MAGNETIC RESONANCE IMAGING (MRI) APPLICATION OF Fe_3O_4 BASED FERROFLUID SYNTHESIZED BY THERMAL DECOMPOSITION USING POLY (MALEIC ANHYDRIDE -ALT-1-OCTADECENE) (PMAO)

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ABSTRACT

The Fe_3O_4 fluid synthesis by thermal decomposition method carried out in organic solvents with high boiling temperatures disposes a possibility of creating high-quality nanoparticles with uniform particle size and high degree of crystallization. In this paper, Fe_3O_4 fluid was prepared by thermal decomposition using poly (maleic anhydride-alt-1-octadecene) (PMAO) as a phase transfer ligand. The crystalline structure, morphology and magnetic property of the as-prepared samples were thoroughly characterized. The results demonstrated that the magnetic Fe_3O_4 nanomaterial was formed in liquid phase with spinel single phase structure, average size of 13-16 nm, and high saturation magnetization (up to 70 emu/g). Iron oxide (Fe_3O_4) nanoparticles coated with biocompatible poly (maleic anhydride-alt-1-octadecene) (PMAO) were synthesized for use as an MRI (magnetic resonance imaging) contrast agent. The spin-lattice (T1) and the spin-spin (T2) relaxation times of the nuclear spins (hydrogen protons) in aqueous solutions of various concentrations of coated ferrite nanoparticles were determined using a nuclear magnetic resonance (NMR) spectrometer. The MRI image was detected with higher contrast in comparison with that before injecting. By comparing with the MRI images taken in T1 weighted the T2 weighted images are clearer. The MRI images of a rabbit taken by the T2 weighted which shows that our coated ferrite nanoparticles can be used as a T2 MRI contrast agent.

Keywords: MRI, superparamagnetic iron nanoparticles, thermal decomposition, ferrofluid, poly (maleic anhydride -alt-1-octadecene).

1. INTRODUCTION

Synthesis of magnetic nanoparticles with higher quality that meet the special requirements of the biomedical applications is one of the most attractive research topics, worldwide[1-4]. In these applications, the magnetic nanoparticles are essential to have not only small particle size, high uniformity and strong magnetism [5, 6], but also high dispersity in water and high biological compatibility. To obtain high-quality magnetic nanoparticles, the synthesis has been carried out in organic solvents that have high boiling points. However, for applications in biomedicine, the magnetic nanoparticles need to be shifted from organic solvents to water solvent. Of these magnetic nanomaterials, Fe_3O_4 has been the most favored for biomedical applications thanks to its biocompatibility and facile synthesis. In particular, research on the application of magnetic nanoparticles as an MRI contrast agent has been intensely reported [3–5]. Gadolinium is the most widely used in commercial MRI contrast agents. With gadolinium-based contrast agents, unpaired electrons in the ion of $[Gd(H_2O)_8]^{3+}$ increase the relaxation of nuclear spins (hydrogen protons). Due to the toxicity of gadolinium, however, only the chelate compounds of gadolinium can be used as contrast agents [7, 8]. Since gadolinium-based contrast agents have a T2 effect that is relatively smaller than the T1 effect, they have mainly been used as T1 contrast agents. Ferrite nanoparticle-based T2 contrast agents, such as Feridex [9-12], have also been developed and are now used clinically to obtain better T2 images.

Many methods have been proposed to synthesize Fe_3O_4 nanoparticles such as co-precipitation, sol-gel, hydrothermal or thermal decomposition [13,14]. Among these methods, thermal decomposition is one of the most widely used methods due to its ability to generate uniform particles with high saturation magnetization [15,16]. In our previous work [17,18], Fe_3O_4 fluid was prepared by thermal decomposition using sodium dodecyl sulphate (SDS) and poly (acrylic acid) (PAA) as phase transfer agent. In this paper, we describe a new and convenient thermal decomposition – based approach to synthesize Fe_3O_4 liquid in which poly (maleic anhydride-alt-1-octadecene) (PMAO) was used as the phase transfer ligand. This hydrophilic coating should improve the stability of magnetic nanoparticles in aqueous solution. Within the scope of this report, we focus only on application-oriented research of Fe_3O_4 magnetic nanoparticles in contrast-enhancement of MRI. In animal experimentation, the signal loss in the MR images of rabbit liver was observed after injecting an aqueous solution of the coated nanoparticles into the rabbit, which shows that our coated ferrite nanoparticles can be used as a T2 MRI contrast agent.

2. EXPERIMENTAL

2.1. Chemicals

Iron (III) acetylacetonate ($Fe(acac)_3$), oleylamine (OLA), oleic acid (OA), dibenzyl ether and poly (maleic anhydride -alt-1-octadecene) were purchased from Sigma-Aldrich.

2.2. Synthesis of Fe_3O_4

The fabrication process of Fe_3O_4 particles by thermal decomposition method is described as follows: The original chemicals include $Fe(acac)_3$: 4 mmol, OA: 20 mmol ~ 6.35 ml and OLA: 20 mmol ~ 6.58 ml were put into the reactor containing 40 ml of dibenzyl ether solvent. The mixture was stirred for 30 minutes before warming to a certain temperature. The temperature increasing rates of 5 °C/min, 7 °C/min and 7°C/min were applied for the varying from 25 – 100 °C, 100 – 200 °C and 200 – 300 °C range, respectively. Then, the solution was cooled naturally to

room temperature and washed with ethanol and centrifuged before dispersing in n-hexane solvent. The samples were then dried to determine the structural characteristics, particle size and magnetic properties.

2.3. Synthesis of Fe_3O_4 magnetic fluid

To make Fe_3O_4 nanoparticles soluble and stable in aqueous solution, a phase transfer process was applied, using poly (maleic anhydride -alt-1-octadecene) (PMAO). To prepare solutions, 0.5 g PMAO was dissolved in 10 ml chloroform and stirred gradually for 5 minutes, and iron oxide NPs after washing was dissolved in 10 mL of n-hexane, ultrasonic vibration for 10 minutes.

The obtained solution was added by dropwise into 10 ml of chloroform PMAO solution dissolved and ultrasonic vibration for 30 minutes. When the reaction finished, the obtained product was cooled to room temperature. Then 10 ml of NaOH solution was added into solution. The NPs were then precipitated by centrifuge. After removing the supernatant, the residue was dispersed in water using ultrasound.

2.4. Characterization of magnetic nanoparticles

The morphology properties of these particles (size and shape) were obtained by using transmission electron microscopy TEM (JEM 1010). The shell – core bonds were analyzed by Fourier transform infrared spectroscopy FT-IR (Nicolet 6700). The saturation magnetization of these samples at room temperature was measured up to the highest magnetic field of 10 kOe using a vibrating sample magnetometer (VSM). Size distribution and stability of magnetic fluids were examined by the Zetasizer (Nano ZS – Malvem – UK).

Contrast enhancement in MRI

The MRI experiments were performed using a Siemens MR spectrometer with magnetic field intensity of 1.5 T.

3. RESULTS AND DISCUSSION

3.1. Morphology and particle size

The morphology and particle size of magnetic nanoparticles before and after phase transfer were evaluated by TEM. It can be seen from Figure 1, the magnetic particles were well dispersed in liquid form with relatively narrow size distribution. Herein, the use of coating material with hydrophilic nature such as PMAO has helped to improve the dispersion of magnetic nanoparticles in aqueous phase [17, 18]. This feature is extremely important to enable the biomedical applications of magnetic particles. On Fig. 1 it is shown clearly, the sample enclosed by PMAO owns the large size of about 16.1 ± 0.8 nm while in comparison with the original size of 13.0 ± 1.5 nm. This results show the enlargement of size of particles after enclosing by polymer.

The fluids prepared using PMAO were also subjected to the measurements of the zeta potential done on the Zetasizer system (Fig. 2a). The zeta potential measurements for the three samples results in the low value of -16.6 mV for Fe_3O_4@SDS [17] and equal -40.9 mV for

Fe_3O_4@PAA [18] while for Fe_3O_4@PMAO this value is shifted to higher and equal + 60.5 mV [19, 20].

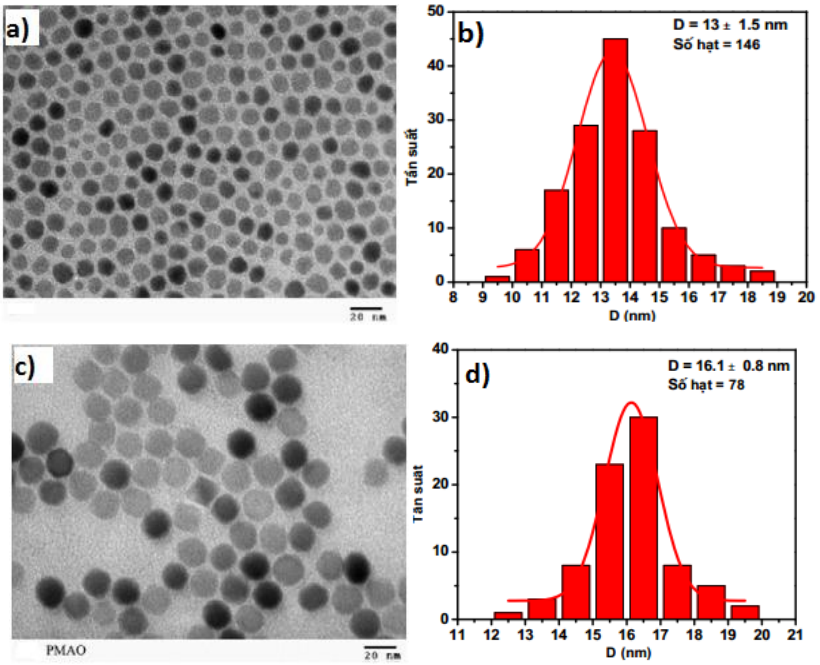

Figure 1. TEM images and particle size distribution of a pre-transfer sample (a, b) and post-transfer samples (c, d).

3.2. FT-IR

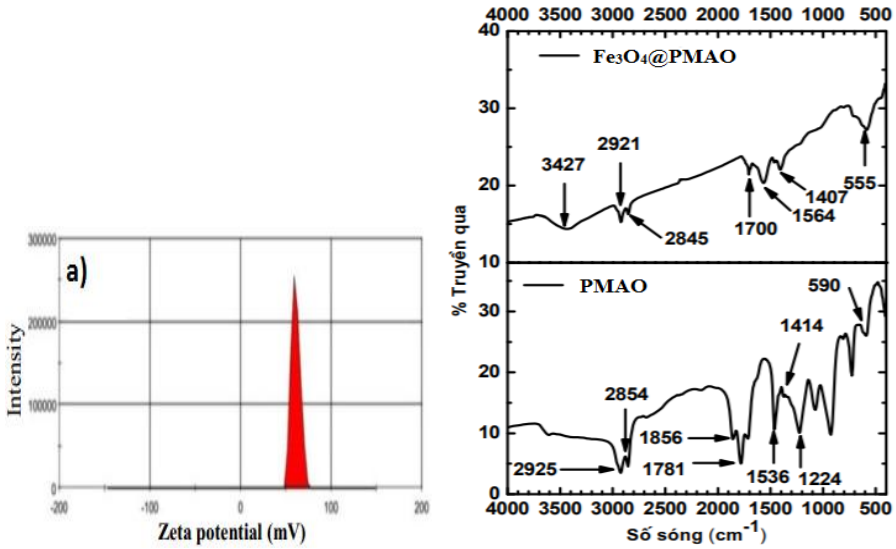

Figure 2. The zeta potential scanning of Fe_3O_4-PMAO (a) and FT-IR spectra of Fe_3O_4 sample, Fe_3O_4-PMAO and poly (maleic anhydride -alt-1-octadecene) (PMAO) sample (b).

FT-IR spectroscopy was performed to confirm the interaction between PMAO and iron oxide nanoparticles (Figure 3). The samples were washed several times with water to remove free

molecules before characterization.

FT-IR spectra were performed to confirm the interaction between PMAO and iron oxide nanoparticles (Fig.2b). Two peaks at 1856 and 1781 cm^{-1} in IR spectrum of PMAO due to vibrations of anhydride ring, are absent in the IR spectrum of Fe_3O_4@PMAO, but two new peaks at 1564 cm^{-1} and 1407 cm^{-1} in this spectrum showed that anhydride ring are opened and changed to be COO^- groups. These changes in IR spectra confirmed the presence of PMAO coating layer on the surface of Fe_3O_4 nanoparticles. Moreover, the strong absorption band at 555 cm^{-1} is associated with Fe-O bond, due to the presence of Fe_3O_4 magnetic nanoparticle. These results are in good agreement with the findings in [21] due to interaction between Fe_3O_4 and PMAO in Fe_3O_4@PMAO.

3.3. Magnetic properties

To study the magnetic characteristics of enclosed samples, their room temperature M(H) curves were measured by the measuring field with the maximum value of 10 kOe. Fig. 3 shows the measured curves of M(H) as well the curves fitted by the Langevin function. From these results, one notes that the M(H) data of the original sample (Fe_3O_4) and enclosed sample Fe_3O_4@PMAO are Langevin-behaved with the accuracy of $R^2 = 0.9969$, thus one can claim that these enclosed samples are superparamagnetic at the room-temperature. The magnetic properties of the original and enclosed samples are characterized by the M(H) curves sketched in Fig. 3. Results showed that magnetization of sample Fe_3O_4@PMAO insignificantly decreased compared to the ungrafted Fe_3O_4 sample (65 and 70 emu/g, respectively, about 7.1% reduced compared to Fe_3O_4 sample). The sample Fe_3O_4@PMAO has been selected to study the effect for high-contrast MRI application.

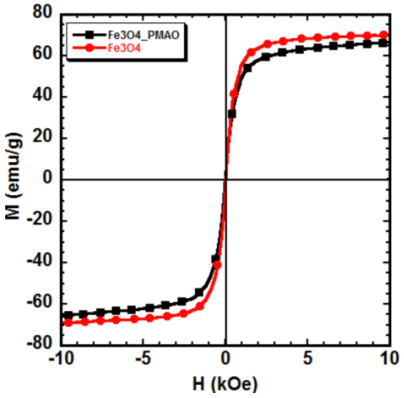

Figure 3. Magnetic hysteresis loops of sample Fe_3O_4 and Fe_3O_4@PMAO. Solid curves are the fitting curves calculated by using the Langevin function.

3.4. Contrast enhancement in MRI

In these studies, the T2 relaxation enhancement effect for our coated sample was also observed in animal experimentation. We obtained abdomen MR images of a Vietnamese white rabbit (2 kg weight) both with and without the injection of the aqueous solution of PMAO-coated nanoparticles. Rabbits were anaesthetized with an intraperitoneal injection of Ketamine Hydrochloride (22 - 50 mg/kg body weight) by intramuscular injection. First, liver, lymph and marrow were scanned with parallel MRI. After that, 6 ml ferrofluid (5 mg/ml) was injected into the rabbits, followed by enhanced MR scanning at the time of 15, 30, and 60 minutes,

respectively [22]. To further investigate the potential usage of the ferrofluid in MR imaging, the T1 longitudinal and T2 transverse relaxation times were measured using the MR spectrometer in 1.5 T field strength. The sequence parameter for (1) T1-weighted image was: TR/TE was 550 ms/20 ms with Coronal, TR/TE was 997 ms/20 ms with AXIAL and for (2) T2-weighted image was: TR/TE was 7500 ms/112 ms with CORONAL. The image was taken in the matrix size of 290 × 290, with the view field of 298 mm × 320 mm. The MR images were obtained using an MRI scanner (1.5 T MR Scanner of Siemens, Vinh International Hospital).

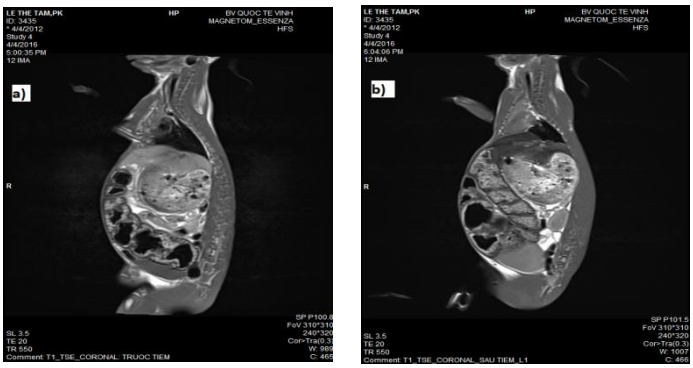

Figure 4. T1-weighted MR images acquired before (a) and after the injection of Fe_3O_4@PMAO (b).

In comparison with post-injection image (Fig. 4a) and 30 minutes after the injection (Fig. 4b), the signal of T1-weighted MR images of abdomen has not changed, since superparamagnetic Fe_3O_4 nanoparticles are T2-type contrast agents in MR imaging.

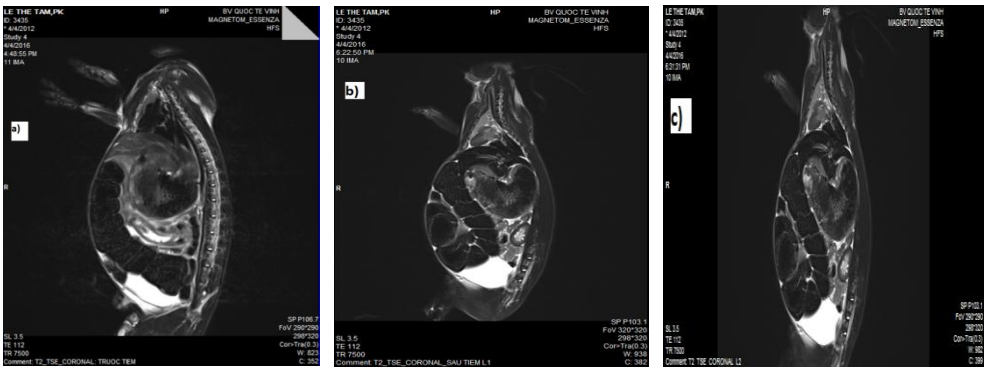

Figure 5. T2-weighted MR images acquired before (a) and at different times after the injection of Fe_3O_4@PMAO (b): 30 min, (c): 60 min).

A T2 image with no contrast agent was taken for reference. Then, after the injection of the contrast agent, the T2 images were taken every 15 minutes. Figure 5(a) shows an abdomen MR image before the injection of the agent. Most parts of this image correspond to the liver. Figure 5(b) shows an image taken 30 minutes after the injection of the agent. In this figure, the liver part of the image is darker than the image without the injection of the agent because the T2 relaxation of nuclear spins in the liver is faster due to the uptakes ferrite nanoparticles by Kuppfer's cells (liver's macrophage cells). The signal intensity after the injection of the agent at the liver (Fig. 5b) is a notable decrease than the signal intensity at the same position before the injection (Fig. 5a), due to the superparamagnetism effect of Fe_3O_4 nanoparticles [23]. This can

be explained that after intravenous injection, Fe_3O_4 nanoparticles are phagocyted by macrophages within lymph nodes. Homogeneous uptake of iron oxide particles in normal lymph node shortens the T2, turning these nodes dark on post contrast images. The water contained in the liver is generally less than that found in surrounding tissues, even less than in a hepatoma (75 % or more) T2-weighted MR images of the abdomen of a Vietnamese white rabbit (a) before and (b) 30 minutes; (c) 60 minutes after the injection of a ferrite nanoparticle agent into the ear vein. Thus, in the T2 image, the liver appears darker than surrounding tissues. The gallbladder has no macrophage cells; thus, it cannot uptake the ferrite nanoparticles. The results of animal experimentation show that our contrast agent of PMAO coated nanoparticles can be used as a T2 agent in MRI.

4. CONCLUSION

In this work, high-quality magnetic fluid was synthesized by thermal decomposition with using poly (maleic anhydride -alt-1-octadecene) (PMAO) as phase transfer ligand. The additional organic coating layer has improved the stability of magnetic particles in aqueous solutions. The average diameter of the coated particles was 16.1 ± 0.8 nm. The result tested on a rabbit showed that the contrast of MRI images taken after injecting the fluid Fe_3O_4@PMAO into the subject is significantly improved. These results show that our agent can be used as a T2 contrast agent in MRI. Further research should clarify whether ferrite-based nanoparticles with different structures or coating materials can also be used as T1 contrast agents.

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