

MATERIALS CHEMISTRY | RESEARCH ARTICLE

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Design, preparation, surface recognition properties, and characteristics of icariin molecularly imprinted polymers

Xiaohe Jia¹, Ni Tan^{1*}, Yi Cheng¹, Wen Zhang¹, Xueming Yan¹ and Xiaolong Li¹

Abstract: Icariin molecularly imprinted polymers (MIPs) were prepared by precipitation polymerization. Prior to the polymerization, computer simulation was performed to sketchily choose the suitable functional monomer and the corresponding polymerization solvent. The optimized synthesis parameters, including the functional monomer acrylamide, the mixture of methanol and acetonitrile (V:V = 3:1) as the polymerization solvent, and the reaction molar ratio (1:6:80) of template molecule, functional monomer and cross-linker, were respectively obtained by single factor analysis and orthogonal design methods. The results of the adsorption experiments showed that the resultant MIPs exhibited good adsorption and recognition abilities to icariin. Scatchard analysis illustrated that the homogeneous binding sites only for icariin molecules were formed in the prepared MIPs.

Subjects: Adsorption Science; Biochemistry; Separation Processing

Keywords: preparation; adsorption; recognition; icariin; molecularly imprinted polymers (MIPs)

1. Introduction

Molecular imprinting technique (MIT) is a new technology developed through simulating the interaction between antigen and antibody *in vivo* which forms selective sites in a polymer matrix with the memory of the template molecule due to shape recognition, hydrogen bonding and hydrophobic interactions (Cirillo, Curcio, Parisi, Puoci, & Iemma, 2011; Zeng, Wang, Liu, Kong, & Nie, 2012). In recent



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PUBLIC INTEREST STATEMENT

Epimedium is one of the Chinese traditional herbal medicines, whose major active ingredient icariin has a wide range of pharmacological activities including regulating cardiovascular, circulatory, genital, and bone marrow systems, stimulating neurite growth, possessing estrogenic function, enhancing bone healing, and treating osteoporosis. However, the extraction of trace amounts of icariin from epimedium with traditional methods is tedious and a bit inefficient. In this paper, a kind of advanced technology (molecular imprinting technique) was tried to extract the trace amounts of icariin in solution. The experimental results showed that the prepared MIPs had high affinity and selectivity to the natural drug icariin, and they were of great application potential to separate and purify icariin from the Chinese medicinal herb epimedium.

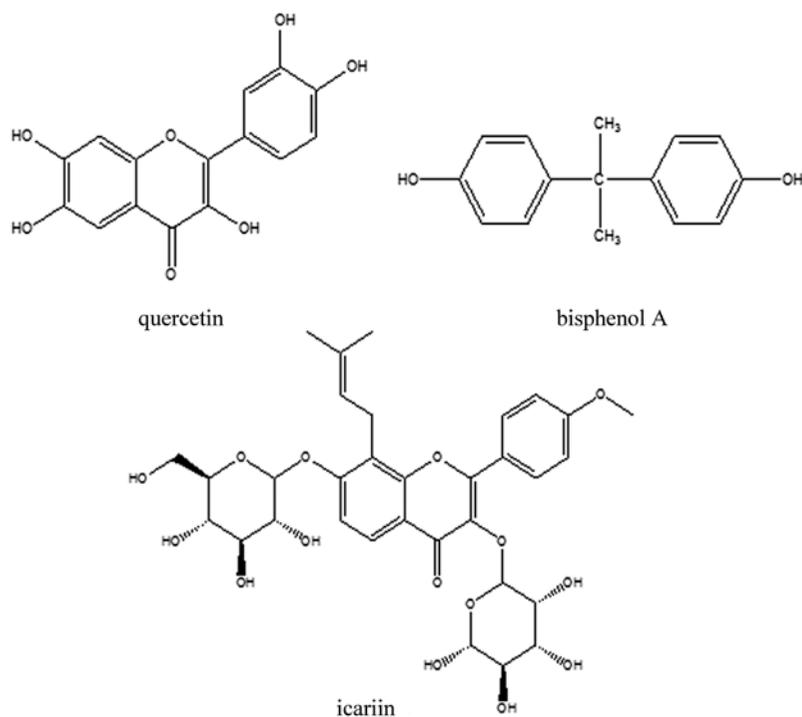
years, MIT has been widely used in many fields including the separation of isomeric compounds and enantiomers (Balamurugan, Gokulakrishnan, & Prakasam, 2012; Xu, Wan, Liang, & Cao, 2008), enzyme-like catalysis (Wulff, 2002), drug delivery systems (Azodi-Deilami, Abdouss, & Javanbakht, 2011; Ruela, Figueiredo, & Pereira, 2014), solid phase extraction (Özcan & Demirli, 2014; Park, Tian, & Row, 2014), supercritical fluid technology (Yoon & Byun, 2013), and the determination of food and environmental samples (Gholivand, Torkashvand, & Malekzadeh, 2012; Lv, Lin, Feng, Zhou, & Tan, 2007).

Molecularly imprinted polymers (MIPs) can be synthesized using MIT by co-polymerizing the functional monomers and the cross-linkers in the presence of template molecules in solution. After polymerization, the templates are removed from the polymeric network, then the three-dimensional cavities complementary to the template in shape, size, and position of the functional groups are obtained, thus the MIPs exhibit a high affinity and selectivity toward the template (Lee, Chuang, Tsai, & Chen, 2010; Lian & Wang, 2013).

The recognition mechanism of MIPs to template molecules is based on many biological processes such as enzyme-substrate, hormone-receptor, and antibody-antigen. However, compared to biological molecules, MIPs have some advantages like easy preparation, good physical and chemical stability, good storage, and reusability with different applications (Davoodi, Hassanzadeh-Khayyat, Asgharian Rezaei, & Mohajeri, 2014; Llorina Rañada, Akbulut, Abad, & Güven, 2014).

The roots and aerial parts of the epimedium are extensively used in Chinese traditional herbal medicine for their reinforcing properties of the liver and kidney, strengthening of tendons and bones, antagonizing rheumatism, anticancer, anti-aging, and enhancing immunity properties for thousands of years (Liang, Wei, Chen, Wang, & Huang, 2012). Its major active ingredient is icariin. The chemical structures of icariin and its structural analogs are shown in Figure 1. It has been reported that icariin has a variety of pharmacological activities including regulating cardiovascular, circulatory, genital and bone marrow systems, stimulating neurite growth, and possessing estrogenic activity. Studies also demonstrated that icariin could enhance bone healing, treat osteoporosis, and inhibit osteoclast differentiation (Hsieh, Sheu,

Figure 1. The chemical structures of icariin and its structural analogs (quercetin and bisphenol A).



Sun, Chen, & Liu, 2010, 2011; Huang, Zhu, & Lou, 2007; Liu, Sun, Tsai, Sheu, & Chen, 2010). However, the extraction of icariin from epimedium with traditional methods is tedious and a bit inefficient. So developing new extraction adsorbent materials with specific selectivity to icariin is very necessary.

Based on the above situation, icariin MIPs were synthesized by precipitation polymerization, employing AM as functional monomers, EDMA as cross-linker, and the mixture of methanol and acetonitrile as the reaction solvent. The synthesized MIPs and non-molecularly imprinted polymers (NIPs) were characterized by scanning electron microscope (SEM) and Fourier transform infrared spectra. Ultraviolet and visible spectrophotometer was used to evaluate the adsorption and selectivity properties of the MIPs. The experimental results showed that MIPs prepared in this work have good selectivity and high affinity to icariin.

2. Experimental

2.1. Materials and reagents

All the reagents used in these experiments were of analytical grade. Acetic acid, methanol, tetrahydrofuran (THF) and acetonitrile (ACN) were obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Acrylamide (AM) was bought from Shantou Xilong Chemical Factory (Shantou, China). Methacrylic acid (MAA) was obtained from Tianjin Fucheng Chemical Reagent Factory (Tianjin, China). 2,2'-azobisobutyronitrile (AIBN) was purchased from Shanghai Shanpu Chemical Factory (Shanghai, China). 4-Vinylpyridine (4-VP) was obtained from Alfa Aesar Chemical Technology Co., Ltd (Tianjin, China). Itaconic acid (IA) was purchased from Shanghai Titan Scientific Co., Ltd (Shanghai, China). Ethylene glycol dimethacrylate (EDMA) was obtained from Sun Chemical Technology Co., Ltd (Shanghai, China). Icariin ($\geq 98\%$), bisphenol A ($\geq 98\%$) and quercetin ($\geq 98\%$) were obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Acetone was obtained from Hengyang Kaixin Chemical Reagent Factory (Hengyang, China).

2.2. Apparatus

SEM (Hitachi, Japan) was used to observe the morphologies of MIPs and NIPs. Fourier transform infrared spectrometer (SH-IMADZU, Japan) was applied to characterize the structures of the samples. UV-vis 8500 spectrometer (Shanghai, China) and high-speed centrifugation (Shanghai, China) were employed to detect the concentrations of icariin in solutions and accelerate the phase separation process, respectively. Vacuum drying oven (Beijing, China) was applied for drying MIPs and NIPs. BG-01C ultrasonic cleaner (Guangzhou, China) was used for degassing the mixed solution before polymerization.

2.3. Computer simulation

At present, many methods for the strategy of optimizing the prime imprinting conditions have been proposed, but most of them are based on empirical trial-and-error approaches (Dong, Li, Guo, & Qi, 2009). With the development of quantum chemistry and computer science, the application of computer simulation technology in the chemical field is widely increasing. As a promising technology, computer simulation is a useful tool in the study of MIPs. In this paper, computer simulation was used to select the proper functional monomer and the suitable polymerization solvent. In the process of molecular simulation, firstly, the models of template, functional monomer and template-monomer complex were set up, all calculations were performed by utilizing Gaussian 09 software in the windows XP operating system. Then the geometry of the complex between the template and functional monomer in gas (or in porogenic solvent) was formed, and it is used as the initial guess to start full optimizations at the level of B3LYP/6-311g(d, p) with the Density Functional Theory (DFT). In order to obtain the stable geometry of the complex (no imaginary frequency), the calculation of vibrational frequency was performed, then the binding energy of the complex was calculated through DFT at B3LYP level with 6-311g (d, p) basis set. For each selected functional monomer, the most stable binding energy (ΔE) of the complex was obtained by the following formula:

$$\Delta E = E_{(\text{template-monomer})} - E_{(\text{template})} - E_{(\text{monomer})} + \text{BSSE} \quad (1)$$

where ΔE is the binding energy, $E_{(\text{template-monomer})}$ is the complex energy, $E_{(\text{template})}$ is the template energy, $E_{(\text{monomer})}$ is the functional monomer energy, and BSSE is the energy of the Basis Set Superposition Error. The stabilization energies of the template and functional monomer in different solvents were calculated with the following equation:

$$E_{(\text{solvation})} = E_{(\text{solvent})} - E_{(\text{gas})} \quad (2)$$

where $E_{(\text{solvation})}$ is the solvation energy, $E_{(\text{solvent})}$ is the energy of the template or monomer in solution, and $E_{(\text{gas})}$ is the energy of the template or monomer in gas.

2.4. Synthesis and characterization of the MIPs

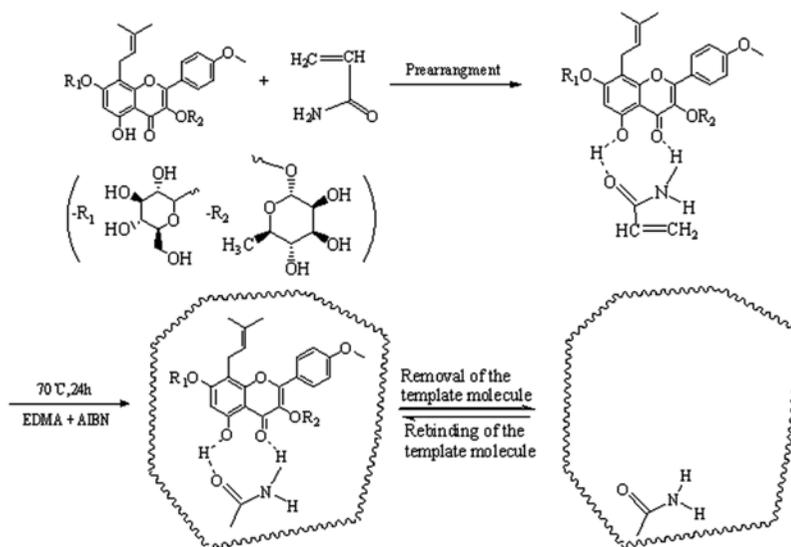
Icariin-MIPs were synthesized by the precipitation polymerization method. The preparation procedure of MIPs are shown in Figure 2. A brief introduction is as follows: 6.3 mg AM (functional monomer) was added into a 25 mL pear-shaped flask where 10.0 mg template of icariin was dissolved in 10 mL mixed solvent of methanol and acetonitrile (ACN) (V:V = 3:1), then the mixtures were shaken in an oscillator at room temperature so as to pre-polymerize. After 6 h, cross-linker EDMA (225 μL) and free-radical initiator AIBN (10.0 mg) were added into the mixture solution, and the new formed mixtures were degassed in an ultrasonic bath for 15 min and sparged with oxygen-free nitrogen for 30 min, respectively. Then the pear-shaped flask was sealed and placed in a water bath at 70°C for 24 h, and the obtained polymers were washed with methanol-acetic acid 7:3 (V:V) to remove the template until no icariin was detected in the elution. Finally, the polymers were continuously washed with methanol to remove residual acetic acid and dried at 60°C in vacuum overnight. In a contrast experiment, the NIPs were prepared in an identical manner with the MIPs but without the addition of the template icariin.

2.5. Adsorption experiments of MIPs and NIPs

The adsorption experiments were performed to study the properties of MIPs and NIPs, then the Scatchard curve was used for analysis of the adsorption mechanism of polymers.

For kinetic adsorption, 40.0 mg of MIPs or NIPs were added into conical flasks which contained 25 mL of 0.0140 mg mL⁻¹ icariin solution, then the flasks were stirred at room temperature, and part of the solution were taken out and centrifuged at the defined time. Finally, the centrifugal liquid was determined by UV-vis 8500 Spectrophotometer. Each of the above mentioned tests was done three times. The absorption capacity and imprinting factor were calculated by the following equation:

Figure 2. The synthesis process of MIPs.



$$Q = (C_0 - C_s)V/W \quad (3)$$

$$\beta = Q_{\text{MIPs}}/Q_{\text{NIPs}} \quad (4)$$

where Q ($\mu\text{g g}^{-1}$) is the absorption capacity of MIPs or NIPs, C_0 (mg mL^{-1}) and C_s (mg mL^{-1}) are respectively the initial and the equilibrium concentrations of icariin in the solution, V (mL) is the volume of the solution, W (mg) is the weight of MIPs or NIPs (da Mata et al., 2014), and β is the imprinting factor.

Static adsorption experiments were carried out as follows: 20.0 mg of MIPs or NIPs were respectively added into conical flasks, then mixed with 10 mL of icariin methanol solutions with the initial concentrations ranging from 0.0035 to 0.0180 mg mL^{-1} . After being stirred at room temperature for 10 h, the solution was centrifuged at 4,000 rpm min^{-1} for 20 min, and the concentration of icariin in the supernatant was determined by UV-vis Spectrophotometer. Each test was done three times. To further investigate the adsorption mechanism of MIPs and NIPs, Scatchard analysis was performed. The Scatchard curve was constructed according to the following equation (Xie et al., 2013):

$$Q/C = (Q_{\text{max}} - Q)/K_d \quad (5)$$

where Q ($\mu\text{g g}^{-1}$) is the absorption capacity of icariin bound to the polymers at equilibrium, C (mg mL^{-1}) is the free concentration of icariin in the solution, K_d (mg mL^{-1}) is the dissociation constant, and Q_{max} ($\mu\text{g g}^{-1}$) is the apparent maximum binding amount of binding sites. The values of K_d and Q_{max} can be calculated from the slope and intercept of the linear line plotted in $Q/[C]$ vs. Q .

The selective adsorption experiments were performed with icariin and its structural analogs (quercetin and bisphenol A). 20.0 mg MIPs or 20.0 mg NIPs were added into 10 mL of methanol solution containing icariin or quercetin (bisphenol A) at concentrations of 0.0140 mg mL^{-1} respectively. After being stirred for 10 h at room temperature, the centrifugal liquid was detected by UV-vis spectrum. The ability for the selective adsorption of MIPs is estimated by the following equations:

$$K_d = C_p/C_s \quad (6)$$

$$\alpha = K_{d1}/K_{d2} \quad (7)$$

$$\gamma = \alpha_1/\alpha_2 \quad (8)$$

where K_d is defined as the static distribution coefficient, which reflects the migration and separation capacity of the solute in the two phase system. C_p ($\mu\text{g g}^{-1}$) and C_s ($\mu\text{g mL}^{-1}$) represent the adsorption capacity of MIPs and the equilibrium concentration of the template molecule in solution, respectively. α is the separation factor, K_{d1} and K_{d2} respectively denote the static distribution coefficients of the template and its analogs. γ is the relative separation factor, α_1 and α_2 respectively refer to the separation factors of MIPs and NIPs.

3. Results and discussion

3.1. The polymerization solvents

Polymerization solvents have a great influence on adsorption performance of the MIPs as well as its imprinting efficiencies, since the solvents not only act as dispersion media but also as the pore forming reagent in the polymerization process (Song, Li, Wang, & Chen, 2009). In the course of selecting the best polymerization solvent, the solvent effect should be taken into account. The stabilization energy of solvent is often used to evaluate the interaction strength of the molecular template with solvent, monomer or cross-linker with solvent (Khodadadian & Ahmadi, 2010). In this paper, prior to the polymerization, computer simulation based on CPCM model was used to calculate the solvation energies of the template and functional monomers in different solvents such as methanol, ethanol, THF, and ACN.

The results are presented in Table 1. As seen, the solvation energies of both AM and icariin were larger in methanol than those in THF. Generally, the larger the solvation energy, the greater the influence on the polymer formation, so theoretically speaking, the best candidate solvent should be THF. However, experimental results listed in Table 2 showed that polymers prepared in methanol had a larger adsorption capacity than those in THF. This could be the reason that the polymers prepared in methanol were microspheres, which need not be ground before use, and those in THF were bulk polymers, whose binding sites were embedded into polymers. In the course of being ground, parts of the binding sites were damaged, so they had lower adsorption capacity. By comparing with the adsorbing capacities of MIPs formed in different polymerization solvents listed in Table 2, the mixture of methanol and acetonitrile 3:1 (V:V) was chosen as the best polymerization solvent.

3.2. Functional monomers

It is known that the selection of the suitable functional monomers is a crucial action in the study of MIPs, because the interaction between the template and functional monomers determines the affinity and selectivity of the MIPs (Yuan et al., 2011). Generally, the main relations between them are non-covalent interactions including H-bonding, van der Waals forces, π - π interactions and electrostatic interactions. The non-covalent interactions are usually more easily employed in the preparation of imprinted polymers because of its rapid recognition kinetics and the simplicity of the elution process. In the icariin molecule, there are many hydroxyl groups and one carbonyl, which can form hydrogen bonds when meeting with suitable functional monomers. At the beginning of this study, we selected the functional monomers by molecular simulation technology, the calculation results are summarized in Table 3. As can be seen, the binding energies of complexes formed with template and different functional monomers were different, namely $\Delta E_{(\text{icariin-AM})} > \Delta E_{(\text{icariin-MAA})} > \Delta E_{(\text{icariin-4-VP})} > \Delta E_{(\text{icariin-IA})}$. Generally, in molecular simulation technologies, monomers with the highest binding scores are subsequently selected to produce full-scale MIPs with hopefully superior recognition properties (Khodadadian & Ahmadi, 2010), so AM was chosen as the best candidate to prepare icariin MIPs. To confirm the accuracy of the theoretical calculations, the MIPs with different functional monomers including acrylamide (AM), methacrylic acid (MAA), 4-Vinylpyridine (4-VP) and itaconic acid (IA) were synthesized respectively, and their adsorbing capacity data are listed in Table 4. It was obvious that the adsorption capacity of the MIPs was the largest ($550 \mu\text{g g}^{-1}$) when AM acted as the functional monomer, and the smallest adsorption capacity ($4.16 \mu\text{g g}^{-1}$) belonged to the MIPs synthesized when IA was used as polymer monomer. The experimental results and theoretical simulation data coincided quite well.

Table 1. The solvation energy of the icariin and AM in different solvents

Solvents	ϵ	$E_{\text{solvation-icariin}}$ (kJ mol ⁻¹)	$E_{\text{solvation-AM}}$ (kJ mol ⁻¹)
Methanol	33.6	100.740	27.935
Ethanol	24.3	99.551	27.594
ACN	37.5	101.074	27.358
THF	7.58	87.999	24.365

Table 2. The adsorbing capacity of MIPs with different polymerization solution^a

Polymers	Porogen	Q_{MIP} ($\mu\text{g g}^{-1}$)	Q_{NIP} ($\mu\text{g g}^{-1}$)	β
MIP ₁	Methanol	515	250	2.06
MIP ₂	Methanol acetonitrile	550	263	2.09
MIP ₃	Methanol acetone	525	258	2.03
MIP ₄	THF	510	247	2.06

^aPolymerization conditions: 12 mL polymerization solution, 10 mg icariin and the ration of [icariin]:[AM]:[cross-link] 1:6:40.

Table 3. Binding energies ΔE of icariin with AM, MAA, 4-VP and IA

Complexes	ΔE (kJ mol ⁻¹)
Icariin—AM	70.890
Icariin—MAA	55.136
Icariin—4-VP	36.757
Icariin—IA	26.255

Table 4. The adsorbing capacity of MIPs and NIPs with different functional monomers^a

Polymers	Functional monomer	Q_{MIP} ($\mu\text{g g}^{-1}$)	Q_{NIP} ($\mu\text{g g}^{-1}$)	β
MIP1	AM	550	263	2.09
MIP2	MAA	508	254	2.00
MIP3	4-VP	445	230	1.93
MIP4	IA	416	223	1.86

^aPolymerization conditions: 12 mL polymerization solution, 10 mg icariin and the ration of [icariin]:[AM]:[cross-link] 1:6:40.

3.3. Optimal conditions for preparing the polymers

In this paper, the single-factor experiments were first performed to sketchily explore the conditions for the preparation of icariin MIPs, then the most suitable experimental parameters were obtained by three factors and three levels of orthogonal design, based on the results of single factor experiments.

3.3.1. The molar ratio between the template molecule and functional monomer

As shown in Table S1, the adsorption capacity of icariin MIPs increased with augmenting the molar ratio between the template molecule (icariin) and the functional monomer (AM). When their molar ratio was 1:6, it attained the largest 550 $\mu\text{g g}^{-1}$, thereafter it decreased, and this was probably the reason that the excess functional monomers had a bad effect on the formation of MIPs.

3.3.2. The molar ratio of the template molecule and cross-linker

The cross-linker plays a key role in the preparation of MIPs, which is employed to maintain the stability and rigidity of the polymer matrix and for the formation of the three-dimensional network structure between the functional monomer and the template under certain conditions. In this experiment, ethylene glycol dimethacrylate (EDMA) was employed as the cross-linker, and the influences of the different ratios between icariin and EDMA on the properties of the prepared MIPs are listed in Table S2. As shown, the relatively suitable molar ratio of template molecule and cross-linker seemed to be 1:60.

3.3.3. Dosage of the polymerization solvent

The dosage of polymerization solvent also has an important influence on the adsorption properties of the synthetic MIPs. In this paper, the mixture of methanol and acetonitrile (ACN) (V:V = 3:1) acted as polymerization solvent, and it was studied that the dosage of the polymerization solvent had an impact on the adsorption properties of the prepared icariin MIPs when the polymerization conditions were 10.0 mg icariin, 6.3 mg AM and 170 μL cross-linker. The results are summarized in Table S3. As seen, when the dosage of the polymerization solvent was 12 mL, the adsorption capacity attained the largest value 605 $\mu\text{g g}^{-1}$, thereafter, it decreased with the increase of the solvent dosage. The reasonable explanation should be that the excessive solvent broke the formation of hydrogen bonds between the template molecule and functional monomer.

3.3.4. Orthogonal design

In the synthesis process of MIPs, the factors including the dosage of solvent, the molar ratio of template molecule, functional monomer and cross-linker and so on, should not be isolated, on the contrary, they interact with each other. Generally, orthogonal design is deemed to the suitable method to define

the optimal synthesis conditions. In this paper, the orthogonal design was performed as follows: firstly, based on the preceding single-factor experiments, the relatively large adsorption capacities were chosen as the horizontal range of factors, then the three factors and three levels of the orthogonal experimental plan was designed in Tables S4 and S5. The experiment results in Table S5 showed that the icariin MIPs (No. 5) possessed the largest adsorption capacity value ($610 \mu\text{g g}^{-1}$). Therefore, the optimal reaction molar ratio of the template molecule (icariin), functional monomer (AM), and cross-linker (EDMA), was 1:6:80.

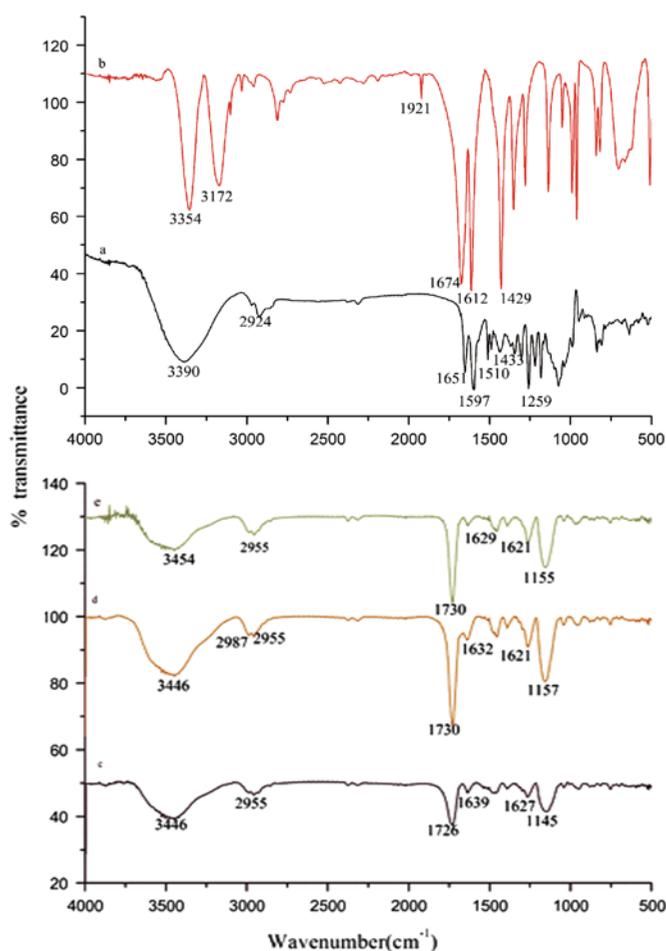
3.4. Characterization of icariin MIPs

3.4.1. FTIR analysis

To illustrate that the icariin-MIPs have been prepared, FTIR analysis was performed. Figure 3(a) showed the FTIR spectrum of icariin, where a strong and broad absorbance peak at $3,390 \text{ cm}^{-1}$ was produced by the stretching vibration of hydroxyl groups, which might form the intra- and/or inter-molecular hydrogen bonds. The absorbance peaks at $1,651$ and $2,924 \text{ cm}^{-1}$ were assigned to $\text{C}=\text{O}$ and CH_3 stretching vibrations, respectively. In addition, the multiple peaks at $1,597$, $1,510$, and $1,433 \text{ cm}^{-1}$ were attributed to cyclohexene skeleton in the icariin molecule. Figure 3(b) was representative of the FTIR spectrum of AM, and the peaks at $3,354$ (and $3,172 \text{ cm}^{-1}$), $1,674$ and $1,612 \text{ cm}^{-1}$ were assigned to the stretching vibrations of N-H , $\text{C}=\text{O}$ and $\text{C}=\text{C}$, respectively.

The FTIR spectrum of MIPs which loaded icariin is shown in Figure 3(c). Compared with Figure 3(a-c) indicated some big shifts happened, that the bands indicative of O-H/N-H shifted from $3,390$, $3,354$, and $3,172$ to $3,446 \text{ cm}^{-1}$, and the peaks on behalf of carboxylate shifted from $1,651$ or $1,674$ to $1,726 \text{ cm}^{-1}$,

Figure 3. FTIR spectra of icariin (a), AM (b), MIPs non removal of the template molecule (c), MIPs removal of the template molecule (d), and NIPs (e).



which reflected the MIPs with hydrogen bonds had been produced. The shifts of the cyclohexane skeleton peaks from 1,597, 1,510, and 1,433 cm^{-1} in Figure 3(a) to 1,639 cm^{-1} , 1,510, and 1,406 cm^{-1} in Figure 3(c), respectively, could introduce many repeated cross-linking units (EDMA) that existed in the prepared MIPs. The FTIR spectra of MIPs with the removal of icariin by complete washing and NIPs are respectively shown in Figure 3(d and e). As seen, the differences of the wave numbers for the adsorption peaks of C=C (1,629 cm^{-1}) and C-O (1,155 cm^{-1}) in Figure 3(e) are lower than those in Figure 3(d) (1,632 and 1,157 cm^{-1}), and the results might also indirectly illustrate that icariin-MIPs had been formed.

3.4.2. Surface morphology of MIPs

The surface morphologies of MIPs prepared in different solvents were investigated by SEM (Figure 4). Compared with NIPs (Figure 4(a)), MIPs (Figure 4(b)) prepared in THF had rough surfaces with more and larger dimension pores, which were obviously favorable for the embedding of icariin molecules. The MIPs prepared in methanol and in the mixture of methanol and acetonitrile (Figure 4(c and d), respectively) both existed in the form of polymer microspheres, so these MIPs had a larger surface, and icariin molecules could be more easily embedded in the pores. Among the above four polymers, the MIPs prepared in the mixture of methanol and acetonitrile were concluded as the most homogeneous and uniformly imprinted.

3.5. Adsorption analysis and recognition mechanism

3.5.1. The study of dynamic and static adsorption

The dynamic curves for the adsorption of icariin onto the MIPs and NIPs are shown in Figure 5, from which it could be seen that both of the adsorption capacities of MIPs and NIPs increased with time at the beginning of the experiment, and reached the absorption equilibrium at 10 and 8 h, respectively, which corresponding values of 609 and 266 $\mu\text{g g}^{-1}$. On the other hand, under the same conditions, all the adsorption capacity data of MIPs were obviously much higher than those of NIPs, and this indicated that the special binding sites had been formed in MIPs, which were of high selectivity and affinity to icariin.

Figure 4. Scanning electron micrographs of the MIPs and NIPs prepared in different porogens, (a) NIPs-THF, (b) MIPs-THF, (c) MIPs-methanol, and (d) MIPs-methanol and acetonitrile.

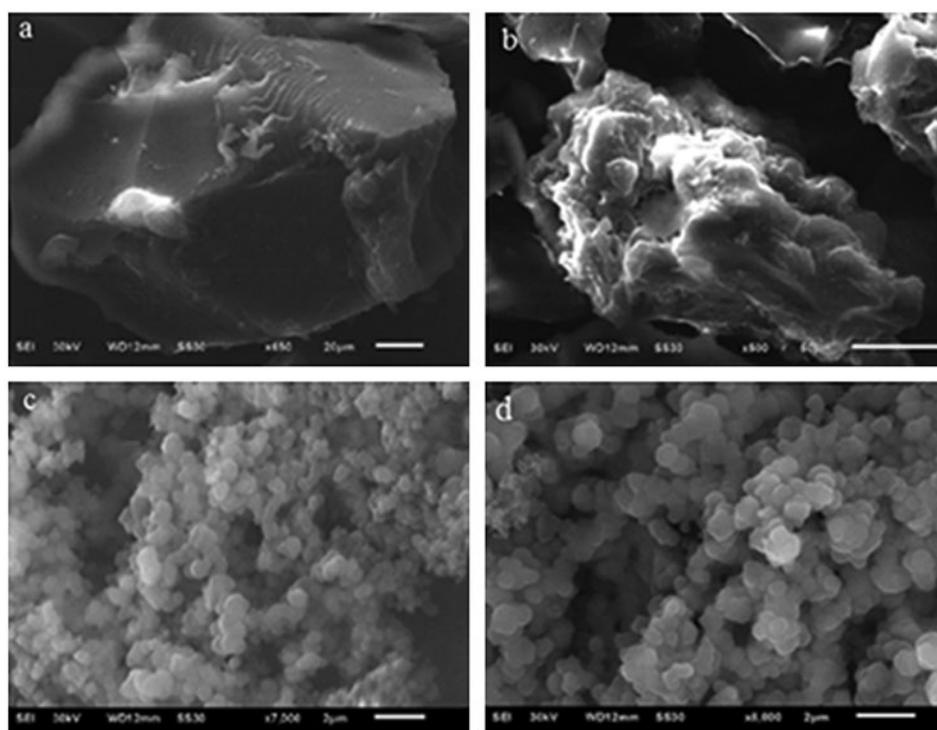


Figure 5. Dynamic curves for the adsorption of icariin MIPs and NIPs.

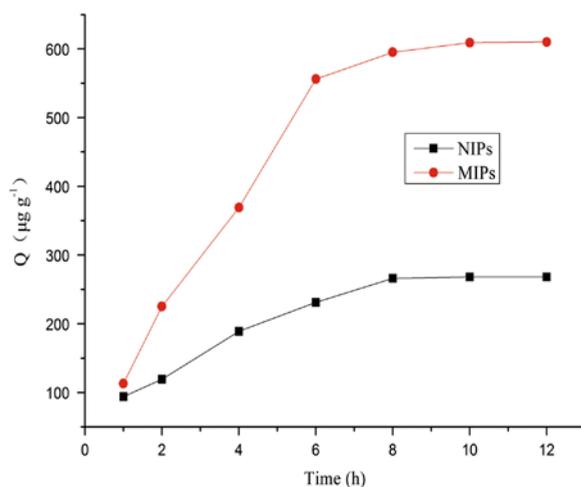


Figure 6. Adsorption isotherms of MIPs and NIPs.

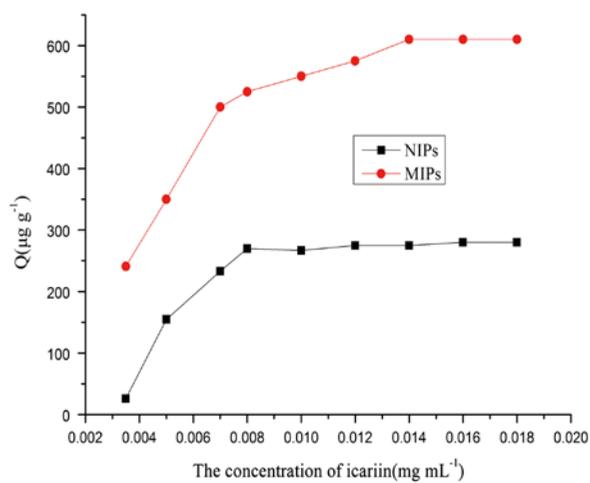


Figure 7. Scatchard plots of MIPs and NIPs.

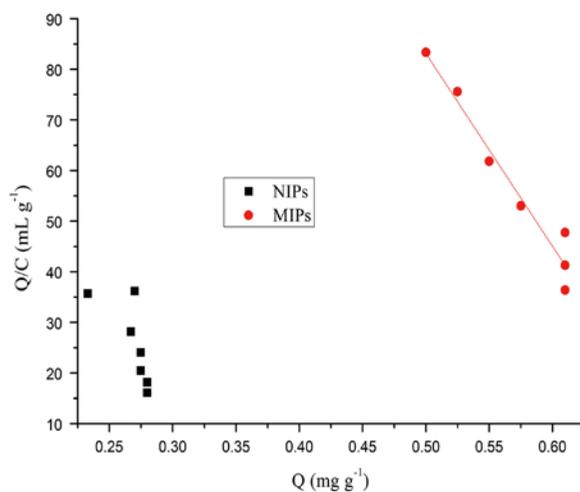


Table 5. Adsorption and selectivity capability of MIPs and NIPs for icariin and its structural analogs

Compounds	icariin		quercetin		bisphenol A	
	MIPs	NIPs	MIPs	NIPs	MIPs	NIPs
C_0 (mg mL ⁻¹)	0.0140	0.0140	0.0140	0.0140	0.0140	0.0140
C_s (mg mL ⁻¹)	0.01278	0.01345	0.01341	0.01348	0.01338	0.01346
Q (μg g ⁻¹)	610	275	295	260	310	270
β	2.22		1.13		1.15	
K_d (mL g ⁻¹)	47.73	20.45	22.00	19.29	23.17	20.06
α	-	-	2.17	1.06	2.06	1.11
γ	2.05				2.04	

The results of static adsorption of MIPs and NIPs are expressed in Figure 6. It could be observed that the adsorption capacities for both MIPs and NIPs increased with higher concentrations of icariin solution. When the concentrations of icariin in methanol were 0.0140 and 0.0080 mg mL⁻¹, MIPs and NIPs reached adsorption equilibrium, respectively. Under the same conditions, all the adsorption capacity data of MIPs were always clearly higher than those of NIPs. It was hypothesized that the binding sites which were complementary in size and shape to icariin were formed in the MIPs. To prove this hypothesis, the obtained data were plotted by the Scatchard formula, the results are shown in Figure 7. It was clear that the plots of MIPs formed a straight line, and the linear equation is $y = -380.6430x + 273.4250$ ($R^2 = 0.9490$). From the slope and intercept of the MIPs Scatchard curve, K_d and Q_{max} were calculated to be 0.0026 mg mL⁻¹ and 0.7110 mg g⁻¹, respectively. This indicated that the homogeneous binding sites had been formed in MIPs, which have specific adsorption to icariin. On the contrary, the plots of NIPs formed an irregular curve, this was because there were no special binding sites produced in NIPs.

3.5.2. The selective adsorption analysis

The results of selective experiment are listed in Table 5. It showed that the adsorption capacity of MIPs for icariin was larger than that for quercetin and bisphenol A, and the separation factor α attained 2.17 and 2.06 respectively. As for NIPs, the adsorption capacities for three substances were almost the same and the separation factors were close to 1, so it was meaningless to separate icariin, quercetin and bisphenol A by NIPs. The relative separation factor γ (2.05 and 2.04) indicated that the prepared icariin MIPs had good selectivity and strong separation capacity to the icariin molecule.

4. Conclusion

In this study, to explore the optimal preparation conditions of icariin MIPs, computer simulation technology, single factor analysis and orthogonal design methods were performed. The optimized synthesis parameters were obtained as follows: acrylamide (AM) as the functional monomer, the mixture of methanol and acetonitrile (ACN) (V:V = 3:1) as polymerization solvent, and the suitable reaction molar ratio of the template molecule, functional monomer and cross-linker as 1:6:80. The adsorption experiments (including the dynamic, the static and the selective) indicated that the prepared MIPs have high affinity and selectivity to the template icariin. Scatchard analysis showed that many binding sites were formed in MIPs, which exhibited specific recognition to icariin. So the prepared MIPs can be used as solid-phase extraction materials for the separation and purification of icariin from Chinese medicinal herbs.

Supplementary material

Supplementary material file for this article can be accessed from <http://dx.doi.org/10.1080/23312009.2015.1059597>.

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