

Relaxivity of Hydrogen Protons of Water Molecules in the Aqueous Solutions of Dextran - and Chitosan - Coated Ferrite Nanoparticles

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Abstract. Ferrite nanoparticles were synthesized at around 4°C~25°C and coated during the synthesis process with biocompatible and biodegradable polymers, dextran or chitosan. The size of ferrite nanoparticles was determined to be 7.51nm by DLS (Dynamic Light Scattering) and TEM (Transmission Electron Microscope). The size of coated nanoparticles was increased up to 128.6nm and 67nm for dextran and chitosan coating, respectively. The relaxivity of T_1 and T_2 of hydrogen proton of water molecule in the aqueous solution of both uncoated and coated nanoparticles were found to be linear with the concentration of nanoparticles in the solution. We found that the relaxivity of hydrogen protons in the solution of coated nanoparticles are much faster (50 to 60 times) than those in the solution of uncoated nanoparticles.

Keywords: ferrite nanoparticles, dextran coating, chitosan coating, relaxivity.

1. Introduction

Recently, along with the development of technologies that enable the manipulation of the shape and size of nanomaterial, their application to the medical field is attracting great interest. Especially, the development of contrast agents for MRI (Magnetic Resonance Imaging), which is widely used in noninvasive diagnosis, is of great interest. Gd (Gadolinium), the paramagnetic material currently used for contrast agents, is not suitable for biological use in its bare state. In order to negate its toxicity that induces cellular dechelation, Gd is usually coupled with ligands such as DTPA and used in a chelated state. As an alternative, Fe (iron) [1] is under development, since it is a stronger magnet and has a stronger influence on the magnetic relaxation time, especially the T_2 . For the Fe-ferrite (Fe_3O_4) nanoparticle to be used as a contrast agent, it needs to have the properties of a super-paramagnet which is achievable by making the size of nanoparticles smaller than a few tens of nanometer [2]. Also, since it is extremely toxic in its bare state, it needs to be coated with biocompatible polymers. Other conditions include absence of side effects, maintenance of chemical stability in the human body, and the excretion from the body in an appropriate amount of time. Several nano-sized ferrite particle synthesis methods, which involve the use of Fe_2^+ and Fe_3^+ in room temperature, exist [3-9]. The polymer materials used for coating are PCL (poly- ϵ -caprolactone), PLA (poly lactide), copolymer [10] of PCL and PLA, dextran [11, 12], chitosan [13], and so on. Dextran and chitosan are hydrophilic, biodegradable, and biocompatible. Especially, chitosan is non-antigenic, non-toxic, and biofunctional, making it an ideal polymer for biological application [14-15]. In our study, synthesis of ferrite, Fe_3O_4 , and the coating of it with biocompatible polymer such as dextran and chitosan were executed simultaneously at 4°C~25°C and approximately pH 7, in the neutral state; this method is similar to the one used for coating ferrite with trypsin [9], which is an enzyme. We studied the relaxation time of the protons of water molecules surrounding the nanoparticles, which were dissolved in water. More specifically, the effect of two factors on the relaxation time was studied: the concentration of nanoparticles and the absence/presence of polymer coating. Results showed that both $1/T_1$ and $1/T_2$ were linearly dependent on the nanoparticle concentration. Polymer coating significantly reduced the effect of nanoparticles on T_1 and T_2 .

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2. Experiment

The coating of ferrite nanoparticles with dextran (SIGMA, Average mol wt 68,800) was performed simultaneously with the synthesis of the ferrite particles. The experimental setup for these processes is shown in Fig.1. 10ml of 0.15M iron chloride aqueous solution ($\text{FeCl}_2 + \text{FeCl}_3$) and 20ml of 2% (w/w) dextran solution were mixed together. When air bubbles were introduced into this mixture by using a pipette, some of the Fe^{+2} ions were oxidized to Fe^{+3} ions, and Fe_3O_4 particles were synthesized. The dextran in the mixture adhered to the particles, but most biocompatible materials, including dextran, were coated stably on ferrite surfaces only when a near neutral pH condition was met. Thus, we adjusted the amount of added NH_4OH solution so that the pH value of the mixture maintained a value of around 7. The coating process was performed inside an ice water bath [7]. The oxidation of iron due to an oversupply of oxygen by the air jet was eliminated at low temperature of about 4°C . Otherwise, when the coating processes were performed at room temperature, a precipitate of iron oxide was observed at the bottom of the cylinder.

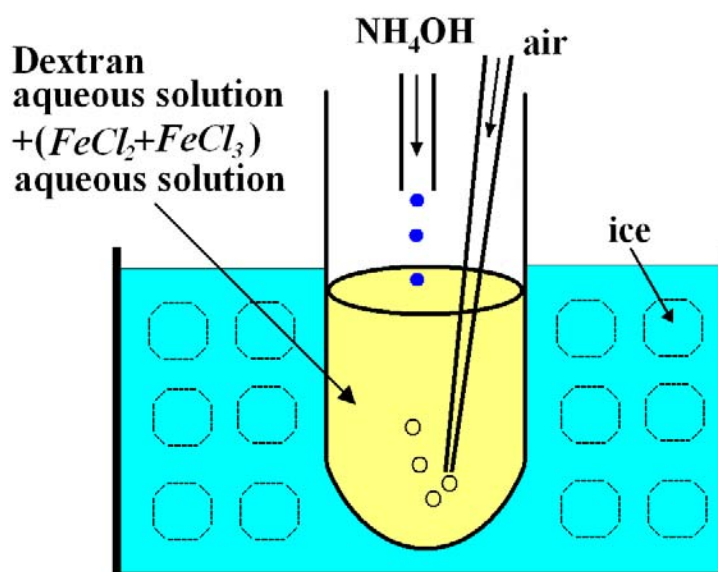


Fig.1: The schematic figure of the experimental setup for synthesizing and coating the ferrite nanoparticles simultaneously is shown here. The dextran coating processes are performed inside the ice water bath at around 4°C and the chitosan coating processes are performed at room temperature.

1% (w/v) chitosan (ALDRICH, low molecular weight, Brookfield viscosity 20.000cps) solution was made by dissolving 0.2g of chitosan in 19ml of water with 1ml of 2N Acetic acid solution (SIGMA). The coating of ferrite nanoparticles with chitosan was performed simultaneously with the synthesis of the ferrite particles by mixing 15ml of the diluted 0.05% chitosan solution with 0.5ml of 0.5M iron chloride solution. The process of coating with chitosan was basically the same as that of dextran coating except the iron bath was not used. The reason for this is that the colloid state of coated particles is not maintained due to high viscosity of chitosan at low temperature.

Dextran coating is formed by hydro-bonding of the carboxy group (COOH) of dextran to the oxygen atom of Fe_3O_4 . On the other hand, the hydro-bonding of the hydroxyl group (OH) of chitosan to the oxygen atom of Fe_3O_4 results in the coating of chitosan on the particles. The OSO_3^- and NH_2^- groups exist on the opposite sides of the hydro-bonding for dextran and chitosan coatings, respectively. Thus, any organic solvent or surfactant is not necessary to maintain the colloidal state in the solution of coated particles [14, 16].

The synthesis of bare ferrite nanoparticles and their physical properties were already described in detail elsewhere [17].

The size distribution of particles was measured by the DLS (Dynamic Light Scattering) method. This is shown in Fig.2. The mean diameter of bare particle was about 7.51nm. On the other hand, the mean diameters of coated particles were increased to 128.6nm and 67nm for dextran and chitosan coatings, respectively. TEM (Transmission Electron Microscope) was used to check how well the coating was formed. This is shown in Fig.3. In the dextran coating, aggregated ferrite particles showed a diameter of about 100nm. On the other hand, separated bare ferrites of a few nm were observed in chitosan coating.

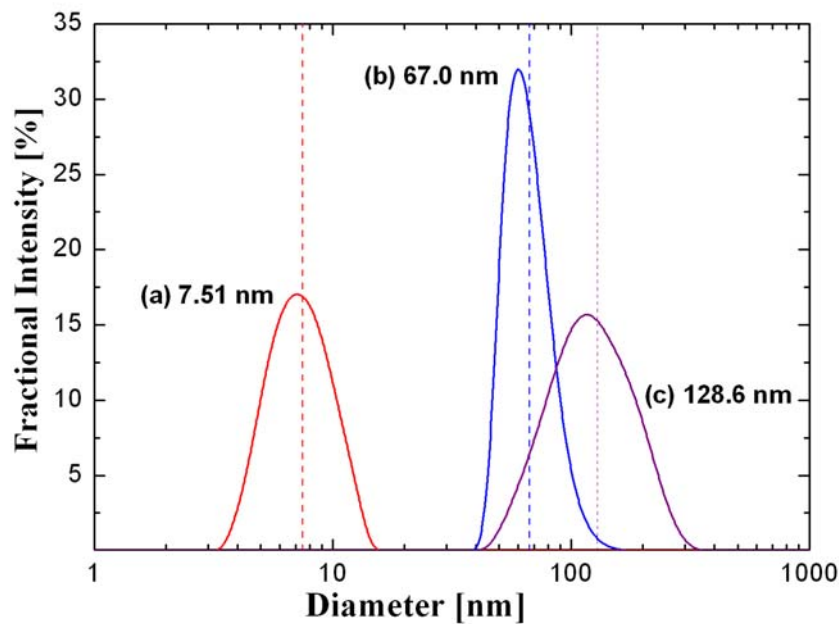


Fig.2: The size distribution of nanoparticles in an aqueous solution by using dynamic light scattering spectrometer is shown in this figure. The mean diameters of the bare (a), chitosan coated (b), and dextran coated (c) ferrite particles are about 7.51nm, 67.0nm, and 128.6nm, respectively.

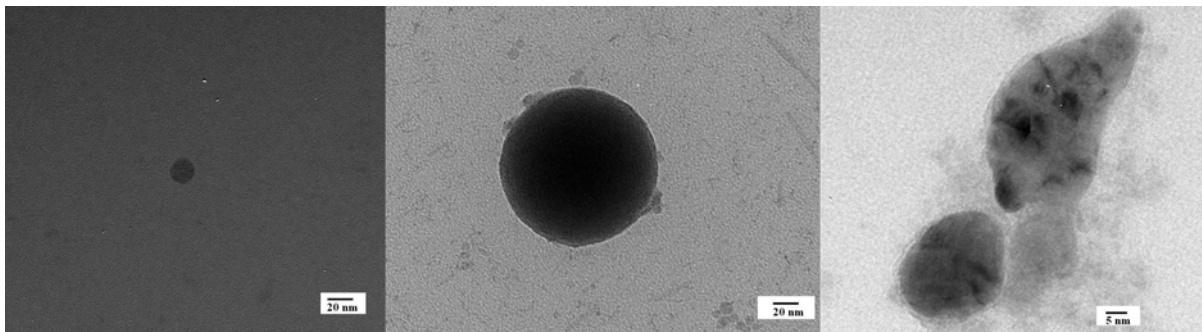


Fig.3: The transmission electron microscope images of the bare (left), dextran coated (center), and chitosan coated (right) ferrite particle are shown here. We can see in this figure that the dextran and chitosan are well coated on the entire surface of ferrite particles.

3. Results and Discussion

The T_1 and T_2 relaxation times of hydrogen protons of water molecules in ferrite nanoparticle solutions at various concentrations were measured using the nuclear magnetic resonance spectrometer (Bruker Advance Digital 400, 400MHz, T_1 : Inversion Recovery T_2 : CPMG). In the solutions, the T_1 and T_2 of the hydrogen protons of water molecules are influenced by the surrounding magnetic iron-ferrite particles, and the relaxation times become shorter than those in pure water (Both the T_1 and T_2 have the same value of about 4 sec in pure water). The spin-lattice relaxation time, T_1 is mainly related to the energy transfer between hydrogen protons and their surroundings. On the other hand, the spin-spin relaxation time, T_2 is related to the dephasing process of the spin vectors of the hydrogen protons.

In MRI, the reduction of relaxation times due to the paramagnetic materials in the contrast agent results in the decrease of MR (Magnetic Resonance) signal, and consequently increases the image contrast. Especially, super-paramagnetic materials like nano-sized ferrite have a very high magnetic susceptibility, and thus generate a large local magnetic field which accelerates the dephasing process of the spin vectors of the hydrogen protons. Consequently, the ferrite based contrast agent will give us a very good contrast in the T_2 weighted image.

The T_{1m} and T_{2m} of hydrogen protons in an aqueous solution of magnetic particles are related to the T_1 and T_2 in pure water as [18-19]

$$1/T_{im} = 1/T_i + R_i C \quad (1)$$

where $i=1$ or 2 , R_i 's denote the relaxivities of T_i 's of hydrogen protons, and C represents the concentration of particles homogeneously distributed in aqueous solution. The plots of $1/T_{im}$ versus C for both bare and coated ferrite particles are shown in Fig.4.

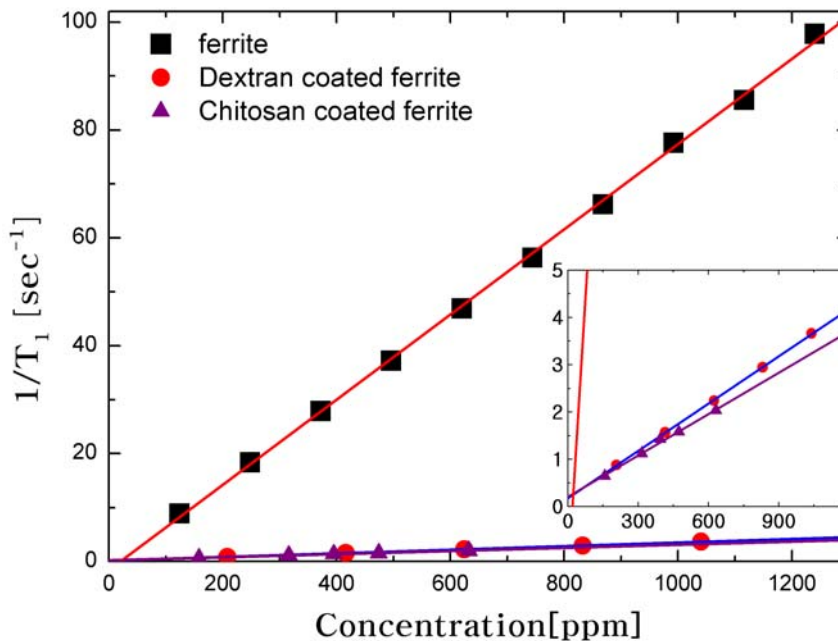


Fig.4: The plots of T_1 versus C for both bare and coated ferrite particles are shown here. The plot for the coated ferrite is blown up as an inset for clarity.

We can see in Fig.4 that the linear dependence of T_1 relaxation rate for both dextran and chitosan coatings are well preserved. The slopes (relaxivities) are 0.07897, 0.00333, and 0.00291 [1/s-ppm] for the

bare, dextran- and chitosan-coated particles, respectively. We can see that the T_1 relaxivity for the bare ferrite particles is decreased by a factor of about 1/23 and 1/27 for the dextran- and chitosan-coated particles, respectively. This result is consistent with our expectation. The distance between the water molecules and the core ferrite of coated particles is much larger than the distance between the bare ferrite and water molecules. Consequently, the relaxivity for the coated ferrite becomes much smaller than that for the bare ferrite. A slightly larger relaxivity for the dextran coating reflects the fact that the aggregated dextran-coated particles shown in Fig.3 generate a slightly larger local magnetic field fluctuation than the dispersed chitosan-coated nanoparticle. The T_2 relaxivities are determined to be 3.7544, 0.07223, and 0.0619 [1/s-ppm] for the bare, dextran- and chitosan-coated particles, respectively (Fig.5). Thus, we can see that T_2 relaxivities for the dextran and chitosan coatings are decreased by a factor of about 1/52 and 1/61, respectively. This means that T_2 is influenced much more by the coating of ferrite nanoparticles than T_1 . The reason for this is that T_2 is more sensitive to the magnetic inhomogeneity than T_1 . To achieve the maximum effect of contrast agent on T_2 , the ferrites should be coated as thinly as possible, since the interaction between magnetic particles and water molecules depends on their distance. The ratio of T_2 to T_1 relaxivity for the bare particle is about 50. This ratio is reduced to about 21 for both dextran- and chitosan-coated particles. Thus, we can see that the change of T_2 relaxivity according to the particle concentration is much greater for both the bare and coated particles. Consequently, we can see that the MRI contrast agent using ferrite superparamagnetic particles is much more effective for the T_2 weighted image than for the T_1 weighted image.

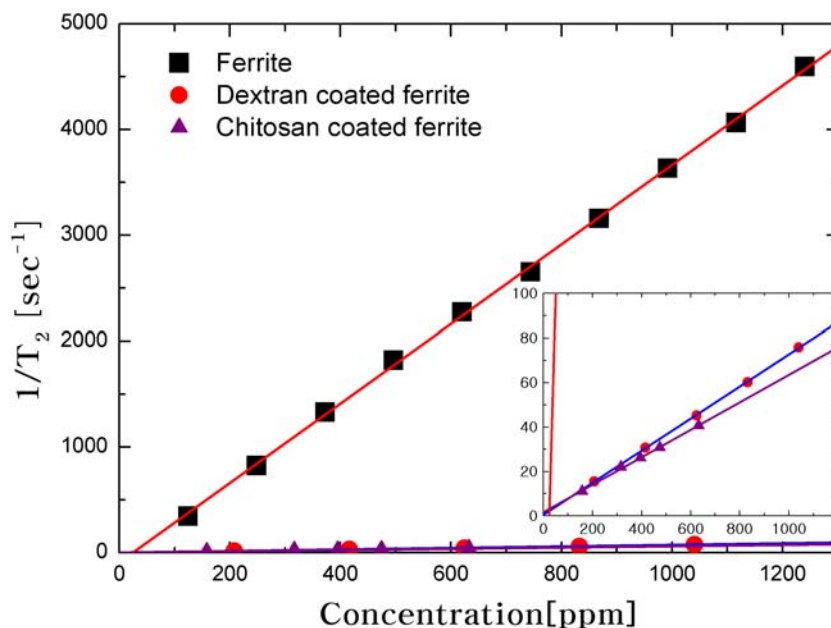


Fig.5: The plots of T_2 versus C for both bare and coated ferrite particles are shown here. The plot for the coated ferrite is blown up as an inset for clarity.

Using our results, we will be able to formulate the relationship between the changes of relaxation times of hydrogen protons and the magnetic particle concentration injected into an experimental body. These outputs will help us develop MRI contrast agents based on magnetic nanoparticles. Animal experiments with coated ferrite solution as an MRI contrast agent are now under way. We hope that a systematic relationship between the concentration of ferrite nanoparticle and the contrast of MRI imaging is obtained. This will let us develop MRI contrast agents whose relaxation times are controllable by adjusting the concentration of magnetic particles.

4. References

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