Chitosan-coated Ferrite ($\text{Fe}_3\text{O}_4$) Nanoparticles as a T\textsubscript{2} Contrast Agent for Magnetic Resonance Imaging

Sungwook Hong
Division of Science Education, Daegu University, Gyeongsan 712-714

Yongmin Chang
Department of Diagnostic Radiology, College of Medicine, Kyungpook National University and Hospital, Daegu 700-721

Ilsu Rhee\textsuperscript{*}
School of Physics and Energy Sciences, Kyungpook National University, Daegu 702-701

(Received 4 August 2009, in final form 30 December 2009)

Iron oxide ($\text{Fe}_3\text{O}_4$) nanoparticles coated with biocompatible chitosan were synthesized for use as an MRI (magnetic resonance imaging) contrast agent. The coating was performed simultaneously with the synthesis of the ferrite nanoparticles. A dynamic light-scattering spectrometer (DLS) and a transmission electron microscope (TEM) were used to measure the average diameter of the coated nanoparticles, which was 67.0 nm. Fourier transform infrared (FT-IR) measurements showed strong bonding of the chitosan molecules to the surfaces of the ferrite nanoparticles. The spin-lattice ($T_1$) and the spin-spin ($T_2$) 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. Using these data, we found that the $T_1$ and the $T_2$ relaxivities of the nuclear spins in aqueous solutions of ferrite nanoparticles were 0.00291 and 0.0691 ppm$^{-1}$sec$^{-1}$, respectively. In particular, the value of the $T_2$ relaxivity was much larger than that of the commercial contrast agent GD-DTPA (gadolinium diethylenetriamine penta-acetic acid). A 31.7\% intensity loss in the $T_2$ image of a rabbit liver was observed after injecting the aqueous solution of coated nanoparticles into the rabbit, which shows that our coated ferrite nanoparticles can be used as a $T_2$ MRI contrast agent.

PACS numbers: 87.61.Pk, 76.60.Es
Keywords: Nanoparticles, $T_2$ contrast agent, MR imaging
DOI: 10.3938/jkps.56.868

I. INTRODUCTION

Medical applications of nanoparticles have attracted a great deal of attention as evidenced by the development of various techniques for the synthesis of nanoparticles with uniform size and shape distributions [1,2]. 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 $[\text{Gd(H}_2\text{O)}_8]^{3+}$ 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 [6,7]. Since gadolinium-based contrast agents have a $T_2$ effect that is relatively smaller than the $T_1$ effect, they have mainly been used as $T_1$ contrast agents. Ferrite nanoparticle-based $T_2$ contrast agents, such as Feridex [8], have also been developed and are now used clinically to obtain better $T_2$ images. Both the gadolinium-based contrast agents (Gd-DTPA, Gd-DTPA-BMA, etc.) and Feridex use dextran as a biocompatible coating material. Other polymers, such as PCL(poly-$\epsilon$-caprolactone), PLA(poly lactide), and chitosan, are also used as coating materials [9,10]. Chitosan is known as an ideal bio-polymeric material, because it has high biocompatibility, disintegration, non-toxicity, and antibacterial and hydrophilic properties [11,12].

In this paper, we report the application of chitosan-coated ferrite nanoparticles as an MRI contrast agent. We used a method to coat chitosan simultaneously with the synthesis of the ferrite nanoparticles. The $T_1$ and the $T_2$ relaxivities of nuclear spins were determined from

\textsuperscript{*}E-mail: ilrhee@knu.ac.kr
Chitosan-coated Ferrite (Fe₃O₄) Nanoparticles as a T₂ Contrast Agent for Magnetic

Sungwook Hong et al.

the T₁ and the T₂ data for aqueous solutions of various nanoparticle concentrations. We found that the T₂ relaxivity of our contrast agent had a larger value in comparison with commercial contrast agents. In animal experimentation, a 31.7% 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.

II. EXPERIMENTS

Chitosan-coated ferrite nanoparticles were formulated by coating chitosan simultaneously with the synthesis of iron ferrite (Fe₃O₄) nanoparticles. The detailed procedures of this synthetic method for the formation of dextran-coated ferrite nanoparticles have been described elsewhere [13].

Chitosan differs from dextran in that it is not dissolvable in water. Thus, the aqueous solution of chitosan (ALDRICH, low molecular weight, Brookfield viscosity 20 cps) was made with the use of acetic acid. A 1%(w/v) aqueous solution of chitosan was formulated by adding 0.2 g of chitosan into a mixture of water (19 mL) and an acetic acid solution (1 mL, SIGMA, 2N). The pH of the mixture of aqueous solutions (0.5 M, 0.15 mL) of iron chloride (FeCl₂ + FeCl₃) and diluted chitosan (0.05%(w/v), 15 mL) was maintained at 6.9 through the slow addition of a solution of NH₄OH. The pH was fixed at 6.9 so that the chitosan dissolved in the solution would conglomerate at a pH higher than 7. Pressurized air was supplied to the above solution to oxidize Fe²⁺ and Fe³⁺ for the formation of Fe₃O₄ [14]. Using this method, the coating of chitosan was formed simultaneously with the synthesis of ferrite nanoparticles. We also prepared bare ferrite nanoparticles to compare their relaxivities with those of the chitosan-coated nanoparticles [15]. In synthesizing bare ferrite nanoparticles, we used TMAOH (tetramethylammonium hydroxide) as a surfactant to maintain the aqueous solution of bare ferrite nanoparticles in a state of colloidal suspension.

A dynamic light-scattering spectrometer (DLS-7000AL, Otsuka Electronics, Japan) was used to determine the average diameters (Fig. 1) of the bare and the coated nanoparticles at 7.51 nm and 67.0 nm, respectively. Figure 2 shows TEM (H-7600, Hitachi) pictures of a bare nanoparticle and of coated particles. As shown by this figure, several bare particles conglomerate to form a coated particle.

The bonding status of chitosan molecules on the surface of the particle could be checked from the wavelength-dependent data of transmittance obtained using an FT-IR spectrometer (Bruker IFS66/FRA-106). Figure 3 shows the FT-IR spectra for pure chitosan and for the bare and coated particles. The absorption lines at 1650 and 1576 cm⁻¹ in the chitosan spectra are due to the absorption by amide I and II, respectively [16]. On the other hand, the absorption line at 1158 cm⁻¹ are due to the absorption by the vibrational motion of the -C-O-C- bond [17]. In the spectra of the coated nanoparticles, it is apparent that the absorption line at 1158 cm⁻¹ has shifted to 1066 cm⁻¹, and the amide absorptions became relatively larger than those in the chitosan spectra. This fact indicates that there was strong hydrogen bonding between the oxygen in Fe₃O₄ and the hydrogen in the amino group (-NH₂) in chitosan, which explains the larger amide absorptions in the spectra of the coated particles. As shown in Fig. 4, in the bonding of chitosan with the particle, the amino group bonded to the particle, but the hydroxyl group (-OH) did not. Consequently, the coated particles were slightly positively charged due to hydrogen’s having a relatively smaller electronegativity than the oxygen. Due to the Coulomb repulsion be-
Fig. 3. FT-IR spectra for pure chitosan, bare ferrite particles, and chitosan-coated ferrite particles. In the spectra of the coated particles, the absorption line at 1158 cm\(^{-1}\) is shifted to 1066 cm\(^{-1}\), and the amide absorptions become relatively larger than those in the pure chitosan spectra. This suggests that the existence of a strong hydrogen bond between the oxygen of Fe\(_3\)O\(_4\) and the hydrogen of the amino group (-NH\(_2\)) in the chitosan, which is why there is larger amide absorptions in the spectra of the coated particles.

Fig. 4. The amino group (-NH\(_2\)) of chitosan is bonded to the particle. However, the hydroxyl group (-OH) of chitosan remains unbonded. Consequently, the coated particles are slightly positively charged. Due to the Coulomb repulsion between these positively charged particles, the aqueous solution of the coated particles remains in a state of colloidal suspension when no organic solvent or surfactant is used.

III. RESULTS AND DISCUSSION

The \(T_1\) and the \(T_2\) relaxation times of the nuclear spins in the aqueous solution of chitosan-coated ferrite nanoparticles were determined using an nuclear magnetic resonance (NMR) spectrometer (Bruker Advance Digital 400, 400 MHz). For the \(T_1\) measurements, the inversion-recovery pulse sequence was used. On the other hand, the CPMG (Carr-Purcell-Meiboon-Gill) pulse sequence was used for the \(T_2\) measurements.

The spin-lattice relaxation time (\(T_1\)) is mainly related to the rate of energy transfer from nuclear spins to the neighboring molecules. The spin-spin relaxation time (\(T_2\)), however, is related to the rate of the dephasing process of nuclear spins due to their neighboring magnetic inhomogeneity.

Relaxivity is the measure of the ability of the MRI contrast agent to increase the relaxation of the surrounding nuclear spins, which can be used to improve the contrast of the MR images. Relaxivity is expressed in units of s\(^{-1}\) per ppm of nanoparticles. The contribution of paramagnetic contrast agents to the relaxation of the nuclear spins is mainly due to both inner and outer sphere processes. The inner sphere process is due to the chemical interchange interaction between the bound water of the paramagnetic agents and the surrounding free water, which eventually increases the relaxation (larger effect on \(T_1\)) of nuclear spins. On the other hand, the outer sphere process occurs when the paramagnetic agents diffuse through the free water. In this process, random fluctuations of paramagnetic agents create local magnetic field inhomogeneity, thus increasing the relaxations (larger effect on \(T_2\)) of nuclear spins [20]. In the clinically-used gadolinium-based contrast agents, gadolinium ions are formed as chelates. Thus, the bound water of the chelates can continuously interact with the surrounding free water and, consequently, increase the \(T_1\) relaxation of nuclear spins. Most gadolinium chelate agents have an inner sphere effect that is larger than the outer sphere effect, therefore, they are used as \(T_1\) contrast agents. On the other hand, the coated ferrite nanoparticle agents are completely surrounded by their coating material, and the chemical interchange interaction (inner sphere process)
Chitosan-coated Ferrite (Fe$_3$O$_4$) Nanoparticles as a T$_2$ Contrast Agent for Magnetic... Sungwook Hong et al.

Fig. 5. Plot of 1/T$_1$ versus the particle concentration for the bare and the coated ferrite particles. The same plot for the Gd-DTPA-BMA is shown here for reference. The plot for the low concentration region is enlarged in the inset for clarity.

does not occur. However, the ferrite nanoparticles have a much larger magnetic moment than gadolinium ions and produce larger magnetic field fluctuations (inhomogeneity). Due to this property of magnetic nanoparticles, they are considered to be ideal T$_2$ contrast agents.

The relaxivities of nuclear spins in the aqueous solution of magnetic nanoparticles can be expressed as [21]

$$\frac{1}{T_{1\text{im}}} = \frac{1}{T_i} + R_i C,$$

where $i = 1$ or 2, and $1/T_i$ represents the relaxivity of nuclear spins with no nanoparticle contrast agent. $R_i$ is denoted as the relaxivity of nuclear spins per ppm of nanoparticles, and $C$ represents the concentration of nanoparticles in the aqueous solution.

Figure 5 shows the 1/T$_1$ as a function of the particle concentration for samples of aqueous solutions of the bare and coated ferrite nanoparticles and for the commercial Gd-DTPA-BMA contrast agent. The slopes of the linear lines fitted to these samples were 0.07897, 0.00291, and 0.00699 ppm$^{-1}$sec$^{-1}$, respectively. The T$_1$ relaxivity for the coated ferrite sample was 27 and 2.4 times smaller than those for the bare ferrite and Gd-DTPA-BMA samples, respectively. These results imply that the T$_1$ relaxation was more influenced by the inner sphere process than by the outer sphere process. A larger T$_1$ relaxivity for the bare ferrite sample can be attributed to the continuous exchange interaction of bound water on the surface of bare particles with the surrounding free water. The slow tumbling rate of paramagnetic particles is known to increase the T$_1$ relaxivity. The results of the enhancements of T$_1$ relaxivity by reducing this tumbling rate have been reported [22,23]. To decrease the tumbling rate, they modified the surface structure of paramagnetic particles either by attaching various ligands or by increasing the molar weight of the particles with additional coatings. The low T$_1$ relaxivity of our coated ferrite particles might be improved by reducing the tumbling rate with structural modifications.

Figure 6 shows 1/T$_2$ as a function of particle concentration for the samples of aqueous solutions of the bare and coated ferrite nanoparticles and of the commercial Gd-DTPA-BMA contrast agent. The slopes of the linear fitting lines for these samples were 3.75438, 0.0619, and 0.00855 ppm$^{-1}$sec$^{-1}$, respectively. The T$_2$ relaxivity for the coated ferrite sample was 60 times smaller than it was for the bare ferrite sample. However, it was 7.2 times larger than it was for the Gd-DTPA-BMA sample, which was expected. This result shows that the ferrite nanoparticles are a more effective T$_2$ agent than the gadolinium-based contrast agents. Compared with the previously reported relaxivity of the dextran-coated ferrite sample [3], we obtained a 10% increase in the T$_2$ relaxivity for the chitosan-coated ferrite sample.

The T$_2$ relaxation enhancement effect for our coated sample was also observed in animal experimentation. We obtained abdomen MR images of a New Zealand white rabbit (2 kg weight) both with and without the injection of the aqueous solution of chitosan-coated nanoparticles. We used an aqueous solution containing 1,237 ppm of the coated particles. A total of 60 $\mu$mol (1.81 mL of the aqueous solution) was given to the rabbit, which corresponded to 2.23 mg of the ferrite. This amount of ferrite injection is about 62% of the ferrite particles used in our previous work [3].

The MR images were obtained using an MRI scanner (1.5 T MR Scanner, GE Medical System). A T$_2$ image with no contrast agent was taken for reference. Then, after the injection of the contrast agent, the T$_2$ images
Fig. 7. T2-weighted MR images of the abdomen of a New Zealand white rabbit (a) before and (b) 20 minutes after the injection of 60 µmol of a ferrite nanoparticle agent into the ear vein. The ratio of the signal intensity at the marked position was measured at I_b/I_a = 0.707. Consequently, the presence of the particles resulted in a 29.3% decrease in the signal intensity.

Fig. 8. Same T2-weighted MR images as in Fig. 7 (a) before and (b) 20 minutes after the injection of the ferrite nanoparticle agent into the ear vein, but at a different angle (lower abdomen region). The ratio of the signal intensity at the marked position was measured at I_b/I_a = 0.660. Thus, the presence of the particles resulted in a 34.0% decrease of the signal intensity. The brighter regions at the bottoms in both figures correspond to the gallbladder. The brightness at the position of the gallbladder did not change even after the injection of the agent because it has no Kupffer cells. See the text for details.

IV. CONCLUSIONS

We formulated chitosan-coated ferrite nanoparticles by using a method that coat the particles simultaneously with their synthesis. The average diameter of the coated particles was 67.0 nm. The effect of the nanoparticles on the relaxations of the nuclear spins in an aqueous solution of ferrite particles was 7.2-fold larger during T2 relaxation than that of a commercial gadolinium-based contrast agent. In animal experimentation, a 31.7% signal loss was observed in the MR image taken 20 minutes after the injection of the nanoparticle agent. 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.
Chitosan-coated Ferrite ($\text{Fe}_3\text{O}_4$) Nanoparticles as a $T_2$ Contrast Agent for Magnetic

---

Sungwook Hong et al.