Chitosan and O-carboxymethyl chitosan modified Fe$_3$O$_4$ for hyperthermic treatment

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Abstract

In this study magnetic fluids were manufactured by the adsorption of chitosan (CS) and O-carboxymethyl chitosan (OCMCS) on Fe$_3$O$_4$ nanoparticles to be used as hyperthermic thermoseeds. Fe$_3$O$_4$ particles were characterized by physico-chemical methods such as: thermogravimetry analysis (TGA), x-ray diffraction (XRD), Raman spectrum, Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM) and vibrating sample magnetometer (VSM). The SEM images and XRD patterns showed that the synthesized Fe$_3$O$_4$ nanoparticles were of single phase and spherical shape with 10–15 nm in diameter. The VSM measurements showed that Fe$_3$O$_4$ particles were superparamagnetic with saturation magnetization of 70 emu g$^{-1}$. The adsorbed layers of CS and OCMCS on the magnetite surface (Fe$_3$O$_4$/CS) and (Fe$_3$O$_4$/OCMCS) were confirmed by FTIR, Raman spectra and SEM. In the ac magnetic field of 80 Oe and 236 kHz, the saturation heating temperatures of the sample Fe$_3$O$_4$/CS and Fe$_3$O$_4$/OCMCS were 100 and 98 $^\circ$C, respectively. At the same concentration of Fe$_3$O$_4$ nanoparticles in suspension, the two magnetic fluids exhibited quite high heating capacity, with different behaviors of concentration dependence. The Fe$_3$O$_4$/CS and Fe$_3$O$_4$/OCMCS nanoparticles would serve as good thermoseeds for localized hyperthermia treatment of cancers.

Keywords: chitosan, nanomaterials, hyperthermia, cancer treatment

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1. Introduction

Magnetite (Fe$_3$O$_4$) nanoparticles (MNPs) with their multifunctional properties such as small size, superparamagnetism, low toxicity, etc, show many potential applications in biomedicine for disease diagnosis and cancer treatment (magnetic resonance imaging, drug delivery system, hyperthermia, etc) [1–3]. To be applied in biomedicine, the MNPs must have long term stability in aqueous solution, particle size of less than 100 nm, and magnetization as high as possible. Apart from those properties, the MNPs have to be encapsulated to avoid the agglomeration or to make them monodisperse in suspension. Various methods have been reported for the preparation of stable dispersion of iron oxide in organic solvents including hexane and decane [4, 5]. For these solvents, the biological applications are greatly restricted because of their poor solubility in aqueous solutions. So, it is essential to modify the MNPs to increase the stability by using stabilizers such as surfactants, oxide or polymer compounds (especially biocompatible polymer) with some specific functional groups.

Owing to its biocompatibility and biodegradability, chitosan (CS) has been used widely in biomedicine. However, one disadvantage of chitosan is that it can
only dissolve in acidic solution (pH < 6) and cannot dissolve in a physiological environment (pH = 7.4). This constrain restricts applications of CS in biomedicine. To overcome this restriction, many derivatives of CS have been manufactured. O-carboxymethyl chitosan (OCMCS) is one of the hydrophilic derivatives which has a backbone structure similar to CS but hydroxyl group (–OH) is replaced by carboxyl group (–COOH). This helps to increase the ability to disperse OCMCS, promising potential applications in biomedicine fields [6–11], in neutral and alkaline solutions.

In our study, magnetic nanoparticles were prepared by co-precipitation and surface modified by CS and OCMCS. The inductive heating property of the magnetic fluids in an ac magnetic field was investigated in order to show application potential of the ferrofluids in cancer treatment.

2. Materials and methods

2.1. Chemicals and materials

Ferric chloride hexahydrate (FeCl₃·6H₂O, >99%), ferrous chloride tetrahydrate (FeCl₂·4H₂O, >99%) and ammonium hydroxide (29.4%) were obtained from Merck. Chitosan was obtained from Sigma. The degree of deacetylation was 90%. O-carboxymethyl chitosan was purchased from Chung-mu Industrial Corp (Korea). All other chemicals were of reagent grade and used without further purification. Deionized water was used throughout the experiments.

2.2. Magnetic nanoparticles preparation and surface modification

Firstly, Fe₃O₄ particles were prepared by co-precipitation from Fe²⁺ and Fe³⁺ solution in N₂ condition at 70 °C. Briefly, Fe²⁺ and Fe³⁺ solutions were added into three-neck flash (with the ratio of Fe³⁺: Fe²⁺ = 2 : 1). Chemical precipitation was achieved under vigorous stirring by adding 2 M NH₄OH solution. During the reaction process, the temperature was maintained at 70 °C and the pH was 10. The precipitates were washed several times with distilled water and ethanol. Finally, Fe₃O₄ powder was obtained after 24 h drying in vacuum condition at 50 °C.

To prepare CS and OCMCS modified Fe₃O₄ fluids, 20 mg of Fe₃O₄ powder was dissolved in 10 ml of 2 mg ml⁻¹ CS solution (pH = 4) or OCMCS solution (pH = 7). Then, the mixture was stirred for 24 h. Magnetic fluids were finally obtained by centrifuging at 5000 rpm for 10 min.

2.3. Characterization and magnetic heating measurement

The morphology and particle size of synthesized Fe₃O₄ particles were examined by using field emission scanning electron microscope FESEM (S-4800 Hitachi). Physical properties of unmodified MNP, CS-modified (MNP/CS) and OCMCS-modified (MNP/OCMCS) solutions were characterized by thermogravimetry analysis –TGA (Setaram Labsys 18), x-ray diffraction XRD (Siemens D-5000), Fourier transform infrared spectroscopy FTIR (Nicolet 6700), micro
3. Results and discussion

3.1. Characterization

3.1.1. Fluid images. The colloidal suspension of the resulted ferrofluids is shown in figure 1, which clearly demonstrates that both CS and OCMCS are good agents for stabilizing aqueous solution of Fe₃O₄ particles. CS and OCMCS stabilized Fe₃O₄ particles show well dispersed appearance, while the unmodified Fe₃O₄ particles precipitate easily.

3.1.2. IR and Raman spectra of CS and OCMCS surface modified magnetites. Figure 2 shows the FTIR and Raman spectra of unmodified, CS and OCMCS surface modified magnetic particles. The peak of 585 cm⁻¹ is the characteristic peak of Fe–O–Fe in the bulk Fe₃O₄, and it can be seen that in IR spectra of Fe₃O₄/CS and Fe₃O₄/OCMCS nanosystems this peak shifts to 567 and 545 cm⁻¹, respectively. In addition, the characteristic peaks of 1654 and 1392 cm⁻¹ for CS shift to 1604 and 1379 cm⁻¹ for the Fe₃O₄/CS and Fe₃O₄/OCMCS conjugates, while the 1554 and 1354 cm⁻¹ peaks of OCMCS shift to 1479 and 1338 cm⁻¹, respectively. These results prove that CS and OCMCS were adsorbed on to the magnetic particles. It can be seen clearly in Raman spectra that the 680 cm⁻¹ peak of Fe–O–Fe in Fe₃O₄ shifts to 670 and 672 cm⁻¹ after covering the MNP by CS and OCMCS, respectively.

3.1.3. Thermogravimetry analysis. The TGA and DTA curves of CS and OCMCS-coated Fe₃O₄ nanoparticles are shown in figure 3. Two weight losses are observed in the CS curve. The initial weight loss of about 1.9% can be attributed to the evaporation of absorbed H₂O between 50 and 150 °C. The weight loss of about 4% in the temperature range.

Raman spectroscopy (Horiba Labram 1B) and vibrating sample magnetometer VSM (homemade). Magnetic inductive heating of the ferrofluids was studied on a system using a commercial generator (RDO HFI 5 kW) to create an alternating magnetic field of frequency of 236 kHz and amplitude of 40–100 Oe. The temperature of the suspension during the heating was measured online by a commercial GaAs optical sensor (Opsens) with accuracy of 0.3 deg.

Figure 4. XRD patterns for the naked Fe₃O₄, CS- and OCMCS-coated Fe₃O₄ particles.

Figure 5. Magnetization hysteresis of the naked, CS- and OCMCS-coated Fe₃O₄ particles.
150–600 °C is assigned as the decomposition of CS layer. Meanwhile, the first weight loss due to moisture evaporation at 50–150 °C in the TGA curve of OCMCS is about 1.5% and the second weight loss of approximately 5% is attributed to the decomposition of OCMCS from 150 to 600 °C. It is obvious that the CS and OCMCS cover layers are quite thin (4–5% of sample’s weight), therefore they should not affect the magnetic properties of Fe₃O₄ nanoparticles.

### 3.1.4. Crystal structure and magnetization.

The XRD diagrams of the unmodified, CS- and OCMCS-modified MNPs are presented in figure 6. The obtained patterns proved that the synthesized Fe₃O₄ particles were of pure single phase with size of tens of nanometers. This result is quite consistent with the above FESEM images. The existence of peaks of Fe₃O₄ appearing in XRD spectra of Fe₃O₄–CS or Fe₃O₄–OCMCS confirms that the encapsulation by CS and OCMCS shell does not affect the magnetism of nanoparticles. Magnetizations versus field of three samples in dry form are shown in figure 7. The magnetization of unmodified Fe₃O₄ particles is about 70 emu g⁻¹. After surface stabilization by CS and OCMCS the particle magnetization was reduced by only 1–2%. This result again proves that the coating process on the magnetic particles does not affect the nature of the Fe₃O₄ core and the polymer coating layers are rather thin.

### 3.1.5. FESEM images of CS and OCMCS covered magnetites.

The sizes of unmodified and CS or OCMCS modified Fe₃O₄ particles were characterized by FESEM image (figure 6). It can be seen from figure 6 that the obtained magnetic particles are almost spherical and diameters of unmodified and OCMCS modified Fe₃O₄ particles are about 20 nm, whereas the size of CS modified particles is about 50 nm.

### 3.2. Magnetic inductive heating capacity of the ferrofluids

The heating curve measurements were carried out for modified magnetite suspensions with different concentrations of Fe₃O₄ particles in deionized water and in the same field conditions, namely of a frequency of 236 kHz and amplitude of 80 Oe (figure 7). The saturation temperature for suspensions of the magnetite NPs modified by two encapsulating materials were gathered in table 1. The

| No. | Concentration Fe₃O₄/CS (mg ml⁻¹) | Tₛ (°C) | dT/dt (°C/s) | Tₛ/Fe₃O₄/OCMCS (°C) | dT/dt (°C/s) |
|-----|---------------------------------|---------|--------------|----------------------|--------------|
| 1   | 1                               | Not determined | 0.36 | 98 | 0.2 |
| 2   | 0.7                             | Not determined | 0.22 | 90 | 0.15 |
| 3   | 0.5                             | 90        | 0.14         | 78.5 | 0.1 |
| 4   | 0.3                             | 59        | 0.05         | 72 | 0.07 |
| 5   | 0.2                             | 53.3     | 0.039        | 60.7 | 0.045 |

### Table 1. Magnetic heating parameters.
saturation temperature of the most dense (as-prepared ferrofluid without any dissolution) magnetic fluids was quite high, namely higher than 98 °C for both modifying materials. It is worth noting that these values are much higher than those heating temperatures reported by Zhao et al [12] for the case of chitosan modifying material. In order to compare the MIH characteristics observed for two encapsulating materials in our experiment, we show in figure 8 concentration dependences of two MIH parameters, i.e. saturation temperature $T_s$ (estimated at heating time of $t = 1500 s$) and initial temperature increase rate $dT/dt$ (at $t = 0 s$). As one can note (from figure 8 and also table 1), in the high Fe$_3$O$_4$ concentration region both the heating parameters $T_s$ and $dT/dt$ of the CS-modified ferrofluid are higher than the corresponding parameters estimated for the case of OCMCS-modified sample. Upon decreasing of MNP concentration by adding more and more water, $T_s$ and $dT/dt$ of the former sample decrease faster than those of the latter one. At $c = 0.3$ mg ml$^{-1}$, $T_s = 59 ^{\circ}$C, $dT/dt = 0.05$ deg s$^{-1}$ and $T_s = 72 ^{\circ}$C, $dT/dt = 0.07$ deg s$^{-1}$, respectively for the CS-modified and OCMCS-modified ferrofluid. Because the cancer cell can be damaged by necrosis and/or ablation in the temperature range of about 40–60 °C while the safe cells survive up to 80 °C [13], we therefore note that for cancer hyperthermia application the OCMCS-modified ferrofluid serves as a better thermoseed. We also suppose the fast decrease of heating parameters observed for the case of normal chitosan encapsulation to be related with decreasing solubility of the ferrofluid when the pH becomes higher.

4. Conclusion

Biocompatible chitosan and O-carboxymethyl chitosan have been demonstrated to be good surfactants for stabilization of magnetic nanoparticles. The particle sizes of Fe$_3$O$_4$ before and after modification were below 50 nm. The highest inductive heating temperatures of the as-prepared Fe$_3$O$_4$/CS and Fe$_3$O$_4$/OCMCS ferrofluids were above 98 °C. The research results also indicated that the heating effect of the Fe$_3$O$_4$/CS and Fe$_3$O$_4$/OCMCS could be tuned by reducing the concentration by a factor of larger than 30 to get the cancer hyperthermia range of temperature (41–46 °C). In this low concentration region, the Fe$_3$O$_4$/OCMCS nanoparticles proved to be a better thermoseed for localized hyperthermia treatment of cancer than the Fe$_3$O$_4$/CS.

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References

[1] Lin M, Li S, Kim H H, Kim H, Lee H B, Muhammed M and Kim D K 2010 J. Mater. Chem. 20 444
[2] Mahmoudi M, Simchi A, Imani M, Milani A S and Stroeve P 2008 J. Phys. Chem. B 112 14470
[3] Shen Z H, Xie M Q, Zhuang L, Deng Y Y, Hu S L and Lin Y Y 2008 Scr. Mater. 59 211
[4] Shaﬁ K V P M, Ulman A, Yan X, Yang N L, Estourbes C, White H and Rafalovich M 2001 Langmuir 17 5093
[5] Fried T, Shemer G and Markovich G 2001 Adv. Mater. 13 1158
[6] Zhu A, Yuan L and Liao T 2008 Int. J. Pharm. 350 361
[7] Li G Y, Huang K, Jiang Y, Ding P and Yang D 2008 Biochem. Eng. J. 40 408
[8] Donadel K, Felisberto M D V, Fávere V T, Rigoni M, Batistela N J and Laranjeira M C M 2008 Mater. Sci. Eng. C 28 508
[9] Li G Y, Huang K, Jiang Y, Ding P and Cheng J 2008 J. Alloys Compd. 466 451
[10] Qu J, Liu G, Wang Y and Hong R 2010 Adv. Powder Technol. 21 461
[11] Chen S, Zhong H, Zhang L, Wang Y, Cheng Z, Zhu Y and Yao C 2010 Carbohydr. Polym. 82 747
[12] Zhao D, Wang X, Zeng X, Xia Q and Tang J 2009 J. Alloys Compd. 447 739
[13] Jordan A, Scholz R, Wust P, Fehling H and Rolix R 1999 J. Magn. Magn. Mater. 201 413