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Lanthanide-doped nanoparticles for hybrid X-ray/optical imaging

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ABSTRACT

Some lanthanide-doped nanoparticles can absorb X-ray radiation and emit in the visible to near infrared spectrum via a down-conversion mechanism, making them a potentially valuable agent for *in vivo* imaging studies. We have studied a series of Gd³⁺ and Eu³⁺ compositions in lanthanide fluorides to optimize the emission from Eu³⁺ upon X-ray excitation. The optimum concentration of Eu³⁺ that produced the most intense emission in NaGdF₄ was found to be 15% molar concentration. The impact of the crystallographic phases (i.e. cubic or hexagonal) on the optical emission was investigated. Furthermore, an attempt to include a sensitizer (i.e Ce³⁺) in NaGdF₄:Eu resulted in a reduction in the emission following X-ray excitation. A surface coating of NaGdF₄:Eu nanoparticles with a gold shell showed a similar decrease in luminescence intensity by a factor of two although the gold shell offers other advantages in biomedical applications.

Keywords: X-ray; imaging; nanoparticle; phosphor; europium; gold

INTRODUCTION

Scintillation materials emit visible photons when irradiated with gamma or X-rays, constituting an extreme form of down-conversion. Lanthanide elements are often the choice for such materials as they have high atomic number and suitable electronic energy states to emit photons in the visible and near infrared (NIR) region of the electromagnetic spectrum. Elements with high atomic number that are included in a host crystal matrix can generate high-energy electrons that are down-converted by appropriate lanthanide sensitizers to result in the emission of visible/NIR photons. High efficiency lanthanide nanophosphors, especially up-converting nanophosphors[1-4] are increasingly finding application in *in vitro* and *in vivo* imaging.

Very recently, this class of nanophosphors has been studied as a molecular imaging probe in radioluminescent imaging.[5-7] The combination of the deep penetration and low scattering of X-rays (compared to optical photons) in tissues and the emission from phosphors in the optical window where tissue has good

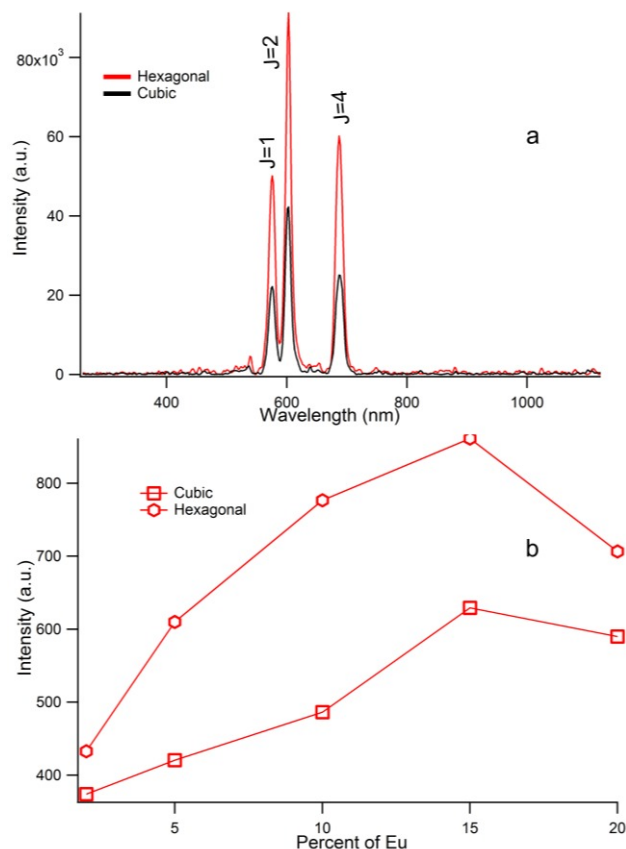


Figure 1 a) X-ray luminescence spectra from NaGdF₄:15%Eu. The emissions corresponding to $^5D_0 \rightarrow ^7F_J$ are plotted. b) The integrated peak intensities of the X-ray luminescence spectrum at different molar concentrations of Eu in NaGdF₄.

transparency can offer detailed imaging at depth and high spatial resolution. Spatial resolution using this approach is largely limited by the dimensions of the X-ray beam. This has opened up avenues for optical tomography, as the excitation region is well-defined by the X-ray beam. Accurate 3D reconstruction of target regions of interest with high spatial resolution is possible by employing X-ray luminescence optical tomography (XLOT).

X-ray activated luminescence benefits from the fact that the excitation region is known (the X-ray beam trajectory), and the resulting optical signal is directly proportional to the luminescent particle concentration.[8] Using this approach, imaging of nanoparticles *in vitro* with tissue phantoms was demonstrated using Gd₂O₂S:Eu particles,[9] and the

same group showed the viability of employing fluoride-based nanoparticles for *in vivo* radio-luminescence imaging.[10] Use of X-ray luminescent nanoparticles *in vivo* would require that the particles

emit efficiently in 600-1100 nm region.

Fluoride-based ALnF₄ (A= alkaline; Ln= lanthanide) materials are preferred hosts for light-emitting lanthanide phosphors, since they have low phonon energy states.[11-13] ALnF₄ is the preferred matrix for down-conversion because it is a wide band gap material with a band gap in the range of 9-10 eV. Such large band gap contributes to luminescence through charge transfer, directly feeding the excited radiative levels of the emitters, e.g., Eu ions.[14] These hosts, when doped with Eu, Tb and Tm, generate visible photons following excitation by ultraviolet (UV) and vacuum ultraviolet (VUV) radiation with high efficiency quantum cutting[15] – the inverse of up-conversion.

Gadolinium serves as an excellent photosensitizer for other down-converting lanthanide emitters.[14, 16] It has an excellent photon absorption cross-section in the VUV range. It is known that singly-doped phosphors, without a photosensitizer, are not efficient down-converters of VUV irradiation.[14] The Gd-Eu combination

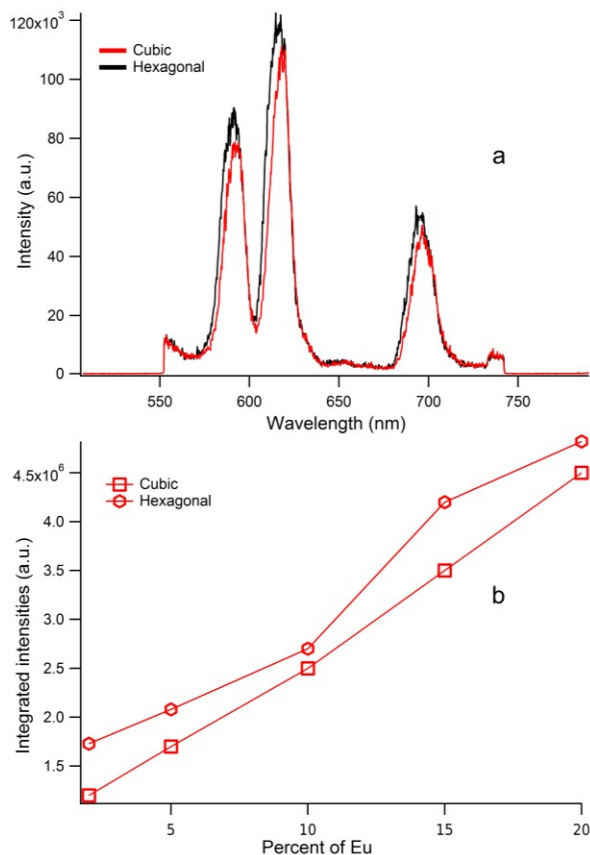


Figure 2 a) Photoluminescence spectra of NaGdF₄:15%Eu in aqueous medium excited at 365 nm. b) Integrated peak intensities of the photoluminescence spectrum at different molar concentrations of Eu doped in NaGdF₄.

is the best studied system for down-conversion of UV radiation, as the emission energy transitions within Gd can resonantly couple to the excited state of Eu. In addition, there is an energy-conserving cross-relaxation mechanism that transfers energy from Gd to Eu.[14, 15] Therefore, for *in vivo* X-ray luminescence imaging, AGdF₄:Eu nanoparticles appears to be an excellent choice, as it might provide an efficient route to down-convert high energy electrons generated by X-rays to vis-NIR photons.

It is known that the efficiency of emission from lanthanides is linked not just to the lanthanide ion crystal symmetry or size,[17-19] but can be improved by core-shell structures that suppress surface quenchers,[20, 21] enhance energy migration,[22] and can provide dye sensitization[23] and plasmonic perturbation.[24-26] We have explored specifically the impact of the crystal phase, the concentration of lanthanide, and the impact of a gold particle coating on the vis-NIR emission from NaGdF₄:Eu nanophosphors.

METHODS

Nanoparticles of NaGdF₄ doped with Eu and Ce were obtained by a citrate method. A transparent aqueous solution containing 4 mL of 0.2M lanthanide chlorides (Sigma Aldrich, MO) and 8 mL of 0.2 M sodium citrate (Sigma Aldrich, MO) was heated to 90 °C. 16 mL of 1M Sodium fluoride (Fischer Scientific, PA) solution was added to the solution, upon which the solution turned whitish. The resulting mixture was heated for 2 h to produce the desired cubic nanoparticles. The gold coated nanoparticles were prepared by adding 0.1% HAuCl₄ to the solution. The nanoparticles were centrifuged and washed in de-ionized water twice before characterization and measurements. To obtain the hexagonal phase of the nanoparticles, the as-synthesized particles were heated in an autoclave (Cole-Parmer, IL) at 230 °C for 1 h and washed twice with de-ionized water.

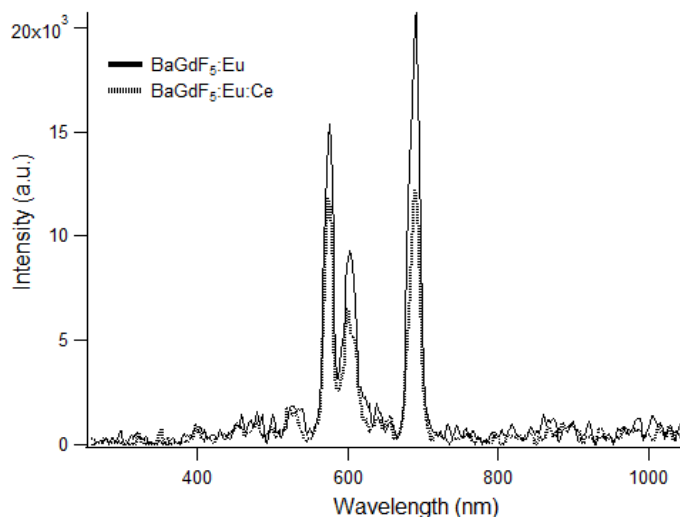


Figure 3. X-ray photoluminescence spectra of BaGdF₅:Eu and BaGdF₅:Eu:Ce.

A UV source (Fischer Scientific, PA) was used for excitation in the study photoluminescence of the nanoparticles. The emission was fed into Acton spectrapro 300i series spectrometer, fitted with a PI-MAX camera (Princeton Instruments, NJ), by an objective and condensing lenses. The spectra were collected using Win32 software provided by Princeton Instruments.

X-ray luminescence was carried out using an X-ray tube (SB80250, Oxford Instruments, Scott's Valley, CA), controlled with commercial software SRI developed by Source-ray, Inc (Bohemia, NY); the tube generates X-ray photons with energy up to 80 kVp. The maximum X-ray tube current is 0.25 mA. In this study, the X-ray tube was set at 75 kVp and 0.24 mA. An electron multiplying charge coupled device (EMCCD) camera (C91003, Hamamatsu) was used to measure the emitted optical photons. The EMCCD camera was set at maximum EM gain and analog gain with photon mode 1. The EMCCD camera was water-cooled to be -92 °C.

To measure its optical emission spectra, the sample is placed inside a transparent plastic cuvette and excited with non-collimated X-ray photons. The EMCCD camera is located orthogonal to the X-ray beam to minimize X-ray photon direct hit on the EMCCD camera. A transparent x-ray shielding glass is placed in the front of the EMCCD camera to protect the camera from high energy X-ray photons. A spectrograph (Inspector V10E, Specim, Oulu, Finland) with a lens is mounted in the front of the EMCCD camera to split light into its wavelength components[27]. The wavelength measurement is calibrated with lasers.

RESULTS AND DISCUSSION

A typical luminescence spectrum of NaGdF₄:Eu nanophosphors with X-ray excitation is shown in Figure 1a. The particles were suspended stably in water at a concentration of 30 mg/mL (Figure 1a) and 3 mg/mL (Figure 1b). From the luminescence intensities of the solutions, as detected by the EMCCD camera, the X-ray luminescence from the cubic phase was observed to be lower than from the hexagonal phase by a factor of two. The doping concentration of Eu in the nanophosphors was studied with a view to optimize the X-ray luminescence. In Figure 1b, the integrated intensities of the three major ⁵D₀→⁷F_J emissions of Eu³⁺(as shown

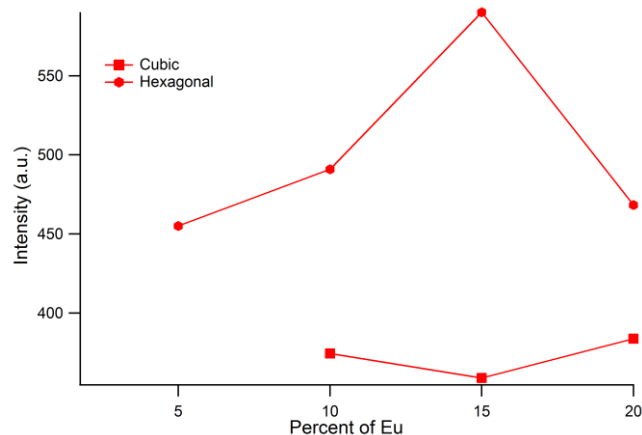


Figure 4 Integrated peak intensities of X-ray luminescence from gold-coated NaGdF₄ containing different molar concentrations of Eu.

in Figure 1a) is plotted against the molar percentage of the Eu. The X-ray luminescence appears to be brightest with 15 molar percent concentration of Eu in NaGdF₄.

We compared the emission of X-ray luminescence with the UV excited (i.e. 365 nm) photoluminescence of the nanophosphors at a concentration of 3 mg/mL. Figure 2a shows the

emission spectrum of NaGdF₄:15%Eu. From the intensities of the spectrum, it is obvious that the difference in the photoluminescence

between hexagonal and cubic crystal phases was negligible. The effect of Eu concentration on the photoluminescence from NaGdF₄ nanoparticles is shown in Figure 2b. The photoluminescence increased linearly with Eu concentration. The photoluminescence results are in contrast to the X-ray luminescence results presented in Figure 1. As in the case of up-conversion, the X-ray luminescence from the hexagonal phase had a higher efficiency in comparison to the cubic phase. However, under UV excitation, the photoluminescence efficiencies from the two phases were comparable. One possible reason could be that the non-radiative, multi-phonon relaxation could be dominant in the cubic structure with X-ray excitation. It can also be attributed to the different conversion efficiencies of an X-ray photon in cubic and hexagonal phases that usually involve many competing energy loss processes[28]. On the other hand, the wavelength of UV excitation matches the electronic excitation of Eu, and the emission from Eu appears to be insensitive to the crystal field difference between the cubic and the hexagonal phase.

The ratios of $^5D_0 \rightarrow ^7F_1$ and $^5D_0 \rightarrow ^7F_2$ transition intensities for cubic and hexagonal phase under X-ray excitation are 0.58 and 0.63, respectively, while for photoluminescence it is 0.72 for both cubic and hexagonal phases. This indicates that the local crystal field surrounding the Eu is different with X-ray irradiation. Furthermore, a comparison of intensities of $^5D_0 \rightarrow ^7F_J$ transitions between the photoluminescence and the X-ray luminescence showed that the $^5D_0 \rightarrow ^7F_4$ transition (~700 nm) dominates the $^5D_0 \rightarrow ^7F_1$ transition (~580 nm) with X-ray excitation, a desirable outcome in terms of *in vivo* applications.

It is proposed that the emission from Eu in Gd-Eu phosphors can involve cross-relaxation between $^6G_J \rightarrow ^6P_J$ (Gd) and $^7F_1 \rightarrow ^5D_0$ (Eu) states or energy transfer leading to radiative decay of the excited electron in Eu[15]. To understand the role of Gd as a photosensitizer, the photoluminescent emissions from cubic and hexagonal

nanophosphors were studied with 255 nm excitation; the wavelength matched well one of the excitation energies in Gd. Under this excitation, no discernible emission from the nanophosphors was observed, indicating that sensitizer played a minimal role in both X-ray luminescence and photoluminescence from NaGdF₄:Eu nanophosphors. Upon changing the host matrix to BaGdF₅, we observed photoluminescence from Eu excited at 255 nm, indicating that the Gd sensitizer played a role in the down-conversion. In addition, we also observed significant X-ray luminescence from this nanophosphor (Figure 3). The addition of Ce (15%), a broadband photosensitizer, resulted in decreased X-ray luminescence (Figure 3). The effect of the host matrix and photosensitizer is being studied in further detail.

Surface defects can diminish the emission of photons by energy traps. Defects can be reduced through surface coating in a core-shell architecture. The potential for a bio-compatible gold coating to improve the vis-NIR emission following X-ray excitation of NaGdF₄:Eu phosphors was examined using our method for adding gold to fluoride phosphors[26]. Figure 4 shows the integrated intensities from all the $^5D_0 \rightarrow ^7F_J$ transitions in Eu. The effect of increasing concentrations on the X-ray luminescence was similar to that observed for uncoated nanophosphors shown in Figure 1b. The gold coating decreased the X-ray luminescence by a factor of two, with hexagonal nanophosphors being efficient in comparison to the cubic. Although, there is a decrease in X-ray luminescence for the gold-coated nanophosphors, the coating provides leverage through biocompatibility and its plasmonic feature can be an advantage in combining imaging with phototherapies.

CONCLUSIONS

X-ray luminescence and photoluminescence of hexagonal and cubic NaGdF₄:Eu nanophosphors in aqueous medium were studied. X-ray luminescence from the hexagonal phase was twice that from the cubic phase. The photoluminescence efficiencies, on the other hand, from these two phases remained the same. Coating of gold shell on these nanophosphors decreased the X-ray luminescence by a factor of two in comparison to uncoated phosphors.

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