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To cite this article: Nguyen Hoang Hai *et al* 2009 *J. Phys.: Conf. Ser.* **187** 012009

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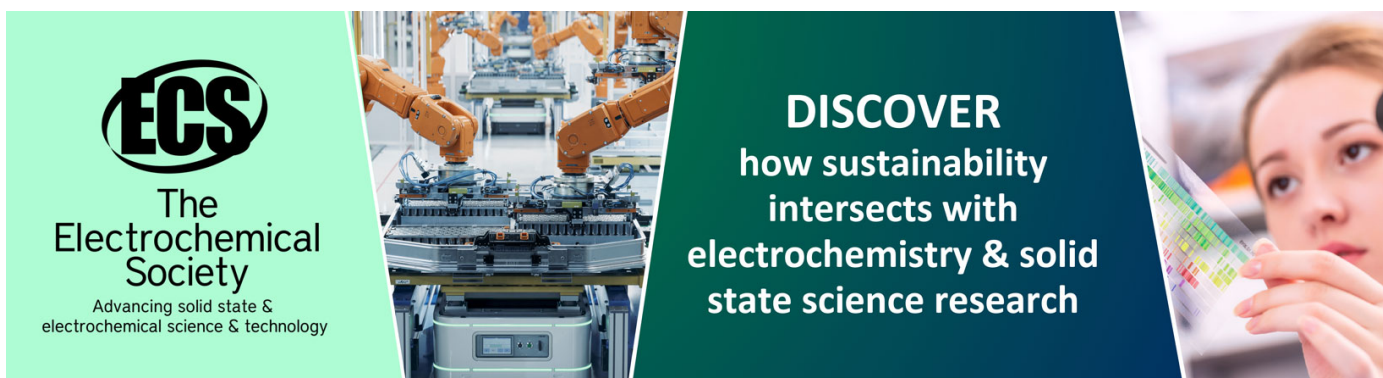
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Preparation of magnetic nanoparticles embedded in polystyrene microspheres

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Abstract. Superparamagnetic particles are widely used for biological applications such as cell separation. The size of the particles is normally in the range of 10 – 20 nm which is much smaller than the size of a cell. Therefore small particles create small force which is not strong enough to separate the cells from solution. Superparamagnetic nanoparticles embedded in Polystyrene microspheres (magnetic beads) are very useful for cell separation. Magnetic beads have been prepared by solvent evaporation of an emulsion. The beads with size of 0.2 μm – 1.0 μm have a saturation magnetization of 10 – 25 emu/g. The change of the amount of surfactants, volatile solvent, magnetic particles resulted to the change of size, magnetic properties of the magnetic beads.

Keyword: Fe_3O_4 , nanoparticles, superparamagnetism, magnetic beads, bio application.

1. Introduction

Superparamagnetic iron oxide nanoparticles embedded in polymer microspheres with appropriate surface chemistry have been widely used experimentally in numerous biological applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and cell separation and labelling [1-6]. All these biomedical and bioengineering applications require that these nanoparticles have a superparamagnetic behaviour with high magnetization values, a narrow particle size distribution, a biocompatibility, and stability under working environments. The typical size of iron oxide nanoparticles is in the range from 10 – 20 nm [7]. Suppose that there is a layer of such nanoparticles coated around a cell, we need a strong external magnetic field gradient to create a strong force to separate the cell from its surrounding medium. One approach to satisfy all requirements in bioapplications is to coat nanoparticles with polymers. Magnetic polymer microspheres should fulfil some criteria to fit further biomedical applications: no sedimentation, uniform size and size distribution, high and uniform magnetic content, superparamagnetic behavior, no toxicity, no iron leaking, high selectivity in case that these particles are used for hyperthermia purposes, and sufficient heat generation at lower frequencies to enhance selective heating. Therefore, magnetite particles homogeneously encapsulated in a hydrophobic polymer which keep away water-soluble components from contacting the magnetite particles are of high interest. Many types of polymers can be used for coating magnetic nanoparticles. Polystyrene is one of the most preferred polymers. There are several reasons to use polystyrene as hydrophobic encapsulation material in biomedical applications, e.g., it is inexpensive and it is a hydrophobic

polymer which allows physical adsorption of antibodies or proteins, it can also be functionalized, e.g., by carboxylic groups which enables covalent binding of antibodies, proteins, or cells [8].

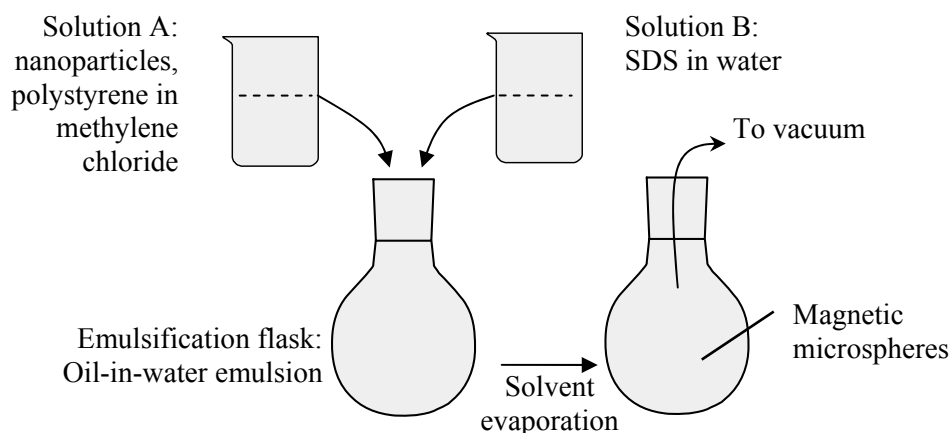


Figure 1. Schematic overview of the four basic steps in microspheres preparation by solvent evaporation.

In Vietnam, nanomaterials, especially nanoparticles, have been paid much attention since 1997. Up to now, dozens of scientific conferences have been organized and hundreds of scientific papers have reported on the preparation, properties and applications of nanomaterials, which have been done by Vietnamese scientists. However, most of the papers focused on the materials with similar properties such as hard/soft nanocomposites [9], CdS/ZnS core/shell structure [10],... This paper will report on another approach to combine nanomaterials with very different properties in one entity – nanoparticles embedded in polymer microspheres. The combination magnetic nanoparticles, quantum dots, metallic nanoparticles, drugs,... in polymer microspheres can create new properties and new applications. There are various techniques, which feature partly competing, partly complementary characteristics, for obtaining iron oxide nanoparticles embedded in polymer microspheres. Many of them are modifications of the three basic techniques: spray-drying [11], phase separation (coacervation) [12], solvent extraction/evaporation [13]. Spray-drying is simple and of high throughput but must not be used for high temperature-sensitive compounds. Moreover, control of the particle size is difficult, and yields for small batches are moderate. Coacervation is normally impaired by residual solvents and coacervating agents found in the microspheres. In addition, it is not well suited for producing microspheres in the low micrometer size range. Solvent extraction/evaporation neither requires elevated temperatures nor phase separation including agents. Controlled particle sizes in the nano- to micrometer range can be achieved, but careful selection of encapsulation conditions and materials is needed to yield high encapsulation efficiencies and a low residual solvent content. Microsphere preparation by solvent extraction/evaporation consists of four major steps: (i) dissolution or dispersion of the bioactive compound often in an organic solvent containing the matrix forming material; (ii) emulsification of this organic phase in a second continuous (frequently aqueous) phase immiscible with the first phase; (iii) extraction of the solvent from the dispersed phase by the continuous phase, which is optionally accompanied by solvent evaporation; (iv) harvesting and drying of the microspheres.

In this paper, we used the solvent evaporation technique to make iron oxide Fe_3O_4 embedded in polystyrene microspheres. This is one of the first advances in this field in Vietnam.

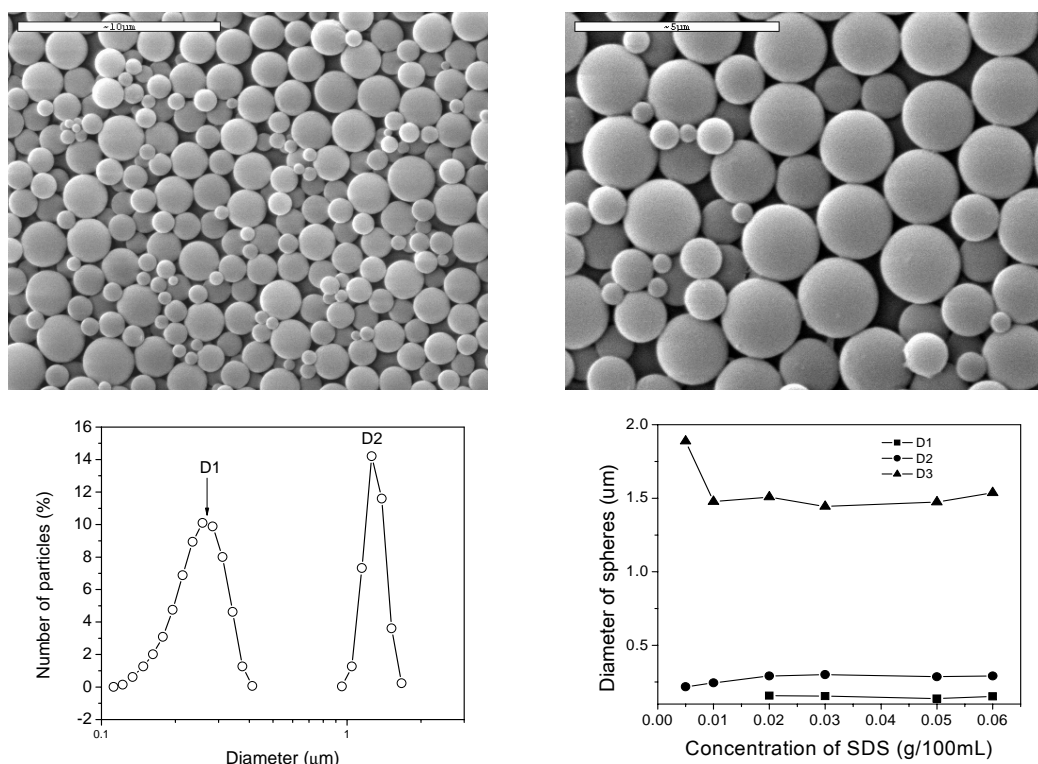


Figure 2. SEM images of the polystyrenes microspheres (top), a typical size distribution of the spheres (bottom left) and the particle diameter of the samples (bottom right).

2. Experimental

Magnetite Fe_3O_4 nanoparticles were synthesized by coprecipitating a $\text{Fe}^{2+}/\text{Fe}^{3+}$ mixed solution (the molar ratio $\text{Fe}^{2+} : \text{Fe}^{3+} = 1 : 2$) with a NH_4OH solution in air [7]. In a typical reaction, 3.98 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (molar concentration 0.1 M) and 10.82 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were dissolved in 200 ml of distilled water. An 18 ml solution of NH_4OH 25 % diluted to 100 ml by water, was then added to the $\text{Fe}^{2+}/\text{Fe}^{3+}$ solution at a rate of one drop per second at room temperature with constant rate. After the reaction, black precipitates of magnetite were washed five times by using distilled water and magnetic decantation to remove all the soluble substances. 0.5 g of magnetic nanoparticles in 20 ml of water were then vigorously mixed with 10 ml of oleic acid (OA, 9-Octadecenoic acid $\text{C}_{18}\text{H}_{34}\text{O}_2$, CAS number: 112-80-1) in order to coat the magnetic nanoparticles with a single surfactant layer of OA (denoted as OA-coated nanoparticles) and to disperse them into a volatile solvent methylene chloride (CH_2Cl_2). The excess OA was washed three times by using magnetic decantation. An amount of 0.1 g of polystyrene was dissolved in 10 ml of methylene chloride by using a magnetic stirrer (IKA RH basic KT/C) at room temperature. The obtained solution was used to disperse 0.01, 0.02, and 0.03 g (correspondingly, three samples with 10, 20, and 30 wt. % of nanoparticles) of OA-coated magnetite nanoparticles to form solution A (figure 1). Solution A was then emulsified with solution B containing water and sodium dodecyl sulfate (SDS) as a stabilizer, which prevents the organic droplets from coalescing. The concentration of SDS was changed from 5 – 60 mg/100 ml. The emulsion process was conducted by mixing solution A and solution B with the help of an overhead stirrer (IKA RW 20) at room temperature. The speed and duration of agitation of stirring affect the size of the droplets, with stronger agitation breaking up the polymer into smaller droplets. Here, we fixed the speed A and the duration time of 2 mins. Methylene chloride was eliminated by using a rotating evaporator (Buchi rotavapor R II) for about 20 mins at 60°C and the microspheres were remained in the flask (figure 1).

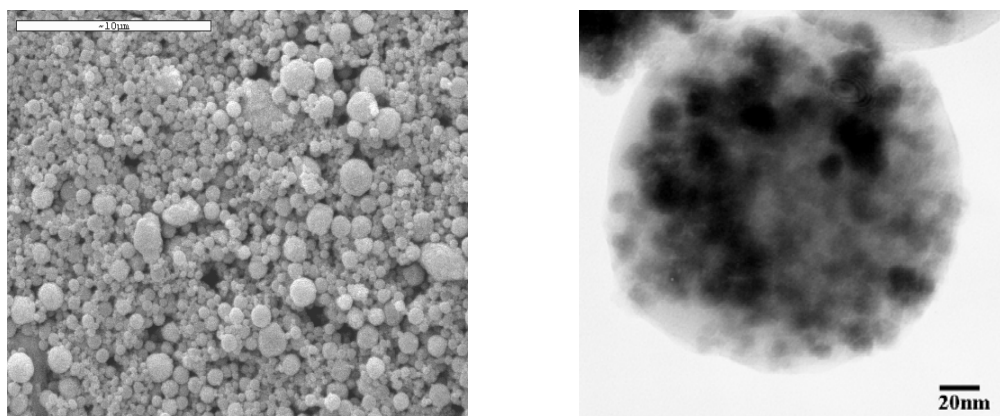


Figure 3. SEM images of the polystyrenes microspheres (top), a typical size distribution of the spheres (bottom left) and the particle diameter of the samples (bottom right).

In the microspheres, magnetite nanoparticles were distributed randomly to form magnetic microspheres (magnetic beads). The microspheres were washed with doubly distilled water and collected by using a centrifuge (Hettich Universal 320, 9000 RPM, 20 mins). Another series of samples in which there was no magnetic nanoparticles have been prepared by a similar method as reference samples. The difference was that, in the solution A, there was only polystyrene dissolved in methylene chloride without magnetic nanoparticles. As a result, the microspheres did not contain magnetic nanoparticles.

The structure of the magnetite nanoparticles was analyzed by using a Bruker D5005 X-ray diffractometer (XRD). Magnetic measurements were conducted by using a DMS-880 sample vibrating magnetometer (VSM) with maximum magnetic field of 13.5 kOe at room temperature. The particle morphology was obtained from a transmission electron microscope (TEM JEM1010-JEOL) and a scanning electron microscope (SEM JEOL 5410 LV).

3. Results and discussion

Figure 2 shows the SEM images of the reference non-magnetic polystyrene microspheres. The shape of the products were almost ideally spherical with very smooth surface. The spherical shape indicated the isotropic property of the oil-in-water emulsion. It is evident that there were two size distributions of the microspheres with different diameters which were denoted by D1 (for small microspheres) and D2 (for big microspheres). In some samples, there were three size distributions with corresponding diameters (denoted by D1, D2, and D3 for the diameter from small to large). The diameters and the size distributions were given in figure 2. Figure 2 (right bottom) presents the diameter of the reference microspheres as a function of SDS concentration. Samples with SDS concentration were smaller than 0.2 had two size distributions and the diameter was too large. Therefore, later on, we chose the concentration of 30 mg of SDS in 100 ml to make magnetic microspheres. When the SDS concentration was equal or larger than 0.2, there were three size distributions and the diameter of the microspheres kept almost constant with SDS concentration. The figure can be explained by the fact that when SDS concentration was high, there was enough SDS to form small oil droplets in the emulsion. After solvent evaporation process, the final microspheres were small.

Figure 3 shows the SEM image of magnetic microspheres. It is not like in the case of the reference microspheres, the shape of microspheres was not ideally spherical with rough surface. This may come from the hydrophobic OA-coated magnetite nanoparticles which were free coated around the microspheres and affected to the surface energy of oil droplets in the emulsion. The TEM image shows the heterogeneous contrast of a microsphere. The dark areas present for the crystalline magnetite nanoparticles and the light areas indicate the amorphous polystyrene.

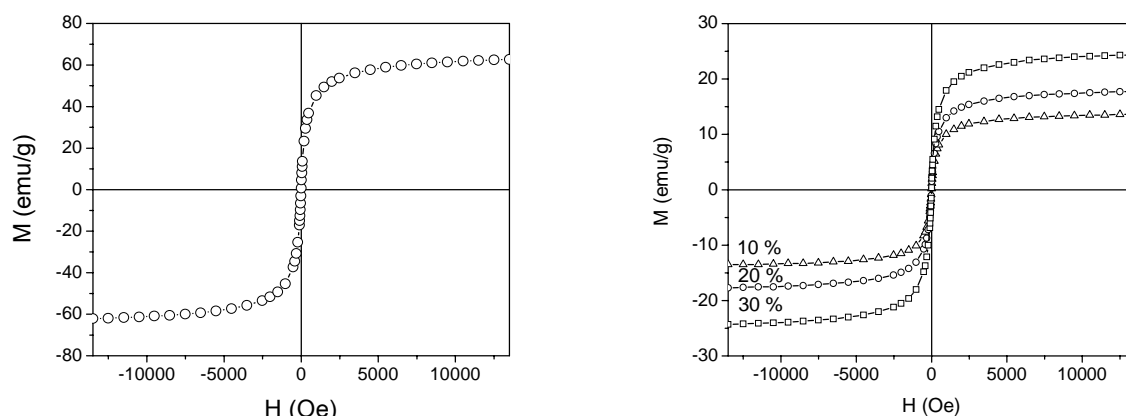


Figure 4. Magnetic curve of magnetite nanoparticles (left) and magnetic microspheres with 10, 20, and 30 wt. % (right) the nanoparticles embedded in polystyrenes microspheres.

Magnetic curves of the magnetite nanoparticles are shown in figure 4. The curve of magnetite nanoparticles shows a superparamagnetic behavior with the saturation magnetization, M_s , of 60 emu/g. The superparamagnetic property did not change when the nanoparticles were embedded in the microspheres. But M_s reduced with the ratio of the nanoparticles in the magnetic microspheres. The values of M_s in magnetic microspheres were higher than expected from the ratio of magnetic nanoparticles. This can be ascribed to the preparation process. During washing by water and magnetic decantation, a commercial magnet was used. The field gradient of the magnet may be not strong to collect microspheres with low magnetization. Therefore, a portion of polystyrene was lost which made the saturation magnetization of the expected values calculated from the ratio smaller than that of magnetic nanoparticles in the microspheres.

4. Conclusion

We have fabricated magnetite nanoparticles embedded in polystyrene microspheres by solvent evaporation techniques. By changing the preparation conditions we could control the diameter, size distribution and saturation magnetization of the final magnetic microspheres. This technique can be applied to make other types of nanoparticles such as metal and semiconducting materials embedded in polymer microspheres.

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Acknowledgments

This work is supported by the Vietnamese Fundamental Research Program for Natural Science (Project 406506) and the Key Project of the Vietnam National University, Hanoi (QGTD.08.05). The authors would like to thank Mr. Nguyen Dang Phu and Mr. Nguyen Minh Hieu for the experimental help.