

# Synthesis and Evaluation of a Metal-Complexing Imprinted Polymer for Chinese Herbs Quercetin

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**ABSTRACT:** In this study, a metal-complexing imprinted polymer has been prepared using the complex of Quercetin and Co(II) (Qu-Co(II)) as template (Quercetin is a kind of flavonoid, which is a class of important active ingredients of Chinese herbs.). The polymers were synthesized in the mixed solution of methanol and tetrahydrofuran by photo- and thermal-initiated polymerization, respectively. The complex ratio between Qu and Co(II), as well as the ternary complexation of Qu, Co(II), and acrylamide, was assessed in differential UV absorption spectrometry. Fourier transform infrared spectra (FTIR) were useful to verify whether the

polymers prepared in two ways had the same molecular structure. The effect of initiation mode was studied by the transmission electron microscopy (TEM) and equilibrium binding experiments. Isothermal adsorption and selectivity experiments showed that molecularly imprinted polymers exhibited high affinity and specific recognition for Qu by introducing cobalt ion. © 2012 Wiley Periodicals, Inc. *J Appl Polym Sci* 000: 000–000, 2012

**Key words:** molecular imprinting; metal coordination; quercetin; Co(II); selective adsorption

## INTRODUCTION

The Chinese herbal has occupied an extremely significant position due to its own unique and notable effect in medical field around the world. The advanced science and technology provide effective means for research deeply of the Chinese herbs active components. However, there are rich diversity of active ingredients and analogues in complex structural herbal, so tremendous difficulties are faced in extraction and separation of them. An effective and rational technology of isolation and purification is a crucial premise to accelerate modernization process of Chinese medicine.

To enhance the molecular selectivity in isolation and purification method, the molecular imprinting technique (MIT) has been developed. Molecularly imprinted polymers (MIPs) are special polymers synthesized by MIT. They are polymerized among crosslinker and complex which was formed between template molecule and function monomer by covalent bond or noncovalent bond. Then MIPs have specific molecular recognition ability and high affinity

toward internal imprinting cavities and selective identification sites complemented to the template after elution.<sup>1</sup> High selectivity, ease of preparation, and economical synthesis of MIT make it competitive with other molecular recognition materials. Because of these advantages, it has been widely used in separation techniques,<sup>2–4</sup> chemical bionic sensors<sup>5,6</sup> and enzyme-like catalysis,<sup>7</sup> clinical drug analyses,<sup>8</sup> and membrane separation technology,<sup>9,10</sup> etc.

The metal coordination interaction has attracted wide attention due to its own advantages. The metal coordination bonds are much stronger and more stable than the hydrogen bonds in water or alcohol system. It is comparatively appropriate to the detection of herbs dissolved easily in high polar solvents. Meanwhile, the drug molecules binding to metal ions have characters of the high specificity and moderate combination and fracture. Obviously, metal-complexing imprinted polymer is quite suitable to be applied in the biological recognition systems.

Flavonoids are important active composition of traditional Chinese medicine and the widespread Quercetin (Qu) may be considered as a major dietary flavonoid. Like most herbal, the separation of Qu encounters similar obstacles.<sup>11</sup> Now there are some polymer materials that could be used for the adsorption of Qu such as macroporous adsorptive resins, polyamides, and silica gel. Macroporous adsorptive resins are a kind of organic polymer adsorbent. It can adsorb selectively effective ingredients of

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traditional Chinese medicine so as to achieve the purpose of the extract refined. Polyamides are considered as common flavonoid compounds adsorbent because of hydrogen bond formation between polyamides and flavonoids. Silica gel is used mainly for the separation of isoflavone, two hydrogen flavonoids, two hydrogen flavonol, and highly methylated (or acetylated) flavonoids and flavonols. Sometimes it can also be activated and used for separation of the larger polarity compounds after adding water. The macroporous adsorptive resins and polyamides are common commercial available materials because of their high adsorption quantity and fine stability. But they still have some disadvantages, such as excess organic residue, poor reproducibility, and complex pre-processing problem. Cobalt ions, as major components of vitamin B12, are one of essential microelements for people. In this article, based on the characteristic of MIT and the advantages of metal coordination, a new metal-complexing imprinted polymer was prepared successfully in methanol using Co(II)-Qu complex as template, the optimal initiation mode and recognition performances were assessed in detail. The research findings establish a solidify craft theoretical and the foundation of experiment for separation and enrichment.

## EXPERIMENTAL

### Materials

Qu, Rutin (Ru), and Naringenin (Na) were bought from Shanxi Huike Botanical Development Co. (Shanxi Province, China, 98%); acrylamide (AA) was obtained from Tianjin Chemical Reagent Research Institute (Tianjin, China); ethylene glycol dimethacrylate (EGDMA) was obtained from Shanghai Haiqu Chemical Co. (Shanghai, China). 2,2-Azo-bisobutyronitrile (AIBN) was purchased from Shanghai Hewei Co. (Shanghai, China); tetrahydrofuran, methanol, acetic acid ethanol, and cobalt chloride were supplied by Tianjin Damao Chemical Reagent Co. (Tianjin, China); all of the materials were of analytical grade. AA and AIBN were recrystallized in ethanol before use.

### Apparatus

All UV-vis spectra and adsorption experiments were performed using an UV-1200 Ultraviolet-visible spectrophotometer (Beijing Rayleigh Analytical Instrument Corp., China); Fourier transform infrared spectra (FTIR) analyses were taken on a TENSOR37-Fourier Transform Infrared Spectrometer (Bruker Co., Germany). An H-7650 transmission electron microscope (Hitachi, Japan) was used to observe the morphology of imprinted polymer. To synthesize,

prepare, and process the products, there were many other used equipments including: WE-1 thermostat water bath oscillator (Tianjin Honour Instruments Inc., China), SK5200 ultrasonic vibration generator (Shanghai Kudos Ultrasonic Instrument, China), TG16-WS(1650D), supercentrifuge (Shanghai Luxian-gyi Gentrifuge Instrument, China.), long-wave (365 nm) ultraviolet lamp (Tianjin Zijin Special Photo Source), Soxhlet extractor, etc.

### Ultraviolet spectrum analysis

A series of test solutions, with a fixed concentration of Qu (0.025 mmol/L) and different dosages of Co(II), were prepared in methanol. The ratios of the molar concentration between Qu and Co(II) among these solutions were: 1 : 0, 1 : 0.3, 1 : 0.5, 1 : 0.7, 1 : 0.9, 1 : 1, 1 : 2, 1 : 3. They were shaken for about 2–3 h by ultrasonic oscillations and then rested for 15 min with the purpose of ensuring the full reaction between Qu and Co(II). The changes in absorbance and difference absorption spectra of these solutions were obtained by UV-spectrometer in the wavelength range 200–600 nm.

A set of test solutions with variant amount of AA and Qu-Co(II) were prepared in methanol in which Qu was kept in fixed concentration (0.025 mmol/L) and molar ratio of Qu/Co(II) was retained as 1 : 2. The absorption spectra of these solutions were determined with corresponding Qu-Co(II) solutions as references by the similar method above.

### Preparation of the polymers

For the preparation of (Qu-Co(II))-imprinted polymer, 1 mmol Qu and 2 mmol Co(II) were dissolved in 15 mL of tetrahydrofuran/methanol ( $v/v = 1 : 3$ ) mixed solution into in a thick-walled glass tube with the ultrasonic oscillation for 2 h. To this AA was added with a further ultrasonic oscillation for 2 h for achieving sufficient interaction of the template molecule, the metal ions, and the functional monomer. Then 40 mmol crosslinking monomer EGDMA and 50 mg AIBN as initiator were added. After shaking 10 min for homogeneity, the mixture was sparged with nitrogen for 15 min to remove the dissolved oxygen, and then immediately sealed under vacuum. The polymerization was allowed to continue in a water bath at 60°C or under ultraviolet lamp (365 nm) at 15°C for 24 h. The resultant bulk rigid polymers were crushed and ground to collect 75  $\mu\text{m}$  particles. Next, the obtained particles were removed by decantation in ethanol, followed by extraction in a Soxhlet apparatus using acetic acid/methanol mixed solution ( $v/v = 1 : 9$ ), in order to remove residual template molecules, until the template could no longer be detected. 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 and distilled deionized water repeatedly and dried to constant weight under vacuum at 60°C (marked as P<sub>Th</sub> and P<sub>Ph</sub>).

Non-imprinted polymers (NIPs) were prepared simultaneously using the same protocol just in the absence of the template molecules or metal ions, denoted hereafter as P<sub>Q-Th</sub>, P<sub>Q-Ph</sub>, P<sub>N-Th</sub>, and P<sub>N-Ph</sub>, respectively. ("Q" stands for no metal ions, "N" stands for no the template molecules Qu and metal ions.)

### Characterization

The prepared polymers as test samples were grinded into fine powder and dried absolutely. Then made them by pressed disc method using KBr and characterized by FTIR. The samples contained Qu, MIPs before elution and after elution, and NIPs by two initiated modes.

An H-7650 transmission electron microscope was used to obtain morphology images of MIPs and NIPs samples. For transmission electron microscopy (TEM) analyses, samples with a certain degree of fineness were put into ethanol to prepare suspension of an appropriate concentration. The power samples were scattered for 15 min in the ultrasonic dispersion and then hanged to professional copper mesh quickly. When ethanol was completely volatilized, the samples could be observed by TEM.

### Adsorption dynamics of the MIPs

A 50 mg of imprinted polymers (P<sub>Th</sub> and P<sub>Ph</sub>) were weighed into 5 mL of methanol solution containing 2 mmol/L of Qu/Co(II) ( $n/n = 1 : 2$ ) in 25-mL conical flasks, respectively. The solutions were oscillated with constant temperature bath at 25°C for 4 h. During this period, samples were collected at different intervals by high-speed centrifuge. After the UV-vis spectrophotometer analysis of each samples, the concentration of Qu in the methanol solution ( $c_t$ ) was obtained. Then the binding amount ( $Q$ ) was calculated according to the eq. (1).

$$Q = (c_0 - c_t) \times V/m \quad (1)$$

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.

### Equilibrium binding experiments

To evaluate the binding properties of the imprinted polymers, equilibrium binding experiments were

carried out. Fifty milligrams of the polymers were weighed into a 25-mL conical flask and mixed with 5 mL of Qu/Co(II) or Qu methanol solution, the concentration of which varied from 0.25 to 7 mmol/L. The mixtures were oscillated with constant temperature bath at 25°C for 12 h, and then the concentration of Qu in the solution was determined and binding amount ( $Q$ ) were calculated using the same method above.

### Adsorption experiment

The dry MIPs (50 mg) were mixed with 5 mL of a fixed concentration of Qu solution, Ru solution, and Na methanol solution (2 mmol/L) in 25-mL conical flasks, respectively. In order to investigate the adsorption of MIPs to different substrates, the same method above was appropriate to calculate dissociation coefficient ( $K$ ) and separation factor ( $\alpha$ ). Dissociation coefficient ( $K$ ) and separation factor ( $\alpha$ ) were calculated based on the following equations [eqs. (2) and (3)], respectively.

$$K = C_p/C_s \quad (2)$$

$$\alpha = K_i/K_j \quad (3)$$

where  $C_p$  and  $C_s$  are the initial substrate concentration ( $\mu\text{mol/g}$ ) and the equilibrium concentration (mmol/L), respectively. And  $K_i$  is dissociation coefficient of the template molecule;  $K_j$  is dissociation coefficient of substrate. Furthermore, the value of  $\alpha$  is 1.0 when  $i = j$ .

## RESULTS AND DISCUSSION

### Study of the ternary coordination between Qu, Co(II), and AA by UV spectrophotometry

UV-vis spectra generated by electronic transitions in organic molecules are a kind of common analytics. Some groups containing valence electron with lower excitation energy could lead to electron transition. These groups called chromophore are confined to certain functional groups. Qu shown in Figure 1 has a high degree of delocalization and intact  $\Pi$  bond conjugated system. Qu exhibits two absorption peaks at 255 nm and 372 nm, respectively belonging to  $n-\pi^*$  (A ring) electronic transition and  $\pi-\pi^*$  (B ring) electronic transition, corresponding to two chromophores: benzoyl chromophore produced the absorption band (II) at 255 nm, while cinnamoyl chromophore generated the absorption band (I) at 372 nm.<sup>12</sup> There are phenolic hydroxyl and enol hydroxy, the 3-hydroxyl-4-ketone in C ring, 3',4'-dihydroxyl in B ring, and 5-hydroxyl-4-ketone as electron donors, in the Qu. When Qu coordinates with metal ions, its whole conjugated system extends and

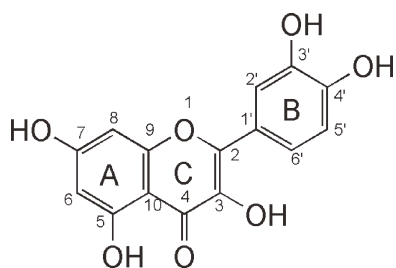


Figure 1 Chemical structure of Qu.

maximum wavelength red-shifts because of the types of hydroxyls.

The coordination between Qu and Co(II) is researched by equimolar continuous change method in methanol. The UV-vis spectra of free Qu and Qu-Co(II) mixtures with different ratios is shown in Figure 2. Band (I) weakened red-shift from 372 nm to 427 nm and then strengthened in the intensity of absorption peak at 427 nm with the addition of Co(II), these are obvious features of the formed complex. According to the structure of Qu and Figure 2, it is deduced that metal ions coordinate with 3-hydroxyl-4-ketone of C ring firstly and 3',4'-dihydroxy subsequently. The coordination makes electron density lower and mobility of  $\pi$  electrons enhanced in conjugated system of the band (I).<sup>13</sup> Because electron transition is excited just by lower energy, the red shift is generated in band (I) for the formation of the absorption peak at 427 nm. Nevertheless, there is a steric hindrance after coordination of 3-hydroxyl-4-ketone and 3',4'-dihydroxyl. 5-Hydroxyl-4-ketone always involved in intra-molecular hydrogen bond-

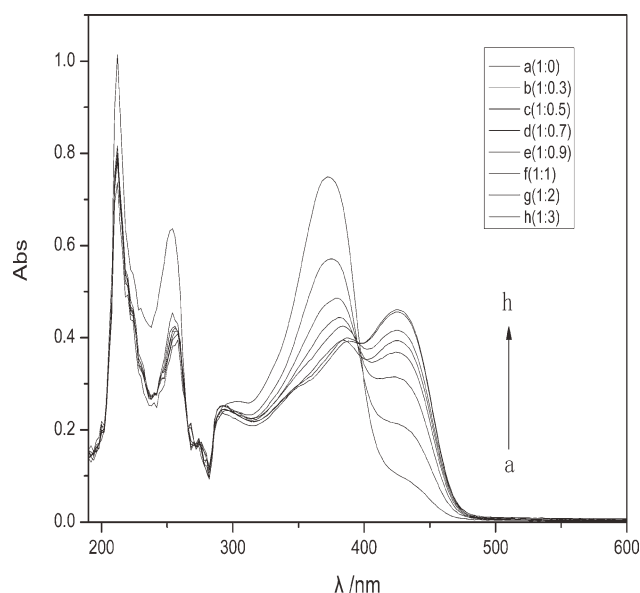


Figure 2 UV-vis spectra of Qu and Qu-Co(II) mixtures with different molar ratios in methanol. \*The (a-h) corresponds to 1 : 0, 1 : 0.3, 1 : 0.5, 1 : 0.7, 1 : 0.9, 1 : 1, 1 : 2, 1 : 3, respectively.

ing. Thus it is difficult to participate in coordination for 5-hydroxyl-4-ketone.

Moreover a complex is generated with Qu and Co(II) in competition, as evident from isosbestic point at 394 nm in Figure 2. When Co(II)/Qu molar ratio reach 2, absorption spectra changes insignificantly, proving that coordinating ability attains saturation. The optimal ratio  $n$  (template molecule) :  $n$  (metal ions-Co(II)) = 1 : 2 is obtained.

In this experiment, the samples were prepared at a fixed concentration (0.025 mmol/L) of Qu-Co(II) (1 : 2) with gradually increasing amounts of AA in methanol. The UV absorption spectra of AA with background correction for Qu-Co(II) (1 : 2) solvent were shown in Figure 3. The addition of AA to the methanol solutions of Qu-Co(II) (1 : 2) resulted in the formation of tertiary complex systems as evident by a decrease in the intensity of maximum absorption peak (257 nm) and the sharp increase in the intensity of maximum absorption peak (220 nm) shifting to the long wavelength region (Fig. 3). The results indicate that the Qu-Co(II) complex formed have a strong interaction with AA in methanol.

### IR spectra studies

The relevant structure and characteristics of molecules can be analyzed by the information from IR absorption spectra. Figure 4 shows the infrared spectrum of prepared polymers. There are four infrared spectral lines in each figure, where a and c correspond to the Qu and blank polymers, while b and d represent the imprinted polymers after and before elution, respectively.

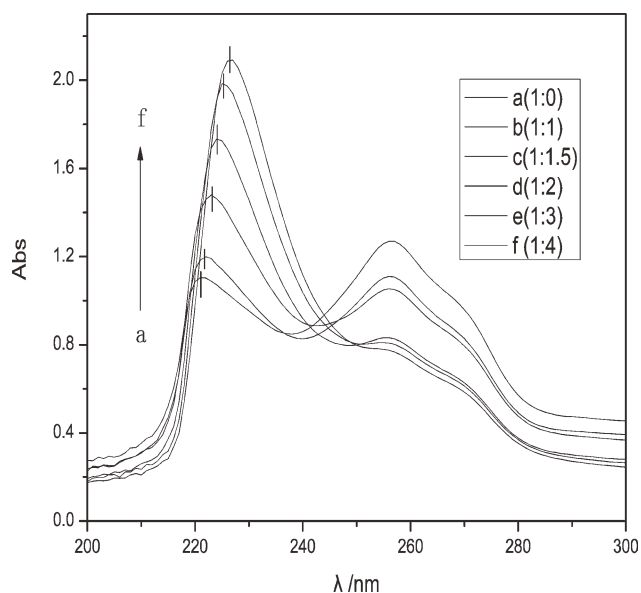
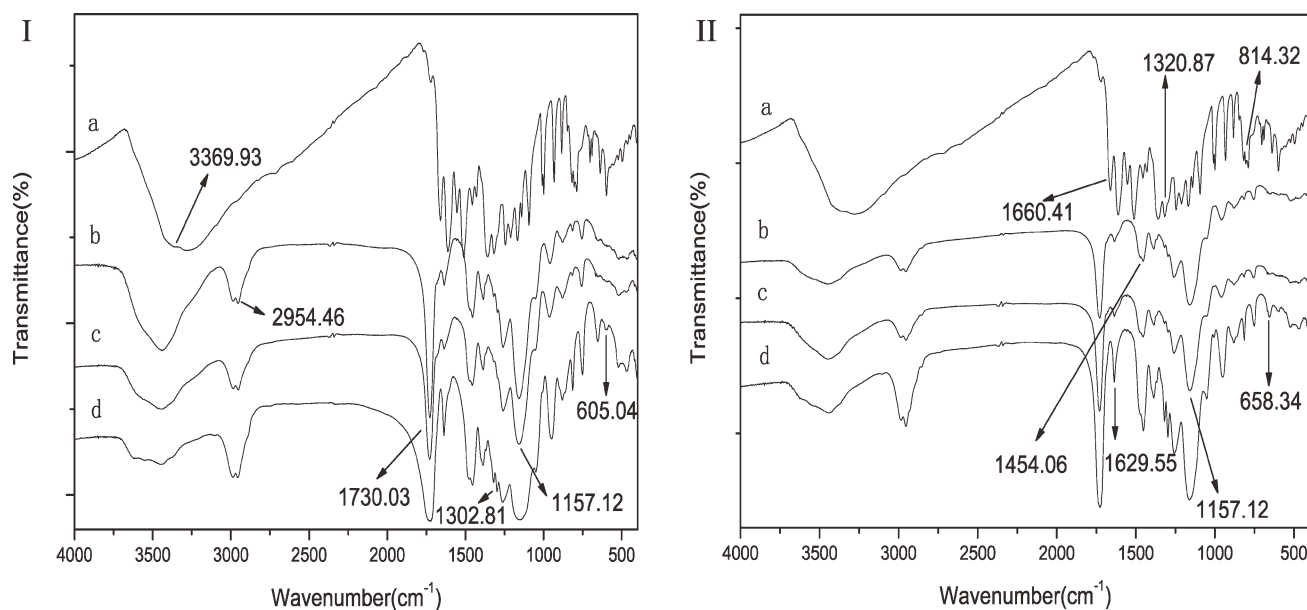


Figure 3 UV-vis spectra of Qu-Co(II) with different Qu-Co(II)/AA molar ratios in methanol. \*The (a-f) corresponds to 1 : 0, 1 : 1, 1 : 1.5, 1 : 2, 1 : 3, 1 : 4, respectively.



**Figure 4** IR spectra of Qu and polymer products. (I) Thermal-initiated mode. (II) Photo-initiated mode. (a) Qu; (b) MIPs after elution; (c) NIPs; (d) MIPs before elution.

Control of the synthetic route of polymers (see Fig. 5) and a further analysis for products' IR was carried on. It can be observed from the Figure 4 that b, c, d lines almost had the similar absorption peak formed in each region. For example, the features at  $2954\text{ cm}^{-1}$  and  $1629\text{ cm}^{-1}$  indicated C—H ( $\nu_{\text{C-H}}$ ) and C=C stretching vibration ( $\nu_{\text{C=C}}$ ), respectively. The bands at  $1730\text{ cm}^{-1}$  resulted from C=O stretching vibrations ( $\nu_{\text{C=O}}$ ) of the EGDMA. The C—O—C ester group of EGDMA ( $\nu_{\text{C-O-C}}$ ) was reflected at  $1157\text{ cm}^{-1}$ . The characteristic stretching C—N mode of the amide groups occurred at  $1454\text{ cm}^{-1}$ . NIPs were prepared by AA and EGDMA with no template molecules or metal ions, so the absorption peak intensity at  $1660\text{ cm}^{-1}$  corresponds to  $\nu_{\text{C=O}}$  of AA in line c is much weaker. MIPs after elution and NIPs had the same functional groups, confirmed by similar line b and line c. The existence of molecular structure above manifested that products of polymers were prepared by EGDMA and AA crosslinking copolymerization under the synthesis conditions and having the functional group (amide functional group) matched with the template removed completely.

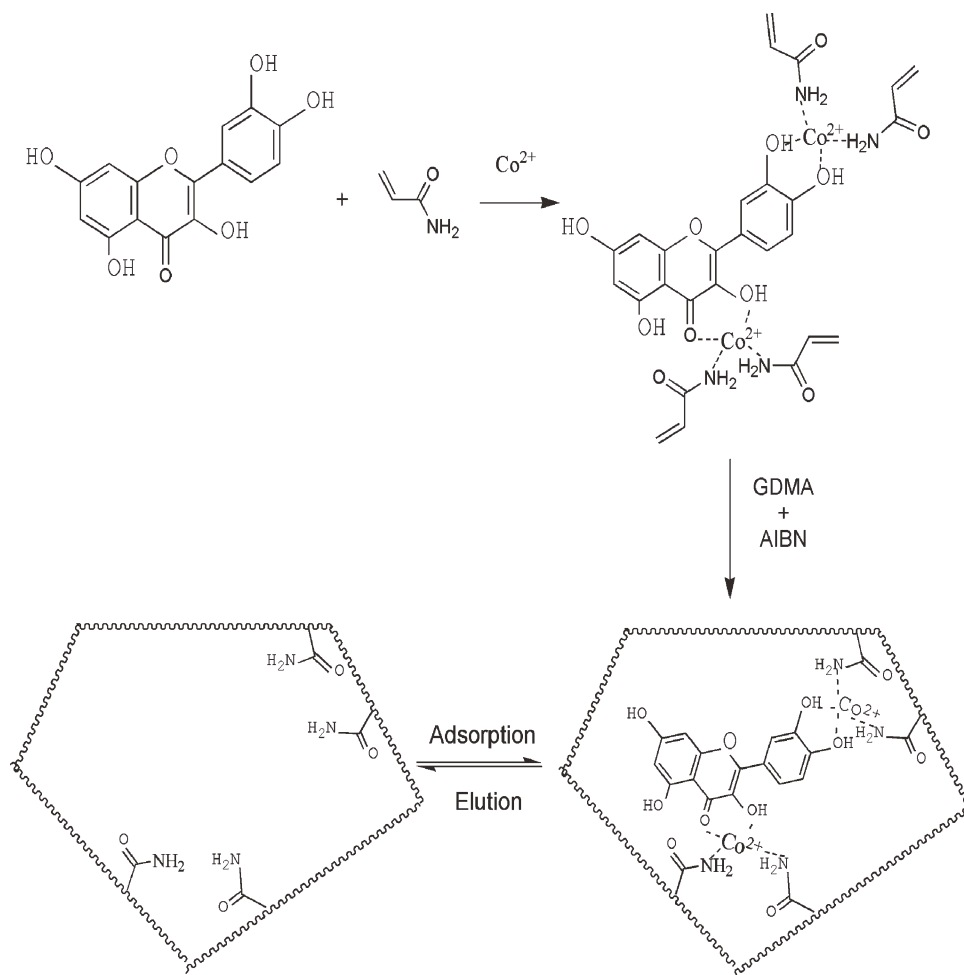
In addition, lines b and d, belonging to MIPs after and before elution, were different. There were obvious absorption peaks at  $1321\text{ cm}^{-1}$  and  $1302\text{ cm}^{-1}$ , and new absorption peaks at  $814\text{ cm}^{-1}$ ,  $658\text{ cm}^{-1}$  and  $605\text{ cm}^{-1}$ . These conformed to the characteristics of Qu. In the spectrum (Fig. 4), there were two characteristic bands which had been identified as Qu located at  $3370\text{ cm}^{-1}$  and  $1660\text{ cm}^{-1}$ , respectively, corresponding to stretching ( $\nu_{\text{O-H}}$ ) modes and  $\nu_{\text{C=O}}$ . As metallic coordination polymers were formed,  $\nu_{\text{O-H}}$  moved toward blue wavelength and  $\nu_{\text{C=O}}$

disappeared. These results suggested that the complex of Qu and Co(II) was successfully fixed into AA and EGDMA copolymer and it could be removed completely to make polymers present high identification and re-adsorption for the imprinting molecule. As can be seen, there was almost no difference between Figure 4 (I) and (II). In other words, polymers synthesized in two initiated modes had the same molecular structure.

### Characterization of the polymers

MIPs had been prepared through radical polymerization in general. The initiation methods in preparing process have influence on the macro-properties and micro-structure of polymer materials to some extent. The morphology and network structure of polymers were studied by transmission electron microscopy (TEM), while the effect of microstructure of polymers on the binding capacities was researched by adsorption kinetic curve. Figures 6 and 7 show the results.

It is known from the four photos in Figure 6 that the high crosslinked polymers synthesized by MIT were porous materials with three-dimensional meshwork structure. However, morphology of MIPs prepared by photo- and thermal-initiated polymerization are markedly different by comparison. Figure 6(a) showed a nonuniform distribution, some large pores with lots of tiny pore structures and many uneven thickness of matrix material. Though Figure 6(c) was not perfect, its uniform size pore structures distribute equably. The whole seemed much more irregularly

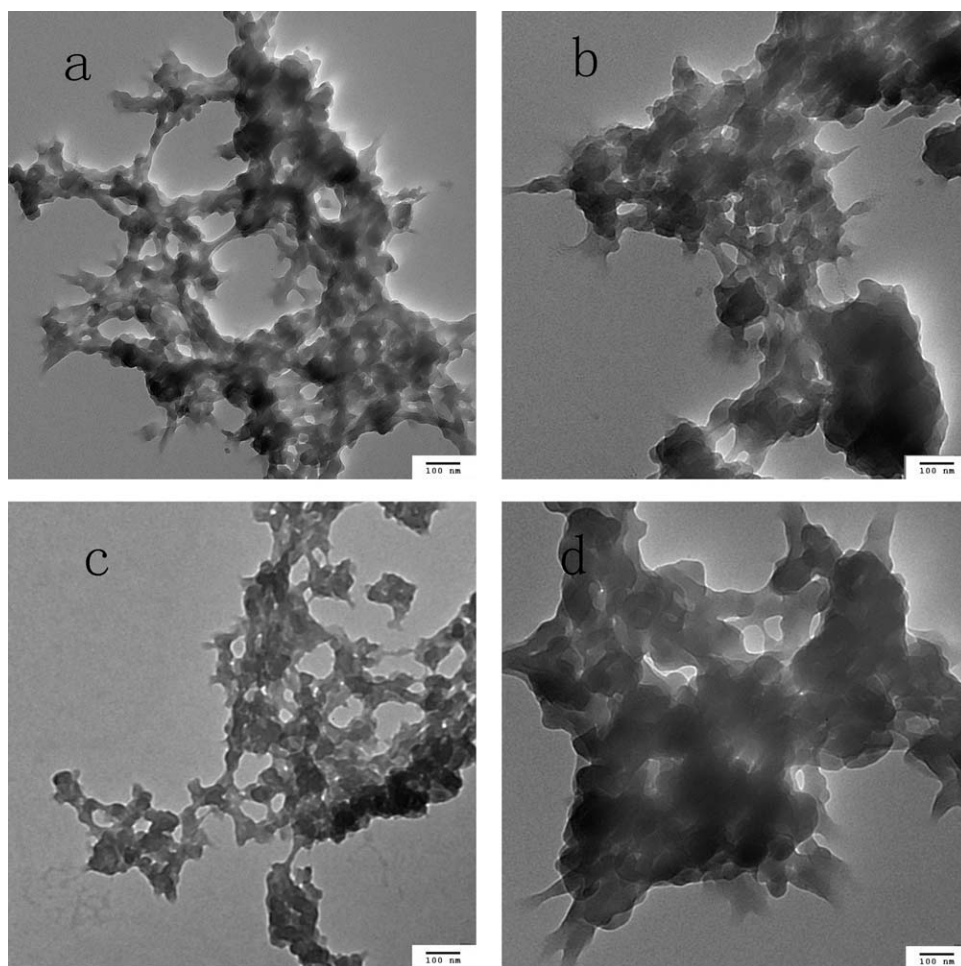


**Figure 5** The schematic representation of Qu-Co(II) molecular imprinting process.

three-dimensionally cross-linked network in Figure 6(c). As shown in image a (in Fig. 6), some large pore structures with uneven thickness of matrix material was formed apparently in the thermal-initiated polymer  $P_{\text{Th}}$ , and the kinetic curve a (in Fig. 7) indicated that the adsorption of  $P_{\text{Th}}$  increased quickly and was very easy to reach saturation with lower adsorption amount in a short period of time. In fact, an actual reaction temperature during the preparation is much higher than original initiation temperature, especially heating thermal initiation. And a high temperature in the polymerization system, which can make prepared polymers in large meshes, results in high vapor pressure of the solvents and stronger effect on the pore formation, relatively. The macroporous structure of materials with a large specific surface area makes the recognition sites in cavities easily accessible. So it helps the template molecule spread into deep parts of MIPs and a high adsorption speed has been obtained. Furthermore, the supra-molecular complex stability, which formed by template and functional monomer, is reduced by high temperature at the initial stage of polymerization. It leads to poor imprinting effect

and low adsorption quantity of  $P_{\text{Th}}$ . In addition, high temperatures result in a decrease in the number of selective recognition points, increasing the number of nonspecific interactions.

By contrast, the molecular imprinting polymer  $P_{\text{Ph}}$ , which was synthesized in photo-initiated polymerization, had advantages in microscopic morphology and sorbent behavior. Photo-initiation in low-temperature is contributed to stable complex formation between template molecule and functional monomer. After elution, MIPs have the matrix matching template molecule in neat shapes and adequate recognition sites. So, low temperatures induce the formation of polymers with higher recognition capacities than those prepared at elevated ones. As the image c shows in Figure 6,  $P_{\text{Ph}}$  presents regular three-dimensionally crosslinked network. This means that fully accessibility of binding sites, excellent mass transport and effective identification. As the kinetic curve b presents in Figure 7, it had the same trendline of change as curve a, but the significant differences in both curves are equilibration time and equilibrium adsorption quantity. Though  $P_{\text{Ph}}$  took longer



**Figure 6** TEM images of (a)  $P_{Th}$ , (b)  $P_{N-Th}$ , (c)  $P_{Ph}$ , (d)  $P_{N-Ph}$  by different initiated modes.

time for Qu-Co(II) adsorption to attain an equilibrium than  $P_{Th}$ ,  $P_{Ph}$  shows higher adsorption amount. And error bars in curve b showed that  $P_{Ph}$  has fine hydrodynamic properties and high adsorption capacity.

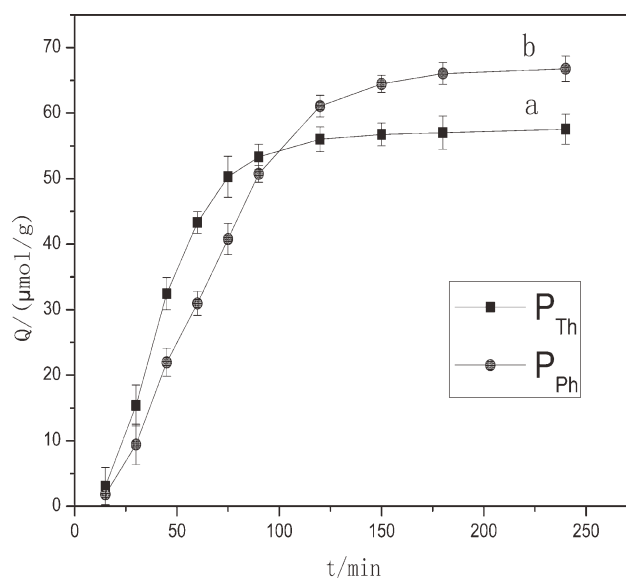
Figure 6(b,d) present separately the morphology of blank polymers  $P_{N-Th}$  and  $P_{N-Ph}$ , distinguished from MIPs. Because the functional monomers arranged random during  $P_{N-Th}$  and  $P_{N-Ph}$  prepared process, the blank polymers have not complete and neat pore volume. Visible, appropriate synthesis approach of initiation mode plays an important role in construction and properties of the Qu-Co(II) imprinted polymers.

#### Adsorption isotherm

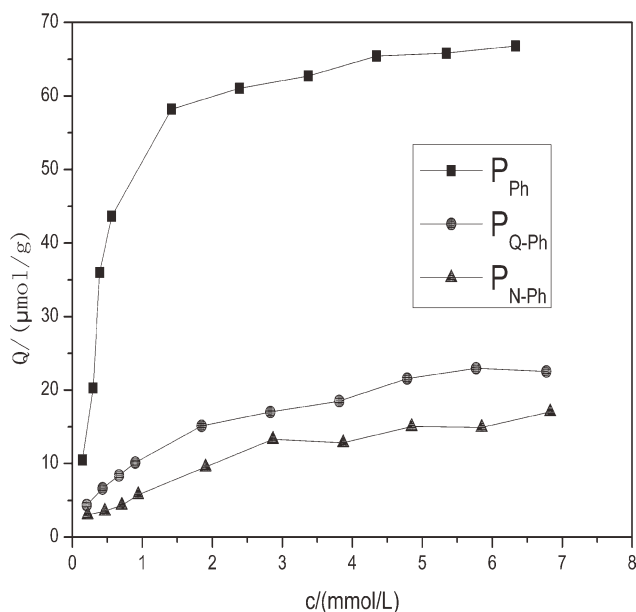
The equilibrium adsorption capacity ( $Q$ ) of series of polymers to Qu-Co(II) and Qu in different concentrations (0.25–7 mmol/L) was investigated by equilibrium binding experiment. The adsorption isotherms were depicted in Figures 8 and 9 for Qu-Co(II) complex and Qu, respectively.

As can be seen, experimental data were fitted by the monolayer adsorption of Langmuir model.<sup>14</sup> By

comparison of the three adsorption isotherms of  $P_{Ph}$ ,  $P_{Q-Ph}$ , and  $P_{N-Ph}$  in Figure 8, it shows an increase in their adsorption abilities when the equilibrium

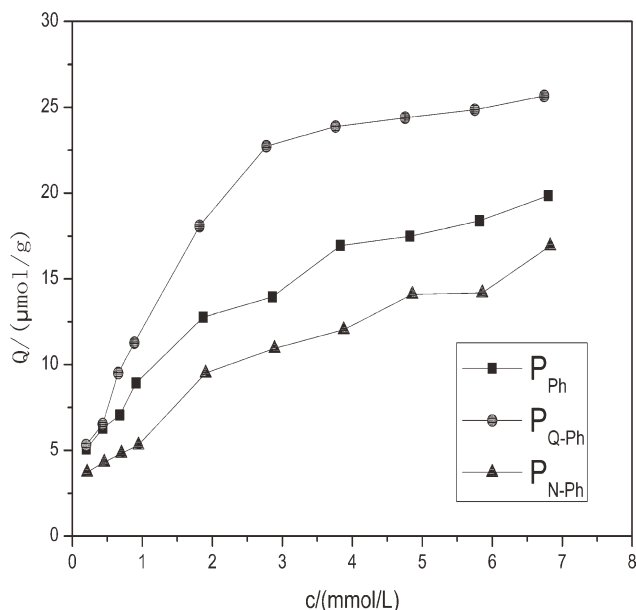


**Figure 7** Adsorption dynamic curves of MIPs. ( $V = 5$  mL,  $C = 2$  mmol/L,  $T = 25^\circ\text{C}$ ).



**Figure 8** Adsorption isotherms of different polymers to Qu-Co(II).

substrate concentration was increased. However, the combination amount of P<sub>Ph</sub> to Qu-Co(II) is always greater than those of P<sub>Q-Ph</sub> and P<sub>N-Ph</sub>, obviously. It is clear that P<sub>Ph</sub> exhibits better binding ability and the adsorption reached saturation in high concentration range. In many of the receptor binding assay,<sup>15</sup> as the curve of nonselective combined volume to the initial concentration increases linearly, it is difficult to reach saturation as a rule. The statement further assures us that adsorption of P<sub>Q-Ph</sub> and P<sub>N-Ph</sub> to the Qu-Co(II) complexes were nonselective, and P<sub>Ph</sub> displays a selective absorption. P<sub>Ph</sub> and P<sub>Q-Ph</sub> were

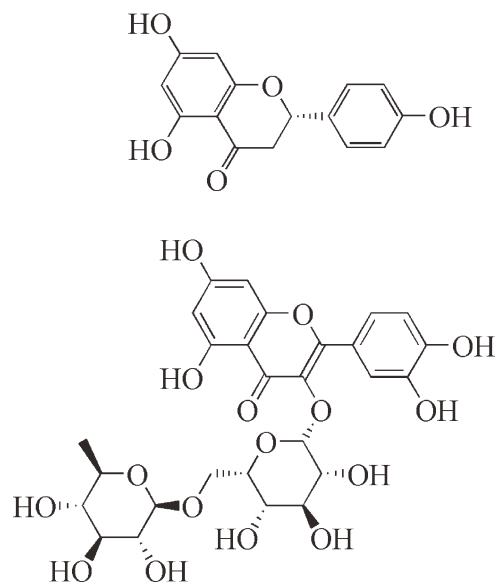


**Figure 9** Adsorption isotherms of different polymers to Qu.

prepared by Qu-Co(II) complexes and Qu as template, P<sub>N-Ph</sub> had no template. Because the structure-activity predetermination and specific identification are notable features of molecular imprinting technology, the corresponding imprinting holes which are complementary with the template have been left in them after elution. In the presence of Co(II) in base solution, Qu-Co(II) complexes formed is the fittest to the specific structure holes of P<sub>Ph</sub>. So P<sub>Ph</sub> showed more impressive adsorption capacity than others. The imprinted polymers are really useful for the separation if cobalt has to be involved.

Meanwhile, as demonstrated, the maximum binding capacity of P<sub>Ph</sub> to the Qu-Co(II) complex is higher than that of P<sub>Ph</sub> to Qu as shown in Figure 9. The reason is that matrix of MIPs is complementary with the template not only about stereo shapes but also functionality sites. The imprinted molecules P<sub>Ph</sub> whose template is Qu-Co(II) complexes had the corresponding cavities and specific binding sites after template extraction. Good selectively binding capacity of P<sub>Ph</sub> depends on these imprinted cavities. This result is in agreement with molecular imprinting effect. Furthermore, P<sub>Ph</sub> identifies Qu-Co(II) complexes rather than Qu through the metal coordination. This indicates that Co(II) plays the role of a regulation to specific recognition of P<sub>Ph</sub>.

In Figure 9, binding capacity of P<sub>Q-Ph</sub> to Qu is far less than that of P<sub>Ph</sub> in Figure 8. P<sub>Q-Ph</sub> was prepared by Qu as template. The molecule realization of P<sub>Q-Ph</sub> is related to the stable supra-molecular complex formed through the noncovalent bond interaction between Qu and AA. The strength of noncovalent complexes directly affects the stability of MIPs and further impacts on imprinting effect of the imprinted



**Figure 10** Chemical structures of Quercetin's structural analogues (Rutin and Naringenin).



**TABLE I**  
**Values of  $K$  and  $\alpha$  of Different Substrates**

| Substrates | $P_{Ph}$ |          | $P_{Q-Ph}$ |          | $P_{N-Ph}$ |          |
|------------|----------|----------|------------|----------|------------|----------|
|            | $K$      | $\alpha$ | $K$        | $\alpha$ | $K$        | $\alpha$ |
| Qu         | 48.73    | 1.00     | 10.82      | 1.00     | 9.71       | 1.00     |
| Ru         | 7.54     | 6.46     | 9.05       | 1.19     | 10.57      | 0.93     |
| Na         | 8.76     | 5.56     | 9.77       | 1.11     | 9.04       | 1.07     |

polymer to template. So the low-polarity solvents are frequently used in the preparation process of noncovalent imprinted polymer. In the experiment, methanol, strong polar solution, may damage or disturb hydrogen bond or electrostatic interaction between Qu and AA. It leads to less binding capacity of  $P_{Q-Ph}$  to Qu than that of  $P_{Ph}$  to Qu-Co(II). Based on comparison of the experimental results and aforementioned analysis found, we could verify that metal coordination bond is stronger than noncovalent bond, such as hydrogen bond, van der Waals force and so on. Metal coordination bond is so stable that it is more adaptive to prepare the imprinted polymers in strong polar solvents. And it also reveals that the interaction between template and functional monomer has great influence on especial selectivity and predominant recognition of MIPs.

### Adsorption selectivity

To further confirm the imprinting effect and extreme selectivity of the Qu-Co(II) MIPs, its ability to resolve structural analogs was also measured by the equilibrium adsorption method. Qu and its analogs Ru and Na, which have almost similar molecular structures (Fig. 10), were used for this study. The obtained  $K$  and  $\alpha$  values are listed in Table I.

As can be seen, the calculation of the separation factor ( $\alpha$ ) of  $P_{Q-Ph}$  and  $P_{N-Ph}$  to Na and Ru are all approximately equal to 1. This shows that adsorption abilities of  $P_{Q-Ph}$  and  $P_{N-Ph}$  are almost the same. It could be attributed to the electrostatic attractions developed among  $P_{Q-Ph}$ ,  $P_{N-Ph}$  and substrates in a nonspecific manner.

Table I also indicates that  $P_{Ph}$  presented highest dissociation coefficient ( $K$ ). So it is confirmed that  $P_{Ph}$  obviously exhibited higher binding affinity for Qu than the structurally related compounds. The imprinting methods create a micro-environment based on shape selection and position of functional groups that identifies the imprinting molecule. Though the chemical structures of Ru and Na are similar with Qu, their 3-hydroxyl and 4-carbonyl cannot form triple complex with Co(II). The space volume of Ru is much larger than Qu because of

glucose and rhamnose joined with 3-hydroxyl in its composition. Na consists of A ring and C ring excluding double bond in the same plane, so the multi-dimensionality of spatial configuration is much stronger. Because the matrix of MIPs is matched to the size of Qu, it is very difficult for the molecules with different dimension to enter the matrix. So MIPs represent weak binding capacity to Ru and Na. From above results, we conclude that the selectivity of prepared MIPs is doubtless and definite.

### CONCLUSIONS

In summary, a metal-complexing imprinted polymer was prepared successfully in two polymerization approaches of thermal- and photo-initiated mode. It is investigated by UV-vis spectra that the formation of metal coordination interaction and optimal ratio  $n$  (Qu) :  $n$  (Co(II)) = 1 : 2. Through TEM and binding assays, polymers synthesized by photo-initiated approach were quite different with the thermal-initiated ones. It was confirmed for MIPs with improved molecular recognition and enhanced specific adsorption in the presence of Co(II) by isothermal adsorption and selective adsorption experiments. These findings provide an important theoretical basis and technical support for the separation and detection of Chinese traditional medicine samples with potential application prospects.

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