Preparation of Magnetic Polystyrene Microspheres with a Narrow Size Distribution

Chengli Yang, Yueping Guan, Jianmin Xing, Junguo Liu, Guobin Shan, Zhentao An, and Huizhou Liu
Laboratory of Separation Science and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, China

DOI 10.1002/aic.10467

A spraying suspension polymerization process for preparation of magnetic polystyrene microspheres with a narrow size distribution was proposed. The magnetite (Fe_3O_4) nanoparticles with hydrophobic shell were first prepared by a modified chemical coprecipitation method. Then the oil solution, composed of monomer styrene, crosslinker divinylbenzene, Fe_3O_4 nanoparticles, and initiator benzoyl peroxide, was sprayed into the poly(vinyl alcohol) aqueous solution by a pressure of nitrogen gas to form droplets. Finally, the droplets would be polymerized rapidly to magnetic polystyrene microspheres at the polymerization temperature. The morphology and magnetic properties of microspheres were examined using a scanning electron microscope and a vibrating sample magnetometer. The results showed that the magnetic microspheres had a narrow size distribution with mean diameter of around 10 μm and smooth surface, and the magnetic microspheres with saturation magnetization of 15.6 emu/g exhibited distinct superparamagnetic characteristics. Powder X-ray diffraction was used to investigate the structure of the magnetite nanoparticles dispersed in the polystyrene matrix. © 2005 American Institute of Chemical Engineers *AIChE J,* 51: 2011–2015, 2005

Keywords: preparation, polystyrene microspheres, superparamagnetic, narrow size distribution, spraying suspension polymerization (SSP)

Introduction

Highly selective magnetic polymer microspheres have been widely used 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 their surfaces for coupling affinity ligands.

Coating or encapsulating magnetic particles with preformed polymers is a conventional method of preparing magnetic microspheres. Various types of magnetic microspheres based on natural polymers,[6] inorganic and organic composite microspheres,[7] and synthetic polymers[8] have been prepared by this method. Another method, which is more widely used, is monomer polymerization. Several kinds of polymerization can be used, such as emulsion polymerization,[9,10] dispersion polymerization,[11,12] suspension polymerization,[13,14] and seed polymerization.[15] Of these methods, suspension polymerization is simple and more suitable for large-scale production of magnetic polymer microspheres with higher saturation magnetization. Conventionally, the droplets of preformed oil phase were dispersed by the mechanical stirring method. The magnetic microspheres obtained, however, are not quite suitable for the biological and medical research because of the inherent inhomogeneity in microsphere size and magnetite content as well as the distortion in shape. Obviously, these defects cannot guarantee uniform behavior of magnetic microspheres in a solution

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C. Yang is also affiliated with Graduate School of the Chinese Academy of Sciences, Beijing 100080, China.

Correspondence concerning this article should be addressed to H. Liu at hzliu@home.ipe.ac.cn.

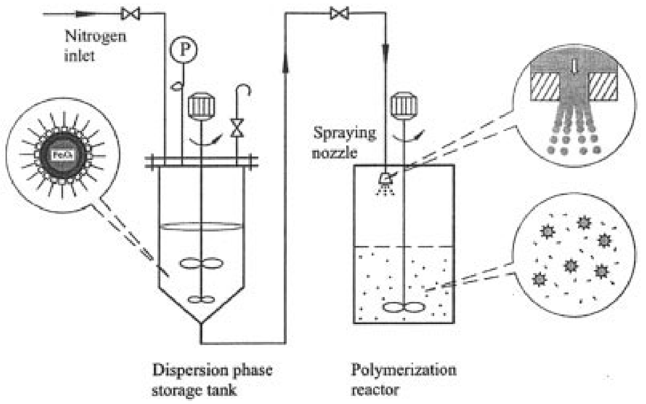

Figure 1. Spraying suspension polymerization (SSP) process.

and under a magnetic field. Ugelstad[16] developed in situ formation of iron oxide inside the preformed porous monodisperse polymer particles produced by the activated swelling method, but the multistep process is very tedious with preparation of monodisperse seed particles in the first step. As a result, the magnetic microspheres prepared by this method are rather expensive.

In the present work, a spraying suspension polymerization (SSP) process was proposed. Magnetic polystyrene microspheres with a narrow size distribution were prepared by the spraying suspension polymerization of styrene in the presence of Fe_3O_4 nanoparticles. The magnetization and structure of the products were characterized by use of a vibrating sample magnetometer (VSM), a scanning electron microscope (SEM), and powder X-ray diffraction (XRD).

Experimental Methods

Materials

Styrene (St) and divinylbenzene (DVB) were reagent grade, distilled under reduced pressure to remove inhibitors before use. Ferric chloride hexahydrate ($FeCl_3$·$6H_2O$), ferrous chloride tetrahydrate ($FeCl_2$·$4H_2O$), NH_3·H_2O, oleic acid, and ethanol were analytical grade and used without any further purification. Poly(vinyl alcohol) (PVA-217: degree of polymerization 1700; degree of hydrolysis 88%) was used as a stabilizer. Benzoyl peroxide (BPO, reagent grade) was used as an initiator in polymerization. Water was purified by distillation followed by deionization using ion-exchange resins. Other chemicals were reagent grade and used as received.

Apparatus

A schematic diagram of the SSP process is shown in Figure 1. A spraying nozzle with pore size of 50 μm was installed over the polymerization reactor. The dispersion phase (oil phase) was stored in the storage tank connected to a nitrogen gas inlet. The aqueous phase, stored in the polymerization reactor, was stirred gently with a mechanical stirrer to prevent the coalescence of droplets.

Preparation of Fe_3O_4 nanoparticles with hydrophobic shell

The Fe_3O_4 nanoparticles with hydrophobic shell were prepared by a modified coprecipitation method, as follows: 0.196 mol $FeCl_3$·$6H_2O$ and 0.098 mol $FeCl_2$·$4H_2O$ were dissolved in 600 mL deionized water in a 2.0-L beaker under nitrogen gas with vigorous stirring at 80°C. Then, 30 mL NH_3·H_2O was added to the solution, after which 20 mL oleic acid was added dropwise into the suspension within 20 min. After several minutes, the magnetic precipitates were isolated from the solvent by a permanent magnet. After the precipitates were washed several times with deionized water, to remove the excess oleic acid, the magnetic Fe_3O_4 nanoparticles coated with oleic acid were obtained.

Preparation of magnetic polystyrene microspheres

A spraying nozzle with pore size of 50 μm was used in the present work. The aqueous phase, where PVA was dissolved in deionized water with constant stirring, was stored in the polymerization reactor. After the aqueous phase was bubbled with nitrogen gas for 30 min, the temperature of the aqueous phase was increased gradually and maintained at 80°C. The Fe_3O_4 nanoparticles with hydrophobic shell were dissolved in a mixture of St, DVB, and BPO, and were used as a dispersed phase (oil phase). The detailed conditions for preparation of magnetic polystyrene microspheres are given in Table 1.

After the pressure of the dispersion phase storage tank was controlled at 0.1 MPa, the oil phase was sprayed through the spraying nozzle into the aqueous phase to form droplets, which were polymerized in about 1 h with gentle stirring. The resulting magnetic polystyrene microspheres were separated by a permanent magnet, washed several times with deionized water and ethanol, and dried in a vacuum for 48 h.

Characterization

The diameter and surface features of the magnetic polystyrene microspheres obtained were investigated by use of a scanning electron microscope (SEM, JSM-6700F, JEOL, Tokyo, Japan). The free-flowing dried microspheres were sprinkled onto aluminum stubs with double-face adhesive tape on the stud, coated under vacuum for 3 min with a thin layer of gold, and examined under a scanning electron microscope. The magnetization curves of samples were measured with a vibrating sample magnetometer (VSM, model-155, Digital Measurement Systems, now ADE Technologies, Milpitas, CA) at room temperature. Powder X-ray

Table 1. Preparative Conditions of Magnetic Polystyrene Microspheres by Spraying Suspension Polymerization (SSP) Process

Oil phase	
Fe_3O_4 nanoparticles (g)	20
Styrene (St) (ml)	85
Divinylbenzene (DVB) (mL)	5
Benzoyl peroxide (BPO) (g)	4
Aqueous phase	
Polyvinyl alcohol (PVA) (g)	20
Deionized water (mL)	1000
Polymerization temperature (°C)	80
Spraying nozzle size (μm)	50
Applied pressure (MPa)	0.1

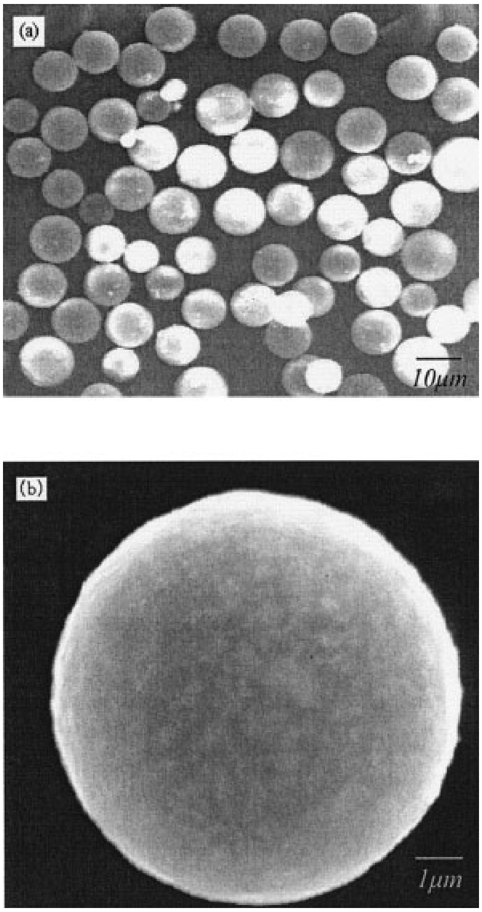

Figure 2. (a) SEM micrographs of overall appearance and (b) outer surface of magnetic polystyrene microspheres.

diffraction (XRD; MAC, MXP21VAHF, Cu–K$_\alpha$) was used to determine the crystal structure of samples.

Results and Discussion

Properties of the SSP process

The SSP process was investigated, and the spraying nozzle was magnified partially in the schematic diagram, as shown in Figure 1. The formation of droplets must pass through three different stages from spraying nozzle to the upper surface of the aqueous solution. At the very beginning, the oil solution was sprayed through a spraying nozzle to form many small rotating posts. Then, the small rotating posts slowly became agglomerate droplets as the distance from the nozzle increased. Finally, the droplets were dispersed to spherical droplets in the headspace, and then entered into the aqueous solution. Because the headspace is at room temperature, and the temperature of the aqueous solution is at 80°C (which is higher than the polymerization temperature of monomer St), the droplets would be polymerized only in the aqueous solution, not in the headspace. Therefore, the distance between the spraying nozzle and the free surface of aqueous solution is considered as a very important factor in controlling the size of droplets. The spraying nozzle installed in the polymerization reactor can be moved up and down, so the size of droplets required will be obtained by adjusting the distance between the spraying nozzle and the upper surface of aqueous solution. It was found that if the nozzle was immersed in the aqueous solution, the oil solution would not be sprayed to form dispersed droplets. Instead, a post-like solid will be formed because of high temperature and high density of the aqueous solution. When the distance between the spraying nozzle and the upper surface of aqueous solution was too short, the oil solution could not be sprayed to small droplets before they entered into the aqueous solution, leading to the formation of large droplets with broad size distribution. On the contrary, if the distance was too long, the diffuse area of droplets sprayed through the spraying nozzle increased quickly as the distance increased, so most of the droplets would hit the reactor wall and enter into the aqueous solution as liquid film. As a result, the sizes of droplets in the aqueous solution will have a rather wide size distribution. To obtain the ideal magnetic microspheres, the distance between the spraying nozzle and the upper surface of aqueous solution is fixed to 20 cm. On the other hand, it was also found that formation of droplets started at a critical spraying pressure. The rate of spraying increases with the increase of pressure above the critical value while the droplets become extra fine. With an excess spraying pressure, the delicate force balance against the interfacial tension was broken, resulting in a broad size distribution of droplets. In the present work, the optimal pressure was controlled at 0.1 MPa.

Preparation of magnetic polystyrene microspheres

Magnetic polystyrene microspheres were prepared by the SSP process. The morphology of the resulting magnetic polystyrene microspheres was observed by SEM, as shown in Figure 2. In the present work, PVA was used as a stabilizer in the aqueous solution to stabilize the droplets. The detailed recipe is shown in Table 1. Nanosized magnetic Fe_3O_4 particles coated with oleic acid were obtained from the coprecipitation method with mixed ferrous and ferric ionic solution. They had an average size of about 8 nm[17] and exhibited superparamagnetic characteristics, as shown in Figure 3a. The Fe_3O_4 nanoparticles coated with oleic acid could be easily mixed with an organic mixture of hydrophobic monomer St, crosslinker DVB, and hydrophobic initiator BPO to form a uniform oil solution with mechanical stirring. Under a particular pressure of nitrogen gas, the oil solution was sprayed through the spraying nozzle into the aqueous solution to form uniform droplets,

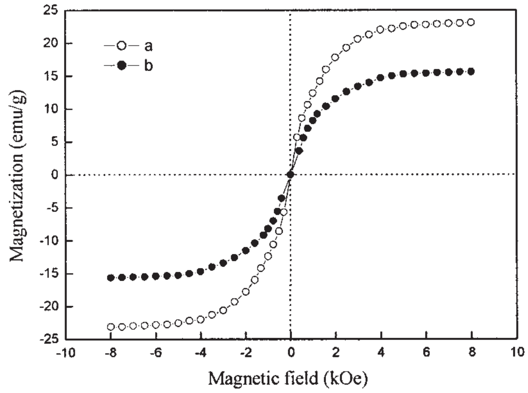

Figure 3. Magnetization curves of (a) Fe_3O_4 nanoparticles coated with oleic acid and (b) magnetic polystyrene microspheres.

which were stabilized by stabilizer PVA dissolved in the aqueous solution. The polymerization temperature of the monomer St is about 70°C. However, the temperature of the aqueous solution in the polymerization reactor was maintained at around 80°C. So the droplets were solidified rapidly after being sprayed from the spraying nozzle as they approached the aqueous solution. The droplets sprayed from the spraying nozzle gained acceleration, which promoted easy entrance into the aqueous solution. Gentle agitation of the solution induced adequate entrainment of the droplets from the interface into the bulk solution and also prevented conglomeration among the droplets. In conventional suspension polymerization, the system usually consists of a hydrophobic monomer, magnetic nanoparticles, water (medium), stabilizer, and hydrophobic initiator. The droplets are formed by a conventional mechanical stirring method, so the coalescence and redispersion of droplets will occur continuously during the polymerization. Therefore, the polymer particles obtained are in the size range of hundreds of micrometers with broad size distribution. Compared with conventional suspension polymerization, the SSP process can cause the droplets to disperse uniformly by spraying dispersion instead of the conventional mechanical stirring dispersion. As a result, the magnetic microspheres prepared by this process have a narrow size distribution.

In suspension polymerization, the droplets are stabilized mainly using water-soluble nonionic macromolecules such as partially hydrolyzed PVA.[18] The stabilizer 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. In the present work, PVA (degree of hydrolysis 88%) was used to decrease the hydrophilicity of the polystyrene surface or increase the charge density of the polystyrene surface so as to enhance the repulsive force between microspheres. An increase of PVA concentration in the aqueous solution increases the viscosity of the reaction medium and will allow more PVA molecules to be diffused and adsorbed at the droplet–water interfaces in a random manner. However, it is found that only a small amount of stabilizer added is adsorbed onto the droplets.[19] Thus, it is necessary to add a large amount of stabilizer to obtain stable droplets.

The droplets sprayed from spraying nozzle were spherical in the headspace. The spherical droplets probably would deform somewhat when they hit the interface of aqueous solution. However, the droplets were stabilized quickly by a large amount of stabilizer PVA dissolved in the aqueous solution, so the deformed droplets quickly reassumed the shape of spherical droplets because of the surface tension. Finally, the droplets were polymerized rapidly at the polymerization temperature to form spherical particles, as shown in Figure 2a. The present work shows that the magnetic microspheres had a narrow size distribution if the magnetic microspheres were prepared with higher concentration of PVA (above 20 wt % of monomer) in the aqueous solution. It can be explained that the time taken for the formation stage of stable droplets becomes shorter with decreasing nucleation time. In addition, in the initial stage of polymerization reaction, free radicals are formed by initiator decomposition at polymerization temperature and subsequent chain growth will occur in the monomer droplets. The aqueous solution was bubbled with nitrogen gas for about 30 min before polymerization, so it could be verified that the oxygen gas in the aqueous solution was eliminated completely. The magnetite (Fe_3O_4) is a strong inhibitor with adsorption of free radicals during polymerization, however, so it seems to decelerate the polymerization rate considerably. Therefore, a large amount of initiator BPO was added (>4 wt % of monomers) in the present work.

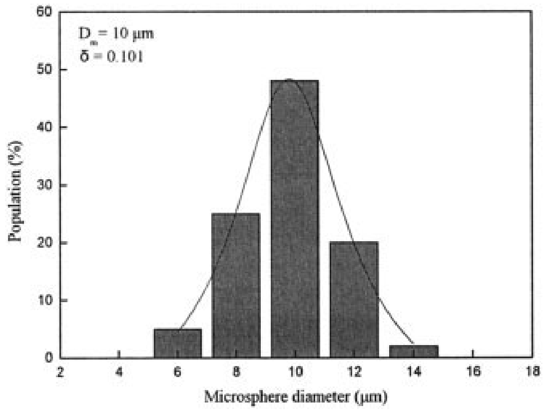

Figure 4. Size distribution curve of magnetic polystyrene microspheres.

Properties of magnetic polystyrene microspheres

Figure 2 shows the SEM micrographs of the overall appearance and outer surface of the magnetic polystyrene microspheres prepared by the SSP process. Figure 2a shows that the magnetic polystyrene microspheres have a narrow size distribution. The surface morphology of magnetic polystyrene microspheres was investigated with SEM microphotography taken under a higher magnification. A smooth spherical structure could be observed from the surface of magnetic polystyrene microspheres, as shown in Figure 2b. The results verified that the experimental recipe used in the present work was suitable for preparing magnetic microspheres with smooth polystyrene surface.

The size distribution has been determined by statistically measuring the size of more than 100 individual microspheres from different regions of the SEM micrographs. The size distribution curve of magnetic polystyrene microspheres is shown in Figure 4. The geometrical average size D_m and geometrical standard deviation δ were determined by fitting the lognormal distribution function to the histogram ($D_m = 10\ \mu$m and $\delta = 0.101$). The value of δ is much smaller than that generally obtained by the conventional suspension polymerization. It is clearly evident that the magnetic polystyrene microspheres have a narrow size distribution with mean diameter of around 10 μm.

The magnetization curves for the Fe_3O_4 nanoparticles coated with oleic acid and the magnetic polystyrene microspheres are shown in Figure 3. Figure 3a shows a typical magnetization curve of the Fe_3O_4 nanoparticles coated with oleic acid. The saturation magnetization was found to be 23.1 emu/g. Both remanence and coercivity were zero, suggesting that such nanoparticles were superparamagnetic. Figure 3b shows a magnetization curve of magnetic polystyrene microspheres. There was no hysteresis in the magnetization, showing that such magnetic microspheres were also superparamagnetic. It can be

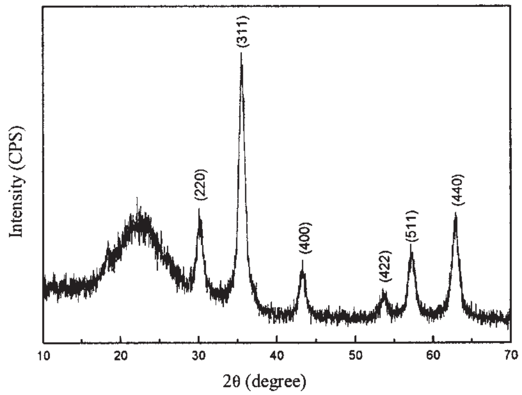

Figure 5. X-ray diffraction of magnetic polystyrene microspheres.

concluded that the Fe_3O_4 nanoparticles still dispersed uniformly in the polystyrene microspheres as 8 nm nanoparticles during polymerization. The saturation magnetization was found to be 15.6 emu/g, which is higher than values reported in other similar works in the literature.[13,14] With such high saturation magnetization, the magnetic microspheres responded very rapidly to a magnetic field, typically being attracted to a permanent magnet from a suspension in about 10 s, leaving a clear supernatant, which could readily be removed by aspiration or decantation.

Figure 5 shows the X-ray diffraction patterns of magnetic polystyrene microspheres. It is found that the standard Fe_3O_4 crystal with inverse cubic spinel structure has six diffraction peaks: {220}, {311}, {400}, {422}, {511}, and {440}. The XRD patterns of magnetic polystyrene microspheres, shown in Figure 5, are in agreement with the standard spectra for magnetite (Fe_3O_4), and there is no other phase such as γ- or α-ferric oxide. The results shown in Figure 5 reveal that magnetic nanoparticles dispersed in the polystyrene matrix are high-purity Fe_3O_4 nanoparticles. The full width at half maximum (FWHM) of each diffraction peak is rather broad, which reveals that the magnetite crystals are in the ultrafine size range (~8 nm)[17] with a single domain structure. As a result, the sample displays superparamagnetic characteristics as shown in Figure 4b. In addition, the broad peak appeared in the range from 16 to 28°, indicating the existence of amorphous polymer for polystyrene.

Conclusion

The results of the present study showed that the magnetic polystyrene microspheres were prepared by the spraying suspension polymerization (SSP) process. Compared with conventional suspension polymerization, the magnetic polystyrene microspheres obtained by this process have a narrow size distribution with mean diameter of around 10 μm, and the magnetic polystyrene microspheres with higher saturation magnetization show distinct superparamagnetic characteristics. Powder X-ray diffraction measurement shows the inverse cubic spinel structure for magnetite nanoparticles in polystyrene microspheres. Importantly, the SSP process can be considered as an inexpensive way of preparing magnetic polystyrene microspheres to be used in biological applications such as cell separation, immobilization of enzymes, and purification of proteins.

Acknowledgments

The authors express thanks to Prof. Jiayong Chen at Institute of Process Engineering, the Chinese Academy of Sciences, for his valuable help in writing of this paper. The authors are also grateful to the National High Technology Research and Development Program of China (Grant 2002AA302211) and the National Natural Science Foundation of China (Grant 20206032) for their partial financial support of this research.

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Manuscript received Mar. 18, 2004, and revision received Nov. 5, 2004.