Studies of magnetite nanoparticles synthesized by thermal decomposition of iron (III) acetylacetonate in tri(ethylene glycol)

Dipak Maity\textsuperscript{a,},*, S.N. Kale\textsuperscript{b}, Ruchika Kaul-Ghanekar\textsuperscript{c}, Jun-Min Xue\textsuperscript{a}, Jun Ding\textsuperscript{a}

\textsuperscript{a} Department of Materials Science and Engineering, National University of Singapore, Singapore 117574, Singapore
\textsuperscript{b} Department of Electronic-Science, Fergusson College, Pune 411004, India
\textsuperscript{c} Interactive Research School for Health Affairs, Bharati Vidyapeeth University, Pune 411008, India

\textbf{A R T I C L E  I N F O}

Article history:
Received 10 February 2009
Received in revised form 6 April 2009
Available online 18 May 2009

PACS:
75.75.+a
81.07.–b
81.16.–c
87.85.Qr
87.85.Rs

Keywords:
Nanoparticle
Magnetite
Thermal decomposition
Superparamagnetic
Hyperthermia

\textbf{A B S T R A C T}

In this paper, water-soluble magnetite nanoparticles have been directly synthesized by thermal decomposition of iron (III) acetylacetonate, Fe(acac)\textsubscript{3}, in tri(ethylene glycol). Size and morphology of the nanoparticles are determined by transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements while the crystal structure is identified using X-ray diffraction (XRD). Surface charge and surface coating of the nanoparticles are recognized using Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectra (XPS) and zeta potential measurements. Magnetic properties are determined using vibrating sample magnetometer (VSM) and superconducting quantum interference device (SQUID) measurements. The results show that as-prepared magnetite nanoparticles are relatively monodisperse, single crystalline and superparamagnetic in nature with the blocking temperature at around 100 K. The magnetite nanoparticles are found to be highly soluble in water due to steric and electrostatic interactions between the particles arising by the surface adsorbed tri(ethylene glycol) molecules and associated positive charges, respectively. Cytotoxicity studies on human cervical (SiHa), mouse melanoma (B16F10) and mouse primary fibroblast cells demonstrate that up to a dose of 80 \( \mu \)g/ml, the magnetic nanoparticles are nontoxic to the cells. Specific absorption rate (SAR) value has been calculated to be 885 and 539 W/gm for samples with the iron concentration of 1 and 0.5 mg/ml, respectively. The high SAR value upon exposure to 20 MHz radiofrequency signifies the applicability of as-prepared magnetite nanoparticles for a feasible magnetic hyperthermia treatment.

\textcopyright 2009 Elsevier B.V. All rights reserved.

1. Introduction

Superparamagnetic (SPM) iron oxide (particularly Fe\textsubscript{3}O\textsubscript{4} and Fe\textsubscript{2}O\textsubscript{3}) nanoparticles have been attracting great attention in the bio-medical field such as magnetic separation, drug delivery, cancer hyperthermia and magnetic resonance imaging (MRI) contrast enhancement [1–6]. The single-step synthesis of highly water-soluble, superparamagnetic and biocompatible magnetite nanoparticles with controlled size and distribution is extremely important to realize the full potential of these materials in biomedicine. To date, the thermal decomposition method is very promising technique to fabricate high-quality superparamagnetic and monodisperse magnetite nanoparticles [7–9]. Typically, this method involves decomposition of Fe(acac)\textsubscript{3} in a high-boiling temperature solvent in presence of stabilizing surfactants such as oleic acid and oleylamine. However, the obtained magnetite nanoparticles are organic soluble which makes them inappropriate for bio-medical applications. Therefore, many groups have further developed the thermal decomposition method to directly synthesize water-soluble magnetic nanoparticles [10,11]. Recently, Cai et al. [12] have synthesized water-soluble magnetite nanoparticles by high-temperature decomposition of Fe(acac)\textsubscript{3} in triethylene glycol (TREG). An obvious advantage of this approach is that no further reducing agent and surfactants are required, which made this process easy to scale-up for mass. The TREG has been used for a triple role as high-boiling solvent, reducing agent and stabilizer to efficiently control the particle growth and prevent interparticle aggregation. Here, we have extended this work and proposed a mechanism for solubility of the as-prepared magnetite nanoparticles in aqueous suspension by exploring surface charge and surface coating of the nanoparticles. Cytotoxicity and hyperthermia studies of the nanoparticles have also been performed to evaluate their potentiality in biomedicine.

2. Experimental section

Absolute ethanol and ethyl acetate were used without purification. Tri(ethylene glycol) (TREG, 99%) and Iron(III) acetylacetonate (Fe(acac)\textsubscript{3}, 97%) were purchased from Sigma-Aldrich.

Water-soluble Fe\textsubscript{3}O\textsubscript{4} nanoparticles were prepared by the thermal decomposition of Fe(acac)\textsubscript{3} in TREG at elevated temperature with out...
using any surfactants [12]. Typically, 2 mmol of Fe(acac)₃ was dissolved in a 20 ml of hydrophilic TREG media and then magnetically stirred under a flow of argon. The solution was dehydrated at 120 °C for 1 h, and then quickly heated to 280 °C and kept at this temperature for 2 h. The black solution was cooled to room temperature by removing the heat source. The nanoparticles were precipitated by addition of 20 ml of ethyl acetate and then isolated by centrifugation. The obtained precipitate was re-dispersed in ethanol and further precipitated by ethyl acetate, followed by centrifugation. This washing procedure was repeated many times and then, one-half of the washed particles were dispersed in water to get a stable aqueous ferrofluid suspension and the other half was dried overnight in oven to obtain dry particles.

Size and morphology of the nanoparticles were determined using transmission electron microscopy (TEM, JEOL 2010) and dynamic light scattering (DLS) (Zetasizer Nano-ZS, Malvern Instruments) while crystal structure was identified by X-ray diffraction (XRD, Bruker D8 Advance). Surface charge and surface coating of the nanoparticles were recognized by Fourier transform infrared spectroscopy (FTIR, Varian 3100), X-ray photoelectron spectra (XPS, Kratos AXIS Ultra DLD) and laser Doppler anemometry (Zetasizer Nano-ZS, Malvern Instruments). Magnetic properties of the nanoparticles were studied by the superconducting quantum interference device (SQUID, Quantum Design, MPMS XL) and vibrating sample magnetometer (VSM, Lakeshore, Model 665).

Cell viability studies were performed by MTT assay on SiHa (human cervical), B16F10 (mouse melanoma) cell lines as well as on mouse primary fibroblasts cells that were isolated from skin epithelium of Swiss albino mice. The cells were seeded at density of 1 × 10⁵ cells/ml in 96-well plates. The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 μg/ml of penicillin/streptomycin followed by incubation at 37 °C in 5% CO₂ overnight. Next day, cells were treated with fresh medium containing the magnetite nanoparticles at concentrations ranging from 2.5–160 μg Fe/ml in each well in triplicates and the plates were further incubated for 24 h. After 48 h, medium was removed and 5 mg/ml MTT solution was added and the cells were incubated for 4 h at 37 °C in 5% CO₂ incubator. The intensity of colored formazan derivative was determined by measuring the absorbance at 570 nm in ELISA microplate reader (BioRad, Hercules, CA). The mean optical density (OD) value of three wells (for each concentration of nanoparticles dosed to the cells) was used for assessing the cell viability that was expressed as percentage of control [\% cell viability = nanoparticle-treated cells / control cells] x 100).

Heating ability of the as-prepared nanoparticles was measured from the time-dependent calorimetric measurements. Four milliliters of the aqueous ferrofluid with the Fe concentration of 0.5–1 mg/ml are subjected to radiofrequency (RF) radiation (generator: 20 MHz, 100 W, capacitatively coupled into a small 5 ml glass bottle) for different times, and the temperature rise was monitored. The temperature increase was compared to that for pure deionized water. The specific absorption rate (SAR) was calculated using the following equation:

\[ \text{SAR} = C \frac{\Delta T}{\Delta t} \frac{1}{\Delta T} \frac{1}{m_r} \]  

where \( C \) is the specific heat of solvent (here \( C_{\text{water}} = 4.18 \text{ J/g °C} \)), \( \Delta T/\Delta t \) is the initial slope of the time-dependent temperature curve and \( m_r \) is weight fraction of magnetic element (i.e. Fe) in the sample [13,14].

### 3. Results and discussions

#### 3.1. Crystal structure

Fig. 1 shows the XRD patterns which match well with the standard XRD patterns for bulk magnetite (JCPDS File no. 19-0629) indicating that the particles are consisting of Fe₃O₄ phase. The average crystallite size is measured using Scherrer’s equation [15] as 10.7 nm.

#### 3.2. Size and morphology

The TEM image in Fig. 2 indicates that the magnetite nanoparticles are well dispersed in water. The average size of the particle is about 11 nm which is very close to the crystallite size obtained from XRD. Inset of Fig. 7 is showing the HRTEM image of a single Fe₃O₄ nanocrystal.
Fig. 3 is showing DLS size distribution plot of the magnetite nanoparticles in an aqueous suspension. The DLS measurement indicates that the nanoparticles are relatively monodisperse in aqueous media. The obtained average hydrodynamic size of the nanoparticles is about 13 nm which is slightly larger than the size measured by TEM (11 nm). This difference is due to the coordination of TREG molecules, which effectively increase the particle size.

3.3. Surface charge and surface coating

Fig. 4 shows flow of the aqueous suspension of as-prepared Fe$_3$O$_4$ nanoparticles when exposed to a very strong permanent magnet. This indicates that the particles are highly soluble in water to form a stable ferrofluid suspension. FTIR, XPS and zeta potential measurements are carried out to identify the surface charge and surface coating which are responsible for the water solubility of the Fe$_3$O$_4$ nanoparticles.

Fig. 5 depicts the FTIR spectra for the particles. The peaks at about 2962–2809, 1632, 1455, 1350, 1251 and 1063 cm$^{-1}$ are due to C–H stretching, O–H stretching, C–H bending, C–O bending and O–H bending vibration, respectively, attributed for adsorbed TREG molecules to the particle surface [16,17]. The broad band between 3600 and 3000 cm$^{-1}$ centered at about 3400 cm$^{-1}$ are due to the O–H stretching vibration attributed for water and TREG molecules adsorbed to the particle surface [11]. In addition, the strong absorption band at about 580 cm$^{-1}$ is due to Fe–O stretching vibration for the Fe$_3$O$_4$ nanoparticles [18].

Fig. 6(a) shows wide scan XPS spectra while Fig. 6(b) and (c) are the deconvoluted O(1 s) and C(1 s) spectra of the Fe$_3$O$_4$ nanoparticles, respectively. The O(1 s) peaks at 531.6 and 533.3 eV are due to O–H and C–O bond which arise from the adsorbed water and TREG molecules, respectively [19,20]. The C(1 s) peaks at 285.5 and 288 eV are due to C–C/C–H and C–O–C bond, respectively, arise from the chemically adsorbed TREG molecules to the particle surface [21,22]. In addition, the Fe(3p3/2), O(1 s), Fe(2p3/2) and Fe(2p 1/2) peaks of Fig. 6(a) at about 55, 530.3, 710 and 724 eV are due to Fe–O bond of the Fe$_3$O$_4$ nanoparticles [18].

Fig. 7 shows the zeta potential distribution plot of the magnetite nanoparticles. The maximum of the plot is at about +40 mV which indicates that surface of nanoparticles is positively charged. The TREG molecules polarizes into R–O$^-$ and H$^+$ ions at elevated reaction temperature (R–OH $\rightarrow$ R–O$^-$+H$^+$, where R=-(CH$_2$–CH$_2$–O)$_3$–H). The R–O$^-$ part of TREG molecules coordinates with Fe$^{2+}$/Fe$^{3+}$ of the Fe$_3$O$_4$ nanoparticle (which is confirmed by FTIR and XPS) while the H$^+$ ions associates with the nanoparticles along with the R–O$^-$ and as a consequence surface of the nanoparticles become positively charged. Thus, the adsorbed R–O$^-$ and the associated H$^+$ ions cause the steric and electrostatic interactions between the nanoparticles (as shown in Fig. 8) and this could be the reason of higher water solubility of the as-prepared Fe$_3$O$_4$ nanoparticles.

3.4. Magnetic properties

Fig. 9 shows the hysteretic $M$–$H$ curve of the magnetite nanoparticles at room temperature. The saturation magnetization ($M_s$) of the magnetite nanoparticles are found to be 65 emu/g which is smaller than that of bulk magnetite (92 emu/g). The decrease in $M_s$ compared to bulk magnetite could be due to the surface effect of the smaller size (11 nm by TEM) magnetite nanoparticles [23–27] and the presence of nonmagnetic TREG molecules adsorbed to the surface of the nanoparticles [16]. Zero coercivity and zero remanance on the magnetization curves
indicates superparamagnetic behavior of the magnetite nanoparticles [26,28].

Fig. 10 shows the temperature dependence of the zero-field-cooled/field-cooled (ZFC–FC) magnetization of the magnetite nanoparticles measured by SQUID under an applied field of 100 Oe. The feature of the ZFC–FC curves is indicating that the nanoparticles are of superparamagnetic in nature [29]. The ZFC curve reached the maximum at about 100 K, which corresponded to the blocking temperature ($T_B$) of the sample. Above $T_B$ the sample is superparamagnetic and below is ferromagnetic. Thus, the as-prepared magnetic nanoparticles are SPM in nature with the blocking temperature at around 100 K.

3.5. Cytotoxicity studies

The cytotoxicity results on the cell lines SiHa, B16F10 and mouse primary fibroblast cells have been shown in Fig. 11 that are
compared with control cells, which is not treated with the magnetite nanoparticles. The observation revealed that cells do not show any cytotoxicity with the magnetite nanoparticles in the range 2.5–80 μg/ml dose. However, at 160 μg/ml concentration, the cell viability is decreased slightly for B16F10 cell lines (~80% viability as compared to control), which could be due to mild cytotoxic effect at higher concentrations in this cell line. But, other two cell lines do not show any cytotoxicity even at 160 μg/ml concentration. Thus, it is evident from the cytotoxicity study that the as-prepared magnetite nanoparticles are biocompatible up to the iron concentration of 80 μg/ml with SiHa, B16F10 and mouse primary fibroblast cells.

3.6. Hyperthermia studies

We now show that the magnetite nanoparticles can also absorb radiofrequency radiation causing heat generation, a property of great interest to hyperthermia applications. We also briefly discuss the possible mechanism of heat generation. It may be noted that generally speaking RF radiation in the frequency range 100–500 kHz is used for hyperthermia. Here we used 20 MHz because the corresponding generator is available to us, just to demonstrate the electromagnetic radiation absorption property of our nanoparticles. Given the fact that there are no resonances in absorption for this material over the range 100 kHz–30 MHz, we expect similar (but quantitatively somewhat different) heating effects at lower frequencies as well. It is useful to mention here that in one of our previous papers on a different material system, we have addressed the safety concern related to the radiofrequency at 20 MHz, and have shown based on experiments in different body tissues and blood that use of this (20 MHz) high radiofrequency is also safe [30].

Fig. 12 shows a significant temperature rise for sample loaded with the magnetite nanoparticles as compared to that of pure deionized water. A systematic increase in the heat generation with increase in the quantity of the dispersed nanoparticles is observed for RF irradiation as a function of time. This confirms the potential applicability of the as-synthesized Fe3O4 nanoparticles for magnetic hyperthermia application. Fine control on the final achievable temperature with a judicious combination of concentration of the nanoparticles and the time of RF irradiation is definitely possible, which is underway. The heating effects in magnetic nanoparticles in alternating fields are due to several types of loss mechanisms such as hysteresis loss, Neel loss and Brownian loss, all being strongly dependent upon the particle size. For SPM particles above the blocking temperature, the process of hysteresis loss is ruled out. However the other two mechanisms, Brownian loss due to the magnetically driven physical rotation of the particle and Neel loss due to the rotation of magnetization vector can still contribute. In addition to the nanoparticle size, the anisotropy energy of the magnetic material and the viscosity of the dispersion medium control the relaxation times of the Neel and Brownian mechanisms, respectively, and thereby their relative dominance in the heating phenomenon.

SAR value has been calculated to be 885 and 539 W/gm of Fe for the sample with iron concentration of 1 and 0.5 mg/ml, respectively, which indicating a higher SAR value as compared to the reported values in the literature [31,32]. Clearly, the use of much higher RF power in our case generates the high SAR value, suggesting that as-prepared magnetite nanoparticles are useful for a feasible hyperthermia treatment.

4. Conclusions

As-prepared magnetite nanoparticles are relatively monodisperse, single crystalline and superparamagnetic in nature. The nanoparticles are highly soluble in water because of the steric and electrostatic interactions between the particles arise from the surface adsorbed tri(ethyleneglycol) molecules and the associated positive charges, respectively. Cytotoxicity studies show that as-prepared magnetite nanoparticles are biocompatible up to the iron concentration of 80 μg/ml. The significant temperature rise of the magnetite nanoparticles upon exposure to 20 MHz radio-frequency confirms their potential applicability for magnetic hyperthermia application.

Acknowledgments

The authors gratefully acknowledge the help of Dr. S.D. Dhole, Department of Physics, University of Pune, for the hyperthermia heating measurement.
References