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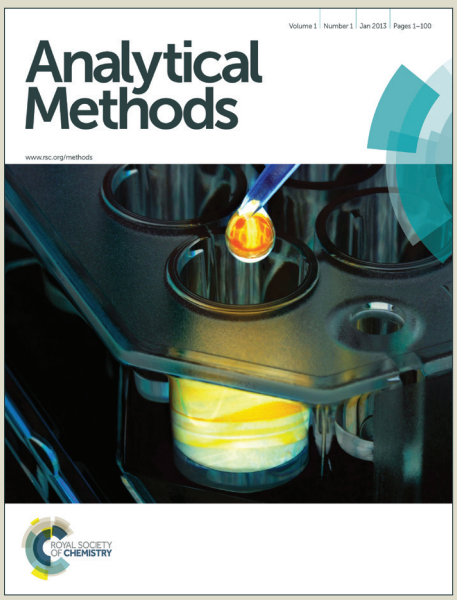

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Preparation and Recognition Characteristics of Alanine Surface Molecularly Imprinted Polymers

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Abstract

Surface Molecularly imprinted polymers (SMIPs) with high performance in selectively recognizing alanine (Ala) were prepared using a facile surface molecular-imprinting technique. The Ala surface molecularly imprinted polymers (SMIPs) were prepared by heating suspension polymerization with methacrylate acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linker on vinyl-SiO_2 surface. The obtained materials were characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). The synthesis conditions for the SMIPs formation were systematically investigated to obtain the highest selectivity and binding capacity. The formation and monodispersity of the SMIPs are mainly affected by the molar ratio of the template, the functional monomer, and compositions of the cross-linker and solvent. In comparison to the imprinted polymers prepared using the traditional polymerizations, the obtained SMIPs exhibited a regular spherical shape and were relatively monodispersed. The template recognition properties were evaluated; the results showed that the maximal sorption capacity (Q_{max}) of the resulting SMIPs was up to 831 μmol g^{-1}, whereas that of non-imprinted polymers was only 341 μmol g^{-1}. A kinetic binding study showed that the sorption capacity reached 49.73% of Q_{max} in 30 min and sorption equilibrium at 60 min. SMIPs have excellent accessibility and affinity toward Ala because the selectivity coefficients of SMIPs for Ala with respect to Gly, His, Glu and Phe were 2.36, 1.79, 2.48, and 2.56, respectively. The regeneration process verified that the SMIPs have admirably stable adsorption capacity toward Ala and can be regenerated up to eight times.

Keywords: Surface molecularly imprinted; Selectivity; Molecular recognition; Alanine.

1. Introduction

As one of the 20 amino acids which formatted the protein of people, alanine existed in many biological samples with complex components, such as serum, saliva and urine, thus the imprinting of the alanine has positive implication for its selective recognition. Molecularly imprinted polymers (MIPs) are smart tailored-made materials to selectively and sensitively recognize small molecules or biologically important compounds. In 1972, the German scientist Wulff successfully synthesized amino acids and their derivatives and compounds with a highly selective carbohydrate covalent imprinted polymer,[1] which raised extensive concern in academics for this technology. Then, the molecularly imprinted polymers of amino acids were prepared using various methods: precipitation polymerization, bulk polymerization, suspension polymerization, surface polymerization, multi-step swelling suspension polymerization, magnetic polymer surface polymerization and sol-gel polymerization. All along, there are many scientists committed to the synthesis of amino acid molecular imprinting, the aim is to extract the separation and enrichment of amino acids from the complex matrix.[2-11] In order to make MIP more widely used and development, the key is to overcome these problems of MIPs: the heterogeneous binding sites, template leakage, low binding capacity, and slow mass transfer of MIPs.[12]

Molecular imprinting on particle surface is the preferred method to overcome the traditional molecular imprinting method. Compared to traditional MIP nanoparticles, imprinting a matrix with binding sites at the surface has many advantages: the sites are more accessible, the mass transfer is faster, and the binding kinetics is faster. Surface-imprinted nanoparticles can be prepared by grafting MIP to or from the surface of preformed support nanomaterials (e.g., silica nanoparticles, magnetite nanoparticles, quantum dots, or carbon nanotubes). Since silica is a non-swelling inorganic material, stable under acidic condition, and has high thermal resistance, silica showed promising characteristics among these support particles. Surface imprinting using silica as the support has been applied to imprint pharmaceuticals[13-18]

and biological macromolecules.[9, 19-29] Regrettably，the surface imprinting technology to prepare amino acid MIP was seldom reported.

Herein, we present a new method to prepare surface molecularly imprinted size-monodisperse spheres. The Ala-imprinted polymer is coated at the surface of Vinyl–SiO_2 spheres. Highly monodispersed 300-nm-sized vinyl-SiO_2 spheres were facilely prepared using a one-step approach in aqueous solution. Then, a highly binding surface molecularly imprinted polymer for Ala was directly synthesized on the surface of vinyl-SiO_2 spheres for the first time. The polymers were characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The functional evaluations of the materials included static and dynamic binding processes, Scatchard analysis, Langmuir analysis, Freundlich analysis, selective binding tests in five types of analogues, and regeneration performance in identical conditions for eight times. The results demonstrated that the Ala-SMIPs spheres had great rebinding capacity, efficient recognizing ability for template, and steady and excellent regeneration property.

2. Experimental

2.1 Materials and Chemicals

Glycine (Gly, purity: 99.5~100.5%) and L-alanine (Ala，99%) were purchased form Sightre Industrial Co. (China). L-Histidine (His, 99%), $L_{(+)}$-glutamic (Glu, 99%) and L-phenylalanine (Phe, 99%) were purchased from Aladdin reagent Co. (China). Ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%) was purified by distillation under vacuum. Azobisisobutyronitrile (AIBN, chemical grade) was purchased from Shanghai No. 4 Reagent & H. V. Chemical Company (China) and purified through recrystallization in ethanol before use. Vinyl-trimethoxysilane (VTMS) was purchased from Nanjing Union Silicon Chemical Co. (China). Acetonitrile (purity≥99.9%) of CHROMASOLV® gradient grade for HPLC was purchased from Sigma-Aldrich Co. Ammonium hydroxide (28%), methacrylate acid

(MAA, purity≥98%), toluene, acetic acid, sodium acetate anhydrous, phenyl isothiocyanate, triethylamine, and n-hexane were purchased from Shanghai Chemical Reagent Co. (China). Doubly distilled water, which was used throughout the experiment processes, was obtained from the laboratory purification system.

2.2 Instrumentation

FTIR spectra were recorded on a Tensor-27 FTIR spectrometer (Bruker, Germany) with a resolution of 2 cm^{-1} and a spectral range of 4000-400 cm^{-1}. The morphologies and structures of Ala-imprinted silica spheres were observed using an SIRION200 SEM (FEI, Holland) and a JEM-2011 TEM (JEOL, Japan) with measurements at 5 and 200 kV, respectively. The Ala amount was analysed using a high-performance liquid chromatography (HPLC) system (Shimadzu, Japan). The HPLC system was composed of a LC-15C pump, a SIL-10AF injector with 50-μL loop, and a SPD-15C dual-wavelength absorbance detector.

2.3 Procedure to prepare Molecularly Imprinted Polymer

2.3.1 Preparation of Vinyl–SiO_2 spheres

Vinyl–SiO_2 spheres were synthesized according to the literature-reported approach with some modifications.[30] VTMS (2.1 mL) was added to 50 mL of doubly distilled water under vigorous stirring until an emulsion formed. After 3 h, $NH_3 \cdot H_2O$ (1 mL) was added to the emulsion, and the mixture was continuously stirred at 25°C for 2 h. The resulting spheres were separated from the reaction medium by centrifugation and washed with anhydrous ethanol and doubly distilled water several times. Finally, they were dried at 60°C for 12 h.

2.3.2 Imprinting of Ala Molecules on the Surface of Vinyl–SiO_2 spheres

To prepare the surface-imprinted polymer, vinyl–SiO_2 spheres (0.5 g) were dispersed in 20 mL of toluene using an ultrasonic bath. MAA (0.3476 g, 4 mmol), EGDMA (1.132 mL, 5.5 mmol), Ala (0.08910 g, 1 mmol), doubly distilled water (5 mL) and AIBN (0.08 g) were subsequently dissolved into this solution. The mixing solution was purged with nitrogen for 30 min while cooling in an ice bath. Polymerization was performed at 40°C for 4 h and subsequently at 60°C for 20 h. The final

polymerization was completed after further aging at 75°C for another 4 h to obtain a high cross-linking density. Magnetically, the solution was continuously stirred at a rate of 300 rpm throughout the experiment. The resultant SMIPs were collected by filtration and washed with a mixed doubly distilled water/methanol solvent (1:9, v:v). The original Ala templates in the imprinted shells were removed using Soxhlet extraction in 200 mL of mixed doubly distilled water/methanol (1:9, v:v) solution until no residual Ala could be detected. Finally, the as-prepared polymers were washed with methanol to neutrality and dried at 60°C for 12 h. For comparison, the surface non-imprinted polymers (SNIPs) were also prepared using the same method, except no template molecule (Ala) was added during the polymerization process.

2.4 Measurements of HPLC[31]

Pre-column Derivatization: To 200 μL samples in 2 mL of amino acids in a centrifuge tube was added a 0.1 mol L^{-1} solution of phenyl isothiocyanate in 100 μL of acetonitrile and 1 mol L^{-1} triethylamine in 100 μL of acetonitrile. The resulting solution was mixed at room temperature for 1 h; then, 400 μL of n-hexane was added, and the solution was mixed in a vortex mixer oscillator for 1 min and allowed to stand for 10 min. The lower solution was drawn with a syringe through a 0.45-μm membrane filter for backward chromatographic analysis.

Chromatographic Conditions: Kromasil C_{18} column (200 mm × 4.6 mm, 5 μm particle size); detection wavelength: 254 nm; injection volume: 2 μL. Mobile phase A: 0.1 mol L^{-1} sodium acetate - acetonitrile (volume ratio of 97:3) solution; mobile phase B: acetonitrile - water (volume ratio 4:1). A linear gradient from 0 to 100% B in 45 min was performed[31]. The mobile phase flow rate was 1.0 mL min^{-1}.

2.5 Measurements of the Recognition Properties of SMIPs

The static and dynamic tests of rebinding Ala molecules were investigated using a reversed-phase HPLC. Ten-milligram aliquots of SMIPs and SNIPs particles were individually suspended in 10 mL of Ala aqueous solution at different concentrations of 0.1-4 mmol L^{-1}. After shaking at room temperature for 12 h to facilitate full adsorption, the amounts of Ala in the supernatants were detected. The binding amount of Ala was determined by measuring the difference between the initial and final amounts in the

solution. Meanwhile, the binding kinetics was performed using 10 mg of SMIPs, which was added to 10 mL of aqueous solution with 2 mmol L^{-1} Ala by monitoring the temporal residual Ala concentration in the solutions at certain time intervals.

2.6 Selectivity of SMIPs

To explore the selectivity of the SMIPs toward Ala, the selective recognition assays of Ala from Ala and its analogues (Gly, Glu, His and Phe) were investigated on SMIPs and SNIPs. Their chemical structures were shown in Figure 1. The mixed solutions contained identical concentration of Ala, Gly, Glu, His and Phe with a series of concentration of 0.1-4 mmol L^{-1}. Then, the adsorption was performed similarly to the aforementioned process, and the final compounds in the residual solutions were analysed using HPLC.

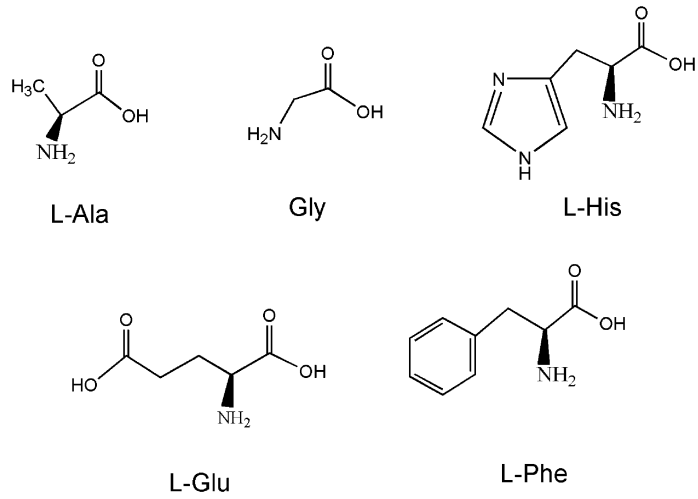


Figure 1. Molecular structures of Ala, Glu, Gly, His and Phe.

2.7 Regeneration of SMIPs

Approximately 100 mg of SMIPs particles were suspended in 30 mL of aqueous solution with an Ala concentration of 0.6 mmol L^{-1}. Then, the adsorption proceeded as previously mentioned. After the adsorption, the material was washed with doubly distilled water 3-4 times and dried at 60°C to constant weight. The sorption–desorption cycle was repeated eight times.

3. Results and Discussion

3.1 Characterization of SMIPs spheres

The FTIR spectra in Figure 2 directly proved the existence of the functional vinyl groups on the surface of silica spheres and the synthetic process of MIPs-coated vinyl-SiO_2 spheres. The IR bands at 3064–2956 cm^{-1}, 1601 cm^{-1} and 1408 cm^{-1} were attributed to the presence of C=C groups on the prepared vinyl–SiO_2 spheres. The strong peaks at 1045 cm^{-1} and 769 cm^{-1} were related to the Si–O–Si vibrations. In addition, from the FTIR spectra of SMIPs and SNIPs, new peaks were observed when new chemical bands were created. The SMIPs and SNIPs showed similar locations and appearances of the major bands. The strong band at 1728 cm^{-1} was the C=O stretch, which suggests that the MIPs were successfully coated on the surface of the silica spheres. Moreover, some new peaks were noticed when we compared spectra b-c with spectrum a (e.g., 1635, 1465, 1412, and 1383 cm^{-1}), and the bands at 1635, 1456 and 1412 cm^{-1} were attributed to the C=C stretching vibration, CH_2 scissor bending vibration and carboxyl symmetry variable angle vibration, respectively. These results confirmed that MAA and EGDMA were successfully grafted onto the surface of the vinyl-SiO_2 spheres by the copolymerization of MAA, EGDMA and the vinyl group of vinyl-SiO_2 spheres.

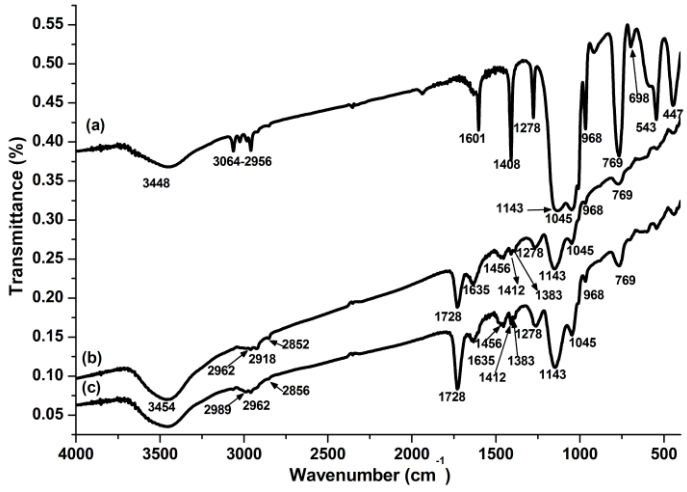


Figure 2. FTIR spectra of (a) vinyl-SiO_2 spheres, (b) SNIPs, and (c) SMIPs.

SEM and TEM images were obtained for the vinyl–SiO_2 spheres and SMIPs. As shown in Figure 3, the vinyl–SiO_2 spheres and SMIPs have a regular spherical shape. The highly spherical morphology and smooth surface suggest that the MIP layer

resulted from the highly selective polymerization of monomers at the surface of silica particles. The obtained vinyl–SiO_2 particles were approximately 300 nm in size and relatively monodispersed. The estimated layer thickness of the SMIPs was approximately 60 nm. The TEM spectra show that the silica cores and polymer shells could be clearly distinguished, which indicates that surface polymerization successfully occurred on the surface of silica spheres, and the core-shell structure formed.

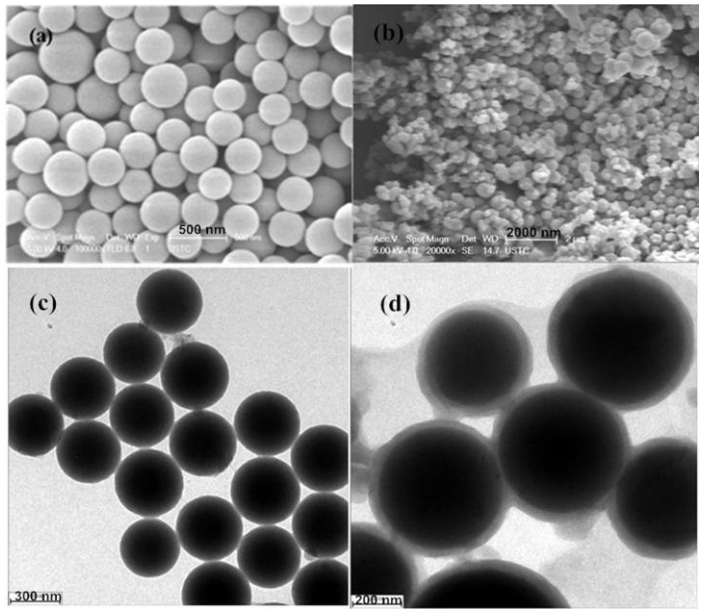


Figure 3. (a) SEM image of vinyl-SiO_2 spheres, (b) SEM image of SMIPs, (c) TEM image of vinyl-SiO_2 spheres, and (d) TEM image of SMIPs.

3.2 Optimization of synthetic conditions

The choice of solvent has an important role in the formation of MIP: the types and doses of the solvent critically affected the formation and morphology of the SMIP. The choice of solvent should follow the following principles: all polymerization reactors have good solubility in the solvents; the solvent should at least form hydrogen bonds with no negative effects, and at best, it can promote the molecular bonding; the solvent must have a low boiling point, be volatile, and easily form a gap. According to this principle, the common solvents to prepare amino acid MIP are methanol, acetonitrile,[32, 33] tetrahydrofuran,[34, 35] chloroform,[35] benzene/water,[2] N, N-dimethylformamide

(DMF),[8] etc. In this experiment, we chose toluene/water as the solvent; here, water was used to dissolve the amino acid, and toluene was used to dissolve the polymeric reactants such as the cross-linking agent and initiator, which belonged to the organic matter. However, water can prevent the formation of hydrogen bond between the monomer and the template molecule, which reduces the numbers of bonding sites in MIPs. Therefore, different volumes of water were chosen to optimize the conditions (Figure 4). Comparing Figure 3 (d), where the solvent was 20 mL of toluene with 5 mL of water, and Figure 4, the results show that when the volume of water was 5 mL, the Ala-SMIPs exhibited the best dispersity and morphology. And when the volume of water was more than 5 mL, the results show that the adhesion among SMIPs particles increased with the increase in water volume. When only water was used as the solvent, from the TEM image of the spherical material, we found no clear but only wrapped deformed polymer. Thus, we selected the solvent consisting of 20 mL of toluene and 5 mL of doubly distilled water.

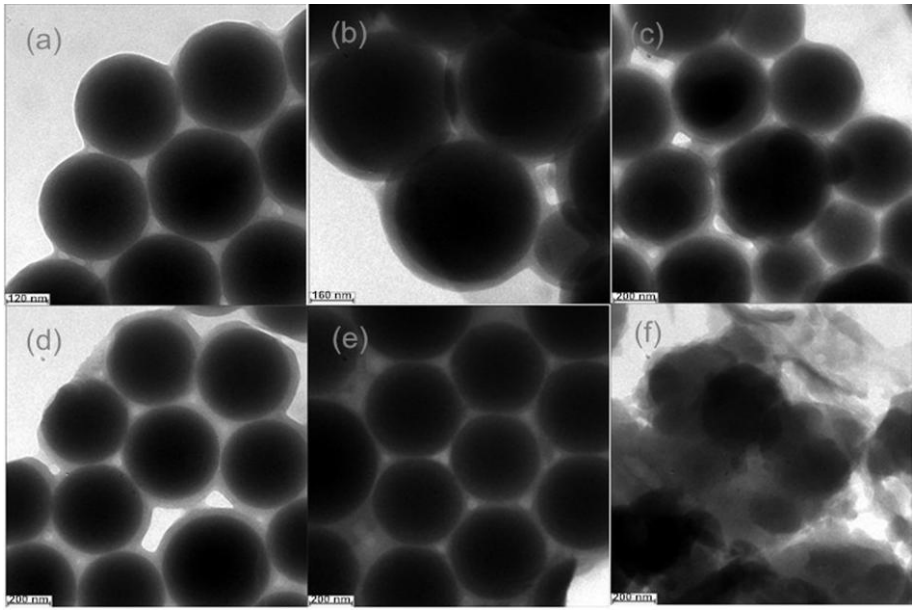


Figure 4. TEM image of SMIPs: (a) 20 mL of toluene and 3 mL of water; (b) 20 mL of toluene and 10 mL of water; (c) 20 mL of toluene and 15 mL of water; (d) 20 mL of toluene and 20 mL of water; (e) 20 mL of toluene and 25 mL of water; (f) 30 mL of water

The molar ratio of the template, functional monomer, and cross-linker also had an important effect on the formation and morphology of the material. The ratio of 1:4:20

is relatively commonly used to prepare MIPs, but it appeared inappropriate in our experiment. Figure 5 shows a larger proportion of the cross-linking agent, which indicates that when the SMIPs had more severe adhesions, the thickness of the imprinting layer increased. With further increase of the cross-linking agent, a regular spherical morphology SMIPs could not be formed. Considering its dispersity, thickness uniformity and other factors, we chose the molar ratio of the template, functional monomer, and cross-linker to be 1:4:5.5.

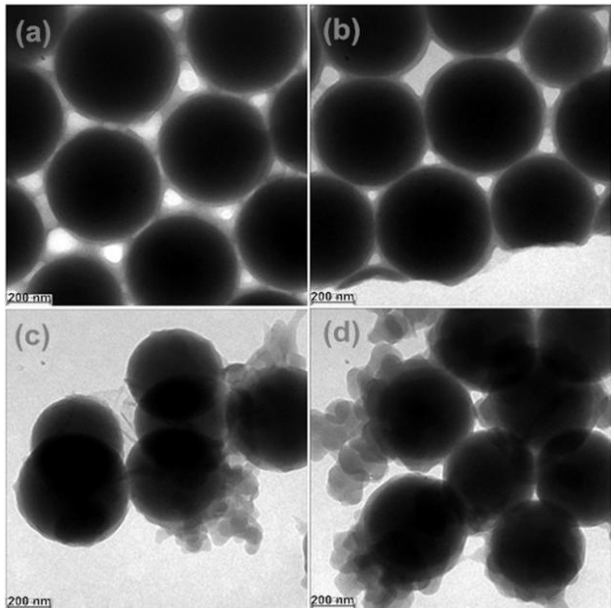


Figure 5. TEM image of SMIPs: (a) 1:4:3; (b) 1:4:10; (c) 1:4:15; (d) 1:4:20.

The amount of initiator significantly affects the SMIPs morphology, as observed in Figure 6. When the amount of AIBN increased, the surface of the silica spheres of the molecularly imprinted layer became thicker, but its adhesion became more serious. Considering the morphology and the issue of eluted template, the final amount of added AIBN chosen was 0.08 g.

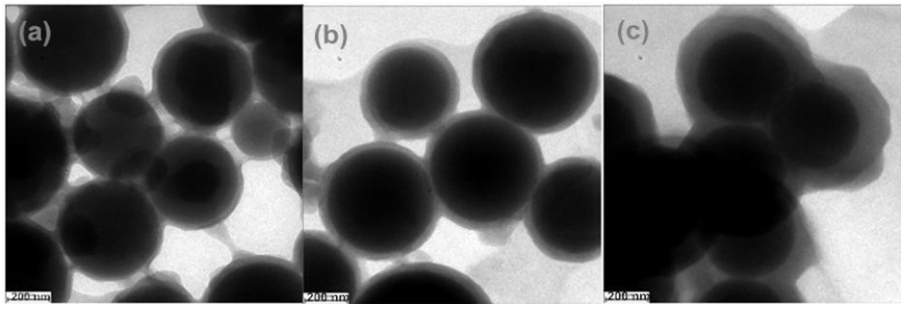


Figure 6. TEM image of SMIPs: (a) AIBN: 0.06 g; (b) AIBN: 0.08 g; (c) AIBN: 0.1 g

Considering the morphology and binding capacity of SMIPs, 20 mL of toluene, 5 mL of doubly distilled water, 0.08 g of AIBN and a template-functional monomer-cross-linker molar ratio of 1:4:5.5 were chosen for the synthesis of the SMIP and SNIP sorbents.

3.3 Evaluation of the Adsorption Character of SMIPs and SNIPs

For further application, the adsorption capacity of the SMIPs and SNIPs sorbents was investigated in aqueous solutions of Ala with different concentrations of 0.1-4 mmol L^{-1}. The sorption capacity Q (μmol g^{-1}) is calculated using the following equation:

$$Q = [(C_i - C_f)V]/W \quad (1)$$

where C_i and C_f are the initial and final template concentrations (μmol L^{-1}) in the solutions, respectively, V (L) is the volume of the bulk solution, and W (g) is the weight of the materials. Figure 7 shows the static binding isotherm of Ala. The adsorption capacities of SMIPs and SNIPs obviously increased with the increase in the initial concentration of Ala. The maximum adsorption of SMIPs for Ala was calculated to be 831 μmol g^{-1}, which was nearly two times as large as that of the corresponding SNIPs (341 μmol g^{-1}). Then, it was concluded that the SMIPs displayed much stronger affinity to Ala than SNIPs, and the sorption capacity of the prepared SMIPs was favourably superior to those reported in the previous literature.[10-12]

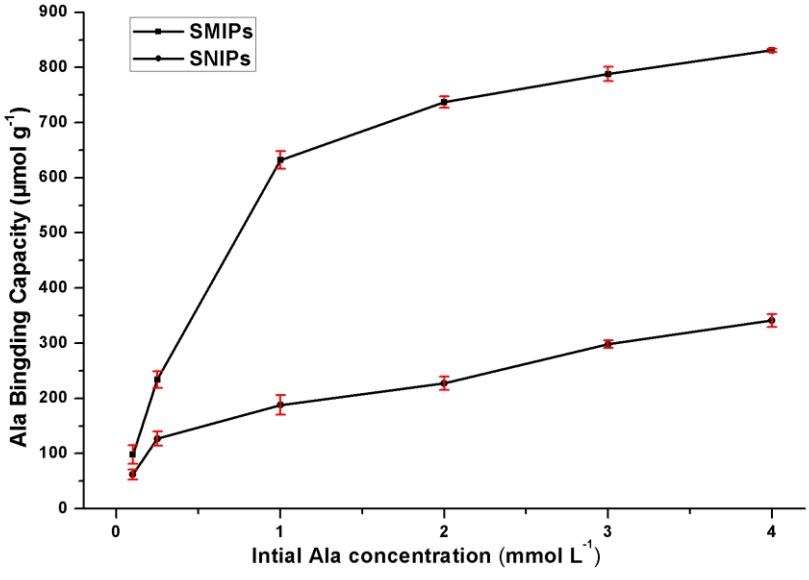


Figure 7. Static adsorption isotherms of the SMIPs and SNIPs sorbents for Ala (0.1-4 mmol L^{-1}).

Four different models, which are Scatchard,[36] Langmuir,[37] Freundlich[38] and Dubinin–Radushkevich (D–R)[39] were fitted and the calculated isotherm constants are presented in Table 1. The binding affinity and theoretical binding site number for the template of the imprinted polymers were primarily estimated using Scatchard analysis with the data of the static adsorption experiment. The Scatchard analysis is provided by the Scatchard equation, which is described as follows:

$$Q_{eq}/C_{eq} = (Q_{max}-Q_{eq})/K_d \quad (2)$$

where Q_{eq} (μmol g^{-1}) is the equilibrium adsorption capacity, Q_{max} (μmol g^{-1}) is the apparent maximum adsorption capacity, C_{eq} (μmol L^{-1}) is the equilibrium concentration of Ala, and K_d (μmol L^{-1}) is the equilibrium dissociation constant. Langmuir equation shows as follows:

$$Q_{eq} = bQ_{max}C_{eq} / (1+bC_{eq}) \quad (3)$$

Q_{max} (mmol g^{-1}) is the complete single-layer adsorption biggest adsorptive capacity, C_{eq} (mmol/L) is the equilibrium concentration, b (L $mmol^{-1}$) is the adsorption equilibrium constant, the b value represents the interaction strength of between adsorbate and absorbent. Freundlich equation:

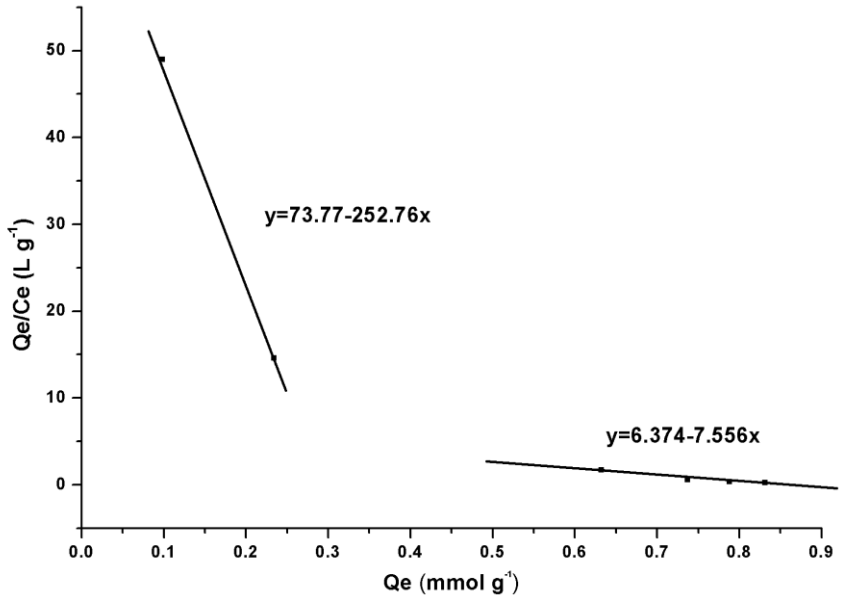
$$Q_{eq} = K_f C_{eq}^{1/n} \quad (4)$$

Q_{eq} is balanced adsorptive capacity (mmol g^{-1}), C_{eq} is the liquid phase equilibrium concentration (mmol L^{-1}), K_f is adsorbs coefficient [(mmol g^{-1}) / (mmol L^{-1})$^{1/n}$], n describes the isothermal misalignment Freundlich index. D-R equation:

$$Q_{eq} = Q_{max}\exp[-\beta(RT\ln(1+1/C_{eq}))^2] \quad (5)$$

Where β is a constant related to the mean free energy of biosorption per mole of the biosorbate (mol^2 J^{-2}), Q_{max} is the theoretical saturation capacity, R (J mol^{-1} K^{-1}) is the gas constant, and T (K) is the absolute temperature. As follows, Scatchard, Freundlich, Langmuir and Dubinin-Radushkevich models were used to fitting the isotherm sorption curve for different concentration of Ala adsorb on SMIPs and SNIPs (see Fig. 8, Fig. 9 and Table 1). In Figure 8, the steeper line exhibits the specific binding sites, and the flatter line exhibits nonspecific binding sites.

According to the slope and intercept of the regression model, K_{d1} for specific binding sites and K_{d2} for nonspecific binding sites for SMIPs were calculated to be 3.956 and 132.340 μmol g^{-1}, respectively. Likewise, an equilibrium dissociation constant for the SNIPs was calculated to be 186.857 μmol g^{-1}, which shows that the SMIPs had high selectivity for Ala. Through comparison of linear correlation coefficients (R^2), the Langmuir isotherm model is found to better fit the experimental data of Ala on SMIPs than others. Langmuir model fitting suggests resveratrol adsorption process having place in a monolayer binding system (r = 0.965). While Langmuir isotherm model is basically used for monolayer adsorption onto a surface with a homogeneous system, Freundlich isotherm model is suitable for multilayer adsorption of heterogeneous system, being increasingly heterogeneous as 1/n value is approaches zero. In this study, 1/n value of 0.226 suggested that although some degree of heterogeneity was present, a more homogeneous surface could be assumed. The Dubinin–Radushkevich isotherm is more general than the Langmuir isotherm since it does not assume a homogeneous surface or constant biosorption potential.Since the correlation coefficient value was low in the Dubinin–Radushkevich model, it was not suitable for the fitting of experiment data of resveratrol on SMIPs.



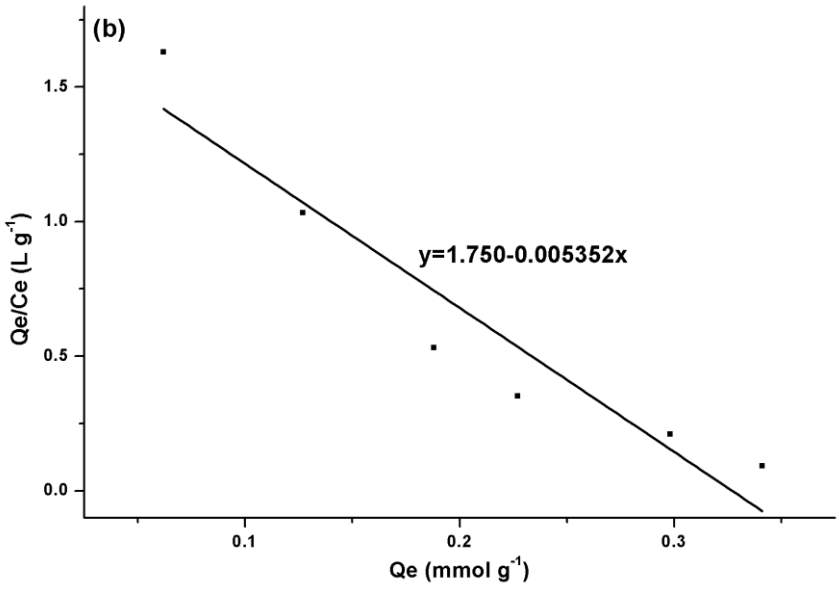


Figure 8. Scatchard plot for Ala in (a) SMIPs sorbent and (b) SNIPs sorbent.

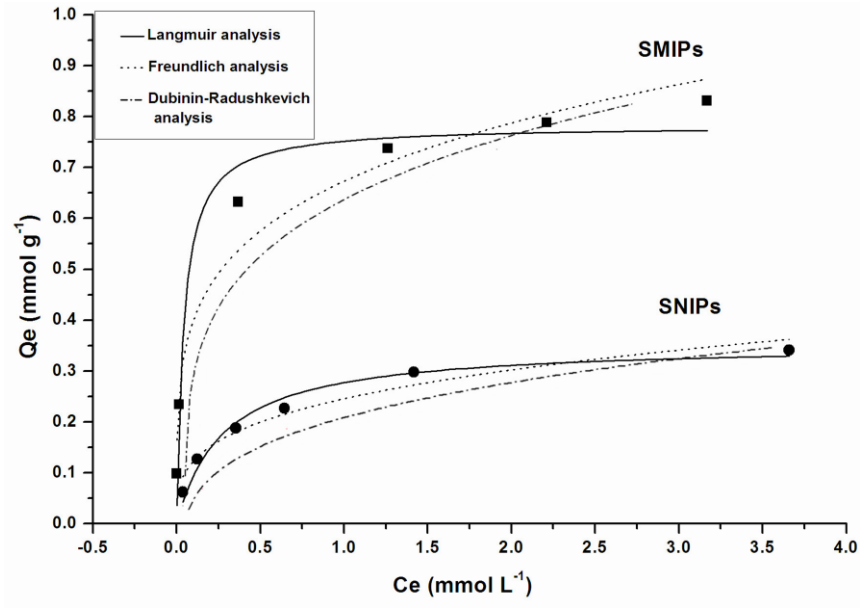


Figure 9. Measured and fitted adsorption isotherms of the Ala on the SMIPs and SNIPs materials.

Table 1. Nonlinear fits of adsorption isotherms of the alanine on the SMIPs and SNIPs materials.

	SMIPs	SNIPs
Scatchard		
Q_{max} (mmol g^{-1})	0.843	0.327
K_{d1} (mmol L^{-1})	3.96×10^{-3}	0.187
K_{d2} (mmol L^{-1})	0.132	/
R^2	0.958	0.886

Langmuir		
Q_{max} (mmol g^{-1})	0.783	0.354
b (L $mmol^{-1}$)	24.0	3.60
R^2	0.965	0.968
Freundlich		
1/n	0.226	0.300
K_f ((mmol g^{-1})/(mmol $L^{-1})^{1/n}$)	0.673	0.245
R^2	0.955	0.949
D-R		
Q_{max} (mmol g^{-1})	0.722	0.324
β (mol^2 J^{-2})	1.61×10^{-8}	0.130×10^{-8}
R^2	0.602	0.609

A dynamic adsorption test of the SMIPs for Ala was performed at different time intervals. The results in Figure 10 indicate an initial rapid increase in the adsorption capacity within a short shaking period of 30 min, and equilibrium was obtained in 60 min. This result implies that the SMIPs with specific recognition cavities at the surface reduced the mass transfer resistance to make it easier for the targets to access and led to rapid binding kinetics; in comparison, the molecularly imprinted polymers that are synthesized using the traditional method take several hours to achieve equilibrium.[40, 41]

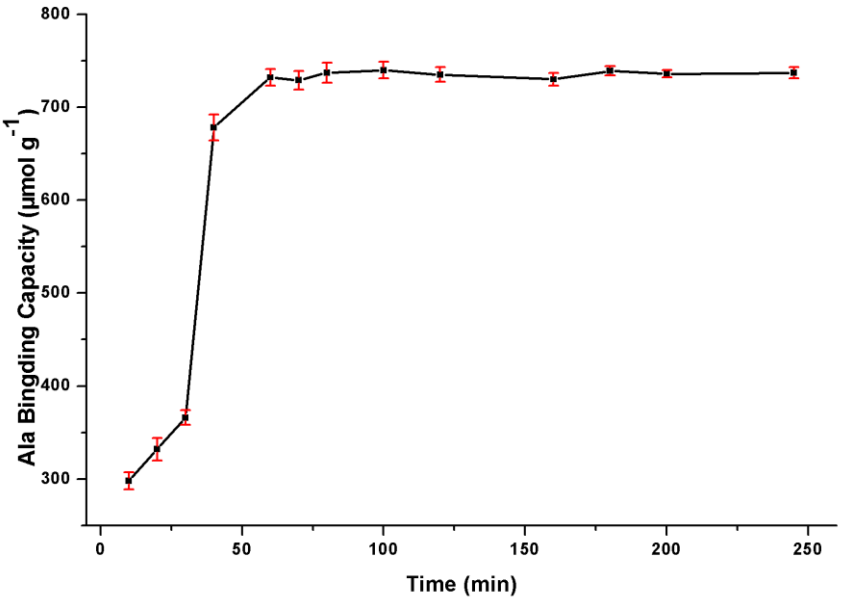


Figure 10. Adsorption kinetics of the SMIPs for Ala (2 mmol L^{-1}).

3.4 Selectivity of SMIPs

The structurally similar compounds (Gly, Glu, His, and Phe) were chosen as the competitive species with Ala for the selective recognition study. The selectivity of the imprinted and non-imprinted sorbents was evaluated using some factors, which were calculated from the following eqs. (5)–(7). [22] The distribution coefficient K_d denote the character of a substance adsorbed by a sorbent, and the selectivity coefficient k of the sorbent represents the difference between two analogues adsorbed by the same sorbent, whereas the relative selectivity coefficient k_0 suggests the difference between two different sorbents.

$$K_d = [(C_i - C_{eq})/C_{eq}](V/W) \quad (5)$$

where C_i and C_{eq} represent the initial and equilibrium concentrations (μmol g^{-1}), respectively, and V (mL) and W (g) are the solution volume and the sorbent mass, respectively.

$$K = K_{dAla}/K_{danalogue} \quad (6)$$

$$K` = K_{imprinted}/K_{nonimprinted} \quad (7)$$

Figure 11 indicates that the SMIPs has higher recognition capability and affinity toward Ala. The absorption capacity of the SMIPs to Ala was 11.73 times that to Phe, 7.94 times that to Glu, 5.04 times that to His and 3.85 times that to Gly. Phe and Glu could hardly be adsorbed on the SMIPs. The material had an affinity to Gly, but the SMIPs and SNIPs have similar binding capacities, so the adsorption was not specific. The $K_{(Ala/analogue)}$ value of the Ala-imprinted silica sorbent in Table 2 shows that the Ala-imprinted silica sorbent had higher selectivity for Ala over the structurally similar compounds. These facts prove the strong interactions between the template and the functional monomer, which favourably forms high-affinity binding sites and improves the polymer selectivity. The relative selectivity coefficient k` values were 2.36, 1.79, 2.48, and 2.56. The Ala imprinted silica sorbent clearly had more significant selectivity than the non-imprinted silica sorbent.

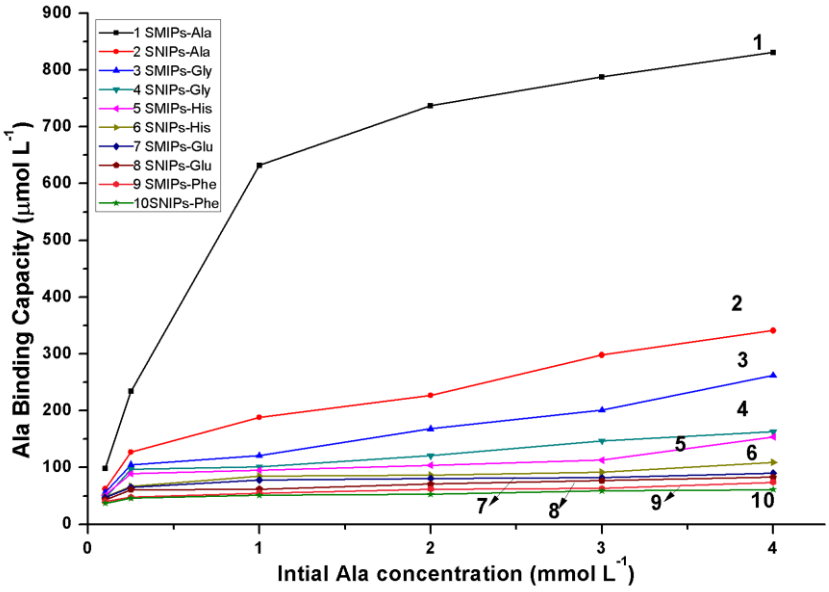


Figure 11. Static adsorption curves of Ala, Gly, His, Glu, and Phe on the SMIPs and SNIPs sorbents (0.1-4 mmol L^{-1}).

Table 2. Competitive binding tests of Ala and four analogues on the Ala-imprinted and non-imprinted silica sorbents (0.25 mmol L^{-1}).

Sorbents	K_d					K				K`	
	Ala	Gly	His	Glu	Phe	Ala/Gly	Ala/His	Ala/Glu	Ala/Phe	(K_{SMIPs}/K_{SNIPs})	
SMIPs	2787.88	724.14	552.80	351.35	237.62	3.85	5.04	7.94	11.73	2.36	1.79
SNIPs	1032.52	633.99	366.12	322.75	225.49	1.63	2.82	3.20	4.58	2.48	2.56

3.5 Regeneration of SMIPs

A sorption–desorption cycle was repeated eight times using the same SMIPs to evaluate its regeneration performance. The results in Figure 12 show that the binding capacity of Ala remained at a high level of over 507 μmol g^{-1} during the cycle, which indicates that the recognition sites are stable and that the material is reusable after a regeneration process. Few studies of the regeneration performance of SMIPs have been reported. Thus, the characteristics of the sorbents were superior to those traditional materials, and the SMIPs can save the pretreatment costs of samples.

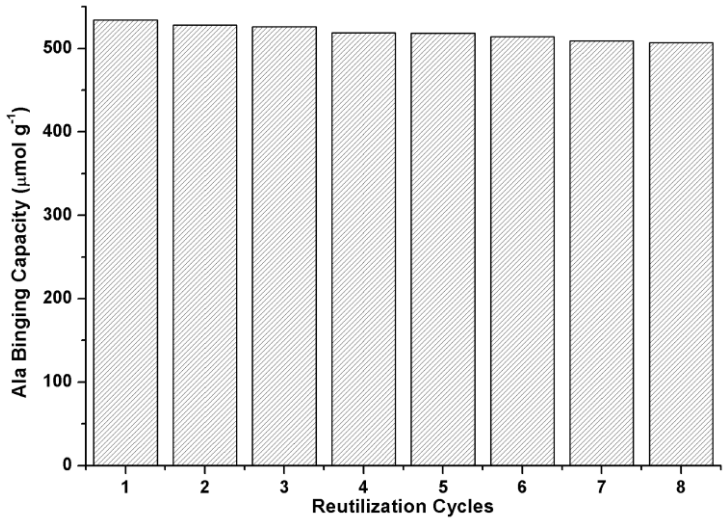


Figure 12. Reuse results of SMIPs for eight times (0.6 mmol L^{-1}).

4. Conclusions

In this work, an Ala–SMIP with a high recognition capability was first directly synthesized using the molecular-imprinting technique on the surface of vinyl–SiO_2 spheres to form a one-step emulsion reaction in aqueous solution. The adsorption properties of the core-shell structure SMIPs were evaluated, and the material shows a large adsorption capacity, fast binding kinetics, and excellent selectivity toward Ala, which were superior to those of the SNIPs. The results demonstrate that surface-imprinted polymer can significantly improve the binding capacity and kinetics of its recognition sites on the surface. The SMIPs also exhibits steady and excellent regeneration performance toward Ala in eight sorption-adsorption cycles. This class of new imprinted materials may become a powerful tool for the study of enrichment and purification of trace Ala from complex matrix samples such as saliva, serum and urine. Because of its nice regeneration performance, it may be available to achieve coupling with SPE-HPLC in the future. The merits make the surface imprinting materials one of the most promising candidates for various applications, including chemical and biochemical separation, recognition elements in bio-sensors, and drug delivery.

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Graphical abstract

Preparation and Recognition Characteristics of Alanine Surface Molecularly Imprinted Polymers

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The Ala-SMIPs has higher recognition capability and affinity toward Ala, and the Ala–SMIPs clearly had more significant selectivity than the SNIPs.