



High Value of Proton Relaxivity Achieved by Graphene Oxide-Cobalt Ferrite Nanoparticle Composite: A Potential Contrast Agent in Magnetic Resonance Imaging

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Abstract | This work investigates the potential of graphene oxide-cobalt ferrite nanoparticle (GO-CoFe₂O₄) composite as image contrast enhancing material in Magnetic Resonance Imaging (MRI). In the present work, GO-CoFe₂O₄ composites were produced by a two-step synthesis process. In the first step, graphene oxide (GO) was synthesized, and in the second step CoFe₂O₄ nanoparticles were synthesized in a reaction mixture containing GO to yield graphene GO-CoFe₂O₄ composite. Proton relaxivity value obtained from the composite was 361 mM⁻¹s⁻¹. This value of proton relaxivity is higher than a majority of reported relaxivity values obtained using several ferrite based contrast agents.

1 Introduction

Magnetic Resonance Imaging (MRI) is an imaging technique that is widely used as a diagnosis tool in medical science owing to its ability to provide soft tissue contrast.¹ MRI is based on the principle of Nuclear Magnetic Resonance (NMR).^{1,2} Despite rendering excellent imaging spatial resolution, MRI technique suffers from limited probe sensitivity.³⁻⁵ It has been demonstrated that the probe sensitivity in MRI can be enhanced using materials that act as image contrast enhancing agents.³⁻⁵ Enhanced image contrast synergistically with high spatial resolution increases the sensitivity of the MRI technique significantly, and facilitates detection of pathologies in their early stages of development.⁶ Contrast in MRI is proportional to the relative difference between the kinetics of dephasing of magnetic moments of water protons (proton relaxivity) present in the vicinity of the infected and healthy tissues.² Controlled functionalization of the contrast agents (which typically are superparamagnetic nanoparticles) by coating with a bio-compatible material that has a preferential affinity for a particular kind of infection causes the particles to selectively adsorb only on the infected sites.⁷ During the MR imaging process,

when an external magnetic field is applied, these adsorbed magnetic nanoparticles significantly alter the magnetic field gradient in the regions adjacent to the infected sites as compared to magnetic field in the vicinity of the non-infected sites that do not contain the functionalized nanoparticles.⁷ A difference in the magnetic field gradient between infected and normal tissue environments then leads to a marked difference in proton relaxivity in the vicinity of the infected and normal sites. This difference in proton relaxivity results in an image contrast, which helps in distinguishing between the infected and non-infected tissues.⁸ In the NMR experiment, water proton relaxivity, in the presence of contrast agent nanoparticles, is extremely sensitive to the size and magnetic moment of the functionalized nanoparticles.^{9,10} This dependence is quantitatively illustrated by equation (1) reproduced here from Ref. [9]:

$$R_2 = \frac{1}{T_2} = \frac{a}{d_{NP}D} \gamma^2 \mu^2 C_{NP} J(w, \tau) \quad (1)$$

where R_2 is overall proton relaxation rate that is equal to the product of proton relaxivity and the nanoparticle concentration, C_{NP} is the magnetic nanoparticle concentration, d_{NP} is the radius of the

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nanoparticles, μ is the magnetic moment of the nanoparticles, D is the diffusion coefficient of the protons, $J(w, \tau)$ is the spectral density function, γ is the gyromagnetic ratio of the protons, and T_2 indicates spin-spin relaxation process which produces negative contrast (darkening of images) in the MRI.¹⁰

One widely investigated potential material for MRI contrast agent application is superparamagnetic ferrite nanoparticles.^{11–13} Several iron oxide nanoparticle based MRI contrast agents such as Feridex and Resovist® have already been approved for clinical usage.¹⁴ Going beyond isolated nanoparticles, researchers have recently illustrated that attachment of ferrite nanoparticles to graphene oxide/graphene/carbon nanotubes results in a significant enhancement of the proton relaxivity values as compared to the proton relaxivity value obtained from isolated nanoparticles. In one such study, Chen *et al.*¹⁵ investigated aminodextran coated Fe_3O_4 nanoparticles-graphene oxide composite materials as T_2 -weighted contrast agent for cellular MRI. Their¹⁵ study clearly illustrates that as compared to free Fe_3O_4 nanoparticles, graphene oxide-nanoparticle composites exhibit significantly improved T_2 -weighted MRI contrast, which according to Chen *et al.*¹⁵ is due to the aggregation of nanoparticles on the graphene oxide sheets. In another study, Wu *et al.*¹⁶ reported that multi-walled carbon nanotube (MWCNT)- Fe_3O_4 hybrids exhibit excellent hydrophilicity at the room temperature, and produce a very high proton relaxivity value of $175.5 \text{ mM}^{-1}\text{s}^{-1}$ in aqueous dispersion. In another study, Lamanna *et al.*¹⁷ introduced various experimental approaches for attaching magnetic nanoparticles to MWCNT. Their¹⁷ report illustrates that, in addition to their role in enhancing contrast in MRI images, the carbon nanotube-nanoparticle composite can also be internalized into tumor cells without showing cytotoxicity. In yet another report, Ma *et al.*¹⁸ produced magnetic nanoparticle-graphene oxide composites with biocompatible surface functionalization. It was observed that these composites are stable in the physiological environments and exhibit no *in vitro* toxicity to cells. Ma *et al.*¹⁸ illustrated the potential of these composites towards targeted drug delivery and photothermal treatments for selective killing of cancer cells in highly localized regions. In this work,¹⁸ *in vivo* MR imaging of tumors in mice was illustrated using functionalized graphene oxide-nanoparticle composites as T_2 contrast agent.

In addition to Fe_3O_4 nanoparticles, nanoparticles of other magnetic spinel ferrites (general formula $\text{M}^{2+}\text{Fe}_2^{3+}\text{O}_4^{2-}$, where $\text{M} = \text{Ni, Zn or Mn}$) are also being explored for their potential use as contrast enhancing agent in MRI.^{12,13,19} There is, however, no

report on the proton relaxivity value obtained in a dispersion of $\text{M}^{2+}\text{Fe}_2^{3+}\text{O}_4^{2-}$ nanoparticles-graphene oxide/graphene/carbon nanotube composite. This report provides proton relaxivity value obtained in a dispersion of graphene oxide- CoFe_2O_4 nanoparticle composite (GO- CoFe_2O_4 composite). Large magnetic anisotropy and high saturation magnetization in case of CoFe_2O_4 provides flexibility in designing contrast agents with tunable properties.²⁰ Whereas, GO which is bio-compatible, hydrophilic and flexible can be functionalized by several drugs.²¹ GO- CoFe_2O_4 nanoparticle composites can therefore, be potentially useful both in locating the pathologies which are in their early stages of development and then curing these pathologies by localized drug delivery.

2 Experiment

In the present work, GO was initially synthesized, followed by the precipitation of cobalt ferrite nanoparticles in the presence of GO to produce GO- CoFe_2O_4 nanoparticle composites.

2.1 Synthesis of GO

GO was synthesized by the Hummer's method²² through the following process. 50 mL of concentrated H_2SO_4 was poured into a beaker containing 2 g of graphite powder and 1 g of NaNO_3 . This reaction mixture was then cooled below 20°C under constant stirring. At this temperature, 6 g of KMnO_4 was slowly added into the reaction mixture while maintaining its temperature below 20°C . After the addition of KMnO_4 , temperature of the reaction mixture was increased to 35°C and was kept for 30 min at this temperature. After 30 min, the reaction mixture was diluted with 80 mL of distilled water. Addition of water raised the temperature of the reaction mixture to 80°C due to exothermic reaction. The reaction mixture was then maintained at this temperature for another 20 min by external heating. After 20 min, mixture was cooled to room temperature. At room temperature, a mixture of 100 mL of water and 3 mL of 30% H_2O_2 was slowly added into the reaction mixture to sediment the as-synthesized GO. After sedimentation, the supernatant was discarded, and the isolated GO was washed with 100 mL of water and 100 mL 30% HCl.

2.2 Synthesis of GO- CoFe_2O_4 nano-composite

200 mg of initially synthesized GO was dispersed in 100 ml of water by sonication for 30 min. Into this dispersion, 0.0457 g of FeCl_3 and 0.0251 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ were added under continuous stirring to dissolve the precursor salts into water. pH

of the reaction mixture was adjusted to 11 by adding 2M NaOH solution. This reaction mixture was then heated to 80°C and maintained at this temperature for 1 hour to precipitate the nanoparticles. The reaction mixture was then cooled to the room temperature and centrifuged to isolate the synthesis product. The synthesis reaction was performed under inert atmosphere.

3 Characterization

X-Ray Diffraction (XRD) profiles from as-synthesized samples were obtained using the X-Pert PAN Analytical machine employing CuK_α radiation source. A 300 keV field emission FEI Tecnai F-30 Transmission Electron Microscope (TEM) was used for obtaining TEM bright field images and selected area electron Diffraction (SAD) patterns from as-synthesized samples. Samples for the TEM based analysis were prepared by drop-drying a highly dilute dispersion of the as-synthesized sample onto an electron transparent carbon coated Cu grid. Magnetic measurement data from the as-synthesized composites was obtained by using a Lakeshore Vibrating Sample Magnetometer (VSM). Spin-Spin relaxation times (T_2) were measured using a field of 1.5T by Siemens Magnetom Avanto scanner. For measuring the T_2 value a single slice with slice thickness of 5 mm was used. The repetition time (TR) was kept static at 3000 ms and echo time (TE) was varied from 22 ms to 352 ms with difference of 22 ms. Mass of graphene oxide in the GO- CoFe_2O_4 composite was determined by Thermal Gravimetric Analysis (TGA) measurement conducted using the TGA NETZSCN STA 403 PC machine. Concentration of iron in dispersions used in the NMR experiment was calculated by Atomic Absorption Spectroscopy (AAS) technique conducted using the Thermo Electron Corporation M-series machine. Raman spectra from as-synthesized samples were obtained using microscope setup (HORIBA JOBIN YVON, Lab RAM HR) consisting of Diode-pumped solid-state laser operating at 532 nm with a charge coupled detector.

4 Results and Discussion

XRD profile obtained from GO synthesized in the first step is provided in Fig. 1(a). The XRD profile reveals a broad diffraction peak at the 2θ position of 10.28°. Interplanar spacing value corresponding to this peak was calculated to be 8.61 nm. This interplanar spacing value is approximately similar to the typical inter-layer spacing of stacked graphene oxide layers.²³ Phase contrast AFM image of as-synthesized GO sheet is shown in Fig. 1(b). Raman spectrum

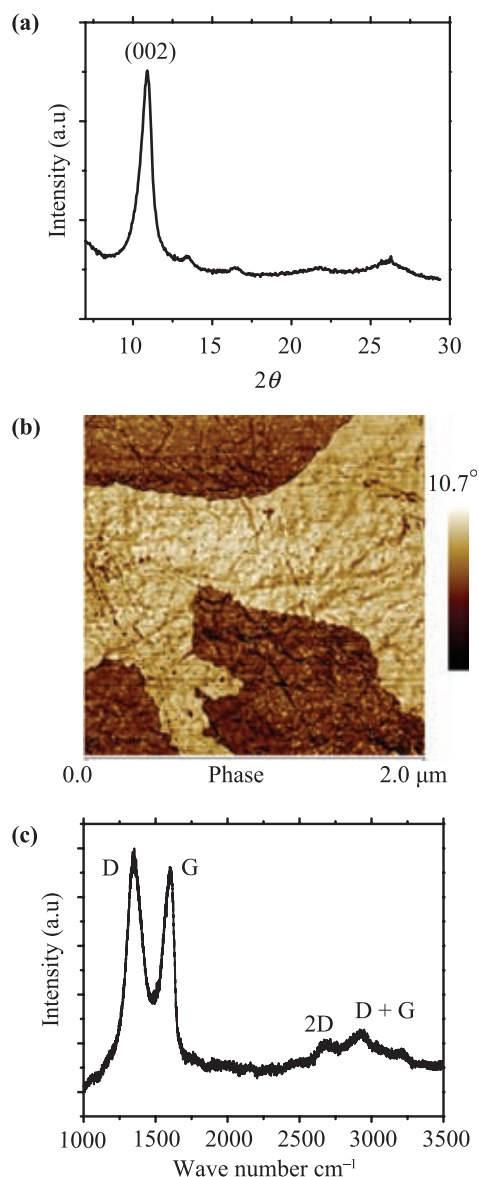


Figure 1: (a) XRD profile obtained from as-synthesized GO, (b) AFM phase contrast image of GO and (c) Raman spectrum obtained from GO.

obtained from as-synthesized GO is provided in Fig. 1(c). The Raman spectrum shows two prominent peaks at $\sim 1355 \text{ cm}^{-1}$ and $\sim 1605 \text{ cm}^{-1}$ corresponding respectively to D and G bands of GO.²³ The results provided in Fig. 1(a, b and c) collectively confirm that the first step of synthesis process produced graphene oxide.

TEM bright field image of GO- CoFe_2O_4 composite is shown in Fig. 2(a). In the TEM micrograph the darkest regions correspond to nanoparticles and the lighter contrast regions show graphene oxide sheet. The darker contrast lines in the micrographs correspond to the ripples in the graphene oxide sheets or its edges. The uniform

base contrast in the TEM micrographs is from the electron transparent amorphous carbon support film that is used to hold the sample. One important observation from TEM based analysis was negligible population of free nanoparticles.

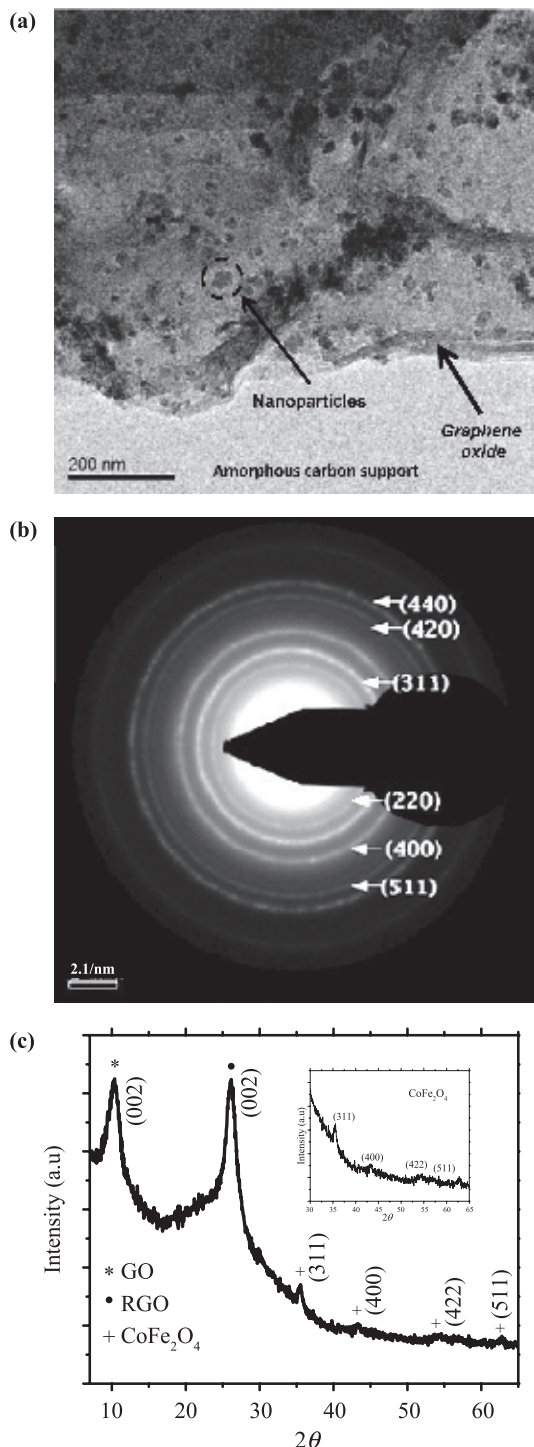


Figure 2: (a) TEM bright field image of GO- CoFe_2O_4 composite, (b) SAD pattern and (c) XRD pattern obtained from GO- CoFe_2O_4 composite.

Approximately all the nanoparticles were found to be attached to the GO sheet. SAD pattern obtained from an agglomerate of nanoparticle is shown in Fig. 2(b). The SAD pattern shows diffraction rings corresponding only to the CoFe_2O_4 phase. TEM bright field image and SAD pattern, therefore, confirm that the second step of the synthesis process resulted in the formation of GO- CoFe_2O_4 nanoparticle composite. XRD profile obtained from GO- CoFe_2O_4 nanoparticle composite produced in the second step of the synthesis process is shown in Fig. 2(c). The XRD profile shows peaks corresponding to the cobalt ferrite phase in addition to the peak corresponding to the GO phase; the insert image shows peaks corresponding to the CoFe_2O_4 phase. XRD result supports the TEM observation that the second step of the synthesis process successfully produced CoFe_2O_4 nanoparticles. In addition to the CoFe_2O_4 and GO phase, the XRD profile also revealed peaks at the 2θ value of 10.13° and 26° . These peaks indicate reduction of GO during the synthesis of the CoFe_2O_4 nanoparticles. Reduction of GO and absence of free nanoparticles collectively indicated that the GO sheet present in the reaction mixture mediated the formation of CoFe_2O_4 nanoparticles by providing a surface for heterogeneous nucleation and growth. Scherrer average size calculated from the full width at half the maximum intensity of the (311) peak was 8.5 nm.

Room temperature magnetic hysteresis curve obtained from the GO- CoFe_2O_4 composite using a 2T applied field is shown in Fig. 3. The magnetic hysteresis curve reveals negligible coercivity with no magnetic saturation till the applied field of 2T indicating that the CoFe_2O_4 nanoparticles in the GO- CoFe_2O_4 composites are superparamagnetic in nature. A small value of magnetic coercivity observed may be because of the presence

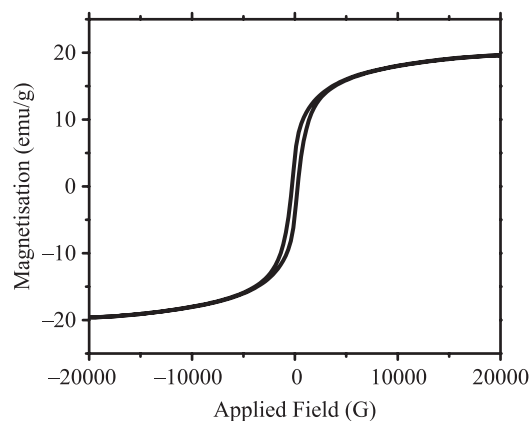


Figure 3: Magnetic hysteresis curve obtained from GO- CoFe_2O_4 composite.

of small fraction of larger sized particles in the GO-CoFe₂O₄ composite.

To investigate the potential of as-synthesized GO-CoFe₂O₄ composite for MRI contrast agent application, proton relaxivity value was obtained from the NMR experiment; the relaxivity value was obtained from the slope of the line fitted to the $1/T_2$ versus iron (Fe) concentrations plot. $1/T_2$ versus Fe concentrations plot, shown in Fig. 4, also shows the T_2 response of the composite for difference concentration of Fe. Value of relaxivity calculated from the Fig. 4 was $\sim 361 \text{ mM}^{-1}\text{s}^{-1}$. It should be noted that this value of proton relaxivity (r_2) obtained in the presence of GO-CoFe₂O₄ composites is extremely high and about an order of magnitude greater than the relaxivity values reported for CoFe₂O₄ nanoparticles of similar sizes in several reported studies provided in Table 1. The present result illustrates the potential of GO-CoFe₂O₄ composites as a potential contrast agent for MRI, and clearly calls for further detailed study and development of this material for biomedical imaging.

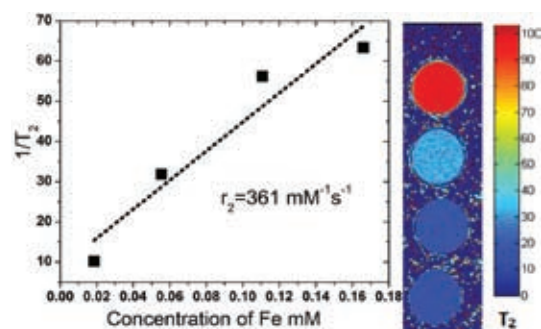


Figure 4: $1/T_2$ versus Fe concentrations plot (left figure). T_2 response of the composite for difference concentration of Fe (right figure).

Table 1: Reported relaxivity values for CoFe₂O₄ nanoparticles.

Synthetic method	Particle size (nm)	r_2 ($\text{mM}^{-1}\text{s}^{-1}$)	References
Co-precipitation	Flower shaped cluster	101	[24]
Organometallic method	6	110	[25]
Organometallic method	11	165	[25]
Co-precipitation	7.5	16.6	[26]
Co-precipitation	8.5	39.8	[26]
Co-precipitation	9.5	55.5	[26]
Co-precipitation	10.5	69.9	[26]
Co-precipitation	11.2	75	[26]

5 Conclusion

GO-CoFe₂O₄ composite was synthesized in a two-step synthesis process. GO was initially synthesized using the Hummer's method, CoFe₂O₄ nanoparticles were then precipitated in the presence of GO sheets to produce the composite. The proton relaxivity value obtained from the GO-CoFe₂O₄ composite was $\sim 361 \text{ mM}^{-1}\text{s}^{-1}$, which is significantly higher than the proton relaxivity value reported for ferrite nanoparticle based contrast agents.

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