>
</tr>
<tr>
<td>MIP14</td>
<td>[COOHavim]Br</td>
<td>3</td>
<td>Methanol/water (4:1, v/v)</td>
<td>Methanol/water (4:1, v/v)</td>
<td>61.52</td>
</tr>
<tr>
<td>MIP15</td>
<td>[COOHavim]Br</td>
<td>5</td>
<td>Methanol/water (4:1, v/v)</td>
<td>Methanol/water (4:1, v/v)</td>
<td>66.83</td>
</tr>
</tbody>
</table>

$^a$ All MIPs are prepared by adding the same amount of SYN (0.2 mmol), functional monomer (0.8 mmol) and AIBN (0.02 mmol) in 25 mL porogenic solvent under a nitrogen atmosphere for 24 h at 60 °C.

$^b$ MIP binding solvent is the solvent in which the static binding experiment was performed.

$^c$ $Q$ is the adsorption capacity of MIP for SYN.

3.2. Adsorption kinetics

To investigate the equilibrium time, the effect of contact time was studied at 25 °C in methanol/water (4:1, v/v) media with an initial SYN concentration of 0.25 mmol L$^{-1}$. Based on the results presented in Fig. 1, it was observed that the adsorption capacity increased with increase in duration and reached the equilibrium value at 240 min. The SYN adsorption was observed to rapidly increase in the first 120 min, and then after 180 min the adsorption capacity had no significant change. This tendency could be explained by the decreasing external active sites and SYN concentration. When the external active sites reached saturation, SYN might overcome the transfer resistance and diffuse from the exterior to the interior of MIP (Yang et al., 2012).

To investigate the kinetic mechanism of the adsorption process, two kinetic models (Eqs. (3) and (4)) were employed to fit the experimental data (Dai et al., 2012; Yang et al., 2012; Yu et al., 2012). The pseudo-first-order model can be expressed as follows:

$$Q_t = Q_e - Q_e e^{-k_1 t}$$

Fig. 1. Adsorption dynamic curves of SYN on the MIP with the fitting to the pseudo-first-order model and the pseudo-second-order model at 25 °C in methanol/water (4:1, v/v) media.
rate constant. The pseudo-second-order model is presented as follows:

\[ Q_t = \frac{K_2 Q_e^2 t}{(1 + K_2 Q_e t)} \]  

(4)

where \( Q_e \) and \( Q_t \) are the amounts of SYN adsorbed on the sorbent at equilibrium and time \( t \), respectively. \( K_2 \) is the pseudo-second-order rate constant.

A comparison of the kinetic models for SYN adsorption onto MIP using the nonlinear regression of the two rate equation (Eqs. (3) and (4)) is also presented in Fig. 1, and the parameters determined from kinetic models are also shown in Fig. 1. As shown in Fig. 1, the pseudo-first-order model (\( R^2 = 0.9892 \)) was a little better than the pseudo-second-order model (\( R^2 = 0.9756 \)) in describing the adsorption process of SYN onto MIP according to the higher correlation coefficients (\( R^2 \)) values. Moreover, the calculated \( Q_e \) values \( (Q_{e,\text{cal}}) \) obtained from pseudo-first-order model were also closer to the experimental \( Q_e \) values \( (Q_{e,\text{exp}}) \) than those from pseudo-second-order model. This meant that the adsorption kinetics described a diffusion-controlled process (Yu et al., 2012).

### 3.3. Adsorption isotherms

To evaluate the adsorption capacity of the MIP and NIP for SYN and the adsorption isotherm experiments were performed at the different SYN concentrations at 25 °C. As shown in Fig. 2, the adsorption capacity of the MIP and NIP increased in the whole concentration range, while the MIP exhibited a higher adsorption capacity compared with the NIP. Then, to design and establish the adsorption system, two classical isotherm models were used to analyze the equilibrium data. The Langmuir model assumed that adsorption took place on a homogeneous surface with identical active sites and uniform energies. The Freundlich model assumed that adsorption occurred on a heterogeneous surface with the exponential distribution of active sites and energies (Yang et al., 2012). The Langmuir model is expressed as: \( (Ren\ et\ al.,\ 2012;\ Yang\ et\ al.,\ 2012)\).

\[ Q_e = \frac{K_L Q_{\max} C_e}{(1 + K_L C_e)} \]  

(5)

where \( C_e \) is the equilibrium concentration, \( Q_e \) is the amount of SYN adsorbed at equilibrium, \( Q_{\max} \) is the theoretical maximum monolayer capacity, and \( K_L \) is the Langmuir constant related to the affinity of the active sites. The Freundlich model is expressed as:

\[ Q_e = K_F C_e^{1/n} \]  

(6)

where \( C_e \) is the equilibrium concentration, \( Q_e \) is the amount of SYN adsorbed on the sorbent at equilibrium, \( n \) and \( K_F \) are Freundlich constants, which are related to the adsorption favorability and adsorption capacity, respectively.

A comparison of the isotherm models for SYN adsorption onto MIP and NIP using nonlinear regression (Eqs. (5) and (6)) are given in Fig. 2. As shown in Fig. 2, the Langmuir and Freundlich models were evaluated to describe the adsorption behavior of SYN on the MIP, the correlation coefficients of 0.8040 and 0.9793 were obtained, respectively. While the Langmuir and Freundlich models were evaluated to describe the adsorption behavior of SYN on the NIP, the correlation coefficients of 0.9838 and 0.9521 were obtained, respectively. Therefore, for MIP the data better fit the Freundlich model than the Langmuir model in terms of the higher correlation coefficients (\( R^2 \)) values, probably exhibited a logarithmic distribution of the binding sites in multilayers in the MIP (Vieira, Zampieri, de Siqueira, Martins, & Figueiredo, 2012; Yang et al., 2012), and the results demonstrated that the active site distribution of the MIPs was heterogeneous. For NIP, the Langmuir model yielded a better fit to the equilibrium data than the Freundlich model in terms of the higher correlation coefficients (\( R^2 \)) values, which demonstrated that the active sites distribution of the NIPs was homogeneous (Dai et al., 2012; Ren et al., 2012).

### 3.4. Recognition selectivity of MIPs and NIPs

To further evaluate the competitive recognition coefficients of the MIPs, OCT and TYR as reference compounds were chosen for

![Fig. 2. Adsorption isothermic curves of SYN binding onto MIP and NIP with the fitting to the Langmuir model and the Freundlich model at 25 °C in methanol/water (4:1, v/v) media.](image-url)
measurement in a mixed solution. Both of them possess similar structures to that of SYN and often coexist in the plant extract.

The selectivity of the sorbents was evaluated by three parameters as the static distribution coefficient ($K_d$), separation factor ($\alpha$) and relative separation factor ($\beta$) herein (Fan et al., 2012; Gu et al., 2010).

$$K_d = \frac{C_p}{C_s}$$

(7)

where $C_p$ is the bound concentration and $C_s$ is the unbound concentration;

$$\alpha = \frac{K_{d1}}{K_{d2}}$$

(8)

where $K_{d1}$ and $K_{d2}$ are the static distribution coefficients of the target molecule (SYN) and competitive one;

$$\beta = \frac{x_{\text{MIP}}}{x_{\text{NIP}}}$$

(9)

where $x_{\text{MIP}}$ and $x_{\text{NIP}}$ are the separation factors of MIPs and NIPs, respectively. $K_d$ reflects the adsorption capacity. The bigger $K_d$ is, the stronger the adsorption capacity will be. The parameter $\alpha$ embodies the selectivity between the target molecule (SYN) and the competitive one. The greater the $\alpha$ value is, the better the competitiveness of the adsorption capacity of the target molecule (SYN) will be. The selective difference between MIPs and NIPs is characterized by $\beta$. The bigger $\beta$ is, the stronger the selectivity resulted from the molecular imprinting will be. The selectivity of MIPs is shown in Table 2. The $\alpha$ value of the MIPs of SYN to OCT is 2.23, and to TYR is 1.99, however, the $\alpha$ value of the NIPs of SYN to OCT is 1.05, and to TYR is 1.02. Hence, we can know that the competitive adsorption capacity of SYN on the MIP is nearly two times larger than the other two competitive compounds and there is not much difference among the three compounds on NIPs. This selectivity of MIPs was nearly two times ($\beta = 2.12$ and 1.95) as high as NIPs, which suggested that the imprinting process significantly improved binding selectivity to the imprinted template (SYN). The extraction of SYN by MIPs is based on the specific recognition capacity even when structurally similar compounds existed. Selectivity coefficient of the MIPs is larger than that of NIPs and the adsorption of the MIPs of SYN is specific. In comparison with conventional sorbent, the selectivities capability of silica gel, reverse-phase silica gel (C18) and macroporous resin (D101) were also investigated (Table 2). The $\alpha$ value of the conventional sorbent of SYN to OCT and to TYR are close to 1, which indicated that the selectivity of the conventional sorbents was low. Therefore, these demonstrate the theoretical feasibility of the MIPs of SYN as sorbent for separating with a relatively high selectivity.

### Table 2

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>$K_d$(mL·g$^{-1}$)</th>
<th>$\alpha$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SYN $K_{d1}$</td>
<td>OCT $K_{d2}$</td>
<td>TYR $K_{d3}$</td>
</tr>
<tr>
<td>MIP</td>
<td>392.73</td>
<td>176.06</td>
<td>197.84</td>
</tr>
<tr>
<td>NIP</td>
<td>164.64</td>
<td>156.16</td>
<td>161.09</td>
</tr>
<tr>
<td>Silica gel</td>
<td>117.81</td>
<td>115.42</td>
<td>116.55</td>
</tr>
<tr>
<td>Reverse-phase silica gel (C18)</td>
<td>119.96</td>
<td>118.26</td>
<td>118.77</td>
</tr>
<tr>
<td>Macroporous resin (D101)</td>
<td>123.40</td>
<td>119.16</td>
<td>120.14</td>
</tr>
</tbody>
</table>

$x_1 = K_{d1}/K_{d2}; \ x_2 = K_{d3}/K_{d2}; \ \beta_1 = x_{\text{MIP}}/x_{\text{NIP}}; \ \beta_2 = x_{\text{MIP}}/x_{\text{NIP}}$. All parameters are measured in methanol/water (4:1, v/v) media.

### 3.5. Selective separation of SYN from the mixture solution containing SYN, OCT and TYR standard and from the extract of Aurantii Fructus Immaturus in methanol–water media

After the evaluation of MIP efficiency, SYN was selectively separated from the mixture solution containing SYN, OCT and TYR standard, and the extract of Aurantii Fructus Immaturus in methanol–water media by a MSPE process described as experimental sections. To validate the selectivity of MIPs, OCT and TYR were chosen as the competitors of SYN in competitive recognition studies. The procedure diagram was shown in Supplementary Scheme S1B. The performances of MIP and NIP were compared.

The chromatograms of the analytes with MSPE by NIPs and MIPs were shown in Fig. 3A and B, respectively. For NIPs, Fig. 3A(b) showed that SYN, as well as OCT and TYR, was saturated on the NIPs, and the ratio of SYN to OCT and TYR in the solution after extraction by NIP is similar to that in the untreated mixture before MSPE. Fig. 3A(c) shows that a large amount of SYN, OCT and TYR was rinsed after being rinsed with an acetonitrile/water mixture (1:9, v/v), the ratio of SYN to OCT and TYR in the rinsing solution is also similar to that in the untreated mixture before MSPE. Finally, as shown in Fig. 3A(d), nearly no SYN was recovered after being eluted by methanol/acetic acid (9:1, v/v). The results of Fig. 3A for NIPs indicated that SYN, OCT and TYR had similar and weak affinity on NIPs, and NIPs had no significant selectivity and enrichment of SYN.

For MIPs, Fig. 3B(b) shows that SYN was bound on the MIP when the standard mixture was extracted by the MIP, while OCT and TYR have been saturated on the MIPs. Fig. 3B(c) shows that there was a large amount of OCT and TYR but nearly no SYN in the rinsing solution after being rinsed with an acetonitrile/water mixture (1:9, v/v). This shows that OCT and TYR are easily rinsed from the MIPs, and SYN could be hardly rinsed by an acetonitrile/water mixture (1:9, v/v) from the MIPs. However, as shown in Fig. 3B(d), when using a stronger eluting solvent of methanol/acetic acid (9:1, v/v), most of SYN was eluted from the MIPs and 85.45% of SYN was recovered. Moreover, there were neither OCT nor TYR in eluting solution, which illustrated the purity of SYN in the mixture significantly increased after MSPE. From the Fig. 3B, we can also see that the SYN's polarity is in the middle of OCT and TYR, however SYN was selectively bound and retained on the MIPs after rinsing with an acetonitrile/water mixture (1:9, v/v), which showed that SYN had a stronger affinity on MIPs and higher imprinting efficiency than both other two competitive compounds on the MIPs due to the selective molecular recognition.

Finally, we employed MIPs in an MSPE format to examine the potential of this approach for the selective enrichment and isolation of SYN from the extracts of stir-baked Aurantii Fructus Immaturus with bran (Fig. 3C) in methanol/water (4:1, v/v) media, and the MSPE protocol of Aurantii Fructus Immaturus sample extracts was similar to that of the SYN standard solution. The results are shown in Fig. 3C(a), OCT, TYR were undetected or present in very low concentrations, and there were a lot of unknown components...
in the untreated extracts in addition to the active ingredients of SYN. However, the relative content of the unknown components in the eluting solution (d) was greatly decreased after MSPE; 82.72% of SYN were recovered from the extracts of the stir-baked Aurantii Fructus Immaturus with bran. Therefore, the MIPs have achieved the desired effect that the SYN was enriched and separated from the initial extracts.

Compared with our previous work in which a SYN MIP was prepared via bulk polymerization and used in acetonitrile media, this work has at least two advantages. Firstly, the method of precipitation polymerization using SYN as a template, [COOHavim]Br as the functional monomer, EGDMA as crosslinker and methanol/water (4:1, v/v) as porogenic solvents. After optimization and due to the limited solubility of SYN in pure water, the MIP has a relatively high adsorption capacity in methanol/water (4:1, v/v). Because the combination of a variety of cations and anions gives a tremendous amount of ionic liquids and makes custom synthesis feasible, this provides a large pool, from which ionic liquids can be selected as the functional monomer for the preparation of MIPs. The obtained polymer showed a good selectivity and high adsorption capacity for SYN. The adsorption process could be described by the pseudo-first-order model, which meant that the adsorption kinetics described a diffusion-controlled process. The equilibrium data fitted well to the Freundlich model, indicating multilayer adsorption. MIPs were then successfully applied as sorbent to purify SYN from the standard mixture of SYN, OCT and TYR, and from the extract of the stir-baked Aurantii Fructus Immaturus with bran. After MSPE, SYN was enriched and separated from the extracts with a relatively high recovery (80–90%), which proved that the method was valid for selective enrichment, purification or removal of SYN in the samples of Aurantii Fructus Immaturus. In our future work, more attention will be paid on the MIP using the specific ionic liquid as the functional monomer which can be used in pure aqueous media.

**4. Conclusions**

In this work, a novel molecularly imprinted polymer using the specific ionic liquid (i.e. [COOHevim]Br), [COOHpvim]Br, [COOH-avim]Br or [COOHHvim]Br) as a functional monomer was prepared for the selective separation of SYN from the extracts of Aurantii Fructus Immaturus in methanol–water media. The MIP is prepared via precipitation polymerization using SYN as a template, [COOH-avim] as the functional monomer, EGDMA as crosslinker and methanol/water (4:1, v/v) as porogenic solvents. After optimization and due to the limited solubility of SYN in pure water, the MIP has a relatively high adsorption capacity in methanol/water (4:1, v/v). Because the combination of a variety of cations and anions gives a tremendous amount of ionic liquids and makes custom synthesis feasible, this provides a large pool, from which ionic liquids can be selected as the functional monomer for the preparation of MIPs. The obtained polymer showed a good selectivity and high adsorption capacity for SYN. The adsorption process could be described by the pseudo-first-order model, which meant that the adsorption kinetics described a diffusion-controlled process. The equilibrium data fitted well to the Freundlich model, indicating multilayer adsorption. MIPs were then successfully applied as sorbent to purify SYN from the standard mixture of SYN, OCT and TYR, and from the extract of the stir-baked Aurantii Fructus Immaturus with bran. After MSPE, SYN was enriched and separated from the extracts with a relatively high recovery (80–90%), which proved that the method was valid for selective enrichment, purification or removal of SYN in the samples of Aurantii Fructus Immaturus. In our future work, more attention will be paid on the MIP using the specific ionic liquid as the functional monomer which can be used in pure aqueous media.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.06.040.

**References**


