

Gd₂O₃ nanoparticles: size-dependent nuclear magnetic resonance

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In this communication, we demonstrate that there is an optimum gadolinium oxide (Gd₂O₃) nanoparticle size of 2.3 nm; in the presence of Gd₂O₃ particles smaller and larger than this critical size, the spin-lattice relaxation rate ($T_1 = 1/r_1$) of water protons at 7.0 T drastically decreases. Since r_1 is directly related to the quality of magnetic resonance imaging, the results presented here have significant implications for clinical diagnostics. Copyright © 2012 John Wiley & Sons, Ltd.

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Magnetic resonance imaging (MRI) is widely used for medical diagnostic purposes (1–14). Among the 7–10 million MRI scans performed each year around the world, 30–50% are performed in the presence of MRI contrast agents (CA) (1,2,15). Contrast agents are magnetic materials that enhance the quality of the MRI images by controlling the spin-lattice (longitudinal, $T_1 = 1/r_1$) and the spin-spin (transverse, $T_2 = 1/r_2$) relaxation times of water protons available in the biological sample under investigation (1,2,12,14). Depending on the chemical composition of a CA, T_1 and T_2 are reduced to different extents (1,12,14,16). The MR signal intensity increases with increasing r_1 while decreasing with increasing r_2 (1,16–18). Contrast agents that increase the MR signal intensity, such as those based on gadolinium (Gd), are known as positive CAs, whilst CAs that enhance r_2 , i.e. those based on iron (Fe), are known as negative contrast agents (15,17,19). MRI images enhanced by negative contrast agents, such as ultra-small superparamagnetic iron oxide [USPIO < 40 nm (2)] particles and superparamagnetic iron oxide [SPIO > 60 nm (2)] particles, suffer from low resolution and the so-called 'blooming artefacts', which make cell delineation difficult (15). Also, synthesizing SPIO of definite size is a daunting task (14). Further, SPIO is specific to liver and spleen (2,14,20) while USPIO is extensively taken up by lymph nodes and bone marrow (2). In contrast, gadolinium-based positive CAs are overwhelmingly popular owing to their higher MR signal intensity, higher resolution, and suitability for all organs (1,15,18,20,21).

Since ionic gadolinium is toxic, currently used gadolinium-based contrast agents are in chelate forms (1,15,21). However, despite the widespread usage of gadolinium chelates as positive contrast agents, they offer limited scope for extended imaging time. This is due to the low molecular weights of gadolinium chelates, which lead to rapid renal excretion (2,19). Also, their inherent structures limit the possibilities for further chemical modifications (18,19). These limitations of the gadolinium chelates and the growing demand for positive contrast agents led to the development of nanoparticulate gadolinium-based contrast agents such as NaGdF₄ (18), gadolinium phosphate (GdPO₄) (13), gadolinium fluoride (GdF₃) (22) and gadolinium oxide (Gd₂O₃) (11,19,23,24). Particulate contrast agents provide the opportunity for extended imaging and their surfaces are readily available for adding further functionalities (11,17,19,20). For example, Bridot *et al.* (19) have demonstrated

that organic dye-functionalized Gd₂O₃ nanoparticles can be used for both MRI and fluorescence imaging. Another advantage of using nano-particulate gadolinium oxide over chelated gadolinium-based CAs is that the former has a much higher X-ray attenuation capability. This makes Gd₂O₃ nanoparticles suitable for computed tomography (CT) and hence for multimodal imaging combining MRI and CT scans (24). In the presence of Gd₂O₃ nanoparticle-based contrast agents, the spin-lattice relaxation of water protons is also significantly enhanced. In the past, Gd₂O₃ particles of 1–40 nm (11,15,17,19,20,24) have been used as CAs and it is generally believed that the smaller the Gd₂O₃ particle is, the better the MR signal is. However, in this communication we experimentally demonstrate that there is an optimal Gd₂O₃ nanoparticle size that provides higher spin-lattice relaxation rate (r_1) of water protons compared with those given by particles smaller or larger than this size. Knowing the optimum size of Gd₂O₃ particles that produces the highest longitudinal relaxation rate has implications in MRI, since the intensity of MR images is directly related to r_1 (17).

In order to find Gd₂O₃ size-dependent spin-lattice relaxation of water protons, samples of gadolinium oxide in the size range 1.5–194.0 nm were prepared using a synthetic route similar to that we reported earlier (25) (for the description of the synthesis route see the Experimental section).

To determine the average size of the as-synthesized particles, characterizations using dynamic light scattering (DLS) and transmission electron microscope (TEM) were carried out. Figure 1(a) shows a representative TEM image corresponding to an average particle size of 2.34 nm (DLS measurement). TEM image reveals particles between 2 and 3 nm and a relatively narrow size distribution. Given that DLS measurement depends on other parameters such as the solvent viscosity, and the refractive indexes of the solvent and particles, agreement between DLS and TEM

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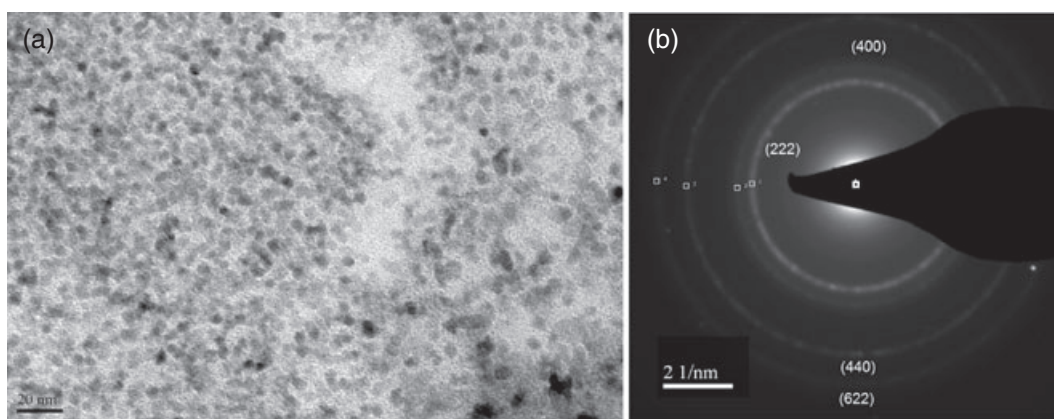


Figure 1. (a) Transmission electron microscope image of Gd_2O_3 nanoparticles. Scale bar is 20 nm. (b) Selected area electron diffraction image.

measurements is very good. Figure 1(b) shows selected area electron diffraction (SAED) data corresponding to the particles shown in Fig. 1(a). It confirms a cubic crystalline structure of typical gadolinium oxide (26,27). To confirm the composition of the nanoparticles involved in this study, we conducted X-ray photoelectron spectroscopy (XPS). Figure 2(a) shows an XPS high-resolution spectrum of the binding energy of the as-prepared sample. Peaks at 142.1 and 147.8 eV denote Gd ($4d_{5/2}$) and Gd ($4d_{3/2}$), respectively.

These results are in good agreement with the previously published XPS data on Gd_2O_3 particles (28). Figure 2(b) shows a Fourier transform infra-red (FTIR) spectrum of citric acid-coated Gd_2O_3 nanoparticles used in this study. In the pure form, citric acid has (C=O) stretching bands at 1715 and 1748 cm^{-1} , while in the case of citric acid stabilized Gd_2O_3 nanoparticles, these two bands completely disappear. Instead, two new bands at 1383 and 1565 cm^{-1} appear, which can be attributed to the symmetric and anti-symmetric stretches of the carboxylate ion (COO^-), respectively (29).

Figure 3 shows longitudinal relaxation data for water protons in the presence of Gd_2O_3 particles of different sizes at 7.0 T. In all the samples, the concentrations of gadolinium were equal. The size of the Gd_2O_3 particles was controlled by the concentration of citric acid (the capping agent). It can be seen that, as the particle size decreases, the r_1 of the water protons gradually increases. This trend continues until the particle size reduces to ~ 3.0 nm. When the particle size decreases below 3.0 nm, the longitudinal relaxation rate of the water protons increases rapidly until the Gd_2O_3 nanoparticles size reaches ~ 2.30 nm. Once the size of the Gd_2O_3 nanoparticles reaches the optimum value (~ 2.30 nm), a further

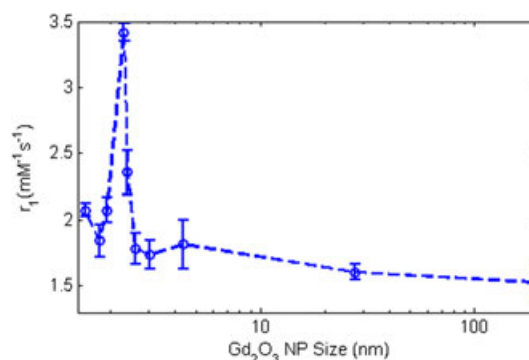


Figure 3. Longitudinal relaxation of water protons in presence of Gd_2O_3 nanoparticles at 7 T.

reduction in the particle size has a negative impact on the spin-lattice relaxation rate of the water protons. Specifically, if the size of Gd_2O_3 particles differs from the optimum size by approximately 1 nm, the rate of the longitudinal relaxation (r_1) varies greatly.

For example, the spin-lattice relaxation rate corresponding to the average particle size of 3.0 nm is approximately 50% less than that of the optimum particle size. This result may have great implications for clinical diagnostics where gadolinium-based contrast agents are used. For instance, it is known that, with the increasing magnetic field, the spin-lattice relaxation rate decreases (16), which implies loss of MR signal intensity. This loss of intensity nullifies the benefits of high spatial resolution and low acquisition time of the high magnetic field MRI scanners. However, by designing the CAs

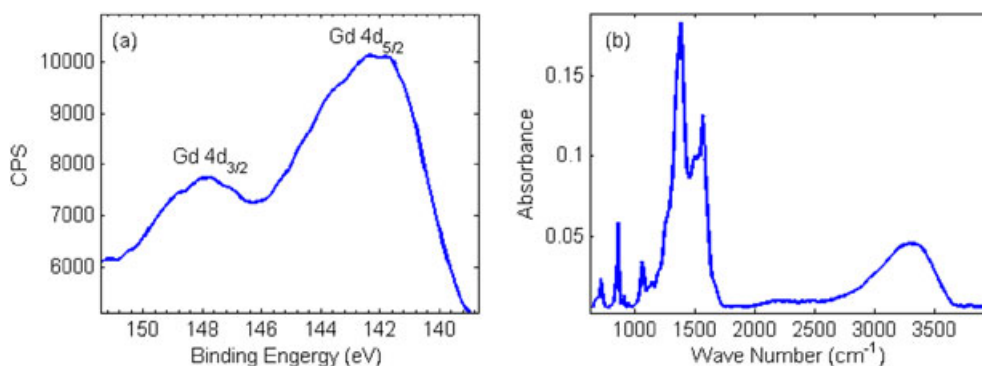


Figure 2. (a) XPS analysis, and (b) FTIR data of the as-synthesized Gd_2O_3 nanoparticles.

with appropriate size, loss of signal owing to a high magnetic field may be compensated for. Also, choosing an appropriate size of contrast agent can reduce the administered dose significantly to attain the same level of contrast. Further, it can also lower the risk of toxicity and can reduce the cost of MRI diagnostics.

At this point it is relevant to mention that we have performed relaxation studies at 7 T only. The question is whether this result is valid for other contrast agents – for instance the iron oxide nanoparticles and other magnetic field intensities such as 1.5 T scanners and 9.4 T imagers. To answer these questions and to clarify the role of temperature, additional studies are underway and we will report the results in a forthcoming article.

Lastly, in addition to the clinical importance, our result also demands theoretical attention. Specifically, existing theory, i.e. the Solomon–Bloembergen–Morgan theory of interpreting relaxation of water protons in the presence of a contrast agent, does not take into account the size and the shape of a contrast agent. Instead, it considers the distance (d) between a water proton and electrons of a contrast agent (1,2). According to this theory, r_1 varies as $1/d^6$, that is r_1 increases exponentially as the distance between a water proton and an electron of a contrast agent decreases. This is clearly in disagreement with our experimental results, although the relationship between d and the size of the nanoparticle is not exactly known. In any case, either the existing theory needs to be modified or a new theory needs to be developed to explain the experimental results presented in this work.

In conclusion, we have experimentally demonstrated that nanoparticulate gadolinium oxide-based positive contrast agent has an optimum particle size of ~2.3 nm at which the longitudinal relaxation of water protons is significantly higher than those of particles smaller or larger than this size. The results reported here show that there is a need for further investigation of other magnetic field intensities and types of nanoparticles used in MRI imaging.

1. EXPERIMENTAL

In synthesizing the gadolinium oxide nanoparticles we have used a slightly modified synthetic route reported earlier by us (25). In brief, 0.1921 g of citric acid (bought from UNIVAR, Australia) was thoroughly dissolved in 20 ml purified water in a sonic bath. Then 0.2636 g of gadolinium chloride (purchased from Sigma Aldrich and used as received) was added in the citric acid-containing beaker in the presence of continuous sonication. After approximately 10 min, 1 ml of 6 M sodium hydroxide (Merck Australia) was gradually added to the beaker in a drop-by-drop manner. Then the overall solution was kept in the sonic bath for approximately 1 h. The resulting Gd_2O_3 particle size was ~2.30 nm. To obtain smaller/larger particles than the previous example, more/less citric acid can be added. TEM and SAED studies of the as-synthesized nanoparticles were carried out using a Philips cm-200 microscope while the DLS measurement was performed in a Malvern Zetasizer nano. XPS and FTIR data were obtained using a SPECS SAGE and a Perkin Elmer-65 spectrometer, respectively. Longitudinal relaxation studies of water protons were performed in a Bruker Avance II BioSpin 300 MHz 7.0 T ultra-shield NMR equipment.

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