

Synthesis and Recognition of Molecularly Imprinted Polymers for Gastrodin Based on Surface-Modified Silica Nanoparticles

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ABSTRACT: A molecular imprinting material with high performance for recognizing Gastrodin (GAS) was prepared by adopting the novel surface molecular imprinting technique. Silica nanoparticles were first modified with 3-methacryloxypropyltrimethoxysilane (KH-570) as a carrier material. Then, GAS surface molecule-imprinted polymer (GAS-SMIP) were prepared by polymerization with methacrylic acid as the functional monomer, ethylene glycol dimethacrylate as the crosslinker. The equilibrium adsorptive experiments indicated that GAS-SMIP had significantly higher adsorption capacity for GAS than its nonimprinted polymers. Scatchard analysis revealed that two classes of binding sites were formed in GAS-

SMIP with dissociation constants of 1.019 and 7.278 $\mu\text{mol/mL}$, and the affinity binding sites of 17.82 and 83.11 $\mu\text{mol/g}$, respectively. The selectivity coefficient of GAS-SMIP for GAS in respect to competition species obtained was 3.455, which revealed GAS-SMIP had excellent selectivity and site accessibility for GAS. Kinetic binding study showed GAS-SMIP adsorbed quickly in the first 40 min, and reached saturation adsorption at 1 h. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 121: 2354–2360, 2011

Key words: Gastrodin; surface molecular imprinting; adsorption; selectivity

INTRODUCTION

Molecularly imprinted polymers (MIPs) were artificially synthesized material to imitate natural molecular recognition. MIPs are extensively applied in segregation analysis, biosensor, catalysis, and other fields for its structure-effect predetermination, specific recognition, and widely practicability.^{1–9} Recent years, a number of novel strategies have developed for improving the performance of MIPs and adapting them to new application areas. Surface imprinting technology¹⁰ that controls templates to be situated at the surface or in the proximity of material surface has received significant attention over the past few years, such as templates immobilizing on surfaces,¹¹ film or surface grafting,^{12,13} supports sacrificing,¹⁴ and imprinting on the surface of core-shell microbeads.¹⁵ The imprinted materials prepared with these approaches have significant advantages compared with the materials by traditional imprinting technology, such as complete removal of templates, good accessibility to the target species, and low mass-transfer resistance. Although the surface imprinting technology still needs to overcome some limitation. For example, immobilizing the template molecules on the

surface of substrate is typically carried out by the covalent interaction, which remains complicated and difficult reproducible. Furthermore, because the area of material's surface is greatly limited, the total amount of recognition sites is very small in the imprinted materials. Accordingly, these products have always rather small rebinding capacity. Therefore, surface imprinted materials with extremely high specific surface area are expected to improve the binding capacity, binding kinetics, and site accessibility of imprinted materials. Imprinting on the surface of silica nanoparticles via the graft polymerization fixed the binding sites maximumly on the surface, improved mass-transport significantly, enhanced recognition ability, and further strengthened the separation efficiency of target molecular.¹⁶ Gastrodin (GAS) is an effective ingredient of a traditional Chinese medicine *Gastrodia*.^{17,18} Because of its good sedative hypnotic effect and low toxicity as a drug for neurasthenia, insomnia, and headaches, it has high research and application value in the health care field as well as with certain effect on maintaining a good learning state for students and keeping sober and defending against aging. At present, the separation of GAS was complex and low efficiency by High Performance Liquid Chromatography (HPLC).^{19–21}

In this article, we applied surface molecular imprinting technology in preparing separation material with great specific recognition and high efficiency, which provided an original method for efficient enrichment

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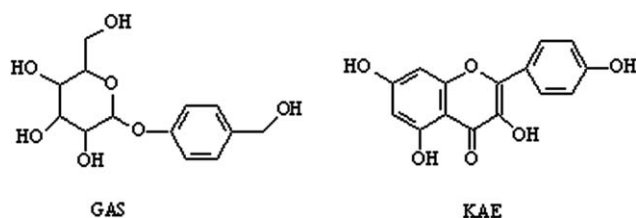


Figure 1 The structures of GAS and KAE.

and purification of GAS theoretically. Silica nanoparticles modified with 3-methacryloxypropyltrimethoxysilane was used as carrier. A kind of novel MIP grafting on silica surface was synthesized by using methacrylic acid (MAA) and GAS as functional monomer and template, respectively. To test the properties of imprinted particles, a series of adsorption studies were conducted. The results demonstrated that GAS surface molecule-imprinted polymer (GAS-SMIP) not only has great rebinding capacity but also was highly effective for recognizing the template.

EXPERIMENTAL

Reagents and instruments

Silica nanoparticles (20 nm) were purchased from Nanjing Haitai nanomaterial Co. (Nanjing, Jiangsu, China). 3-Methacryloxypropyltrimethoxysilane (KH-570) was obtained from Jiangsu Chenguang Co. (Zhenjiang, Jiangsu, China). GAS (98%) and Kaempferol (KAE; 98%) were purchased from Nanjing Sorun Herbal Technology Development Co. (Nanjing, Jiangsu, China). MAA was obtained from Shanghai Sinopharm Chemical Co. (Shanghai, China). Ethylene glycol dimethacrylate (EDMA) was purchased from Shanghai Haiqu Chemical Co. (Shanghai, China). 2,2'-Azobisisobutyronitrile (AIBN) was purchased from Shanghai NO.4 reagent and H. V. Chemical Co. (Shanghai, China). MAA and EDMA were distilled under vacuum before used. AIBN was purified by recrystallization from ethanol. All other reagents used were all of analytical chemical grade. The structures of GAS and KAE were shown in Figure 1.

The instruments used in this study were as follows: UV-2450 Ultraviolet-visible Spectrophotometer (Shimadzu, Japan); SHZ-82 constant temperature bath oscillator (Zhongda Instrument, Jiangsu, China); KQ2200DB ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Jiangsu, China); YXJ-II high-speed centrifuge (Zhongda Instrument, Jiangsu, China); fourier transform infrared spectra (FT-IR) (Nicolet, America); Element analysis instrument (CE, Italy).

Preassembling test

A series of solutions were prepared containing a fixed concentration of GAS (0.5 mmol/L) and vari-

ous amounts of MAA in methanol (molar ratio of GAS/MAA was 1 : 0, 1 : 2, 1 : 4, 1 : 6, 1 : 8, 1 : 10, 1 : 12, 1 : 16, respectively). The changes in absorbance and difference absorption spectra of these solutions were determined with corresponding methanol solutions of MAA as references.

Procedures for the preparation of the GAS-SMIP

Introduction of vinyl groups on the surface of silica nanoparticles

The activated silica (AS) was obtained by drying in vacuum at 60°C to eliminate surface water and activate the surface silanol groups for silanization.

Dried activated silica (6 g) was dispersed in absolutely dry toluene (150 mL) in three-necked round-bottomed flask under the atmosphere of nitrogen with continuous stirring. Then, KH-570 (4.5 mL) and triethylamine (1 mL) were added into the flask. The reaction was carried out at 90°C for 24 h. The beads were then separated from the mixture via centrifugation and washed with toluene, acetone, and methanol, respectively. The modified silica nanoparticles MPS-SiO₂ were dried under vacuum at 80°C for 24 h.

Synthesis of GAS-SMIP and nonimprinted polymers (SNIP)

The template GAS (0.25 mmol) and the functional monomer MAA (1.5 mmol) were dissolved in methanol (10.0 mL) in a glass vial (50 mL volume). After shaking for 6 h, MPS-SiO₂ (0.160 g), EDMA (7.5 mmol) as the crosslinking agent, and AIBN (20 mg) as the initiator were added to this solution. The mixture was fully stirred at room temperature for 6 h, followed by purging thoroughly with nitrogen for 10 min, and then sealed under vacuum. The polymerization was started by thermal initiation in a water bath at 50°C for 6 h, and then continued at 60°C for 24 h.²² The product particles were washed repeatedly with a mixed solvent of methanol and acetic acid ($V_{\text{CH}_3\text{OH}} : V_{\text{CH}_3\text{COOH}} = 90 : 10$) to remove the template GAS and the remaining monomer. GAS-SMIP was obtained by filtrating and drying. As a control, SNIP were prepared in the absence of the template and treated with the same method.

Binding experiments

The capacity of the adsorbent for GAS was determined in batch mode, involving adsorption isotherm, adsorption dynamics. A certain amount GAS-SMIP (50.0 mg) or SNIP (50.0 mg) was accurately taken into a 5-mL centrifuge tube, and then 3.0 mL of GAS solution in methanol with varies concentrations was added. The mixture was agitated in a shaken bed. At different time intervals, the mixture

was centrifuged at 4000 rpm for 10 min, and the supernatant solutions were collected after filtrating with microporous membrane and diluted with methanol to a certain times. Finally, the concentrations of KAE were determined by UV spectrophotometer. The adsorption quantity (Q), which was defined as μmol of template molecule GAS bound per gram polymers, was calculated by the following eq. (1).

$$Q = (C_0 - C)V/W \quad (1)$$

where Q ($\mu\text{mol/g}$) is the adsorbed amount of GAS, C_0 ($\mu\text{mol/mL}$) is the initial concentration of GAS, C ($\mu\text{mol/mL}$) is the supernatant solution's concentration of GAS, V (mL) and W (g) are the volume of the solution and the amount of the polymer, respectively.

The binding parameters of GAS-SMIP are mainly estimated by Scatchard analysis with the data of static adsorption experiment. Scatchard equation is described as follows:

$$Q/C = (Q_{\text{max}} - Q)/K_d \quad (2)$$

where Q ($\mu\text{mol/g}$) is the adsorbed amount of GAS, Q_{max} ($\mu\text{mol/g}$) is the apparent maximum adsorption capacity, C ($\mu\text{mol/mL}$) is the equilibrium concentration of GAS, and K_d ($\mu\text{mol/mL}$) is the equilibrium dissociation constant.

Selectivity experiments

To examine the recognition selectivity of GAS-SMIP nanoparticles toward GAS, KAE was selected as a contrast substance in this study. The adsorption capacity of the template or the competition species was calculated as above. The distribution coefficient for each substance was calculated according to eq. (3),

$$K_D = C_P/C \quad (3)$$

where K_D represents the distribution coefficient (mL/g), C_P ($\mu\text{mol/g}$) is the equilibrium combining quantity, and C ($\mu\text{mol/mL}$) is the equilibrium concentration.

The selectivity coefficient of GAS-SMIP for the combining of GAS with respect to the competition species KAE is evaluated by α , which is defined as

$$\alpha = K_{D_i}/K_{D_j} \quad (4)$$

where α is the selectivity coefficient, i and j represent the template and competition species, respectively. The value of α allows an estimation of selectivity of GAS-SMIP for GAS.

A relative selectivity coefficient α' can be defined as expressed in eq. (5), and the value of α' can indi-

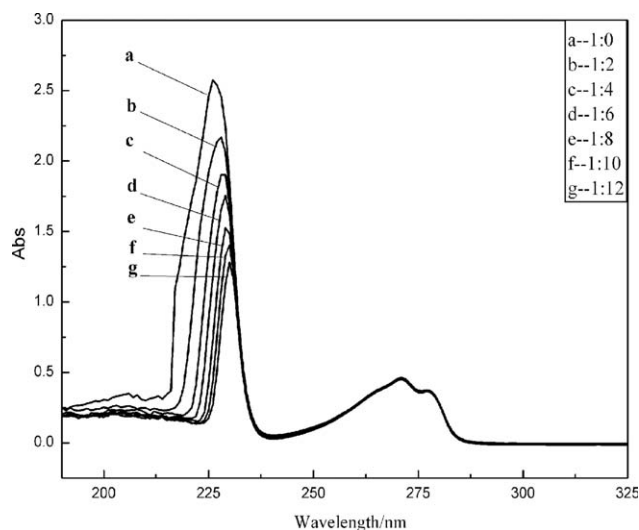


Figure 2 Absorption spectra of the GAS in the presence of various concentration of MAA in methanol. Concentration of GAS: 0.5 mmol/L, corresponding pure MAA solutions as blanks.

cate the enhanced extent of adsorption affinity and selectivity of imprinted material for the template molecule with respect to nonimprinted material.

$$\alpha' = \alpha_{\text{GAS-SMIP}}/\alpha_{\text{SNIP}} \quad (5)$$

where $\alpha_{\text{GAS-SMIP}}$ is the selectivity coefficient of GAS-SMIP for GAS with respect to the competition species KAE and α_{SNIP} is the selectivity coefficient of SNIP for GAS with respect to the same competition species KAE.

RESULTS AND DISCUSSION

UV analysis

It is well known that the principle of molecular imprinting depends on the preservation of the host guest structure in the polymerization solution into a polymer matrix, so it is crucial that template molecule and functional monomers form stable complexes by the self-assembly process at the prepolymerization stage.²³ MAA is frequently used as functional monomer for imprinting basic template species. It is a kind of acidic functional monomer which serves as not only a proton donor but also a proton acceptor. Its carboxyl group may have typical interaction with the hydroxyl group of GAS by forming hydrogen bond. Here, we characterized the interactions between the template GAS and the functional monomer MAA at the prepolymerization stage by UV-vis spectroscopic analysis and it was shown in Figure 2.

It was clearly observed that the maximum absorbance of the mixture solutions was declining and the

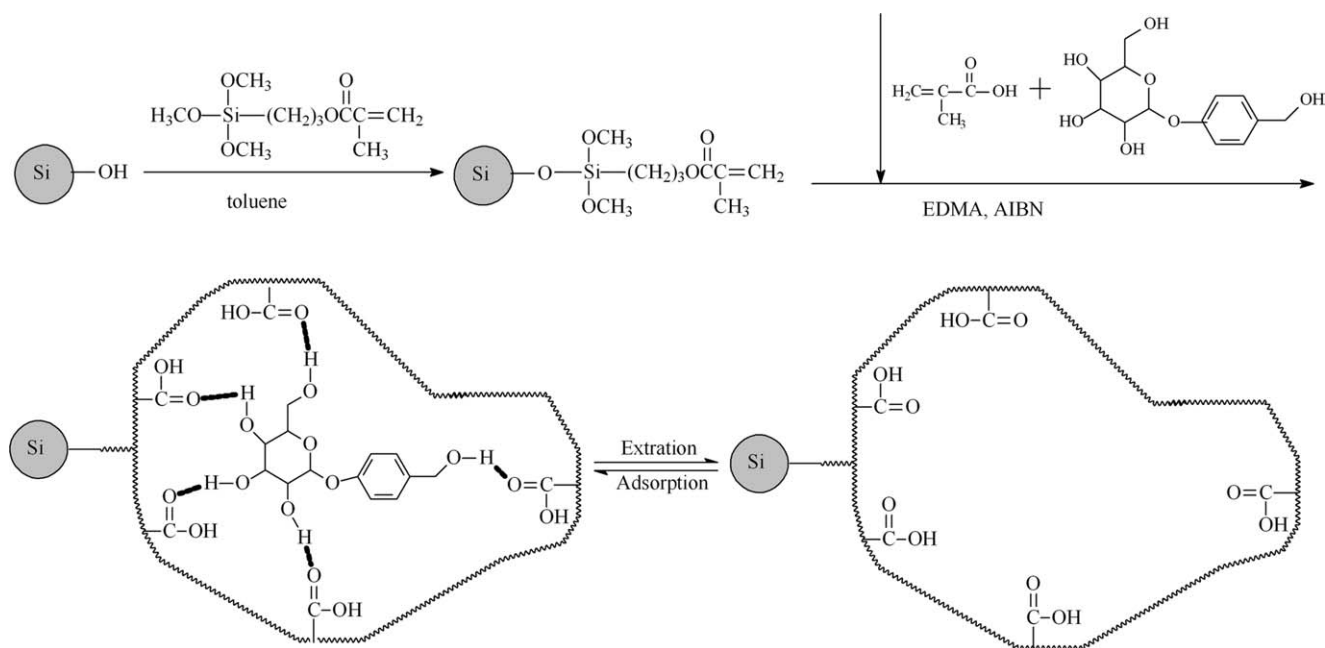


Figure 3 Schematic illustration for synthesis of GAS-SMIP.

maximum absorption wavelength showed bathochromic effect with increasing concentration of MAA. The change in the absorption spectra indicated that GAS interacted with MAA by hydrogen bond and the interaction was getting stronger when the concentration of MAA increased further. However, excessive assembly of functional monomers may lead to nonfunctional monomer residues, which increases the nonselective binding sites, on the other hand, too large amount of functional monomer may lead to their own association, leading to the decrease of selective binding sites. The molar ratio of the template and functional monomer-1 : 6 was chosen for the synthesis of the GAS-SMIP.

Preparation of the GAS-SMIP

In this study, 3-methacryloxypropyltrimethoxysilane (KH-570) was chosen as the intermediate to link activated silica and polymers. After activated silica was chemically modified with KH-570, polymerizable double bonds were attached chemically on the surface of the silica particles. As the interaction between GAS and MAA reached stably, the modified silica particles, crosslinking agent EDMA were added. Then, with the initiator AIBN, the graft polymerization was carried out on the surface of the silica particles. GAS molecules were enveloped in the crosslinking networks. As the template molecules were washed away, a great deal of GAS caves will be reserved in the imprinted polymer coated on the surface of silica particles, and the GAS imprinted material GAS-SMIP was obtained. The whole preparation process of GAS-SMIP is shown in Figure 3.

Characteristic of elemental analysis and FT-IR spectra

Elemental analysis was used to confirm the grafting of polymer particles. Table I summarizes elemental analysis results of the silica samples following each functionalization step. With the highest grafting rate, the modified silica particles was obtained with a carbon content of 5.444% and a hydrogen content of 1.104%. According to the carbon and hydrogen contents of the MPS-SiO₂ and SiO₂(AS), 3-methacryloxypropyltrimethoxysilane was bonding on the surface of the activated silica, of which the graft density was calculated about 0.648 mmol/g. In this process, the activated silica gave an estimated 6.10 mmol/g capacity of silanol groups. The capacity of functionalized silanol groups was 4.156 mmol/g when the three methoxy groups of the silane reagent were supposed to react, and thus, 68.1% of silanol groups were still not functionalized.

FT-IR spectra of the four kinds of nanoparticles-SiO₂(AS), MPS-SiO₂, GAS-SMIP, and SNIP were determined with KBr pellet method, and their FT-IR spectra were shown as a, b, c, and d in Figure 4,

TABLE I
Elemental Analysis of SiO₂(AS), MPS-SiO₂, GAS-SMIP, and SNIP

Samples	C%	H%
SiO ₂ (AS)	0.000	0.610
MPS-SiO ₂	5.444	1.104
GAS-SMIP	53.580	6.284
SNIP	53.150	6.231

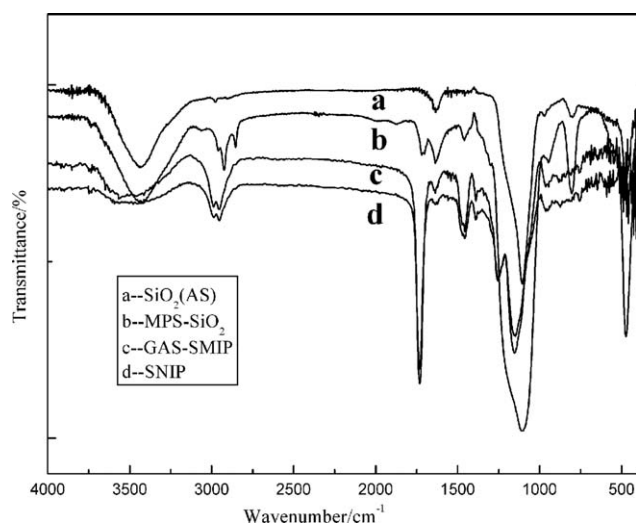


Figure 4 FT-IR spectra of $\text{SiO}_2(\text{AS})$, MPS-SiO_2 , GAS-SMIP , and SNIP .

respectively. The observed features around 1057 and 971 cm^{-1} indicated Si—O—Si and Si—O—H stretching vibrations, respectively. The bands around 792 and 465 cm^{-1} resulted from Si—O vibrations. When compared with the spectra of a and b, the stretching vibration of O—H appearing at around 3450 cm^{-1} indicated that silanol groups were not silanized completely, which was corresponding with the elemental analysis. The vinyl groups introduced onto the surface of the activated silica by immobilization of a long chain group played a space-shield effect on the surrounding silanol groups, which was why so many silanol groups were still not derivatized.

The spectrum of MPS-SiO_2 showed the new bands at 2960 cm^{-1} and 1711 cm^{-1} , which were attributed to the stretching vibration absorption of saturated C—H band and carbonyl C=O in ester groups, respectively. The infrared data indicated that the sur-

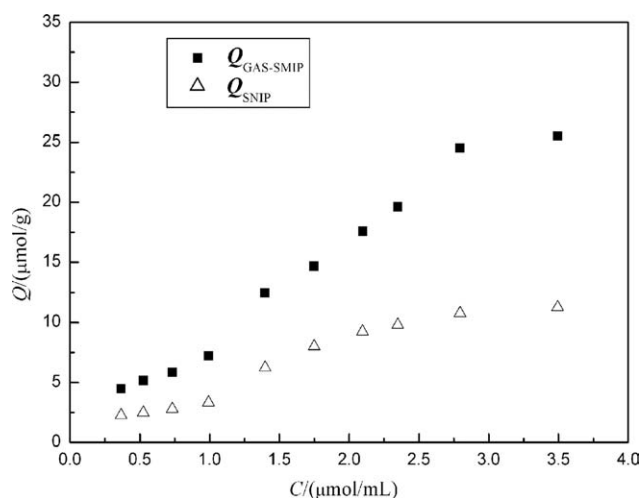


Figure 5 Adsorption isotherm of GAS-SMIP and SNIP .

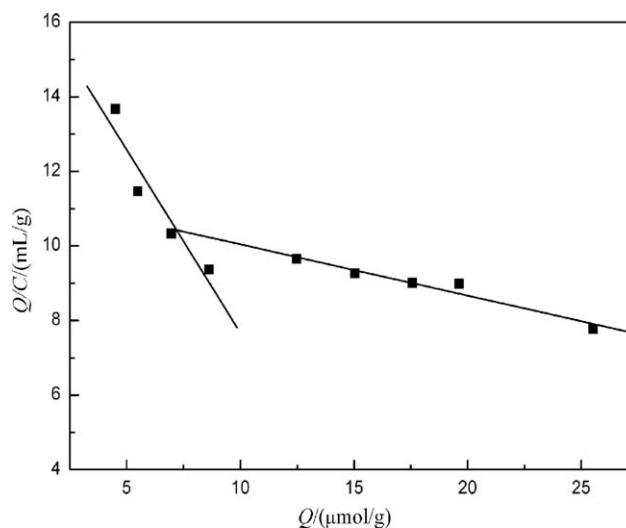


Figure 6 Scatchard analysis of GAS-SMIP .

face of $\text{SiO}_2(\text{AS})$ had been modified by silane reagent. Imprinted and nonimprinted material showed similar locations and appearances of the major bonds. When compared with modified silica particles, the spectra of the imprinted and nonimprinted polymers showed that the stretching vibration absorption of saturated C—H band was strengthened greatly as well as carbonyl C=O in ester groups resulted from polymerization crosslinking process. This fact suggested that the polymerization had been successfully carried out.

Adsorption capacity of GAS-SMIP for GAS and Scatchard analysis

The template recognition ability of GAS-SMIP was investigated in static equilibrium binding experiments. The adsorption capacities of the polymers for GAS with various initial concentration of the template were shown in Figure 5.

As shown in Figure 5, GAS-SMIP exhibited a higher capacity for GAS than SNIP at either low or high concentration. GAS at low concentration was not enough to saturate the specific binding cavities. The binding amount of the polymers increased gradually with increase of GAS initial concentration and the difference of adsorption between GAS-SMIP and SNIP was getting larger with the increasing concentration of the template. The results indicated that the two polymers with almost the same element were remarkably different in their space-structure. The nonselective physical adsorption occurred between SNIP and GAS . In contrast, the GAS-SMIP adsorbed much more template than that of SNIP because GAS-SMIP had generated specific recognition sites in imprinting cavities.

Scatchard analysis was used to evaluate the binding affinity and the theoretical binding site number for template of the molecularly imprinted material. Figure 6 showed the Scatchard plot of the adsorption

TABLE II
The Results of the Scatchard Analysis

Binding site	Linear equation	K_d ($\mu\text{mol/mL}$)	Q_{max} ($\mu\text{mol/g}$)
Higher affinity site	$Q/C = 17.48 - 0.9812Q$ ($r^2 = 0.8569$)	1.019	17.82
Lower affinity site	$Q/C = 11.42 - 0.1374Q$ ($r^2 = 0.9337$)	7.278	83.11

r : relative coefficient.

of GAS-SMIP for GAS. There were two distinct linear sections in the plot suggesting that two types of binding sites existed in the imprinted polymers in respect to the adsorption for GAS: one was of high selectivity or affinity with a high binding energy, and the other was of low affinity with a low binding energy. The K_d and Q_{max} values were calculated from the slopes and intercepts of the two linear portions of the Scatchard analysis, and the results are listed in Table II.

Adsorption dynamics

Adsorption dynamic studies were carried out for GAS-SMIP particles to investigate the adsorption process. It can be seen from Figure 7 that adsorption capacity of GAS-SMIP particles was observed to increase quickly in the first 40 min, and then the rate of adsorption increased slowly with time extension. After about 1 h, adsorption process reached equilibrium. At the early time, a large number of imprinted cavities existed on the surface of the imprinting material, so the template GAS was easy to reach the specific binding sites. When the recognition sites were filled up, the rate of adsorption dropped significantly and adsorption process achieved equilibrium gradually.

Selectivity of GAS-SMIP

To examine the recognition selectivity of GAS-SMIP nanoparticles toward GAS, KAE was selected as a

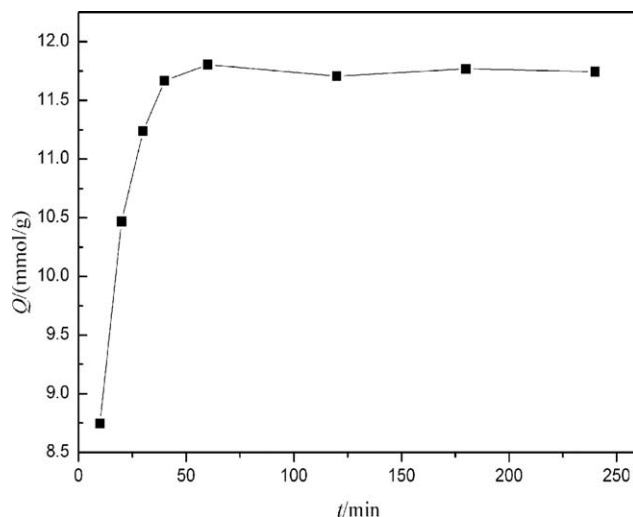


Figure 7 Adsorption dynamics of GAS-SMIP.

contrast substance in this study. KAE is an effective ingredient of plants. It contains hydroxyl groups and has the same molecular weight as GAS.

Figure 8 illustrates the adsorption amounts of GAS-SMIP and SNIP for GAS and KAE, respectively. GAS-SMIP showed a higher adsorption capacity to GAS than KAE, whereas the adsorption capacity of SNIP for the two substrates was almost the same. The results indicated that GAS-SMIP differed from SNIP in the space structure obviously. Therefore, the high adsorption ability of GAS-SMIP for the template GAS stemmed mainly from existence of cavities, which were perfectly complementary both in shape and functional groups with GAS. GAS-SMIP had not only strong effect binding for template but also steric effect hindering nontemplate from being adsorbed. KAE had less chance to be adsorbed on the GAS-SMIP. The binding capacity of SNIP for the substrates was due to physical adsorption, which was nonspecific adsorption and had no selectivity.

As shown in Table III, distribution coefficient and selectivity coefficient of the imprinting polymers lied on the structure of substrate. The distribution coefficient of the imprinting material for GAS is very high and the selectivity coefficient is 3.455, which were displaying an excellent binding selectivity. When compared with GAS-SMIP, SNIP had considerably less binding for the selected substrates. The above facts

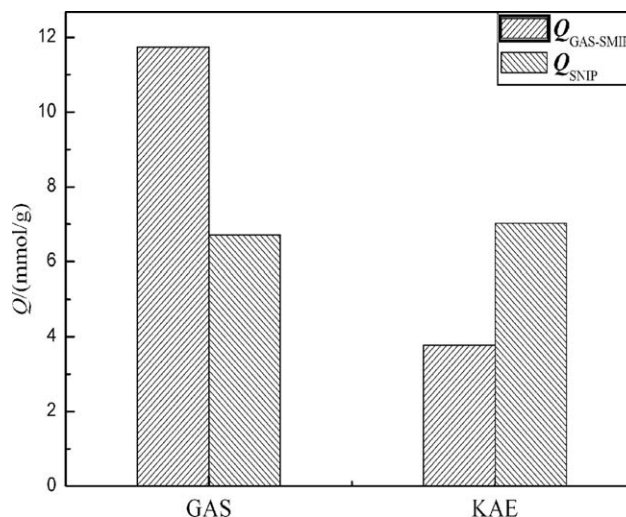


Figure 8 Adsorption amounts of the polymers for GAS and KAE.

TABLE III
Distribution Coefficient and Selectivity Coefficient
Data of the Polymers

Samples	$K_{D\text{ GAS}}$	$K_{D\text{ KAE}}$	$\alpha_{\text{GAS-SMIP}}$	α_{SNIP}	α'
GAS-SMIP	19.53	5.654	3.455	0.9234	3.741
SNIP	10.43	11.30			

clearly revealed that GAS-SMIP had high recognition selectivity and binding affinity for the template GAS.

CONCLUSIONS

In this article, GAS-imprinted material GAS-SMIP with high performance has been prepared successfully by using the intermolecular forces of the hydrogen bonds between GAS and the grafted macromolecules. GAS-SMIP had high adsorption capacity, selectivity, and good site accessibility for GAS, and it also had significantly good mass transport property. This class of new imprinted materials like GAS imprinted on the surface of modified silica particles GAS-SMIP is significant for the study of separation and enrichment of GAS from complex plant samples.

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