

PREPARATION OF Fe_3O_4 /PSt MAGNETIC PARTICLES IN THE PRESENCE OF MAGNETIC FLUID IN ETHANOL/WATER MIXTURE*

Xiao-bin Ding^{a*}, Zong-hua Sun^a, Guo-xiang Wan^a and Ying-yan Jiang^b

^a Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, China

^b Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

Abstract Fe_3O_4 /Polystyrene(PSt) magnetic particles with core/shell structure have been prepared in the presence of Fe_3O_4 magnetic fluid in ethanol/water medium by dispersion polymerization of styrene. A Fe_3O_4 particle formation mechanism was proposed. According to this mechanism, the size of particle nuclei is determined by the extent of aggregation of Fe_3O_4 /oligomer. Magnetic particles with diameter ranging from 5 to 200 μm were prepared under different reaction conditions. Some polymerization parameters such as the concentration of monomer, stabilizer, initiator, and ethanol which affect particle size and size distribution are discussed and their effect on particle formation are explained by the proposed mechanism.

Keywords Fe_3O_4 magnetic fluid, Magnetic particle, Polystyrene, Polymerization, Mechanism

INTRODUCTION

Magnetic polymer microspheres with functional groups such as hydroxyl and carboxyl groups or unfunctionalized groups have been applied to the studies of cell labeling, cell separation, enzyme immunoassay, target drug, etc^[1]. Several advantages of using magnetically separable particles as supports for biological molecules were recognized, for instance, when used as solid supports in immunoassays or other competitive binding assays, magnetic particles permit homogeneous reaction conditions and facilitate separation of bound analytes from unbound ones. Compared to centrifugation, centrifugal separation are time-consuming, require expensive and energy-consuming equipment and pose radiological, biological and physical hazards. Magnetic separations, on the other hand, are relatively rapid and easy, requiring simple equipment.

Different methods have been applied in the preparation of magnetic particles. Coating or encapsulation of inorganic magnetic powder (i.e. iron oxide) with preformed polymers including natural and synthetic polymers is the simplest and oldest method to prepare polymer magnetic particles, e.g. Fe_3O_4 /cellulose, Fe_3O_4 /polyacrylamide, Fe_3O_4 /nylon and Fe_3O_4 /BSA (bovine serum albumin) particles have been prepared by this method^[2-4]. However, the particles obtained by this method are not ideally suitable for biological research, because the particle size is difficult to control, and easy to distort. Recently, preparation of polymer magnetic particles has been reported. Danil^[5] prepared magnetic particles by emulsion polymerization; Iman^[6] prepared magnetic particles having diameter between 104 and 295 μm by a modified suspension polymerization of St; Ugelstad^[7] developed a novel method to prepare magnetic microspheres based on their own

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** Corresponding author: Xiao-bin Ding (丁小斌), E-mail: ioczhang@mail.sc.cninfo.net

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methods of "activated" swelling method. The other methods include polymerization of glutaraldehyde in the presence of ferrofluid^[8] and polymerization of monomer initiated by γ -ray irradiation^[9]. We have also prepared $\text{Fe}_3\text{O}_4/\text{PSt}$ by polymerization of St in the presence of Fe_3O_4 powder (dried state) and used the particles to study the immobilization of a neutral protease^[10].

In this study, $\text{Fe}_3\text{O}_4/\text{PSt}$ magnetic particles with core/shell structure have been prepared in the presence of Fe_3O_4 magnetic fluid by dispersion polymerization of St. A $\text{Fe}_3\text{O}_4/\text{PSt}$ particle formation mechanism was proposed. Some parameters such as the concentration of monomer, stabilizer, initiator and ethanol, which may affect particle size, have been discussed in detail.

EXPERIMENTAL

Materials

Styrene (St) was treated with sodium hydrate solution to remove the inhibitor and stored at 4°C. All other materials were analytical grade, and used without further purification, including polyethylene glycol (PEG, $M_w = 4000$), ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), potassium persulfate (KPS), sodium hydrate (NaOH), hydrogen peroxide (H_2O_2 , 30%) and ethanol etc.

Preparation of Magnetic Fluid

Fe_3O_4 magnetic fluid was prepared by the precipitation-oxidation method as follows: A mixture of 70.0 g PEG and 3.0 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was dissolved in 140 mL of distilled water in a 250 mL round-bottom flask equipped with a condenser, a nitrogen inlet, and a stirrer. Nitrogen was bubbled into the solution and the mixture was stirred for 30 min. 20 mL of an aqueous solution containing 0.6 mL H_2O_2 was added to the system. Reaction was allowed to proceed for 6 h at 50°C at pH 13, which was regulated by 3 mol/L NaOH solution. The resulting magnetic fluid was dialyzed and purified repeatedly by magnetic field separation, decantation, and redispersion.

Preparation of $\text{Fe}_3\text{O}_4/\text{PSt}$ Magnetic Particles

$\text{Fe}_3\text{O}_4/\text{PSt}$ magnetic particles were prepared by polymerization of styrene in the presence of Fe_3O_4 magnetic fluid. Polymerization was carried out in a 250 mL round-bottom four-necked flask equipped with a stirrer, an inlet of nitrogen, a condenser. The required amount of ingredients was placed into the flask. The polymerization was allowed to proceed for 12 h at 70°C under stirring. The products were dialyzed and purified by repetitive magnetic field separation, decantation, and this purification procedure was repeated after 48 h by immersion into 1 mol/L HCl solution.

The average size and size distribution of $\text{Fe}_3\text{O}_4/\text{PSt}$ particles were obtained using an electron microscope (AMRAY-1000), followed by statistical tabulation on a computer. The content of Fe_3O_4 in $\text{Fe}_3\text{O}_4/\text{PSt}$ particle was determined by atomic absorption spectrometry (4100ZL, USA). The size and morphology of magnetic fluid particles were observed under a transmission electron microscope (JEM-100cx, Japan). PEG absorbed on the surface of particles was analyzed by infrared spectroscopy (MX-1E, USA), and thermogravimetry (TGA-7, USA).

RESULTS AND DISCUSSION

Fe_3O_4 Magnetic Fluid and $\text{Fe}_3\text{O}_4/\text{PSt}$ Magnetic Particle Morphology

Figure 1 shows the morphology of the Fe_3O_4 particles. The average diameter was about 50 nm. Some of them formed multiparticle aggregates because of the magneto-dipole interparticle interactions.

In Fig. 2, two absorption bands were observed at 1080 cm^{-1} , and 1630 cm^{-1} , the former bands being assigned to the C—O stretching vibration of PEG, the latter to the C=O, which indicates that some of the hydroxyl groups of PEG were changed to carboxyl groups by the oxidation of H_2O_2 . The amount of PEG absorbed on the surface of Fe_3O_4 was about 10 mg/g.

Figures 3 and 4 are the scanning electron micrograph and histogram of $\text{Fe}_3\text{O}_4/\text{PSt}$, showing

that these particles are polydispersed.

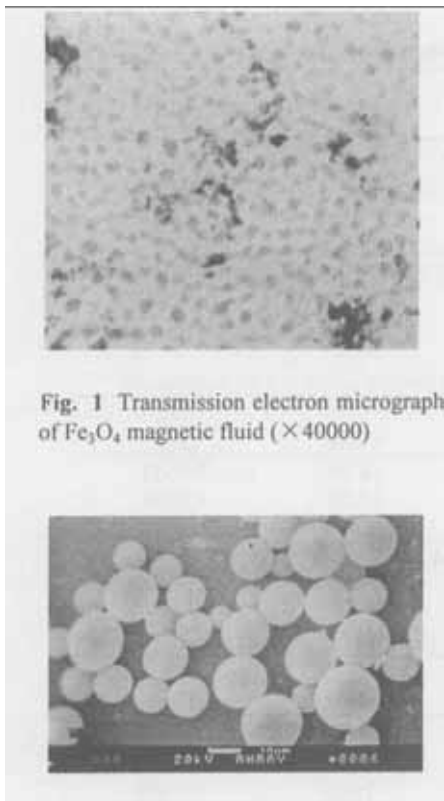


Fig. 3 Scanning electron micrograph of Fe₃O₄/PSt particles
Polymerization conditions: 4.92wt% PEG, 15.75 wt% St, 3.00 wt% KPS, 8.20 wt% Fe₃O₄ magnetic fluid, ethanol/H₂O(mL/mL) = 52/39

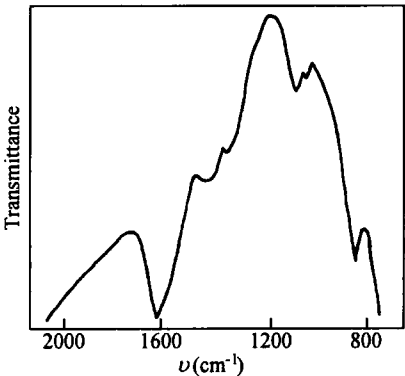


Fig. 2 IR spectra of Fe₃O₄ magnetic fluid

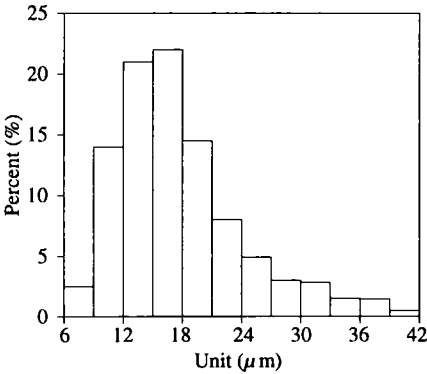


Fig. 4 Histogram of Fe₃O₄/PSt particles
Polymerization conditions: 4.92 wt%PEG, 15.75 wt% St, KPS 3.00 wt% 8.20 wt% Fe₃O₄ magnetic fluid, ethanol/H₂O(mL/mL) = 52/39

Effect of Polymerization Conditions

The monomer concentration could affect particle size in several ways: (a) by changing the polymerization rate, thus changing the rate of nuclei formation; (b) by changing the amount of monomer absorbed on the surface of Fe₃O₄; (c) by changing the solubility of the oligomer chains in the medium and (d) by changing the viscosity of reaction system. Our experimental results show that as the concentration of St was increased from 8.55 to 18.94 wt%, the average diameter increased from 11.10 to 42.60 μm. Higher concentration gave rise to a wider range of size distribution and resulted in the formation of some coagulum. These results are shown in Table 1.

Tseng et al.^[11] proposed that the aggregation nucleation process in dispersion polymerization may be controlled by the adsorption rate of stabilizer/costabilizer. If the generation rate of the oligomeric radicals is much faster than the adsorption rate of the stabilizer, the oligomers would tend to aggregate and form larger particles of various sizes before enough stabilizer is absorbed to stabilize them. In our experiment, we obtained the expected results (Table 2).

The effect of initiator concentration on Fe₃O₄/PSt particle size was also investigated. Polymerizations were performed with four different KPS concentrations. These results are shown

in Table 3. As the KPS concentration was increased from 0.6% to 4.0 wt%, the average size was increased from 3.91 to 58.08 μm .

Table 1. Effect of monomer concentration on $\text{Fe}_3\text{O}_4/\text{PSt}$ particle size and size distribution^a

Wt% (based on total)	Size (μm)			SD ^b (μm)
	Max	Min	Mean	
8.55	41.66	2.68	11.10	6.7252
12.30	55.74	4.74	14.28	9.0769
15.75	92.57	6.78	17.10	7.3818
18.94	160.11	11.11	42.60	25.553

a. 4.92 wt% PEG and 8.20 wt% magnetic fluid (based on total), 3.00 wt%, KPS (based on monomer), ethanol/water (mL/mL)= 52/39;

b. SD: Standard Deviation

Table 2. Effect of PEG concentration on $\text{Fe}_3\text{O}_4/\text{PSt}$ particle size and size distribution^a

Wt% (based on total)	Size (μm)			SD (μm)
	Max	Min	Mean	
1.64	64.05	5.91	18.46	8.9333
4.92	55.74	4.74	14.28	9.0769
6.56	40.18	1.50	5.54	6.0774

a. 12.30 wt% St and 8.20 wt% magnetic fluid (based on total), 3.00wt% KPS (based on monomer), ethanol/water (mL/mL) = 52/39

Table 3. Effect of KPS concentration on $\text{Fe}_3\text{O}_4/\text{PSt}$ particle size and size distribution^a

Wt% (based on total)	Size (μm)			SD (μm)
	Max	Min	Mean	
0.6	22.40	1.93	3.91	2.0603
2.0	50.54	2.40	10.56	7.9134
3.0	55.74	4.74	14.28	9.0769
4.0	144.35	17.35	58.08	25.501

a. 12.30 wt% St, 4.92 wt% PEG and 8.20 wt% magnetic fluid (based on total), ethanol/water (mL/mL) = 52/39

A series of experiments were designed to investigate the effect of ethanol on the $\text{Fe}_3\text{O}_4/\text{PSt}$ particles. These results are shown in Table 4. An interesting phenomenon has been found that ethanol has a greater effect on $\text{Fe}_3\text{O}_4/\text{PSt}$ particle size and size distribution. As the concentration of ethanol was increased from 10.0 to 50.0 wt%, the average size was decreased from 187.32 to 15.37 μm , and the size distribution also became narrow. When the ethanol concentration was further increased to 60.0 wt%, no $\text{Fe}_3\text{O}_4/\text{PSt}$ particles were obtained, but black Fe_3O_4 accumulated granules about 1 mm were obtained instead.

Table 4. Effect of ethanol concentration of $\text{Fe}_3\text{O}_4/\text{PSt}$ particle size and size distribution^a

Wt % (based on total)	Size (μm)			SD (μm)
	Max	Min	Mean	
10.0	400.13	50.13	187.32	98.431
20.0	234.66	34.66	97.20	35.984
30.0	67.57	9.66	26.96	8.4860
50.0	46.99	4.78	15.37	6.8228
60.0	—	—	—	—

a. 12.30 wt% St, 8.20 wt% magnetic fluid, 4.92 wt% PEG and 74.60 wt% (ethanol+water) (based on total), 3.00 wt% KPS (based on monomer)

$\text{Fe}_3\text{O}_4/\text{PSt}$ Particle Formation Mechanism

Figure 5 gives a schematic representation of the mechanism for $\text{Fe}_3\text{O}_4/\text{PSt}$ particle formation and

growth in the ethanol/water system. Before polymerization begins, Fe₃O₄ magnetic particles will disperse in the medium, and part of the monomer, stabilizer and initiator will be absorbed by the surface of the Fe₃O₄ magnetic particles (Fig. 5A). Upon heating, the initiator decomposes and the free radicals react with the monomer to form Fe₃O₄/oligomeric radicals. The Fe₃O₄/oligomeric radicals could be formed in several ways: (1) free radicals generated by initiator react with the monomer absorbed by the surface of Fe₃O₄ particles; (2) Fe₃O₄ particles capture the oligomer radicals generated in the solution and (3) PEG absorbed on the surface of Fe₃O₄ particles forms radicals by hydrogen abstraction and initiates the monomer, as shown in Fig. 5B. At a critical chain length, the oligomers precipitate and form Fe₃O₄/oligomers, which aggregate and absorb stabilizer to form stable particle nuclei (Fig. 5C). Once the particles are formed, they absorb monomer at the surface and grow further through polymerization within the monomer-swollen particles until the monomer is consumed (Fig. 5D).

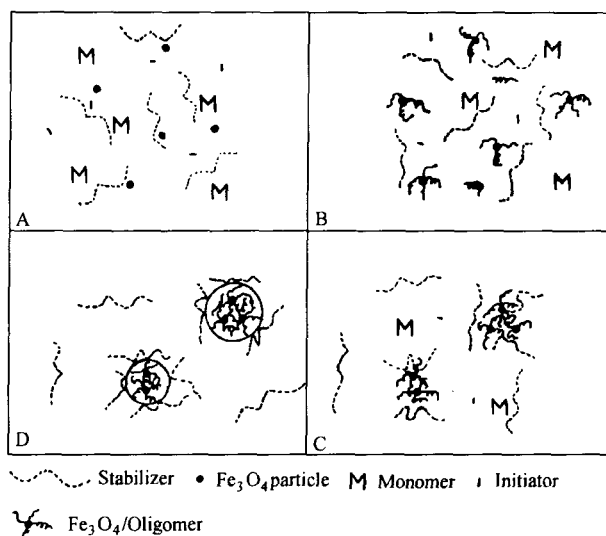


Fig. 5 Schematic representation of Fe₃O₄/PSt magnetic particle nucleation and growth in ethanol/water medium

According to this proposed mechanism, the length of the precipitating oligomer chains depends on the solubility of the oligomer chains in the medium. The size of particle nuclei is determined by the extent of aggregation, which results from the competition between the aggregation of Fe₃O₄/oligomers and the stabilization by absorbing stabilizer. This competition depends on the concentration of Fe₃O₄/oligomers and the rate of adsorption of the stabilizer.

An increase in monomer concentration increases the amount of Fe₃O₄ particles that adsorbs St and the reaction system, it also increases the solubility of oligomer and the concentration of Fe₃O₄/oligomer. All of these effects tend to increase the extent of aggregation and thus increase the average size of Fe₃O₄/PSt particles (Table 1).

With increasing stabilizer concentration, the rate of adsorption of the stabilizer increases, which results in reducing the extent of aggregation and thus reduce the particle size. On the other hand, at low stabilizer concentration, the rate of adsorption of the stabilizer is low, which results in a high rate of uncontrolled aggregation and thus generate larger and more widely distributed dispersion particles (Table 2).

Increasing the initiator concentration causes an increase in the rate of polymerization and the concentration of Fe₃O₄/oligomers, thus increasing the rate of aggregation of the Fe₃O₄/oligomers and the coagulation rate of the unstable nuclei to form larger particle nuclei. It may, therefore,

generate particles of larger size and wider size distribution (Table 3).

Ethanol play an important role in the polymerization system and no satisfactory explanation can be presently offered as to why increasing the ethanol concentration further to 60.0 wt% cannot produce $\text{Fe}_3\text{O}_4/\text{PSt}$ particles. We will discuss this interesting phenomenon in our future work.

CONCLUSION

$\text{Fe}_3\text{O}_4/\text{PSt}$ magnetic particles with core/shell structure were prepared in the presence of Fe_3O_4 magnetic fluid in ethanol/water mixture by dispersion polymerization of styrene. As the monomer or initiator concentration was increased, the particle size of $\text{Fe}_3\text{O}_4/\text{PSt}$ increased. On the other hand, as the stabilizer concentration increased, the particle size of $\text{Fe}_3\text{O}_4/\text{PSt}$ became smaller. Ethanol has an interesting effect on the $\text{Fe}_3\text{O}_4/\text{PSt}$ particles. A $\text{Fe}_3\text{O}_4/\text{PSt}$ particle formation mechanism was proposed. According to this mechanism, the size of particle nuclei is determined by the extent of aggregation of Fe_3O_4 /oligomers.

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