Fluorescent Magnetic Gadolinium Oxide Nanoparticles for Biomedical Applications

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Abstract

The unique properties of nanostructures are attracting tremendous interest because they offer a promising role in diagnostics and therapeutics. Here Eu³⁺ doped Gd₂O₃ nanoparticles have been fabricated by simple co-precipitation route without the use of any precipitating agent or surfactant at room temperature. The Gd₂O₃:Eu³⁺ nanoparticles of size ~4 nm synthesized exhibit superparamagnetic nature with susceptibility, χ = 6.1.10⁻⁴ emu/g Gauss with red luminescence in the visible region at 395 nm excitation. XPS studies depict that, Gd (3d⁵/₂) and Gd (3d₃/₂) peaks at 1228.10 and 1195.33 eV, respectively.

Introduction

Nanocrystals have emerged as attractive alternatives to commonly used organic lumophores because of their high quantum yields and the spectral tunability that can be achieved through synthetic control. Stable colloids of Magnetic Nanoparticles (MNP) in physiological medium, also known as Biocompatible Magnetic Fluids (BMFs) have been used as potential candidates for diagnosis and therapy in clinical, biomedical, and biotechnological applications. The main emphases of these works were on cancer therapy and early detection [1-3]. A wide variety of magnetic nanomaterials has been synthesized and used for biomedical applications. Small changes in the band structure of a solid, originating from nanoscale size, are expected to have dramatic effects on the luminescent and magnetic properties, simultaneously. These small nanoparticles, also known as Quantum Dots (QDs), represent a new form of highly fluorescent agents that can expand the range of possible fluorescent studies [4,5].

Rare-Earth (RE) ions doped inorganic QDs are one of the most promising materials for a variety of applications such as in solid-state lasers, lighting, displays and biolabels [6]. QDs allow multiplexed detection, are resistant to photo-bleaching, and can be tracked over extended periods of time [7,8]. QDs can also be engineered to attach the biological molecules and have great potential for detecting and characterizing certain diseases, such as cancer, at the single cell or tissue level [9,10]. Recently, nanoparticles made of RE oxides have been investigated as emerging materials for fluorescent labeling [11-13], due to their large stokes shift, sharp emission spectra, long lifetime and flexibility of excitation wavelengths. As RE oxides are used as commercial phosphors, it can be predicted that biological labels based on these materials will have better photo-stability than the commercially available polystyrene nanoparticles doped with lanthanide chelates.

On the other hand, the magnetic interactions at the nanoscale are generally strong and can be tailored to give key materials for hyperthermia and other applications such as good contrast agents in MRI for early diagnosis as diagnostic tools or guided drug delivery for effective control and prevention of cancer. The magnetic properties of rare earths QDs are very sensitive to the structure and filling of the conduction bands because these originate entirely from their incomplete 4f shells. Several nanoparticles viz Gd₂O₃, GdPO₄, GdF₃, etc are extensively synthesized and studied by various research groups. Among these nanoparticles, particularly Gd₂O₃ nanoparticles have been synthesized by numerous approaches and studied thoroughly. These nanoparticles have been tagged to fluorescence dyes for bioimaging purposes. These particles are known to be internalized by different cell types (macrophages, fibroblasts, lymphocytes). As a result, the labeled cells can be visualized by MRI and fluorescence imaging. The circulation of these particles after intravenous injection in mice and rats has been monitored by fluorescence and magnetic resonance imaging [14]. To overcome these shortcomings, Eu³⁺ doped Gd₂O₃ nanoparticles were then synthesized. But the synthesized nanoparticles were in the size range of 10-100 nm [15]. Due to high luminescence efficiency and long lifetime, Gd₂O₃:Eu³⁺ nanoparticles have been used as fluorescent markers for variety of immunosensing applications [16]. Recently, anomalous thermalization phenomenon resulting from the restricted phonon relaxation has been observed in Gd₂O₃:Eu³⁺ nanotubes [17].

Gd₂O₃ nanoparticles are reported as potential contrast agents for MRI because they accelerate the T₁ and T₂ relaxation...
processes of water protons within their surroundings and have proven to show positive contrast effect T1-weighted imaging [18]. Furthermore, since Gd3+ is a known contrast agent for Magnetic Resonance Imaging (MRI), Gd2O3:Eu3+ nanoparticles may function as both fluorescence and MRI labels [19]. Because of a large number of unpaired electrons in the gadolinium ion, complex species of Gd3+ ion have been commonly used as an MRI contrast agent for positive intensity images. They displayed the potential applications combining with fluorescence to achieve multi-imaging in vivo [20]. Besides these, the non-toxicity, water-solubility, biocompatibility and tissue-specific targeting are requirements to be met; undoubtedly a challenging task from the point of view of chemical synthesis. Recent improvements in nanoparticle synthesis have led to the establishment of reliable methods for synthesizing Eu3+ doped Gd2O3 nanoparticles of very fine and narrow size distributions [21,22].

These nanoparticles may be employed in biomedical applications such as targeted drug delivery or as MRI contrast agents.

Experimental details

The analytical grade gadolinium (III) nitrate hexa hydrate, Gd(NO3)3·6H2O, 99.9% (REO), europium (III) nitrate hexahydrate, Eu(NO3)3·6H2O 99.99% procured from Sigma Aldrich (Alfa Aesar) Germany, whereas oxalic acid dehydrate, ethanol and methanol were procured from E. Merck, India. 10 mM stock solutions of metal nitrates were prepared by dissolving appropriate amount of metal nitrates (Eu3+ for 5% doping) in double distilled water. Simultaneously, 10 mM solution of oxalic acid was prepared separately. Now, 50 mL of Gd(NO3)3·6H2O stock solution and 50 mL Eu(NO3)3·6H2O stock solution were allowed to mix together homogeneously for 45 minutes. 50 mL of oxalic acid solution was added to the above metal nitrate solution. After 30 minutes of stirring at room temperature the solution was heated at 100°C with constant stirring till visible precipitate appeared. Precipitate was centrifuged, washed several times with absolute ethanol and de-ionized water. Precipitated slurry was kept in air for 24 hours to get powders of the sample.

Characterization used

The prepared magnetic nanoparticles of Gd2O3:Eu3+ were thoroughly characterized by X-Ray Diffraction (XRD), Energy Dispersive X-Rays (EDX) and Transmission Electron Microscopy (TEM) in order to elaborate structural properties in precise manner. XRD was performed on Rigaku D/max-2200 PC diffractometer operated at 40 kV/40 mA, using CuKα radiation with wavelength of 1.54 Å in the wide angle region from 20°C to 60°C 28 scale. The size and morphology of prepared nanoparticles were found using a transmission electron microscope (model Tecnai 30 G2S-Twin electron microscope) operated at 300 kV accelerating voltage by dissolving the as-synthesized powder sample in ethanol and then placing a drop of this dilute ethanolic solution on the surface of a carbon coated copper grid. Core level X-Ray Photoelectron Spectroscopy (XPS) studies were performed using AlKα radiation. Room temperature magnetization measurement was carried out using a Vibrating Sample Magnetometer (VSM) (ADE Magnetics, USA) up to an applied field of 1.75 T with pressed pellets of prepared powdered samples. Photoluminescence (PL) studies were performed for λex = 390 nm on a Perkin Elmer LS 55 luminescence spectrophotometer using a Xenon discharge lamp as the excitation source at room temperature, equivalent to 20 kW for 8 microsecond duration.

Results and Discussions

Characterizations of synthesized Gd2O3:Eu3+ nanoparticles

Figure 1 shows the XRD pattern of the Gd2O3:Eu3+ nanoparticles synthesized by co-precipitation method. XRD spectra showed broad peaks at the positions of 26.15°, 31.94°, 35.14°, 40.46°, 42.03°, 47.97°, 52.50°, 53.60° and 59.07°, which were in excellent agreement with the standard JCPDS file for Gd2O3 (JCPDS 76-0155, a = b = c = 10.79 Å) and indexed as the body centered cubic structure of Gd2O3 having space group 123(199). All available reflections of the present phases were fitted with the Gaussian distribution. The broadening of XRD peaks (i. e. Scherrer’s broadening) gave clear indication of nanosized formation of Gd2O3. Particle size d’ of Gd2O3:Eu3+ was estimated by Scherrer’s Equation.

\[ d = \frac{0.9\lambda}{BCos\theta} \]

where, d is the particle size, λ is the wavelength of radiation used, θ is the Bragg angle and B is the Full Width at Half Maxima (FWHM) on 2θ scale.

For this Gd2O3:Eu3+ sample, average particle size using Scherrer’s equation was of the order of ~5 nm. Furthermore, in addition to the Gd2O3 phase, a new phase at 45.78° is also observed in the XRD spectra. This new phase in the XRD spectra corresponds to body centered cubic phase of Eu2O3 (521) (matched with JCPDS 74-1988), which may be due to the formation of Eu2O3 from remaining un-reacted Eu3+ ions present in the reaction solution.

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Figure 2: TEM image of (a) non sonicated sample, (b) sonicated sample, (c) HRTEM image and (d) the SAED pattern for the Gd₂O₃:Eu³⁺ under study.

Figure 2 shows the TEM image of (a) non sonicated sample, (b) sonicated sample. The morphology of the sample was found to be nearly spherical in nature having diameters ~4 nm. After sonication step well dispersed particles were observed. Figure 2 clearly shows that the diameters of these spherical nanoparticles were in agreement with those obtained using XRD results.

Figure 2(c) show TEM image of Gd₂O₃:Eu³⁺ nanoparticles inset of Figure 2 shows the SAED pattern of the nanoparticles. While inset of Figure 2 shows the Selected Area Electron Diffraction (SAED) pattern. Broad diffused rings along with few diffraction spots are observed in SAED, indicating mixture of amorphous and poly-crystalline nature, which is obvious due to nano-sized nature of the prepared sample.

In order to confirm the doping and the involved valance states of gadolinium and europium in the synthesized Gd₂O₃:Eu³⁺ nanoparticles, EDX measurements were performed. Figure 3 shows the EDX spectra of Gd₂O₃:Eu³⁺ nanoparticle. From the similarity of the Gd and Eu peak intensity line traces, it is clear that after the synthesis process, gadolinium and europium are homogenously distributed inside the nanoparticle. From the EDX line traces it can also be concluded that Eu³⁺ was successfully substituted into the crystal structure of Gd₂O₃. The estimated amount of Eu³⁺ ions was ~4.7%. EDX measurements on single nanoparticle found that gadolinium and europium are homogeneously distributed throughout the Gd₂O₃:Eu³⁺ nanoparticle. Furthermore, these findings are also in accordance with the obtained XPS results as shown in Figure 4. The obtained XPS spectra consists of Gd (3d), O (1s), and Eu (4s, 4p, 3d) states.

Figure 4: XPS survey spectra of the Gd₂O₃:Eu³⁺ nanoparticle.

The Gd (3d) level consists of a spin orbit split doublet, with the Gd (3d⁵/₂) and Gd (3d³/₂) peaks at 1228.10 and 1195.33 eV, respectively. The peaks consisting spin singlet at 984.6 and 541.4 eV corresponds to the oxygen in the Gd₂O₃, and the prominent peak at 541.4 eV is, as expected, a contribution from the O (1s) state. Whereas, the small peak, consisting of spin triplet, at 371.52(4s1/₂), 292.19(2p1/₂) and 174.79(3d³/₂) eV correspond to the Eu³⁺ states. The line shape and peak positions are in good agreement with standard XPS look-up data sheet, confirming that the sample consists of Gd₂O₃ with Eu³⁺ as an impurity (doping).

Figure 5a shows the dependence of magnetization at room temperature with applied magnetic field (M-H loop) for the prepared Gd₂O₃:Eu³⁺ nanoparticle. A clear strong paramagnetic nature with paramagnetic term, susceptibility χ = 6.1*10⁻⁴ emg/g Gauss was recorded. The narrow hysteresis (almost zero in present case) implies a small amount of dissipated energy in repeatedly reversing the magnetization which is important for quick magnetization and demagnetization of the nanomagnet synthesized. The strong paramagnetic behavior can be attributed to the presence of small magnetic dipoles located at the surface of nanocrystals, which interacts with their nearest neighbors inside...
Figure 5: (a) Room temperature M-H loop (b) temperature dependence of the field-cooled and zero field-cooled magnetization (M) at a field of 5 kOe, showing super-paramagnetic nature of the as synthesized Gd$_2$O$_3$:Eu$^{3+}$ nanoparticles. Inset shows the response of liquid suspension of Gd$_2$O$_3$:Eu$^{3+}$ nanoparticles to the external magnet.

Consequently, the interchange energy in these magnetic dipoles making other neighboring dipoles oriented in the same direction. In nanocrystals, surface to volume ratio increases, so the population of magnetic dipoles oriented in the same direction will increase at the surface. Thus, the sum of the total amount of dipoles oriented along the same direction will increase subsequently. In short the crystal surface will be usually more magnetically oriented. One possibility for the observed behavior could be due to nano sizes (~4 nm) of the studied samples, which may lead to super-paramagnetic behavior if individual nanoparticle turns into a single ferromagnetic domain. In order to confirm the tangible rationale behind the observed MH behavior, a detailed magnetization study is crucial to support. Furthermore, our results show that the prepared Gd$_2$O$_3$:Eu$^{3+}$ nanoparticle of mean size 5 nm will have ~250 Gd-atoms, showing almost highest density of Gd-atom for any earlier reported Gd-based contrast agents (paramagnetic ions nm$^{-3}$). Previous computational studies, based on small cluster of Gd$_{12}$O$_{18}$ have shown that the Gd$_{12}$O$_{18}$ could exhibit superparamagnetic nature as in case of iron oxide nanoparticles, and thus is consistent with our experimental observations.

Temperature dependence of the Field-Cooled (FC) and Zero Field-Cooled (ZFC) Magnetization (M) at a field of 5 kOe is shown in Figure 5b. FC and ZFC magnetization measurements were performed from 77 to 300 K. The FC is obtained by measuring the magnetic moment while heating the sample in a magnetic field (5 kOe), after it was previously cooled in the same magnetic field. The ZFC is also obtained by measuring the magnetic moment while heating the sample in the same magnetic field as for FC, but after the sample has been cooled in the absence of a magnetic field. By taking the difference between these two quantities (denoted as $\Delta M = M(\text{FC})-M(\text{ZFC})$), the para- and diamagnetic contributions to the magnetization can be subtracted. Therefore, a nonzero difference between the FC and ZFC curve in the present sample indicates some type of ferromagnetic ordering taking place, which could be due to genuine ferromagnetic interactions between the magnetic dopants or spin glass behavior or super-paramagnetic behavior below the blocking temperature of the sample. Inset of Figure 5b shows response of external magnet on the synthesized Gd$_2$O$_3$:Eu$^{3+}$ samples in liquid phase. It can be clearly seen that the accumulation of suspended magnetic nanoparticles appear near the surface where external magnet is placed. This is due to attractive force acting on these suspended magnetic nanoparticles towards the external magnet. The energy density gradient is greatest at the magnet’s poles, thus, all the magnetic nanoparticles present in the solution, collect near the pole of external magnet.

In order to study the luminescence behavior of the synthesized super-paramagnetic nanoparticles, excitation spectra was recorded (spectra not shown here). Excitation spectra showed an
intense peak at 390 nm. Based on this observation, the emission is recorded for 390 nm excitation wavelength and is depicted in Figure 6. Inset of Figure 6 shows photograph of liquid suspension of synthesized \( \text{Gd}_2\text{O}_3: \text{Eu}^{3+} \) nanoparticles in (A) UV lamp and (B) ordinary light. Intense bright red color emission is observed under UV lamp. The emission spectra consist of number of peaks ranging from 535 nm to 700 nm, associated with forced electric dipole transition from excited \( ^5\)D\(_{0} \rightarrow ^7\)F\(_{J} \) (\( J = 0, 1, 2, 3, 4 \)) levels of \( \text{Eu}^{3+} \) activator as labeled in the Figure 6.

The strongest red peak at 615 nm, in Figure 6, was due to \( ^5\)D\(_{0} \rightarrow ^7\)F\(_{J} \) transition. The 592 nm orange emission was originated due to \( ^5\)D\(_{0} \rightarrow ^7\)F\(_{J} \) transition is typically because of magnetic dipole transition. The ratio of red and orange peak intensity (R/O value) strongly depends on local symmetry of activator ion \( \text{Eu}^{3+} \). Lower symmetry of crystal field near the activator ion \( \text{Eu}^{3+} \) will result in higher R/O value. Due to higher degree of disorder near the surface, nanomaterials generally gives better chromaticity. These are the main reasons behind getting better chromaticity in synthesized \( \text{Gd}_2\text{O}_3: \text{Eu}^{3+} \) nanoparticles. From Figure 6 it is also clear that gadolinium did not affect the characteristic emission spectra of \( \text{Eu}^{3+} \) ion (dopant ion) and acts as a host. Along with its super-paramagnetic nature, the luminescence behavior of the synthesized \( \text{Gd}_2\text{O}_3: \text{Eu}^{3+} \) nanoparticles significantly enhance the possibilities or their biomedical implications and opens new era for researchers in future. Besides having strong paramagnetic nature and luminescence characteristic, these \( \text{Gd}_2\text{O}_3: \text{Eu}^{3+} \) nanoparticle possesses non aggregating behavior. The size of nanoparticles in the range of ~4 nm is additional factors for their in vivo applications. Since the nanoparticles of this range can be easily excreted out of the kidneys, this makes them suitable for \textit{in vivo} applications such as MRI contrast agents and as vehicles for targeted drug delivery.

Conclusions

Super-paramagnetic \( \text{Gd}_2\text{O}_3: \text{Eu}^{3+} \) nanoparticles of size ~4 nm are synthesized using a simple co-precipitation technique and characterized thoroughly by several techniques in order to elaborate their functional properties in a very precise manner in view of their applications as MRI contrast agent. However, further studies are required to confirm that these particles do not cause any stenosis or thrombosis, which will contribute in making them suitable for \textit{in vivo} applications such as MRI contrast agents and as vehicles for targeted drug delivery.

Notes

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