Journal of Magnetism and Magnetic Materials 323 (2011) 1216–1222

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

Preparation of magnetite aqueous dispersion for magnetic fluid hyperthermia

Teppei Kikuchi [a], Ryo Kasuya [a], Shota Endo [b], Akira Nakamura [b], Toshiyuki Takai [b], Nils Metzler-Nolte [c], Kazuyuki Tohji [a], Jeyadevan Balachandran [d],*

[a] Graduate School of Environmental Studies, Tohoku University, 6-6-20, Aramaki, Aoba, Aoba-ku, Sendai 980-8579, Japan
[b] Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-cho, Aramaki-Aoba, Aoba-ku, Sendai 980-8575, Japan
[c] Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Universitätstrasse 150, D-44801 Bochum, Germany
[d] Department of Material Science, School of Engineering, The University of Shiga Prefecture, 2500, Hassaka-cho, Hikone-City, Shiga 522-8533, Japan

ARTICLE INFO

Available online 23 November 2010

Keywords:
Magnetic fluid hyperthermia
Surface modification
Thermal decomposition

ABSTRACT

An aqueous magnetic suspension was prepared by dispersing amphiphilic co-polymer-coated mono-dispersed magnetite nanoparticles synthesized through thermal decomposition of iron acetylacetonate ($Fe(acac)_3$) in a mixture of oleic acid and oleylamine. The average diameter of narrow-size-distributed magnetite nanoparticles varied between 5 and 12 nm depending on the experimental parameters such as reaction temperature, metal salt concentration and oleic acid/oleylamine ratio. Though the as-synthesized particles were coated with oleate and were dispersible in organic solvent, their surfaces were modified using amphiphilic co-polymers composed of poly(maleic anhydride-alt-1-octadecene) and polyethylene glycol-methyl ether and made dispersible in water. Infrared spectra of the sample indicated the existence of $-COOH$ groups on the surface for further conjugation with biomolecules for targeted cancer therapy.

1. Introduction

Magnetic fluid hyperthermia is a type of cancer therapy in which cancer cells are killed by raising the cell temperature above 43 °C, utilizing the heat dissipated from magnetic nanoparticles exposed in an alternating current magnetic field [1,2]. Since magnetic nanoparticles work as thermal seeds in human tissues, high heating efficiency and tumor-specificity are required to decrease dosage. Among various magnetic nanoparticles, magnetite (Fe_3O_4) has been considered suitable due to its biocompatibility, ease of synthesis and heating properties. Generally, the heat dissipation of magnetite nanoparticles (MNPs) is caused due to the delay in the relaxation of the magnetic moment through either rotation within the particle (Néel relaxation) or rotation of the particle itself (the Brown relaxation) [3]. Since particle rotation is dependent on the viscosity of the suspending media, higher viscosity of surrounding medium inhibits rotation and the heat dissipated through Brownian relaxation will decrease in highly viscous medium [4] such as tumor tissues. We have reported the heating efficiency of MNPs with various diameters in low and high viscosity media, and concluded that the physical dimension of the particle to achieve maximal heat dissipation through Néel relaxation was around 12 nm [5].

Although nanoparticles could be synthesized through conventional coprecipitation of divalent and trivalent iron ions in alkaline media [6], their maximum size is limited to around 10–12 nm in diameter and also their size distribution is very wide. On the other hand, thermal decomposition of organometallic compounds [7] has been proposed and successful demonstrations of the synthesis of monodispersed particles have been reported. Though production of monodispersed particles in a wide size range by manipulating the precursor, solvent and surfactant [8,9,10,11] through higher temperature decomposition of organic precursors has been claimed, large-scale synthesis of MNPs in the size range 10–15 nm has been limited. Furthermore, the synthesis of monodispersed particles with diameters above 11 nm has also not been realized while maintaining the monodispersity. Thus, the authors believe that any step towards the effective use of MNPs for magnetic hyperthermia should begin with the development of an efficient synthesis scheme to prepare MNPs with reasonably distinct magnetic properties. Recently, a solvent-free thermal decomposition technique has been developed to synthesize high-quality MNPs [12]. Unlike the case of decomposition of highly toxic organometallic salts, this method proposes the decomposition of iron acetylacetonate in the presence of surfactants such as oleylamine and oleic acid [12]. However, neither the formation scheme nor the synthesis of particles with average diameter over 12 nm has been reported. Thus, in this paper, we report the results of the study attempting to understand the magnetite formation scheme and also the conditions suitable to achieve particles with diameters larger than 11 nm. Then, we also report the results of the study undertaken to prepare aqueous magnetite suspension, which is another task that has to be met prior

---

* Coreresponding author. Tel.: +81 749 28 8352; fax: +81 749 28 8486.
E-mail address: jeyadevan.b@mat.usp.ac.jp (J. Balachandran).

0304-8853/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jmmm.2010.11.009

to implementing effective hyperthermia. To realize the same, the particle surface has to be modified with organic moieties not only enabling the particles to disperse in water, but also suppress the phagocytosis of macrophage cells [13]. For example, MNPs modified with polyethylene glycol (PEG) have longer blood circulation time. This is due to the stealth properties of PEG molecules. In addition, selective targeting of tumor cells has been performed by conjugating MNPs and biomolecules such as cell-penetrating peptides [14] and antibodies [15]. Thus, we have focused on the preparation of aqueous suspension dispersing MNPs by modifying the particles with amphiphilic co-polymers containing PEG chain to increase the dispersibility in aqueous solutions. We also identified the chemical species on the particle surface to confirm the existence of functional groups that are needed for linking with biomolecules for cell targeting.

2. Experimental

2.1. Synthesis of magnetite nanoparticles (MNPs)

Synthesis of MNPs was carried out in a nitrogen atmosphere. In a typical synthesis procedure, 1.06 g of iron (III) acetylacetonate (Fe(acac)$_3$, Dojindo Japan) was added to a 34.2 mL of oleic acid (Sigma-Aldrich) and oleylamine (Sigma-Aldrich) mixture whose oleic acid/oleylamine ratio was 0.82. Then, the suspension was heated at 120 °C with vigorous stirring. After aging for 1 h, the solution was quickly heated to specific temperatures (200, 250, 300, 320 °C) and kept at these temperatures for 2 h. After cooling the suspension to room temperature, the particles were recovered using a magnet and washed several times with acetone and ethanol. Finally, the recovered particles were dried and re-dispersed in chloroform.

2.2. Conjugation of amphiphilic co-polymers to MNPs

Amphiphilic co-polymers (PMAO – PEGME) were obtained as follows [16]. 1.0 g of poly(maleic anhydride-alt-1-octadecene) (PMAO, M_n= 30,000–50,000, Sigma-Aldrich) and 100 g of polyethylene glycol-methyl ether (PEGME, M_W=5000, Sigma-Aldrich) were added to chloroform and reacted at room temperature for 15 h using 200 μL of concentrated sulfuric acid as a catalyst. The reaction scheme is shown in Fig. 1. The solution obtained after the reaction was neutralized by adding 1.0 mol/L of aqueous solution of NaOH. Then, 50 mg of MNPs and the PMAO – PEGME were mixed in chloroform and stirred overnight at room temperature. The resulting PMAO – PEGME-coated particles were gathered by centrifugation and washed several times with chloroform. The particles were then dried and re-dispersed in water.

2.3. Characterization

Crystalline phases were identified by X-ray diffractometry (XRD), using CuK$_\alpha$ radiation. The particle morphology and microstructure were observed by transmission electron microscopy (TEM). Mean particle diameter was obtained by measurement of 500 particles from TEM images. Fourier transform-infrared (FT-IR) spectra of as-synthesized magnetite powder using oleylamine and a mixture of oleic acid/oleylamine and the spectra of amphiphilic PMAO – PEGME co-polymer conjugated magnetite were collected from films of dried solution on KBr crystals.

3. Results and discussion

3.1. Synthesis of MNPs

The XRD profiles of samples confirmed the presence of magnetite with spinel structure (Fig. 2). Also, the positions and relative intensities of all the diffraction peaks match well with the standard profiles. The full width at half maximum (FWHM) of the (3 1 1) peak was used to estimate the average particle size using Scherrer's formula. The FWHM increased with the decrease in MNP diameter. Furthermore, the lattice parameters calculated from XRD patterns ranged between 8.348 and 8.410. The lattice parameters were close to that of maghemite (8.346) when the particles were synthesized in oleylamine alone, or synthesized with high metal-salt concentration at the reaction temperature above 300 °C. Otherwise, the values were quite close to the standard lattice parameter of magnetite (8.396). Fig. 3(a), (b), (c) and (d) shows the TEM images of MNPs synthesized using 1.06 g (3 mmol) of Fe(acac)$_3$ at the oleic acid/oleylamine ratio of 0.82 and 200, 250, 300 and 320 °C, respectively. The TEM images indicated that the MNPs had a very narrow size distribution. When the reaction temperature was 200 °C, the average particle size was estimated to be about 5 nm, as shown in Fig. 3(a). On increasing the reaction temperature from 200 to 250 °C, the average particle size increased from 5 to 11 nm. In contrast, the average size of the particles aged at 320 °C (Fig. 3(d)) was smaller than that of the sample reacted at 300 °C (Fig. 3(c)). This suggests that the particle size can be controlled up to 11 nm by adjusting the reaction temperature from 250 to 300 °C. However, neither the reason for the decrease in particle diameter of the samples synthesized at 320 °C nor the role of various reagents

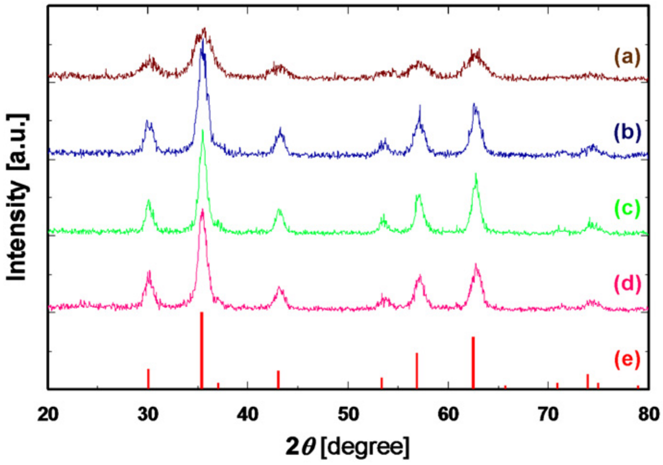

Fig. 2. XRD patterns of MNPs synthesized at (a) 200 °C, (b) 250 °C, (c) 300 °C and (d) 320 °C using 1.06 g of Fe(acac)$_3$ at the oleic acid/oleylamine ratio of 0.82. (e) The standard magnetite XRD peaks (PDF 19-0629).

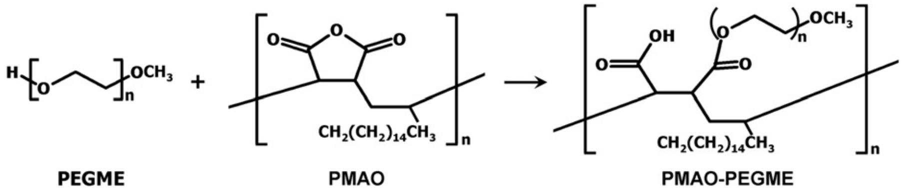

Fig. 1. Synthetic scheme of amphiphilic PMAO – PEGME co-polymers.