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Preparation and Characterization of a Metal-Complexing Imprinted Polymer for Improved Quercetin Recognition

Juanjuan Shan[a]; Bing Wang[a]
[a] State Key Laboratory of Hollow-Fiber Membrane Materials and Membrane Processes (Tianjin Polytechnic University), School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin, P.R. China

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Preparation and Characterization of a Metal-Complexing Imprinted Polymer for Improved Quercetin Recognition

Juanjuan Shan and Bing Wang
State Key Laboratory of Hollow-Fiber Membrane Materials and Membrane Processes (Tianjin Polytechnic University), School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin, P.R. China

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This paper describes the rational design and testing of a new metal-complexing imprinted polymer with enhanced selectivity for Quercetin (Quercetin is a kind of flavonoid which is an important active ingredient of Chinese herbs.). The complex of Quercetin and Cu(II) (Qu-Cu) was used as a template molecule and the mixed solution of methanol and tetrahydrofuran was used as the porogenic solvent. UV–visible spectra was useful to assess the rational ratio of Quercetin to Cu(II). And the evaluation of the various polymers by binding assays revealed that the optimal ratio of the functional monomer to the template was 4:1. The effect of porogenic solvent on molecularly imprinted polymers (MIPs) was studied by transmission electron microscopy (TEM) and equilibrium binding experiments. MIPs were also characterized by Fourier transform infrared spectra (FT-IR). The adsorption behavior for substrates indicated that compared with Quercetin's structural analogs (Rutin and Naringenin), MIPs exhibited significant adsorption selectivity for Qu in the presence of Cu(II). The separation factors were 5.38 and 7.23, respectively.

Keywords adsorption behavior; Cu(II); enhanced selectivity; metal-complexing imprinted polymer; quercetin

INTRODUCTION

Molecular imprinting, which is an attractive method for obtaining selective recognition, has been applied in various fields such as those of separations, catalysis, and sensors (1–3). Molecularly imprinted polymers (MIPs) have gained great attention due to their enormous possibilities of applications (4,5). These polymers are prepared by inducing selective recognition sites in a polymer matrix using a template molecule in a casting procedure. This highly cross-linked polymer is a rigid network and generates a defined spatial configuration. The template molecule is consequently removed from the network leaving behind cavities which are complementary to the template in size, shape, and chemical functionality. Therefore, MIPs are able to rebind the template with high affinity and specificity. And MIPs have been proved to be highly resistant to time and use (6). Because of these advantages, it has been widely used in separation techniques (7,8), sensor applications (9) and enzyme-like catalysis (10), and clinical drug analyses and membrane separation technology (5,11), especially in sample enrichment for the determination of Chinese traditional medicine (12).

Flavonoids are important active components of traditional Chinese medicine and Quercetin (Qu) may be considered as a major dietary flavonoid most commonly present in nature. However, there are also other flavonoids having similar structures with Qu, so enormous difficulties are presented in the separating process of Qu from its natural analogues. Molecular imprinting technology provides a new approach to solve these difficulties, especially the technology based on metal complexing. And Qu has five hydroxyl groups which make it hard to dissolve in the non-polar or weakly polar solvent, so it is imperative that MIPs developed are capable of functioning under polar conditions.

As is known that metal ion complex widely occurred in biological identification system (13), for example, heme and chlorophyll are the complexes of iron and magnesium, respectively. On the other hand, the antioxidant action of Qu (Fig. 1) appears to be a combination of a reaction with free radicals and metal ion complex. As metal chelator, the flavonoids play an important role in both the bioavailability and toxicity of a variety of metals (14).

Keeping all these comments in mind, this article describes the development of a metal-complexing imprinted polymer using Qu-Cu as a template molecule in a strong polar solvent (methanol and tetrahydrofuran mixed solution). At the same time, the optimal amount of the functional monomer was investigated by binding assays. The effects of the porogenic solvent on the surface morphology and binding capacity of MIPs were also studied. Batch rebinding studies of MIPs for Qu-Cu had been

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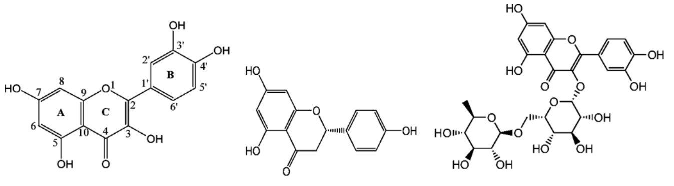
Address correspondence to Bing Wang, State Key Laboratory of Hollow-Fiber Membrane Materials and Membrane Processes (Tianjin Polytechnic University), School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300160, P.R. China. E-mail: bingwang666@yahoo.com.cn



Quercetin (Qu) Naringenin (Na) Rutin (Ru)

FIG. 1. Chemical structures of Qu and Qu's structural-related products.

performed in detail. The ability of MIPs for the recognition of its analogue (Rutin (Ru) and Naringenin (Na)) was also evaluated. The obtained results revealed that the imprinted polymer produced presents high selectivity to Qu-Cu. This work is of great significance in the separation and detection applications of Quercetin. Meanwhile, investigating the function of metal-complexing in a molecularly imprinted technique may promote further understanding of the process and mechanism of a molecular imprinting technique and biological identification.

EXPERIMENTAL

Materials

Quercetin (Qu), Rutin (Ru), and Naringenin (Na) were obtained from Shanxi Huike Botanical Development Co. (Shanxi, China). Methacrylic acid (MAA) [functional monomer] and copper sulfate pentahydrate (Cu(II)) were purchased from Tianjin Chemical Reagent Research Institute (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) [cross-linker] was supplied by the Shanghai Haiqu Chemical Co. (Shanghai, China). 2,2-Azo-bis-isobutyronitrile (AIBN) from Shanghai Hewei Co. (Shanghai, China) was used as a free radical initiator. Tetrahydrofuran, methanol, acetic acid, and ethanol were obtained from Tianjin Damao Chemical Reagent Co. (Tianjin, China). All of the materials were of analytical grade and purified by vacuum distillation or recrystallization to remove the inhibitor prior to use.

Apparatus

UV-vis spectra and binding assays were performed using an UV-240 ultraviolet-visible spectrophotometer (Shimadzu, Japan). FT-IR analyses were taken on a TENSOR37-Fourier Transform Infrared Spectrometer (Bruker Co., Germany). An H-7650 Transmission Electron Microscope (Hitachi Ltd., Japan) was used to observe the morphology of the imprinted polymer. In the polymer processing, some other equipments were used, including: WE-1 thermostat water bath oscillator (Tianjin Honour Instruments Inc., China), SK5200 ultrasonic vibration generator (Shanghai Kudos Ultrasonic Instrument Co. Ltd. China), TG16-WS (1650D) supercentrifuge (Shanghai Luxiangyi Gentrifuge Instrument Co. Ltd., China.), Soxhlet extractor, etc.

UV-vis Spectrometry of Qu-Cu Complex

A set of solutions with a different amount of Cu(II) and Quercetin(Qu) were prepared using methanol as solvent in which Qu was kept at a fixed concentration (0.02 mmol/L). The ratio of molar quantities between Qu and Cu(II) among these solutions were 1:0,1:0.4,1:0.6,1:1,1:2. After ultrasonic oscillation for 2 h, these solutions were placed for 10 min aiming at achieving sufficient interaction between Qu and Cu(II). Then the changes of different absorption spectra of these solutions were obtained by UV-spectrometer under the wavelength range 250 ~ 550 nm.

Preparation of Metal-Complexing Imprinted Polymers

Quercetin(Qu)(338.27 mg)and copper sulfate pentahydrate (Cu(II)) (499 mg) were dissolved in a certain tetrahydrofuran/methanol mixed solution (V/V = 1:3) (Table 1) in a borosilicate test tube with the ultrasonic oscillation for 2 h. To this MAA was added and the mixture suffered a further ultrasonic oscillation for 2 h. Then 40 mmol of EGDMA (7928 μL) and 50 mg quantity of AIBN were added. The mixture was shocked for a further 10 min to ensure complete dissolution of all components. The mixture was sonicated for 10 min and sparged with N_2 for 10 min to degas it. The tubes were plugged under vacuum and gradually heated to 60°C in a water bath and held for 12 h. Following polymerization, the resultant polymers were removed and ground to a fine powder with a mortar and pestle. Then the ground particles were to pass through a 74 μm molecular sieve. Superfine particles were

TABLE 1
Polymers produced by the combination of different functional monomer amount and porogenic solvent amount

Polymer	Template	Template: Functional monomer*	Porogenic solvent amount (mL)
P_1	Qu-Cu	1:4	10
P_2	Qu-Cu	1:4	15
P_3	Qu-Cu	1:4	20
P_4	Qu-Cu	1:1	15
P_5	Qu-Cu	1:2	15
P_6	Qu-Cu	1:3	15
P_7	Qu-Cu	1:5	15
P_8	Qu-Cu	1:6	15
P_9	Qu-Cu	1:7	15
P_{10}	Qu	1:4	15
P_{11}	–	1:4	15

*Template: Functional monomer represents the molar quantity ratio of template to MAA.

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removed by repeated washing in ethanol. The resulting particles were extracted using the Soxhlet method and washed with acetic acid/methanol mixed solution (V/V = 1:9) until the template could no longer be detected in the elution. Then 0.1 mmol/L EDTA solution was used to remove the residual metal ions on polymers. Finally, the particles were washed with pure methanol numerous times and dried to constant weight under vacuum at 60°C.

In order to verify that rebinding to the template molecule was due to molecular recognition and not to non-specific binding, a control(non-imprinted polymer, NIP) was prepared following the same procedure, including washing, but with the omission of the template molecule. So there are eleven sets of MIPs and NIP given in Table 1.

IR Spectra Studies

Qu was grinded to a molecularly imprinted polymer before elution and after elution, and NIP was grinded into a fine powder respectively, and then the samples were made by the squash method using KBr. After drying, they were characterized by Fourier transform infrared spectra (FT-IR).

Transmission Electron Microscopy (TEM) Analyses

To investigate the effect of porogenic solution on MIPs, TEM assay was carried out. Molecular imprinted polymers with certain fineness were put into ethanol to prepare a suspension of an appropriate concentration. After ultrasonic dispersion of 15 min, powder samples were hung on to the copper mesh quickly. When ethanol was completely volatilized, they were put in the TEM for observation.

ADSORPTION BEHAVIOR STUDIES

Adsorption Dynamics of MIPs

To investigate the adsorption dynamic of MIPs, 50 mg different polymers (P_1, P_2, P_3) were placed in 5 mL of Qu/Cu(II) solutions in 25 mL conical flasks, respectively. The molar ratio between Qu and Cu(II) in the solutions were all 1 : 2, and the concentrations of Qu in all solutions were 2 mmol/L. The mixtures were oscillated with constant temperature bath at 28°C for different time (20, 30, 40, 60, 80, 120, 160, 200, 240 min), and then the solutions at different time were separated by centrifuge. After that the concentrations of the upper clear liquids were determined by UV-vis spectrophotometer at 280 nm, respectively. The binding amount (Q) was calculated according to the Eq. (1).

$$Q = (c_0 - c_t) \times V/m \tag{1}$$

where, c_0 is the initial concentration of Qu (2 mmol/L), c_t is Qu concentration (mmol/L) at different time, V and m are the volume of the substrate solution (5 mL) and the weight of dry polymer (50 mg) in the adsorption experiment, respectively.

Isothermal Adsorption Experiments

The dry MIPs (50 mg) were placed in a 25 mL conical flasks and mixed with 5 mL of a known concentration of Qu/Cu or Qu solution (0.25 ~ 6 mmol/L). The mixtures were oscillated with constant temperature bath at 28°C for 10 h, and then the concentration of Qu in the solution was determined and the binding amount (Q) was calculated using the same method as above.

Study of Selectivity Adsorption to Different Substrates

The dry MIPs (50 mg) were placed in 25 mL conical flasks and mixed with 5 mL of a known concentration of Qu solution, Ru solution, and Na solution (2 mmol/L), respectively. In order to investigate the adsorption of MIPs to different substrates, the same method as above was adapted to calculate the dissociation coefficient (*K*) and the separation factor (α). The dissociation coefficient (*K*) and separation factor (α) were calculated based on the following Eqs. (2) and (3), respectively.

$$K = C_p/C_s \tag{2}$$

$$\alpha = K_i/K_j \tag{3}$$

where C_p and C_s are the initial substrate concentration (μmol/g) and the equilibrium concentration (mmol/L), respectively. And K_i is the dissociation coefficient of the template molecule; K_j is the dissociation coefficient of the substrate. Furthermore, the value of α is 1.0 when i = j.

RESULTS AND DISCUSSION

UV-vis Spectroscopic Study

Functional groups which could absorb visible ultraviolet are called chromophore in organic molecule. And chromophore having valence electron with lower excitation energy could result in electron transition (15). The UV-vis spectrum of free Quercetin (Qu) and Qu-Cu with different ratios in methanol is described in Fig. 2. Like most flavonols, Qu exhibits two major adsorption bands in the region of 200 nm to 400 nm, at 280 nm (band II) representing A ring (benzoyl chromophore) and 367 nm (band I) representing B ring (cinnamoyl chromophore). The spectra of A ring and B ring are related to n-π^* electron transition and π-π^* electron transition respectively. There are several possible groups as electron donor to form complexes with the metal ion. And they are 3-OH and 4-oxo groups of C ring, 3′,4′-dihydroxy groups of B ring, and 5-hydroxy-4-oxo groups, respectively (16).

As can be seen from Fig. 2, band I is shifted to the long wavelength region with the increase of Cu(II), and intensity

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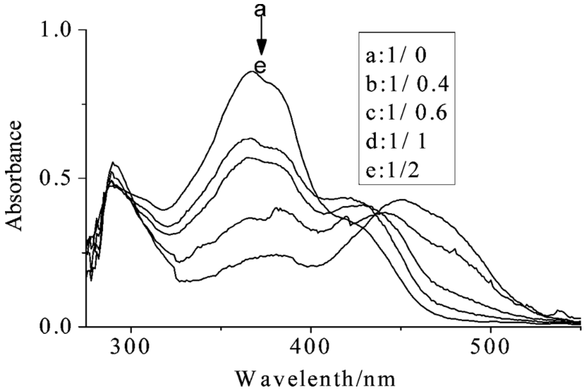

FIG. 2. UV–vis spectra of free Quercetin and Qu-Cu mixtures with different ratios in methanol.

increase and bathochromic shift are observed in band II. When the Cu(II)/Qu ratio is 2, the characteristic absorption peak of Qu-Cu complex is clearly observed at 451 nm. Given the structure of Qu and the change of UV–vis spectra, 3-OH and 4-oxo groups are the first sites involved in the complex process and 3′,4′-dihydroxy groups subsequently take part in coordination. Coordination makes the conjugate system prolonged and electron density lower which promotes π electron fluidity. So there is lower energy to excite electron transition which results in obvious bathochromic shift of band I. The 5-hydroxy-4-oxo groups are not involved in the coordination because 5-hydroxy has less acidic proton (17) and there is steric hindrance after the coordination of 3-OH and 4-oxo groups. Based on the above, the Qu-Cu complex is formed and this confirms that using Qu and Cu(II) complex as template is rational and the optimal ratio of Cu(II) to Qu is 2.

Monomer-Template Ratio Studies

In order to identify the optimal monomer-template ratio, a series of MIPs (Table 1) were prepared by changing the amount of functional monomer (MAA) under the condition of keeping Qu/Cu(II) = 1:2. And then binding assays were carried out. The results were shown in Fig. 3. As can be seen, with the increase of the monomer-template ratio, the equilibrium binding capacity of MIPs increases gradually and when the ratio reaches 4:1 it reaches the maximum. After that, continuing to enlarge the monomer-template ratio, the equilibrium binding capacity begins to decrease. This is because the amount of functional monomer is not enough to form stable imprinting sites at low ratio and stable imprinting sites will increase with the increase of functional monomer. However, excessive functional monomer will result in the increase of non-specific binding sites and self-association. These two factors will decrease specific binding sites and result in higher mass transfer resistance. So the optimal ratio is *n* (Qu-Cu):*n* (MAA) = 1:4 for MIPs preparation.

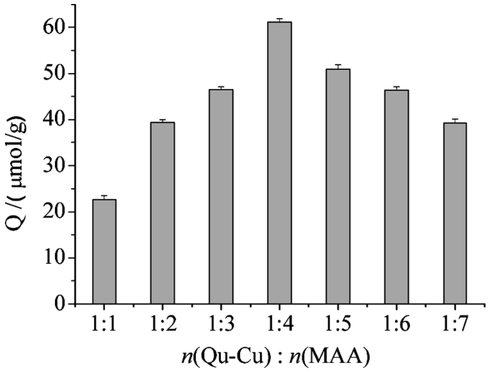

FIG. 3. Binding capacity of MIPs with different ratios of Qu-Cu to MAA.

TEM Analyses

The functions of a porogenic solvent are to dissolve all of the components of the polymer and to allow the arrangement of pre-polymerization complex. TEM analyses are necessary to evaluate its effect on MIPs. Figure 4 gives the results. By comparing the three photos of P_1, P_2, and P_3, there are significant differences of morphology with different porogenic solvent amount. In fact, the amount of porogenic solvent is equated with the content of the functional monomer and cross-linker. Less porogenic solvent (P_1) will result in higher solution concentration and viscosity in polymerization. And this will make it hard to completely diffuse the solution into the matrix to form large pore structures. Less porogenic solvent will also affect the wrapped degree of network to the pre-polymerization complex. As shown in the first photo, a chunk of non-porous structure in polymer is formed with uneven thickness of matrix material which indicates that the degree of imprinting sites embedding is severe and this is not of any benefit to the mass transfer process. However, excessive porogenic solution (P_3) makes reaction concentration much lower, thus slowing the free radical initiated polymerization rate. Furthermore, the polymerization degree of products decreases which makes the material structure loose. Thus the polymer with excessive porogenic solvent has some disadvantages such as low

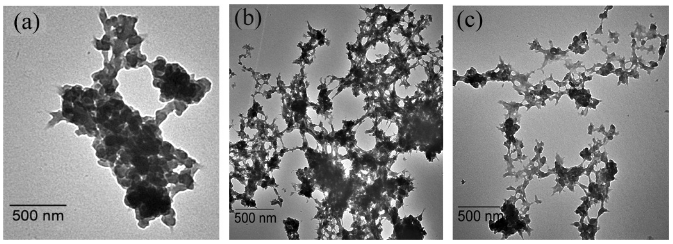

FIG. 4. TEM images of (a) P_1, (b) P_2, (c) P_3 with different porogenic solvent amount.

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hardness, low mechanical strength, and poor physical performance. As can be seen from Fig. 4, P_2 shows the three-dimensionally cross-linked network irregularly in which the presence of internal pores could greatly increase the surface area of MIPs and reduce imprinting embedding and achieve effective identification. Based on the above and the adsorption dynamic assays, the porogenic solvent amount of P_2 is the best.

IR Spectra Studies

Figure 5 presents the infrared spectrum of prepared polymers. Important information can be obtained by comparing Quercetin and the prepared polymers. The main stretching modes of infrared spectra of Fig. 5(b), (c), and (d) are almost the same. For example, the characteristic stretching $\upsilon_{\text{C-H}}$ mode occurs at about $2959\,\text{cm}^{-1}$, $\upsilon_{\text{C=O}}$ of MAA and EGDMA at about $1729\,\text{cm}^{-1}$, $\upsilon_{\text{C=C}}$ at about $1636\,\text{cm}^{-1}$, and $\upsilon_{\text{C-O-C}}$ at about $1160\,\text{cm}^{-1}$. As can be seen, there is almost no difference between Fig. 5(b) and (d). In other words, there are similarities in both the MIP and NIP. The results indicate that EGDMA and MAA have achieved cross-linking copolymerization under the synthesis conditions and the template could be completely removed. Furthermore, MIPs after elution and NIP have the same functional groups but little difference in the micro environment.

In addition, as observed in Fig. 5(a), Quercetin has two main characteristic stretching $\upsilon_{\text{O-H}}$ modes at $3379\,\text{cm}^{-1}$ and $3290\,\text{cm}^{-1}$, and $\upsilon_{\text{C=O}}$ at $1669\,\text{cm}^{-1}$. As can be seen from Fig. 5(c), when the complex of Quercetin and Cu(II) is formed, there is only one $\upsilon_{\text{O-H}}$ which also shifts to a high wavenumber and $\upsilon_{\text{C=O}}$ disappears. By comparing Fig. 5(c) with Fig. 5(d), there are obvious stretching modes at $1636\,\text{cm}^{-1}$ and $1600\,\text{cm}^{-1}$, and new stretching modes exhibit at $1358\,\text{cm}^{-1}$, $1316\,\text{cm}^{-1}$, $932\,\text{cm}^{-1}$, $820\,\text{cm}^{-1}$ and $636\,\text{cm}^{-1}$ which are complementary to Quercetin. All the changes of spectra indicate that the template molecule is fixed by MAA and EGDMA in copolymerization and the polymers could achieve elution, recognition, and rebinding which thus presents an advantage for the study of binding behaviors. Based on the UV-vis and IR spectra studies, synthesis processing and the recognition mechanism of MIPs are shown in Fig. 6.

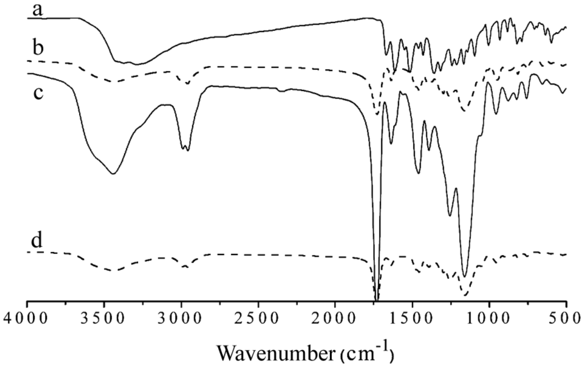

FIG. 5. IR spectra of Quercetin and prepared polymers. (a: Quercetin, b: P_{11}, c: P_2 without elution, d: P_2).

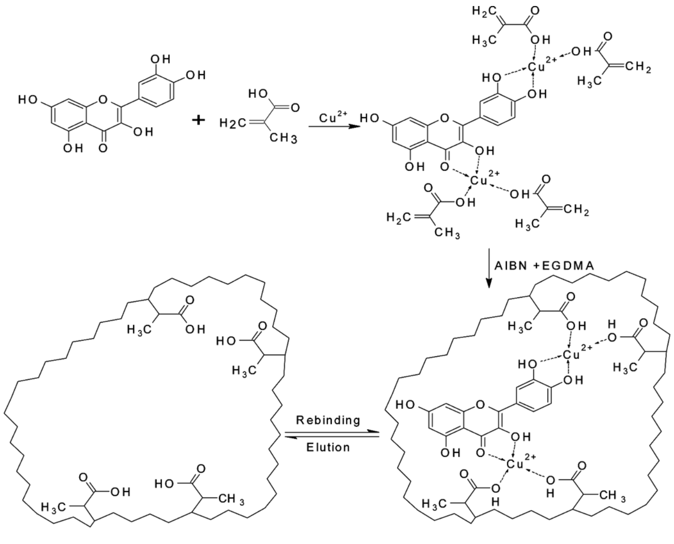

FIG. 6. The schematic representation of Qu-Cu molecular imprinting process.

Adsorption Dynamics of MIPs to Qu-Cu

The adsorption of MIPs to Qu-Cu was carried out with 50 mg P_1, P_2, and P_3, respectively. The results are shown in Fig. 7. It can be seen that the adsorption of P_2 to Qu-Cu has a rapid increase in the first 1 h, and then it increases slowly with the time extension. The adsorption process reached equilibrium at last. Thus it can be explained that stereo cavities of MIPs could not be well distributed when they were copolymerized by a cross-linker and functional

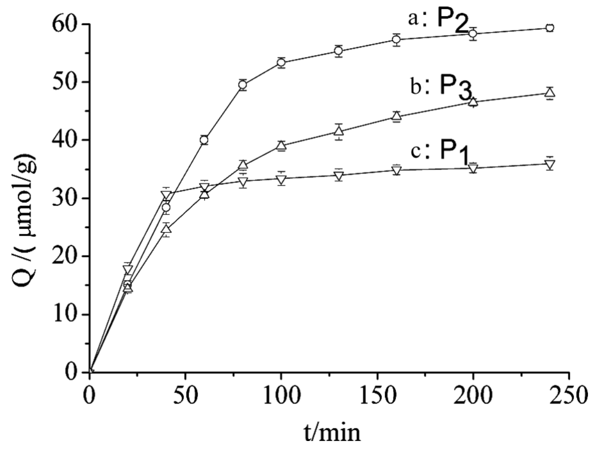

FIG. 7. Adsorption dynamic curves of MIPs. (V = 5 mL, C = 2 mmol/ L, T = 28).

monomer. So the depths of cavities are different from each other and the shallow cavities are of benefit for the adsorption of MIPs to Qu-Cu which presents as a rapid adsorption at the initial experiment. However, when these shallow cavities are saturated, the adsorption rate would decrease because there is stereo hindrance for the mass transfer of Qu-Cu into the deeper cavities. This trend indicates that there are some channels of MIPs for the template molecule which further proves that the porogenic solvent was of importance in the process of producing MIPs. The porogenic solvent may contribute to the multiple cavities of MIPs and decrease the embedding of imprinted cavities. On the other hand, the porogenic solvent provides an environment in which substrates could enter into the inside of material and improves the accessibility of recognition sites.

So the amount of porogenic solvent plays an important role in the matrix of MIPs and the matrix would affect the distribution of the material's specific surface area and volume. So it will influence the degree of embedding of imprinted cavities. A less amount of porogenic solvent will cause a lumpy structure of material and more embedding of imprinted cavities which makes against mass transfer. So the dynamic adsorption curve of P_1 went upward slowly and was apt to reach saturation in a short time. However, a larger amount of the porogenic solvent results in looseness of the structure and big specific surface area which makes recognition sites easy to access. So it helps the template molecule spread into deep cavities of MIPs and the adsorption rate is relatively fast. But excessive porogenic solvent will influence the copolymerization degree and reduce the rigidity of the backbone of MIPs leading to unfavorable mass transfer. Recognition sites also decrease and the adsorption amount diminishes. The adsorption curve of P_3 is in line with the above statements. So MIPs have the high adsorption ability only when the amount of the porogenic solvent is appropriate, like P_2.

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Adsorption Isotherm

The adsorption capacity (Q) of the series of polymers to Qu-Cu and Qu in different concentrations (0.25–6 mmol/L) was investigated by an equilibrium binding experiment. Fig. 8 and Fig. 9 show the isotherms for adsorption to Qu-Cu complex and Qu, respectively. As can be seen, adsorption isotherms of metal complexing molecularly imprinted polymers conform to monolayer adsorption of Langmuir model (18,19). By comparison of the three adsorption isotherms of P_2, P_{10} and P_{11} in Fig. 8, with the increase of initial concentration of Qu-Cu their adsorption ability to Qu-Cu are all increased and the adsorption amount of P_2 to Qu-Cu is always bigger than that of P_{10} and P_{11}. It is obvious that P_2 shows better binding ability and the adsorption reached saturation in a high concentration range. In many binding assays of the receptor, the relation curve of the binding amount and initial concentration is hard to achieve saturation because of the linear increase of non-selectivity. Based on that statement, we can judge that the adsorption of P_{10} and P_{11} to Qu-Cu is non-selectivity and P_2 shows a selective adsorption.

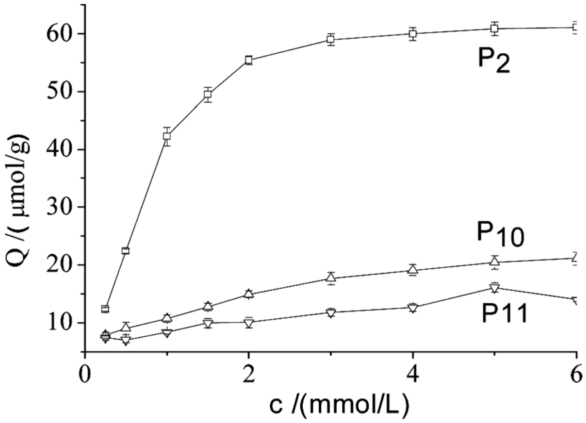

FIG. 8. Adsorption isotherms of different polymers to Qu-Cu with different initial concentrations.

As observed in Fig. 9, the maximum binding capacity of P_2 to Qu is smaller than that of P_2 to Qu-Cu in Fig. 8. The reason is that the molecularly imprinted polymer P_2 whose template is Qu-Cu could represent a specific recognition capacity only by the metal coordination. In other words, P_2 recognizes the metal complex Qu-Cu and not single Qu. This indicates that Cu(II) plays the role of a regulation to a specific recognition of P_2. This result is in agreement with the molecular imprinting effect which is described as the following: the matrix of MIPs is complementary with the template not only about the stereo shape but also the functionality sites, and this matrix makes MIPs a good selectively binding capacity.

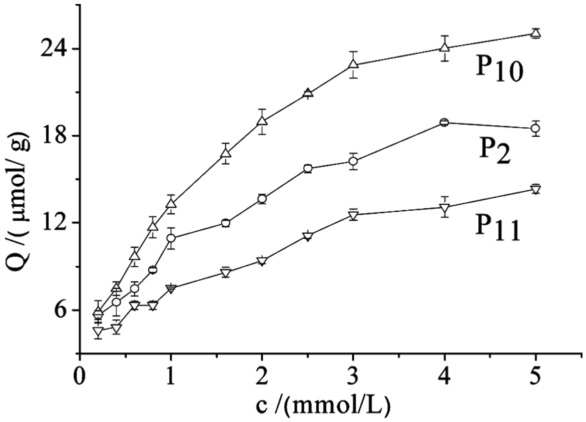

FIG. 9. Adsorption isotherms of different polymers to Qu with different initial concentrations.

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As can be seen from Fig. 8, the binding capacity of P_{10} to Qu is always smaller than that of P_2. P_{10} whose template is Qu, is formed by the non-covalent complexes between Qu and MAA. The strength of non-covalent complexes directly affects the stability of MIPs and further influences the imprinting effect of MIPs to the template. In the experiment, methanol, a strong polar solution, may disturb or destroy the hydrogen bond interaction or electrostatic interaction between Qu and MAA. So the binding capacity of P_{10} to Qu is always smaller than that of P_2. The result also further illustrates that the metal coordination bond is stronger than the non-covalent bond, such as the hydrogen bond and the van der Walls force and so on, and the metal coordination bond is relatively stable, and this also further shows that the interaction between the template and the functional monomer is of significance to specific selectivity and high affinity of MIPs to the template.

Selectivity Evaluation of MIPs

To gain better insight into the selectivity capacity of MIPs, the structural analogs of Quercetin (Qu), Naringenin (Na), and Rutin (Ru) (Fig. 1) were chosen for the study. Table 2 gives the results.

The data in Table 2 show that P_2 exhibits high selectivity for Qu compared to its structure-related products (Na and Ru). This indicates that P_2 presents specific adsorption to Qu. This is because that P_2 has the matrix matching Qu-Cu in shape and reactive groups. As can be seen, the separation factor (α) of P_{10} and P_{11} to Na and Ru are all close to 1.00 and this shows that P_{10} and P_{11} present non-specific adsorption to substrates.

Given the MIPs processing and the structures of these products, when there is Cu(II), Qu, Cu(II), and the functional monomer achieve pre-polymerization and then form a triple complex. After elution MIPs have left behind the matrix and binding sites complementary to the template molecule. Because 3-hydroxyl of Ru is joined with glucose and rhamnose, the space volume is bigger than that of Qu. In addition, A ring and B ring of Na are not in the same plane, so the multi-dimensionality of spatial configuration is more strong. Furthermore, 3-hydroxyl and 4-carbonyl of these two structures cannot form a triple complex with Cu(II). Based on the above, spatial configuration and binding sites of Ru and Na cannot match the matrix of MIPs and they are all difficult to diffuse into the matrix. So MIPs represent bad binding capacity to Na and Ru.

TABLE 2
Values of K and α of different substrates

	P_2		P_{10}		P_{11}	
Substrate	K	α	K	α	K	α
Qu	43.24	1.00	11.29	1.00	10.12	1.00
Na	5.98	7.23	8.98	1.26	8.21	1.23
Ru	8.03	5.38	8.14	1.39	10.99	0.92

CONCLUSIONS

The aim of this study is to rationally design metal-complexing imprinted polymers with improved recognition for Quercetin by introducing Cu(II). This has been successfully synthesized. FT-IR spectra studies show that MIPs have chemical groups complementary to the template which create conditions for specific recognition of MIPs. Furthermore, the effect of the porogenic solvent amount on polymer morphology and binding capacity are evaluated by TEM and binding assays. The optimal amount of functional monomer is also investigated by binding assays and the optimal ratio n (template molecule): n (functional monomer) = 1:4 is obtained. Isothermal adsorption and selective adsorption experiments are carried out which indicate that the metal-complexing imprinted polymers have high affinity and specific recognition for the complex of Quercetin and Cu(II) and the addition of Cu(II) improves adsorption of MIPs for Quercetin. The study presents a supplement to the separation, enrichment, and determination of Chinese traditional medicine in polar solvents which have considerable application prospects.

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