

Water-Compatible Imprinted Polymers Based on CS@SiO₂ Particles for Selective Recognition of Naringin

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ABSTRACT: Molecularly imprinted polymers are being proposed for the development of novel biorecognition elements for active components. In this study, an imprinted chitosan coated silica nanoparticles (I-CS@SiO₂) polymer was prepared by a simple procedure, in which, naringin (NG) with antioxidant activity, acted as a template, silica as a matrix and CS as a functional polymer. The binding properties were discussed by the equilibrium binding experiment method. Experiments show that the adsorption characteristics of I-CS@SiO₂ are better than that of nonimprinted polymer. It exhibited high selectivity for NG when compared with the nonimprinted polymer, with an imprinting factor α of 1.74. Scatchard analysis of the I-CS@SiO₂ indicated that there was a class of binding sites during the I-CS@SiO₂ recognizing NG: The dissociation constant of K_D is 0.016 mmol L⁻¹, the maximum apparent binding capacity of B_{max} is 6.56 μ mol g⁻¹. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 40491.

KEYWORDS: biopolymers and renewable polymers; molecular recognition; separation techniques

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INTRODUCTION

Naringin (NG) is of the flavonoid family, whose members are widely found in plants such as fruits and vegetables. NG exhibits interesting pharmacological and biological activity including antiallergy, antiviral, anti-inflammatory, and anti-cancer properties.^{1,2} The extraction or separation of active components from plants is usually tedious and inefficient, because of their poor affinities and selectivity to conventional separation materials (e.g., silica gel, modified silica gel, or polyamides). Much research has been directed toward the variation of binding materials to achieve selective interaction with the target molecule. Molecular imprinting could potentially be used to prepare separation materials for extracting NG.³⁻⁵

Molecular imprinting, which was first proposed by Wulff and Sarhan,⁶ and much expanded by the work of Mosbach and co-workers,⁷ has proven to be a versatile avenue to prepare synthetic receptors with tailor-made recognition sites.⁸⁻¹¹ Molecularly imprinted polymers (MIPs) with affinity and specificity, compared with other recognition systems, possess many promising characteristics, such as low cost and easy synthesis, high stability to harsh chemical and physical conditions, and excellent reusability. Thus, MIPs have received increasingly attentions in many fields, particularly as selective adsorbents for solid-phase extrac-

tion,¹²⁻¹⁶ chromatographic separation,^{17,18} ligand binding assays,^{19,20} chemical sensors.²¹⁻²⁴ And they are being proposed for the development of novel biorecognition elements for active components in aqueous media. Water incompatibility, the primary concern of imprinting fraternity,²⁵ severely hampers the applications of MIPs in aqueous media. Efforts are being made to improve the water compatibility of MIPs, for an instance, silica-surface-confined grafting procedure.²⁶

As the recognition of flavonoids in aqueous media is a bit challenging due to poor affinities, there is growing interest in producing MIPs.²⁷⁻³⁰ The recognition of NG was performed in aqueous medium using β -cyclodextrin (β -CD) as functional monomer.^{29,30} But it was difficult to find an excellent cross-linker. Within this context, we tried to exploit an alternative for preferential adsorption of NG on molecular imprinting technique in aqueous medium. Chitosan (CS) is a natural abundant biopolymer and possesses unique chemical and biological properties such as nontoxic, biocompatible, and biodegradable. Therefore, CS has been widely used in medicine and food industries.³¹ CS was used as functional polymer for the imprinted membrane, unfortunately, it showed a big swelling degree and lower selective property in an aqueous environment.³² To improve the physical/chemical properties of CS, formation of CS-silica hybrid materials maybe provides an

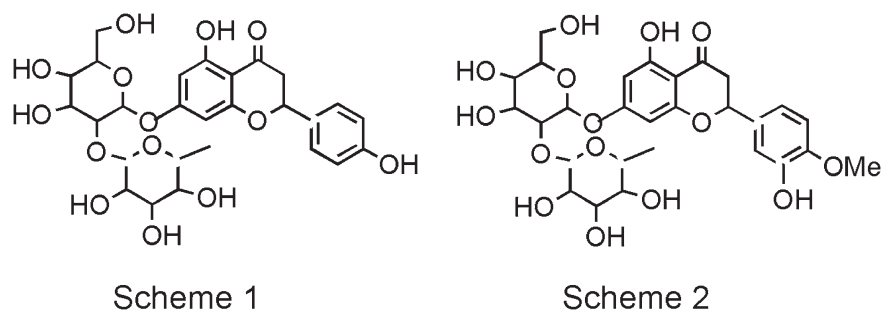


Figure 1. Schemes of naringin (Scheme 1) and hesperidins (Scheme 2).

efficient approach.³³ Herein we introduced CS coated silica nanoparticles (CS@SiO₂) as an alternative NG molecular imprinting functional material in aqueous medium. The structure and morphology of the resulting imprinted particles (I-CS@SiO₂) were characterized by X-ray diffraction (XRD), scanning electron microscope (SEM), thermogravimetric analysis (TGA), and FT-IR. The NG recognition properties were examined by single-naringin or competitive batch rebinding experiments and rebinding kinetics study.

EXPERIMENTAL

Materials

CS (with an *N*-deacetylation degree of 90%) and silicon dioxide were purchased from Guoyao Chemicals. Naringin (Figure 1, Scheme 1) and hesperidins (HD, Figure 1, Scheme 2) ($\geq 98\%$) were supplied by Nanjing Zelang Medicine. Other reagents used for the following investigations were of analytical grade.

Instruments

X-ray diffraction (XRD, Philips) studies were performed to investigate sample phase in $2\theta = 10^\circ$ to 80° . The morphologies of the polymers were examined by JSM-7500F scanning electron microscope (SEM). The characteristic functional groups were analyzed by means of NICOLET 380 FT-IR (USA Nicolet Co.). The thermal stability of the polymer was carried out using TGA/SDTA851e thermal gravimetric analysis (TGA, Sweden Mettler-Toledo).

Preparation of NG Imprinting CS@SiO₂ (I-CS@SiO₂)

The preparation of I-CS@SiO₂ particles was schematically shown in Figure 2. CS coated silica nanoparticles were prepared as reported elsewhere.³⁴ In our work, the I-CS@SiO₂ was prepared as follows: 1.0 g CS was dissolved in 60 mL 2% (m/v) acetic acid solution. Then add 5 mL DMF solution of naringin (0.6 g) with stirring for 2 h to obtain the prepolymerization solution. 5.0 g silica nanoparticles were then introduced to the

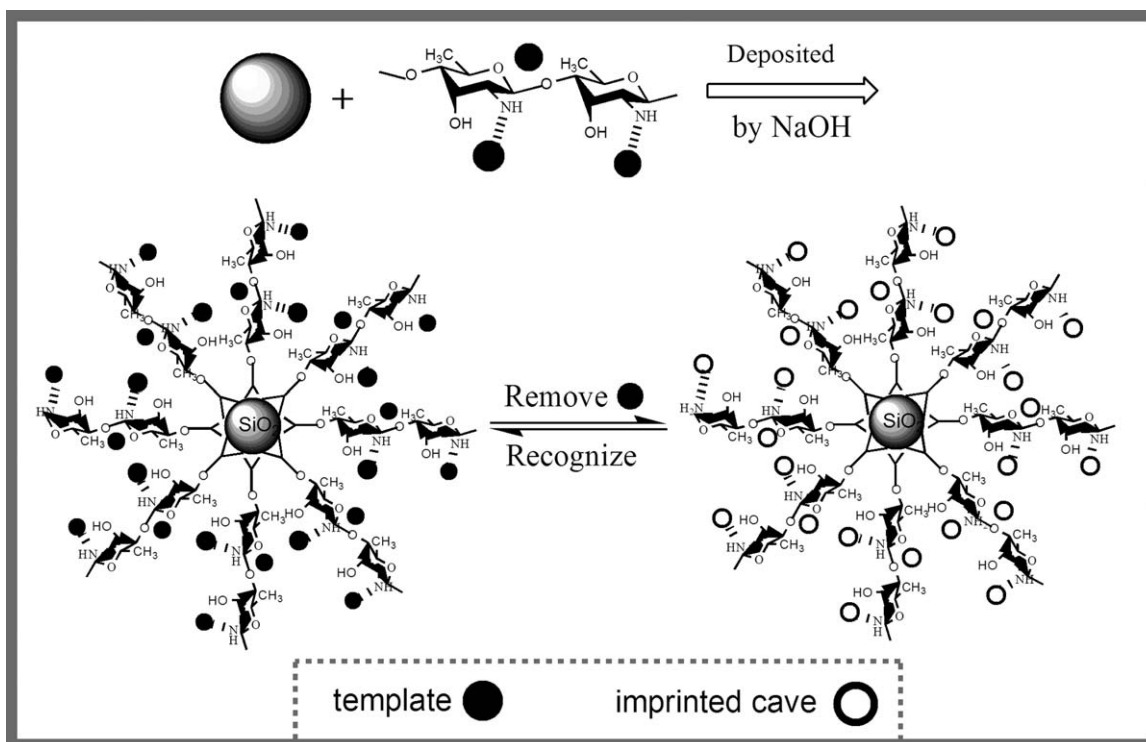


Figure 2. Schematic diagrams showing the preparation of I-CS@SiO₂ particles.

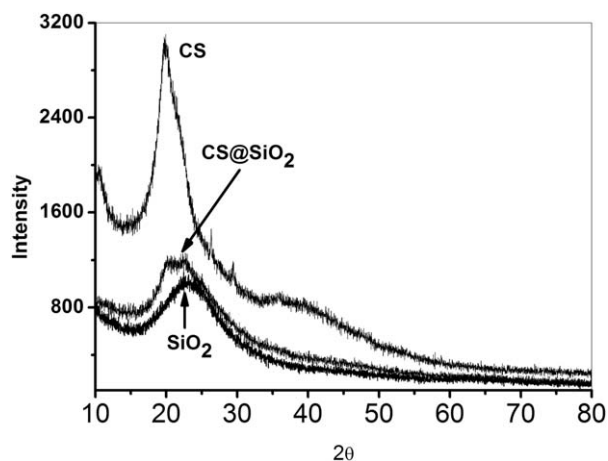


Figure 3. XRD patterns of SiO₂, CS and I-CS@SiO₂.

mixture under magnetic stirring. To obtain the CS@SiO₂ particles, 4 mol L⁻¹ NaOH solutions were added dropwisely to the mixture until pH = 13. The product was filtered, and washed thoroughly with distilled water until a neutral pH was achieved, and template molecular NG was removed from the particles using ethanol as an elate in the ultrasonic bath till no naringin was detected by UV measurements (283 nm). The final was lyophilized to obtain white I-CS@SiO₂ particle.

The nonimprinted polymer (N-CS@SiO₂) was also prepared by the same method as the preparation of I-CS@SiO₂, just without the addition of the template molecule NG.

Batch Rebinding Assay

Batch experiments were performed to examine the binding kinetics and binding equilibrium, and were carried out at room temperature and 100 rpm on an orbital shaker with ~0.2 g of the I-CS@SiO₂/N-CS@SiO₂ in a 250 mL flask containing 50 mL ethanol–water solution (4:6, v:v). In the kinetic binding experiments, 20 mg L⁻¹ NG solution was used, and in the isotherm experiments the initial NG concentration range was 0.014–0.14 mmol L⁻¹.

The concentration of NG was determined by UV–vis. absorption spectroscopy (TU-1900, Beijing PERSEE) at 283 nm. The binding amount B_{bound} ($\mu\text{mol g}^{-1}$) was calculated according to the following equations:

$$B_{\text{bound}} = \frac{(C_0 - C_t) \times V}{W} \times 10^3 \quad (1)$$

where C_0 was the initial NG concentration (mmol L⁻¹), C_t was the residual NG concentration (mmol L⁻¹) after a given time, V was the volume of NG solution (L), and W was the weight of I-CS@SiO₂/N-CS@SiO₂ (g).

All results were reported as the average of three different experiments. After the binding test, polymers were re-extracted with ethanol to regain the initial performance. For comparison, blank membranes were subjected to the same procedure.

Selectivity of Polymers

Selective recognition ability of I-CS@SiO₂/N-CS@SiO₂ was investigated using the structure analogue HD. The selectivity analyses were carried out by shaking a mixture of NG and HD

(20 mg L⁻¹ each) for 48 h. The others were the same as those for the NG binding experiment above. The mixture was filtered through a 0.45 μm syringe filter, and the concentration of the substrate in solution determined by high performance liquid chromatography (HPLC) with a Prostar 240 pump (USA Varian Co.). Chromatography conditions were Microsorb-MV C-18 column (250 \times 4.6 mm), UV detection at 283 nm, acetonitrile: 2% acetic acid (38 : 62) as mobile phase, flow rate: 1.0 mL min⁻¹. A standard curve was obtained using an external standard.

RESULTS AND DISCUSSIONS

Preparation and Characterization of I-CS@SiO₂

The SiO₂ particles had large surface-to-volume ratios. After the coating, more functional groups of CS on the SiO₂ particles were supposed to be exposed. The imprinted structure was fixed with the crosslinking agent. After removing the imprinting molecules NG, the specific cavities were left in the CS network. The hydrogen bonds between CS and NG improved the combination stability between imprinting molecules and functional polymer.

Figure 3 shows the X-ray diffraction patterns of SiO₂, CS, and I-CS@SiO₂. All of them were amorphous substance and exhibited a broad halo diffraction pattern without the resolved peaks. This indicated that the coating process of CS has no effect on the structure of the encapsulated SiO₂ nanoparticles, except for the physical cover. It was further verified by the FT-IR spectra of CS and I-CS@SiO₂. As shown in Figure 4, the spectrum of CS showed the absorption peaks at about 3428 cm⁻¹ for the –NH₂ and –OH groups in CS, at about 2920 cm⁻¹ for the aliphatic C–H stretching vibration, 1629 cm⁻¹ for the absorption peaks of the rest NHCO in CS, 1384 cm⁻¹ for C–H bending vibration absorption, and 1154 cm⁻¹ for the C–O–C group. In comparison with the CS, a strong absorption peak of SiO₂ at 470 cm⁻¹ was occurred in the spectra for CS@SiO₂ [Figure 4(b)]. Obviously, chitosan was loaded on the carrier of SiO₂ particles.

Mohammad³⁵ reported that the morphology of imprinted polymer would greatly affect the molecular imprinting behaviors. SEM images were used to compare I-CS@SiO₂ polymers with

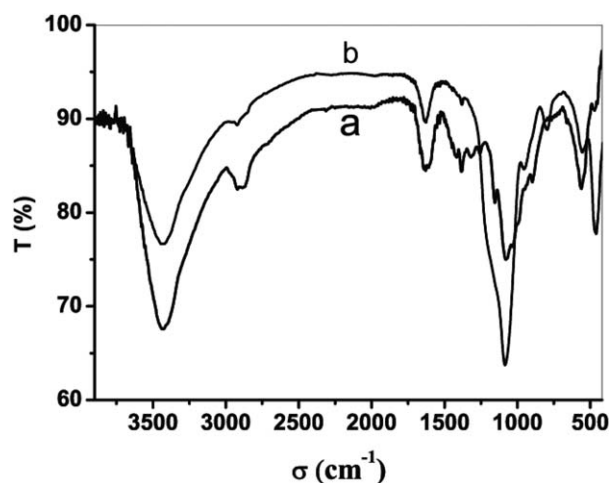


Figure 4. FT-IR spectra (a) CS and (b) I-CS@SiO₂.

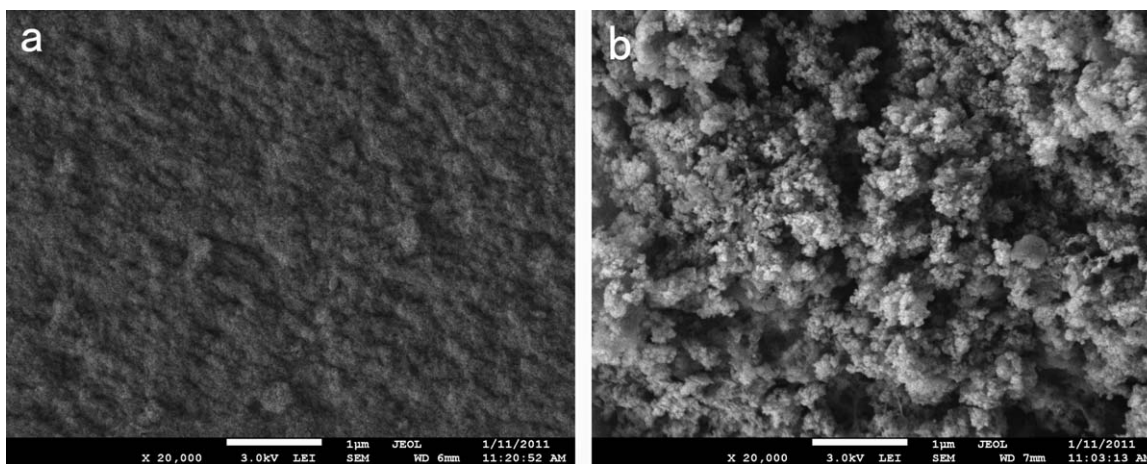


Figure 5. SEM micrographs (a) N-CS@SiO₂ and (b) I-CS@SiO₂.

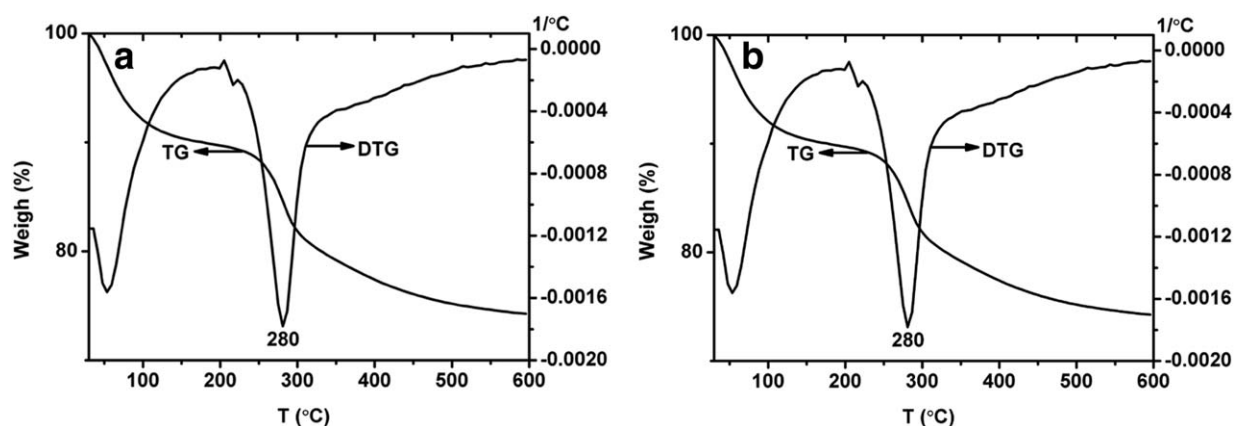


Figure 6. TG-DTG curves of polymers (a) N-CS@SiO₂ and (b) I-CS@SiO₂.

the N-CS@SiO₂. As shown in Figure 5, N-CS@SiO₂ was dense, while I-CS@SiO₂ was loose. Loose structure may lead to increase the free volume and to improve the binding capacity of the imprinted polymer.

The thermal behavior of I-CS@SiO₂ and N-CS@SiO₂ polymers were illustrated by TG-DTG thermal stability curves. As shown in Figure 6(a) (N-CS@SiO₂), the weight decreased substantially in the low temperature region, which was attributed to the removal of water. Except for the first step, there was a weight loss step, giving a total loss of ~15%. A sharp peak at 280°C in the DTG curve was attributed to the decomposition of CS. After 550°C, the TG curve was flattened, as the decomposition of all organic species was completed, and the remaining weight was solely pure SiO₂ inorganic phases. The thermal stability of I-CS@SiO₂ after the NG elution [Figure 6(b)] was almost the same to N-CS@SiO₂.

Evaluation of Imprinting Efficiency

Binding Kinetics. To investigate the time-dependent adsorption kinetics of I-CS@SiO₂, binding tests were performed at various incubation times (5, 12, 24, 36, 48, 60, and 72 h) in the 4 : 6 v/v ethanol/water mixture containing NG concentration at 20 mg L⁻¹. Curves for the binding of NG by I-CS@SiO₂ and N-CS@SiO₂ were shown in Figure 7. Their binding kinetics profiles for NG were

similar: As the incubation time increased, the uptake amount of NG increased. Due to the binding sites were existed in MIPs, the amount of bound NG for I-CS@SiO₂ was far more than that for N-CS@SiO₂ at any time, that is, I-CS@SiO₂ exhibiting more high specific binding capacity.

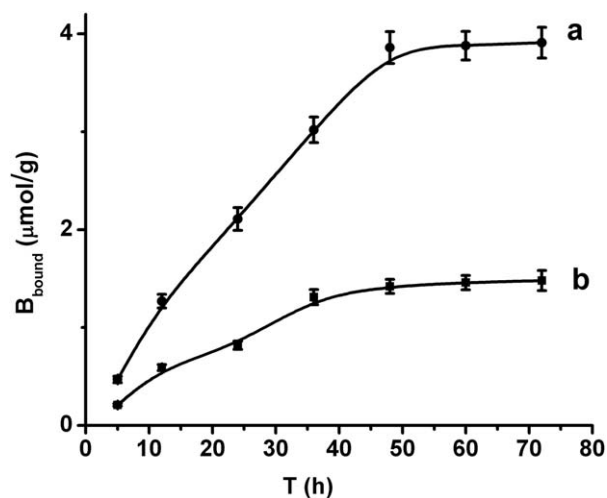


Figure 7. Curves of binding dynamics with error bars: (a) I-CS@SiO₂ and (b) N-CS@SiO₂.

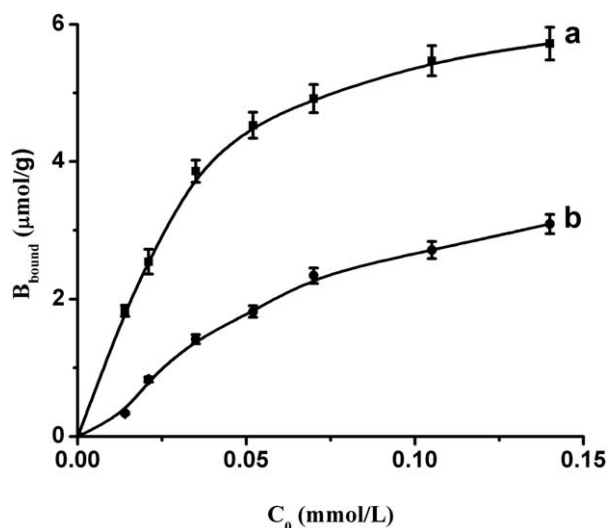


Figure 8. Binding NG isotherms of polymers: (a) I-CS@SiO₂ and (b) N-CS@SiO₂.

Compared with traditional MIPs, surface imprinted polymers possess higher binding capacity, faster mass transfer and binding kinetics. However, the I-CS@SiO₂ reached the final equilibrium after 48 h, and exhibited slow binding kinetics. It may be due to the impact of heterogeneous binding sites, often existing in the MIPs via noncovalent interactions because the prepolymerization step is a nonwell defined process, which results in the formation of complexes with different ratios of template to monomer, and then leads to different binding sites. On the other hand, excess monomers are used to form template–monomer complexes, which also might cause the formation of nonselective binding sites.²⁵

Binding Isotherms. The binding amounts of NG on I-CS@SiO₂ and N-CS@SiO₂ at room temperature for 48 h were presented in Figure 8. The binding amount of NG on I-CS@SiO₂ was higher than N-CS@SiO₂ within the tested concentrations. Clearly, the greater amount of NG bound by I-CS@SiO₂ compared with N-CS@SiO₂ proved the imprinting efficiency again.

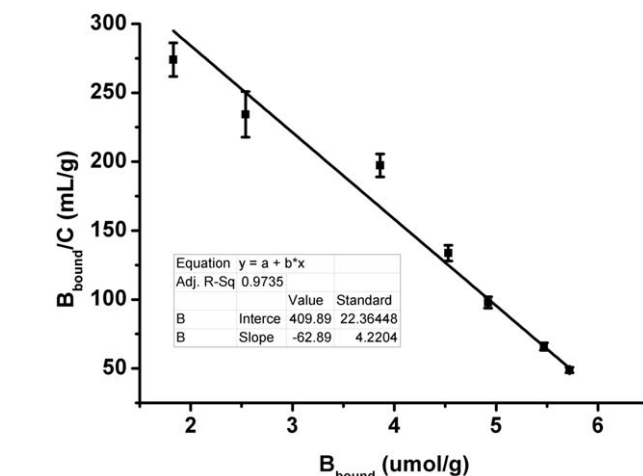
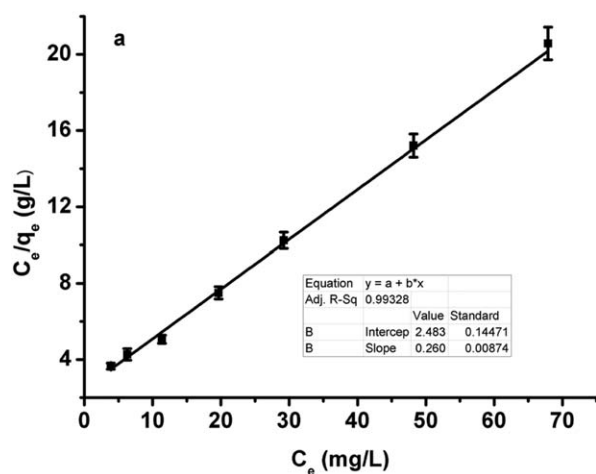


Figure 9. Scatchard plot to estimate the binding nature of NG/I-CS@SiO₂.

In the molecular imprinting, the binding characteristic of MIPs was evaluated with Scatchard model.³⁶

$$\frac{B_{\text{bound}}}{C} = \frac{B_{\text{max}} - B_{\text{bound}}}{K_D} \quad (2)$$

where B_{bound} was the amount of the template bound to polymer, $\mu\text{mol g}^{-1}$; C was the template concentration at equilibrium, $\mu\text{mol mL}^{-1}$; B_{max} was apparent maximum number of binding sites with high affinity and K_D was association constant.

The association constant and binding site density were estimated graphically from a linearly raising section of the binding isotherm. This was done by plotting the binding isotherm in a Scatchard format (shown in Figure 9). The Scatchard analysis suggested that there was a class of binding sites during MIPs recognition of NG. The calculated dissociation constant K_D and apparent maximum number B_{max} of binding sites with affinity were $0.016 \text{ mmol L}^{-1}$ and $6.56 \mu\text{mol g}^{-1}$, respectively.

Adsorption isotherms were important for describing how the adsorbates will interact with adsorbent. Thus, the correlation

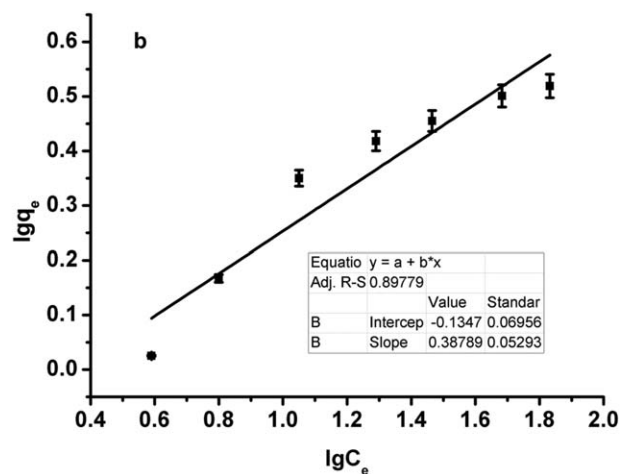


Figure 10. The data fitting to the Langmuir and Freundlich equations for the adsorption of NG on I-CS@SiO₂.

Table I. Adsorption of Various Substrates on Polymers

Substrate	$k_{D,I-CS@SiO_2}$	β_1	$k_{D,N-CS@SiO_2}$	β_2	A
NG	0.789 ± 0.052		0.454 ± 0.044		1.74 ± 0.20
HD	0.395 ± 0.037	2.00 ± 0.23	0.357 ± 0.039	1.27 ± 0.18	1.10 ± 0.16

of equilibrium data using either a theoretical or empirical equation was essential to practical adsorption operation. Langmuir equation (3) and Freundlich equation (4) were employed in the present study. The Langmuir model assumed that the surface of the adsorbent was homogeneous, covered with only one type of adsorption site that these sites were localized, and that there were no adsorbate–adsorbate interactions. The associated adsorption energy distribution was a Dirac distribution. The Freundlich isotherm model was frequently used to account for heterogeneous binding sites in molecularly imprinted polymers.

$$\frac{C_e}{q_e} = \frac{1}{Q} C_e + \frac{1}{Qb} \quad (3)$$

$$\lg q_e = \frac{1}{n} \lg C_e + \lg k \quad (4)$$

where q_e was equilibrium adsorption quantity, mg g^{-1} ; C_e was equilibrium concentration, mg L^{-1} ; Q was saturated adsorption capacity, mg g^{-1} ; b , n , k were the constant.

It can be seen from the curve of Figure 10 that Langmuir equation fitted well for the NG adsorption on the I-CS@SiO₂ polymer under the concentration studied. According to the linear form of Langmuir equation, the plot in the pattern of C_e/q_e versus C_e gave a linear fit with 'R' value of 0.99328. The average values of the Langmuir parameters Q and b were 3.84 and 0.105, respectively. To the linear form of Freundlich equation, the plot in the pattern of $\lg q_e$ versus $\lg C_e$ gave a linear fit with 'R' value of 0.89779. The Langmuir isotherm was preferable, and this further confirmed that homogeneous binding sites are more than heterogeneous binding sites. However, the 'R' value

of Freundlich equation is a bit big, which is consistent with the binding kinetics data as discussed above.

Selectivity Analysis. The selectivity of polymers was indicated by the selector β ($\beta = k_{D,i}/k_{D,j}$ where i and j were the imprinted molecule NG and competition HD, respectively) and imprinting factor α ($\alpha = k_{D,I-CS@SiO_2}/k_{D,N-CS@SiO_2}$, where a larger α indicates better imprinting). k_D was the static distribution coefficient, where $k_D = C_p/C_s$. C_p was the amount of the substance (NG or HD) binding the polymers (I-CS@SiO₂ or N-CS@SiO₂), and C_s was the amount of substance remaining in solution.

Based on the estimated values of β and α with the standard deviations in Table I, it showed that selector β of I-CS@SiO₂ is 2.00 ± 0.23 , which exhibited slightly better selectivity for NG in the presence of the other substrate than N-CS@SiO₂ ($\beta = 1.27 \pm 0.18$). In addition, compared with the imprinting factor α of HD ($\alpha = 1.11 \pm 0.16$), the imprinting factor α of NG is 1.74 ± 0.20 , which suggested that imprinted cavities played an important role in recognition. As shown in Figure 11, the imprinting factor of NG (4#) is slightly lower than that for the imprinted β -cyclodextrin nanofiber with in-situ generated silica as pore-forming agent (5#),³⁷ comparable to the values for the imprinted 5-P (AN-co-IA) membrane (3#)⁴ and the imprinted β -cyclodextrin nanofiber (2#),³⁰ but better than the one for the imprinted β -cyclodextrin polymer (1#).²⁹

CONCLUSIONS

In this study, CS coated silica nanoparticles (CS@SiO₂) was developed as an alternative NG molecular imprinting functional material in aqueous medium, which exhibited an imprinting factor α of 1.74 ± 0.20 . However, due to the impact of heterogeneous binding sites, the I-CS@SiO₂ exhibited slow binding kinetics. To increase binding homogeneity is under a way to enhance the binding kinetics via changing ratios of template to monomer and the prepolymerization time, etc. We will further conduct the extraction of the NG active component from plants in aqueous media with the help of the imprinted particles.

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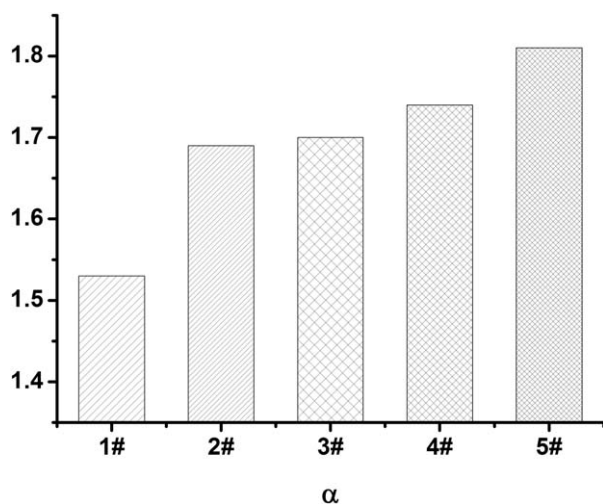


Figure 11. Comparison of the values of α for the different NG-imprinting polymers.

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