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Preparation and characterization of magnetic P(St-co-MAA-co-AM) microspheres

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Abstract

In this study, magnetic polymer-coated microspheres were prepared by the microemulsion polymerization of styrene (St), methacrylic acid (MAA), acryamide (AM) in the presence of emulsifiers with the size of 1–5 μm. The magnetic material (i.e. Fe_3O_4) coated with oleic acid used in the preparation of the microspheres was synthesized in a classical co-precipitation procedure. The morphological and magnetic properties of the microspheres were investigated by different techniques (i.e. TEM, TGA, optical microscopy, vibrating sample magnetometer). The results indicated that the magnetic microspheres were superparamagnetic, well shaped spheres, mono-dispersed with abundant functional groups on the surfaces of the magnetic microspheres and good thermal stability. The microspheres could be linked well with the avidin and FITC antibody.

Keywords: Microspheres; Magnetic property; Functional groups

1. Introduction

In recent years, there has been a noticeable trend towards the use of the magnetic polymer microspheres for various aspects in biotechnology and biomedical engineering, such as cell separation, immobilized enzyme, protein separations, target drug, and biochemical assays, etc. [1–5].

The magnetic microspheres usually consist of a magnetic core and polymer shell. The magnetic core contains small magnetic nanoparticles of mean diameter typically less than 30 nm, such as Fe_3O_4, nickel, etc. The microspheres have high magnetic content, and can be rapidly separated from the mixtures or to special site easily by a magnetic extraction. The polymer shell is around the magnetic core, which enables modification and protects from particle aggregation and binding to ligands.

Some methods have been reported for the preparation of magnetic microspheres. Magnetic microspheres are mostly obtained by coating magnetite with a polymer by solvent evaporation [6,7], chemical metal deposition inside the matrix [8], emulsion polymerization [9,10], dispersion polymerization [11], suspension polymerization [12], microemulsion polymerization [13], and Ugelstad's method of multistep swelling of template particles and polymerization [14,15].

In our previous studies, magnetic Fe_3O_4 nanoparticles have been prepared in water phase and oil phase [16–18]. The polymer-coated composite magnetic microspheres with –COOH groups located on the surface have been synthesized by the microemulsion polymerization [19]. In this study, we added the monomer AM, and prepared the polymer-coated composite magnetic microspheres with $–NH_2$ groups located on the surface successfully. The magnetic microspheres could be linked well with strep avidin, which can conjugate biotin. On the other hand, the protein could be linked with these microspheres. This paper reports the experiments of the magnetic microspheres linked with avidin and protein, and it also reports the characterizations, including morphology, size, magnetic properties and stabilization of the particles.

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2. Experiment and measuring method

2.1. Material

Styrene (St) washed with a 10% NaOH aqueous solution to remove the inhibitors and divinyl benzene (DVB) was used as co-polymerization monomers. Methacrylic acid (MAA) and acryamide (AM) were used as functional monomers. Sodium dodecyl sulfate (SDS) and cetyl alcohol (CA) were used as emulsifier and co-emulsifier, respectively. Benzoyl peroxide (BPO) and potassium persulfate (KPS) were used as initiators.

2.2. Experiment

In order to obtain homogeneous microspheres with high magnetite content, a two-step process was developed. In the first step, hydrophilous magnetite particles with a diameter of about 10 nm were synthesized in a classical co-precipitation procedure, and at the same time the nanoparticles were coated with oleic acid, which finally were dispersed in St very well. In the second step, the polymer coated magnetite particles were obtained by microemulsion polymerization of St, DVB, MAA and AM in the presence of emulsifiers. In this step, BPO was dissolved in the mixed magnetic organic solution of surface-modified magnetic iron oxide nanoparticles, St, DVB, MAA, AM, forming the ferrofluids. This solution was called A. Secondly, SDS, CA were blended and dissolved in water. The solution was called B. When B was agitated in a four-necked reactor for about half an hour, A was dripped evenly. After 30 min, a solution of KPS was added. The emulsion was washed by ethanol and water after 3 h of polymerization, and then the expected microspheres were obtained. The two-step synthesis route is shown in Fig. 1.

2.3. Measuring methods

The morphological characterizations of the magnetic microspheres were carried out with a transmission electron microscopy (TEM, Jeol-2010, Japan). The samples were diluted and mounted on carbon coated copper grids, and then TEM images were obtained. This characterization was also determined from optical micrographics of the microspheres taken with an optical microscopy (XSP-8C, Shanghai, China).

The magnetism can be observed clearly using a magnet of magnetic field about 3000 G s. The magnetic properties of the microspheres were evaluated using a vibrating sample magnetometer (TM-VSM 2050HGC VSM). A certain amount of magnetic microspheres was placed in the magnetometer. The magnetic properties were then determined by applying an increasing magnetic field over the sample, and the results were used to calculate the magnetism of the microspheres.

The degree of the thermal stability of the magnetic microspheres was carried out with a thermogravimetric analysis—Fourier transfer infrared (TGA-FTIR STA 409 + EQUINOX55) under nitrogen atmosphere. The temperature range was between 30 and 800 °C with a heating rate of 10 k min^{-1}.

The fluorescence microscope was used to investigate the avidin linked microspheres. The reagent kit HCG SERozyme was used to check the microspheres' capacity of conjugation with FITC.

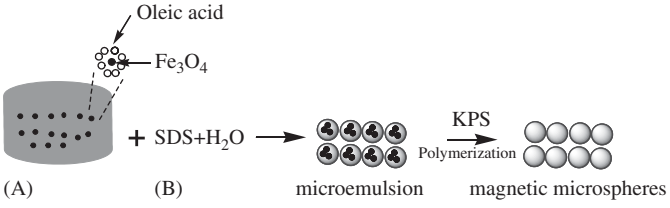

Fig. 1. Formulation of polymer coated magnetite nanoparticles. In the first step, hydrophobic magnetite nanoparticles were produced and dispersed in styrene. In the second step, magnetic microspheres were obtained by the microemulsion polymerization.

3. Results and discussion

3.1. Morphology of the particles

The magnetic nanoparticles can be dispersed in St very well. The structures of these particles were analyzed by TEM. Fig. 2 shows that the magnetic nanoparticles were dispersed in St very well, the length of a magnetic nanoparticle is about 20 nm and the width is about 5 nm. From Fig. 3, one can see that the magnetic nanoparticles are dispersed throughout the interior of the microspheres.

The morphology of the microspheres was investigated using the optical micrographics, which is shown in Fig. 4(a). From Fig. 4(a), one can see that the microspheres are well shaped spheres and mono-dispersed in water. Fig. 4(b)

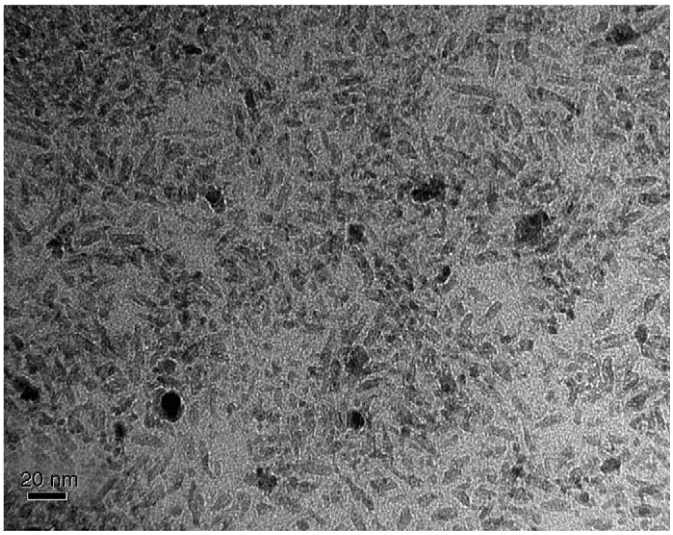

Fig. 2. TEM image of St Ferro-fluid.