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Rapid Synthesis of Uniform Magnetic Microspheres by Combing Premix Membrane Emulsification and in Situ Formation Techniques

Jian Yang,^{†,‡} Dong-Xia Hao,[†] Cai-Xia Bi,^{†,‡} Zhi-Guo Su,[†] Lian-Yan Wang,^{*,†} and Guang-Hui Ma^{*,†}

National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China, and Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Uniform magnetic poly(styrene-co-2-hydroxyethyl methacrylate) (PST-HEMA) microspheres were fabricated by a novel route combining premix membrane emulsification of double emulsion and an in situ magnetization technique. The fabrication involved, first, the preparation of uniform double W/O/W emulsions with ferrous ferric solution as the internal water phase and dichloromethane dissolved with PST-HEMA as the oil phase by premix SPG membrane emulsification. Then, the inner ferrous ferric solution was in situ magnetized into nanoparticles by adding the ammonia solution into the external water phase. This W/O/S emulsion was finally solidified into microspheres by solvent evaporation. Results showed that the in situ magnetization technique could effectively overcome the encapsulation difficulties in traditional synthesis routes due to poorer affinity between iron oxide nanoparticles and polymer, and facilitated the enhancement of encapsulation efficiency with a high magnetization up to 14.23 emu/g.

Introduction

Magnetic polymer microspheres have attracted extensive attention in the past decades.^{1–5} The magnetic properties provide such microspheres with rapid and low-cost separation in external magnetic field. It lead to various utilizations of magnetic polymer microspheres in the fields of cell isolation, enzyme immobilization, immunoassay, protein separation, biochemical assays and drug targeting, etc.^{6–10}

Different methods to prepare the magnetic microspheres with an inorganic magnetic core and polymer shell have been developed, which can be summarized as following three routine routes: (1) Magnetic nanoparticles dispersed in a monomer were entrapped into polymer microspheres during polymerization, including emulsion polymerization, dispersion polymerization, suspension polymerization, seed polymerization, and miniemulsion polymerization.^{2,11–13} (2) Magnetic nanoparticles were adsorbed into polymer microspheres by adjusting the electric charge.^{14–16} (3) Ferrous ferric solution was adsorbed into the polymer microspheres, and then magnetic nanoparticles were formed by increasing the pH value inside these microspheres.^{1,17} The former two routes involved separated steps, i.e., first preparation of the iron oxide nanoparticles, and then encapsulation or adsorption of the iron oxide nanoparticles into the matrix of polymer microspheres. Because of the poor affinity between inorganic nanoparticles and the polymer, both of these methods have difficulties to enhance the encapsulation or adsorption of inorganic magnetic nanoparticles and resulted in low encapsulation and weak magnetic properties.¹⁸ The third route could avoid the above affinity problem by adsorption of ferrous ferric solution into the polymer microspheres, and then oxidation of them into magnetic nanoparticles inside the microspheres. However, the poor absorption of inorganic ferrous ferric solution into the microsphere also makes the third route difficult to improve the magnetic properties of microspheres.¹⁷ Inspired by

high entrapment efficiency in the W/O/W double emulsion and solvent evaporation method,^{19,20} we designed a novel route to prepare uniform magnetic microspheres, i.e., first, the preparation of coarse double emulsions with ferrous ferric solution as the internal water phase and screening it into a uniform emulsion by premix membrane emulsification, then adding the ammonia solution into the external water phase to in situ magnetization of the ferrous ferric solution into nanoparticles, and finally solidifying the emulsion into microspheres.

Compared to traditional routes involved with the direct encapsulating of nanoparticles, this novel route adopted an indirect way by, first, encapsulating the ferrous ferric solution in double emulsion and, then, transforming it into iron oxide nanoparticles by diffusion of the hydroxyl ion from the external water phase. Because of the high entrapment efficiency of double emulsion and fast diffusion of alkali into inner ferrous ferric solution, the poorer affinity between iron oxide nanoparticles and the polymer matrix of microspheres could be effectively overcome. Thus, the fabrication of magnetic microspheres could be sped up and the encapsulation of magnetic nanoparticles could be enhanced effectively.

2. Materials and Methods

2.1. Chemicals. Arlacel 83 (sorbitan sesquioleate, Sigma, U.S.A.), PO 310 (tetraglycerol pentaoleate), PO 500 (hexaglycerin penta ester), MO 310 (tetraglycerol monooleate, Sakamoto Yakuhin Kogyo, Japan), Span 80 (sorbitan monooleate), and Span85 (sorbitantrioleat, Beijing Chemical Reagents Company, China) of biochemical grade were selected as oil emulsifiers. Tween 20 (polyethylene glycol sorbitan monolaurate, Sigma, U.S.A.) was used as a surfactant in the external water phase. Poly(vinyl alcohol) (PVA-217, average degree of polymerization 1700, degree of hydrolysis 88.5%) was provided by Kuraray (Tokyo, Japan) and was used as a stabilizer in the external water phase. FeCl₃·6H₂O, FeCl₂·4H₂O, ammonia solution (25%), TEA (triethylamine), and DCM (dichloromethane) of analytical grade were purchased from Beijing Chemical Reagents Company (China). All other reagents were of reagent grade and used as received. Poly(styrene-co-2-hydroxyethyl methacrylate) (PS-HEMA, 95:5, $M_w = 1.69 \times$

* To whom correspondence should be addressed. Telephone: 86-10-82627072 (L.-Y.W.), 86-10-62557095 (G.-H.M.). Fax: 86-10-82627072. E-mail: ghma@home.ipe.ac.cn (G.-H.M.), wanglianyan@home.ipe.ac.cn (L.-Y.W.).

[†] Chinese Academy of Sciences.

[‡] Graduate University of Chinese Academy of Sciences.

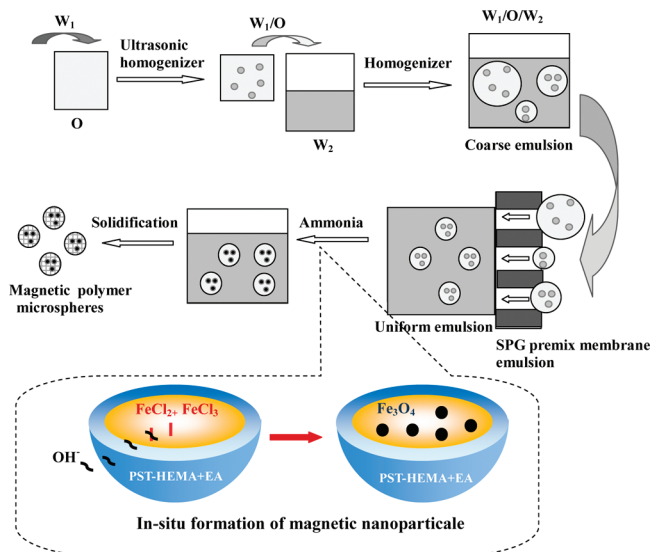


Figure 1. Schematic illustration of the preparation of magnetic polymer microspheres.

10^5 kDa), used as a wall polymer, was synthesized using soap-free emulsion polymerization in our lab. Styrene (St, reagent grade) was distilled under a vacuum and stored in a refrigerator until use. Hydroxyethylmethacrylate (HEMA, reagent grade) was used without further treatment. Potassium persulfate (reagent grade) was used as an initiator.

2.2. Preparation of Magnetic PS-HEMA Microspheres.

The schematic illustration for the preparation of magnetic polymer microspheres is shown in Figure 1. The ferrous chloride and ferric chloride dissolved into deionized water were used as the internal water phase (W_1). PS-HEMA dissolved into DCM containing an emulsifier was used as the oil phase. PVA-217 dissolved into deionized water was used as the external water phase (W_2). At first, W_1 was dispersed into O using an ultrasonic homogenizer to form the primary emulsion of W_1/O . Then, this primary emulsion was dispersed into W_2 to form a double emulsion of $W_1/O/W_2$. After that, the alkali solution was added into W_2 , and the solution diffused from W_2 through O into W_1 . The double emulsion of $W_1/O/W_2$ was changed to $S/O/W_2$ due to the formation of magnetic nanoparticles by a coprecipitation reaction of ferrous and ferric solutions with alkali solution in the internal water phase. Finally, the $S/O/W_2$ was solidified into polymer microspheres containing magnetic nanoparticles by evaporation of DCM. The standard recipe for preparation of magnetic polymer microspheres is shown in Table 1. The preparation conditions were the same with this standard recipe unless specially described.

2.2.1. Preparation of Primary Emulsion (W_1/O). A certain amount of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved into 2.0 mL of deionized water as the internal aqueous phase (W_1). A certain amount of PS-HEMA was dissolved into 50 mL of dichloromethane (DCM) containing an emulsifier as the oil phase (O). Then, W_1 was mixed and emulsified into O by an ultrasonic homogenizer (35 W, 1 min) to form a W_1/O primary emulsion. The stability of the primary emulsion has an important influence on encapsulation and magnetization of the polymer microspheres; a more stable primary emulsion will lead to a higher encapsulation efficiency and magnetization. In order to obtain a stable primary emulsion, five kinds of emulsifiers in the oil phase were used to prepare the primary emulsion.

2.2.2. Preparation of Uniform Double Emulsion ($W_1/O/W_2$). The primary emulsion (W_1/O) was immediately poured into 350 mL of the external aqueous phase (W_2) containing 3.5 g

Table 1. Standard Recipe for the Preparation of Magnetic Polymer Microspheres

| phase/ingredient | amount (g) |
|---|-------------------|
| (W ₁) Internal Water Phase | |
| $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ | 0.65 (1.20 mol/L) |
| $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ | 0.24 (0.60 mol/L) |
| water | 2.0 |
| (O) Oil Phase | |
| PS-HEMA | 0.40 |
| emulsifier (Span 85, Span80, etc.) | 0.20 |
| DCM | 50 (mL) |
| (W ₂) External Water Phase | |
| PVA 217 | 3.5 |
| Na_2SO_4 | 3.5 |
| Tween 20 | 0.7 |
| water | 350 |
| alkali (ammonia) | 30 (mL) |

of PVA, 3.5 g of Na_2SO_4 , and 0.7 g of Tween 20, which was then re-emulsified into a coarse $W_1/O/W_2$ emulsion by a homogenizer. This coarse emulsion was then quickly pressed through the SPG membrane and dispersed into the uniform emulsion by the premix membrane emulsification method.

2.2.3. Formation of Magnetite Nanoparticles. The above double emulsion was transferred into a three-neck glass flask under an agitation rate of 150 rpm by a four-moon shaped impeller, and then an ammonia solution (25%, 30 mL) was added into the double emulsion slowly (5 mL/min) under a nitrogen atmosphere. Ammonia diffused from the external water phase through the oil phase into the internal aqueous phase, and subsequently magnetic nanoparticles were formed by a coprecipitation reaction of FeCl_2 and FeCl_3 with ammonia in the internal water phase. The reaction was kept at room temperature for 2 h under a nitrogen atmosphere. As a result, the $W_1/O/W_2$ emulsions were changed to an $S/O/W_2$ emulsion.

2.2.4. Formation of Magnetic Polymer Microspheres. The organic solvent of above $S/O/W_2$ emulsion was evaporated at room temperature for 24 h under gentle stirring at a rate of 180 rpm. After the solvent was evaporated, the solid magnetic polymer microspheres were obtained. Finally, the magnetic polymer microspheres were separated with the external magnetic field and washed three times with hydrochloric acid, deionized water, and ethanol.

2.3. Measurement of Primary Emulsion Stability. A total of 0.5 mL of ferric solution was added into 12.5 mL of the oil phase containing PS-HEMA and an emulsifier, and then these two solutions were mixed and emulsified by an ultrasonic homogenizer at 35 W for 1 min to form a W_1/O primary emulsion. Then, the primary emulsion was poured into a test tube and kept still to observe whether the phase separation occurred or not. The stability was expressed by the elapsed time until the phase separation was observed. A longer elapsed time means a more stable primary emulsion.

2.4. Characterization of Magnetic PS-HEMA Microspheres.

2.4.1. Magnetic Properties of Microspheres. The magnetization of magnetic polymer microspheres was measured by vibrating sample magnetometer (VSM; model-155, Digital measurement System, Inc.). The maximum applied field was 8 kOe, and the measurement temperature was kept at 290 K.

2.4.2. Morphology and Microstructure of Microspheres. The surface morphology of magnetic polymer microspheres was observed by a scanning electron microscope (SEM; JSM-6700F, JEOL, Japan). The specimens for SEM observation were prepared by mounting the sample on metal stubs with double-sided conductive adhesive tape and coating it with a thin gold

film (approximately 60 nm in thickness) under a reduced pressure below 5 Pa with a JFC-1600 fine coater (JEOL, Japan). Then, this sample was put into the SEM to observe the morphology of the microspheres.

A transmission electron microscope (TEM; H-600, Hitachi, Japan) was used to observe the microstructure of microspheres and the distribution of magnetic nanoparticles in microspheres. The specimens for TEM observation were prepared by dropping the alcohol-diluted sample onto an ultrathin carbon film supported by a copper mesh, and then the copper mesh grid was dried with light. Then, the sample was put into a sample cell to observe the microstructure of the microspheres and distribution of magnetic nanoparticles in the microspheres.

The volume-average diameter and the size distribution of the magnetic polymer microspheres were measured by a dynamic light scattering analysis (DLS; Malvern 2000E apparatus, U.S.A.). The term "SPAN" was used to present the width of the particle size distribution and is expressed as

$$\text{SPAN} = \frac{D90 - D10}{D50}$$

The values D10, D50, and D90 are the diameters at which 10%, 50%, and 90% of the microspheres, respectively, have a smaller diameter than the values D10, D50, and D90.

2.4.3. Fe Content of Microspheres. A certain weight of magnetic polymer microspheres (W_0) was put into a ceramic crucible, and then it was incinerated in a Muffle furnace at a temperature of 800 °C for 6 h. At this temperature, the wall material of the microspheres as PS-HEMA was burnt into carbon dioxide and steam, and the magnetic nanoparticles of Fe_3O_4 became Fe_2O_3 . The weight of the residual substance (Fe_2O_3) was measured and labeled as W_1 . The molecular weights of Fe and Fe_2O_3 were 56 and 160 g/mol, respectively. The Fe content of magnetic polymer microspheres was calculated as follows:

$$\text{Fe content (\%)} = \frac{W_1 \times 56 \times 2}{W_0 \times 160} \times 100$$

2.4.4. Encapsulation Efficiency. The Fe encapsulation efficiency was calculated from the following formula:

$$\text{encapsulation efficiency (\%)} = \frac{\text{total amount of Fe in microspheres}}{\text{total amount of Fe added}} \times 100$$

3. Results

3.1. Effect of Oil Emulsifier Type on the Stability of the Primary Emulsion. The stability of the primary emulsion is critical for improving the encapsulation efficiency of microspheres prepared from a system of double emulsion. Because double emulsion is a distinct thermodynamic, unstable system, the inner water phase is easily separated from the primary emulsion of W_1/O , which will lead to difficult or less encapsulation of W_1 into microspheres. In addition, because DCM was utilized as the oil phase in this study, the higher density of DCM than that of the internal water phase will increase layering between the DCM phase and internal water phase. Fortunately, this phase separation could be retarded by an emulsifier in the oil phase, which could provide effective protection for separated water droplets and prevent their aggregation. Therefore, the choice of a suitable emulsifier in the oil phase for this novel route was especially important to enhance the stability of the primary emulsion and encapsulation capacity.

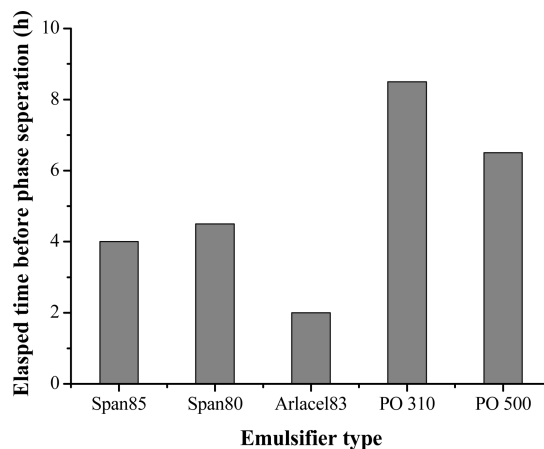


Figure 2. Effect of the type of oil-soluble emulsifier on the stability of primary emulsion.

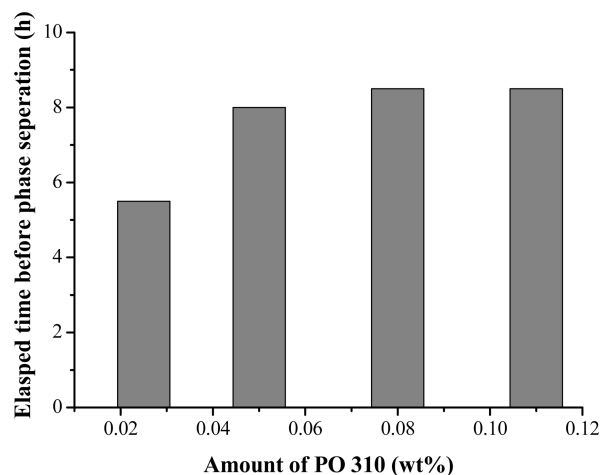


Figure 3. Effect of the amount of PO 310 on the stability of primary emulsion.

The effects of various emulsifiers including Span 85, Span 80, Arlcel 83, PO 310, and PO 500 on the stability of the primary emulsion were investigated in preparation of the primary emulsion (W_1/O), with a fixed concentration of 0.25 wt % of DCM. It showed in Figure 2 that the elapsed time before phase separation of the primary emulsion prepared by PO 310 and PO 500 was longer than others, which meant that the primary emulsion prepared by both of them was more stable than the others. It can be speculated that the emulsifiers of PO 310 and PO 500 had a better ability to emulsify because these two emulsifiers exhibited better affinity with the water phase than the others, which was related with the chemical structures of emulsifiers. The emulsifiers PO 310 and PO 500 are a kind of glyceryl ester, while others are sorbitan ester, and glyceryl has better affinity with the water phase than sorbitan because of a smaller steric effect. Further, because the stability of the primary W/O emulsion resolved with PO 310 in the oil phase is a little better than that with PO 500, PO 310 was chosen as the emulsifier in the oil phase during preparation of the primary emulsion of W_1/O in this route. Further, the effects of the PO 310 amount on the stability of the primary emulsion were investigated, as seen in Figure 3. It showed that, when the amount of PO 310 reached above 0.05 wt %, the primary emulsion could keep enough stability. So in our future experiments, the concentration of PO 310 in the oil phase was fixed at 0.05 wt %.

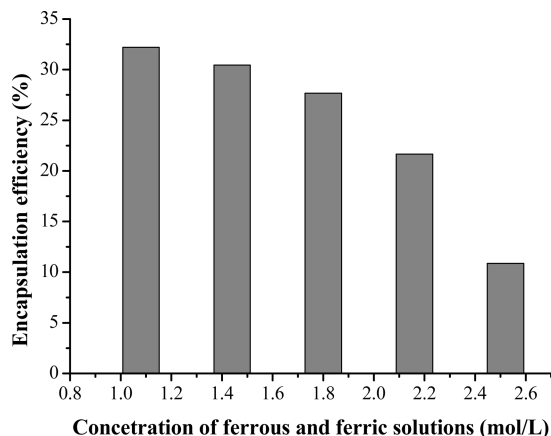


Figure 4. Effect of the total concentration of ferrous and ferric solutions on the encapsulation efficiency of magnetic polymer microspheres.

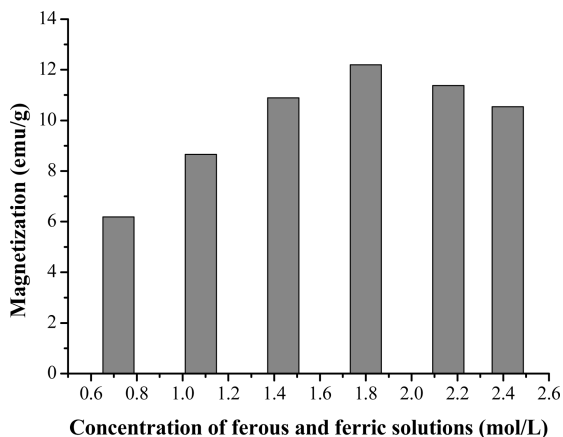


Figure 5. Effect of the total concentration of ferrous and ferric solutions on magnetization of magnetic polymer microspheres.

3.2. Effect of Total Concentration of Ferrous and Ferric Solutions on Encapsulation Efficiency and Magnetization of Microspheres. Since the ferrous and ferric concentration in the internal water phase would cause a difference in osmotic pressure between the internal and external water phases, the total concentration of ferrous and ferric solutions in the internal water phase would determine the encapsulation efficiency and magnetization of polymer microspheres. In this study, the different concentrations of ferrous and ferric solutions from 1.08 to 2.40 mol/L were investigated with a fixed Fe(II)/Fe(III) molar ratio of 1:2 because the stoichiometric ratio of Fe(II)/Fe(III) in the chemical equation is 1:2. Their effects on the encapsulation and magnetization of magnetic polymer microspheres are shown in Figures 4 and 5. Figure 4 showed that the encapsulation efficiency declined with an increase in the total concentration of ferrous and ferric solutions. It can be speculated that the increase in the total concentration of ferrous and ferric solutions lead to an increase in the osmotic pressure between the internal and external water phases and thus cause the decline of the encapsulation efficiency of the microspheres. A higher osmotic pressure would lead to more diffusion of the external water phase into the internal water phase, and this would further result in gradually thinning until breakage of the oil film, which finally decreases the encapsulation efficiency of magnetic polymer microspheres.

Comparatively, Figure 5 showed that the magnetization first increases and then tends to decline with an increase in the total concentration of ferrous and ferric solutions before and after it reaches 1.8 mol/L. This up and down tendency of magnetization

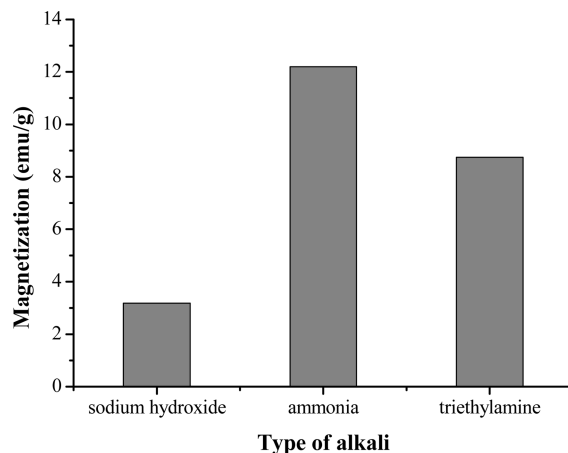


Figure 6. Effect of alkali type on the magnetization of magnetic polymer microspheres.

might come from two controlling factors, i.e., the dynamic reaction extent for the generation of Fe_3O_4 nanoparticles and the encapsulation efficiency of the microspheres. When the total concentration of ferrous and ferric solutions was less than 1.8 mol/L, the magnetization of microspheres was mainly affected by the reaction extent for generation of the Fe_3O_4 nanoparticles. The yield of Fe_3O_4 nanoparticles increased with an increase in the total concentration of ferrous and ferric solutions, which resulted in an increase in magnetization.

Because the higher concentration of ferrous and ferric solutions in the internal water phase would lead to a higher osmotic pressure, it would drive more alkali diffusely into the internal water phase from the external water phase to react with ferrous and ferric solutions, which finally results in a higher yield of Fe_3O_4 nanoparticles. However, when the concentration of ferrous and ferric solutions continues to increase above 1.8 mol/L, the encapsulation efficiency become the controlling factor to determine the magnetization. The rapid decrease of encapsulation efficiency of microspheres led to the decrease of magnetization.

3.3. Effect of the Type of Alkali on the Magnetization and Surface Morphology of Microspheres. Because double $\text{W}_1/\text{O}/\text{W}_2$ emulsion was utilized in this novel route, the magnetic nanoparticles of Fe_3O_4 should be synthesized by adding alkali in the external water phase and the diffusion of alkali through the oil phase into the internal aqueous phase to a coprecipitation reaction with FeCl_2 and FeCl_3 . Different alkali exhibited different abilities of diffusion and reaction extents with ferrous and ferric solutions, and thus led to different magnetizations and morphologies of magnetic polymer microspheres.

Three kinds of alkali solution were investigated to prepare magnetic polymer microspheres, including inorganic alkali of sodium hydroxide and organic alkali of ammonia and triethylamine. The effects of alkali type on the magnetization and morphology of magnetic polymer microspheres are shown in Figures 6 and 7, respectively. Figure 6 showed that ammonia solution can obtain the maximum magnetization, but sodium hydroxide solution caused the minimum one. It can be explained that organic alkali of ammonia and triethylamine could be dissolved both in the water and in the oil phase of dichloromethane, and thus more easily diffused from the external water phase through the oil phase into the internal water phase to synthesis Fe_3O_4 nanoparticles. Therefore, the magnetization of microspheres by ammonia and triethylamine showed much improvement over that by sodium hydroxide. However, due to poorer compatibility of sodium hydroxide with the oil phase,

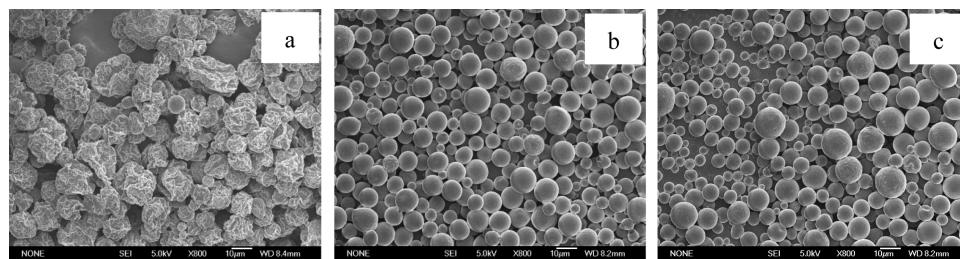


Figure 7. SEM microphotographs of magnetic polymer microspheres prepared by different alkali (a, sodium hydroxide; b, ammonia; c, triethylamine).

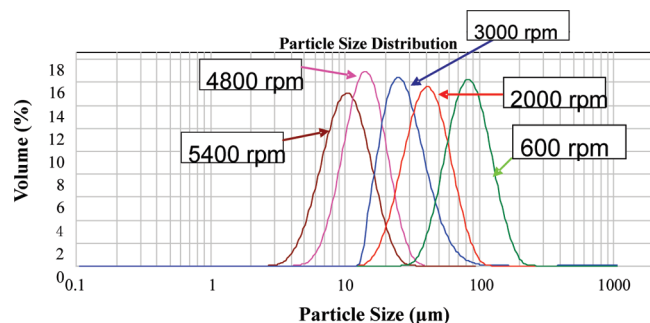


Figure 8. Size distributions of magnetic polymer microspheres under different re-emulsification stirring rates.

the diffusion of sodium hydroxide through the oil phase into the internal water phase was retarded and thus resulted in decreasing Fe_3O_4 magnetic nanoparticles. Besides, sodium hydroxide would result in the salting-out of the PVA-217 stabilizer from the external aqueous phase and thus spoil the stability of the double emulsion. Finally, the magnetic microspheres prepared by sodium hydroxide present the irregular shrinkage and aggregation shown in Figure 7. Finally, considering ammonia with a stronger alkalinity and better solubility in the internal water phase than other alkalis, ammonia was regarded as an optimal alkali solution in this route to prepare magnetic polymer microspheres.

3.4. Effect of the Stirring Rate of Re-Emulsification on the Size and Magnetization of Microspheres. In this route, we also found that the stirring rate of re-emulsification to form the $W_1/O/W_2$ emulsion has an important effect on the size distribution, encapsulation efficiency, and magnetization of the final magnetic polymer microspheres. Stirring rates of re-emulsification from 600, 2000, 3000, and 4800 to 5400 rpm were each investigated. As shown in Figure 8, the size and size distribution of the microspheres can be controlled by the rate of homogenization in the preparation of $W_1/O/W_2$. A high rate of homogenization formed a violent shearing field and caused a high shear stress, which makes the W_1/O phase disperse into smaller $W_1/O/W_2$ emulsion droplets, so correspondingly prepared smaller microspheres. When the rate of homogenization increased from 600 to 5400 rpm, the size of the magnetic polymer microspheres decreased from 80 to 10 μm , and the SPAN values were all around 1.0. On the other hand, re-emulsification was a process to disperse the primary emulsion to external water by shear stress. High shear stress would disperse the primary emulsion to the double emulsion with a smaller volume-mean size; however, more of the internal water phase would overflow to the external water phase during this dispersion process, which would lead to a lower encapsulation efficiency and magnetization of the magnetic polymer microspheres. The encapsulation efficiency of the magnetic polymer microspheres decreased from 36.4% to 29.9% by increasing the stirring rate, as shown in Figure 9, and the magnetization

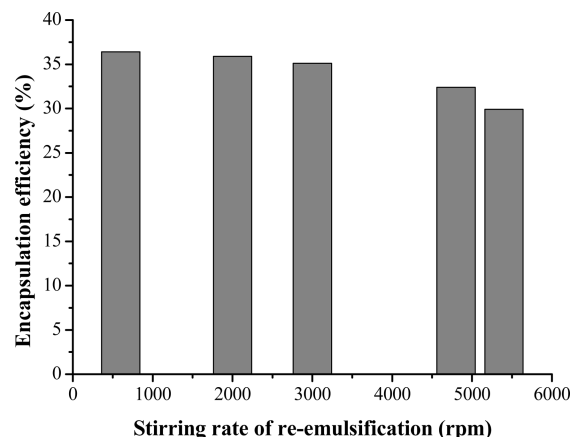


Figure 9. Effect of re-emulsification stirring rate on the encapsulation efficiency of magnetic polymer microspheres.

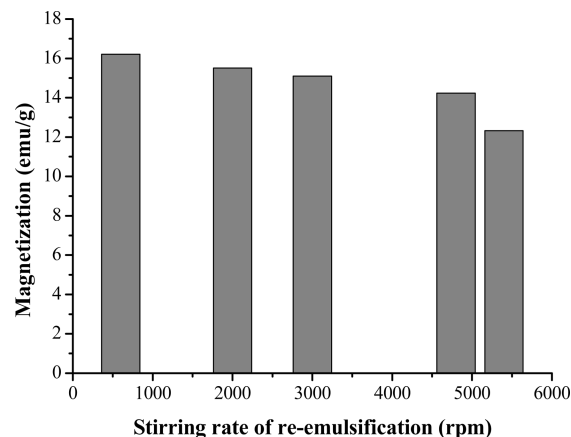


Figure 10. Effect of re-emulsification stirring rate on the magnetization of magnetic polymer microspheres.

decreased from 16.20 emu/g to 12.3 emu/g, as shown in Figure 10. Therefore, the re-emulsification stirring rate should not be too high in order to satisfy the requirement of both encapsulation efficiency and magnetization.

3.5. Morphology and Magnetization of Magnetic Polymer Microspheres. The magnetic polymer microsphere was successfully prepared by use of the following optimal conditions, 0.05 wt % PO 310 as the emulsifier, 1.8 mol/L of the total ferrous and ferric solutions, a molar ratio of 1:2 between the ferrous and ferric ions, and a re-emulsification stirring rate of 4800 rpm. Figure 11 shows a TEM image of several microspheres, in which magnetite nanoparticles looked darker. According to the presence of both dark and light domains inside each microsphere, it can be concluded that the magnetic nanoparticles were well-dispersed in the whole matrix of microspheres. A magnetization curve of the microspheres in Figure 12 also shows that the microspheres are great superparamagnets with 14.23 emu/g and no hysteresis, which

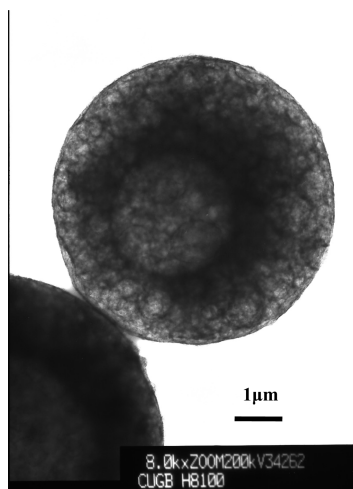


Figure 11. TEM microphotographs of magnetic polymer microspheres prepared under optimal conditions.

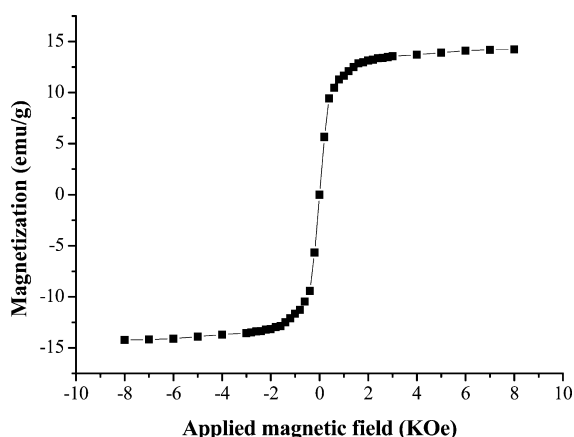


Figure 12. Magnetization curves of magnetic polymer microspheres prepared under optimal conditions.

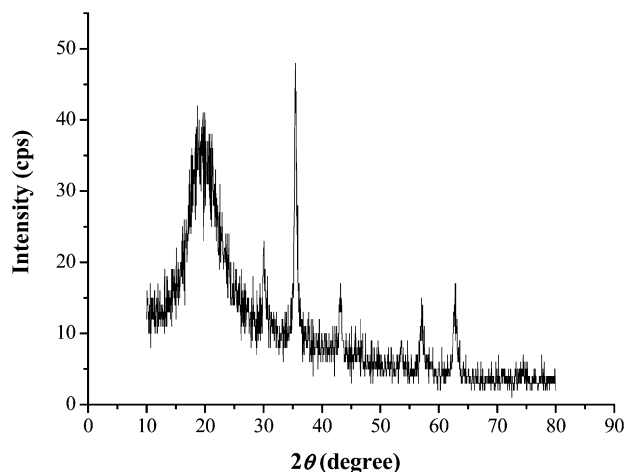


Figure 13. XRD of magnetic polymer microspheres prepared under optimal conditions.

demonstrated that a very homogeneous in situ reaction of magnetic nanoparticles happened with the inner microspheres and minimal agglomeration of the magnetite nanoparticles. The crystalline structures of nanoparticles in microspheres were further characterized by X-ray diffraction (XRD) patterns. As shown in Figure 13, the patterns of in situ formed nanoparticles can be easily indexed to the standard Fe_3O_4 crystal with its six diffraction peaks: $\{2\ 2\ 0\}$, $\{3\ 1\ 1\}$, $\{4\ 0\ 0\}$, $\{4\ 2\ 2\}$, $\{5\ 1\ 1\}$,

and $\{4\ 4\ 0\}$, and no other peaks of impurities were detected. Therefore, it can be concluded that the nanoparticles dispersed in the PST-HEMA microspheres matrix are mainly composed of Fe_3O_4 and the spinal structure.

4. Conclusions

The uniform magnetic PS-HEMA microspheres were successfully fabricated by premix membrane emulsification of a double emulsion and in situ magnetization techniques. We first prepared the $W_1/O/W_2$ emulsion with ferrous and ferric solutions as the internal water phase (W_1), DCM dissolved with a shell polymer of PST-HEMA and a surfactant of PO 310 as the oil phase, and deionized water dissolved with PVA-217 as the external water phase (W_2). Then, the in situ coprecipitation reaction of ferrous and ferric solutions into magnetic nanoparticles was performed in the internal water phase by diffusion of the alkali solution from the external to internal water phase. Finally, the obtained $S/O/W_2$ emulsion was further solidified into microspheres by solvent evaporation. In this route, the critical factors to control the structure and magnetic properties of the final microspheres came from the type of emulsifier in the oil phase, the concentration of ferrous and ferric solutions in the internal water phase, and the type of alkali solution added in the external water phase. The final microspheres with better morphology and higher magnetization of 14.23 emu/g were obtained with the following conditions, i.e., ammonia solution as alkali in the external water phase, 1.80 mol/L of ferrous ferric solution in the internal water phase, and PO 310 as the surfactant in the DCM oil phase. This route can also be utilized in other inorganic–polymer composite microspheres when the inorganic particles encapsulated by the polymer can be synthesized by the chemical coprecipitation reaction.

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