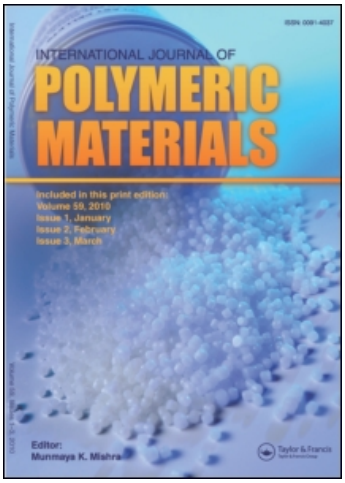

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Preparation and Properties of Uniform-Sized Polymer Beads Imprinted with N-CBZ-L-Phenylalanine

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A series of uniform-sized polymer beads molecularly imprinted for N-carbobenzoxy-L-phenylalanine (N-Cbz-L-Phe) were prepared by a two-step swelling and suspension polymerization method using polystyrene latex particles as precursors, 2-vinypyridine and α-methylacrylamide as host functional monomers and diethylene glycol diacrylate as crosslinker. The uniformly sized polystyrene precursors were synthesized by emulsifier-free emulsion polymerization. The obtained materials had an obvious imprinting effect to the template N-Cbz-L-phenylalanine. The imprinted polymer also had some adsorption to the molecules, which were similar to the template molecule configuration. The adsorbing dynamics of the polymer to the template molecule, and the effects of crosslinking degree of the polymer and the environmental temperature on the absorbing properties were also discussed.

Keywords: molecularly imprinted polymer, uniform-sized polymer beads, amino acid derivative, adsorption, selective separation

INTRODUCTION

Molecularly imprinted polymer materials can be utilized as a separation medium [1–4] for specific recognition of the target molecule.

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Usually, nonaqueous bulk polymerization techniques [5] and a classical suspension polymerization [6] are used to obtain molecularly imprinted polymers. However, for the former method, the obtained block polymers should be crushed, sieved, and classified before their use as the separation medium, sensor, and artificial antibody. And the latter method affords beads that have rather broad particle size distributions, preventing their use directly for chromatography medium.

In the search for uniformly sized beads, Ugelstad [7] developed a technique termed the activated multi-step swelling and polymerization method. In recent years, many researchers applied the method in the molecular imprinting technique for different separation objects [8–14].

As is well-known, amino acids and their derivatives are chiral, and with few exceptions only one enantiomer is of interest. So selective separation materials for purifying amino acid and its derivatives is a very important study topic all the time [2–4].

In this work, a uniform-sized molecular imprinted polymer (U-MIPs), which was imprinted with N-Cbz-L-phenylalanine (N-Cbz-L-Phe), was prepared using a two-step swelling and suspension polymerization method. The polymer was characterized by transmission electron microscope (TEM), scanning electron microscope (SEM), Fourier transform Raman spectrometry (FT-Raman), and UV spectrophotometer measurements. The effects of host functional monomers and the degree of crosslinking on the adsorbing capacity of the molecular imprinted polymer were also discussed.

EXPERIMENTAL

Materials

Styrene and α-methylacrylamide (α-MAM), analytical grade, were purchased from the First Chemical Reagent Factory (Tianjin, China); diethylene glycol diacrylate (DEGDA) was purchased from Institute of Chemical Reagents (Tianjin, China), 2-Vinylpyridine (2-VP) was purchased from Sigma (packed in Switzerland). All these monomers were purified before use under reduced pressure to remove the polymerization inhibitors. Potassium peroxydisulfate and α,α-Azobisisobutyronitrile, analytical grade, were recrystallized before use. Amino acid derivatives (N-Cbz-L-Phe, N-Cbz-D-Phe, N-tert-butyloxy-Phe (N-t-Boc-L-Phe), N-Cbz-tryptophan (N-Cbz-Trp), N-Cbz-L-tyrosine (N-Cbz-Tyr), N-Cbz-L-serine (N-Cbz-L-Ser), and N-Cbz-L-leucine (N-Cbz-L-Leu) were purchased from Sigma. All the other chemicals were used as received.

Preparation of Uniform-Sized Molecularly Imprinted Polymer

Uniformly sized polystyrene latex particles utilized as the shape precursor were prepared by emulsifier-free emulsion polymerization as described elsewhere [15–17].

Preparation of uniformly sized, N-Cbz-L-Phe imprinted polymer beads by a two-step swelling and polymerization method was carried out as follows: A water dispersion of the uniformly sized polystyrene latex particles, 0.7 ml (6.0 wt%), was mixed with an emulsion prepared from 0.2 ml dibutyl phthalate as activating solvent, 0.04 g α, α-azobisisobutyronitrile, 0.2 g sodium dodecyl sulphate and 5 ml of distilled water by sonication for 5 min. The first-step swelling was carried out at room temperature with slow stirring for 18 h until the oil droplets in the added emulsion had completely disappeared, the first-step was finished.

An emulsion prepared with functional monomer, crosslinker, template molecule, polyvinylalchol (mean degree of polymerization = 1750; saponification value = 88–89 mol%) used as stabilizer and 60 ml water, by sonication for 5 min was added to the dispersion liquid of swollen particles. The swelling was carried out at room temperature until the oil droplets disappeared.

After the second-step swelling, the polymerization procedure was started in a 250 ml four-necked reaction flask at 50°C under nitrogen atmosphere with slow stirring (240 rpm) for 24 h. The supernatant liquid was then discarded after sedimentation of the beads. Polymer beads were washed with THF 3 times, and separately extracted for 24 h with water and methanol repeatedly to remove the suspension stabilizer, SDS, and template. Finally the polymer beads were dried in vacuum for 72 h before use.

The blank polymer was prepared in the same procedure as mentioned earlier, without the addition of template molecule.

Characterization

The diameters of polystyrene latex particles were determined by transmission electron microscope (TEM) (Hitachi-600, Japan). The sample was prepared by dropping 0.1 ml of diluted latexes (0.5–1.0 wt%) on a copper grid and then allowed to dry in a desiccator.

The morphology and the diameters of the U-MIP beads were observed with a Hitachi X-650 (Japan) scanning electron microscope after coating with gold.

FT-Raman spectrum was obtained using a RFS100/S (Bruker, German) spectrometer using Nd: YAG diode-pumped solid-state laser (wave length = 1064 nm) and liquid nitrogen cooled detector.

The adsorption capacity was measured by UV spectrophotometer (UV-9100). Different concentrations of amino acid derivative solutions were detected by UV spectrophotometer. And their characteristic curves of absorbency vs. concentration were obtained. Five mg imprinted polymer was placed in a tapered flask with stopper, and then added 5 ml template or other similar molecule's solution using ethyl acetate as solvent. The flask was shaken constantly in a shaking apparatus in a thermostated water bath for 12 h. The beads were filtered on a Teflon membrane (1.5 μm pore diameter), and the absorbency of the supernatant was measured by UV spectrophotometer at a proper wavelength. Based on the difference of the former and later solution concentration, the authors used the characteristic curve to calculate the adsorption capacity.

RESULTS AND DISCUSSION

Preparation of U-MIP

The transmission electron micrograph of polystyrene precursors synthesized by emulsifier-free emulsion polymerization [18, 19] is shown in Figure 1. The mean diameter of the obtained polystyrene particles is about 0.7 μm with the particle diameter distribution of 1.004. The monodispersed polystyrene particles were the starting precursors used to prepare the final imprinted crosslinked polymer beads. Once the step-swelling was completed, free radical suspension polymerization was initiated by heating. Figure 2 is the SEM photograph of the final MIP beads. Figure 2 shows that the size monodispersity of the polystyrene precursors has been transferred to the final MIP beads through the swelling and polymerization process. The average diameter of the final polymer beads was 5.4 μm (as seen in Figure 2). The average diameters and diameter distribution of polymer beads were obtained from data processing of the SEM photograph of MIP beads; the sample capacity was 100. It can be seen from Figure 3 that more than 95% particles were in the range of 5–6 μm, the diameter distribution factor U ($U = d_W/d_N$; d_W, weight average diameter; d_N, number average diameter) was 1.02. The diameter distribution of MIP particles is so narrow that it can be considered to be uniform-sized [20–21].

The FT-Raman spectrum of MIP (sample P2) is shown in Figure 4. The strong peak between 2800–3000 cm^{-1} is caused by the flexural vibration of the carbon-hydrogen bonds. The flexural vibration of the carbonyls and carbon-oxygen bonds in the crosslinker are responsible for the peaks at 1726.02 cm^{-1}, 1295.74 cm^{-1}, and 991.00 cm^{-1}, respectively. The peak at 1049.11 cm^{-1} is the dissymmetry vibration of the

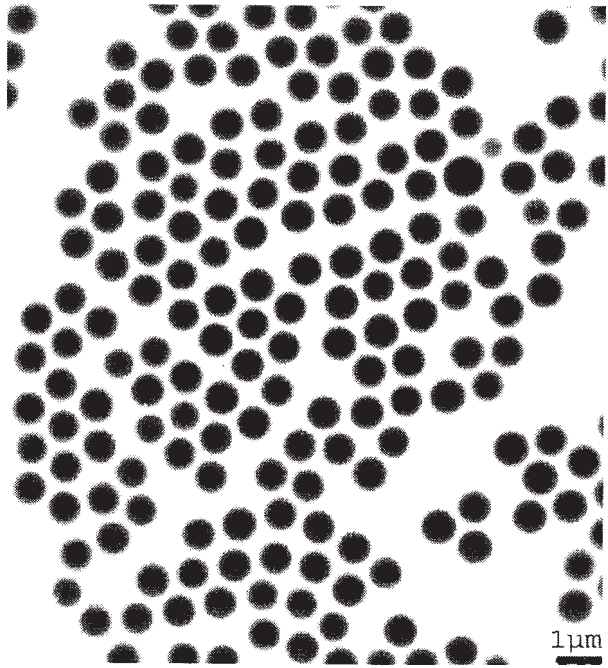

FIGURE 1 TEM microphotograph of the polystyrene precursors (×9000 times).

carbon-oxygen-carbon bond in the crosslinker, whereas the peak at 868.23 cm^{-1} is attributed to the symmetry vibration of it. The characteristic vibration peak, which is caused by the carbon nitrogen double bond of pyridine, is between 1680–1610 cm^{-1}. And the peak at 1449.60 cm^{-1} is created by the flexural vibration of the carbon-carbon bonds in the aromatic rings. Thus, the conclusion can be drawn that the functional monomer 2-VP and the crosslinker DEGDA had participated in the polymerization, and the polymerization process was fully carried out.

The Adsorption Capacity of U-MIP

Data from Table 1 show that the adsorption capacity of U-MIP is higher than the blank polymer P0, while P2, which used 2-VP as functional monomer, shows the highest adsorption capacity (58.40 mg/g). The indicates that after removing the template molecules, many imprinted cavities will be left on the surface of the MIP beads, Thus,

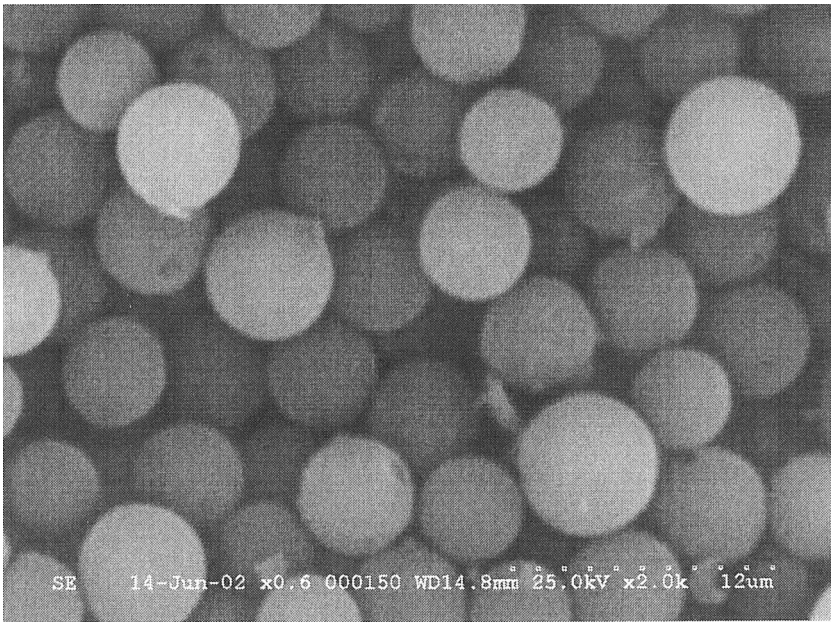

FIGURE 2 The SEM morphologies of molecular imprinted polymer prepared by multi-step swelling and suspension polymerization method (×2000 times).

in the adsorbing process the template molecules will enter into these selective cavities and then reach a high adsorption capacity.

The adsorption capacity of P2, using 2-VP as functional monomer, is more than P1 that was co-polymerized with two kinds of functional monomers (2-VP, α-methylacrylamide) and P3 that used α-methylacrylamide as function monomer. This is probably because much stronger hydrogen bonds may be formed between the strong basic group pyridine in 2-VP and the hydrogen of the carboxyl group in the N-Cbz-L-Phe molecule.

Influence of Crosslinking Degree

The influence of crosslinking degree on the adsorption capacity of U-MIP is shown in Figure 5. The cross-linking degree was defined as the wt% content of the cross-linker in the whole amount of the reaction monomers.

Figure 5 shows that there is a maximal point at the crosslinking degree of 38.8%. This indicates when the crosslinking degree is low, the polymer cannot keep the shape of the imprinted cavities

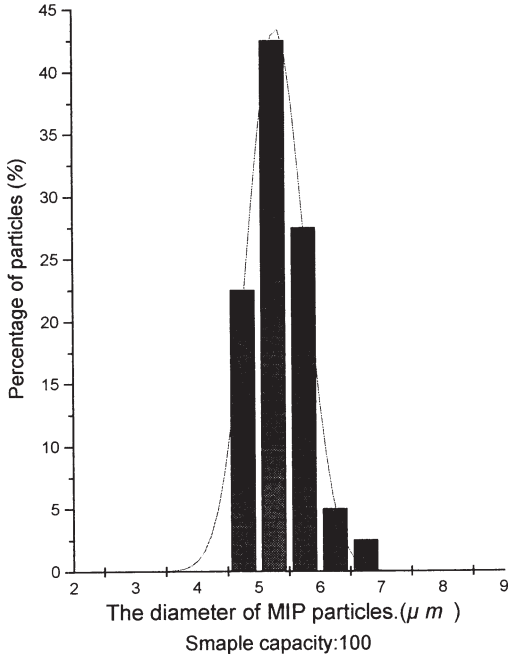

FIGURE 3 Particle size distribution of the U-MIP beads.

due to its poor rigidity, which results in low adsorbing capacity. However, when the crosslinking degree turns too much higher, it is difficult for the template molecule to be eluted from U-MIP, therefore the number of the imprinted cavities remaining on the surface of the imprinted beads is decreased, which lowers the adsorption capacity.

Adsorbing Dynamics of the MIP

The MIP used 2-VP as host functional monomer (P2) showed a remarkable imprinting effect on the template molecule, so in the following experiment, P2 was selected as the sample.

The adsorption dynamic curve is shown in Figure 6. It can be seen from Figure 6 that within one hour, the adsorbance rapidly increases. At the point of one hour, the adsorbance has reached 95.3% of the equilibrium adsorbance. Two hours later, the adsorption process reaches equilibrium. This phenomenon illuminates that the U-MIP combines very rapidly with the template molecules. This is very

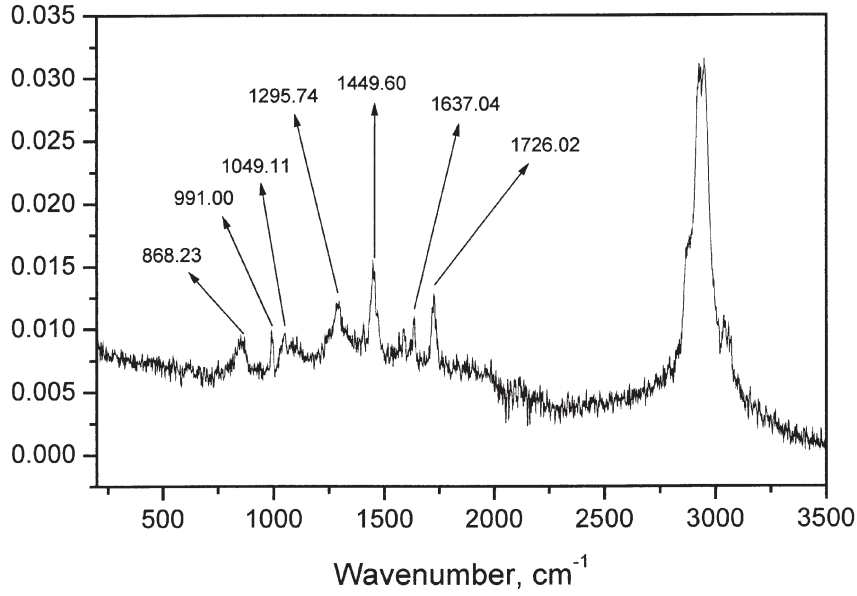

FIGURE 4 The FT-Raman spectrogram of molecular imprinted polymer (sample P2).

important for the application of the MIP in fast analysis and some other fields. For comparison, the MIP prepared by the bulk polymerization [22], at one hour its adsorbance reached only 82% of the adsorbing capacity.

TABLE 1 The Compositions of the Imprinted Polymers and Their Adsorption Properties to the Template Molecule

Sequence number	Template (mmol)	2-VP (mmol)	α-MAM (mmol)	DEGDA (mmol)	Capacity (mg/g)	K_D (l/g)
P0	\	1	1	10	8.35	0.12
P1	0.5	1	1	10	37.57	0.89
P2	0.5	2	\	10	58.40	2.70
P3	0.5	\	2	10	27.8	0.53
P4	0.25	1	1	10	37.5	0.89
P5	0.25	\	2	10	27.83	0.53
P6	0.5	1	1	5	11.13	0.16
P7	0.5	1	1	15	34.78	0.77
P8	0.5	1	1	20	23.65	0.42
P9	0.5	1	1	30	5.57	0.07

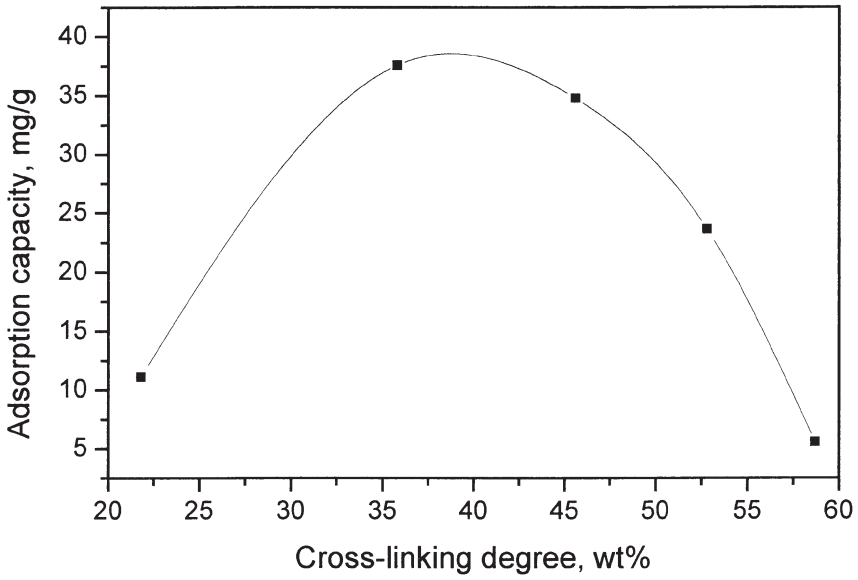

FIGURE 5 The influence of crosslinking degree of the molecule imprinted materials on the adsorption capacity (adsorption time—8 hours; sample quantity: 5 mg; T = 25°C).

Influence of Environmental Temperature

The adsorbing ability of MIP to the template molecule at several environmental temperatures is shown in Figure 7. The adsorption time was 12 h, and the environmental temperatures selected were 30°C, 40°C, 50°C, 60°C, and 70°C.

It can be seen from Figure 7 that below 50°C, the adsorption capacity of the MIP increases with increasing temperature, whereas over 50°C the adsorption capacity declines with the increase of temperature. This is probably because the increase of temperature under 50°C benefits the diffusion of the template molecules, so the adsorption capacity of U-MIP increased with increasing temperature. When the temperature was over 50°C, the higher temperature destroyed the hydrogen bond formed between the U-MIP and the template molecule, thus the adsorption capacity declined. This result agrees that the MIP should prepared in a relatively low temperature in order to avoid the breakage of the pre-interaction between the functional monomer and the template molecule [23].

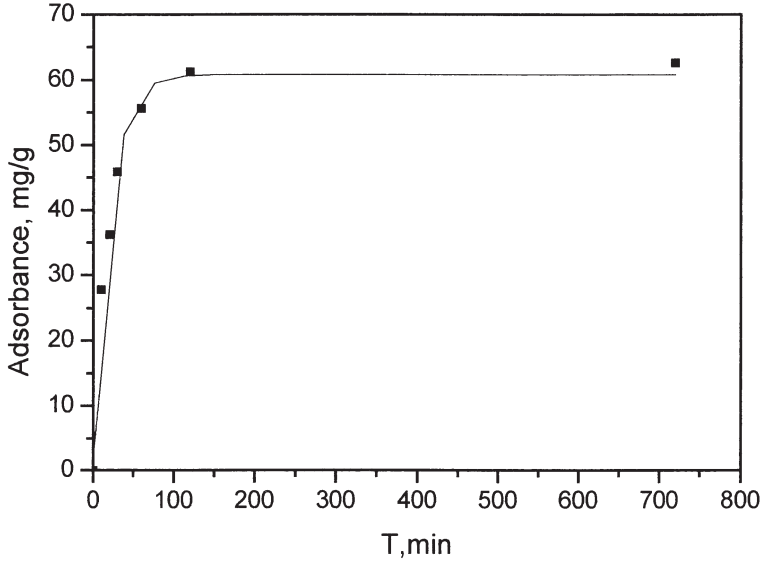

FIGURE 6 Influence of adsorbing time on the adsorbance (C = 0.08 mg/ml; the sample quantity 5 mg; T = 25°C).

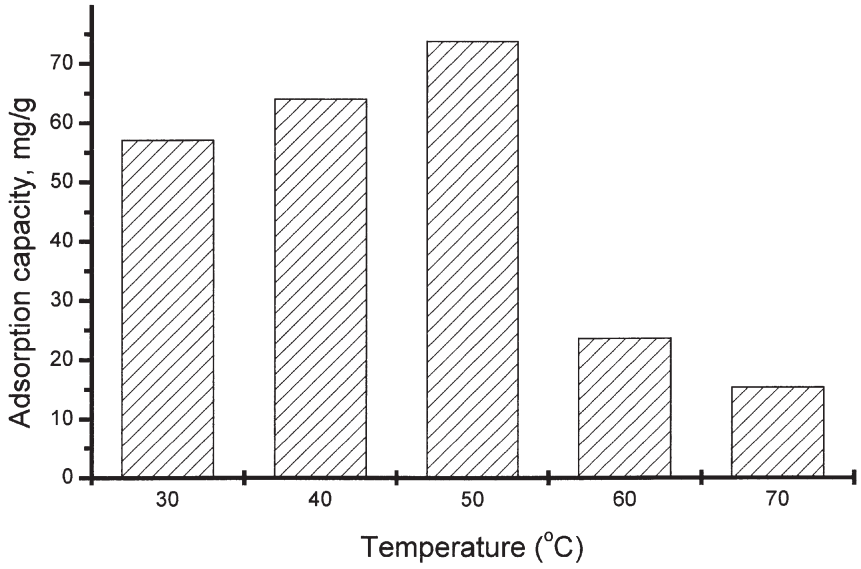

FIGURE 7 The adsorption capacity of MIP at different temperatures (C = 0.08 mg/ml; adsorption time—8 hours; sample quantity: 5 mg).

The Selectivity of U-MIP

The distribution coefficient K_D is defined as $K_D = C_p/C_s$, where C_p = concentration of substrates on the polymer (mg/g) and C_s = concentration of substrates in the solution (mg/l).

The special selectivity of U-MIP was investigated using six types of structurally related amino acid derivatives as substrates. The amino acid derivatives selected were: N-Cbz-D-Phe, N-t-Boc-L-Phe, N-Cbz-Trp, N-Cbz-L-Tyr, N-Cbz-L-Ser, and N-Cbz-L-Leu.

It can be seen from Figure 8 that the U-MIP exhibits high selectivity for the template molecule, N-Cbz-L-Phe, compared to all the other tested substrates. At the same time, because N-Cbz-D-Phe belongs to the same group of the template molecule, with N-t-Boc-L-Phe, its distribution coefficient K_D is higher. And the K_Ds of the U-MIP to N-Cbz-Trp and N-Cbz-L-Tyr are relatively lower. This is probably because although the protecting groups of N-Cbz-Trp and N-Cbz-L-Tyr are the same as the template molecule's, their basic structures

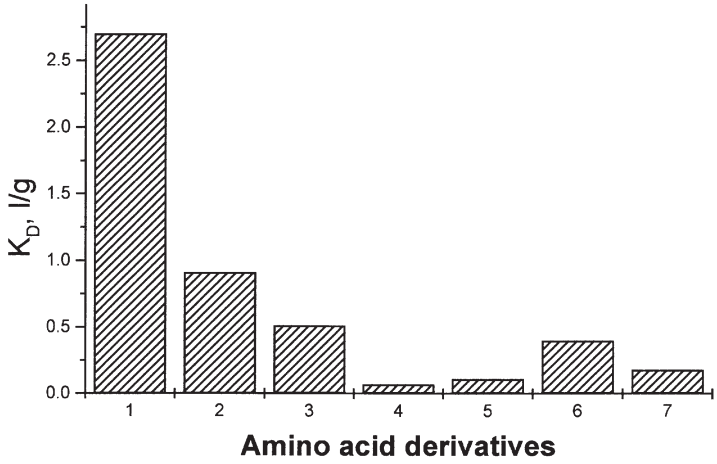

FIGURE 8 The distribution coefficients of U-MIP of different amino acid derivatives (C = 0.08 mg/ml; adsorption time—8 hours; sample quantity: 5 mg).

are different from the template molecule. Because the cavities formed on the surface of U-MIP are matched to the size of the template molecule, it is very difficult for the molecules with different dimensions to enter the cavities; therefore the K_Ds of U-MIP to them are correspondingly lower. The amino acid derivatives, N-Cbz-L-Ser and N-Cbz-L-Leu, belong to the aliphatic family. And their molecular dimensions in solution are different than those of template molecule, compared to N-Cbz-Trp and N-Cbz-L-Tyr, the K_Ds of U-MIP to them are higher.

CONCLUSION

Uniform-sized, molecularly imprinted polymers, with N-Cbz-L-Phe as the template molecule, were prepared using a two-step swelling and suspension polymerization.

The crosslinking degree and the types of monomer selected influence the adsorption ability of U-MIP. 2-vinylpyridine (2-VP), which is strongly basic, is fit to be the functional monomer for the template molecule, and a crosslinking degree at about 40% for U-MIP showed the highest adsorption ability.

The adsorption process of U-MIP reached equilibrium in less than two hours, which is faster than the equilibrium of MIP prepared by bulk polymerization. The U-MIP for N-Cbz-L-Phe prepared in the authors' lab exhibits an obvious selectivity to the template molecule, but it can also adsorb molecules which are somewhat similar. Only at a proper environmental temperature U-MIP has strong adsorption ability to the template molecule, because the hydrogen bond between the functional monomer and the template molecule will be destroyed at high temperature.

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