Magnetic Polymer Microspheres with Azidocarbonyl Groups: Synthesis, Characterization and Application in Protein Immobilization

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Received 29 February 2008; accepted 21 November 2008
DOI 10.1002/app.29755
Published online 17 February 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A novel magnetic polymer microsphere with amide groups and carboxyl groups was synthesized and reported here. The azidocarbonyl groups were derived from amide groups and linked to the proteins to investigate their immobilization capacity. The morphology, size, functional groups and magnetic properties of magnetic microspheres were characterized by optical microscopy, particle size analyzer, atom force microscopy, magnetic force microscopy, fourier transform infrared spectrometer, vibrating-sample magnetometer and thermal gravimetric analysis. The results indicated that the magnetic polymer microspheres had a well spherical shape with the size ranging from 1 to 10 μm, highly reactive functional groups, superparamagnetism and strong magnetic responsibility with saturation magnetization of 18.443 emu/g and Fe_3O_4 content around 21%. The immobilization capacity (η) was over 70%. The novel azidocarbonyl magnetic polymer microspheres showed potentials to be a good magnetic support and promising applications in bioseparation and biomedical fields. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2383–2390, 2009

Key words: magnetic microspheres; copolymerization; functionalization of polymers; azidocarbonyl group; protein immobilization

INTRODUCTION

In recent years, considerable attentions have been focused on magnetic polymer microspheres, composed of inorganic magnetic cores and polymeric shells, due to their unique structure and performance.[1] Compared with inorganic materials, polymer shells provide a variety of surface functional groups that can be tailored to specific proteins. Besides multitudinous characteristics of the conventional polymer microspheres, magnetic polymer microspheres can be rapidly and easily separated from the mixtures under a submagnetic field due to their magnetic property. Conventional separation and purification methods (i.e., centrifugation, precipitation, chromatography, electrophoresis, etc) not only usually involve laborious procedures and costly equipments, but also can result in malformation (i.e., decomposition, inactivation or deformation) of the biomolecules.[2] The attractive advantages of magnetic polymer microspheres include simplicity, rapidity, high purity and efficiency, high sensitivity and specificity, as well as low costs. In addition, magnetic polymer microspheres can be recovered and reused after separation process, which is ideal for large-scale operations and automated tests. Therefore, magnetic polymer microspheres have been widely applied in cell separation, enzyme immobilization, protein purification and drug targeting,[3–6] as well as bioaffinity chromatography, environment or food analysis, organic or biochemical synthesis and wastewater treatment.[7–10]

There are couples of ways to prepare magnetic polymer microspheres. Two-step swelling method reported by Ugelstad et al.[11,12] is commercially available. However, the procedure is complicated and the products are expensive. Monomer polymerization is a simple and most commonly used method, including suspension, emulsion, and dispersion polymerization.[13–15] Suspension and dispersion polymerization are usually used to prepare the micron-sized magnetic polymer microspheres. The disadvantage of suspension polymerization is that it usually generate polydisperse magnetic polymer microspheres with large size and broad size distribution.[16,17] Although dispersion polymerization can provide monodisperse magnetic polymer microspheres, it suffers from some drawbacks, including

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Contract grant sponsor: National Natural Science Foundation of China; contract grant numbers: 30772658, 30570494.

harsh reaction conditions, low magnetite content and unsuitability for massive production. The emulsion polymerization (especially emulsifier-free emulsion polymerization) is relatively simple and suitable for massive production of magnetic polymer microspheres with high magnetite content. However, the magnetic polymer microspheres obtained by this method are usually less than 1 μm in size.[18–20] With the increasing interest in the use of micron-sized magnetic polymer microspheres in biomedical engineering, development of micron-sized magnetic polymer microspheres with strong magnetic responsibility is desirable.

Currently, there are several commercial available magnetic beads (such as Dynal beads). But most of them are without functional groups crosslinked to proteins. Surface functional groups are usually introduced onto magnetic polymer microspheres by two ways, copolymerization of functional monomers and chemical modification of the performed polymer microspheres. The method of chemical modification is relatively tedious with a multistep procedure,[21] while copolymerization of functional monomers is relatively simple by a one-step reaction.[22] Furthermore, the magnetic polymer microspheres with different surface functional groups can be obtained from copolymerization of various functional monomers. At present, polymer microspheres with hydroxyl, aldehyde, carboxyl and amino groups are often used as supports for protein covalent immobilization.[23–26] However, these functional groups always need to be activated by functional agents (i.e., carbodiimide, glutaraldehyde, etc) before they can be covalently linked to proteins.[27,28] This method involves laborious operations, high costs and low binding capacity for proteins. Inman et al. reported that the azidocarbonyl group was highly reactive and could rapidly react on proteins under mild conditions without any crosslinking agent.[29]

In the current work, we prepared a novel micron-sized magnetic poly (styrene/acrylamide/acrylic acid) [P (St/AM/AA)] microspheres by the improved emulsifier-free emulsion polymerization with slight emulsifiers (less than its critical micelle concentration), in the presence of organic solvent, electrolyte and Fe_3O_4 magnetic nanoparticles, and the amide groups and carboxyl groups were incorporated into magnetic polymer microspheres. Then the azidocarbonyl groups were derived from amide groups by hydrazinolysis and azido-reaction. Subsequently, bovine serum albumin was selected as a model protein to be covalently immobilized onto the azidocarbonyl magnetic polymer microspheres to investigate their immobilization capacity. Finally, hepatitis B surface antigen (HBsAg) and goat anti-rabbit IgG were conjugated with the azidocarbonyl magnetic polymer microspheres and the commercial Estapor magnetic microspheres (as the control), respectively. Results revealed that the magnetic polymer microspheres had higher immobilization capacity than the commercial Estapor magnetic microspheres.

EXPERIMENTAL

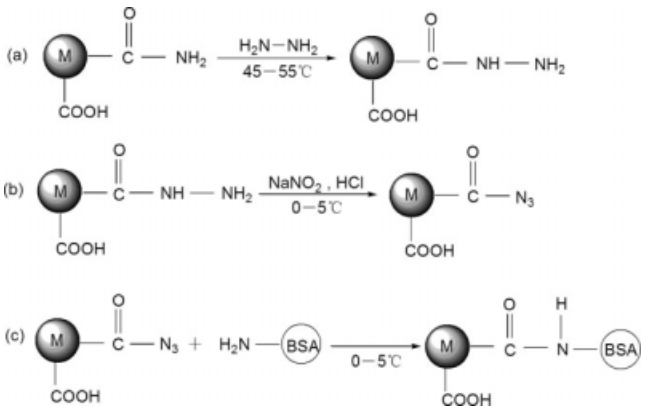
Materials

Styrene (St, Chinese Medical Chemicals Company Limited, Shanghai, China) was distilled under reduced pressure to remove the inhibitor. Acrylamide (AM, Amresco Fraction, America), acrylic acid (AA, Third Chemical Plant, Tianjin, China), potassium persulfate (KPS, Chemical Plant, Xi'an, China), polyethylene glycol (PEG, Mw = 4000, Tiantai Chemicals Company Limited, Tianjin, China) and sodium dodecylsulfate (SDS, Sanland Chemicals Company Limited, Xiamen, China) were of analytical grade and used without further purification. Fe_3O_4 magnetic nanoparticals (20 nm) were supplied by Wuhan Jiayuan Quantum Dots Company of China. Bovine serum albumin (BSA, fraction V, Code 10738328) was purchased from Roche in Germany. Estapor magnetic microspheres (Merck, France), hepatitis B surface antigen (HBsAg, 5.6 mg/mL) and goat anti-rabbit IgG (10 mg/mL) were provided by Zhengzhou Autobio of China. All the other chemicals used in this work were analytical reagents and were used as received. All water used was deionized water prepared by a Milli-Q system (Millipore, New Bedford, MA).

METHODS

Preparation of magnetic P (St/AM/AA) microspheres

The magnetic P (St/AM/AA) microspheres were synthesized using the improved emulsifier-free emulsion polymerization by a two-step process. In the first step, 0.2 g Fe_3O_4 magnetic nanoparticles were dispersed in 10 mL aqueous solution of 0.5 g PEG and 0.1 g KPS. The obtained mixture as the aqueous phase was sonicated for 30 min by an ultrasonic cleaner (KS-3200DE, Kunshan, Jiangsu, China), and then was kept at room temperature for 20 h. After that, the mixture of St (9 mL) and ethyl alcohol (5 mL) as the oil phase was added dropwise into the aqueous phase to disperse for 20 min. In the second step, a certain volume of water and ethyl alcohol (80 mL) was introduced into a three-necked flask and heated to 70°C. Then, under an atmosphere of N_2 and mechanical agitation, 1.5 g AM, 0.1 g KPS and 0.5 mL SDS solution (1%) were added into the dispersion medium. After 10 min, "the homogeneous mixture" obtained in the first step was added



Scheme 1 Procedure for formation of the azidocabonyl magnetic P (St/AM/AA) microsoheres and immobilization of BSA.

dropwise. Subsequently, another 0.1 g KPS and 0.2 g NaCl were added to the system. After 4 h, 0.5 mL AA was added. The reaction was maintained at 70°C for 6 h. The product was brown in color. To remove the surfactant and other impurities, the resulting magnetic polymer microspheres were washed with ethanol and deionized water for several times by a magnet. Afterwards, they were dried under vacuum at room temperature.

Hydrazinolysis of magnetic P (St/AM/AA) microspheres

The mixture of magnetic polymer microspheres suspension and hydrazine (5 : 1, v/v) was placed in a three-necked flask and was stirred for 7 h at 45–50°C. The reaction is illustrated in Scheme 1(a). Afterwards, the resulting magnetic polymer microspheres were washed with deionized water by a magnet until the pH was 7.0. The 5% suspension was obtained and stored at 4°C.

Preparation of azidocarbonyl magnetic P (St/AM/AA) microspheres

The 5% suspension obtained by hydrazinolysis above was put in a three-necked flask, and then the pH was adjusted to 1–2 with 1*M* HCl solution under stirring. Subsequently, 0.1*M* $NaNO_2$ solution was added into the suspension till the color of potassium iodine starch test paper changed. The reaction was carried out for 30 min at 0–5°C. [Scheme 1(b)] The product was directly used in the immobilization of protein.

Immobilization of protein and determination of immobilization capacity

BSA was adopted as a model protein to examine the immobilization capacity of the magnetic polymer microspheres. First, the product obtained by the azido-reaction above without further purification was placed into a three-necked flask and the pH of the suspension was adjusted to eight with 1*M* NaOH solution at 0–5°C under stirring. Then, a certain volume of 3.5*M* BSA buffer solution of pH 8.0 was slowly added into the suspension. The system was stirred for 7 h at 0–5°C. [Scheme 1(c)] Finally, the product was magnetically separated and the resultant magnetic polymer microspheres were washed with PBS solution of pH 7.2 for three times. All supernatants were collected and the concentration of BSA in the supernatants was determined by ultraviolet-visible spectrophotometer (ultrospec 2100pro, Amersham Biosciences, Sweden). The immobilization capacity (η) of the magnetic polymer microspheres was calculated as follows:

$$\eta = \frac{C_1V_1 - C_2V_2}{C_1V_1} \times 100\% \quad (1)$$

Or

$$\eta = \frac{C_1V_1 - C_2V_2}{M} (\mathrm{mg/g}) \quad (2)$$

Here, C_1 and V_1 are the concentration and volume of the total BSA added. C_2 and V_2 are the concentration and volume of the whole supernatants, respectively. M is the amount of the magnetic P (St/AM/AA) microspheres added in the hydrazinolysis reaction.

In addition, HBsAg and goat anti-rabbit IgG were immobilized onto the azidocarbonyl magnetic P (St/AM/AA) microspheres by the same method as BSA, and also they were conjugated with the commercial Estapor magnetic microspheres according to the method reported by Liu et al.[30] The immobilization capacities were examined via the same method as BSA.

Characterization of magnetic P (St/AM/AA) microspheres

Morphology and size distribution

The morphology of magnetic P (St/AM/AA) microspheres was observed with a transmission electron microscope (TEM, JEM-100SX, Japan) and an atomic force microscope (AFM, NTEGRA Prima, Russia). The structure of the magnetic polymer microspheres was evaluated from the magnetograms taken with a magnetic force microscope (MFM, NSG10/Co, Russia). The operation processes were as follows: The aqueous suspension of magnetic polymer microspheres was dropped onto a piece of copper screen. After drying at room temperature, TEM micrographs of magnetic polymer microspheres were taken. A 10 μL suspension of magnetic polymer microspheres

dispersed by dehydrated alcohol was dropped onto a piece of mica. After drying at room temperature, AFM micrographs and magnetograms of magnetic P (St/AM/AA) microspheres were taken. The size and size distribution of magnetic P (St/AM/AA) microspheres were measured by particle size analyzer (Mastersizer 2000, British).

Functional groups

The functional groups of magnetic P (St/AM/AA) microspheres were evaluated by a Fourier transform infrared spectrometer (FTIR, IRpresitge-21, Japan). In a typical procedure, about 0.25 mg dry magnetic P (St/AM/AA) microspheres were thoroughly mixed with IR-grade KBr (0.1 g) and pressed (10 ton) into tablet form, and then the spectrum was recorded. Similarly, the FTIR spectra of Fe_3O_4 magnetic nanoparticles and P (St/AM/AA) microspheres were also recorded.

The capacity of the surface carboxyl groups was estimated by acid-base titrimetric analysis. And the hydrazide group content was determined according to the method reported by Inman et al.[29]

Magnetic properties

The magnetization of magnetic P (St/AM/AA) microspheres was characterized from the hysteresis loop recorded by a vibrating sample magnetometer (VSM, Lakeshore 7307, America). A certain amount of magnetic P (St/AM/AA) microspheres was placed in the magnetometer, and the magnetic properties were measured by applying an increasing magnetic field over the sample at room temperature.

The content of Fe_3O_4 magnetic nanoparticles coated into magnetic P (St/AM/AA) microspheres was calculated by the TGA curves which were examined by a thermal gravimetric analysis (TGA/SDTA 851, Switzerland) under nitrogen atmosphere and the temperature ranging from 25 to 700°C with a heating rate of 10°C/min. Meanwhile, the thermal stability of magnetic P (St/AM/AA) microspheres was evaluated by its TGA curve.

RESULTS AND DISCUSSION

Synthesis of magnetic P (St/AM/AA) microspheres

Micron-sized magnetic P (St/AM/AA) microspheres with amide groups and carboxyl groups were synthesized by the improved emulsifier-free emulsion polymerization in the presence of organic solvent, electrolyte, and Fe_3O_4 magnetic nanoparticles. Primarily, we successfully prepared micron-sized (2 to 5 μm) nonmagnetic P (St/AM/AA) microspheres using a modified method of emulsifier-free emulsion polymerization by the introduction of organic solvent and electrolyte. However, experimental results revealed that it was difficult to obtain magnetic P (St/AM/AA) microspheres by this method. So far, the mechanism of emulsifier-free emulsion polymerization in the presence of Fe_3O_4 magnetic nanoparticles is still not clear. It is possible that the presence of Fe_3O_4 magnetic nanoparticles made the polymerization complicated.[18,19] On one hand, the stability of the system was weakened and the coagulum was easily generated, which was overcome by adding a slight emulsifier SDS (less than its CMC 10.0×10^{-3} *M*) into the polymerization system in this study. As a result, the micelles couldn't form and the composite magnetic particles could be stabilized. On the other hand, Fe_3O_4 magnetic nanoparticles in the polymerization system seemed to considerably decelerate the kinetics of the reaction because the magnetite was a strong inhibitor with adsorption of free radicals, so that, the initiator KPS content was increased by 1% of the total amount of monomers.

In the present work, AM was selected as a functional monomer because the amide groups could be easily converted into the highly reactive azidocarbonyl groups which can rapidly react on proteins under mild conditions without any crosslinking agent. AA was adopted not only because it made the stability of magnetic particles enhance and the amount of coagulum reduce, but also it improved the hydrophilicity and binding efficiency of magnetic polymer microspheres. Otherwise, it is vital that Fe_3O_4 magnetic nanoparticles are pretreated with poly (ethylene glycol) having suitable chain length.[31] First, the Fe_3O_4 magnetic nanoparticles wrapped with PEG can improve the affinity with initiators and monomers and enhance the adsorption of initiators and monomers. Second, the flexible PEG chains on the surface of "incipient latex particles" may hinder agglomeration of latex particles. Third, PEG as an amphipathic dispersion stabilizer could make Fe_3O_4 magnetic nanoparticles suspend in the Styrene for a longer time. The experimental results suggested that this method for preparation of magnetic polymer microspheres was feasible and compatible for massive production with simple procedures and low costs.

Characterization of magnetic P (St/AM/AA) microspheres

Morphology and size distribution

The TEM micrograph and AFM micrographs (top view, 3D graphics) of magnetic P (St/AM/AA) microspheres are shown in Figure 1(a–c). As it can be seen, the magnetic polymer microspheres are approximately spherical with a smooth surface.

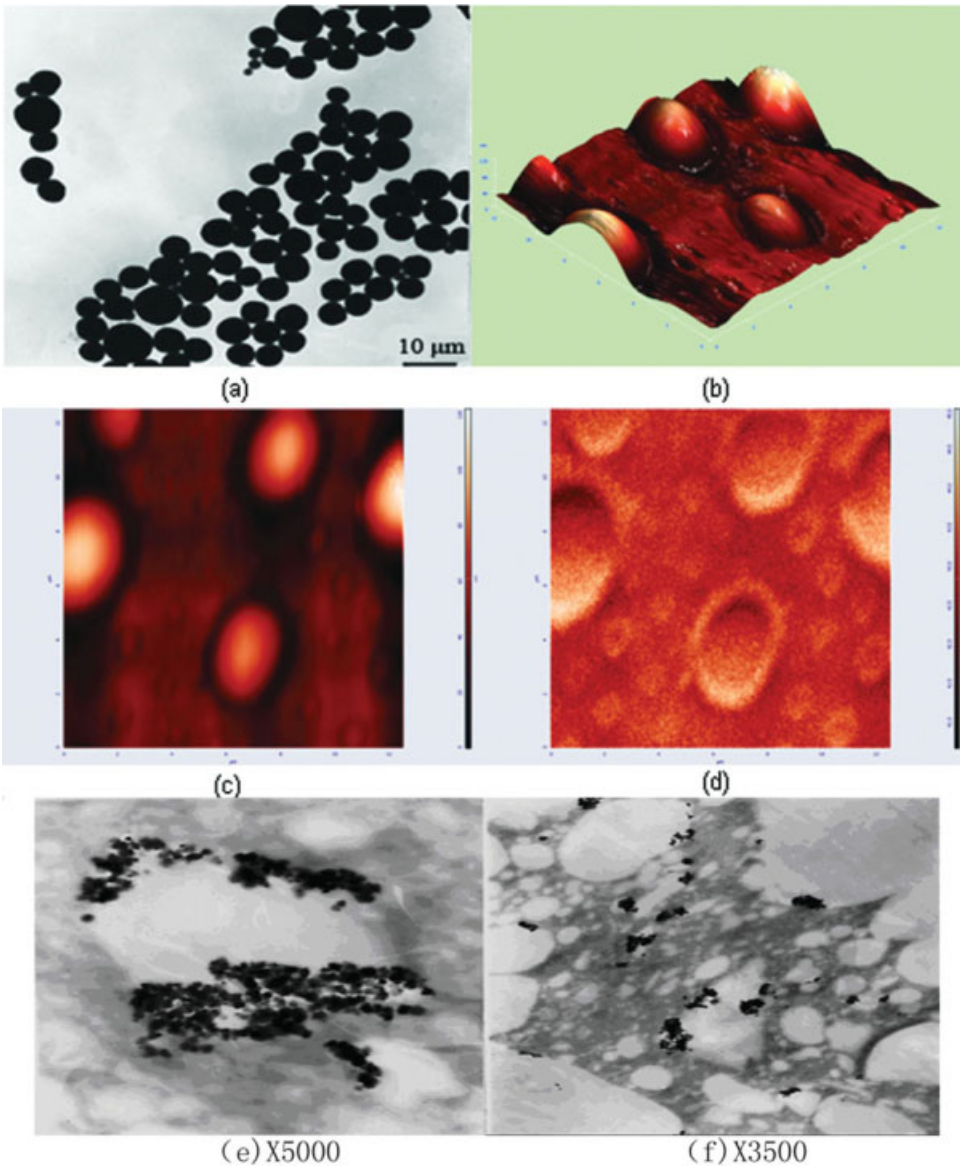


Figure 1 TEM micrograph (a), AFM micrograph (3D graphics) (b), AFM micrograph (top view) (c) and magnetogram (d) of the magnetic P (St/AM/AA) microspheres. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 1(d) shows the magnetogram of magnetic P (St/AM/AA) microspheres. According to the principle of MFM, it can be deduced that the external toroid structure is caused by the copolymer of St, AM and AA and the internal hollow structure is caused by Fe_3O_4 magnetic nanoparticles, which can prove that Fe_3O_4 magnetic nanoparticles locate in the interior of magnetic P (St/AM/AA) microspheres.

The size distribution was obtained from particle size analyzer. Figure 2 shows the size distribution of the resultant magnetic P (St/AM/AA) microspheres without any treatment. It can be seen that the magnetic polymer microspheres have a size ranging

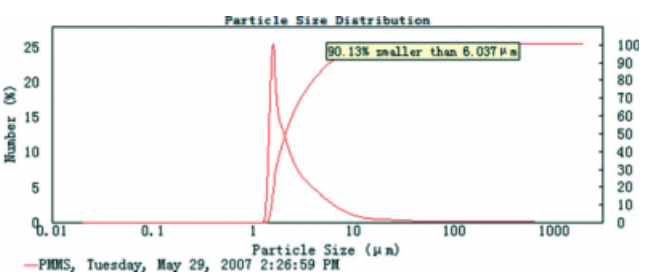


Figure 2 The curves of particle size distribution of the magnetic P (St/AM/AA) microspheres. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

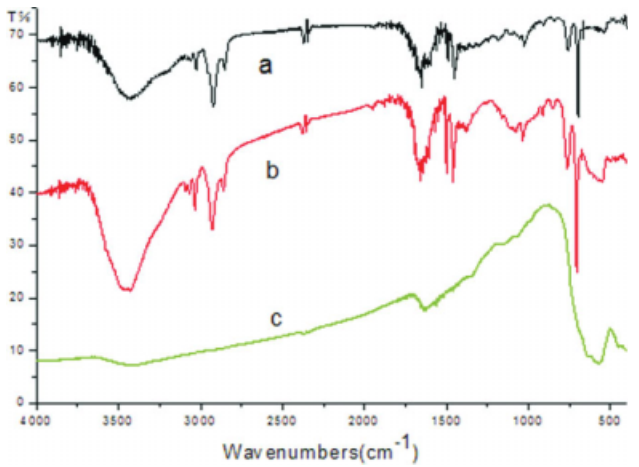


Figure 3 FTIR spectra of (a) P (St/AM/AA) microspheres, (b) magnetic P (St/AM/AA) microspheres and (c) Fe_3O_4 magnetic nanoparticles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

from 1 to 10 μm, 91% of which are smaller than 6 μm and 50% of which are less than 2 μm.

Functional groups

The FTIR spectra of P (St/AM/AA) microspheres, magnetic P (St/AM/AA) microspheres and Fe_3O_4 magnetic nanoparticles are shown in Figure 3. In Figure 3(c), the peak at 561 cm^{-1} is assigned to the stretching vibrations of Fe-O bond of Fe_3O_4 magnetic nanoparticles. In Figure 3(b), the peak at 540 cm^{-1} is also attributed to the Fe-O bond vibration of Fe_3O_4, while no peak of Fe-O bond vibration appears in Figure 3(a). So it can be proved that Fe_3O_4 magnetic nanoparticles exist in the magnetic P (St/AM/AA) microspheres. Compared with that in Figure 3(a), the FTIR spectrum in Figure 3(b) has a similar shape except the peak at 540 cm^{-1}, which confirms that the structure of magnetic P (St/AM/AA) microspheres is similar to the one of P (St/AM/AA) microspheres. All characteristic absorption peaks of benzene ring appear in their spectra. In addition, the wide peak at 3462 cm^{-1} is assigned to the overlapping stretching vibrations of O-H and *N*-H bonds. The absorbing peaks at 1652 and 1627 cm^{-1} are attributed to the vibration of C=O bond and *N*—H bond, respectively. Meanwhile, the absorbing peaks of C—N bond also appear at 1029 and 1068 cm^{-1}. These thoroughly confirm the existence of carboxyl groups and amide groups. In the spectrum of Fe_3O_4 magnetic nanoparticles, the wide peak at 3462 cm^{-1} is also attributed to the stretching vibrations of O—H bond, which is assigned to OH^- absorbed by Fe_3O_4 magnetic nanoparticles. In addition, according to the quantitive analysis, the capacity of the surface carboxyl groups is 182.5 μmol/g, and the hydrazide group content is 309.3 μmol/g.

Magnetic properties

Figure 4 shows the magnetization curves of magnetic P (St/AM/AA) microspheres and Fe_3O_4 magnetic nanoparticles. From Figure 4(a), we can see that the saturation magnetization (Ms) of magnetic P (St/AM/AA) microspheres is 18.443 emu/g, which is higher than those of other similar works reported,[2,21,27,28] this is due to the higher Fe_3O_4 content of the magnetic P (St/AM/AA) microspheres.[32,33] Otherwise, the coercivity (Hc) and remnant magnetization (Mr) are nearly zero. Therefore, it can be fully confirmed that the magnetic polymer microspheres have strong magnetic responsibility and excellent superparamagnetism, which is concerned with the properties of Fe_3O_4 magnetic nanoparticles. It can be clearly seen from Figure 4(b) that the saturation magnetization (Ms) of Fe_3O_4 magnetic nanoparticles is 50.391 emu/g, which is higher than Ms of the P (St/AM/AA) microspheres, the main reason is the increase in particle size and their large surface-to-volume ratio reported.[32] and the coercivity (Hc) and remnant magnetization (Mr) are nearly zero with the same well superparamagnetism.

From the TGA curves (Fig. 5), it can be seen that the shapes of curve (a) and curve (b) are similar. The conspicuous stage of mass loss at the temperature between 350 and 450°C is attributed to thermolysis of the P (St/AM/AA) copolymer. The mass loss of P (St/AM/AA) microspheres is nearly 100% at 599°C, while the mass loss of magnetic P (St/AM/AA) microspheres is only 79% at 599°C. The two curves are steady after 600°C. In addition, from curve (c), we can see that the mass loss of Fe_3O_4

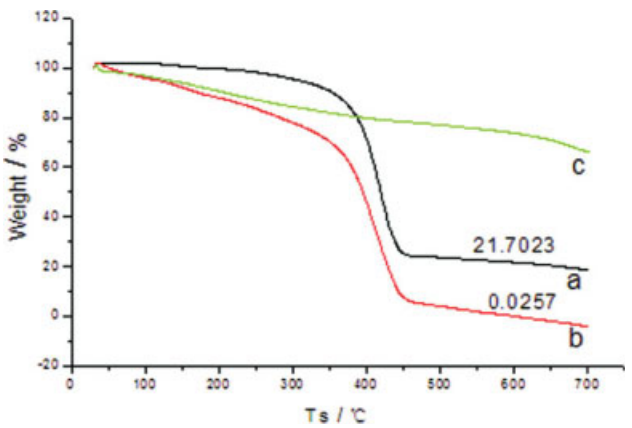


Figure 4 Magnetization curves of (a) magnetic P (St/AM/AA) microspheres and (b) Fe_3O_4 magnetic nanoparticles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

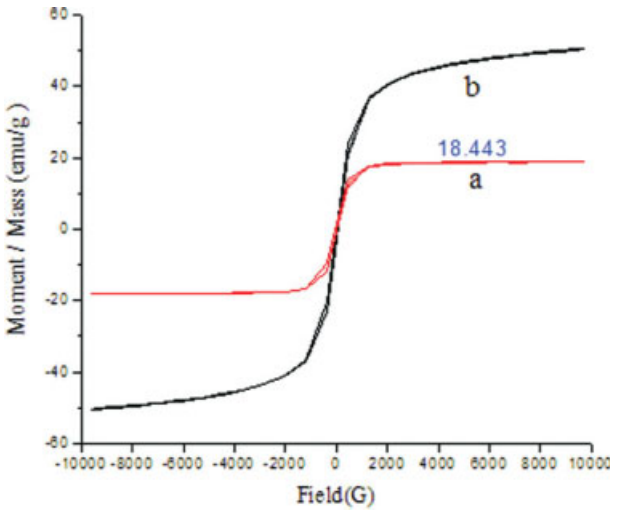


Figure 5 TGA curves of (a) magnetic P (St/AM/AA) microspheres, (b) P (St/AM/AA) microspheres and (c) Fe_3O_4 magnetic nanoparticles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

magnetic nanoparticles is very slight. In theory, it is impossible for Fe_3O_4 to decompose at the temperature ranging from room temperature to 700°C, so the loss in weight is attributable to the moisture absorbed by Fe_3O_4 magnetic nanoparticles and the other impurity. To sum up, it can be estimated that the content of Fe_3O_4 in magnetic P (St/AM/AA) microspheres is around 21%. This method to determine the magnetite content is feasible according to Yanase et al.[18] Moreover, the magnetic P (St/AM/AA) microspheres start degrading at about 350°C with a good thermal stability.

Modification of magnetic P (St/AM/AA) microspheres and immobilization of protein

Protein immobilization on various supports has been extensively applied in many areas, such as solid phase diagnostics, biosensors, extracorporeal therapy, bioseparation and so forth. Currently, the methods of protein immobilization include physical adsorption and covalent immobilization. Compared with physical adsorption, covalent immobilization can not only eliminate or significantly reduce leakage of proteins through increased bond strength, but also increase stability and control of availability of protein binding site. However, this method requires the supports with surface functional groups suitable for coupling. At present, only limited work has been reported for preparation of magnetic polymer microspheres with surface functional groups. Magnetic polymer microspheres with amino groups are commonly studied, as the amino group is stable and easy to be activated.[27,28,34] But they are usually covalently linked to proteins by functional agents. This method suffers from some drawbacks. On one hand, the method involves laborious operations, high costs, low specificity and binding capacity for proteins. On the other hand, it is difficult to obtain the polymer microspheres with amino groups directly by means of monomer polymerization. Liu et al.[30] reported a method to prepare the polyaminostyrene latex by nitration and reduction, and the amino groups were converted into the diazo groups by the diazo-reaction. The diazotized polystyrene latex could be covalently coupled to phenol and imidazole groups of antibodies without any crosslinking agent. This method for protein immobilization has the superiorities of rapidness, sensitivity, very high specificity and relatively high binding capacity (20–40%). However, the process for preparation of polyaminostyrene latex is complex and uneasy to control. Especially, the nitration is very easy to destroy the morphology of microspheres. Comparatively, the polymer microspheres with amide groups can be obtained more easily.[20]

In the present work, we prepared the magnetic polymer microspheres with amide groups by the incorporation of the monomer AM in the polymerization process. Afterwards, the amide groups were converted into the azidocabonyl groups by the two-step simple reaction, and BSA was covalently combined to the azidocabonyl magnetic polymer microspheres by peptide bonds at low temperature. As shown in Table I, with the increase of total amount of BSA, only negligible differences are observed for the amount of BSA immobilized and the maximum is 27 mg per gram of magnetic microspheres, while

TABLE I
Amount of BSA Immobilized Onto Magnetic P (St/AM/AA) Microspheres

Amount of microspheres (mg)	Total amount of BSA (mg)	Amount of BSA immobilized (mg/g)	Immobilization capacity (%)
100	3.5	26	74
100	7.0	27	38
100	18	24	13
100	20	24	12

TABLE II
Amount of HBsAg and Goat Anti-rabbit IgG Immobilized by 25 mg Magnetic P (St/AM/AA) Microspheres

Antigen/antibody	Total amount (mg)	Amount of immobilized (mg/g)	Immobilization capacity (%)
HBsAg	0.84	26	77
Goat anti-rabbit IgG	0.75	27	90

TABLE III
The Control of Immobilization Capacity of Magnetic P (St/AM/AA) Microspheres and Estapor Magnetic Microspheres (Goat Anti-rabbit IgG and HBsAg as Model Proteins)

	Immobilization capacity (%)	
The control sample	Goat anti-rabbit IgG	HBsAg
P (St/AAM/AA) magnetic microspheres	90%	77%
Estapor magnetic microspheres	50%	47%

the immobilization capacity (η) is remarkably decreased and the maximum is 74% as the total amount of BSA is 3.5 mg. As the initial application, hepatitis B surface antigen (HBsAg) and goat anti-rabbit IgG were conjugated with the azidocarbonyl magnetic polymer microspheres, respectively. From Table II we can see that the amount of HBsAg and goat anti-rabbit IgG immobilized is 26 and 27 mg per gram of magnetic microspheres respectively. This result implies that the method for protein immobilization is favorable to antibody and antigen. In Table III, the results show that the immobilization capacity of the magnetic P (St/AM/AA) microspheres is higher than that of the commercial Estapor Magnetic Microspheres. All of these data demonstrate that the azidocabonyl group is highly reactive as reported by Inman et al.[29] and this method for protein immobilization has fairly high immobilization capacity. The further application development is in progress.

CONCLUSIONS

A novel magnetic P (St/AM/AA) microsphere with azidocarbonyl groups was prepared by the improved emulsifier-free emulsion polymerization and two-step simple reaction. Protein could be quickly covalently immobilized to the azidocarbonyl magnetic polymer microspheres without any cross-linking agent. The magnetic P (St/AM/AA) microspheres had a well spherical shape, relatively narrow size distribution with the size ranging from 1 to 10 μm, strong magnetic responsibility and excellent superparamagnetism, highly reactive surface functional groups as well as high immobilization capacity. All of these results indicated that this kind of magnetic polymer microsphere could be used as a good support in biosperation and biomedical fields.

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