Preparation and Characterization of Micron-Sized Magnetic Microspheres by One-Step Suspension Polymerization

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ABSTRACT: Micron-sized magnetic microspheres with different functional groups were prepared by one-step suspension copolymerization of styrene, divinyl benzene, and methyl methacrylate in the presence of oleic acid-coated magnetic nanoparticles. In the present work, we used benzoyl peroxide as an initiator and poly(vinyl alcohol) (PVA-1788; degree of polymerization: 1,700; degree of hydrolysis: 88%) as a stabilizer. We also added acrylamide (AM) monomer in the aqueous phase and methacrylic acid (MAA) in the oil phase. The morphology and properties of the resulting magnetic microspheres were examined by optical micrographs (OMs), vibrating-sample magnetometer (VSM), and Fourier transform infrared spectrometer (FTIR). The results showed the three products have uniform and spherical form with superparamagnetism and well dispersion. Moreover, we found that monomer AM had a little contribution to the copolymerization, and MAA could strikingly decrease the diameter of the final microspheres. The magnetic microspheres with functional groups could be linked well with the IgG-FITC. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1331–1335, 2007

Key words: core-shell; suspension copolymerization; one-step; magnetic microspheres; big size; MAA

INTRODUCTION

Magnetic microspheres have been more concerned because of their wide usage in the fields of biotechnology, biomedical diagnostics, and bioengineering such as in cell isolation,[1] enzyme immobilization,[2] protein separation and purification,[3] immunoassay,[4] and guided site-specific drug delivery.[5] Over the past two decades, many attempts have been made to prepare composite microspheres with inorganic magnetic core and polymer outer shell, which provide favorable biocompatibility, high magnetic susceptibility, appropriate size distribution, and abundant functional groups on the surface for coupling affinity ligands. Most of the experiments adopted the monomer copolymerization method, such as seed,[6] dispersion,[7] suspension,[8,9] emulsion,[10] miniemulsion polymerization,[11,12] etc., among which the suspension polymerization is more apt to synthesize big-sized microspheres.

Recently there are many reports about suspension polymerization.[13–15] But microspheres with size of D_m >20 μm have been less concerned. Methacrylic acid (MAA) or acrylamide (AM) as copolymerization monomer has not been reported in one-step suspension polymerization.

In the present work, we synthesized magnetic microspheres by one-step suspension polymerization. The magnetic microspheres were examined by optical micrographs (OMs), vibrating-sample magnetometer (VSM), and Fourier transform infrared spectrometer (FTIR). The results showed that they had good morphology and properties. We present a detailed analysis about the influence of monomer MAA and AM on suspension polymerization. This article also reports the magnetic microspheres linked with the IgG-FITC, which has potential application in the fields of biomedical diagnostics, biotechnology, and bioengineering.

EXPERIMENT AND MEASURING METHODS

Experiment

Magnetic microspheres were prepared by one-step suspension polymerization. Copolymerization was

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TABLE I
Experiment Recipes

Sample	Aqueous phase	Oil phase
A	PVA-1788, water	Oleic acid-coated magnetic nanoparticles, St, DVB, MMA, BPO
B	PVA-1788, water, AM	Oleic acid-coated magnetic nanoparticles, St, DVB, MMA, BPO
C	PVA-1788, water	Oleic acid-coated magnetic nanoparticles, St, DVB, MMA, BPO, MAA

performed in a three-rocked, round-bottomed flask reactor fitted with mechanical stirrer, reflux condenser with a silicon oil seal at its top, and a nitrogen gas inlet tube. Surface-modified magnetic nanoparticles with size of 13 nm were previously obtained by a typical chemical coprecipitation method.[16–18]

Copolymerization is described as follows. First, the aqueous phase consisted of PVA (-1788 as stabilizer) and water, which were evenly stirred for half an hour at 40°C. The oil phase consisted of magnetic nanoparticles, benzoyl peroxide, monomer St, DVB, and methyl methacrylate (MMA), which were treated with an ultrasonic homogenizer for 15 min and then immersed in the reactor at a certain rate. The two phases were maintained under stirring at 80°C during 2 h with a stirring speed of 500 rpm. Then the temperature was lowered to 60°C and polymerization was carried out for 2 h. The whole process was performed under no oxygen atmosphere. The resulting materials were thoroughly washed with ethanol and hot deionized water several times, with the help of magnetic field to remove the excess stabilizer and other impurities. Finally, big-sized magnetic microspheres were obtained. Based upon different monomers added, we did three experiments and the detailed recipes are given in Table I.

Measuring methods

The morphology of magnetic microspheres was measured by OM (XSP-8C; Shanghai, China). Magnetic properties were analyzed by VSM. We used FTIR to show the functional groups of microspheres. And hydrophile–hydrophobe of the surface was measured by optical contact angle measuring device (JC2000A; Shanghai, China). The microspheres' capacity of conjugation with the IgG-FITC was investigated by the fluorescence microscope.

RESULTS AND DISCUSSION

Morphology of magnetic microspheres

The morphological characterizations of the three kinds of magnetic microspheres were indicated by optical microscopy. Figure 1 shows that all particles have uniform and spherical form, no aggregation, and have a well dispersion with narrow distribution. Samples A and B (added AM) have no difference under optical microscopy, with similar size of about 40 μm [Fig. 1(A,B)]. Whereas, the particles produced by sample C (added MAA) strikingly decrease in size to 12 μm [Fig. 1(C)] compared with samples A and B.

The reason for size decreasing is the addition of monomer MAA. As a rule, the maximum diameter of monomer droplets controlled by dispersion depends on the balance of driving forces for diffusion between the stress and droplet interfacial tension, and can be written as W_{ec}

$$W_{ec} = \rho \upsilon^2 d_{p\,max}/\sigma$$

where σ is drop interfacial tension, υ is stirring velocity, and $d_{p\,max}$ is the maximum droplet diameter.

In general, W_{ec} is a constant. In suspension polymerization solution, the viscosity of oil-phase dispersion is low in the stage of cool agitation before and initial polymerization. At this phase, the interfacial

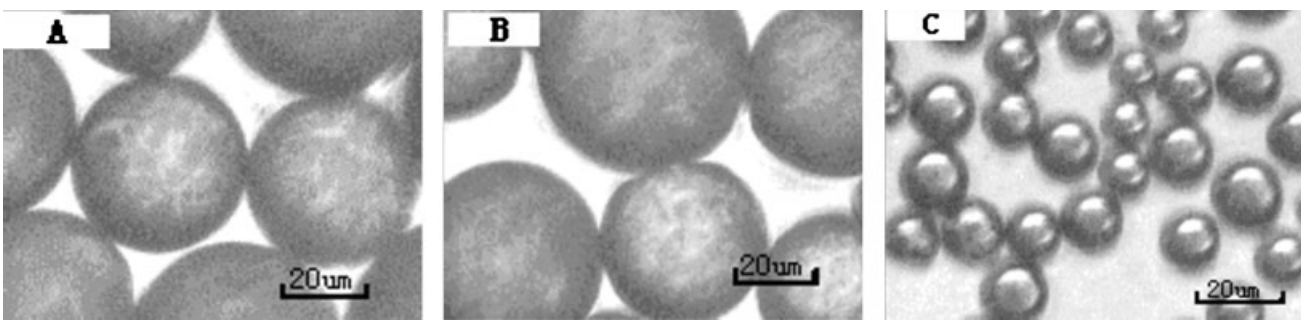

Figure 1 Optical micrographs of the magnetic microspheres: (A) sample A (PSt-MMA), about 40 μm; (B) sample B (PSt-MMA-AM), about 40 μm; (C) sample C (PSt-MMA-MAA), about 12 μm.

tension (or interfacial energy) is dominant to influence the droplet size. In the present work, we select MAA, which is hydrophobic–hydrophilic—organic molecule as polymerization monomer. MAA added can be automatically adsorbed on the interface between liquid (aqueous phase)–liquid (monomer droplets), and form a directional sequence with the hydrophilic groups inserting aqueous phase and the hydrophobic groups inserting oil droplets. That is, to say, the hydrophobic groups with small free energy substitute for the water molecule with bigger free energy. The droplet interfacial energy is dramatically decreased, namely significantly decreasing the interfacial tension, promoting the dispersion of droplets. According to the earlier formula, along with reduce of interfacial tension (σ), the maximum droplet diameter ($d_{p\ \max}$) decreases, leading to the decrease of the final particle size. Comparing Figure 1(A) with Figure 1(C), we can see that the particle size largely decreases because of the addition of monomer MAA in sample C. However, the monomer AM has little influence on the particle size in sample B [Fig. 1(B)]. We think the reason is that the polymerization occurs at the interior of the oil droplets and AM is a water-soluble monomer; it could not enter the oil droplet, and thus make no contribution to the interfacial tension of droplets.

A conclusion could be drawn from Figure 1 that the three magnetic microspheres have a uniform size with well dispersion. In the suspension polymerization, to obtain the narrow size distribution, the two aspects must be assured. On the one hand, the oil phase should be dispersed into the water to form uniform size droplets. On the other hand, in the stage of particle growing, it is very important to improve the stability of suspension polymerization solution, to hinder the droplet coalescence and slower

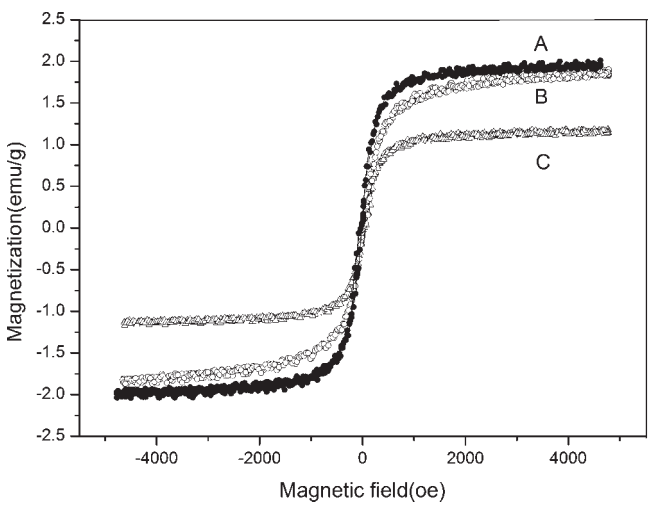

Figure 2 The magnetization loops of the samples are compared. (A) Sample A (PSt-MMA); (B) sample B (PSt-MMA-AM); (C) sample C (PSt-MMA-MAA).

TABLE II
Magnetic Properties of Microspheres

Sample	Particle size (μm)	M_s (emu/g)	M_r (emu/g)	H_c (Oe)
A	40	1.888	0.029	5.490
B	40	1.749	0.031	6.248
C	12	1.191	0.092	12.651

the velocity of particle growing. In the present work, two modifications were adopted. First, we selected vertical stainless steel buffleplates with a four-paddle stirrer, combined with continued agitation. So the monomer phase obtained strong and even forces, and was dispersed into uniform size droplets. Then, the droplets were driven out and entered the cycle area, where the droplets coalescence easily occurs. The position of the stirring blade in the container will influence the time taken by the droplets driven out to return to the blade area, and becomes an important factor for controlling the particle size. In the present work, we found that the combination of a stirring blade rooted at 0.4 of the solution depth and an appropriate stirring velocity could reduce the difference of the initial droplets formed in different areas of the container, furthermore shorten the cycle time, and effectively hindered the droplet coalescence, leading to the production of the magnetic microspheres with narrow size distribution. Second, the concentration of the dispersion stabilizer is another factor to influence the coalescence and redispersion of droplets. In this article, we used partially hydrolyzed PVA (-1788, water-soluble nonionic macromolecules) as the dispersion stabilizer, which usually performs the dual function of providing sites for nucleation of droplets and also providing colloidal stability to the growing droplets as a result of their adsorption at the droplet–water interfaces. PVA (degree of hydrolysis: 88%) can decrease the hydrophilic of the polystyrene surface and increase the charge density of the polystyrene surface so as to enhance the repulsive force between microspheres. The present work shows that the magnetic microspheres had a narrow size distribution if the mass proportion among monomers:PVA:water was in the range of 5 : 1.5 : 100 to 8 : 1.5 : 100.

Magnetic properties

The magnetic properties of three samples were analyzed with a VSM magnetometer. And three typical hysteresis loops of magnetic microspheres, which were measured in powdered state, are shown in Figure 2, and the saturated magnetization (M_s), the residual magnetizations (M_r) per gram of samples, and the coercivities (H_c) are given in Table II. From Figure 2, we know that all samples have nearly

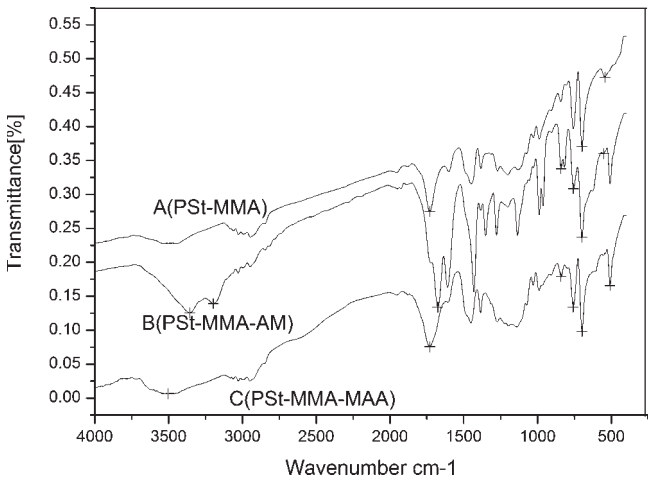

Figure 3 FTIR of the magnetic microspheres is compared. (A) Sample A (PSt-MMA); (B) sample B (PSt-MMA-AM); (C) sample C (PSt-MMA-MAA).

superparamagnetic behavior with immeasurable remanence and little coercivity. The saturated and residual magnetization of sample A, which is 1.888 and 0.029 emu/g, have a little difference with that of sample B, which is 1.749 and 0.031 emu/g. However, they were more than that of sample C, which is 1.191 and 0.092 emu/g. As to the magnetization, on the one hand, magnetic microspheres of small size (sample C) had less content of magnetic nanoparticles per gram than that of big size (samples A and B). So the magnetic susceptibility of the magnetic microspheres of small size was correspondingly reduced. On the other hand, a dead magnetic layer mainly made of the large fraction of low-density polymer coating was formed over the surface layer due to strong interaction between the atoms on the surface and the molecular chain, which prevented the coupled dipoles from aligning along the magnetic orientation. And the dead magnetic layer of small-sized microspheres (sample C) was thicker than that of big-sized (samples A and B). Thus there is a tendency toward lower magnetization values and higher coercivity.[19]

Surface functional groups

The presence of organic groups was confirmed by FTIR spectra as shown Figure 3. Comparison is made among three kinds of magnetic microspheres. The spectrum B is for magnetic microspheres with $—NH_2$. The absorbing peaks at 3356.12 cm^{-1} (N—H), 3197.02 cm^{-1} (N—H), and 1675.09 cm^{-1} (C=C) suggest the existence of $—CONH_2$ offered by AM. The spectrum C is for magnetic microspheres with —COOH. The absorbing peaks at 3504.99 cm^{-1} (—OH) and 1729.87 cm^{-1} (C=O) suggest the existence of —COOH offered by MAA. Besides, the three spectrum all have the absorbing peaks in the neighborhood of 840, 757, 698 (the single substitute peak of benzene), and 550 cm^{-1} (Fe_3O_4).

Surface wetting behavior

In the present work, we studied the surface wetting behavior of the magnetic microspheres, measured by optical contact angle measuring device (JC2000A). If $0^\circ < \theta < 90^\circ$, the microspheres are hydrophilic. If $90^\circ < \theta < 180^\circ$, the microspheres are hydrophobic. First, the dry, powdery samples were pressed into a form of tablets, which were compact with no big interstice between the microspheres. Then a drip of water was dripped on the tablet. And the contact angle θ between liquid phase and solid phase was measured, as shown in Figure 4. It is clear that sample A is hydrophobic [Fig. 4(A)], while samples B and C are hydrophilic [Fig. 4(B,C)]. In general, the contact angle is interrelated with the functional groups on the surface of the magnetic microspheres. And the θ decreases with the increase of hydrophilic functional groups. In sample A, all polymerization monomers are oil soluble with no water-soluble functional groups on the surface of microspheres. So the θ_a is 109°, suggesting hydrophobic. In the monomers of samples B and C, there are water-soluble functional groups, offered by AM and MAA, respectively. Therefore, the corresponding θ_b and θ_c are 78° and 62°, suggesting hydrophilic. The fact of $\theta_c < \theta_b$

Figure 4 The contact angle (θ) pictures of the magnetic microspheres: (A) sample A (PSt-MMA); (B) sample B (PSt-MMA-AM); (C) sample C (PSt-MMA-MAA).

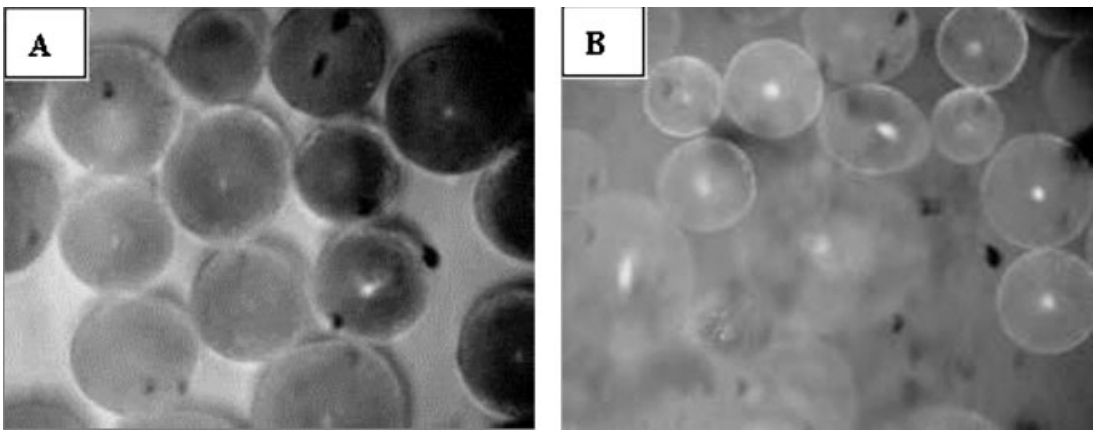

Figure 5 Fluorescence microscopic pictures of the fluorescence-labeled IgG-linked microspheres. (A) Sample B (PSt-MMA-AM); (B) sample C (PSt-MMA-MAA).

suggests that the amount of —COOH groups on the surface of magnetic microspheres in the sample C is higher than that of $—NH_2$ in sample B.

Conjugation with the IgG-FITC

To investigate the feasibility of $—NH_2$ groups and —COOH groups on the surface of the magnetic microspheres, we performed the experiments of antibody adsorption to samples B and C. To covalently attach antibody IgG to the magnetic microspheres, the functional groups on the surface were activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and *N*-hydroxysuccinimide ester solution. Then the magnetic microspheres were modified with a fluorescence-labeled IgG. Finally the magnetic microspheres were washed using Tris (pH 7.4) and BSA with the help of magnetic field. The IgG-modified microspheres were observed with the fluorescence microscope. From Figure 5, we can see almost all of the microspheres are linked with antibody, giving quite good properties in biological adsorption. And we can also see that the conjugation effects are much better for the sample C [Fig. 5(B)], compared with that of sample B [Fig. 5(A)]. The reason is that the surface of sample C has more functional groups, which can adsorb fluorescence-labeled IgG. The microspheres can also be used to attach other antibody, to immobilize enzymes, and to separate cells, which are in progress.

CONCLUSIONS

Three kinds of big-sized magnetic microspheres with different functional groups were prepared by one-step suspension polymerization. The measuring results showed the three products have uniform and spherical form with superparamagnetism, and well dispersion. The mean diameter is around 50 μm for magnetic microspheres with $—NH_2$ or not, 20 μm for —COOH. We found that monomer AM had a little contribution to the magnetic microspheres size; as contrast, MAA added could strikingly decrease the diameter of the final microspheres. The surface functional groups of $—NH_2$ and —COOH were measured by FTIR. The obtained magnetic microspheres with functional groups could be linked well with the IgG-FITC. The experiments of surface wetting behavior and conjugation with the IgG-FITC showed the potential applications of the magnetic microspheres in the fields of biological science and technology.

References

1. Kronick, P.; Gilpin, R. W. J Biochem Biophys Methods 1986, 12, 73.
2. Li, X. H.; Sun, Z. H. J Appl Polym Sci 1995, 58, 1991.
3. Abudiab, T.; Beitle, R. R. J Chromatogr A 1998, 795, 211.
4. Josephson, L. U.S. Pat. 4,672,040 (1987).
5. Cupta, P. K.; Hung, C. T. Life Sci 1989, 44, 175.
6. Lee, J.; Senna, M. Colloid Polym Sci 1995, 273, 76.
7. Hora'k, D. J Polym Sci Part A: Polym Chem 2001, 39, 3707.
8. Cocker, T. M.; Fee, C. J.; Evans, R. A. Biotechnol Bioeng 1997, 53, 79.
9. Lee, Y.; Rho, J.; Jung, B. J Appl Polym Sci 2003, 89, 2058.
10. Kondo, A.; Kamura, H.; Higashitahi, K. Appl Microbiol Biotechnol 1994, 41, 99.
11. Liu, Z. L.; Yang, X. B.; Yao, K. L. J Magn Magn Mater 2006, 302, 529.
12. Liu, Z. L.; Ding, Z. H.; Yao, K. L. J Magn Magn Mater 2003, 265, 98.
13. Macho, V.; Fabíni, M.; Rusina, M.; Bobula, S.; Harustiak, M. Polymer 1994, 35, 5773.
14. Blondeau, D.; Bigan, M.; Despres, P. React Funct Polym 1995, 27, 163.
15. Alvarez, J.; Alvarez, J.; Hernández, M. Chem Eng Sci 1994, 49, 99.
16. Liu, Z. L.; Liu, Y. J.; Yao, K. L. J Mater Synth Process 2002, 10, 83.
17. Liu, Z. L.; Wang, X. J Mater Sci 2004, 39, 2633.
18. Liu, Z. L.; Wang, H. B.; Lu, Q. H. J Magn Magn Mater 2004, 283, 258.
19. Kaiser, R.; Midkolczy, G. J. Appl Phys 1970, 41, 1064.