Preparation and Characteristics of Tryptophan-Imprinted Fe_3O_4/P(TRIM) Composite Microspheres with Magnetic Susceptibility by Inverse Emulsion–Suspension Polymerization

Shulai Lu,[1,2] Guoxiang Cheng,[1] Honggang Zhang,[1] Xingshou Pang[1]

[1]*School of Materials Science and Engineering, Tianjin University, Tianjin 300072, People's Republic of China*
[2]*Research Institute of Jilin Petrochemical Company, PetroChina Company Limited, Jilin 132021, People's Republic of China*

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ABSTRACT: Tryptophan-imprinted Fe_3O_4/P(TRIM) composite microspheres with magnetic susceptibility (MS-SMIPs) were prepared by inverse emulsion–suspension polymerization, according to the principle of molecular imprinting technique, using magnetite Fe_3O_4 particles as magnetically susceptible component, methacrylic acid (MAA) and acrylamide (AM) as functional monomers, trimethylolpropane trimethacrylate (TRIM) as polymeric matrix components, and hydroxy ethyl cellulose (HEC) as dispersant. The external morphology and the inner structure of MS-SMIPs were observed by SEM. SEM photographs showed that the resulting MS-SMIPs were regularly spherical in external morphology and had a large quantity of spherical microvoids inside. The effects of the amount of Fe_3O_4 on particle size and morphology of MS-SMIPs were investigated in detail. The results indicated that the amount of Fe_3O_4 affected particle size distribution and morphology of MS-SMIPs obviously. The magnetic characteristics of MS-SMIPs were measured by vibrating sample magnetometer, and the results showed that the resulting MS-SMIPs had a certain magnetic response to external magnetic fields. Adsorption properties, molecular recognition selectivity, and regeneration recognition selectivity of MS-SMIPs were investigated using tyrosine and phenylalanine as control molecules, and characterized by high performance liquid chromatography. It was shown that the resulting MS-SMIPs exhibited a good recognition selectivity for tryptophan, and the relative separation factor (β) was 2.75, and MS-SMIPs also exhibited higher regeneration recognition selectivity, and the separation factor was 1.83 and 1.80 in first regeneration and second regeneration, respectively. The effect of the amount of functional monomers on molecular recognition selectivity was investigated, and the mechanism of imprinting and recognition was analyzed. J Appl Polym Sci 99: 3241–3250, 2006

Key words: molecularly imprinted polymeric microspheres; tryptophan; morphology; magnetic responsibility; molecular recognition selectivity

INTRODUCTION

Molecularly imprinted polymers (MIPs), prepared by molecular imprinting technique (MIT), provide a means of creating specific recognition and combination similar to those in biological systems, such as antibodies to antigens, enzymes to zymolytes,[1–3] and have exhibited extensive application prospects in enantiomer separation,[4–6] antibody binding mimic,[7–9] enzyme mimic,[10,11] biomimic sensor,[12,13] control of equilibrium shifting of chemical reaction,[14] byproduct removal,[15] and so on[16–19]

Up to now, there are many approaches to prepare MIPs, but MIPs are generally prepared in the form of monolith, which are then ground and sieved to the appropriate particle size. The grinding and sieving process is time-consuming, and MIP particles are usually irregular in shape. As an alternative, MIP particles can be prepared directly by suspension polymerization in the form of spherical particles of controlled diameter.[20,21] The spherical particles can be used directly after being prepared, and merely the templates are removed via extraction. Moreover, they have many advantages in properties, such as their regular shape, large specific surface area, their designed function, and so on. The spherical particles prepared by such MITs are referred to as spherical molecularly imprinted polymers (SMIPs).[22] Their preparation methods and applications are becoming the research focus.

Correspondence to: G. Cheng (gxcheng@tju.edu.cn).
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When magnetically susceptible components, such as Fe, Co, Ni, or their oxides are encapsulated inside SMIPs, the resulting composite microspheres (MS-SMIPs) will have magnetically susceptible characteristics, and then they can be separated conveniently from the system they are located, in the presence of external magnetic fields,[23] and will be applied in the separation of analogical compounds, chiral compounds, byproducts, trace compounds, and other separation areas.

Polymeric matrices are very important in preparation of MIPs,[24] because of using different crosslinking monomer as polymeric matrix, the resulting MIPs would have different crosslinking degree, which affect the swelling degree of MIPs and the shape of the "imprinted cavities" directly in operating environment, and thus affect molecular recognition specificity. Divinylbenzene and ethylene glycol dimethacrylate are difunctional monomers, while trimethylolpropane trimethacrylate (TRIM) is a trifunctional monomer. The crosslinking degree of MIPs prepared by these crosslinking monomers are different obviously, and the structure, the size, the shape, and the amount of the resulting "chemical pores" are different necessarily.

TRIM is a kind of high viscosity monomer, whose viscosity comes up to 108 mPa s (measured by the present writer using NDJ-79 Rotation Viscosimeter at 24.4°C). Our research results showed that morphology of MS-SMIPs was irregular, prepared by using conventional suspension polymerization, because its high viscosity affected the dispersion of monomer droplet, and thus affected the formation of microspheres, and the advantage of MS-SMIPs would not exert thoroughly, because their irregular shape affected their movement velocity. So, we can say that the use of TRIM in preparation of MS-SMIPs was restricted by conventional suspension polymerization.

The so-called inverse emulsion–suspension polymerization (IESP) is an improved suspension polymerization method to prepare composite microspheres, which includes three steps, namely inverse emulsion (IE) polymerization, preparation of IE, and suspension polymerization. The details are as follows: (1) Fe_3O_4 particles were added into aqueous solution of acrylamide (AM) and *N*,*N*′-methylene bisacrylamide (MBA), and stirred to make them disperse uniformly, then IE polymerization was carried out to obtain Fe_3O_4/P(AM-MBA) inverse polymer emulsion (IPE); (2) IPE and TRIM were mixed together, and then disposed by ultrasonic dispersion to obtain IE; (3) the resulting IE was used as dispersed phase, to carry out suspension polymerization and realize molecular imprinting, and then MS-SMIPs were obtained.

The difficulty for conventional suspension polymerization to disperse high viscosity monomers into droplets was overcome by IESP; meanwhile, the compatibility of Fe_3O_4 particles with monomers and polymers was solved through pre-encapsulation of Fe_3O_4 particles, by IE, in this method. In addition, the resulting MS-SMIPs did not precipitate easily in aqueous recognition system and in favor of adsorption for analytes, because they had a large quantity of spherical microvoids inside, and hence had low density.

Try-imprinted MS-SMIPs (Try-SMIPs) were prepared by IESP, proposed by the present writer, using TRIM as polymeric matrix components, Fe_3O_4 particles as magnetically susceptible component, MAA and AM as functional monomers (FMs), and HEC as dispersant. The preparation process and the affecting factors of particle morphology of Try-SMIPs, the characteristics of the resulting MS-SMIPs, including magnetic response property, adsorption property, molecular recognition selectivity, and regeneration recognition selectivity were investigated in detail.

EXPERIMENTAL

Materials

Magnetite Fe_3O_4 particles (Fe_3O_4, 0.5–1.0 μm) were obtained from the Institute of Chemistry and Metallurgy, Chinese Academy of Sciences. Tryptophan (Try), tyrosine (Tyr), and phenylalanine (Phe) were all purchased from Beijing Xinjingke Biotechnology Co. Ltd. [their structures were illustrated in Figs. 9(a)–9(c)]. TRIM was purchased from Tianjin No.1 Chemical Reagent Factory. Methacrylic acid (MAA), AM, MBA, toluene, potassium persulfate (KPS), sodium bisulfite ($NaHSO_3$), ethanol, acetone, methanol, acetic acid, and 2,2′-azobisisobutyronitrile (AIBN) were all analytical reagents, and Span80 and hydroxy ethyl cellulose (HEC) were chemical reagents. All materials were used without further purification. Double-distilled water was used throughout.

Preparation of MS-SMIPs

The preparation process of MS-SMIPs by IESP includes three steps, i.e., (1) IE, (2) preparation of IE, and (3) suspension polymerization. The detailed schematic diagram of IESP was presented in Figure 1.

IE polymerization

(a) AM (9 g), MBA (1 g), and distilled water (20 mL) were added into a 50-mL beaker to obtain an aqueous solution, and then Fe_3O_4 (1.0 g) was added into the solution. (b) KPS (0.1 g), $NaHSO_3$ (0.05 g), and distilled water (20 mL) were added into another 50-mL beaker. (c) Toluene (100 mL) and Span80 (1.0 g) were added into a 250-mL three-necked round-bottom flask, equipped with a reflux condenser, nitrogen inlet, and stirrer. When Span80 was dispersed uniformly,

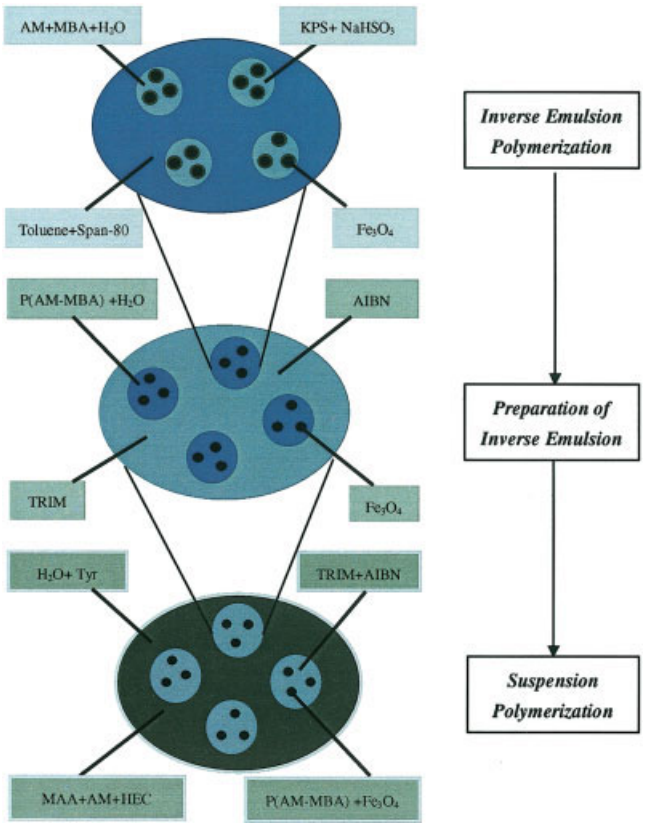

Figure 1 Schematic diagram of the preparation of MS-SMIPs by IESP method. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and (a) and (b) were added into it. The stirrer speed was maintained at 280 rpm, and the reaction system was purged with nitrogen for 10 min, and the reaction lasted for 2 h at room temperature. After 24-h standing, toluene in the upper layer was removed, and then IPE was obtained.

Preparation of IE

AIBN (0.1 g) and TRIM (10 mL) were added into a 50-mL beaker. When AIBN was dissolved completely, IPE (6–10 mL) was added into it. Ultrasonic dispersion (JY-BIIultrasonic oscillator) was used to disperse the mixture, and then IE was obtained.

Suspension polymerization

Suspension polymerizations were carried out in a 250-mL three-necked round-bottom flask, equipped with a reflux condenser, nitrogen inlet, and stirrer. The flask was immersed in a thermostatical water bath at the reaction temperature. The stirrer speed was maintained at 200 rpm. A typical procedure may be given as follows: HEC (0.12 g) was dissolved in distilled water (80 mL) in the flask, and then IE was added. Try (1 mmol) was added into distilled water (50 mL), MAA (8–16 mmol) and AM (4–8 mmol) were added into it after Try was dissolved, followed by stirring this mixture for 30 min, and then transferred into the flask. Rising the temperature of the water bath, the reaction system was purged with nitrogen for 10 min prior to reach the reaction temperature. The reaction lasted for 12 h at 70°C. When polymerization was finished, the product was cooled down to room temperature and filtered through a filter screen, and then MS-SMIPs were obtained. The nonimprinted magnetically susceptible composite microspheres (Non-SMIPs) were prepared in the same manner in the absence of templates.

Elution of templates

The resulting MS-SMIPs were transferred into a 500-mL beaker and washed with acetone, ethanol, and distilled water successively, and then dipped in 50 mL methanol/acetic acid (9/1, v/v) for 24 h, and then were filtered and washed with distilled water repeatedly, until the washing water was neutral. MS-SMIPs were dried using a vacuum oven at 80°C to constant weight. Non-SMIPs were disposed by the same method.

Regeneration reuse of MS-SMIPs

MS-SMIPs that have adsorbed analytes were dipped in methanol/acetic acid (9/1, v/v) for 24 h, and then MS-SMIPs were filtered and washed with distilled water repeatedly, until the washing water was neutral. MS-SMIPs were dried using a vacuum oven at 80°C to constant weight.

Determination of adsorption capacity and molecular recognition selectivity

The experiments were carried out using Tyr and Phe as control molecules. The procedure was given as follows:

1. Each of Try, Tyr, and Phe of 0.25 mmol was added into 100 mL distilled water with stirring, and then 2.5 mmol/L of mixed amino acid solution was obtained.
2. One gram MS-SMIPs, wetted by distilled water, was added into 5 mL mixed amino acid solution, immersing and shaking for 24 h at 25°C. Samples were taken to analyze the concentration of the mixed amino acid solution at regular intervals, until adsorption equilibrium reached, and then the adsorption capacity of different time and static equilibrium adsorption capacity (SEAC) of MS-SMIPs were determined, according to the concentration of the solution.

3. Adsorption capacity–adsorption time curve of MS-SMIPs was drawn using adsorption time as abscissa and adsorption capacity as ordinate.
4. The mixed amino acid solution was diluted to 2.0, 1.5, 1.0, 0.5 mmol/L in turn, and then step (2) was repeated, respectively.
5. Adsorption capacity–analyte concentration curve of MS-SMIPs was drawn using analyte concentration as abscissa and SEAC as ordinate.
6. Static distribution coefficient (K_D), separation factor (α), and relative separation factor (β) were calculated and applied to evaluate molecular recognition selectivity.

Scanning electron microscope

The surface morphology and the size of MS-SMIPs were characterized by Hitachi S-3000N scanning electron microscope (SEM).

Energy spectrum

The distribution of the encapsulated Fe_3O_4 inside MS-SMIPs was studied by using SEM and EDAX-PHOENIX energy spectrum probe equipped in it.

Vibrating sample magnetometer

Magnetic properties of MS-SMIPs and Fe_3O_4 were measured by using LDJ-9600 vibrating sample magnetometer (America LDJ company).

Liquid chromatography

The molecular recognition selectivity of MS-SMIPs and Non-SMIPs was evaluated by chromatographic analysis of the concentration of the mixed amino acid, using LC-10A liquid chromatography (column C18, 250×4.6 mm^2, UV absorbance at 214 nm, flow rate of carrier liquid 1.0 mL/min).

RESULTS AND DISCUSSION

Particle size and morphology of MS-SMIPs

Effect of the amount of Fe_3O_4 on particle size and morphology

Magnetic content in MS-SMIPs was expected to encapsulate as much as possible, so that they have magnetic responsibility as strong as possible. For this reason, the effect of the amount of Fe_3O_4 (in terms of volume of IPE) on particle size and morphology was investigated.

Figures 2(a,d) were SEM photographs of MS-SMIPs, prepared by different amounts of Fe_3O_4. Figure 2 indicated that the morphology of the resulting particles changed from single particle to congeries, along with an increase in the amount of Fe_3O_4, and the more the amount of Fe_3O_4 the more serious the aggregation was. When the amount of IPE was 6 mL, the resulting MS-SMIPs were all regular single particles; when the amount of IPE was 8 mL, the resulting particles congregated to some extent, only minute amount of particles were single, and the great mass of them were "double particles," resembling "tumbler" or "earth-nut" in shape; when the amount of IPE was 9 mL, the resulting particles congregated to a great extent, particles were farewell to single, and the great mass of them were "double particles," congeries of "several particles" occurred, the particle distribution began to become broad; and when the amount of IPE was 10 mL, the gained particles congregated seriously, the number of "double particles" decreased obviously and most of them were "polyparticles," and the particle distribution became broader than others.

It was shown experimentally that the particles of congeries have become an organic whole, although they were still regularly spherical in appearance, and could not be separated by manual labor grinding. Thus, the formation of aggregation occurred presumably in the course of polymerization, instead of polymerization that has been finished.

The imaginable reason for the formation of aggregation was due to the increase in the amount of IPE, the amount of aqueous phase in IE increased, and thus the formation of IE became more difficult, and resulted in the increase of the amount of Fe_3O_4/P(AM-MBA) composite exposed to particle surface. Subsequently, the particles congregated to form congeries in the course of polymerization due to the action of static magnetic attraction and could not be separated. The fact that the particle size distribution became broad with the increase of the amount of IPE was considered by the same reason, namely, the difficulty to form IE increased with the increasing of the amount of aqueous phase in IE.

Inner structure and formation mechanism of MS-SMIPs

A large particle was broken mechanically, and then a hemispherical fragment was selected to observe the inner structure by SEM, and SEM photograph of section of MS-SMIPs was shown in Figure 3.

It could be seen in Figure 3 that there were a lot of spherical microvoids inside and were all regularly spherical. It was clear that the formation of spherical microvoids were mainly due to the combination of IE with suspension polymerization in preparation of MS-SMIPs by using IEPS. Aqueous IPE, acted as the dispersion phase of IE, was dispersed into tiny "waterpolos" by ultrasonic dispersion in the preparation of IE, then the tiny "waterpolos" were fixed subsequently in microspheres through polymerization, and then magnetic

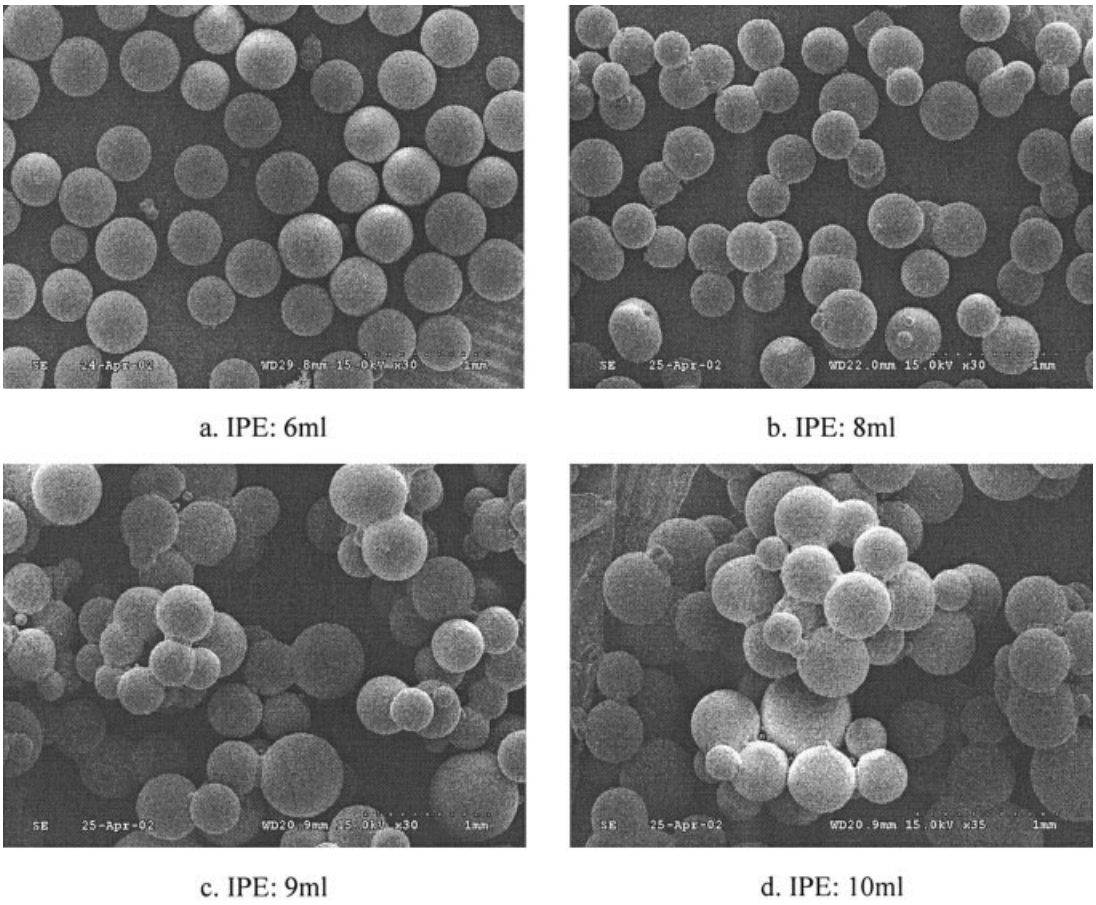

Figure 2 SEM photographs of MS-SMIPs, prepared by different amount of Fe_3O_4 (stirring speed 200 rpm, temperature 70°C).

polymeric microspheres with a large quantity of tiny "waterpolos" inside were obtained. When microspheres were dried, the water in tiny "waterpolos" ran away through interspace of polymer, and then spherical microvoids formed. In other words, the spherical microvoids were the position occupied once by the tiny "waterpolos". This speculation could be confirmed by the result of energy spectrum analysis being shown in Figure 4. Figure 4 indicated that there were Fe_3O_4 particles adhered to the inner wall of spherical microvoids. Therefore, these spherical microvoids could be regarded as "imprints," caused by the tiny "waterpolos" in a wide sense.

Magnetic responsibility of MS-SMIPs

Magnetic hysteresis loop was a vital character of magnetic materials. It reflects the response ability of the

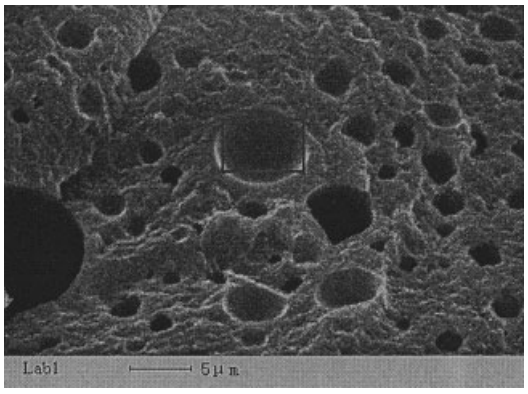

Figure 3 SEM photograph of a section of MS-SMIPs.

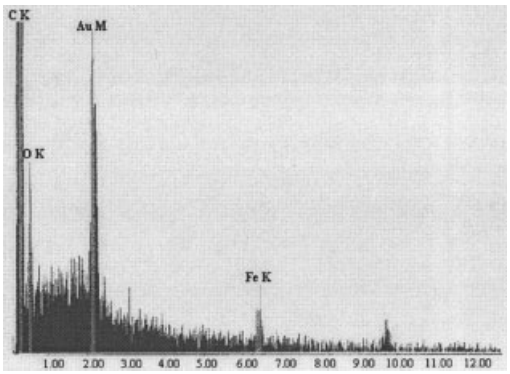

Figure 4 Energy spectrum analysis for the selected region of Figure 3.

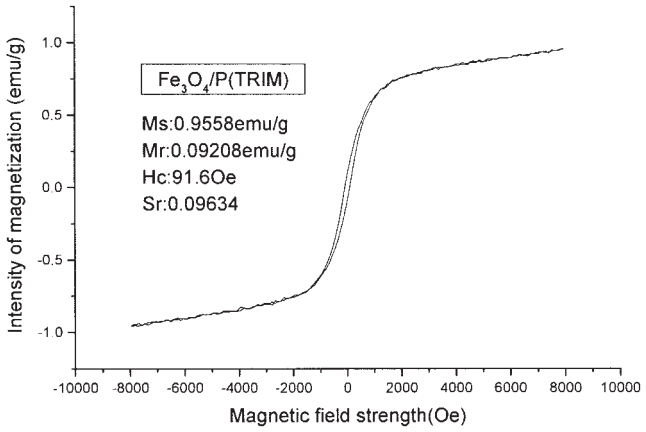

Figure 5 Magnetic hysteresis loop of MS-SMIPs.

magnetic materials to the change of external magnetic field (denoted by magnetic field strength) first, and it characterizes the ability of magnetic materials to keep magnetic field strength, when the external magnetic field was removed (denoted by coercive force, H_c).

Figures 5 and 6 were magnetic hysteresis loop of MS-SMIPs and Fe_3O_4, respectively.

Comparing Figure 5 with Figure 6, it could be seen that the magnetic hysteresis loop of MS-SMIPs was similar to that of Fe_3O_4 in shape, and they were all slimly closed curve. The main magnetic characteristic parameters of the resulting MS-SMIPs were changed largely compared with magnetic material, used as magnetically susceptible component (Fe_3O_4) in them, but the saturation magnetization (M_s, maybe being called maximum magnetization was more suitable here, because it did not reach saturation, see Fig. 5 of MS-SMIPs, which still indicated that MS-SMIPs possess a certain magnetic response property. Magnetic remanence (M_r) of MS-SMIPs was very small in favor of the redispersion when the external magnetic field was removed.

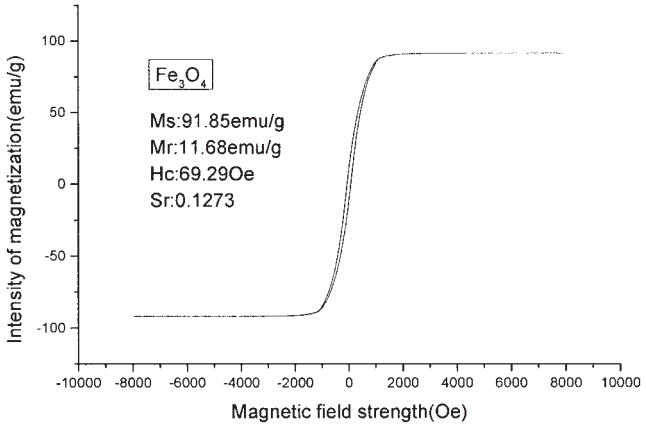

Figure 6 Magnetic hysteresis loop of Fe_3O_4 particles.

TABLE I
Static Equilibrium Adsorption Capacity of MS-SMIPs

MS-SMIPs	Adsorption time (h)	Q_∞ (10^3 mmol/g)		
		Try	Tyr	Phe
Try-SMIPs	24	6.45	3.05	2.40
Non-SMIPs	24	3.55	2.95	2.60

Adsorption properties of MS-SMIPs

Adsorption capacity could indicate the adsorption ability of adsorbent, and the adsorption rate could illustrate the speed of adsorption process to reach adsorption equilibrium, and so the adsorption capacity and adsorption rate were used to characterize the adsorption properties of MS-SMIPs in this article.

Seac of MS-SMIPs

There are two methods being used to determine the adsorption capacity of adsorbent, i.e., static method and dynamic method. Static method is dipping the adsorbent in a certain concentration of analyte solution to make them contact with analytes for a long time. In this article, static method was used to determine SEAC (Q_∞) of MS-SMIPs to the mixed solution of Try, Phe and Tyr (each of them was 2.5 mmol/L). Q_∞ (mmol/g) was the adsorption capacity, when adsorption reaches equilibrium, and was expressed as follows:

$$Q_\infty = (C_{S0} - C_S) \times V/m \quad (1)$$

where C_{S0} is the initial concentration of analytes (mmol/L), C_S is the analyte concentration when adsorption reached equilibrium (mmol/L), V is volume of analyte solution (mL), and m is the amount of MS-SMIPs (g).

In this experiment, $C_{S0} = 2.5 \times 10^{-3}$ mmol/mL, $V = 5$ mL, $m = 1$ g, and so the following equation was obtained, and the results were shown in Table I.

$$Q_\infty = 5 \times (2.5 - C_S) \quad (2)$$

It was shown in Table I that Q_∞ of Try-SMIPs to Try was much higher than that of Tyr or Phe, while Q_∞ of Non-SMIPs to Try was also higher than that of Tyr or Phe, but the difference between them was very small. This result indicated that molecular imprinting resulted in the increase of SEAC.

Adsorption capacity–adsorption time curve of MS-SMIPs

Adsorption capacity–adsorption time curve is a chief means to study the adsorption behavior of adsorbents,

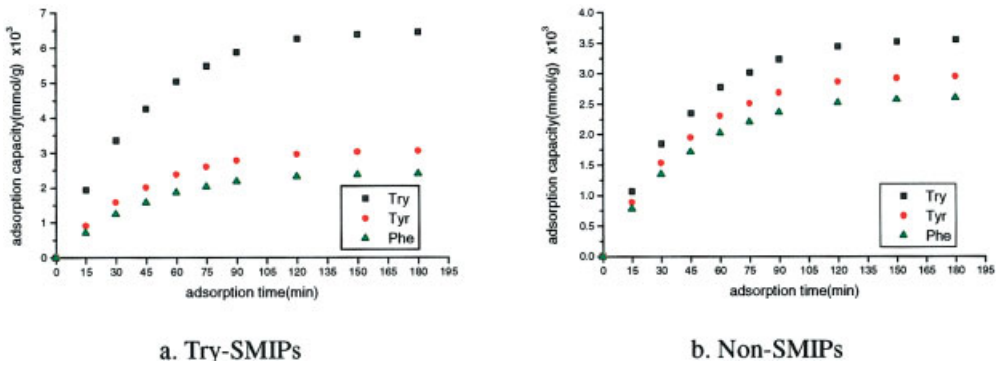

Figure 7 Adsorption capacity–adsorption time curve of MS-SMIPs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

for it reflects the adsorption rate and instantaneous adsorption capacity of adsorbents. In this experiment, adsorption capacity–adsorption time curve of MS-SMIPs to analytes (each of Tyr, Phe, and Try was2.5 mmol/L) was drawn and shown in Figure 7.

Figure 7 showed that the change tendency of adsorption capacity–adsorption time curve of Try-SMIPs and Non-SMIPs were the same, i.e., adsorption capacity increasing with adsorption time lasting, and adsorption capacity increasing rapidly in the beginning, and then increasing rate slowed down in late stage. Adsorption time reaching adsorption equilibrium was the same also, i.e., 150 min.

Adsorption capacity–analyte concentration curve of MS-SMIPs

As mentioned earlier, adsorption capacity–adsorption time curve reflects mainly the rate of adsorption course, while adsorption capacity–analyte concentration curve reflects principally the degree of adsorption course. To a certain adsorption system, equilibrium adsorption capacity is a function of concentration (adsorption in liquid phase) and temperature. When temperature is fixed, the equilibrium adsorption capacity is a monodrome function of concentration. So, when temperature is kept constant, adsorption capacity–analyte concentration curve can be drawn, according to the corresponding relation between equilibrium adsorption capacity and analyte concentration. The result of adsorption was shown in Figure 8.

From Figure 8, it could be seen that the change tendency of adsorption capacity–analyte concentration of the resulting MS-SMIPs were the same, namely, SEAC increased with the increase of analyte concentration, and their corresponding relation was close to linear relationship, probably due to the very dilute inherence of mixed amino acid solution.

Molecular recognition selectivity of MS-SMIPs

Molecular recognition selectivity of MIPS can be evaluated by using K_D, α, and β, calculated according to adsorption experiment results.[25] K_D, α and β are defined as follows:

$$K_D = C_P / C_S \tag{3}$$

where C_P is the amount of analyte absorbed by per gram of MS-MIPS when adsorption reached equilibrium, C_P is equal to Q_∞ (mmol/g), i.e., $C_P = Q_\infty$; C_S is the initial concentration of analyte solution (mmol/mL).

K_D reflects the adsorption capacity of MS-MIPS. According to eqs. (2) and (3), eq. (4) was obtained.

$$K_D = 5 \times (2.5 - C_S)/C_S \tag{4}$$

$$\alpha = K_{D1} / \sum K_{D2} \tag{5}$$

where K_{D1} and K_{D2} are the static distribution coefficient of templates and control molecules, respectively. α indicates the molecular recognition selectivity for MS-MIPS to templates. In general, the larger the value of α, the better the recognition selectivity is.

$$\beta = \alpha_1 / \alpha_2 \tag{6}$$

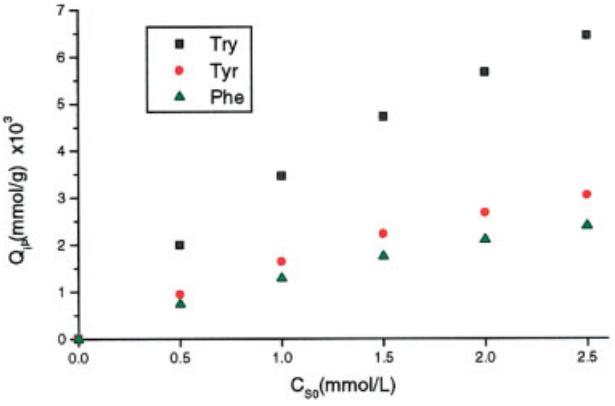

Figure 8 Adsorption capacity–analyte concentration curve of Try-SMIPs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

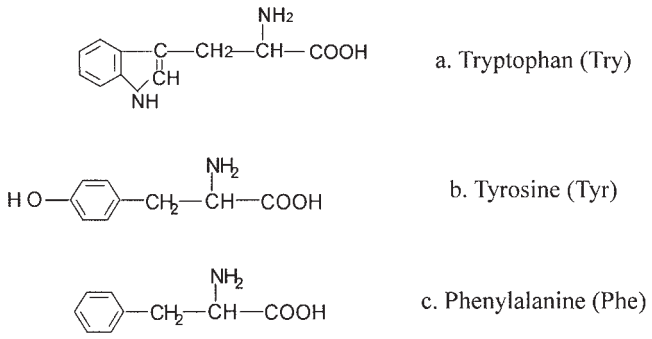

Figure 9 Molecular structure of template and competitive amino acid.

where α_1 and α_2 are the separation factors of Try-SMIPs and Non-SMIPs. β shows the difference between imprinted polymers and nonimprinted polymers. The larger the value of β, the higher the molecular recognition selectivity that resulted from imprinting.

Figure 9 shows the molecular structure of Try, Tyr, and Phe. It could be seen that these three kinds of amino acids were similar in structure, and they all contained the benzene ring (the only kind of amino acid containing benzene ring in 20 kinds of natural amino acid), and so the derivatization of them could not be detected by ultraviolet detector and could be detected easily by HPLC.

Molecular recognition selectivity of the resulting MS-SMIPs were listed in Table II

It could been seen clearly by Table II that the separation factor of Try-SMIPs ($\alpha = 2.03$) was much higher than that of Non-SMIPs ($\alpha = 0.76$), and indicated that the resulting MS-SMIPs had a higher molecular recognition selectivity to their templates. It could also be seen that relative separation factor of Try-SMIPs was high ($\beta = 2.75$), showing that "imprinting" did improve the recognition selectivity of polymer microspheres.

Effect of amount of FMs on molecular recognition selectivity

The amount of FMs has obvious effect on molecular recognition selectivity of MIPs, because it relates directly the concentration of complex formed by FMs with template molecules (TMs) in the imprinting system.[26] In this experiment, the effect of mol ratio of FM (MAA : AM = 2 : 1, mol ratio) to TM on molecular recognition selectivity was investigated and shown in Table III.

TABLE III
Molecular Recognition Specificity of Try-SMIPs Prepared Using Different Amount of Functional Monomers

β	FM : PM (mol ratio)
2.03	4 : 1
2.75	5 : 1
2.58	6 : 1
2.26	7 : 1
1.98	8 : 1

It could be seen that the difference of FM : TM resulted in the difference of molecular recognition selectivity of Try-SMIPs. When FM : TM was 5 : 1, molecular recognition selectivity was the highest; When FM : TM was 6 : 1, molecular recognition selectivity was also relatively high; but when FM : TM was lower than 5 : 1 or higher than 6 : 1, molecular recognition selectivity obviously decreased. These results indicated that the amount of FM did have obvious effect on molecular recognition selectivity. It was known in Figure 9 that Try had three functional groups, while MAA and AM were difunctional monomers, and so the theoretical mol ratio of FM : TM should be 3 : 1. When the amount of FM : TM was relative lower (4 : 1), the practical mol ratio of FM : TM was lower than 3 : 1, because the water solubility of FM resulted in the loss of FM. While FM : TM was too much (7 : 1 or 8 : 1), FM was still excess although part of FM was lost because of their water solubility, and resulted in the increase of random arrange of functional groups, and thus brought about the increase of nonspecific adsorption.

Imprinting and recognition mechanism of MS-SMIPs

The reason why MIPs can recognize their TMs was due to the existence of "imprinting cavities" with fixed size,

TABLE II
The Results of Molecular Recognition Specificity of Try-SMIPs

MS-SMIPs	Analyte	C_S (10^3 mmol/mL)	C_P (10^3 mmol/g)	K_D (mL/g)	a	β
Try-SMIPs	Try	1.21	6.45	5.33		
	Tyr	1.89	3.05	1.61	1.90	2.75
	Phe	2.02	2.40	1.19		
Non-SMIPs	Try	1.79	3.55	1.98		
	Tyr	1.91	2.95	1.54	0.69	2.75
	Phe	1.98	2.60	1.31		

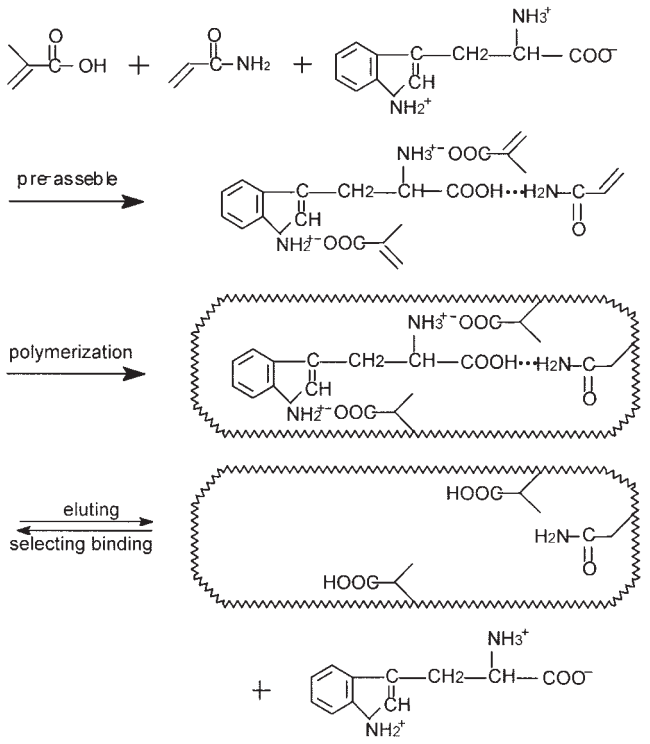

Figure 10 Schematic diagram of imprinting and recognition mechanism of Try.

shape, and arrangement of functional groups complemented to the templates in MS-SMIPs. TMs can bond with the functional groups therein when they enter the cavities. While control molecules can not form binding as strong as TMs, for their size cannot match the cavities or their functional group position do not correspond to functional groups in cavities, and thus cannot bring about specific binding same as TMs.

Figure 10 shows the schematic diagram of imprinting and recognition mechanism. It illustrated that the imprinting and recognition mechanism of MS-SMIPs to their templates distinctly. It could be seen obviously that the molecular imprinting process includes the mixing and the preassembling of the FMs with templates, the polymerization of the FMs with the polymeric matrix components, and the eluting process of templates from the surface of MS-SMIPs. While molecular recognition process was only one step, i.e., selecting binding. To Try-SMIPs, there were three functional groups in "imprinting cavities", and so they could interact with Try to form three-site interaction (binding site) when Try entered into the cavities, but could only interact with Tyr or Phe to form two binding sites when Tyr or Phe entered into the cavities. Obviously, the interaction of three binding sites was stronger than that of two binding sites. To Non-SMIPs, although there were also functional groups in them, there were not "imprinting cavities" in them, and the arrangement of functional groups was random, and so the interaction of functional groups with the three kinds of amino acid was almost the same. Therefore, Non-SMIPs did not have molecular recognition selectivity.

Regeneration recognition selectivity of MS-SMIPs

MS-SMIPs should be reused repeatedly through regeneration, because they were used mainly in separation area. For this reason, the regeneration recognition selectivity of the resulting MS-SMIPs was investigated, and the results were listed in Table IV.

Table IV indicated that SEAC (Q_∞) of MS-SMIPs in first regeneration decreased in a certain extent, and the decrease in percentage was 11.9% compared with the first use, while separation factor (α) had only a little change, the decrease percentage was 3.68%. In second regeneration, Q_∞ decreased a little again, the decrease percentage was 15.0% and 3.52%, relative to first use and first regeneration, respectively, and α only decreased by 1.64%. The reason for the adsorption capacity decrease was probably related to the loss of "imprinting cavities" during regeneration process, which needed to dip, wash, dry, etc. In summary, regeneration had relatively large effect on SEAC, and obviously had no effect on separation factor. The results also indicated that the resulting MS-SMIPs had certain regeneration recognition selectivity, and could be used repeatedly.

CONCLUSIONS

Tryptophan-imprinted Fe_3O_4/P(TRIM) composite microspheres with magnetic susceptibility (MS-SMIPs) were prepared by IESP, using Fe_3O_4 particles as magnetically susceptible component, MAA and AM as

TABLE IV
Regeneration Recognition Specificity of MS-SMIPs Prepared by IPE-IESP

Reuse time	Q_∞(mmol/g)/ change proportion	α/change proportion
First use	6.45	1.90
First regeneration	5.68/11.9%	1.83/3.68%
Second regeneration	5.48/15.0%/3.52%	1.80/5.26%/1.64%

FMs, TRIM as polymeric matrix components, and HEC as dispersant.

The resulting MS-SMIPs were regularly spherical in external morphology, and had a large quantity of spherical microvoids inside. The formation mechanism of spherical microvoids resulted from tiny "waterpolos" that was elaborated, namely, the spherical microvoids was the position occupied once by the tiny "waterpolos".

The results indicated that the amount of Fe_3O_4 affected the morphology and particle size distribution of MS-SMIPs obviously.

The resulting MS-SMIPs had a certain magnetic responsibility, and the saturation magnetization was 0.9558 emu/g.

It was shown that Try-SMIPs exhibited a good recognition selectivity for Try, and the relative separation factor (β) was 2.75; MS-SMIPs also exhibited higher regeneration recognition selectivity, and the separation factor (α) was 1.83 and 1.80 in first regeneration and second regeneration, respectively.

The amount of FMs obviously had effect on molecular recognition selectivity of MS-SMIPs, and the best mol ratio of FMs to TMs was 5–6 : 1.

The mechanism of molecular imprinting and molecular recognition of Try was described in detail. It was confirmed that the three-site interaction between Try and FMs was stronger than the two-site interaction between Tyr or Phe and FMs.

References

1. Wulff, G. Angew Chem Int Ed Engl 1995, 34, 1812.
2. Holliger, P.; Hoogenboom, H. R. Trends Biotechnol 1995, 13, 7.
3. Kempe, M.; Glad, M.; Mosbach, K. J Mol Recognit 1995, 8, 35.
4. Glad, M.; Reinholdsson, P.; Mosbach, K. React Polym 1995, 25, 47.
5. Joshi, V. P.; Karode, S. K.; Kulkarni, M. G.; Mashelkar, R. A. J Eng Appl Sci 1998, 53, 2271.
6. Wistuba, D.; Schurig, V. J Chromatogr A 2000, 875, 255.
7. Vlatakis, G.; Andersson, L. I.; Mueller, R.; Mosbach, K. Nature 1993, 361, 645.
8. Haupt, K.; Mosbach, K. Trends Biotechnol 1998, 16, 468.
9. Ye, L.; Mosbach, K. React Funct Polym 2001, 48, 149.
10. Ohkubo, K.; Urata, Y.; Honda, Y.; Yasuhiro, N.; Yoshinaga, K. Polymer 1994, 35, 5372.
11. Lele, B. S.; Kulkarni, M. G.; Mashelkar, R. A. React Funct Polym 1999, 39, 37.
12. Liang, Ch. D.; Peng, H.; Zhou, A. H.; Nie, L. H.; Yao, Sh. Zh. Anal Chim Acta 2000, 415, 135.
13. Haupt, K.; Mosbach, K. Chem Rev 2000, 100, 2495.
14. Brüggemann, O. Anal Chim Acta 2001, 435, 197.
15. Ye, L.; Ramstrom, O.; Mosbach, K. Anal Chem 1998, 70, 2789.
16. Ramstrom, O.; Ye, L.; Krook, M; Mosbach, K. Anal Commun 1998, 35, 9.
17. Shi, H.; Tsai, W.-B.; Ferrari, S.; Ratner, B. D. Nature 1999, 398, 593.
18. Hirayama, K.; Burow, M. Chem Lett 1998, 8, 731.
19. Randall, H.-F. S.; Harry, M. W.; Huval, C. C. Macromolecules 2001, 34, 1548.
20. Whitcombe, M. J.; Flores, A.; Cunliffe, D.; Vulfson, E. N. J Appl Polym Sci 2000, 77, 1841.
21. Lai, J. P.; Lu, X. Y.; Lu, Ch. Y.; Ju, H. F.; He, X. W. Anal Chim Acta 2001, 442, 105.
22. Kempe, M.; Mosbach, K. J Chromatogr A 1995, 691, 317.
23. Lu, Sh. L.; Cheng, G. X.; Pang, X. Sh. J Appl Polym Sci 2003, 89, 3790.
24. Sellergren, B.; Lepisteo, M.; Mosbach, K. React Polym 1989, 10, 306.
25. Zhang, L. Y.; Cheng, G. X.; Fu, C. Polym Int 2002, 51, 687
26. Sellergren, B.; Lepisteo, M.; Mosbach, K. J Am Chem Soc 1988, 110, 5853.