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Journal of Magnetism and Magnetic Materials 293 (2005) 328–333
Journal of magnetism and magnetic materials
www.elsevier.com/locate/jmmm

# Preparation and characterization of magnetic chitosan particles for hyperthermia application

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Available online 3 March 2005

**Abstract**

The size and shape of magnetic chitosan particles were found to be dependent on both the barium ferrite/chitosan (BF/C) ratio and viscosity of a chitosan solution. The saturation magnetization of magnetic chitosan particles varied directly with the BF/C ratio, while coercivity remained almost constant. Notably, incorporated chitosan was shown to exert substantial activity with regard to low cytotoxicity and high heating rate.


*Keywords:* Barium ferrite; Coercivity; Heating rate; Hyperthermia; Chitosan; Saturation magnetization; Toxicity

## 1. Introduction

Magnetic particles have been increasingly used in the fields of bioscience and medicine since the 1970s. Due to unique magnetic features not present in other materials, magnetic particles can be utilized almost exclusively in some special medical techniques, most notably, separation for purification and immunoassay, drug delivery and targeting, magnetic resonance imaging (MRI), and hyperthermia [1-4]. Loss of hysteresis under an alternative magnetic field is a very important property exhibited by magnetic particles, as it enables effective hyperthermia [5]. The use of magnetic particles to induce hyperthermia in biological tissues is an important factor for tumor therapy. Hyperthermia is a therapeutic procedure, which is used to raise the temperature of a region of the body affected by cancer to 42–46 °C. This method involves the introduction of ferromagnetic

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0304-8853/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jmmm.2005.02.027

or superparamagnetic particles into tissues, and their subsequent irradiation with an alternating electromagnetic field.

The biological application of magnetic particles first requires a previous evaluation of toxicity. The toxicity of magnetic particles appears to be very dependent on either physical state or chemical composition [6]. It has been reported that some magnetic particles exhibit moderate to severe toxicity [7,8]. Moreover, it can be expected that magnetic particles may release toxic substances into surrounding tissues more readily under an alternating electromagnetic field. Thus, it becomes necessary to absolutely minimize the possibility of the release of toxic substances under therapeutic circumstances. In an attempt to ameliorate this problem of toxicity, we attempted to utilize magnetic chitosan particles in this study.

Chitosan is the alkaline deacetylated product of chitin, which is derived from the exoskeleton of crustaceans [9]. It has been shown to be a potentially useful biomaterial, and is used as a wound dressing material, drug delivery vehicle, and candidate for tissue engineering, owing to its good biocompatibility and low toxicity. Among various applications, drug delivery application is most closely connected with hyperthermia. Chitosan has a variety of surface functional groups which can be tailored toward drug delivery. If magnetic chitosan particles are able to release chemotherapeutic drugs during hyperthermia, the hyperthermic effect will increase. However, there have, as yet, been few investigations regarding the properties of magnetic chitosan particles in hyperthermia applications.

In this study, magnetic chitosan particles were prepared and characterized for hyperthermia application. A crosslinking reagent was not used, due to its toxicity. The surface morphology, swelling rates, magnetic properties, heating rates, and cytotoxicity of magnetic chitosan particles were evaluated.

## 2. Experimental

By dissolving chitosan powder (Sigma, St. Louis, MO, USA) in 10 ml of 2% acetic acid solution, 0.5–2.0% chitosan solutions were prepared. Barium ferrite (Aldrich, Milwaukee, WI, USA)-chitosan solutions with different weight ratios (1, 5, and 10) of barium ferrite/chitosan (BF/C) were dispersed via ultrasonication. The barium ferrite-chitosan solution was added dropwise to a 1 M NaOH solution with a syringe, resulting in the formation of magnetic chitosan particles. These were separated by filtration, washed several times with water and ethanol, and then completely dried at 40 °C under vacuum.

The size of the magnetic chitosan particles was measured using an optical microscope (OM; Hirox, Tokyo, Japan). The average size and standard deviation of the 10 particles in the optical micrographs were evaluated to determine values for size.

The morphological characterization of the magnetic chitosan particles was performed using a scanning electron microscope (SEM; Hitachi, Tokyo, Japan). The surfaces were gold-coated with a thickness of 300 Å using an ion sputter device (Eiko, Tokyo, Japan), and then examined using SEM at an accelerating voltage of 3 kV, and digital images were captured at varying degrees of magnification.

The degree of swelling exhibited by the magnetic chitosan particles was evaluated by placing the size-measured particles in pH 7.4 phosphate buffered saline (PBS; Gibco, Grand Island, NY, USA) and incubating at 37 °C for 7 days. After 7 days, the particles were removed from the PBS, and measured for size increases due to water absorption. Five magnetic chitosan particles were prepared for the evaluation of swelling degree. The swelling degree ($S$) was assessed according to the following equation:

$$S(\%) = [(S_f - S_i)/S_i]100,$$

where $S_f$ is the size of the swollen particles and $S_i$ is the initial size of the particle.

The magnetic properties of the magnetic chitosan particles were assessed with a vibrating sample magnetometer (VSM; Lake Shore Cryotronics, Westerville, OH, USA). The magnetic properties of the particles were evaluated in terms of saturation magnetization and coercivity. A certain amount of magnetic chitosan particles was balanced and placed in the magnetometer. The magnetic properties of the particles were then

determined by applying an increasing magnetic field to the particles.

Changes in temperature of the magnetic chitosan particles under an alternating magnetic field at various frequencies were observed using a heating system designed in our laboratory. The temperature change of the particles was determined by measuring the temperature of 1 ml double-distilled water containing barium ferrite particles and magnetic chitosan particles under an alternating magnetic field, with a frequency of 2.38 MHz. The weights of the magnetic chitosan particles for the temperature change measurement were calculated according to BF/C ratio, in order to fix the amount of the barium ferrite in the magnetic chitosan particles to 10 mg.

The cytotoxicity of the magnetic chitosan particles was evaluated using an agar diffusion test. L929 cells, mouse fibroblast cell line, were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were cultured in RPMI Medium 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (PS; Gibco, Grand Island, NY, USA) up to a cell concentration of $1 \times 10^5$ cells/ml in a monolayer state under a humidified 5% $CO_2$ atmosphere at 37 °C. The agar media was prepared from 50% agar (Gibco BRL, Paisley, Scotland) and 50% culture medium supplemented with 5% FBS. The agar media were allowed to gel at room temperature for 30 min. Each agar medium was then stained with a neutral red solution for 1 h. A couple of barium ferrite particles and magnetic chitosan particles were laid onto an area of $5 \times 5\,mm^2$ of each petri-dish filled with the agar medium, with a negative (polyethylene) and positive (polyvinylchloride) control, after removal of the staining solution. Each petri-dish was subsequently incubated under a humidified 5% $CO_2$ air atmosphere at 37 °C, for 24 h. The decolorization and lysis indices were determined using a light microscope (LM; Olympus, Tokyo, Japan). The decoloration index was determined by the grade of decolorizing cell zone, and the lysis index was measured by the grade of cell lysis underneath the particles.

## 3. Results and discussion

When the barium ferrite-chitosan solution was added dropwise to a 1 M NaOH solution with a syringe, sphere-shaped magnetic chitosan particles instantly formed in the NaOH solution. Among the prepared chitosan solutions used, the 1.5% chitosan solution had the most appropriate viscosity for forming sphere-shaped magnetic chitosan particles via the dropping method. Thus, we used a 1.5% chitosan solution to prepare the magnetic chitosan particles. After drying, the magnetic chitosan particles showed a decrease in size due to the evaporation of absorbed water, while retaining morphological properties such as spheric shape and smooth surface. Fig. 1 shows the size of the barium ferrite particles and magnetic chitosan particles after drying. The size of the magnetic chitosan particles varied directly with the BF/C ratio. Thus, the size and shape of the magnetic chitosan particles was dependent on both the BF/C ratio and viscosity of a chitosan solution.

Fig. 2 shows the surface morphology of the barium ferrite particles and the magnetic chitosan particles. On the surface of the barium ferrite particles, barium ferrite microparticles have conglomerated slightly in some places. On the surface of the magnetic chitosan particles, the barium ferrite microparticles conglomerated in a compact fashion, according to decreases in the BF/C ratio. In addition, the surface of the magnetic chitosan

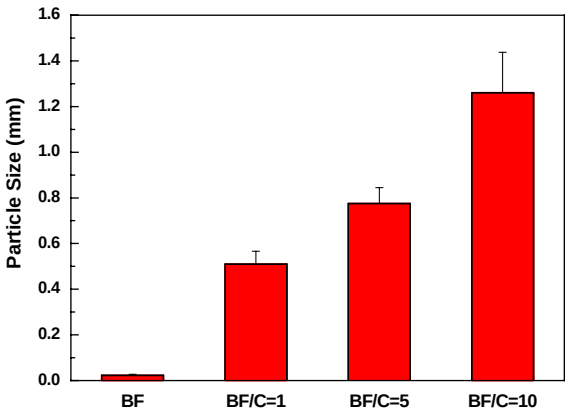


Fig. 1. Size of barium ferrite particles and magnetic chitosan particles after drying.

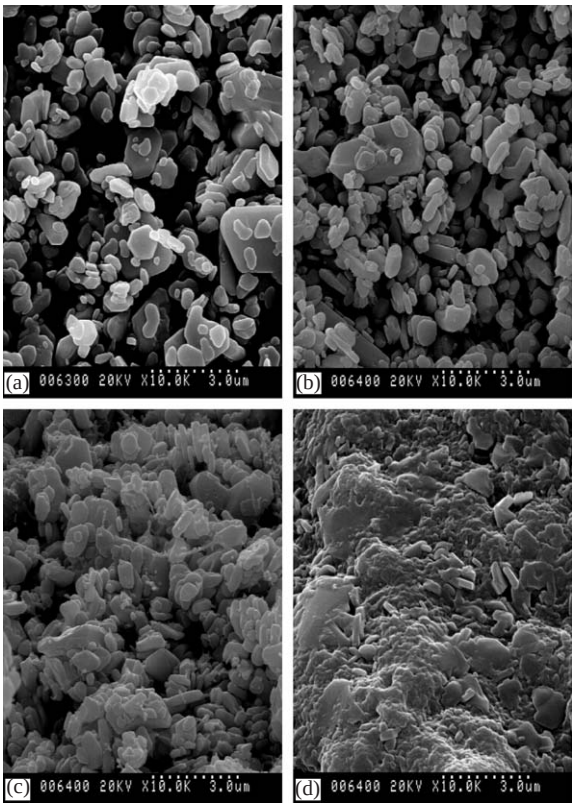


Fig. 2. Surface morphology of (a) barium ferrite particles and magnetic chitosan particles with the BF/C ratio of (b) 10, (c) 5, and (d) 1.

particles reveals that the amount of chitosan mixed with the barium ferrite microparticles increased with decreases of the BF/C ratio, which is as expected. On the surface of the magnetic chitosan particles with a BF/C ratio of 1, the barium ferrite microparticles were completely covered with chitosan.

Fig. 3 shows the swelling degree of the magnetic chitosan particles 7 days after immersion in pH 7.4 PBS at 37 °C. The swelling degree of the magnetic chitosan particles decreased with increases in the BF/C ratio. The swelling of the magnetic chitosan particles was due to the absorption of water by the chitosan. However, the swelling degree of the magnetic chitosan particles was much less pronounced than that of the magnetic alginate particles studied in our laboratory in prior experiments [10].

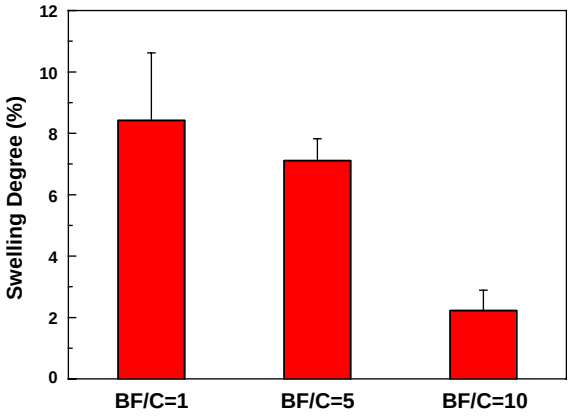


Fig. 3. Swelling degree of magnetic chitosan particles 7 days after immersion in pH 7.4 PBS at 37 °C.

Fig. 4 and Table 1 show the magnetic properties of the barium ferrite particles and magnetic chitosan particles. As the BF/C ratio of the magnetic chitosan particles decreased, the saturation magnetization value of the magnetic chitosan particles decreased. The saturation magnetization values of the magnetic chitosan particles were lower than that of the barium ferrite particles. Because the weights of all particles used for the measurement of magnetic properties were constant, the decrease of saturation magnetization values was due to increases in the amount of chitosan incorporated in the magnetic chitosan particles, i.e. decreases in the amount of barium ferrite. In addition, the coercivity values of the magnetic chitosan particles were similar to those of the barium ferrite particles. This indicates that the high coercivity value of barium ferrite in the magnetic chitosan particles was maintained, irrespective of the amount of incorporated chitosan. The magnetic chitosan particles also exhibited ferromagnetic properties, in a manner similar to that of the barium ferrite particles. Magnetic particles with ferromagnetic properties easily agglomerate into a lump due to magnetic attraction. It is expected that the chitosan covering the surface of the magnetic chitosan particles may interfere with the formation of these lumps.

Fig. 5 and Table 1 show the changes in temperature of the barium ferrite particles and magnetic chitosan particles under an alternating

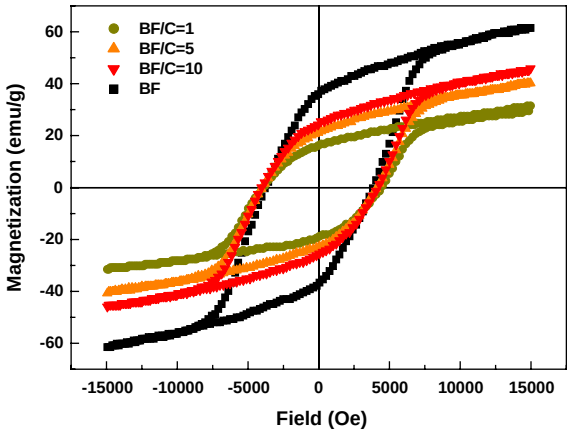


Fig. 4. Magnetization curve of barium ferrite particles and magnetic chitosan particles.

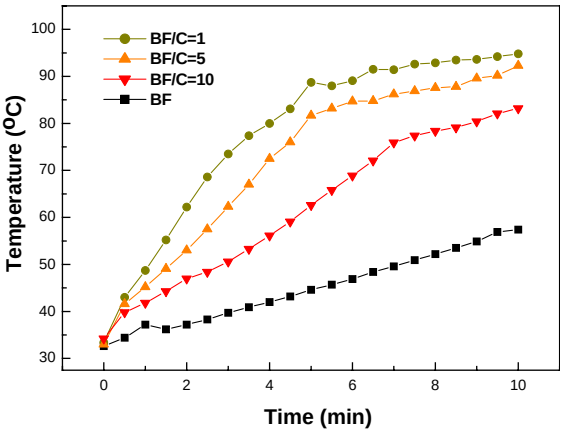


Fig. 5. Temperature change of barium ferrite particles and magnetic chitosan particles under an alternating magnetic field.

Table 1
Magnetic properties of barium ferrite particles and magnetic chitosan particles

| Specimens | Saturation magnetization (emu/g) | Coercivity (Oe) | Temperature change (°C/min) |
|---|---|---|---|
| Barium ferrite | 61.45 | 3779 | 2.48 |
| BF/C = 1 | 31.54 | 3922 | 6.14 |
| BF/C = 5 | 40.20 | 3978 | 5.93 |
| BF/C = 10 | 45.85 | 4035 | 4.9 |

magnetic field. As the BF/C ratio of the magnetic chitosan particles decreased, the temperature change of the magnetic chitosan particles increased. The temperature change of the magnetic chitosan particles was higher than that of the barium ferrite particles. Because the amount of barium ferrite in the magnetic chitosan particles was fixed for the purposes of temperature change measurements, these differences in the temperature change values of magnetic chitosan particles can be considered to be due to incorporated chitosan. Magnetic particles must be placed under an alternating magnetic field for a certain time period, in order to increase temperatures to 42–46 °C for hyperthermia. As this time period gets longer, the normal tissues surrounding the tumor tissues sustain more damage from this magnetic field. Thus, the reduction of this time period is necessary for the preservation of normal tissues. In the magnetic chitosan particles, the time period necessary for increasing temperatures in the range of 42–46 °C was shorter than that required for the barium ferrite particles. As the BF/C ratio decreased, this time period also decreased. The barium ferrite microparticles in the magnetic chitosan particles with lower BF/C ratios were conglomerated in a more compact manner, as is shown in Fig. 3. We believe that this degree of conglomeration has an effect on the heating rate of the magnetic chitosan particles. In addition, because the magnetic chitosan particles with lower BF/C ratios tended to be smaller, the total surface area of the magnetic chitosan particles immersed in the double-distilled water increased. We also surmise that this increase of total surface area had a major influence on the increases in heating rates. The heating rates of the magnetic particles under an alternating magnetic field are related to the loss of hysteresis during the reorientation of magnetization [11]. Thus, we suggest that the degree to which the magnetic microparticles conglomerate, as well as the loss of hysteresis, are closely connected to the heating rates of the magnetic polymer particles.

Table 2 shows the cytotoxicity of barium ferrite particles and magnetic chitosan particles, as well as control values, which were determined by an agar diffusion test. The magnetic chitosan particles showed relatively low cytotoxicity for the L929

Table 2
Cytotoxicity of barium ferrite particles and magnetic chitosan particles, as well as controls, determined by agar diffusion test

| Specimens | Response index (zone index/lysis index) | Cytotoxicity |
|---|---|---|
| Barium ferrite | 2/4 | Moderate |
| BF/C = 1 | 0/0 | None |
| BF/C = 5 | 1/1 | Mild |
| BF/C = 10 | 1/3 | Mild |
| PVC (positive control) | 4/5 | Severe |
| PE (negative control) | 0/0 | None |

cell line, compared to the moderate cytotoxicity exerted by the barium ferrite particles. However, the cytotoxicity of the magnetic chitosan particles increased slightly as the BF/C ratio increased. In general, the surface conditions of the materials which are in direct contact with cells or tissues have the greatest influence on toxicity. This would suggest, then, that the amount of barium ferrite left uncovered by chitosan on the surface of the magnetic chitosan particles is more closely related to cytotoxicity than is that of the barium ferrite incorporated into the magnetic chitosan particle. Clearly corroborating this premise, the magnetic chitosan particles completely covered with chitosan as shown in Fig. 2(d) were found to be noncytotoxic.

## 4. Conclusions

The magnetic chitosan particles were prepared by dropping well-mixed barium ferrite-chitosan solution into a NaOH solution. The size (0.2–1 mm) of the magnetic chitosan particlesvaried directly with the BF/C ratio. The surface morphology of the magnetic chitosan particles showed that the barium ferrite microparticles conglomerated in a compact fashion, according to decreases in the BF/C ratio. The magnetic chitosan particles soaked in pH 7.4 PBS for 7 days exhibited almost no swelling, whatsoever. The saturation magnetization of the magnetic chitosan particles varied directly with BF/C ratio, while the coercivity remained almost constant. The heating rate of the magnetic chitosan particles under an alternating magnetic field increased with decreases in the BF/C ratio. The magnetic chitosan particles exhibited relatively low cytotoxicity overall. The incorporated chitosan appeared to exert a sizeable influence with regard to low cytotoxicity and high heating rate. Therefore, magnetic chitosan particles do, indeed, appear to be promising as a potential magnetic support for hyperthermic applications. Further experimentation is now in progress to decrease the average size of magnetic chitosan particles, utilizing the spray method.

## Acknowledgements

This work was supported by Grant no. R01-2001-000-00157 from the Basic Research Program of the Korea Science & Engineering Foundation.

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