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Designing and Preparing of Quercetin Surface-Imprinted Material and Its Molecular Recognition Characteristics

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ABSTRACT: Quercetin is an important compound of flavonoids. In this work, quercetin molecule surface-imprinted material with high performance was prepared using a novel surface-imprinting technique of "synchronously graft-polymerizing and imprinting." The modified micron-sized silica gel particles containing amino groups were used as matrix, methacrylic acid (MAA) was used as functional monomer, and *N*,*N*′-Methylenebisacrylamide (MBA) was used as crosslinker. In dimethyl formamide solution of quercetin, MAA molecules arranged automatically around the template quercetin molecule by right of hydrogen bonding interactions of two type, ordinary hydrogen bond and π-type hydrogen bond. By initiating the surface-initiating system of $-NH_2/S_2O_8^{2-}$, the graft/crosslinking polymerization of MAA on SiO_2 particles and the quercetin molecule surface-imprinting were simultaneously carried out, forming quercetin molecule surface-imprinted material MIP-PMAA/SiO_2. With another two flavonoids, rutin and genistein, as contrasting substances, the molecule recognition character of the quercetin molecule surface-imprinted material MIP-PMAA/SiO_2 was investigated with batch and column methods. The experimental results show that the imprinted material MIP-PMAA/SiO_2 possesses special recognition selectivity and excellent binding affinity for quercetin molecule. The binding capacity of MIP-PMAA/SiO_2 for quercetin is 0.325 mmol/g, and its selectivity coefficients for quercetin relative to rutin and genistein are 7.69 and 4.40, respectively. The main conditions of imprinting process affect the property of MIP-PMAA/SiO_2 greatly, and the optimal molar ratio of monomer MAA to crosslinker MBA is 7 : 1 and appropriate molar ratio of monomer MAA to template quercetin is equal to 6 : 1. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41112.

KEYWORDS: grafting; molecular recognition; separation techniques

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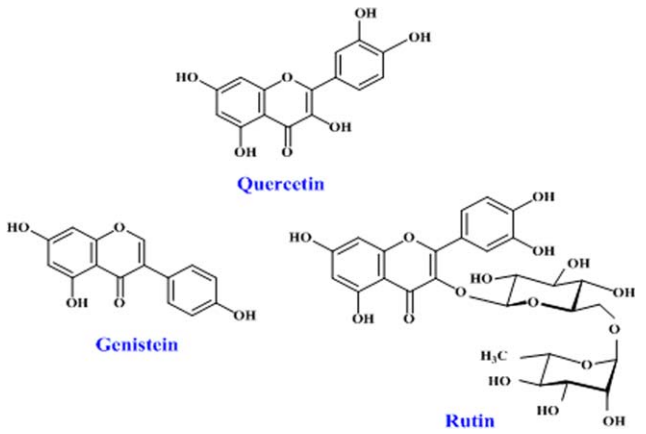
INTRODUCTION

Molecular imprinting is a technique for creating polymeric matrices containing tailor-made receptors. As the products of molecular imprinting, molecularly imprinted polymers (MIPs) are a kind of synthesized smart polymeric materials, in which a great deal of highly specific micro-cavities designed for a target molecule (i.e., template molecule) is distributed, and these cavities are complementary to the target molecule in shape, size, and functionality. Therefore, MIPs have specific molecular recognition ability and high binding affinity for the template molecule,[1–4] so that MIPs are often called artificial antibodies or receptors. MIPs also possess other advantages such as anti-interference property, thermal and chemical stabilities, and robustness, so they are called "plastic antibodies." These characteristics allow imprinted materials to be used as recognition elements in separation, chiral resolution, catalysis, bioassay, sensors, drug delivery, and so on. In particular, in recent years, the molecular imprinting solid-phase extraction (MISPE) technique with MIPs as highly selective solid extraction sorbents have been widely used in separation, purification, preconcentration, and determining.[5–8]

Flavonoids are a diverse group of polyphenolic compounds present in plants, and they are polyphenolic compounds that contain a 15-carbon flavone skeleton and represent a large group of secondary plant metabolites. They have various physiological and biological activities, including anti-oxidative, radical scavenging, anti-inflammatory, anti-allergic, and anti-carcinogenic properties.[9–11] It has been found that flavonoids can generate particular interest with regard to human health effects including antioxidant activities, protection of cardiovascular diseases, cancer prevention, and so on,[12,13] and so the extraction and separation of flavonoids have attracted much attention of researchers. Quercetin is the most active compound in flavonoid family owing to its proposed beneficial effects in a wide range of diseases, for example, cardiovascular and inflammatory disorders and cancer therapy,[10,14] and it widely occurs in leaves, fruits, and flowers of many plants. The crude extracts of flavonoids are first obtained from plant tissue with solvent



Scheme 1. Molecular structures of three kinds of flavonoids. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

extraction method including various strengthened solvent extraction method such as microwave-assisted extraction and ultrasound-assisted extraction and so on, and then the target flavonoid component is gained through further separation and purification steps. There are several methods for the separation of crude extracts, such as liquid–liquid extraction, macroporous resin adsorption, various column chromatography techniques with general solid materials as well as high-speed counter-current chromatography.[15–18] These methods have some disadvantages, such as a lack of selectivity, inefficiency, taking longer to handle, and/or more solvent consumption. Recently, MISPE have been developed for the separation of natural products, and it has been also applied in the separation of flavonoids from extracts.[19,20] However, these imprinted materials used in the separation of flavonoids are almost all of the imprinted polymers prepared with the conventional method, entrapment way. The MIPs prepared by entrapment way have some disadvantages,[21,22] such as small binding capacity, poor site accessibility, slow mass transfer, and irregular materials shape and so on, and they seriously affect the performance of MIPs. Developing molecule surface-imprinting technique is an effective approach to enhance the performance of MIPs.

In this work, a new surface-imprinting technique of "synchronously graft-polymerizing and imprinting" put forward by our group[23,24] is introduced into the preparation of quercetin-imprinted material. Methacrylic acid (MAA) was used as functional monomer, micron-sized silica gel particles were used as matrix, and quercetin surface-imprinted material was prepared by right of two intermolecular interactions between MAA and quercetin molecule, ordinary hydrogen bond and π-type hydrogen bond. With another two flavonoids, rutin and genistein, which are structurally similar to quercetin and whose structures of the three kinds of flavonoids are schematically presented in Scheme 1, as contrast substances, the molecular recognition characteristics of the quercetin surface-imprinted material was researched in depth.

The experimental results show that such research is successful, and the prepared quercetin surface-imprinted material has very high recognition selectivity for quercetin relative to two contrast substances. As far as we know, the quercetin surface-imprinted materials as well as other flavonoid surface-imprinted materials are still rarely reported. It is significant to introduce the new surface-imprinting technique into the preparation of flavonoid substance surface-imprinted materials, and it can enhance the efficiency of solid extraction for the more effective separation of flavonoids, namely the separation of flavonoids can be effectively realized by MISPE with flavonoid surface-imprinted materials as solid phase extractants. Such investigation is significant in the molecular design and preparation of imprinted polymer materials with high performance, and it is valuable in the separation and purification of expensive natural medicines.

EXPERIMENTAL

Materials and Instruments

Silica (about 125 μm of diameter), γ-aminopropyltrimethoxysilane (AMPS), ethylene dimethacrylate, ammonium persulfate (APS), quercetin, rutin, and genistein were purchased from Chinese companies. MAA was obtained from Beijing Chemical Reagent Company, and was purified by distillation under vacuum before use. Other reagents were also purchased from Chinese companies.

The instruments used in this study were as follows: Perkin-Elmer 1700 infrared spectrometer (Perkine-Elmer Company); LEO-438VP scanning electronic microscope (SEM, LEO Company, UK); THZ-92C constant temperature shaker equipped with gas bath (Shanghai Boxun Medical Treatment Equipment Factory, Shanghai, China); P1201 high performance liquid chromatograph (HPLC, Dalian Elite Analytical Instruments, Dalian City, China); and STA 449 thermogravimetric analyzer (TGA, Netzsch Company, Germany).

Preparing Grafted Particles PMAA/SiO_2 and Examining Interaction Between Grafted PMAA and Quercetin

The goal of this work was to prepare quercetin-imprinted material with MAA as functional monomer, and so it is firstly needed to examine the interaction between MAA and quercetin. For this, the grafted particles PMAA/SiO_2 were firstly prepared using surface-initiated graft-polymerization method, and the adsorption action of the grafted PMAA toward quercetin was investigated, namely the examination of the interaction between MAA and quercetin is substituted by examining the interaction between the grafted PMAA and quercetin. According to the procedure described in Ref. 25, the grafted particles PMAA/SiO_2 were prepared: (1) the micro-sized silica gel particles were surface-modified with coupling agent 3-(aminopropyl)trimethoxysilane (AMPS), and amino groups were introduced onto the surfaces of silica gel particles, namely the modified particles AMPS-SiO_2 were obtained; (2) by the initiating of amino group/persulfate system, the surface-initiated graft-polymerization of MAA was conducted, obtaining the grafted particles PMAA/SiO_2. The grafting degree of PMAA on PMAA/SiO_2 particles was determined by acid–base titration and by TGA; The FTIR spectrum of the grafted particles PMAA/SiO_2 was determined to characterize their structure.

Quercetin solutions of *N, N*-dimethyl formamide (DMF) with different concentrations were prepared in a range of 1–7 mmol/L,

and the isothermal adsorption tests of PMAA/SiO_2 for quercetin were performed, obtaining adsorption isotherms.

Preparation and Characterization of Quercetin Surface-Imprinted Material MIP-PMAA/SiO_2

In consideration of quercetin solubility, the surface-imprinting of quercetin was conducted in a DMF solution. In a four-necked flask equipped with a mechanical agitator, a reflux condenser, a thermometer and a N_2 inlet, 50 mL of DMF, 1.48 g of quercetin, and 2.5 mL of MAA were added. The added quercetin and MAA were allowed to interact for a period of time under stirring. And then 0.5 g of modified particles AMPS-SiO_2, and 0.65 g of crosslinker MBA were added, followed by bubbling nitrogen for 30 min. The temperature of the system was raised to 30°C, and 0.028 g of the initiator APS was added. The graft/cross-linking polymerization reaction was performed under N_2 atmosphere at 30°C for 12 h. The product particles were collected by filtering, and were fully soaped and washed with a diluted aqueous solution of NaOH to remove the template molecule quercetin. The resultant particles were dried under vacuum, and these particles were namely quercetin surface-imprinted material MIP-P(MAA-*co*-MBA)/SiO_2 (simplified as MIP-PMAA/SiO_2). For comparison, the non-imprinted material NMIP-PMAA/SiO_2 was also prepared in the absence of template quercetin under the same conditions as the preparation of MIP-PMAA/SiO_2.

The surface-imprinted material MIP-PMAA/SiO_2 was characterized by using several methods. (1)The FTIR spectrum of MIP-PMAA/SiO_2 particles was determined to characterize their chemical structure; (2) Their morphology change was observed with SEM; (3) The grafting degree of the copolymer P(MMA-*co*-MBA) on MIP-PMAA/SiO_2 particles was determined by TGA (by subtracting the weight loss rate of AMPS-SiO_2 from that of MIP-PMAA/SiO_2), and the determination result indicated the grafting degree of P(MMA-*co*-MBA) on MIP-PMAA/SiO_2 particles was 32.18 g/100 g. At the same time, the grafting degree of P(MMA-*co*-MBA) on the non-imprinting material NMIP-PMAA/SiO_2 was also determined, and it was 31.24 g/100 g, showing that the grafting degrees of both particles were similar.

Researching Binding Character of MIP-PMAA/SiO_2 for Quercetin

Evaluating Binding Property of MIP-PMAA/SiO_2 with Batch Method. In a constant temperature oscillator, the adsorption kinetics experiment of MIP-PMAA/SIO_2 for quercetin was first conducted, and the time of equilibrium adsorption was about 3 h. On this basis, the isothermal adsorption experiments were carried out. DMF solutions of quercetin of 20 mL with different concentrations were placed into a number of conical flasks with cover, and about 0.05 g of MIP-PMAA/SiO_2 weighted accurately particles was added into these solutions, respectively. These mixtures were shaken in a constant temperature oscillator for 3 h, and the binding process was allowed to reach equilibrium. After standing, the quercetin concentrations of the supernatants were determined by UV spectrophotometry at 339 nm. The equilibrium binding amount of quercetin on MIP-PMAA/SiO_2, Q_e (mmol/g), was calculated according to eq. (1), and the isotherm binding curve was plotted.

$$Q_e = \frac{V(C_0 - C_e)}{m} \quad (1)$$

where C_0 (mmol/mL) and C_e (mmol/mL) are the initial and equilibrium concentration of quercetin, respectively, V (mL) is the volume of quercetin solution, and m (g) is the mass of the used adsorbent MIP-PMAA/SiO_2 particles.

Rutin and genistein are another two flavonoids, and their chemical structures are similar to that of quercetin to a certain extent. Therefore, in this work, rutin and genistein were selected as contrast substances to investigate the molecular recognition property of the quercetin surface-imprinted material MIP-PMAA/SiO_2 and to evaluate its solid phase extraction ability for quercetin from the crude extract. The DMF solutions of rutin and genistein were also prepared, and the binding property of MIP-PMAA/SiO_2 for rutin and genistein were also tested with batch method like as for quercetin, and the isotherm binding curves were also plotted.

Evaluating Binding Property of MIP-PMAA/SiO_2 with Column Method. The imprinted particles MIP-PMAA/SiO_2 (about 1 g) was packed into a piece of glass pipe with an internal diameter of 1.0 cm, and the bed volume (BV) of the packed column was 2 mL. A DMF solution of quercetin with a concentration of 6 mmol/L was allowed to flow gradually through the packed column at a rate of five BV per hour (5 BV/h) in countercurrent manner. The effluents with one volume (1 BV) interval were collected, and the concentrations of quercetin in these effluents were determined. The concentration as function of BV was figured, namely the dynamic binding curve was plotted, and the break binding amount and saturated binding amount were calculated with the data of the concentration and bed number of these effluents. Similarly, the dynamic binding behaviors of rutin and genistein on MIP-PMAA/SiO_2 column were tested, and their dynamic binding curves were also figured.

Competition Binding Experiments. Two binary mixed solutions, quercetin/genistein and quercetin/rutin, were prepared with DMF as solvent, and the concentration of each component in the two binary solutions was 4 mmol/L. The binary solutions (40 mL) were placed in two conical flasks with cover, respectively, and 0.1 g of the imprinted articles MIP-PMAA/SiO_2 weighted accurately was added. The competition binding experiments were carried out in a thermostatic oscillator for 3 h. After centrifugal separation, the equilibrium concentration of each substance in the two supernatants was determined through HPLC, and the distribution coefficient for each substance was calculated according to eq. (2).

$$K_d = \frac{Q_e}{C_e} \quad (2)$$

where K_d represents the distribution coefficient (L/g) of a substance; Q_e(mmol/g) is its equilibrium combining quantity; C_e (mmol/L) is its equilibrium concentration.

The selectivity coefficient k of MIP-PMAA/SiO_2 particles for quercetin relative to a certain competition substance, rutin or genistein, can be obtained from the distribution coefficient data according to eq. (3). The value of k represents the recognition selectivity of MIP-PMAA/SiO_2 for quercetin, and it marks the

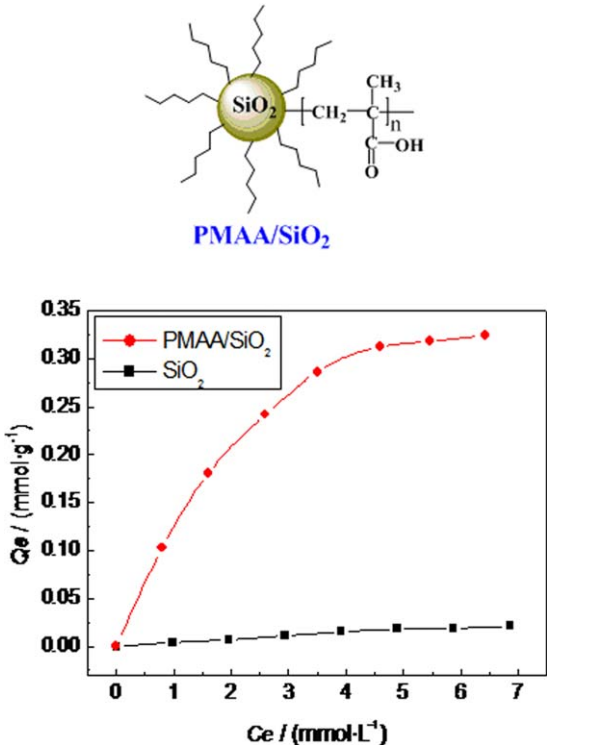


Figure 1. Chemical structure of grafted particles PMAA/SiO_2 and their adsorption isotherm for quercetin. Solvent: DMF; Temperature: 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ability of the imprinted material to separate quercetin from the crude extract.[26,27] Therefore, the selectivity coefficient k is one of the most parameters for evaluating the molecular recognition property.

$$k = \frac{K_d(\text{Quercetin})}{K_d'} \quad (3)$$

where $K_d(\text{Quercetin})$ is the distribution coefficient of quercetin, whereas K_d' represents the distribution coefficient of a certain competition substance.

RESULTS AND DISCUSSION

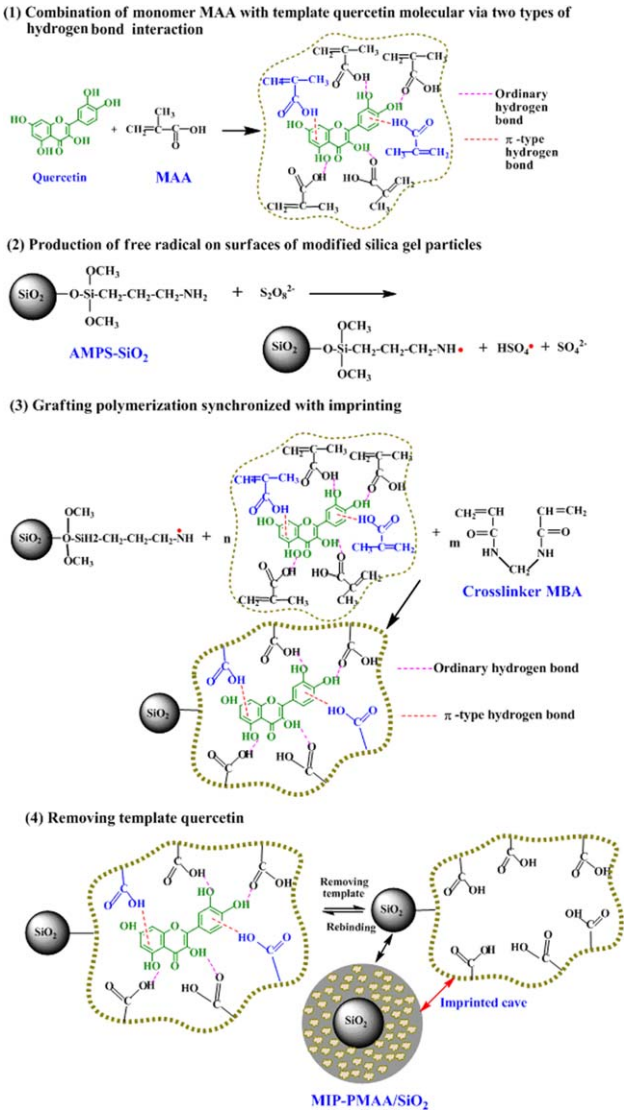
Interaction Between Grafted Macromolecule PMAA and Quercetin

As mentioned, in order to examine the interaction between the monomer MAA and the template quercetin, the grafted particles PMAA/SiO_2 were first prepared through surface-initiating polymerization, and the structure characteristic of PMAA/SiO_2 can be seen in Figure 1. The isothermal adsorption experiment of PMAA/SiO_2 particles for quercetin was performed in DMF solution, and Figure 1 gives the adsorption isotherms of SiO_2 particles and PMAA/SiO_2 particles for quercetin, respectively.

Figure 1 displays that silica gel particles nearly do not adsorb quercetin. However, the grafted particles PMAA/SiO_2 have very strong adsorption ability for quercetin, and the adsorption capacity actually gets up to near 0.32 mmol/g (98 mg/g), suggesting that there are strong interactions between the grafted macromolecule PMAA and quercetin. It also demonstrates that there exist strong interactions between the monomer MAA and the template quercetin, and this is the base of performing molecular imprinting of quercetin with MAA as functional monomer. It can be seen from Scheme 1 that quercetin molecule contains five phenolic hydroxyl groups that not only are the ordinary hydrogen bond donor but also the ordinary hydrogen bond acceptor, and at the same time quercetin molecule contains two benzene rings that are the donor of π-type hydrogen bond as well as one carbonyl group that is the acceptor of the ordinary hydrogen bond. While in the grafted macromolecule PMAA, there is a great deal of hydroxyl groups of carboxyl group that not only act as the donor of the ordinary hydrogen bond but also act as the acceptor of the ordinary hydrogen bond, whereas the carbonyl groups of carboxyl group in the grafted macromolecule PMAA can act as the ordinary hydrogen bond acceptor. Therefore, it appears that a great deal of hydrogen bonding with two types, ordinary hydrogen bond and π-type hydrogen bond (π-type hydrogen bond is formed by the hydroxyl group of carboxyl group of the grafted PMAA with the aromatic ring of quercetin molecule), may form between PMAA/SiO_2 particles and quercetin molecules, leading to the very strong adsorption action of PMAA/SiO_2 particles for quercetin. The interaction status between monomer MAA and quercetin is similar to the situation above, and this is exactly why MAA can be used as functional monomer for effectively performing the molecular imprinting of quercetin. The interaction model of MAA and quercetin can be observed in Scheme 2 below. Furthermore, the interaction mechanism between the following imprinted particles and quercetin as well as between the following non-imprinted particles and quercetin is the same as the interaction mechanism between PMAA/SiO_2 particles and quercetin.

Preparation and Characterization of Quercetin Surface-Imprinted Material

Chemical Preparation Processes for Quercetin Surface-Imprinted Material MIP-PMAA/SiO_2. (1) By right of the hydrogen bonding of two types, ordinary hydrogen bond and π-type hydrogen bond, the monomer molecules of MAA are first combined around quercetin molecule automatically. (2) A redox surface initiation system is constituted by the amino group (primary amine group) on the modified particles AMPS-SiO_2 and APS in the solution, and so primary free radicals are generated on the surfaces of AMPS-SiO_2 particles. (3) These free radicals on the surfaces of the particles initiate monomer MAA around quercetin molecule as well as crosslinker MBA to produce graft/cross-linking polymerization, and a thin layer of crosslinked copolymer forms on the surfaces of silica gel particles. Meanwhile, the template molecules, quercetin molecules, are enveloped in the crosslinking networks, namely, quercetin surface-imprinting is realized. (4) After washing away the template molecules, large numbers of quercetin surface-imprinted caves will remain within this thin polymer layer on the surfaces of silica gel particles so that the quercetin surface-imprinted material MIP-PMAA/SiO_2 is obtained. The procedure described above can be schematically expressed in Scheme 2. It needs to be pointed that in the diagram, the red dotted line represents



Scheme 2. Schematic expression of chemical process to prepare MIP-PMAA/SiO_2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

π-type hydrogen bond, whereas the magenta dotted line indicates ordinary hydrogen bond.

Characterization of Quercetin Surface-Imprinted Material MIP-PMAA/SiO_2. ***Infrared spectrum.*** Figure 2 gives the infrared spectra of three particles, SiO_2 particles, the grafted particles PMAA/SiO_2 particles and quercetin surface-imprinted particles MIP-PMAA/SiO_2.

As compared with the spectrum of SiO_2 particles, in the spectrum of PMAA/SiO_2, there is an obvious absorption band of carbonyl group of carboxyl group at 1718 cm^{-1}, indicating the realization of the graft-polymerization of MAA on silica gel particles. In the spectrum of MIP-PMAA/SiO_2 particles, except for the absorption band of carbonyl group of MAA unit, two new absorption bands appear at 1680 and 1559 cm^{-1}, and they are attributed to the stretching vibration band of carbonyl group of amide (amide I band) and the in-plane bending vibration absorption of N-H bond of amide (amide II band), respectively, and they come from the MBA unit of the grafted crosslinked copolymer forming in the surface imprinting process. The above spectrum facts demonstrate that the monomer MAA and the crosslinker MBA have produced graft/cross-linking polymerization on the surfaces of silica gel particles in the presence of the template quercetin, and the quercetin surface-imprinted material MIP-PMAA/SiO_2 has been obtained. It needs to be pointed that all characteristic absorptions of PMAA/SiO_2 and MIP-PMAA/SiO_2 particles are weaker by the affecting of the strong absorption background of silica gel particles.

Morphology. Figure 3(A,B) present the SEM images of raw silica gel particles and the imprinted particles MIP-PMAA/SiO_2, respectively. It is obvious that the surfaces of raw silica gel particles are rough and scraggy, whereas the surfaces of the imprinted particles of MIP-PMAA/SiO_2 particles become smoother and flatter because of the coating and filling up action of the crosslinking copolymer P(MAA-*co*-MBA) layer.

Binding and Recognition Characteristics of MIP-PMAA/SiO_2 for Quercetin

Binding Isotherm. By using the non-imprinted material NMIP-PMAA/SiO_2 and the imprinted material MIP-PMAA/SiO_2, the static adsorption experiments (batch method) were performed in DMF solutions of three kinds of flavonoids, quercetin, rutin and genistein, respectively. Figure 4 gives the adsorption isotherms of NMIP-PMAA/SiO_2 for three substances, whereas Figure 5 gives the binding isotherms of MIP-PMAA/SiO_2 for the three substances.

Figure 4 indicates that the non-imprinted material NMIP-PMAA/SiO_2 can produce strong adsorption action for the three kinds of flavonoid compounds, and the adsorption capacities are in range of 0.27–0.32 mmol/g. Apparently, for the three flavonoids the difference of the adsorption capacities is very small, and it comes from their refined structural differences that cause the small interaction differences of host-guest. In a word, the non-imprinted material NMIP-PMAA/SiO_2 has no adsorption selectivity for the three flavonoid compounds. However, Figure 5 displays that the binding properties of the imprinted material

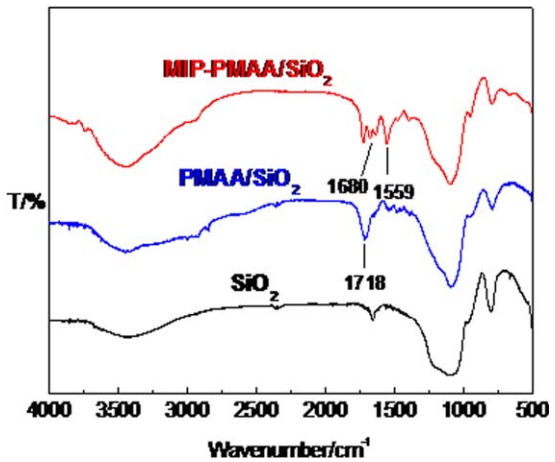


Figure 2. Infrared spectra of three particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

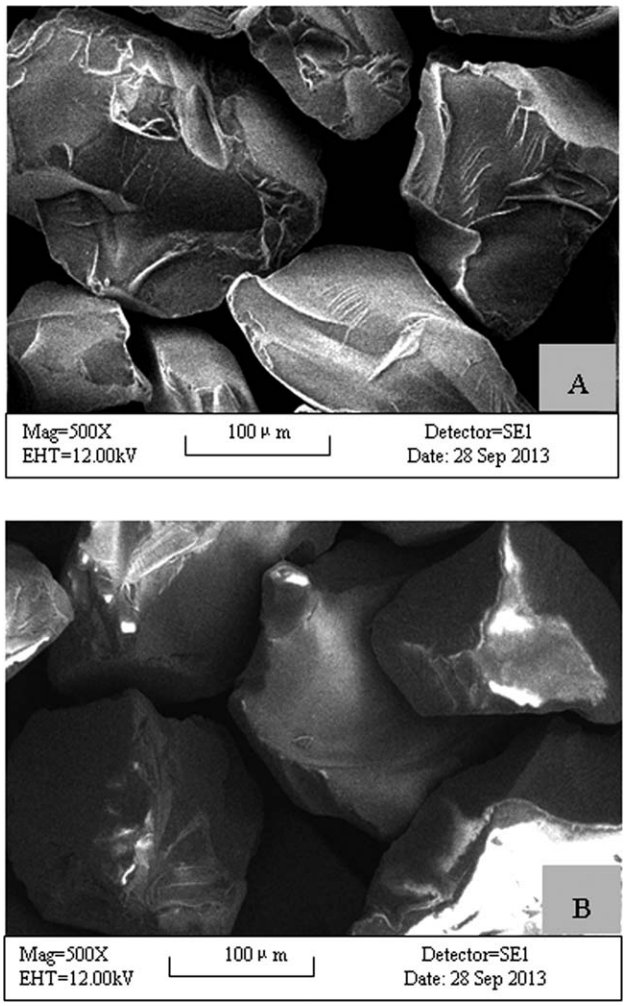


Figure 3. SEM photographs of SiO_2 and MIP-PMAA/SiO_2 particles.

MIP-PMAA/SiO_2 for the three flavonoid compounds have changed substantially. The binding capacities of MIP-PMAA/SiO_2 for the two contrast substances have largely decreased. The binding capacity of rutin decreases from 0.271 mmol/g to 0.074 mmol/g, and that of genistein decreases from 0.313 mmol/g to 0.1 mmol/g, respectively. However, the binding capacity of quercetin still remains high, and it is 0.325 mmol/g. The above facts fully demonstrate that the quercetin surface-imprinted material MIP-PMAA/SiO_2 possesses special recognition selectivity and excellent binding affinity for quercetin molecule. The reason for this can be explained as follows. For MIP-PMAA/SiO_2, a great quantity of quercetin molecule-imprinted caves is distributed within the thin polymer layer on the surfaces of silica gel particles. These caves are highly matched with quercetin molecule in size, steric shape, and combination sites, and it leads to the specific recognition ability and strong binding action of MIP-PMAA/SiO_2 particles toward quercetin molecule. However, these caves are non-matched with rutin and genistein molecules. It can be seen that rutin molecule is larger than that of quercetin, and as a result, rutin molecule is difficult to enter into the quercetin molecule-imprinted caves. While for genistein, its molecular structure is different from that of quercetin to a certain

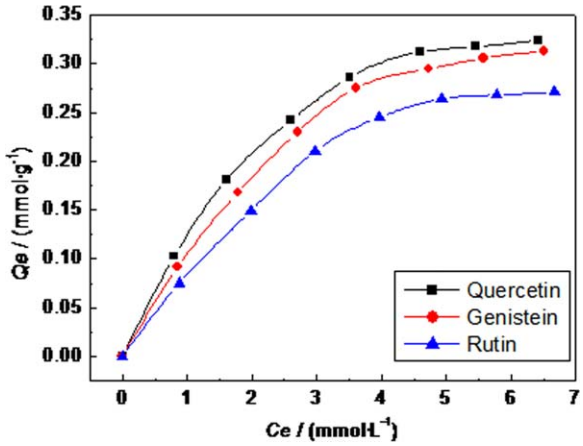


Figure 4. Adsorption isotherms of NMIP-PMAA/SiO_2 for three flavonoids. Solvent: DMF; Temperature: 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

degree. Therefore, the quercetin molecule-imprinted caves are difficult to accept the molecules of the two contrast substances, resulting in their low binding capacity. Besides, by the way, the data processing result shows that all of the adsorption isotherms of NMIP-PMAA/SiO_2 for three substances and the binding isotherms of MIP-PMAA/SiO_2 for three substances fit the Langmuir equation.

Dynamic Binding Curve. By using the non-imprinted material NMIP-PMAA/SiO_2 and the imprinted material MIP-PMAA/SiO_2, the dynamic adsorption experiments (column method) were also performed. Figures 6 and 7 give the dynamic adsorption curves of NMIP-PMAA/SiO_2 and the dynamic binding curves of MIP-PMAA/SiO_2 for the three flavonoids, quercetin, rutin, and genistein, respectively.

It can be seen in Figure 6 that for the column packed with NMIP-PMAA/SiO_2 particles, as the three flavonoid solutions with the same concentrations flow upstream through the column, respectively, their leaking volumes are very similar, and

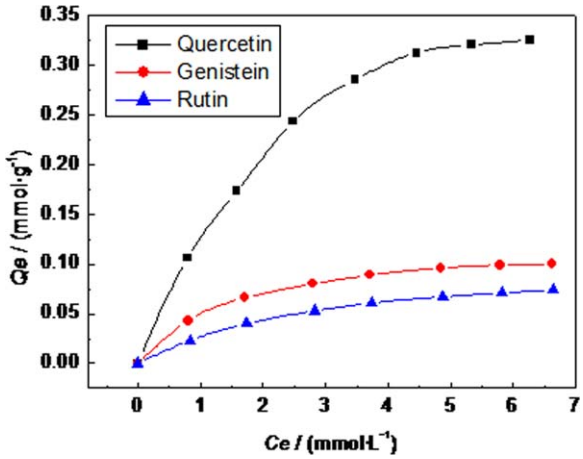


Figure 5. Binding isotherms of MIP-PMAA/SiO_2 for three flavonoids. Solvent: DMF; Temperature: 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

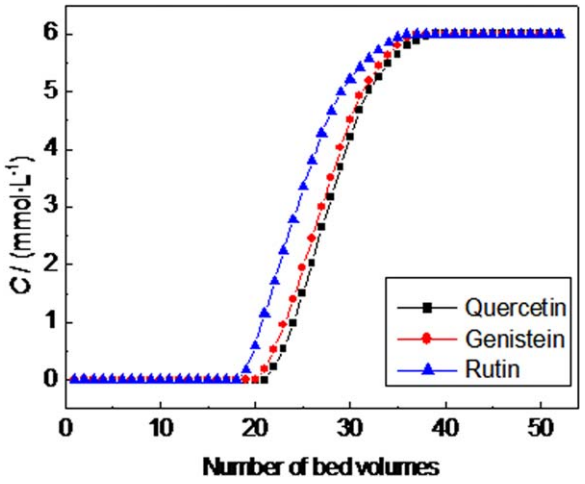


Figure 6. Dynamic adsorption curves of NMIP-PMAA/SiO_2 for three flavonoids. Solvent: DMF; Temperature: 25°C; BV: 2 mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

are 22 BV for quercetin solution, 19 BV for rutin solution, and 21 BV for genistein solution, respectively. This fact again displays that the adsorption properties of the non-imprinted material NMIP-PMAA/SiO_2 for the three flavonoids are similar, and it has no adsorption selectivity.

However, it can be found in Figure 7 that as the three flavonoid solutions with the same concentrations flow upstream through the column packed with MIP-PMAA/SiO_2 particles, the leaking curves have fundamentally changed. The leaking volumes of rutin and genistein solutions significantly decrease, and they are 5 BV for rutin solution and 7 BV for genistein solution, respectively. Such low leaking volumes mean that once the solutions of the two contrast substances go through column, the leaking phenomenon occurs immediately, clearly showing that the column packed with MIP-PMAA/SiO_2 particles does not recognize and bind the two contrast substances basically. By calculating, for rutin the leaking and saturated adsorption amounts are only

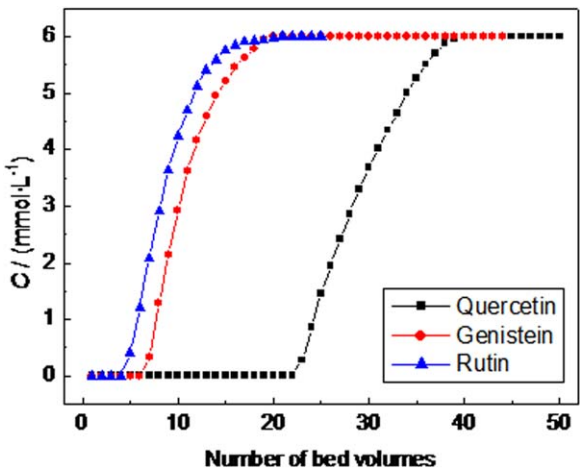


Figure 7. Dynamic binding curves of MIP-PMAA/SiO_2 for three flavonoids. Solvent: DMF; Temperature: 25°C; BV: 2 mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Distribution Coefficient and Selectivity Coefficient Data for Quercetin/Rutin System

Adsorbent	NMIP-PMAA/SiO_2		MIP-PMAA/SiO_2	
Adsorbate	Quercetin	Rutin	Quercetin	Rutin
K_d (L g^{-1})	0.091	0.086	0.10	0.013
k	1.058		7.69	

about 0.048 mmol/g and 0.099 mmol/g, and for genistein they are only 0.072 mmol/g and 0.124 mmol/g. However, as quercetin solution flows through the column of MIP-PMAA/SiO_2, the leaking volume still remains high (23 BV), and by calculating, the leaking and saturated adsorption amounts reach up to 0.264 mmol/g and 0.342 mmol/g, respectively, displaying the special recognition selectivity and excellent binding affinity of the imprinted material MIP-PMAA/SiO_2 for the template quercetin. The results of above dynamic binding experiments still arise from high non-matching of the imprinted caves on MIP-PMAA/SiO_2 particles with rutin and genistein molecules, but these imprinted caves are high matched with quercetin molecule.

Selectivity Coefficients of MIP-PMAA/SiO_2 for Quercetin Molecule. Two binary mixed solutions with DMF as solvent, quercetin/genistein and quercetin/rutin, were prepared and the competitive adsorption experiments of MIP-PMAA/SiO_2 particles in the two solutions were conducted. In Tables I and II, the data of the distribution coefficients K_d of each substance and the selectivity coefficients k of MIP-PMAA/SiO_2 for quercetin molecule are summarized.

It can be found from the data in Tables I and II that the selectivity coefficients of the non-imprinted material NMIP-PMAA/SiO_2 for quercetin relative to the two contrast substances are closed to 1. This fact implies that all of the three flavonoids have the same competitive adsorption abilities on NMIP-PMAA/SiO_2 particles so that this material has no recognition selectivity for quercetin. However, as the quercetin surface-imprinted material is used in the competitive adsorption experiments, relative to rutin, the selectivity coefficient of MIP-PMAA/SiO_2 for quercetin is 7.69, and relative to genistein, it is 4.4, again displaying the high recognition selectivity of MIP-PMAA/SiO_2 for quercetin. The results of the competitive adsorption experiments still rely on that there is a great deal of the imprinted caves that are matched with quercetin molecule within the thin polymer layer on the surfaces of MIP-PMAA/

Table II. Distribution Coefficient and Selectivity Coefficient Data for Quercetin/Genistein System

Adsorbent	NMIP-PMAA/SiO_2		MIP-PMAA/SiO_2	
Adsorbate	Quercetin	Genistein	Quercetin	Genistein
K_d (L g^{-1})	0.088	0.084	0.11	0.025
k	1.048		4.40	

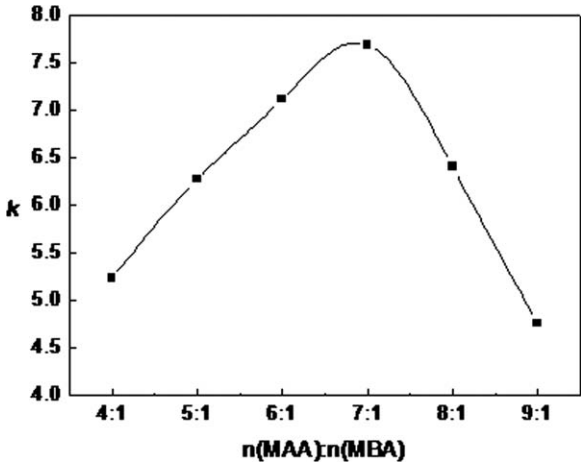


Figure 8. Selectivity coefficient of MIP-PMAA/SiO_2 as function of molar ratio of MAA to MBA.

SiO_2 particles, and these caves can effectively recognize and accept quercetin molecules.

Effects of Main Factors on Imprinting Process

Effect of Used Amount of Crosslinker. During the imprinting process, through changing the molar ratio of monomer MAA to crosslinker MBA, the surface-imprinting of quercetin was carried out with different used amount of crosslinker MBA. Figure 8 presents the selectivity coefficients of MIP-PMAA/SiO_2 for quercetin relative to rutin as a function of the molar ratio of MAA to MBA. Figure 8 displays that as the molar ratio of MAA to MBA is equal to 7 : 1, the prepared imprinted material MIP-PMAA/SiO_2 has the highest selectivity coefficient for quercetin, implying the molar ratio of 7 : 1 is appropriate.

Effect of Ration of Monomer to Template. During the imprinting process, the molar ratios of monomer MAA to template quercetin were changed, and the surface-imprinting of quercetin was conducted under different molar ratio conditions, obtaining different MIP-PMAA/SiO_2 particles. Figure 9 gives the selectivity coefficients of MIP-PMAA/SiO_2 for quercetin relative to rutin as a function of the molar ratio of MAA to quercetin.

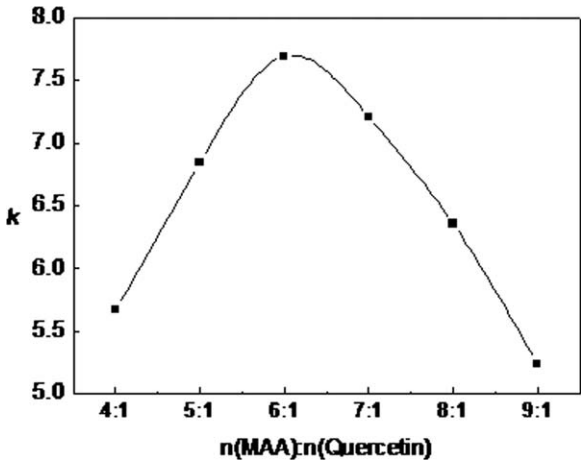


Figure 9. Selectivity coefficient of MIP-PMAA/SiO_2 as function of molar ratio of MAA to quercetin.

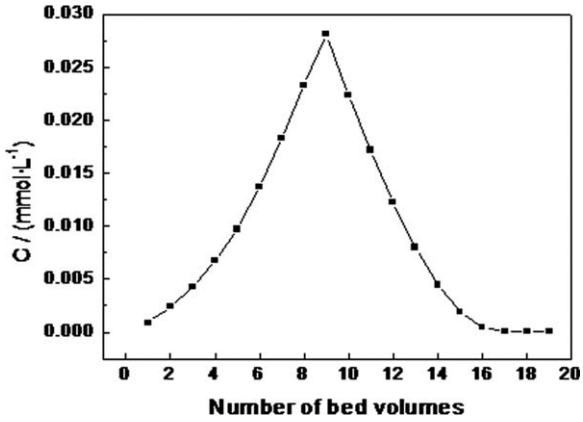


Figure 10. Elution curve of quercetin from MIP-PMAA/SiO_2 column.

Figure 9 shows that as the molar ratio of MAA to quercetin is equal to 6 : 1, the prepared imprinted material MIP-PMAA/SiO_2 has the highest selectivity coefficient, suggesting the molar ratio of 6 : 1 is the optimum ratio. This result may associates with the interaction status between one quercetin molecule and six monomer molecules, and it can be seen from Scheme 2.

Elution Property MIP-PMAA/SiO_2

With a diluted aqueous solution of NaOH (pH < 11) as the eluent, the elution experiments were carried out for the MIP-PMAA/SiO_2 particles that had adsorbed quercetin in a saturated state. The eluent was allowed to upstream pass through the column packed with MIP-PMAA/SiO_2 particles, and the dynamic desorption curve is presented in Figure 10.

It can be seen in Figure 10 that this desorption curve is cuspidal and without trailing, indicating that the template molecules combined in the packed column are easy to be washed off. By calculating, the desorption ratios of quercetin in 16 BV and 20 BV reach 97.5% and 99.2%, respectively, displaying the excellent elution property. The imprinted caves are distributed within the thin polymer layer on the surfaces of MIP-PMAA/SiO_2 particles, and the diffusion resistance is little for the eluting of quercetin, leading to that the combined quercetin molecules are easy to be desorbed or eluted. It demonstrates that the recovering and reusing of the surface-imprinted material MIP-PMAA/SiO_2 is feasible and very convenient.

CONCLUSIONS

A quercetin molecule surface-imprinted material was prepared successfully by using the novel molecular surface-imprinting technique of "synchronously graft-polymerizing and imprinting." In DMF solution, monomers MAA are combined around the template quercetin molecule by right of hydrogen bonding of two type, ordinary hydrogen bond and π-type hydrogen bond. On this basis, quercetin molecule surface-imprinting was smoothly carried out on the surfaces of silica gel particles by the surface-initiated graft/croslinking-polymerization of MAA, resulting in the quercetin molecule surface-imprinted material MIP-PMAA/SiO_2. The selectivity coefficients of MIP-PMAA/SiO_2 for quercetin relative to rutin and genistein are 7.69 and 4.40, respectively, and it is obvious that such material can effectively recognize and bind quercetin molecule. In

order to obtain MIP-PMAA/SiO_2 with high performance, the main conditions of imprinting process should be controlled. The optimal molar ratio of monomer MAA to crosslinker MBA is 7 : 1, and appropriate molar ratio of monomer MAA to template quercetin is equal to 6 : 1.

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