Synthesis of the Artemisinin-Imprinting Polymers on Silica Surface and Its Adsorption Behavior in Supercritical CO_2 Fluid

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Molecular imprinting polymers (MIPs) for artemisinin were prepared by using 3-aminopropyltriethoxysilane and calix[4]arene bonded on silica particle surface as the functional monomers, tetraethoxysilicane as cross-linker, and artemisinin as template. The MIPs were characterized by Fourier Transform Infrared Spectroscope and SEM. Their adsorption capacities were evaluated by static adsorption experiments. The MIPs showed high adsorption capacity and good selectivity for artemisinin. The maximum adsorption capacity of MIPs for artemisinin was 40.0 mg/g. The imprinting factor and the selective factor of the artemisinin-imprinting polymers was 2.0 and 1.5, respectively. The imprinted film coating onto the silica surface showed a fast kinetics for recognizing and binding templates. Especially, mass transfer reaches the equilibrium within 3.5 h and the adsorption capacity of MIPs for artemisinin reached 120.0 mg/g in supercritical CO_2 fluid. © 2011 American Institute of Chemical Engineers *AIChE J,* 57: 3514–3521, 2011
Keywords: molecular kwimprinting polymers, artemisinin, calix[4]arene, supercritical CO_2 fluid

Introduction

Molecular imprinting technology (MIT) is a method to obtain the polymers, which have spatial structure and binding sites matching with template molecules. It comes from the Pauling's[1] theory that synthesized the antibody from the antigen. From 1970s, MIT developed rapidly, because Wulff et al[2] synthesized macromolecules with high selectivity to template molecules. There are three ways to synthesize the molecular imprinting polymers (MIPs), including the covalent, the noncovalent, and the semi-covalent. A template molecule interacts with one or two functional monomers to form an ordered complex. The complex is fixed by using a cross-linker, and the template is eluted from the polymers.[3] The MIPs prepared by traditional method make the template molecules are deeply embedded in the polymers resulting in mass transfer difficulty.[4,5] Hence, it is necessary to overcome the drawback of traditional method. In recent years, many research groups are interesting in surface MIPs with more accessible bonding sites.[6–8]

Artemisinin is an endoperoxide sesquiterpene lactone from Artemisia annual discovered by china researchers in the early 1970s,[9,10] which has good therapeutic effect for malaria and low toxicity, compared with other antimalarial drugs such as chloroquine, primaquine, and pyrimethamine.[11,12] Thus, it was recommended as an antimalarial drug by World Health Organization (WHO).[13] Nowadays, the main source of artemisinin is extracted from leaves of Artemisia annual in which its content is only 0.001–0.8% (w/w).[14] However, the market demand is expanding,[15] and the traditional extraction method is time-consumed and multi-steps process. It is necessary to find a more effective

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extraction method. Preparation of the MIPs for artemisinin is a new attempt. The structure of artemisinin with an endoperoxide bridge is unstable.[16] Noncovalent method could be used to prepare the MIPs by one lactone bone (—O—C=O) interacting with monomer with hydrogen bonds. Calixarene is a cyclic oligomers discovered in 1940s. Until the beginning of 1980s, its conformation and performance were confirmed. It can pack a variety of neutral molecular to form host-guest complex,[17] thus were consider as third host-guest compound after cyclodextrins and crown ethers.

Molecular imprinting has high selectivity as a bioseparation method, but the process has slow mass transfer, low adsorption capacity. Usually, process is carried out in organic solvents. Supercritical fluid has relative high selectivity and strong permeation diffusion effect. If molecular imprinting separation process is carried out under the condition of supercritical fluid, the process should have faster mass transfer, higher selectivity and friendly environment without organic solvent. The process integrates advantage of supercritical fluid and molecular imprinting technique and overcomes their drawbacks, then develops a novel point, and becomes a potential bioseparation technique.

In this article, the MIPs for artemisinin have been prepared by using 3-aminopropyltriethoxysilane (APTS) and calix[4]arene as monomers, artemisinin as template, and tetraethoxysilicane (TEOS) as cross-linker. Calix[4]arene was grafted on the surface of silica particle. The artemisinin-imprinting polymers show high adsorption capacity and good selectivity for template. The mass transfer behavior of the polymers was improved under supercritical CO_2 fluid, and some interesting results have been obtained. Combination of the MIT with supercritical fluid extraction technology shows promising application in preparation of artemisinin.

Materials and Methods

Reagent

Porous Silica (80–100 mesh) was used as the support medium to prepare the surface artemisinin-imprinting sorbent. TEOS, APTS, and γ-Glycidoxypropyltrimethoxysolane (GPTMS) were purchased from Hangzhou guibao Chemical (Hangzhou, China). Tetrachlorosilane was purchased from Aladdin (Shanghai) Reagent (Shanghai, China). Artemisinin and artemether (purity $\geq$ 99.5%) were purchased from Chengdu okay plant and chemical (Chengdu, China). The mobile phase used for the HPLC experiments was a mixture of methanol and water (V:V = 80:20), and was filtered through a 0.22 μm filter prior to use. All reagents were analytical grade or better. Calix[4]arene was prepared by our lab. CO_2 (purity $\geq$ 99.99%) was purchased from Shanghai Wujing Chemical (Shanghai, China).

Apparatus

The high-performance liquid chromatographic systems consisted of two LC-20AD pumps and a SPD-20A ultraviolet detector (Shimadzu, Japan). All separations were achieved on an analytical reversed-phase Shimadzu ODS-SP column (4.6 $\times$ 150 mm^2 long) at a flow rate of 1.0 ml/min at room temperature. The UV detector was operated at 217 nm. Super Phase Monitor SPM20 (Thar Technologies) was applied to the determination of the adsorption behavior of imprinting polymers with artemisinin under the condition of supercritical CO_2 fluid.

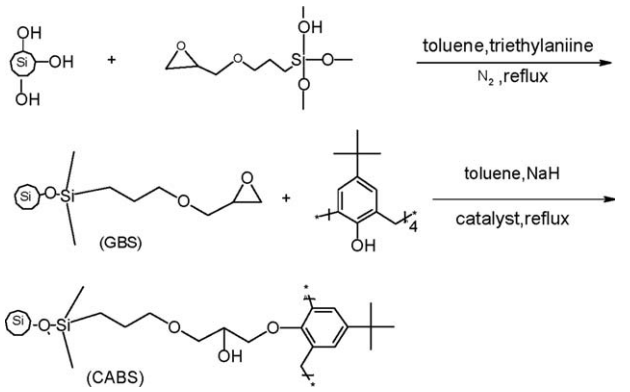

Figure 1. The process of binding calix[4]arene on silica surface.

Preparation of imprinting polymers with artemisinin on silica particle surface

The surface of silica particles was activated according to the method previously reported by Liu Y.[18] Ten gram of silica particles were dipped in a mixture of 50% (V:V) nitric acid aqueous solution for 12 h under stirring, then the silica particles were filtrated, and washed with double-distilled water. Finally, the silica particles were dried under vacuum at 110°C for 12 h.

Calix[4]arene was prepared following Muzaffer Iqbal's[19] method published in 1985. 12.5 ml formaldehyde (37% w/v), 0.24 g NaOH, 20.0 g *p*-tert-butylphenol, and 3–5 ml water were mixed, and then, the mixture was heated to 120–125°C for 2 h. An orange solid was produced. The solid was dissolved in 160 ml diphenyl ether, and the solution was refluxed for 2 h at 240°C. Then, the reaction mixture was cooled with 200 ml ethyl acetate for 0.5 h and was filtrated. Finally, the solid product was washed by ethyl acetate, acetic acid, and ethyl acetate in turn. A white calix[4]arene crude material was obtained, and it was purified by toluene.

The calix[4]arene was grafted on silica surface (CABS) according to the reported literature.[20,21] The preparation process is shown in Figure 1. With 100 ml toluene, 12.5 ml GPTMS, 10 g activated silica particles, and 0.25 ml triethylamine (as a catalyst) were mixed, and the mixture was refluxed for 8 h at 80°C with nitrogen protection. Then the mixture was filtrated, and the solid product was washed with toluene and acetone, and then dried under vacuum at 100°C for 8 h. The GPTMS grafted silica particle was obtained (GBS).

With 100 ml anhydrous toluene, 2.0 g calix[4]arene and 0.2 g NaOH were mixed. After shaking for 30 min at 80°C with nitrogen protection, 5.0 g GBS and 1.0 g tetrabutyl ammonium bromide (as the phase transfer catalyst) were quickly added into it. The reaction was carried out for 24 h with shaking at 80°C under the protection of nitrogen. The product was filtered and washed with toluene, acetone, distilled water, dimethylformamide, and acetone. Finally the CABS was dried at 100°C for 8 h under vacuum.

The preparative process of the MIPs is shown in the Figure 2. To prepare the MIPs with artemisinin on silica

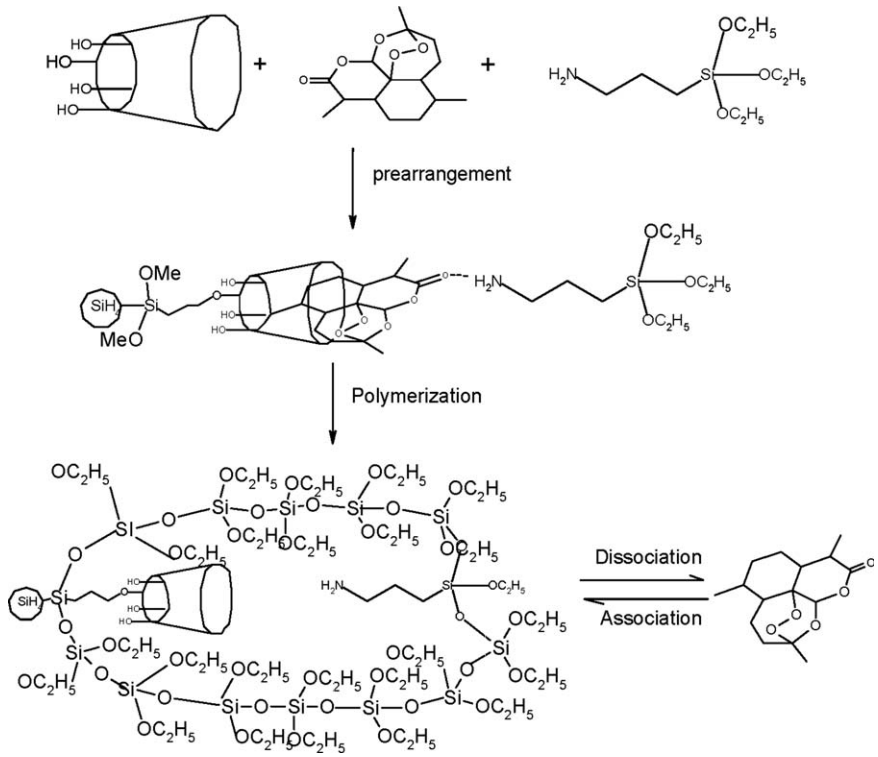

Figure 2. The process of prepare the MIPs.

particle surface, 0.1410 g artemisinin was dissolved in 100 ml petroleum ether, then 2 mmol APTS and 2 g CABS were added into it. After incubating for 16 h at room temperature, 10 mmol TEOS as cross-linker and 3 ml 1M acetic acid solution were added into it and the mixture reacted for 24 h with shaking at room temperature. After that, the product was filtered and washed with methanol, and dried for 12 h at 80°C under vacuum. The artemisinin MIPs on silica particle surface were obtained. The nonimprinting polymers were prepared in the same way without artemisinin. To remove the template molecules, the artemisinin-imprinting silica particle was extracted by Soxhlet method by using the mixture of methanol, acetic acid, and acetone at the volume ratio of 70:10:20 as solvent, and refluxing for 48 h. Then MIPs/NIPs were washed by methanol and dried for 12 h at 80°C under vacuum.

Characteristics of MIPs

The structure of activated silica and MIPs were characterized by FT-IR spectra installed on a VEVTOR-IR instrument (Nicolet). The morphology of the MIPs was observed by using the scanning electron microscope (SEM, JSM-6360LV, JEOL, Japan).

Static adsorption test

The static adsorption experiment was used to determine the adsorption capacity of artemisinin imprinting polymers. With 50 mg MIPs or NIPs, 5 ml artemisinin petroleum ether solutions with different concentrations (0.1, 0.3, 0.5, 0.7, 0.9, 1.2, and 1.5 mg/ml) were mixed and the mixture were shaken for 24 h at 30°C in shaker. Then, 1 ml solution was taken out and the artemisinin concentration was determined by HPLC. The adsorption experiments were performed in triplicate.

The adsorption kinetics experiment was done in triplicate to evaluate the adsorption rate of the imprinting polymers for the template molecules. One gram MIPs were mixed with 100 ml petroleum ether solution with 100 mg artemisinin. Ten samples were taken out at different time (15, 30, 60, 90, 120, 240, 360, 480, 720, 1200, and 1440 min), and the concentration of artemisinin in the solution was determined by HPLC.

Selective recognition ability of imprinting polymers was investigated by using the mixture of artemisinin and analogue artemether at 1.0 mg/ml. The structure of the artemisinin (a) and artemether (b) were shown in Figure 3. The artemether was selected as the structure analogues due to only a methyl group difference compared with artemisnin.

Adsorption experiment in supercritical CO_2 fluid

CO_2 were used as supercritical fluid. The work principle of the instrument was shown in Figure 4. The adsorption kinetics experiment was done in the supercritical CO_2 fluid. The imprinting polymers and solid artemisinin were weighted accurately, and then they were added into the reaction chamber. A specified amount of pre-pressurized CO_2 was pumped into the reaction chamber. After the system reached the desired temperature (40°C) set on controller 2, hydraulic hand pump was used to control the system pressure (20 ± 0.1 MPa). Hundred microliter sample (triplicate) was taken out from quantity sample tube, connected to the system, every 30 min interval, after the artemisinin was completely dissolved in supercritical CO_2. Then amount of artemisinin was

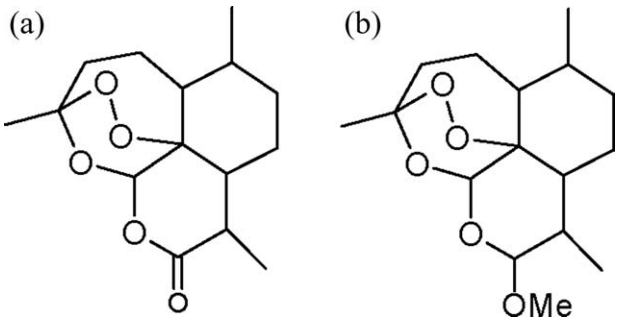

Figure 3. The structure of artemisinin (a) and artemether (b).

analyzed. In the experiment, the choice of the pressure and the temperature were according to the studies of Gong and Cao.[22]

The adsorption experiments were done as the following steps: artemisinin added into the reaction chamber ranged from 0.1 to 0.5 g, and 0.2 g MIPs was added into the chamber. Then, CO_2 was pumped into the chamber. When the artemisinin in the supercritical CO_2 was dissolved completely, 100 μl sample was taken out as the initial point, and three samples were taken out at 4 h, and determined by HPLC. In this study, the Peng-Robinson equation of state (PR-EOS)[23] was used to calculate the molar volume of pure supercritical CO_2.

$$P = \frac{RT}{v-b} - \frac{a}{v(v+b)+b(v-b)} \tag{1}$$

$$a = \frac{0.45724R^2T_c^2}{P_c} * [1 + m(1 - T_r^{0.5})]^2 \tag{2}$$

$$b = \frac{0.0778RT_c}{P_c} \tag{3}$$

$$m = 0.37464 + 1.54226\omega - 0.26991\omega^2 \tag{4}$$

$$\omega = 0.225$$

P is the experimental pressure (Pa), T is the experimental temperature (°C), v is the molar volume (m^3/mol).

In the experiment, the pressure and temperature of supercritical CO_2 fluid were set as 20 MPa and 40°C. The parameter Tc, Pc, and ω of supercritical CO_2 fluid is 31.3°C, 7.38 MPa, and 0.225, respectively, and the molar volume of pure supercritical CO_2 was calculated by the PR equation, the data is shown in Table 1.

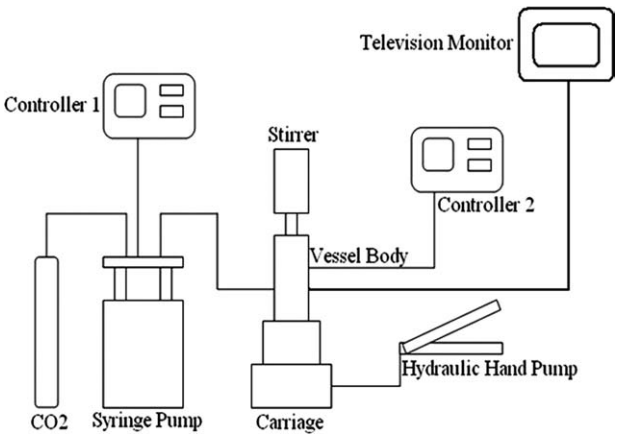

Figure 4. Experimental apparatus.

Table 1. The Parameter of the Experiment from PR Equation

T(°C)	Tr	m	α	a	b*10e5	υ*10e5
40	1.029	0.7079	0.9800	0.3887	2.667	5.294

From the Table 1, the molar volume of pure supercritical CO_2 (υ) is 5.29 $\times$ 10^{-5} m^3/mol. In this work, the volume of liquid CO_2 pumped into the chamber was recorded at a certain temperature. According to recorded temperature, the density of liquid CO_2 could be looked up. Then the mole number of CO_2 in the chamber could be calculated and volume of supercritical CO_2 fluid in it could be known. Moreover, the weight of artemisinin in each experimental operation was also known, so the artemisinin concentration in supercritical CO_2 fluid can be calculated by the formula (5):

$$y^2 = \frac{m^2}{\upsilon\rho_1^L v_1^L / M_1} \tag{5}$$

where y_2 is the concentration of artemisinin in the supercritical CO2, m_2 is the weight of artemisinin (g), υ is 5.29 $\times$ 10^{-5} m^3/mol, ρ_1^L is the density of liquid CO_2 (kg/m^3), v_1^L is the volume of the liquid CO_2 pumped into the chamber and M_1 is the molecular weights of CO_2 (44 g/mol).

Selective recognition ability of imprinting polymers under the condition of supercritical CO_2 fluid was performed by using the mixture of artemisinin and analogue artemether at different concentration.

Results and Discussion

Characteristics of MIPs

MIPs and activated silica were characterized by FI-IR (shown in Figure 5). The activated silica and MIPs showed their anticipated character. The peak around 3425.98 cm^{-1} indicated —OH, and 1100.00 cm^{-1} indicated Si—O—Si. Comparing characteristic of MIPs with those of active silica, the spectrum around 2922.83 cm^{-1} is the vibration of C—H, which belongs to the tert-butyl of the calix[4]arene and the spectrum 1458.27 cm^{-1} is the vibration of phenyl which belonging to the calix[4]aren. The spectrum 3425.98 cm^{-1} and 1635.43 cm^{-1} are the characteristic peaks of N—H. The result indicated that MIPs were prepared expectably. The SEM images in Figure 6 show that MIPs particles have larger volume, and the surface of MIPs particles was roughness and coated with the polymers, compared with activated silica. There were internal pores in activated silica, while these pores were filled by printed polymer in MIP. It could be considered that adsorption not only exists in surface layer on silica particles, but also in internal pores filled MIP.

Adsorption behavior of MIPs

Adsorption Kinetics. The kinetic process of MIPs adsorbing artemisinin was shown in Figure 7. The adsorption capacity of MIPs or NIPs related to the template or analogue are calculated by

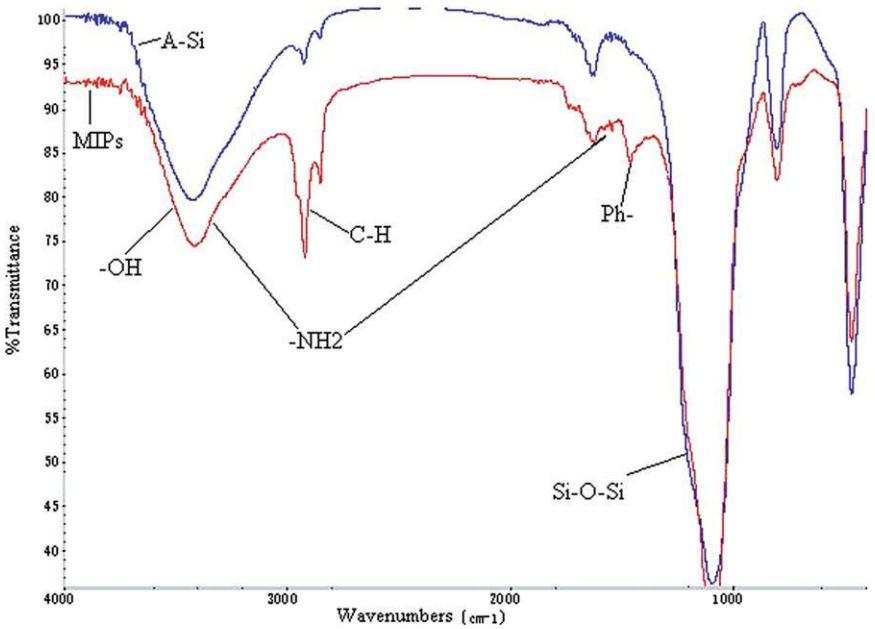

Figure 5. FT-IR spectra of activated silica and MIPs.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$Q = \frac{(C_0 - C_t) * V}{W} \tag{6}$$

where Q (mg/g) is the adsorption capacity, V (ml) is the volume of adsorption solution, W is the weight of MIPs or NIPs, C_0 (mg/ml) is the initial concentration of the template or analogue, and C_t (mg/ml) is the equilibrium concentration of the template or analogue.

The adsorption quantity is average of the three batches experiment, and the standard deviation of the experiment was within ± 2.0. The error bars were shown in the Figure 7. The result indicated that 55% of the total amount of artemisinin loaded is bound to the recognizing sites within 15 min, and the absorption capacity increase with the adsorption time. After 4 h, the adsorption rate became slower and reached equilibrium in 10 h. This means that binding sites on imprinting polymers were occupied by artemisinin. As the MIPs were formed on porous silica surface, at the beginning, the binding sites on the silica surface were quickly bounded with templates. Therefore adsorption capacity increased rapidly in the first 4 h. Since the mass transfer of the internal pore was slower than on the surface, the binding sites in the pores were occupied slowly. The difference of adsorption quantity changed was about 3.0 mg/g from 4–10 h.

Adsorption Isotherm. The adsorption ability of imprinting polymers for artemisinin was investigated by preparing adsorption isotherm. The adsorption capacity was an important factor to evaluate the imprinting polymers. The range of artemisinin concentration was from 0.1–1.5 mg/ml. The adsorption isotherm was shown in the Figure 8 with the error bars, and the standard deviation of the experiment was within ± 2.0. The imprinting polymers had a much higher adsorption capacity than nonimprinting polymers. The maximum adsorption capacity of MIPs reached 42 mg/g when the concentration is 1.5 mg/ml. The number is nearly twice value of nonimprinting polymers. This indicated that the imprinting polymers had higher adsorption ability for the artemisinin.

The Imprinting Factor and Selective Factor. The competitively selective adsorption of imprinting polymers was investigated by using the mixture of artemisnin and artemether at 1.0 mg/ml.

The specific recognition ability of MIPs was evaluated by imprinting factor (α):

$$\alpha = \frac{Q_{MIPs}}{Q_{NIPs}} \tag{7}$$

where Q_{MIPs} and Q_{NIPs} are the adsorption capacity of template or analogue bound on MIPs or NIPs.

The selective factor β was defined as

$$\beta = \frac{\alpha_{tem}}{\alpha_{ana}} \tag{8}$$

Where α_{tem} is the imprinting factor related to template and α_{ana} is the imprinting factor related to the analogue.

The adsorption quantity, imprinting factor and selective factor obtained in the competitive experiment were shown in Table 2. The imprinting factor (α) reflected the recognizing ability. A high α value indicates that the imprinting polymers have a good recognition ability compared with nonimprinting polymers. In this experiment, $\alpha = 2.0$. This demonstrates that imprinting sites can selectively bind artemisinin, and the imprinting polymers had high adsorption capacity for templates. The selective factor $\beta = 1.5$ indicated that the imprinting polymers had higher selectivity for artemisinin than for the analogue artemether. It could be believed that artemisinin could be separated from analogue artemether in a chromatography column packed imprinting polymers.

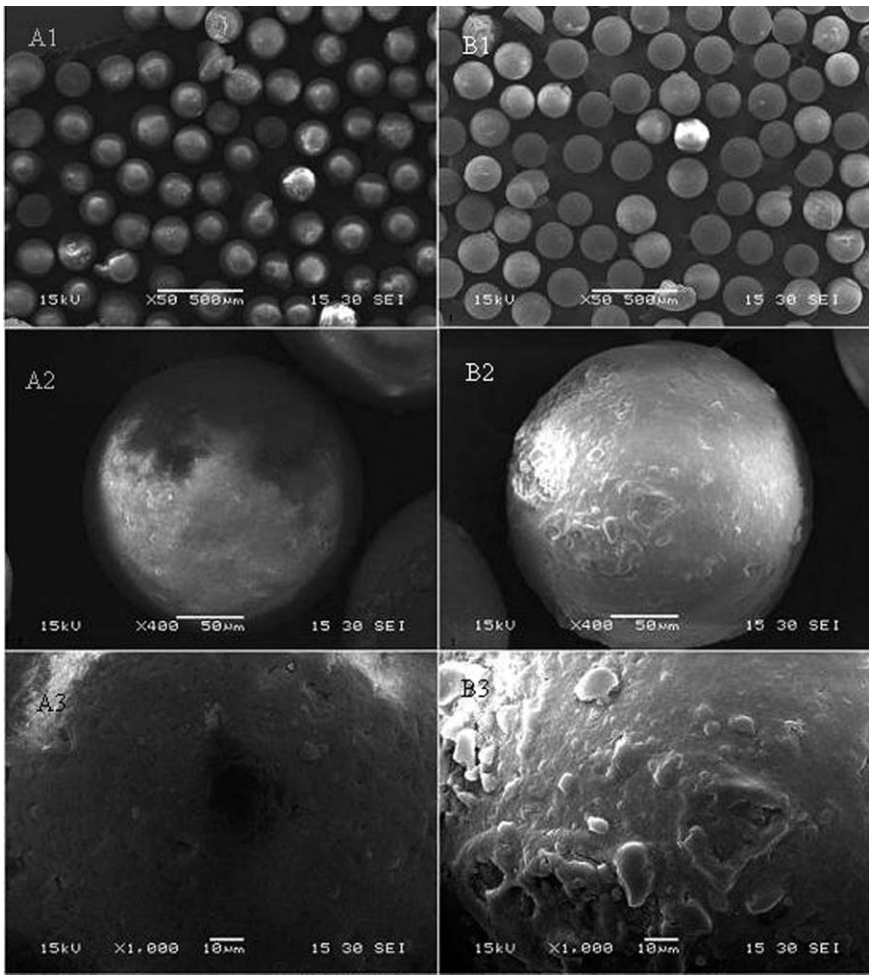

Figure 6. The SEM images of A-Si (A1,A2,A3) and MIPs (B1,B2,B3).

Adsorption experiments in supercritical CO_2 fluid

Adsorption Kinetics. In the experiment, 0.0400 g artemisinin and 0.2000 g MIPs were added into the reaction chamber, and then the chamber was adjusted to the desired pressure and temperature (20 MPa and 40°C). The taken samples at different time were analyzed by HPLC. As shown in the Figure 9 the artemisinin adsorbed on MIPs could reach equilibrium within 3.5 h, and the adsorption quantity was 115.0 mg/g. This value was nearly three folds

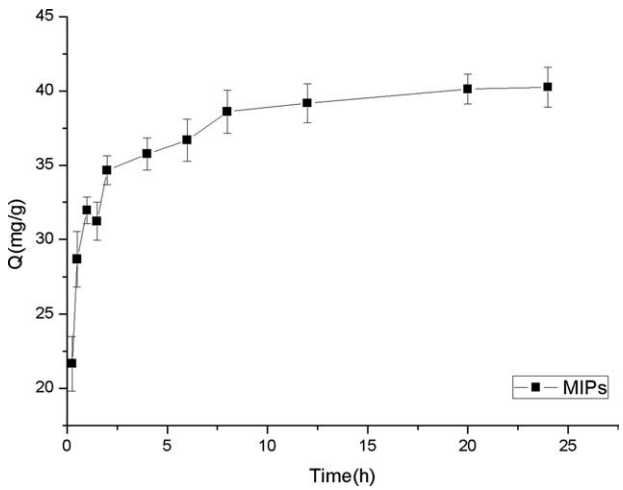

Figure 7. Adsorption kinetic curves of MIPs.

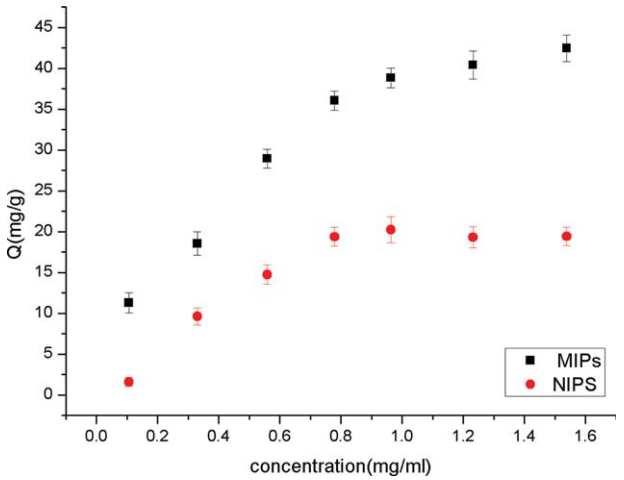

Figure 8. The adsorption isotherm of MIPs and NIPs on artemisinin.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 2. Imprinting Factor and Selective Factor of Artemisinin

	$Q_{AR(mg/g)}$	$Q_{AMe(mg/g)}$	$\alpha = Q_{MIPs}/Q_{NIPs}$		$\beta =$
			α_{AR}	α_{AMe}	α_{AR}/α_{AMe}
MIPs	40.39 ± 1.65	14.27 ± 1.29	2.007	1.329	1.509
NIPs	20.12 ± 1.81	10.69 ± 1.05			

of the adsorption quantity under the condition of normal organic solvents. If the amount of artemisinin added into the chamber increased, the adsorption capacity should also be increased. The equilibrium time shorten 6.5 h, compared with the equilibrium time under the condition of normal organic solvents. It is known to all, supercritical fluid has stronger dissolving ability and penetrability. This means that the mass transfer in supercritical fluid is much faster than the liquid. The inner binding sites in imprinting polymer would be quickly occupied by the template. Thus under the condition of SCF, the equilibrium of the imprinted polymers could be rapidly reached and adsorption capacity increased greatly.

Adsorption Isotherm. The amount of artemisinin, MIPs, and CO_2 added into the reaction chamber were shown in Table 3. The adsorption capacity was calculated by formula (6). The adsorption isotherm in supercritical fluid system was shown in the Figure 10. From the adsorption isotherm, the adsorption capacity increased with the artemisinin concentration. When the concentration of artemisinin is 2.4 mg/ml, the adsorption capacity was higher than 120 mg/g. If the weight of the artemisinin added into the chamber were increased further, the dissolving time would increase, and long dissolving time would cause much error in the experiment. In the experiment, all the artemisinin was completely dissolved in 5 min to reduce the error.

The Selective Experiment in Supercritical Fluid. To the reaction chamber, 0.05 g artemisinin, 0.05 g artemether, and 0.15 g MIPs/NIPs were added and CO_2 was pumped into it. Adsorption quantity was shown in the Table 4. It could be seen from the Table 4 that the adsorption quantity of MIPs related to template is higher than the analogue and adsorption quantity of MIPs related to artemisinin is higher than the NIPs related to artemisinin. Concretely, imprinting factor is 2.38 and selective factor is 2.27. Compared with the condition in petroleum ether, the MIPs have higher selectivity for artemisinin in the supercritical fluid systems.

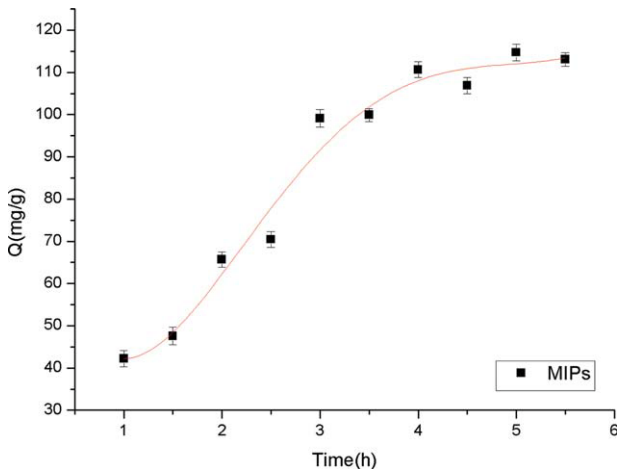

Figure 9. The adsorption kinetics of MIPs on artemisinin in SCF system.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3. The Amount of Artemisinin, MIPs and CO_2 used in the Adsorption Experiment

Material	Artemisinin (g)	MIPs (g)	CO_2 (g)
1	0.0098	0.2074	16.76
2	0.0198	0.1919	16.75
3	0.0302	0.2024	16.46
4	0.0402	0.2016	17.13
5	0.0496	0.1947	17.15

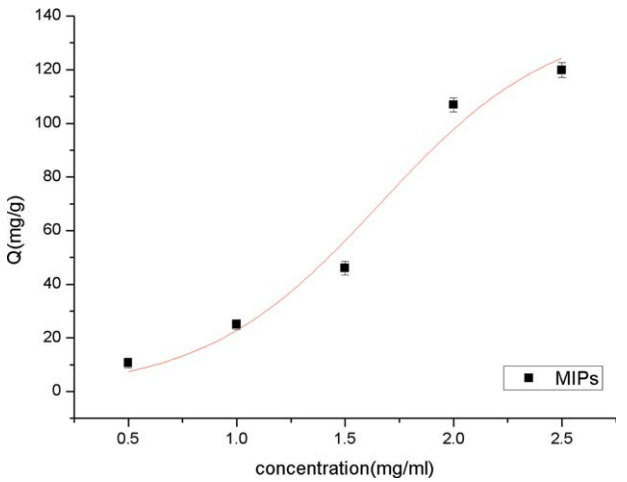

Figure 10. The adsorption isotherm of MIPs in supercritical system.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Conclusion

In this work, calix[4]aren as a monomer was grafted onto the silica surface. silane was used as cross-linker to prepare the imprinting polymers with artemisinin. The MIPs were prepared expectably and it had high adsorption capacity and high selectivity for artemisinin. In the supercritical fluid systems, the MIPs had higher adsorption capacity and could rapidly reach the equilibrium.

Surface MIPs under the condition of supercritical fluid show many advantages such as faster mass transfer, higher selectivity, and higher adsorption capacity. Therefore, the surface imprinting technology combined with the supercritical

Table 4. Imprinting Factor and Selective Factor of Artemisinin in SCF

	$Q_{AR(mg/g)}$	$Q_{AMe(mg/g)}$	$\alpha = Q_{MIPs}/Q_{NIPs}$		$\beta =$
			α_{AR}	α_{AMe}	α_{AR}/α_{AMe}
MIPs	115.5 ± 2.13	40.27 ± 1.76	2.385	1.047	2.278
NIPs	48.43 ± 1.80	38.46 ± 1.52			

fluid technology is a promising method for separation of the active ingredients from Traditional Chinese Medicine.

Acknowledgments

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