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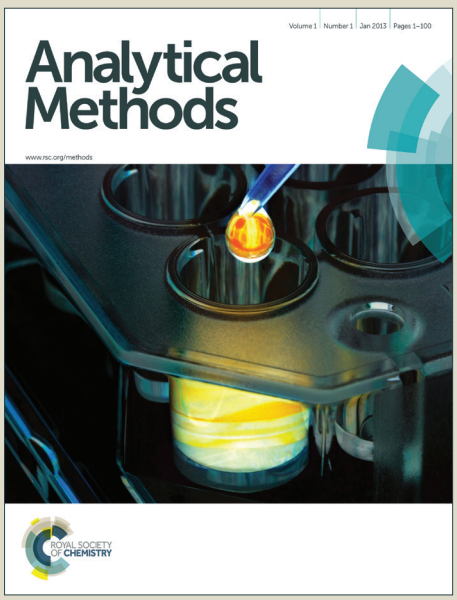

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Silica-Based Surface Molecularly Imprinting for Recognition and Separation of Lysozyme

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Abbreviations: APTES, 3-aminopropyltriethoxysilane; BSA, bovine serum albumin； BHb, bovine hemoglobin; FT-IR, fourier transform infrared spectroscopy; Lyz, lysozyme; MIP,

molecularly imprinted polymer; NIP, non-imprinted polymer; OVA, ovalbumin; PB, phosphate buffer solution; SDS, sodium dodecyl sulfate; TEM, transmission electron microscope; TEOS, tetraethoxysilane; TG, thermogravimetric analysis;

Key Words: Surface molecular imprinting / Silica-based / Sol-gel /Lysozyme/ Recognition

Abstract

A highly selective surface molecular imprinting inorganic polymer composed of tetraethoxysilane and 3-aminopropyltriethoxysilane has been prepared by a sol–gel process on silica submicroparticles in aqueous solution under simple and mild conditions. Lysozyme (Lyz, pI 11, MW 14.4 kDa) is used as the template protein. Product polymers were characterized by FT-IR, TEM, and TG techniques to verify the successful synthesis of the MIP on the surface of silica submicroparticles. The adsorption behavior of MIP was evaluated by adsorption capacity, imprint factor and adsorption model. It was found that both MIP and NIP could adsorb template protein quickly and easily owing to the low mass transfer resistance of thin shell and their adsorption equilibrium could be reached in 5h. Compared with NIP, MIP showed stronger adsorption capacity for template protein Lyz under all experimental conditions. The mechanism for static adsorption of Lyz onto MIP was found to follow Langmuir adsorption model which was further used to calculate the maximum adsorption capacity Q_{max} and gave a result of 90.33 mg/g in theory. The MIP adsorbent could be reuse twice without significant loss in adsorption capacity. MIP indicated excellent recognition and binding affinity toward Lyz, whose selectivity factor β for Lyz relative to reference proteins bovine serum albumin (BSA, pI 4.9, MW 69.0 kDa), bovine hemoglobin (BHb, pI 6.9, MW 65.0kDa) and ovalbumin (OVA, pI 4.7, MW 43.0kDa) were 2.36, 2.22, and 2.24, respectively. It was shown that the shape memory and the size effect were the major factors for the recognition. This imprinted inorganic polymer was used to specifically adsorb the Lyz from the protein mixture, which demonstrated its potential selectivity.

Introduction

Molecular imprinting is a technique for preparing recognition sites with predetermined selectivity which are tailor-made in situ by copolymerization of functional monomers and cross linkers in the presence of template molecule [1–2]. After removal of the template molecule from the polymer network, specific recognition sites that are complementary to the template in terms of their size, shape, and functionality are exposed [3]. Because of its excellent advantages like specific recognition, high stability, low cost and its reusability, MIP material has been successfully applied to the fields of biosensors[4-5], solid-phase extraction [6–8], separation[9-10],catalysis [11–12], and drug delivery[13]over the past decades. Despite the attractive features of MIP material with specificity, it has been largely limited to small molecules. The imprinting of biological macromolecules and proteins still remains to be a challenge.due to their incompatibility of these targets with organic solvents that are typically used for imprinting.

Protein molecularly imprinted technique is a method for separation which use the protein as template to prepare the molecularly imprinted polymers (MIP). The MIP has a spatial structure complementary with template, so it can specifically recognize the target protein. Because protein has characteristics of large molecular size, the high flexibility of conformation, the complex surface structures, there are still many challenges to imprint protein [14]. Therefore, it is necessary to study deeply about the preparation of protein imprinted polymers. The protein imprinted polymers can be used as cell scaffold material, antibodies and enzymes, which can substitute for natural biological structure [15-17]. For the above reason the protein MIPs have a widely application in biomedical field.

However, due to the thick polymeric network, the MIPs prepared by the conventional bulky

polymerization technique had some disadvantages especially for protein imprinting, such as poor site accessibility to the target molecules and low rebinding capacity [18-19]. The protein template molecules were entrapped deeply in the matrices, the elution was difficult, the diffusion barrier for the template molecules was higher, the rate of mass transfer was lower, and the template molecules were not easy to bind with recognition sites [20-21]. These drawbacks can be effectively avoided by surface molecular imprinting approach, which provides an alternative way for improving mass transfer, increasing affinity binding and decreasing high diffusion barrier of the template by fixing MIP on a support substrate [22]. For surface molecular printing, TiO_2 [23], Fe_2O_3 [24], and carbonaceous materials [25] had already been used as solid support materials. However, SiO_2 was the most commonly used solid support materials reported by numerous studies [26-30].

In this work, a highly selective surface molecular imprinting polymer for Lyz was synthesized on the support of silica submicroparticles by a sol−gel process which could create the uniform and small sizes of the particles and offer high surface area for the regeneration of template. APTES and TEOS were chosen as functional monomer and cross linker, respectively. The MIP was characterized by FT-IR, TEM, TG techniques. Imprinting efficiency of MIP was investigated by static adsorption tests. The resulting imprinted polymer (MIP) showed better template affinity than the corresponding non-imprinted polymer (NIP). The selectivity of the obtained polymer was evaluated over competitive compounds, and the results demonstrated that the MIP was capable of selective recognition of Lyz.

Compared with the conventional method, most of the recognition sites generated for surface imprinting would be on the thin outer shell layer, with a greatly increased accessibility and effectiveness. Moreover submicron structured MIPs have a small dimension with extremely high

surface-to-volume ratio, so that most of template molecules are situated at the surface or in the proximity of the material's surface. Obviously, submicro-sized surface imprinting MIPs are expected to improve the binding capacity, binding kinetics and accessibility of the recognition sites, especially for protein molecular imprinting in which the mass transfer limitation may be a more pronounced problem.

This surface molecular imprinting polymer was shown to be promising for regeneration and can be potentially employed as a generally applicable methodology for protein imprinting.

Experimental sections

Instrumentation

A UV-2450 UV-vis spectrophotometer (Shimadzu, Japan) was used to determine the absorbance of protein. FTIR spectra were recorded on a Spectrum One FTIR spectrometer (Perkin Elmer, U.S.). Thermal gravimetry (TG) curves of samples were acquired by STA409 thermal gravimetric analyzer (Netzsch, Germany). Virtis freeze drier (YiKang Experimental Equipment Co. Ltd，Beijing, China) was employed to get the freeze-dried polymers. After being dried, the samples were imaged under a JEOL JEM-3010 transmission electron microscopy.

Chemicals and reagents

3-aminopropyltriethoxysilane (APTES) was supplied by Aladdin chemistry Co., Ltd. (Shanghai, China). Tetraethoxysilane (TEOS) was bought from Shanghai Titan chemistry Co., Ltd. (Shanghai, China).Acetic acid, anhydrous ethanol, ammonium hydroxide, lysozyme (Lyz, pI 11, MW 14.4 kDa), bovine serum albumin (BSA, MW 69kDa，pI 4.9), bovine hemoglobin (BHb, MW

65kDa, pI 6.9), ovalbumin (OVA, pI 4.7, MW 43.0kDa) were all purchased from Guoyao chemical reagents company Co., Ltd. (China). Phosphate buffer solution (pH 7.0, 10 mM) was used as the working medium. All chemicals used were of analytical grade and used directly without further purification. Ultrapure water (18.25MΩ cm^{-1}) used throughout the experiment was obtained from the laboratory purification system.

Preparation of Silica Submicroparticles

Silica submicroparticles were synthesized by the hydrolysis of TEOS with ammonium hydroxide following a procedure described by Stober et al [31]. In this experiment, 4.5 mL of TEOS was resolved in a mixture containing total 48.9 mL of aqueous ammonia (25% concentrated ammonia in water), 9 mL of H2O and 37.6 mL of anhydrous ethanol. The system was stirred at room temperature for 5 h, resulting in the formation of white silica colloidal suspension. The silica submicroparticles were centrifugally separated from the suspension and washed with anhydrous ethanol several times until eluent became neutral. Finally, the SiO_2 submicroparticles were dried under vacuum overnight.

Preparation of Imprinted and Non-imprinted Polymers

The surface molecular imprinting inorganic polymers were synthesized by a sol–gel process on silica submicroparticles polymerized with APTES and TEOS in aqueous solution. At first, 400 mg silica submicroparticles were dispersed in mixture solution containing 20 mL of ethanol and 20 mL of phosphate buffer solution (pH 7.0, 10 mM) by ultrasonic vibration for 10 minutes. Then, 0.1 g of template protein Lyz and 0.62 mL of APTES were added into the suspension under

stirring for 2 h to obtain the completely self-assembled composites on silica submicroparticles. Finally, 1.24 mL of TEOS and 1 mL of HAc (1.0 mol L−1) were added to the mixture suspension at room temperature under stirring for 18h to obtain particles with a high cross-linking structure. After reaction the obtained polymer was first washed with SDS-HAc (10%w/v: 10%v/v) to remove the template molecules for several times until no Lyz could be detected by a UV-vis spectrophotometer at 278 nm and then washed with ultrapure water to remove SDS. The product polymer was dried at 60 °C for 12 h under vacuum. The non-imprinted polymer (NIP) was prepared and treated by the same conditions without the addition of template protein Lyz. The synthesis of MIP was shown in Fig 1. For comparison, several polymers with different cross-linking degrees were prepared by adjusting the reactant ratio of the volume of TEOS and APTES which were summarized in table 1.

Characterization of polymers

The MIP and NIP were characterized by FT-IR spectra and TEM. FT-IR spectra (4000–400 cm−1) in KBr were recorded using a Spectrum One FTIR spectrometer (Perkin Elmer, U.S.). The morphologies of the polymers were determined by a JEOL JEM-3010 transmission electron microscopy. The polymers were coated with a thin layer of gold under vacuum and photographed by the TEM. The average particle sizes of the particles were determined on the TEM images based on a weighted-averages method. Thermal gravimetry (TG) curves of polymers were acquired by using a thermoanalysis instrument under dynamic nitrogen atmosphere at a heating rate of 10 °C min−1.

Determination of Equilibrium Swelling Ratio

The freeze-dried silica-based polymers and silica submicroparticles were prepared by virtis freeze drier after frozen totally in refrigerator. The freeze-dried polymers with specific weight were immersed in phosphate buffer solution (pH 7.0, 10 mM) at room temperature overnight. After equilibrium, the polymers were taken out and isolated by centrifugation at 12 000 rpm for 30 min. Measurement of the polymers swollen weight (M_s) was made after the supernatant was removed. Subsequently, the polymers were dried at room temperature in vacuum box and weighed again to obtain the dry weight (M_d).The Swelling Ratio (SR) was defined by the following formula:

$$SR = \frac{M_s - M_d}{M_d} \quad (1)$$

Where M_d and M_s Represent the weight of the dry and swollen samples respectively.

Static adsorption experiments

The freeze-dried imprinted and non-imprinted polymers (10.0 mg) were incubated in 5.0 mL of a series of different concentrations of Lyz solution for 24 hours. All the binding experiments were conducted by the incubator shaker (200 r/min, 25˚C). After adsorption equilibrium, samples were centrifuged with a centrifuge at 8000 rpm for 8 min. The concentration of Lyz in the supernatant was detected by an UV-2450 UV-vis spectrophotometer (Shimadzu, Japan) at the wavelength of 278 nm used phosphate buffer solution (pH 7.0, 10 mM) as solvent under room temperature.

The amount of adsorbed protein was evaluated from the following formula:

$$Q = \frac{(C_0 - C)V}{M} \quad (2)$$

Where Q is the mass of protein adsorbed onto a unit amount of dry polymer (mg /g), C_0 and C are the initial and equilibrium concentrations of protein solutions respectively (mg /mL), V is the initial

volume of the solutions (mL), and M is the mass of dry polymer (g).

The imprinting factor α was defined by following formula:

$$\alpha = Q_{MIP}/Q_{NIP} \quad (3)$$

Where Q_{MIP} and Q_{NIP} are tested adsorption capacities for MIP and NIP towards a template protein.

All of the measurements were carried out in triplicate, and the average values were chosen.

Results and discussion

Synthesis of Silica Submicroparticles and Polymers

Highly schematic representation of the preparation process for Lyz imprinted silica-based polymers was shown in Fig. 1, which involved the following several steps: first, preparation of silica submicroparticles; second, template protein prepolymerized with APTES on silica submicroparticles; third, polymerization after addition of TEOS and HAc; finally removal of template. APTES was chosen as the functional monomer, HAc (1.0 mol L−1) and TEOS were used as a catalyst and cross linking agent, respectively. In this work, SDS-HAc (10%w/v: 10%v/v) is selected as eluent to remove the Lyz from MIPs and the eluate was detected by UV–vis spectrophotometer at the wavelength of 278 nm for the quantitative analysis of Lyz eluted. The result validated that 51.03% of Lyz was successfully washed away from MIPs. The result confirms the efficient removal of the template for the MIPs. After the template molecules were removed, a great number of tailor-made cavities for Lyz were left on the surface of silica submicroparticles. The uniform and small sizes of the production obtained would offer high surface area for further recognition of template protein.

To further determine the structure of the silica-based MIP, FT-IR spectra of SiO_2 particles (a), NIP (b) and MIP(c), were compared in Fig. 2. The wide and strong adsorption band around 3441

cm−1 could be ascribed to stretching vibrations of O−H. The observed features near 470 cm-1 and 1070 cm-1 were the vibration peak and the anti-vibration peak of Si-O-Si, respectively. A characteristic feature of MIP and NIP compared with silica submicroparticles were the special N−H band around 1558 and 1417 cm-1 from –NH2 group and the C−H band around 2924 cm−1 and 2852 cm−1 from –CH2 group. This FT-IR spectrum indicated that the APTES have been successfully grafted onto the surface of silica submicroparticles after imprinting.

A series of polymers with varied degrees of cross-linking were prepared according to the Table 1. The adsorption capacities of the imprinted and non-imprinted polymers used different amounts of TEOS as cross-linker were tested. The results indicated that the polymers MIP_2 employed 1.24 mL of TEOS as cross-linker proved to have relatively better adsorption capacities for Lyz. The recognition sites may be wrapped in the internal and the protein may be not easy to be eluted by washing with a high cross-linking degree. Besides, polymers with a relatively low cross-linking degree may have a variable three-dimensional structure which is unfavorable for rebinding. Therefore, the MIP_2 with best adsorption capacity were selected in the following experiments.

Characterization of the Imprinted Polymers by TEM and TG

In order to investigate the network structure, the images of SiO_2, MIP and NIP observed by TEM were shown in Fig. 3. From the results of the TEM pictures, uniform regular spherical particle structure ranging from 0.42 to 0.52 um in size could be obviously observed in all of particles, which verified the successful formation of desired material shape and morphology. It could be clearly observed that the average diameters among the SiO_2, MIP and NIP had no significant difference. This meant the modified polymer layer were very thin which could facilitate

mass transfer and recognition in both elution process and rebinding process.

On the basis of the different thermal stability between the silica submicroparticles and the polymers, TGA analysis was used to further study the relative composition of the silica core and the polymers shell. As shown in Fig.4, when the temperature was changed from room temperature to 1000℃, the weight loss of silica was slight and approximately 10.0%, while the decrease of solvent or water in weight was 6.0% at 200 ℃, and the other decrease in weight was 4.0% at 200－700℃ (Fig. 4a). For silica @MIP, the weight loss was 25.0% at 700℃, while the decrease of solvent or water in weight was about 12.0% at 200℃ which was two times compared with that of silica possibly due to the existing of imprinted cavity absorbing more water, and the other decrease in weight was about 13.0% for the decomposition of imprinted polymer coatings at 200－700℃ (Fig. 4c). If the mass retention of silica at 700℃ is used as the reference, there exists near 15.0 wt% difference in the weight retentions at 700 ℃ between silica and silica @MIP. It indicated that the method applied for MIP coating in this work was excellently effective. The grafting yield of MIP coating to silica was 15.0 wt%. In addition, the weight loss trend of silica @NIP (Fig. 4b) was similar to silica @MIP and gave a result of 21.5%. Such distinction was due to the existence of floccules-like imprinting coating on the surface of silica submicroparticles during the imprinting process.

Determination of Equilibrium Swelling Ratio

The swelling ratio values of the SiO_2 particles, silica-based MIPs and NIPs were investigate by immersion in PB overnight until swollen equilibrium, which were determined based on the amount of water uptake. It was found that the SR value for NIPs was 1.96, which was lower than that

obtained with MIPs, where the SR value was 2.71. It is reasonable that the SR value for SiO_2 particles is the least only 1.03. Such results could possibly be due to the formation of binding cavities on the surface of the MIPs, which enhanced water penetration and higher water uptake. Higher SR value for MIP could lead to lower mass transfer resistance, which is important for loading and release of the template in binding and extracting process respectively.

Adsorption Kinetics

In the evaluation for adsorption kinetics, the adsorption capacities of the imprinted and non-imprinted polymers were tested as a function of time. The experiments were performed 10 mg of MIP or NIP in 5.0 mL of 0.8 mg/mL initial Lyz concentration on the shaker (200 r/min, 25˚C) for incubation. The kinetics curves were shown in Fig.5. From the Fig.5, we can see that the adsorption capacities of the polymers for Lyz increased as time goes by. The absorbance of MIP for Lyz had a rapid increase in 2 h, and then slowed down with the time extension, finally reached equilibrium after 5 h. This might be explained that Lyz was easy to reach the surface imprinting sites on the Lyz-imprinted polymers at the starting. With the saturation of the sites, Lyz began to diffuse onto the surface of Lyz-imprinted polymers nonspecifically. Obviously, the imprinted polymers adsorbed more template protein compared to the non-imprinted polymers under the same conditions. This result also confirmed the fact that recognition sites with the specific shape and the orientation of functional groups successfully formed in the imprinted polymers network during the imprinting process. The relatively higher adsorption capacity of the MIP compared with NIP could be attributed to the hydrogen bonding interactions between functional groups of MIP and template protein, such as amide group, amino group, and hydroxyl group. In addition, MIP needed longer time than NIP to

get equilibrium because it contained the deep caves left by template molecules.

Adsorption Isotherms

To investigate the binding capacity for Lyz of both MIP and NIP, the adsorption isotherms were tested in the range of 0.2–1.1 mg/mL initial concentration of Lyz. The adsorption experiments were accomplished as the previous procedures. The effect of the initial Lyz concentrations on the adsorption capacities of the imprinted and the non-imprinted polymers prepared by the same conditions were investigated under room temperature and the results were showed in Fig. 6. As the figure showed, the equilibrium adsorption capacities of the MIP and NIP rose with the increase of the initial Lyz concentrations from 0.2 to 0.8 mg/mL. Then the curve became flat and reached thermodynamic equilibrium in the concentration region of above 0.8 mg/mL. Taking into account that Lyz was insoluble in water with high concentration, the concentration of 0.8 mg/mL was chosen as the optimal condition for the following experiments. It also found that the MIP had comparatively higher Lyz adsorption capacities than the NIP in all the tested Lyz solutions, and the imprinting factor α reached 2.53. In our study, the high adsorption capacity for Lysozyme may be explained by the formation of specific imprinting sites with complementary size and shape in MIP to hold and fix the Lysozyme molecular around MIP. The remaining silanol groups on the surface of the silica cores might play a important role on enrichment of the templates Lysozyme from the prepolymerization solution before polymerization and co-operation with the functional and cross-linking monomers to create the imprinting sites while polymerization process. Additionally, the advantages of the micro-sized MIP with big specific surface area and surface energy also attributed to enhance the adsorption capacity for Lysozyme. However, as for NIPs, the non-specific adsorption has dominant effect for lacking of

recognition sites. Therefore, the binding amount of Lyz is much lower. In addition, the MIP could be reused twice without weakening the binding capacity significantly.

The adsorption experiment data of these polymers could be further processed according to Langmuir adsorption equation to estimate the binding parameters which was proposed by Irving Langmuir [32].This equation was well known for its preferably use at saturation conditions and more homogeneous adsorbents such as the molecularly imprinted materials [33-34]. The equation was listed as following:

$$C_e/Q=C_e/Q_{max}+1/(KQ_{max}) \quad (4)$$

Where C_e is the adsorption equilibrium concentration for Lyz, Q is the theoretical maximum adsorption capacity for polymers, Q_{max} is the saturated adsorption after adsorption equilibrium, and K is the Langmuir adsorption equilibrium constant. A highly linearized plot of C_e /Q versus C_e was made. The Langmuir equation fits well for Lyz adsorption on MIP within the concentration range studied (correlation coefficient, r^2 = 0.99882). The corresponding K and Qmax was 17.41 mL/mg and 90.33 mg/g which were obtained from the slope and intercept of Langmuir equation simulated curve. The result evidenced a relatively high adsorption affinity of our synthesized MIP for Lyz.

Selectivity of the Polymers

In this experiment, BHb, BSA, OVA were selected as reference proteins with a test concentration of 0.8 mg/mL to study the selectivity of MIP for Lyz. This choice was based on the fact that reference proteins had a various shape, isoelectric point and molecular weight compared to the template Lyz. The adsorption capacities results of the MIP and NIP for different proteins were illustrated in Fig. 7. As shown in Fig. 7, MIP displayed a highest adsorption capacity for Lyz among

the four test protein substrates, indicating the high adsorption selectivity for Lyz. Moreover, a comparison of the adsorption of the MIP and NIP for each protein substrate suggested that the MIP hardly showed an imprinting effect for BHb, BSA, OVA as both MIP and NIP nearly had the same adsorption capacity for them.

The selectivity factor β was used to evaluate the specific selectivity and defined by following formula:

$$\beta = \alpha_{Lyz}/\alpha_{RP} \qquad (5)$$

Where α_{Lyz} and α_{RP} were tested imprinting factor of polymer for template protein Lyz and particular reference proteins. The values of imprinting factor α and selectivity factor β for Lyz, BHb, BSA and OVA were easily obtained by simple calculations and presented in Table 2. It was observed that α values of MIP for Lyz relative to BHb, OVA and BSA were much higher, which meant that the imprinted polymer possessed pronounced adsorption selectivity for the template Lyz as compared with other reference proteins. In addition, the β values for BHb, BSA and OVA were far more than 1.0, which again indicated the MIP had higher adsorption selectivity than that of the NIP. The preferred adsorption for the template Lyz might be owing to the existence of memory recognition sites formed in MIP during imprinting process, which were perfectly complementary fit with the template Lyz both in shape and size. So the different spatial orientation of reference proteins did not match the imprinted cavities and its access to the binding sites might be limited by the steric hindrance of polymer chains, thus the adsorption capacity is relatively low and mainly comes from physical adsorption associated with nonspecific interactions.

Conclusions

In our research, a new kind of surface molecular imprinting polymer for template protein Lyz used APTES as functional monomer and TEOS as cross-linker had been successfully synthesized based on supporting matrices silica submicroparticles. The optimal binding conditions were investigated and gave a result of adsorption equilibrium time 5 h and initial protein concentrations 0.8 mg/mL with maximum experimental adsorption capacities of 84.13 mg/g and 33.19 mg/g for MIP and NIP respectively. Batch binding experiments demonstrated product material offered fast adsorption kinetic rate and MIP had significantly higher adsorption affinity for the template Lyz compared with NIP in all cases. The equilibrium adsorption isotherms of Lyz on MIP could be well fitted by the Langmuir adsorption model. At the same time, the MIP for Lyz exhibited good selectivity over reference proteins with all separation factors larger than 2 by direct adsorption of the single reference protein. The results of all the experiments suggested that specific recognition sites which were sterically complementary to the template molecules Lyz were formed in the process of the imprinting step, which was an essential favorable factor during the binding process. In addition, the MIP could be reused twice without weakening the binding capacity significantly. All the abovementioned properties such as easy preparation, fast mass transfer rate, satisfied adsorption capacity, easy separation and specific recognition capability to the template protein Lyz made this method and MIP material a promising prospect for the application in the field of rapid protein separation and enrichment for the near future.

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Table captions

Table 1 Preparation different degrees of cross-link polymers

Table 2 Values of imprinting factor α and selectivity factor β for Lyz, BHb, BSA and OVA

Polymers	SiO_2(g)	Lyz (g)	APTES(mL)	TEOS(mL)	HAc (mL)	Ethanol (mL)	PB(mL)
MIP_1	0.400	0.100	0.620	0.620	1.000	20	20
MIP_2	0.400	0.100	0.620	1.240	1.000	20	20
MIP_3	0.400	0.100	0.620	1.860	1.000	20	20
NIP_1	0.400		0.620	0.620	1.000	20	20
NIP_2	0.400		0.620	1.240	1.000	20	20
NIP_3	0.400		0.620	1.860	1.000	20	20

Table 1 preparation different degrees of cross-link Polymers

Protein	Lyz	BHb	BSA	OVA
Imprinting factor α	2.53	1.14	1.07	1.13
Selectivity factor β		2.22	2.36	2.24

Table 2 Values of imprinting factor α and selectivity factor β for Lyz, BHb, BSA and OVA

Figure captions

Figure 1.Schematic representation of the molecular imprinting procedures.

Figure 2.FT-IR spectra of SiO_2 (a), NIP (b) and MIP(c).

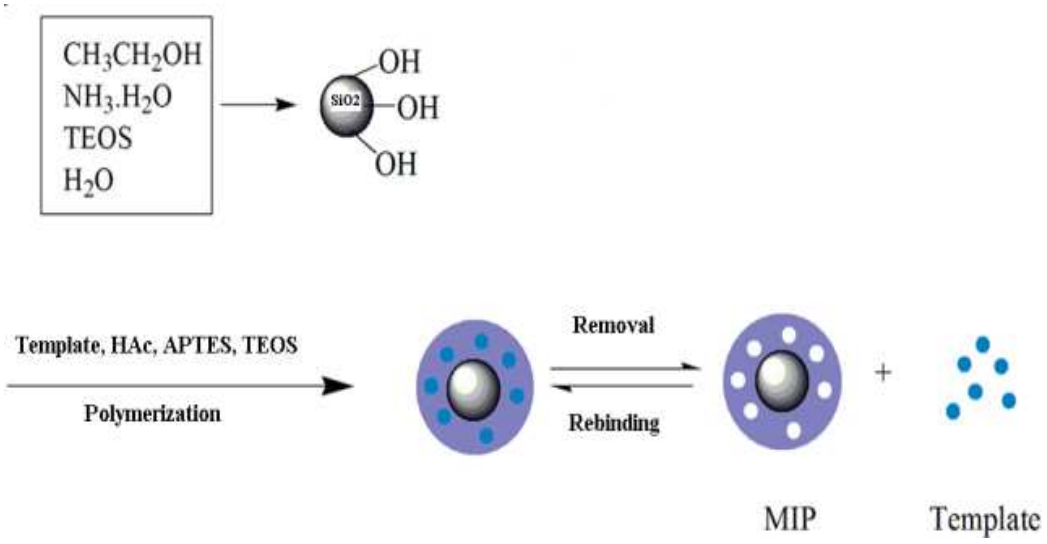
Figure 3.TEM images of SiO_2 (a), MIP (b) and NIP(c).

Figure 4.TGA Curves of SiO_2 (a), NIP (b) and MIP(c).

Figure 5.Binding kinetics curve of the Lyz on MIP and NIP.

Figure 6.Adsorption isotherms of the Lyz on MIP and NIP.

Figure 7.Selective adsorption between reference proteins and Lyz on MIP and NIP.



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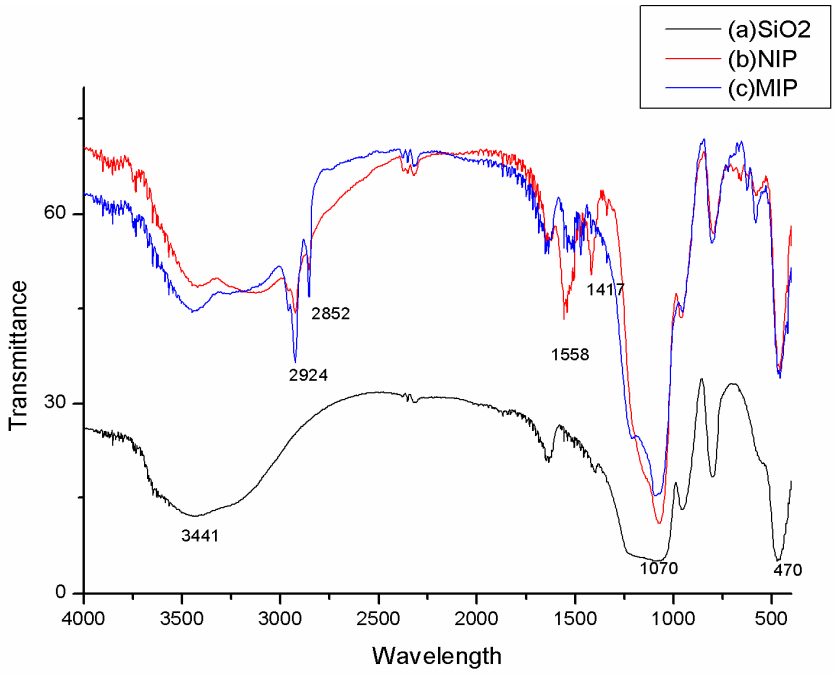

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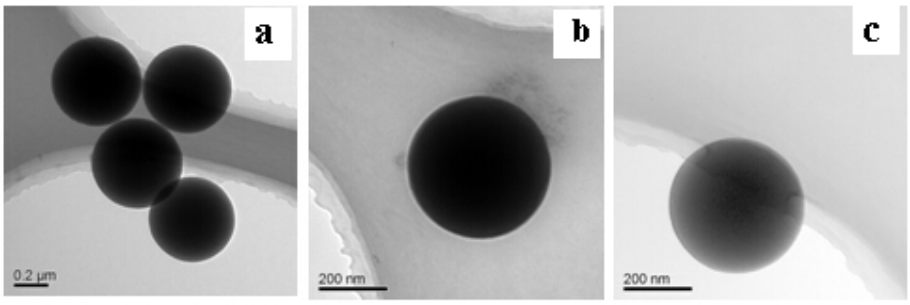

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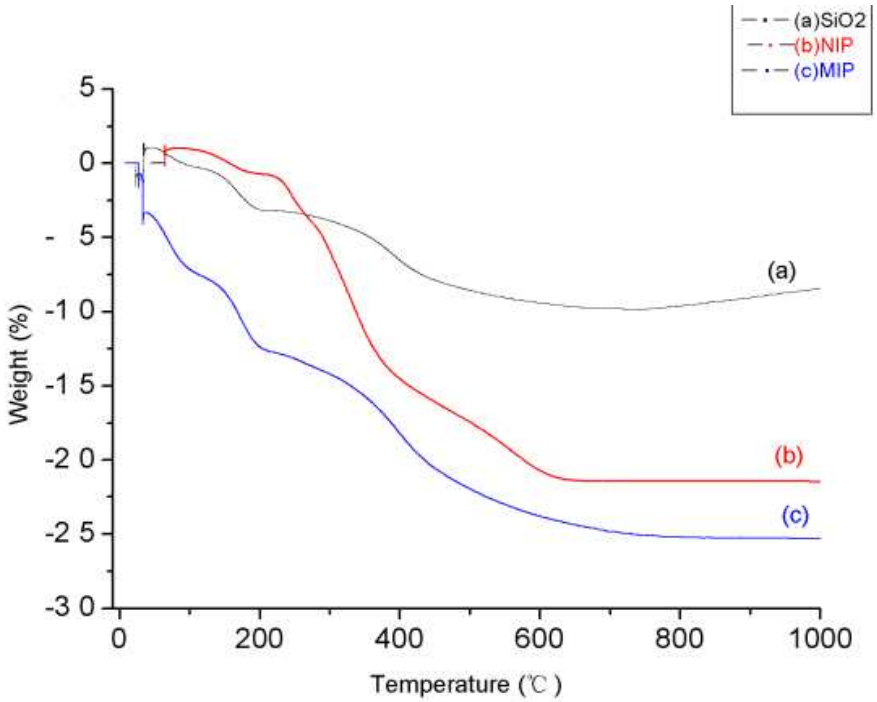


Figure 4.TGA Curves of SiO_2 (a), NIP (b) and MIP(c).

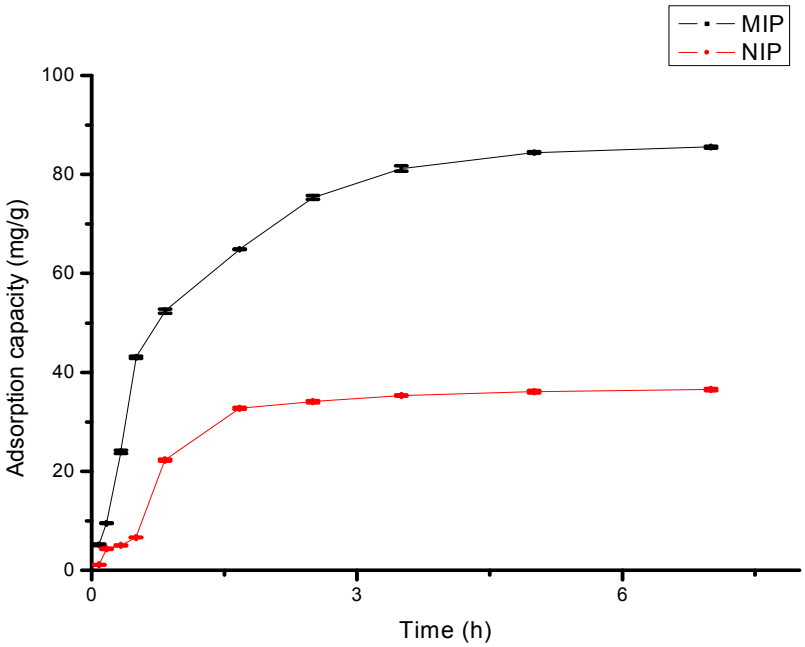


Figure 5.Binding kinetics curve of the Lyz on MIP and NIP (mean±s; n=3).

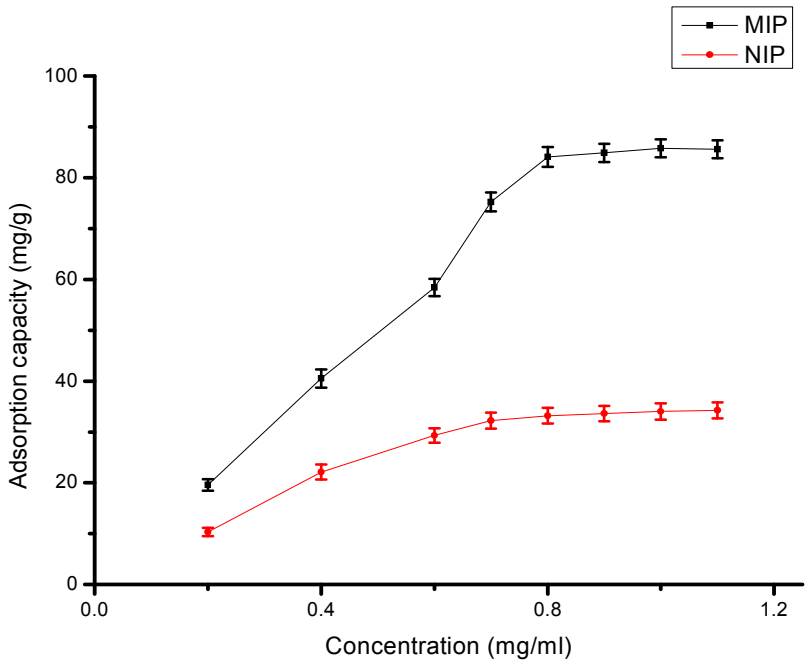
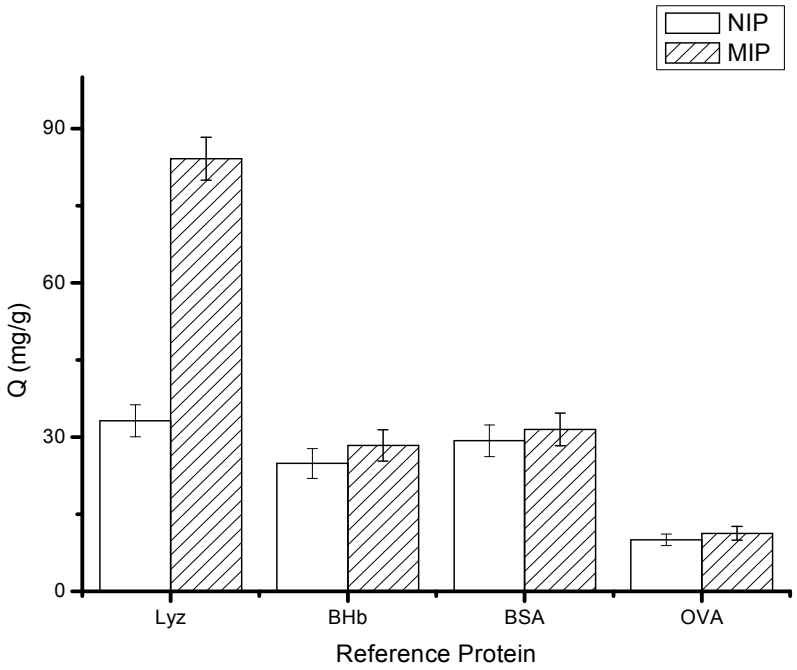


Figure 6.Adsorption isotherms of the Lyz on MIP and NIP (mean±s; n=3).



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