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Glycine Nitrate Process for the elaboration of Eu\textsuperscript{3+}-doped Gd\textsubscript{2}O\textsubscript{3} bimodal nanoparticles for biomedical applications

Graziella Goglio, a, d Gaganpreet Kaur, a Sonia L.C. Pinho, b c Nicolas Penin, a Alexia Blandino, a Carlos F.G.C. Geraldes, a Alain Garcia, a and Marie-Helene Delville, a d

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Monoclinic and cubic structures of were selectively synthesized via Glycine Nitrate Process via a fine control of the temperature synthesis through the crucial fuel--oxidant ratio. The cubic phase is obtained in fuel-rich conditions while stoichiometric conditions induce the simultaneous formation of cubic and monoclinic polymorphs. Samples were subjected to appropriate sintering to obtain highly crystalline and carbon free materials. The average nanoparticle size determined by TEM for these nanopowders (23 nm) is in agreement with the average crystallite sizes obtained from XRD Rietveld analysis, showing that the particles are monocrystalline. Both the EELS analysis and the photoluminescent studies show that the europium doped NPs are highly luminescent with Eu\textsuperscript{3+} homogeneously distributed over the whole material as well as over the two gadolinium crystallographic sites of the cubic phase. These fluorescent NPs exhibit relaxivities which also define them as potential T1 contrast agents for further biomedical applications.

With the advent of nanotechnology, nanoparticles (NPs) are used as contrast agents in MRI. Among the different MRI contrast agents (CA), the most frequently used as clinical MRI contrast ones are gadolinium (Gd)-based contrast agents since this metal ion has seven unpaired 4f electrons. With a S ground state electronic structure it couples a large magnetic moment with a long electron spin relaxation time (of the order of 10\textsuperscript{3} s at the magnetic field strengths of interest for MRI applications). Recently, more and more research demonstrated that gadolinium oxide (Gd\textsubscript{2}O\textsubscript{3}) nanoparticles can act as T\textsubscript{1} CAs to enhance MRI signal intensity remarkably due to the availability of more Gd\textsuperscript{3+} ions at the surface to interact with water. 

The synthesis of such nanosized particles can be carried out by various methods such as the polyol,\textsuperscript{15-17} sol lyophilisation,\textsuperscript{18,19} sol-gel,\textsuperscript{20} precipitation,\textsuperscript{21-23} hydro/solvothermal route,\textsuperscript{24-26} sonochemistry,\textsuperscript{27} spray pyrolysis,\textsuperscript{28,29} aqueous self-combustion methods,\textsuperscript{30-32} etc. Aqueous self-combustion can be the most appropriate synthesis route as this can yield powder of high phase purity and excellent chemical homogeneity. Among these combustion routes, the Glycine Nitrate Process (GNP) was first proposed in 1990 by Chick et al. to elaborate chromite and manganese powders.\textsuperscript{33} This solution combustion process is particularly suitable for the preparation of simple and complex oxide materials since the homogeneity of the aqueous solution of the metallic salts is preserved in the combustion residue. Moreover, it takes advantage of exothermic, fast, and self-sustaining chemical reactions between metal nitrates and the glycine reducing agent. As a consequence, it is particularly suited to the elaboration of uniform crystalline particle of ceria\textsuperscript{34} or alumina\textsuperscript{35} with superfine dimensions. To the best of our knowledge, GNP preparations of nanosized Gd\textsubscript{2}O\textsubscript{3} are scarce.\textsuperscript{36-38} [52,53]

**Introduction**

During the past few decades, (MRI) and Light Microscopy (LM) have emerged as leading and powerful techniques for biomedical imaging.\textsuperscript{1,4} Light microscopy uses fluorescent dyes to visualize the tissues with high resolution. Magnetic Resonance Imaging generates exquisite three dimensional images of tissues and organisms measuring proton relaxation of water.\textsuperscript{5-10} It efficiently differentiates normal and unhealthy tissues using exogenous contrast agents. Though, it possesses 10-20 times lower resolution compared to light microscopy.\textsuperscript{11} These techniques have individual unrivalled advantages but also suffer from some limitations. Their respective advantages can be unified by designing of bimodal probes. These bifunctional/bimodal probes can comprise among others\textsuperscript{1} MRI contrast agent and fluorescent markers. On one hand, contrast agents can either be paramagnetic\textsuperscript{1, 12-14} T\textsubscript{1} (Gadolinium based complexes)\textsuperscript{15-17} or superparamagnetic T\textsubscript{2} agents (Iron oxide)\textsuperscript{18-26} and on the other hand, lanthanides can be excellent fluorescent markers.\textsuperscript{11, 27-28}

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In general, a molar ratio glycine/nitrate equal to 1.1 (i.e. twice the stoichiometric ratio) was chosen and further calcination is systematically mandatory to induce crystallinity. In fact, the success of GNP depends on the right understanding of the influence of each synthetic parameter. As a consequence, the main goal of this work was to optimize and validate the suitability of the GNP to easily and efficiently prepare undoped or Eu-doped Gd$_2$O$_3$ crystallized nanoparticles and to check the distribution of europium into the material. The synthesis was first optimized on undoped Gd$_2$O$_3$, varying the glycine/nitrate ratio. The optimized parameters were then used to synthesize the solid solution Eu$^{3+}$ (2.5 to 20 at.-%) -doped Gd$_2$O$_3$ solid solution. The water proton nuclear relaxation and luminescent properties of these NPs as powders and after internalization in Hela cells were studied to assess their efficiency as Ln$^{3+}$ based bimodal agents for MRI and optical imaging.

Results and Discussion

Elaboration of Gd$_2$O$_3$ nanoparticles

In the case of the GNP synthesis of Gd$_2$O$_3$, the redox combustion reaction is the following:

$$2 \text{Gd(NO}_3\text{)}_3 + n \text{H}_2\text{N(CH}_3\text{)}_2\text{CO}_2\text{H} + (2.25n - 7.5) \text{O}_2 \rightarrow \text{Gd}_2\text{O}_3 + 2n\text{CO}_2 + 2.5n \text{H}_2\text{O} + (3 + 0.5n) \text{N}_2$$

While the nature of the produced gases is still controversial,[54] propellant chemistry concepts and thermodynamic calculations showed that CO$_2$, H$_2$O and N$_2$ are the most stable products with respect to other theoretically acceptable combinations that might be considered, including the formation of NO and CO.$^{[55]}$ For a given fuel, the reaction is governed by the maximum reaction temperature reached during the combustion. This temperature depends itself on the oxidising/reducing character of the G/N (Glycine/Nitrate) ratio quantified by the so-called $\varphi$ elemental stoichiometric coefficient. $\varphi$ is the ratio between the total valencies of fuel and the total valencies of oxidisers, these valencies being considered according to Jain’s method.$^{[56]}$ Assigning the +4, +1, +3 valencies to the C, H, Gd$^{3+}$ reducing elements, respectively, the -2 valency to O$^{2-}$ oxidizer and considering nitrogen with the valence 0, $\varphi$ is then calculated according to the following equation:

$$\varphi = n \left(2 \times 4_{(C)} + 5 \times 1_{(H)} + 3_{(N)} - 2 \times 2_{(O)}\right) / \left[2 \times (3_{(Gd)} + 3_{(O)} - 3 \times 2_{(O)})\right]$$

Then $\varphi = 0.3 \times n$.

The G/N molar ratio (named G/N = n/6) is equal to 0.555 $\varphi$. The reaction is considered stoichiometric when the total valency of reducing elements balances the valency of oxidizing ones. In this case, $\varphi_{\text{stoich}}$ is equal to 1 which involves $\varphi_{\text{stoich}}$ equals to 3.333 and G/N$^{\text{stoich}}$ equals to 0.555, no additional oxygen being needed (2.25n - 7.5 = 0). The adiabatic flame temperature ($T_{\text{flame-adi}}$) is expected to increase with $\varphi$ but, due to the heat dissipation by the gaseous products of the reaction and to the energy needed to burn the excess of organic matter, the experimental flame temperature ($T_{\text{flame-exp}}$) is lower than $T_{\text{flame-adi}}$ all the more that $\varphi$ increases. For a given $\varphi$ value, higher or equal to 1, $T_{\text{flame-exp}}$ reaches a maximum and then decreases.$^{[57]}$

The influence of $\varphi$ on the formation of Gd$_2$O$_3$ was checked and the stoichiometric conditions ($\varphi = 1, \text{G/N = 0.555}$) were at first chosen. They should favor the one-step elaboration of crystalline nanoparticles due to highly exothermic conditions (in particular no excess of organic matter to burn). The as-obtained materials are white ashes, which is consistent with the expected color for Gd$_2$O$_3$. The X-ray diffraction (XRD) pattern evidences the formation of a high crystalline biphasic sample consisting of a mixture of cubic and monoclinic Gd$_2$O$_3$ polymorphs (see Figure 1). The Lorentzian shape of the diffraction peaks and their Full Width at Half Maximum (FWHM) are consistent with nanometric crystallite sizes. It is well known that Gd$_2$O$_3$ undergoes a cubic → monoclinic structural transition at 1200 °C$^{[58]}$ the heat provided by the reaction inducing this polymorphic transition.

![Figure 1. XRD patterns of undoped Gd$_2$O$_3$ obtained a) in stoichiometric conditions (G/N=0.555); and b) in fuel-rich conditions (G/N = 1.1). In this last case sample was 1) not annealed, or annealed under air at 2) 450 °C/4 hr, 3) 600 °C/1 hr and 4) 800 °C/30 min.](image-url)
was submitted to 450 °C during 4 hours, the obtained material (Gd$_2$O$_3$-G/N) exhibited a brown colour and was still amorphous with lower but still high carbon content (Table 1). Crystallization occurred at 600 °C; the crystallite size was 10 nm and 20 nm at 600 °C and 800 °C, respectively (Table 1). Both materials (Gd$_2$O$_3$-600 and Gd$_2$O$_3$-800) are white single phases with a cubic symmetry (Ia-3 space group). Furthermore, the thermal treatment at 800 °C with a short duration induced a significant decrease of carbon content (Table 1).

Table 1. Color, organic content and crystallite size deduced from XRD Rietveld refinements (the profile was fitted using Thomson-Cox-Hastings function) for undoped and 5 at% Eu-doped Gd$_2$O$_3$ elaborated with G/N = 1.1 as a function of annealing conditions.

<table>
<thead>
<tr>
<th>Annealing conditions under air atmosphere</th>
<th>Organic matter elemental analysis (weight%)</th>
<th>Crystallite size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd$_2$O$_3$-450°C</td>
<td>450 °C 4 hours</td>
<td>C</td>
</tr>
<tr>
<td>Gd$_2$O$_3$-600°C</td>
<td>600 °C 1 hour</td>
<td>0.9</td>
</tr>
<tr>
<td>Gd$_2$O$_3$-800°C</td>
<td>800 °C 30 min</td>
<td>0.4</td>
</tr>
<tr>
<td>Eu:Gd$_2$O$_3$, 100% 3+ at% Eu</td>
<td>800 °C 30 min</td>
<td>0.5</td>
</tr>
</tbody>
</table>

The TEM characterization was performed on all the samples. TEM images of Gd$_2$O$_3$-raw and Gd$_2$O$_3$-800 are given in Figure 2. On the raw sample, small nanoparticles with sizes lower than 10 nm and poorly defined morphologies are dispersed in a prevailing organic matrix.

![TEM images of undoped a) Gd$_2$O$_3$-raw and b) Gd$_2$O$_3$-800. The size distribution of Gd$_2$O$_3$-800 obtained by TEM experiments is presented on c).](image)

The annealed sample Gd$_2$O$_3$-800 was characterized by faceted nanoparticles rather homogeneous in size and morphology which is characteristic of an excellent crystallinity. However, despite its short duration, the thermal treatment has probably enhanced some sintering between the particles, as evidenced on Figure 2b. The hydrodynamic diameter of aggregates ranges between 340 and 490 nm, as evidenced by DLS experiments (see Figure S1, supplementary information S1), this value being consistent with the TEM image. The NPs average size is 23 ± 8 nm, which reveals that they are monocrystalline because the particles size is equivalent to the crystallite size. This morphology was also observed by Jacobsohn et al. on europium-doped Gd$_2$O$_3$ elaborated by GNP combustion process in fuel-rich conditions, followed by annealing at 1,000 °C. The best synthesis GNP conditions consisted in a GNP process followed by a thermal treatment at 800 °C during 30 minutes under air. The as-obtained material is a highly crystallized powder made of faceted nanoparticles that are 20-25 nm in size. One should notice that the crystallite size obtained in such conditions is equivalent to that observe by Ningthoujam et al. in the case of nanoparticles elaborated by GNP process under less reducing conditions (G/N = 0.8). The authors claim crystallite size of 24 nm after annealing at 500 °C during 1 hour under air. This clearly indicates that T$_{\text{time-exp}}$ decreases between 0.8 and 1 values of G/N. However, in this case, the morphology is far from regular and the authors do not discuss the presence of carbon residue which, according to our experience and results (annealing at 450 °C during 4 hours), might not be negligible in such conditions. One should notice that carbon has to be removed in order to get reliable optical measurements in the case of europium doped Gd$_2$O$_3$. For these reasons, the annealing temperature must not be too low.

**Elaboration of Eu$^{3+}$-doped Gd$_2$O$_3$ nanoparticles**

This overall optimized synthesis process (GNP with G/N = 1.1 followed by a thermal treatment at 800 °C during 30 minutes under air) was also used to elaborate europium-doped gadolinium oxide with doping rates ranging from 2.5 to 20 at% in Eu and the parent phase Eu$_2$O$_3$. All samples are single-phase with white color exhibiting the cubic symmetry (Figure 3a), even for the highest europium doping.

![XRD patterns of parent phases Gd$_2$O$_3$ and Eu$_2$O$_3$ and of europium-doped Gd$_2$O$_3$, all materials being elaborated by GNP (G/N = 1.1)](image)
followed by a thermal treatment under air at 800 °C during 30 minutes. b) A Vegard’s law is observed on the whole solid solution.

The XRD patterns are slightly shifted towards lower angles when the doping rate increases due to the difference of ionic radii between Eu (0.947 Å) and Gd (0.938 Å).[60] The variation of the cell parameter as a function of the doping rate follows a Vegard’s law which is consistent with the formation of a solid solution.

Figure 3b). Neither the carbon content (0.5 weight%) nor the crystallite size depend on the doping rate, all the characteristics of Gd$_2$O$_3$800 being recovered in doped samples (as an example see Table 1 for 5% doped Gd$_2$O$_3$). TEM characterizations confirm that the substitution of gadolinium by europium does not induce any change in the morphology of the nanoparticles as shown on Figure 4.

These monocrystalline faceted nanoobjects exhibit sizes ranging from 20 to 30 nm that remain agglomerated whatever the doping rate. The morphology and size of nanoparticles are rather homogeneous. No residual coating by carbon residue was detected. The size and morphology are consistent with those observed by Li and Hong[61] on europium doped gadolinium oxide elaborated by aqueous combustion route using an excess of urea as reducing agent followed by annealing at 800 °C for two hours.

High resolution TEM images confirm the high crystallinity of the nanoparticles with a size ranging from 20 to 30 nm (as evidenced for 20% doping on Figure 5a), a nice sequence of atomic planes being observed in each crystallite from one surface to another (SI, Figure S2).

The presence of amorphous grains boundaries confirms the sintering effects during annealing. The distribution of europium in the materials has been checked by EELS mappings on the most doped gadolinium oxide (20 at.%). It appears obvious from these images that there is a systematic co-localization of both gadolinium and europium and no specific segregation at the grain boundaries. Moreover, the semi quantitative EELS analysis indicated an atomic content of europium of 20% which is fully consistent with the targeted composition and confirmed by ICP/OES. The superposition of europium and gadolinium signals on b) evidences the homogeneous distribution of both elements into the particles.

Figure 5- a) HRTEM image and b) EELS chemical mapping of the selected area of Gd$_2$O$_3$ with 20 at% of Eu$^{3+}$ revealing the distribution of both cations (gadolinium in green and europium in red). Qualitative elemental EELS mappings on c) gadolinium and d) europium. The superposition of europium and gadolinium signals on b) evidences the homogeneous distribution of both elements into the particles.

Photoluminescence properties of Gd$_2$O$_3$:Eu nanocrystals

Trivalent europium in as-prepared Gd$_2$O$_3$ nanoparticles and those annealed at 800 °C for 30 min in the air presents an intense red emission. Figure 6 shows the corresponding (PL) emission spectra of doped Eu$^{3+}$-Gd$_2$O$_3$ annealed at 800 °C for 30 min for the different doping rates under a 260 nm excitation (corresponding to the excitation in the charge transfer band) (from 2.5 to 20 at.%).

They exhibit very sharp peaks corresponding to the intra-4f transitions of Eu$^{3+}$ which take place between the $^1$D$_0$→$^3$F$_J$ ($J = 0$-6) levels according to 581 ($^1$D$_0$→$^3$F$_0$), 587, 592, 599 ($^1$D$_0$→$^3$F$_1$), 611 ($^1$D$_0$→$^3$F$_2$ cubic symmetry), 650 nm ($^1$D$_0$→$^3$F$_3$) and 706 nm ($^1$D$_0$→$^3$F$_4$) transitions.[62]

Figure 6. Emission spectra of Eu$^{3+}$ doped Gd$_2$O$_3$ nanoparticles with various Europium doping rates, excitation wavelength at 260 nm. Inset: magnification of the 580-605 nm region. Relative intensities.

The 4f→4f transitions have essentially electric-dipole (ED) and magnetic dipole (MD) characters. ED transitions require a change
of parity of the electron wave function and, then, they should be strictly forbidden within the 4f configuration (due to the Laporte selection rule). On the opposite, MD transitions between these levels are permitted. For Ln3+ ions localized in crystalline sites without inversion symmetry, however, a mixing of opposite-parity states into the 4f level occurs resulting in a relaxation of the Laporte selection rule and then in the observation of intra-4f-4f forced ED transitions. For Eu3+ the maximum of the intensity emission occurs at 611.2 nm for all the compositions, which is due to the electric dipole forced transitions. The high luminescence efficiency of the samples is the confirmation of defect free and highly crystalline samples.

The intensity of this peak at 611.2 nm (3D0→3F2) is much higher than that at 591 nm (3D0→3F1) which is a magnetic dipole allowed transition but with a low rate; the electric dipole forced transition (normally forbidden) depends on the surrounding environment and the local structure symmetry and to a lesser extent on the crystal field, whereas the magnetic dipole allowed transition is independent on the surrounding environment according to Judd–Ofelt theory.[65,66] In cubic Gd2O3, Eu3+ ions occupy Gd3+ sites, which have C2 and S6 site symmetries. Both sites exhibit an octahedral coordination. C2 has two different bond distances: four equal equatorial Eu-O bonds and two equal apical Eu-O bonds. S6 presents all equal bond distances (Eu-O) and an inversion element (i). According to this theory, the greater the intensities ratio 3D0→3F2/3D0→3F1, the higher is the asymmetric surrounding of Eu3+ in the host matrix. The intensity of the 3D0→3F2 transition is 20 times higher than that of the 3D0→3F1. Hence, the dominance of 3D0→3F2 over 3D0→3F1 essentially proves the non-centrosymmetric environment of Eu3+ in the Gd2O3 host lattice and as a consequence that in Gd2O3, more Eu3+ ions occupy the C2 site as compared to the S6 site giving rise to highly intense electric dipole transition. Additionally, this high ratio implies the increasing covalence of Eu-O bonds in the sample. The localization of Eu3+ in the S6 crystalline site of Gd3+ is shown by the presence of the 581.8 nm peak corresponding to the highest component of the 3D0→3F1 transitions of Eu3+ in this site (Figure 6 inset).

Figure 7 shows the (PLE) excitation spectra obtained by monitoring the 3D0→3F2 main transition of Eu3+ at 611.2 nm in Eu3+:Gd2O3 nanocrystals at room temperature for the different europium doping rates.

The main characteristics are as follows: (i) a broad excitation band which extends from 200 to ~300 nm, resulting from both the host and the charge-transfer-band (CTB) excitation; This Eu-O charge transfer absorption corresponds to an electron transfer from the 2p orbital of O2− to 4f Eu3+ orbitals; its intensity and energy position depend on degree of covalency between O2− and Eu3+ and on the coordination environment around Eu3+[65]; (ii) two relatively weak peaks at 277 nm and 280 nm. They result from the 4f–4f absorption transitions of Gd3+ (5S2→5I, Gd3+ transitions set) and can be regarded as the results of Gd3+→Eu3+ energy transfer[61] (see SI Figure S3); (iii) a lot of weak peaks in the higher wavelength region (350–500 nm), they correspond to direct 4f→4f Eu3+ absorption lines with a resulting emission much less intense than that of the CT band.[66,67]

In these excitation spectra, the broad UV band systematically increases in intensity and exhibits a red-shift of the wavelength from 248 nm to 263 nm when the concentration of Eu3+ increases from 2.5% to 20%. This red shift is linked to the higher increase of diffusion reflectance (r) at the excitation lines.

The photoluminescence intensity is a function of the Eu3+ concentration in the NPs. As usual, we observed that the PL intensity is improved, as a result of an increase in the number of luminescent centers; however as soon as the Eu3+ activator concentration exceeds a certain threshold concentration (5%), the PL intensity decreases again. This phenomenon corresponds to the concentration quenching (Figure 8). Among the material the Eu3+—Eu3+ distances decrease with Eu3+ concentration; as a consequence Eu3+ ions are more in interaction with each other and the luminescence intensity decreases due to a deactivation process of the luminescent centers via a very efficient resonant energy transfer among activators until non radiative relaxations occur at a defect or impurity places.[69]

From Figure 7, it can be noticed that comparing NPs with 2.5 and 5% Eu3+ doping there is an inversion in the intensities of the CTB and 4f–4f excitation transitions. This is confirmed in Figure 8 where the maximum of the intensity is reached for 2.5% Eu3+ when the excitation is performed in the CTB and 5% when it is performed in 4f–4f bands. This result can be explained by the huge difference in the strength of the absorption for these two kinds of excitation mechanisms.

All these assumptions are also illustrated by UV–vis. absorption spectra of undoped and Eu3+ doped (from 0 to 20%) Gd2O3 NPs measured by using diffuse reflectance (Figure 9). An intense
absorption band at 235–275 nm is observed for the europium doped samples. These spectra clearly show that a soon as 2.5% Eu\(^{3+}\) are introduced into the material, 70% of the absorption in the BTC already takes place. The emission of the doped NPs is then really efficient since it is observed even if the absorption peaks due to Eu\(^{3+}\) cannot be seen on Figure 9.

Figure 9. UV–vis absorption spectra of Eu\(^{3+}\):Gd\(_2\)O\(_3\) for 0% to 20% doping rate.

**Internalization of NPs by Hela cells**

Two types of NPs were chosen for further studies for MRI and cytotoxicity: Gd\(_2\)O\(_3\) and Eu:Gd\(_2\)O\(_3\) (5 at% europium as suggested by the emission spectra). The internalization of the NPs in Hela cells was studied for two different incubation times: 2h and 6h. The internalization kinetics of both NPs are quiet similar, nevertheless Eu:Gd\(_2\)O\(_3\) was significantly \((P < 0.0001\) at 2 h) faster than Gd\(_2\)O\(_3\). After 6h, both types of NPs Gd\(_2\)O\(_3\) and Eu:Gd\(_2\)O\(_3\) presented the same internalization efficiency and this time range was established for the subsequent studies. To quantify the real amount of NPs internalized by Hela cells at different timings, inductive coupled plasma mass spectrometry (ICP-MS) was used (Figure 10). For these studies Hela cells were exposed to Gd\(_2\)O\(_3\) (50 μg/mL 9.08 × 10\(^{14}\) NPs/mL) and Eu:Gd\(_2\)O\(_3\) (5%) (50 μg/mL 1.1 × 10\(^{15}\) NPs/mL) for 2 h and 6 h. The results indicate a time-dependent increase in the uptake of the NPs by Hela cells. Both type of NPs after 6h have an uptake of approximately 6 × 10\(^{9}\) NPs per cell.

Figure 10. Quantification of NPs in Hela cells determined by ICP-MS analysis. Cells were incubated with Gd\(_2\)O\(_3\) (50 μg/mL 9.08 × 10\(^{14}\) NPs/mL) and Eu:Gd\(_2\)O\(_3\) (5%) (50 μg/mL 1.1 × 10\(^{15}\) NPs/mL) for 2 h and 6 h. After the incubation, the cells were washed, trypsinized and finally freeze-dried. The concentration of NPs was normalized per cell. The results are expressed as Mean ± SEM \((n = 3)\). *denotes statistical significance \((P < 0.001)\).

Additionally, to preliminary assess the biological effect of such NPs, the cell metabolism was evaluated after exposing Hela cells (1 x 10\(^{4}\) cell/well) to various concentrations of NPs (from 10 to 100 μg/mL) for 24h and 48h (Figure 11). Cell metabolism was assessed by ATP production using a CellTiter-Glo ® Cell Viability Assay. Hela cells exposed to concentrations of 100 μg/mL for the different NPs showed significant impaired ATP production relatively to controls for 24h (Eu\(^{3+}\):Gd\(_2\)O\(_3\): 54.3 ± 2.7% and Gd\(_2\)O\(_3\): 65.0 ± 6.7%) and for 48h (Eu\(^{3+}\):Gd\(_2\)O\(_3\): 48.8 ± 2.2% and Gd\(_2\)O\(_3\): 73.4 ± 6.3%). Similar findings have been reported in previous studies for different sized Gd\(_2\)O\(_3\) NPs.\(^{[13, 68-69]}\) For concentrations below 100 μg/mL, the ATP production is equal or even higher than that of the controls for both types of NPs indicating an absence of cytotoxicity of these NPs at these concentrations for this type of cells. Further tests should be performed to assess in more detail the cytotoxicity of these particles in other cell lines.

Figure 11. Effect of NPs on Hela cell metabolism: the cytotoxicity of NPs was evaluated by Hela cell metabolism. Hela cells were incubated with Gd\(_2\)O\(_3\) and Gd\(_2\)O\(_3\):Eu (5%) for 24 h (a) or 48 h (a). The results are expressed as Mean ± SEM \((n = 3)\).

Owing to the magnetic properties of Gd\(^{3+}\) ions, doped and undoped Gd\(_2\)O\(_3\) nanoparticles were tested as a T\(_1\) magnetic resonance imaging contrast agent. Table 2 shows the water \(^1\)H relaxivity values \((r_1\) and \(r_2\)) and the \(r_1/r_2\) values, measured at 20 MHz and at 25 °C and 37 °C, for clear dispersions of the Gd\(_2\)O\(_3\) and Eu:Gd\(_2\)O\(_3\) nanoparticles in water and compares with literature data.\(^{[70-79]}\)

The proton relaxivities \((r_1)\) were obtained from the observed linear dependence of the relaxation rates \(R_1\) \((R_1 = 1/T_1, i = 1, 2)\) on the concentration of the Gd\(^{3+}\) ions in the samples, using at least four independent measurements at concentrations between 0 and 1 mM, (Table 2) (See also SI Figures S4 and S5). The \(r_1\) values are quite low for all the samples studied, with slightly larger \(r_2\) values. The value of the \(r_2/r_1\) parameter is lower than 2, indicating that the materials could be useful for \(T_1\)-weighted (positive contrast) imaging. With regard to the longitudinal relaxivity, there is no significant difference with the data reported for analogous Gd\(_2\)O\(_3\) materials (Table 2). As demonstrated by different authors,\(^{[71-79]}\) the \(r_1\) and \(r_2\) relaxivities of the nanoparticles decrease with, among others, the increase in particle size, explaining our low \(r_1\) measurements. The \(r_1\) relaxivity can have contributions from the inner-sphere and outer-sphere mechanisms. The inner-sphere mechanism refers to the number of water molecules directly coordinated to the Gd\(^{3+}\) ions on the surface of the NPs, and therefore the surface to volume ratio of the nanoparticles (S/V), as already reported, plays an important role. The comprehensive study by Luo et al.\(^{[72]}\) shows that \(r_1\) is given by the product of three factors: \(i\) the longitudinal relaxivity of inner-sphere water molecules directly coordinated to Gd\(^{3+}\), \(ii\) the hydration number and \(iii\) the surface to volume ratio. Depending on the magnetic field, the \(r_1\) values can go up to 12 s\(^{-1}\)mM\(^{-1}\) (1.5 T) or 10 s\(^{-1}\)mM\(^{-1}\) (3 T), with an optimal particle size of 1.6 nm and 3.8 nm, respectively (Table 2). The low \(r_1\) values we obtained are also attributed to the annealing temperature which leads to an anhydrous cubic phase, working essentially through an outer-sphere relaxation mechanism. Indeed, high temperature treatment of the particles prevents the hydrated surface state, leading to less efficient \(T_1\) relaxation.\(^{[73]}\)

Table 2. Water \(^1\)H relaxivities, \(r_1\) \((i = 1, 2)\), determined at 20 MHz (0.47 T), at 25 °C and 37 °C, for aqueous suspensions of Gd\(_2\)O\(_3\), Gd\(_2\)O\(_3\):Eu, compared with some of the values from the literature see also the review by Lux et al for additional data.\(^{[79]}\)
### Materials

<table>
<thead>
<tr>
<th>Materials</th>
<th>$r_2$ [s−1mM⁻¹]</th>
<th>$r_3$ [s−1mM⁻¹]</th>
<th>$r_2/r_1$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Gd}_3\text{O}_7$</td>
<td>0.92±0.02</td>
<td>1.10±0.02</td>
<td>1.43±0.01</td>
<td>This work[6]</td>
</tr>
<tr>
<td>Eu:Gd$_3$O$_7$ (5 at%)</td>
<td>1.27±0.03</td>
<td>1.44±0.04</td>
<td>2.11±0.01</td>
<td>This work[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (10 nm)</td>
<td>3.489</td>
<td>-</td>
<td>-</td>
<td>70[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (50 nm)</td>
<td>0.315</td>
<td>-</td>
<td>-</td>
<td>70[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (270 nm)</td>
<td>0.145</td>
<td>-</td>
<td>-</td>
<td>70[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (1.6 nm)</td>
<td>12.4</td>
<td>-</td>
<td>-</td>
<td>71[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (75 nm)</td>
<td>7.98</td>
<td>13.75</td>
<td>1.72</td>
<td>73[6]</td>
</tr>
<tr>
<td>Gd$_3$O$_7$ (1.3 nm) cubic</td>
<td>24</td>
<td>60</td>
<td>2.5</td>
<td>78[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (3.8 nm)</td>
<td>9.76±0.18</td>
<td>16.2±0.3</td>
<td>3.95</td>
<td>72[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (7.1 nm)</td>
<td>5.5±0.2</td>
<td>-</td>
<td>-</td>
<td>76[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (4 nm)</td>
<td>6.4</td>
<td>9.1</td>
<td>1.42</td>
<td>75[6]</td>
</tr>
<tr>
<td>Eu:Gd$_3$O$_7$ (4 nm)</td>
<td>6.3</td>
<td>8.8</td>
<td>1.39</td>
<td>75[6]</td>
</tr>
<tr>
<td>$\text{Gd}_3\text{O}_7$ (2.9 nm) different ligand sizes</td>
<td>12.1 0.5</td>
<td>33.18</td>
<td>11.92</td>
<td>2.74 22.07</td>
</tr>
</tbody>
</table>

Relaxivity data measured at [a] 0.47 T (20 MHz); [b] 4.7 T (200 MHz) in 0.5% agarose; [c] 1.5 T (64 MHz); [d] 3 T (128 MHz); [e] 1.41 T (60 MHz); [f] 7 T.

On the other hand, the transversal relaxivity ($r_2$) values reported by Park et al[71] are quite large and the $r_2/r_1$ ratio is over seven, which corresponds to a dominant $T_2$-weighted (negative contrast) mechanism. Our $r_2$ values are quite smaller and in the range of the $r_1$ values. As already reported, the transversal relaxivity might depend on the time interval between two consecutive 180° pulses ($τ_ϕ$) in a CPMG pulse sequence. This dependence is influenced by particle size[73] the magnetic field[74] as well as particle composition[73-74]. The magnetic field plays an important role since $r_2$ increases with the increase of the magnetic field. When we compare the results obtained by Park et al.[71] and our results it is necessary to have this dependence in mind since they use a magnetic field 3 times higher.

### Conclusions

It has been demonstrated that monoclinic and cubic structures of Eu$^{3+}$ doped and undoped Gd$_3$O$_7$ can be synthesized via Glycine Nitrate Process. The crucial role of fuel - oxidant ratio (and then of the experimental flame temperature that is reached during self-combustion) on the nature of the crystallographic phases of these nanopowders was illustrated. Cubic phase is obtained in fuel-rich conditions while a biphasic sample (cubic and monoclinic polymorphs) is formed for stoichiometric conditions.

The as-synthesized samples were subjected to various thermal treatments to eliminate the organic impurities originating from glycine. The appropriate temperature to obtain highly crystalline and carbon free compound is found to be 800°C (with short duration). The average crystalline size determined by TEM for these nanopowders is found to be 23 nm approximately. This is in agreement with the results obtained from XRD Rietveld analysis. Both the EELS analysis and the photoluminescent studies approve, as reported, that doped Europium materials to be highly luminescent with Eu$^{3+}$ homogeneously distributed over the whole material and the two crystallographic sites of the cubic phase of the gadolinium cation. This excellent chemical homogeneity is provided by the chosen GNP conditions, the excess of glycine ensuring the chelation of cations. These NPs also exhibit relaxivities which define them as T1 contrast agents for further biomedical applications. Works are now in progress to perform disaggregation of nanoparticles according to the sequence planetary ball milling / acidic treatment / size sorting, this procedure being previously successfully optimized for manganites.[57]

### Experimental Section

**Synthesis of Gd$_3$O$_7$ and Gd$_3$O$_7$:Eu$^{3+}$ nanoparticles by GNP**

Gd$_3$O$_7$ and Gd$_3$O$_7$:Eu$^{3+}$ (2.5 to 20 at.-%) nanoparticles were elaborated according to the following protocol. Gadolinium nitrate and europium nitrate solutions (0.1M) were previously prepared from Gd(NO$_3$)$_3$.6H$_2$O (Alfa Aesar, 99%) and Eu(NO$_3$)$_3$.6H$_2$O (Alfa Aesar, 99%), respectively, dissolved in de-ionised water and titrated by inductively coupled plasma optical emission spectrometry (ICP.OES 720ES Varian). For the doped compound, Gd(NO$_3$)$_3$ and Eu(NO$_3$)$_3$ were mixed in stoichiometric conditions in order to get about 2 g of doped oxide. Glycine (Sigma Aldrich, 99%) was dissolved in the Gd or Gd:Eu nitrate solution with various glycine/nitrate molar ratio (G/N) (0.55 and 1.1 for undoped sample and 1.1 for europium doped samples). As-prepared glycine - metallic nitrate solutions were heated on a hot plate at 120 °C while stirred at 260 rpm using magnetic needle. When a viscous gel-like liquid was formed, temperature was increased up to around 200 °C. Self-ignition then occurred which led to the production of voluminous red-hot ashes accompanied by the emission of large amounts of gases.

These as-synthesized materials with G/N = 1.1 were submitted to thermal treatment. In this case, after careful manual grinding, they were calcinated in an alumina boat into a tubular furnace at temperatures T ranging from 450 to 800 °C for durations from 30 min to 4h under air. Samples were directly introduced into the furnace at T.

**Characterizations**

Elemental analyses were performed on the starting solutions and on doped Europium compound by inductively coupled plasma optical emission spectrometry (ICP/OES 720ES Varian) and mass spectroscopy (ICP/MS Agilent 7500series).

Bulk elemental combustion analyses of C, H, N elements were performed on a Thermofisher Flash EA 112 analyzer.
The X-ray diffraction (XRD) patterns were recorded at room temperature on a Brucker Apex II powder diffractometer in the Bragg-Brentano geometry, using Cu-Kα radiation (1.54056 Å). The data collections were made in the 10-80° 20 range with a 0.02° step. The diffraction data were analyzed using the Fullprof program implementing the Rietveld method.[81] The phase composition analysis was based on data from ICSD.[82] In order to evaluate the mean crystallite size, the Thompson-Cox-Hastings pseudo-Voigt profile was used to resolve the instrumental, strain and size contributions to peak broadening. The instrumental contribution was determined by measuring a NIST LaB₆ SRM660a standard reference material.

Morphology of the nanoparticles was observed by Transmission Electron Microscopy (TEM) on a JEOL JEM 2000FXII apparatus (acceleration voltage 200 kV). A drop of diluted (ethanol) sample was deposited on a 300-mesh carbon-coated copper grid and let to evaporate at room temperature. The size of particles is measured using the software Image J.

High resolution transmission electron microscopy (HRTEM) and Electron Energy Loss Spectroscopy (EELS) observations were performed using a field emission gun JEOL 2200 FS operating at 200 kV, equipped with an in-column filter (Omega Filter). The spatial resolution is 0.19 nm. The EELS maps were obtained by the three window method on the N₁s edge at 133eV and 140 eV for Europium and gadolinium, respectively. A semi-quantification EELS was also performed on the edges M₂M₃ 1131/1161 eV and M₂M₄ 1185/1217 eV for Eu and Gd, respectively.

Optical characterizations were carried out on powder materials. Luminescence spectra were recorded on a SPEX Fluorolog FL 212 spectrofluorimeter (Horiba Jobin Yvon). The excitation source is a 450 Watt xenon lamp, excitation spectra were corrected for the variation of the incident lamp flux, as well as emission spectra for the transmission of the monochromator and the response of the photomultiplier (Peltier cooled Hamamatsu R928P photomultiplier). Diffuse reflectance spectra of luminescent powders were also recorded on the SPEX FL 212 spectrofluorimeter but in diffuse (not focalized) and synchronous mode (simultaneous scanning of excitation and emission monochromators in order to collect the diffuse reflexion of the incident radiation without catching the fluorescent photons).

1H longitudinal and transverse relaxation times (T₁ and T₂ respectively) of aqueous suspensions of nanoparticles were measured at 20 MHz on a Bruker Minispec mq20 relaxometer, at 37 °C. T₁ values were measured using the inversion recovery pulse sequence, while T₂ values were measured using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. The time interval between two consecutive refocusing pulses (τCP) in the train of 180° pulses applied was 0.8 ms. All the experimental values were corrected for the diamagnetic contributions of water under the same conditions.

Cell culture

HeLa cells (5 × 10⁵ cells/well) were plated in 6 well plates and left to adhere overnight. Then the cells were incubated with S 1 and S 10 (50 µg/ml) for 2 and 6h. After incubation, in order to remove non-internalized NPs, the cells were washed three times with PBS, dissociated with trypsin [0.2% (w/v) in PBS], centrifuged, counted and washed again with PBS. Afterwards, the samples were freeze-dried and the presence of Gd and Eu in the samples was evaluated by ICP-MS. For this purpose, the samples were digested overnight in the presence of Nitric acid (0.1 mL, 68%, (w/v)), then, aqueous nitric acid solution (9.9 mL, 2% (w/v)) was added. The samples (n = 4) were analyzed by ICP-MS for the quantification of internalized Gd and Eu.[83]

Supporting Information

The Supporting Information provide data on the DLS histogram of Gd₂O₃:300 dispersed in ethanol; the HRTEM of Eu:Gd₂O₃ with 20 at% doping showing the nice sequence of atomic planes and a FFT of the zone delimited in red on HRTEM image: as well as Excitation spectra of Eu³⁺ doped Gd₂O₃ nanoparticles with various Europium dopings, monitoring at 611.2 nm emission and the proton relaxation rates R₁ vs. Gd(III) concentration measured at 20 MHz (a) 25°C and (b)37°C for 2 aqueous solutions of samples: Gd₂O₃ and Gd₂O₃:Eu.

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References

Glycine Nitrate Process via a fine control of the flame temperature gave highly crystalline Eu$^{3+}$ doped and undoped Gd$_2$O$_3$ of 23 nm size. In doped samples, the Eu$^{3+}$ is homogeneously distributed over the whole material as well as the two Gd crystallographic sites of cubic phase. Gd$_2$O$_3$:Eu$^{3+}$ NPs are highly luminescent and exhibit relaxivities which also define them as potential T1 contrast agents for further biomedical applications.


Keywords: aqueous self-combustion / Glycine Nitrate Process / Gd$_2$O$_3$:Eu$^{3+}$ / bimodal contrast agents.