Synthesis and Characterization of Composite Magnetic Microspheres of Artemisia Seed Gum and Chitosan

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Received 11 November 2005; accepted 28 August 2006
DOI 10.1002/app.25438
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this study, composite magnetic microspheres of artemisia seed gum and chitosan were prepared in a well-shaped spherical form with a size range of 230–460 μm by the suspension crosslinking technique for use in the application of magnetic carrier technology. The magnetic material used in the preparation of the composite microspheres was prepared by precipitation from $FeCl_3$ and $FeSO_4$ solution in basic medium. The morphological, magnetic properties, and the functional groups of the microspheres were characterized by different techniques (i.e., SEM, magnetometry, and FTIR). The results demonstrated that the stirring rate of the suspension and the Fe_3O_4/chitosan ratio are the most effective parameters for the average of the size distributions and the magnetic quality of the microspheres, while the amount of artemisia seed gum and Tween-80 have no significant effect on these properties. The best magnetic quality of the composite magnetic microspheres is around 4.08 emu/g microspheres at 10 KG magnetic field intensity. The thermal stability of the composite magnetic microspheres was measured by using DSC methods. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 3045–3049, 2007

Key words: composite magnetic microspheres; artemisia seed gum; chitosan; properties; functional groups; stability

INTRODUCTION

In all biochemical processes, procedures such as separation, purification, and unit operation (i.e., centrifugation, precipitation, mixing, or the application of the high pressure) have to be performed, and these operations can result in malformation (i.e., decomposition, inactivation, or deformation) of the biomolecules. Magnetic carrier technology appears to be a significant alternative for the solution of these biomolecule malformations. With this technology, magnetic carriers are used as the support materials and they can be easily separated from the reaction medium and stabilized in a fluidized bed reactor by applying a magnetic field. On the other hand, the use of magnetic particles can reduce the capital and operational costs. The most popular application of magnetic carrier technology is wastewater treatment, immobilization of enzymes or other biomolecules, and the preparation of immunological assays.[1–5]

Magnetic carriers are most commonly manufactured from polymers, since they have a variety of surface functional groups, which can be tailored to specific applications. In the literature, different types of natural and synthetic polymers (i.e., calcium alginate, polystyrene, polyacrylamide, polyvinyl alcohol, nitrocellulose, and polyvinyl butyral) have been used in the preparation of magnetic carriers. Artemisia seed gum and chitosan can be used as base materials for magnetic carriers. They are familiar natural polymers and very cheap biopolymers too. In this study, composite magnetic microspheres were prepared and characterized for application in magnetic carrier technology (in particular, enzyme immobilization and bioaffinity chromatography). The suspension crosslinking technology was used for the production of composite magnetic microspheres and glutaraldehyde was used as a crosslinker.[6] The effects of some reaction conditions on the average of the size distributions and on the magnetic properties are evaluated (the stirring rate of the heterogeneous medium, the Fe_3O_4/chitosan ratio). We used selected parameters in this evaluation. The functional groups and thermal stability of the composite magnetic microspheres were also identified.

MATERIALS AND METHODS

Materials

Artemisia seed gum and chitosan were obtained from Yuhuan (Zhejiang, China). Acetic acid glacial solution was used as solvent for the chitosan polymers and glutaraldehyde was used as the crosslinker. The suspension medium was composed of a mixture of petroleum ether and mineral oil, and Tween-80 was added as an emulsifier. All chemicals were of analytical grade and no further purification was required.

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Journal of Applied Polymer Science, Vol. 103, 3045–3049 (2007)

Preparation of magnetic fluid

Magnetic fluid was synthesized as follows: 35% (w/v) ferrous sulfate solution, 54% (w/v) ferric chloride solution, and 36% (w/v) sodium hydroxide solution were prepared using distilled water. Then the ferric salt and ferrous salt were mixed, stirred, and heated. When the temperature reached 55°C, the alkaline solution was added. The mixture was stirred for 30 min, and then 5 g of polyethylene glycol-10,000 (PEG-10,000) was added. The temperature was raised to 80°C and maintained for 30 min. The mixture was then neutralized while cooling, and the magnetic fluid was prepared.

Preparation of composite magnetic microspheres

In a typical procedure, 1% suspension (w/t) of artemisia seed gum was prepared using distilled water containing a certain amount of magnetic fluid. Then, 1.5% chitosan solution, prepared using 2% acetic acid glacial solution, was added.[7] The mixture was added dropwise to the dispersion medium, which was composed of mineral oil, petroleum ether (15 : 45, v/v), and emulsifier (Tween-80, 0.7 mL). During this process, the dispersion medium was stirred at 1000 rpm at room temperature. Twenty minutes later, 1 mL glutaraldehyde was added to the dispersion medium and stirring was maintained for about 1 h. At the end of this period, the composite magnetic microspheres were collected using a magnet and washed consecutively with petroleum ether and acetone. The microspheres were dried in an oven at 40°C for two days. In this part of the study, the stirring rate of the suspension medium and the Fe_3O_4 content/chitosan ratio were changed in an investigation of the effects of these parameters on the average of the size distributions of composite magnetic microspheres. There parameters were varied in the range of 500–1000 rpm and 1 : 3–1 : 1 mL/g, respectively.

Characterization of composite magnetic microspheres

Morphology

The morphological characterization of the composite magnetic microspheres was carried out with a scanning electron microscope (SEM, HITHCHI, S-450). A certain amount of composite microspheres was dropped onto a sample holder and placed in a vacuum oven at room temperature to dry. The samples were coated with gold, and then SEM micrographs were obtained.[7,8]

The average of the size distributions

The average of the size distributions of the composite magnetic microspheres was also determined from SEM micrographs of the microspheres. The average size and standard deviation of the microspheres (three to four micrographs, each containing ~10–20 microspheres) in the micrographs were evaluated.

Magnetic properties

The degree of magnetism of the composite magnetic microspheres was evaluated using a vibrating-sample magnetometer (7304, VSM). A certain amount of the microspheres was balanced and placed in the magnetometer. The degree of magnetism of the microspheres was then determined by applying an increasing magnetic field over the microspheres, and the results were used to calculate the magnetic quality of the microspheres. The most effective parameters determining the magnetic quality of the microspheres were the stirring rate and the magnetic materials contents of the microspheres, and these parameters were varied in the range of 500–1000 rpm and 1 : 3–1 : 1 mL Fe_3O_4/g chitosan, respectively.[9]

DSC methods

DSC was performed using a PerkinElmer DSC instrument with intracooler. The samples were purged with the atmosphere of nitrogen. The heat flow rate was recorded from 25 to 500°C, at a rate of 10°C/min. Indium (m.p. = 156.8°C) was used as the standard reference materials to calibrate the temperature and energy scales of the DSC instrument.[10–12]

Functional groups

FTIR spectra were recorded and after processing we also record the FTIR spectra of the composite magnetic microspheres to identify the functional groups of the composite magnetic microspheres. The FTIR spectra were obtained using a FTIR spectrophotometer (Alpha-centauri). In a typical procedure, around 0.25 mg of composite magnetic microspheres was thoroughly mixed with IR-grade KBr (0.1 g) and pressed (10 ton) into a tablet form and the spectrum was then recorded.

RESULTS AND DISCUSSION

Morphology of the composite magnetic microspheres

The morphology of the composite magnetic microspheres was investigated using SEM. Scanning electron micrographs are shown in Figure 1. As can be seen, the composite magnetic microspheres are well-shaped spheres with a rather rough surface. Figure 1(c) shows the microstructure of the surface of the microspheres, and it can be clearly seen that Fe_3O_4 particles

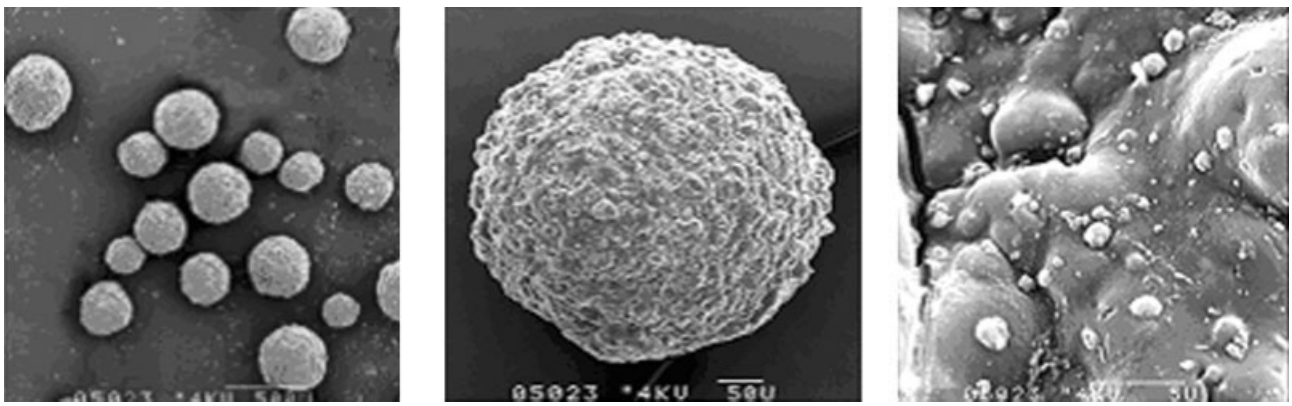

Figure 1 Scanning electron microscopy of composite magnetic microspheres. (a) 70 times; (b) 360 times; (c) 8600 times.

was achieved on the surface of the microspheres and the microspheres were porous.

The average of the size distributions

The stirring rate of the suspension medium and the Fe_3O_4/chitosan ratio were selected and evaluated as being the most effective parameters determining the average of the size distributions of the composite magnetic microspheres and the results are summarized in the following subsections.

Stirring rate

The stirring rate of the suspension medium was varied between 500 and 1000 rpm for investigation of the effects of the stirring rate on the average of the size distributions. The volume/mass ratio was fixed at 1 : 3 mL/g during the preparation of the microspheres with different stirring rates. The results obtained are shown in Figure 2, and it can be seen that the size of the microspheres decrease with the increasing stirring rate. This trend can be explained by the energy transfer differences for different stirring rates. When the stirring rate is increased, the energy transferred to the suspension medium is increased and the polymer solution can be dispersed into smaller droplets and the size is reduced.

Fe_3O_4/chitosan ratio

The Fe_3O_4/chitosan ratio was varied in the range 1 : 3–1 : 1 (mL/g). The stirring rate was fixed at 700 rpm. The size of the microspheres decreased with increasing Fe_3O_4 content, as shown in Figure 3. There are may be two reasons for this phenomenon. First is that the dispersion of the materials into microspheres is more difficult as the Fe_3O_4/chitosan ratio increases, and second is that the increase of water phase in the heterogeneous medium.

Magnetic properties

The magnetic properties of the composite magnetic microspheres were evaluated using a vibrating-sam-

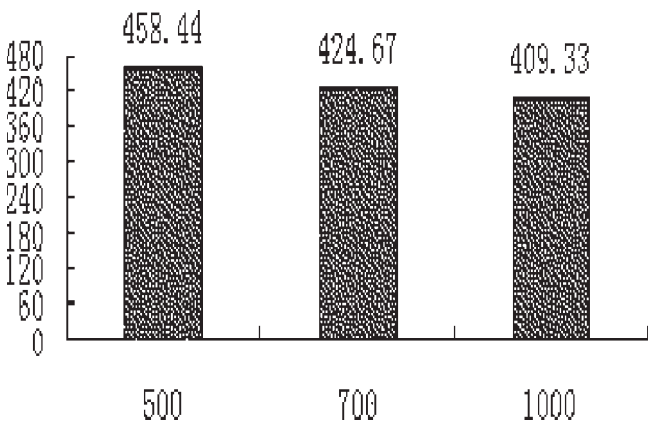

Figure 2 Effects of stirring rate on the size/size distribution. Axis of horizontal: stirring rate (rpm); axis of vertical: microsphere size (μm).

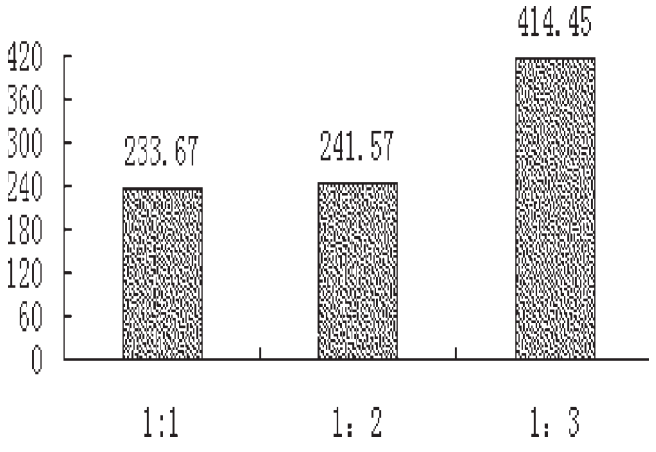

Figure 3 Effects of Fe_3O_4/chitosan ratio on the size/size distribution. Axis of horizontal: Fe_3O_4/chitosan ratio (mL/g); axis of vertical: microsphere size (μm).

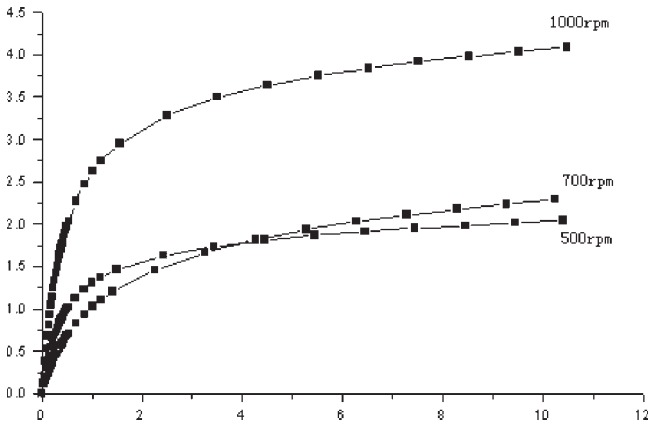

Figure 4 Effects of stirring rate on the magnetic properties. Axis of horizontal: magnetic field intensity (KG); axis of vertical: emu/g microspheres.

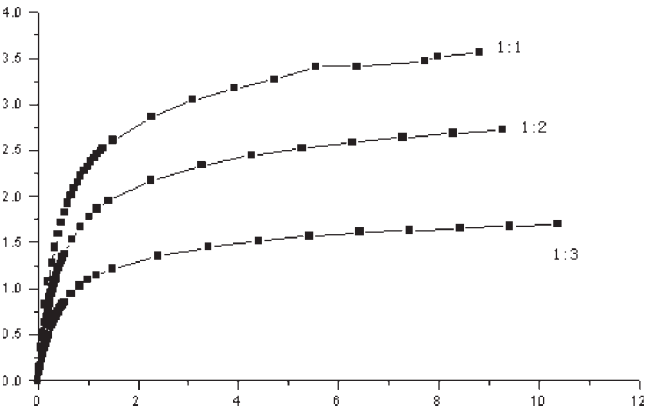

Figure 5 Effects of Fe_3O_4 content on the magnetic properties. Axis of horizontal: magnetic field intensity (KG); axis of vertical: emu/g microspheres.

ple magnetometer. The most effective parameters determining the magnetic properties were the stirring rate of the suspension medium and the Fe_3O_4/chitosan ratio, as in the case of the average of the size distributions evaluation. The results obtained are summarized in the following subsections.

Stirring rate

The stirring rate of the suspension medium was varied between 500 and 1000 rpm for the investigation of the effects of stirring rate on the magnetic properties of the composite magnetic microspheres. The results are shown in Figure 4. As can be seen, in all cases, a 10 KG magnetic field was found to be sufficient to excite almost all of the dipole moments of 1.0 g composite magnetic microspheres. The maximum magnetic quality (i.e., ~4.08 emu/g microspheres) was achieved at the highest stirring rate. This behavior can be explained by the change in microspheres size with a change in stirring rate. This indicates that the microspheres sizes decrease by the increasing stirring rate, as discussed earlier, and magnetic excitation is easier in the case of a smaller microspheres size.

Fe_3O_4/chitosan ratio

The Fe_3O_4/chitosan ratio was varied in the range of 1 : 3–1 : 1 (mL/g) for investigation of the effects of the Fe_3O_4/chitosan ratio on the magnetic properties. The results are shown in Figure 5. As can be seen, the magnetic quality increases significantly with Fe_3O_4 content. It is easy to explain this behavior by the relationship between emu and the Fe_3O_4 content. Furthermore, similar behavior for this parameter had been reported previously.[13,14]

DSC analysis

Figure 6 shows the results obtained from DSC analyses of composite magnetic microspheres. Composite magnetic microspheres show that a strong transition was found at 300°C and this may be due to the faster decomposition of the microspheres. And chitosan microspheres show a strong transition at 200°C.[10] The reason for this phenomenon may be the crosslinking of artemisia seed gum and chitosan. Artemisia seed gum and chitosan are two single systems. When the crosslinker makes them a composite system, the free energy decreases. So, the thermal stability becomes stronger than that of Artemisia seed gum and chitosan. The results demonstrated that the composite magnetic microspheres have a better thermal stability than that of chitosan microspheres.

Functional groups

The functional groups of materials are very important for diverse applications, especially for biotechnological purposes. Therefore, the present functional groups should be kept even if the shape (or geometry) is changed into a new form (i.e., microspheres or membrane). FTIR spectra of the materials and of the form processed into microspheres were recorded. The spec-

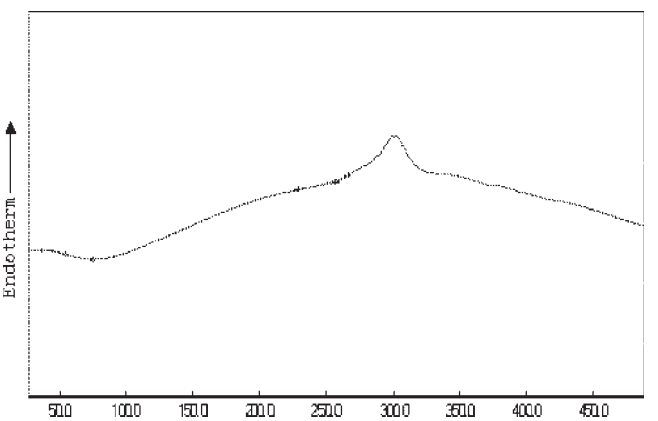

Figure 6 DSC of composite magnetic microspheres. Axis of horizontal: temperature (°C).

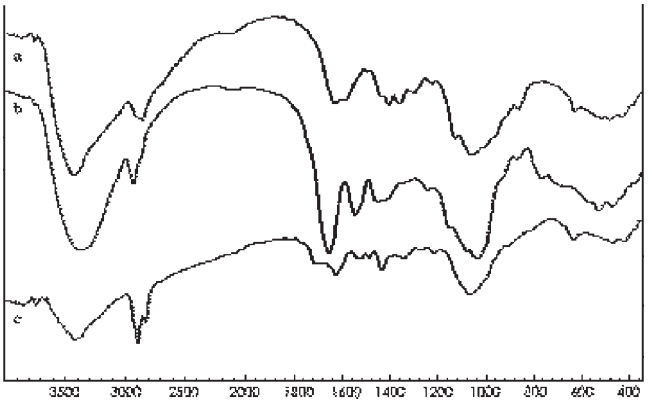

Figure 7 FTIR spectrum of (a) chitosan; (b) artemisia seed gum; (c) composite magnetic microspheres.

tra are shown in Figure 7 for all forms (i.e., artemisia seed gum, chitosan, and composite magnetic microspheres). The OH groups present in artemisia seed gum and chitosan are clearly seen at 3400 cm^{-1} and they are also present in the case of the microspheres forms. In addition, the carbonyl bands at around 1700 cm^{-1} show glutaraldehyde crosslinking in the case of the microspheres forms. The other bands are similar for both materials and microspheres forms. It implied that the magnetic composite microspheres produced reactive aldehyde groups and these then react with the free amino groups of enzyme. The composite magnetic microspheres can be used for industrial application of the immobilization of enzymes. Therefore, enzyme was mostly immobilized on the surface of the activated magnetic carriers by covalent binding.

CONCLUSIONS

In various kinds of biochemical and biotechnological applications (such as immobilization of biomolecules, wastewater treatment, affinity chromatography, and drug delivery systems), the separation and recovery steps are difficult and expensive with respect to conventional techniques. Magnetic carrier technology is a very promising alternative to enhance the operational performance of these steps. The most important parameters in magnetic carrier technology are the economy and physicochemical characteristics of the carriers.

In this study, well-shaped spherical composite magnetic microspheres were produced using a very cheap biopolymer (i.e., artemisia seed gum and chitosan). The composite magnetic microspheres were evaluated based on morphology, size/size distribution, magnetic properties, functional groups, thermal stability. The results show that composite magnetic microspheres can be produced in the size range of 230–460 μm by changing the operational parameters (i.e., stirring rate and Fe_3O_4/chitosan ratio). The most important aspect for the magnetic carrier is a sufficient magnetic field intensity to excite all the dipole moments of each gram of magnetic carrier. This value defines the magnetic quality of the carriers and is in the range of 8–20 KG for various applications. The magnetic field intensity for composite magnetic microspheres was found to be in the range of 8–12 KG in this study. Therefore, composite magnetic microspheres are promising, as a potentially good magnetic support to be employed in magnetic carrier technology with good economical aspects and good magnetic quality.

This study was carried out under the auspices of the foundation of Gansu key laboratory of polymer material.

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