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Eu³⁺ doped α -sodium gadolinium fluoride luminomagnetic nanophosphor as a bimodal nanoprobe for high-contrast *in vitro* bioimaging and external magnetic field tracking applications†

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Abstract

Herein, we introduce a novel strategy for the synthesis of Eu³⁺ doped α -sodium gadolinium fluoride (α -NaGd_{0.88}F₄:Eu_{0.12}³⁺) based luminomagnetic nanophosphors using hydrothermal route. The synthesized nanophosphor has exceptional luminescent and paramagnetic properties in a single host lattice, which is highly desirable for biomedical applications. This highly luminescent nanophosphor with an average particle size $\sim 5 \pm 3$ nm enables high-contrast fluorescent imaging with decreased light scattering. *In vitro* cellular uptake is shown by fluorescent microscopy that envisages the characteristic hypersensitive red emission of Eu³⁺ doped α -sodium gadolinium fluoride centered at 608 nm (⁵D₀-⁷F₂) upon 465 nm excitation wavelength. No apparent cytotoxicity is observed. Furthermore, time- resolved emission spectroscopy and SQUID magnetic measurements successfully demonstrate a photoluminescence decay time in microseconds and enhanced paramagnetic behavior respectively, which promises the applications of nanophosphors in biomedical studies. Hence, the obtained results strongly suggest that this nanophosphor could be potentially used as a bimodal nanoprobe for high-contrast *in vitro* bio-imaging of HeLa cells and external magnetic field tracking applications of luminomagnetic nanophosphors using permanent magnet.

Graphical Abstract

Demonstration of a highly luminescent-paramagnetic α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor as a bimodal nanoprobe for high-contrast *in vitro* bio-imaging and magnetic tracking applications.

†Electronic Supplementary Information (ESI)

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1 Introduction

Bifunctional nanomaterials having both luminescent and magnetic properties play a vital role in modern biotechnology. These bifunctional nanomaterials are highly desired for many biomedical applications such as biolabeling with external magnetic field tracking of nanoparticles for targeted drug delivery, biosensing and bioimaging with magnetic resonance imaging (MRI) contrast agents.¹⁻³ Although these kind of materials merely exist in nature, yet many research groups worldwide have current attention to synthesize these nanomaterials for desired biomedical applications.¹⁻⁶ In this context, various types of materials exhibiting both luminescent and magnetic properties in a single entity such as semiconducting core-shell quantum dots⁷, hybrid two dimensional nanomaterials⁸ and core-shell nanocomposites⁹ etc. have been synthesized and investigated for bioimaging applications, in the recent past. All of the materials investigated in the past suffer from some disadvantages that really challenges and are obstacle for their practical applications. For instance, quantum dots may possess high contrast imaging capabilities, but their applicability is still a challenge due to issues like high toxicity, optical blinking, low penetration depth, low quantum efficiency and high radiation dose.¹¹⁻¹² While other hybrid core shell nanostructures also suffers from photobleaching and low chemical stability.¹³ Compared to above mentioned nanomaterials, rare earth based bi functional nanophosphor is an another important class of materials, that has shown ability to be used in biomedical applications due to their high photochemical stability, sharp emission bands, long luminescent lifetimes, lower photo bleaching potential, low toxicity, high chemical stability and desirable magnetic properties.¹⁴⁻¹⁷ Recently, these features coupled with lanthanide doped rare earth nanophosphors such as oxides, fluorides and vanadates have gained much attention due to their unique bifunctional properties. Among all these, sodium rare-earth fluoride (NaREF_4 , RE= Y, Gd, La etc.) was considered to be a highly efficient and most promising host lattice, because it is well established that these nanophosphors have lower phonon energy that decreases the non-radiative relaxation probability and consequently increases the luminescent quantum yields.¹⁴ In present investigation, particularly for the luminomagnetic nanophosphor, NaGdF_4 doped with suitable trivalent rare earth ions show potential paramagnetic characteristics in addition to the highly-efficient photoluminescence properties. Hence rare-earth doped NaGdF_4 nanophosphor could be a better alternative to be explored thoroughly for the proposed next generation bimodal nanoprobe for bioimaging and external magnetic field tracking applications.

In the present manuscript, we have investigated the bi- functional luminomagnetic α -NaGd_{1-x}F₄:Eu³⁺ ($x = 0.03 - 0.3$) nanophosphor synthesized by facile hydrothermal method for *in vitro* fluorescent bioimaging applications using HeLa cells with external magnetic field tracking capability under permanent magnet. We found that this nanophosphor exhibits highly intense narrow red emission peak with paramagnetic characteristics. The red emitting ultra-fine downshift nanophosphor with high quantum yield (>83%) was purposely synthesized for easy detection of nanophosphor due to red emission during bioimaging applications and to minimize the light scattering because of its ultra small size. Further, the cytotoxicity measurements with MTT assays, *in vitro* fluorescence imaging of HeLa cells and potential paramagnetic properties of α -NaGd_{1-x}F₄:Eu³⁺ nanophosphor assures its suitability as a bimodal nanoprobe for next generation bioimaging applications, which is discussed and described in details.

2 Experimental

2.1 Materials

Gd₂O₃ (99.99%), Eu₂O₃ (99.99%), HCl (35.5%) NaOH (>98%, GR grade), NH₄F (>98%, GR grade), C₂H₅OH (GR grade), cyclohexane (GR grade) and polyvinyl alcohol (GR grade), were all purchased from Sigma-Aldrich and used as starting materials without further purification.

2.2 Synthesis of the luminomagnetic nanophosphor

In present investigation, α -NaGd_{1-x}F₄:Eu³⁺ ($x = 0.03 - 0.3$) nanophosphor was synthesized using a facile hydrothermal method. We have varied the growth temperature (500-1200 °C), time (1-6 hrs.) and doping concentration ($x = 0.03-0.3$) in order to find the optimum parameters.

Initially, Gd₂O₃ and Eu₂O₃ were taken according to stoichiometric formula and mixed well in a beaker with minimum quantity of De-Ionized (DI) water. A few drops of concentrated HCl were added to dissolve the oxides and the solution was then heated at 100°C for one hour with continuous stirring to form metal chloride solutions. Further, the above metal chloride solution was taken and mixed well with NH₄F (1 M) solution in a molar ratio of 1:4 to form metal fluoride mixture precursor. Then 10 ml of 1M aqueous solution of NaOH was added to the above formed metal fluoride mixture precursor. Afterwards, polyvinyl alcohol (PVA) solution was added to above solution mixture in volume ratio of 2:1. The pH of the final solution was monitored and maintained at ~3 by addition of few drops of 4M NaOH. This was one of the critical parameter for tailoring the size. Finally, the polymer caged sodium metal fluoride solution was transferred into a 50 ml Teflon-lined autoclave and heated at 180 °C for 4 h. The obtained fine white powder were collected by centrifugation, washed with water and ethanol several times, and sintered at 800±1°C for 3 h in air, a white colored nanophosphor fine powder were synthesized. The yield of the material was over 75 % with a high degree of homogeneity.

2.3 Characterizations

As-synthesized nanophosphor was thoroughly characterized using a number of different techniques. For phase purity identification, gross structural characterization was performed by X-ray diffraction (XRD) employing Rigaku: MiniFlex, operating at 30kV and 15 mA with Cu K α = 1.5406 Å radiation in the 2 θ range from 10 ^{0} - 80 ^{0} with a step size of 0.02 ^{0} in Bragg–Brentano geometry. Accurate lattice parameters were obtained by a least-squares fitting method using computer-based unit-cell refinement software.^{2,4} The surface topography was characterized using field emission scanning electron microscopic (FESEM) image with a microscope of Carl ZEISS-SUPRA 40 VP equipped with energy dispersive x-ray analysis (EDAX) facility for elemental analysis. The microstructural studies were carried out using high-resolution transmission electron microscopy (HRTEM, Model No. Technai G20-twin, 300 kv with super twin lenses having point and line resolution of 0.144 nm and 0.232 nm, respectively). The X-ray photoelectron spectroscopy (XPS) provides information about the binding states of different atomic species within tens of nanometers of the surface of the material. The XPS analysis of the sample was carried out in an ultrahigh vacuum (UHV) chamber equipped with a hemispherical electron energy analyzer (Perkin Elmer, PHI1257) using a non mono- chromatic Al K α source (1486.6 eV) with a base pressure of 4 $\times 10^{-10}$ Torr at room temperature. Binding energies were calibrated against the binding energy of the C 1s peak at 284.6 eV; gold was deposited on a portion of the sample and taken as a standard to take care of the shifts due to charging etc. The photoluminescence (PL) and time-resolved spectroscopic measurements were carried out using an Edinburgh Instruments spectrometer (Edinburgh, FLS – 920), where a xenon lamp acts as the source of excitation. To estimate the absolute luminescence quantum efficiency of the α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor, an integrating sphere equipped with an Edinburgh spectrometer (Model FLS920) instrument has been used for measuring the integrated fraction of luminous flux and radiant flux with the standard method.¹⁸ The PL mapping of the nanophosphor was performed using a WITec alpha 300R+ confocal PL microscope system, employing 375 nm diode lasers as a source of excitation. A Quantum Design MPMS (SQUID magnetometer) was used to investigate the magnetic properties of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺. A room-temperature $M(H)$ loop was traced with an applied field.

2.4 Biocompatibility

HeLa cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM), high-glucose medium (Invitrogen) containing 4.5 g L⁻¹ D-glucose, 4 mM L⁻¹ glutamine and 10 mg L⁻¹ sodium pyruvate, supplemented with 10% fetal bovine serum (FBS), 100 IU mL⁻¹ penicillin and 100 μ g mL⁻¹ streptomycin, in a humidified incubator at 37 °C with 5% CO₂. Each well of a 96-well cell culture plate was plated with 4 $\times 10^3$ cells with culture medium (100 μ L) and incubated overnight. The next day, α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor in cell culture medium was added to each well in different concentrations ranging from 0 to 200 μ g mL⁻¹ in triplicate. After 24 and 48 h of incubation, the medium containing α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor was removed and the cells were washed gently with warm, sterile phosphate buffer solution (PBS, 500 μ L). To each well, MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) reagent (200 μ L, 0.5 mg mL⁻¹ in medium) was added and the plate was returned to the incubator for 4 h. After incubation, medium with MTT reagent was removed and dimethyl sulfoxide (DMSO, 200 μ L) was

added to each well. The cells were subsequently incubated for 5 min and the optical density of solubilized formazan salts was assessed at 570 nm in a Tecan Infinite M200 microplate reader (Mannedorf, Switzerland).

2.5 *In vitro* Bio-imaging

The cells were cultured and maintained in DMEM as described above. For bioimaging, 1×10^4 cells were plated in each well of a four-well sterile chamber slide (Nunc, USA) with culture medium (500 μ L). After overnight culture, α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ (50 μ g mL⁻¹) was added to the culture medium and incubated under regular cell culture conditions. After overnight incubation the medium with α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor was removed from the cells and washed twice with phosphate buffer (1 mL). Cells were fixed using 1% paraformaldehyde and mounted with Vectashield antifade mounting medium with 4',6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Inc., CA). Cellular imaging (Figure 8) was performed using a Nikon Eclipse 90i microscope equipped with a Cool SNAP HQ2 CCD camera (Photometrics, AZ). A Nikon Intensilight C-HGFI lamp (wavelength range 380-600nm) was used as the fluorescence light source. The excitation wavelength (465nm) of nanophosphor falls within this range. The fluorescent images were taken under red channel that works as a filter having a range to pick fluorescent images under red colour region only.

3 Results and discussion

In the typical synthesis of α -NaGd_{1-x}F₄:Eu_x³⁺ ($x = 0.03$ – 0.3) nanophosphor, a modified hydrothermal method was used. The main aim of the modification in present method was to avoid agglomeration of the ultrafine nanophosphors, which is required for the proposed biomedical applications. In this context, polyvinyl alcohol (PVA) was used which forms a carbon network of cages of PVA around the nucleus of the nanophosphor during the course of its growth.⁴ This can completely avoid nanophosphor agglomeration even at high temperatures.⁴ We have also introduced this concept earlier for other host lattices and successively achieved the non-agglomerated ultrafine nanophosphors.⁴ This method provides large scale synthesis of α -NaGd_{1-x}F₄:Eu_x³⁺ ($x = 0.03$ – 0.3) nanophosphor with a narrow size distribution without agglomeration of nanoparticles. The concentration of europium ion in NaGd_{1-x}Eu_xF₄ was varied from $x = 0.03$ – 0.3 (α -NaGd_{0.88}F₄:Eu_{0.12}³⁺) and $x = 0.12$, was found to be the optimum value for the synthesis of high-quality nanophosphors with high brightness at 800 °C sintering temperature for 3 h. Fig. 1a shows the powder XRD pattern of the α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor. The observed diffraction pattern from the sample can be indexed to pure cubic phase with space group Fm3m possessing lattice parameters $a = b = c = 5.5000 \pm 0.0020$ Å, which are comparable with joint committee on powder diffraction standards (JCPDS) database file no 27-0697. Absence of any impurity peak indicates that the sample is of high purity. By using the Scherrer's equation the average crystallite size of the grains was determined to be ~ 5 nm. Inter-planer spacing is found to be 3.18 ± 0.0012 Å corresponding to (111) plane, which was further supported by FESEM and TEM/HRTEM characterizations.

The surface morphology was examined using FESEM. Fig. 2a demonstrates the SEM image of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor. The SEM image shows that the synthesized nanophosphor is almost spherical shaped nanostructure. The magnified version of the SEM image (Fig. 2a) of the spherical shaped nanostructure is shown in Fig. 2b, which clearly demonstrates the dimension of the spherical shaped nanophosphor, which is in the range of nanometers. Energy dispersive X-ray analysis spectrum is shown in Fig. S1 (†ESI) as well as qualitative analysis is shown in Fig. 2c as a histogram, confirms the presence of the Na, Gd, Eu and F in the α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor. The chemical composition and formation of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor was derived from X-ray photo electron spectroscopy (XPS) studies as shown in Figs. S2(†ESI) and S3(†ESI). The microstructural analysis of the synthesized nanophosphor was done by TEM/HRTEM. Fig. 2d shows the typical TEM micrograph of the α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ spherical shaped nanophosphor.

The magnified version of TEM image (Fig 2d) of the spherical shaped nanostructure is shown in Fig. 2e, which are in the range of nanometer with diameter 5 ± 3 nm. The TEM results clearly demonstrate the non-agglomerated ultrafine spherical shaped nanophosphor. A typical HRTEM micrograph of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor shows the well resolved lattice fringes without distortion as shown in Fig. 2f, which suggests the good quality of the crystal. The estimated interplaner spacing of the nanophosphor is ~ 3 Å, which corresponds to the (111) plane (JCPDS card no. 27-0697). Selected area electron diffraction (SAED) pattern is shown in the inset of Fig. 2f, which shows the spotty polycrystalline diffraction rings corresponding to the specific (111), (200), (220) and (311) planes of the cubic phase α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ lattice. The HRTEM and SAED results are in good agreement with the XRD results.

The particle size distribution and non-agglomeration are further confirmed by dynamic light scattering measurements in two different mediums: ethanol and DI water as shown in Fig. S4a and S4b (†ESI). These results of DLS show that the nanophosphor is non-agglomerated in nature with size of the order of 5 ± 3 nm, that further support the results of HRTEM and XRD.

In order to optimize the PL intensity and high brightness of α -nanophosphor for good brightness, the Eu concentration (mol) in the nanophosphor was varied from 0.03-0.3. Also the growth temperature and growth time were varied from 500-1200°C and 1-6 h, respectively. It has been observed that $x = 0.12$ (12 mol %), temperature of 800°C and time of 3 h were found to be optimum for the synthesis of nanophosphor with high intensity photoluminescence. The PL intensity was found to increase from $x = 0.003$ -0.12 and then decreased thereafter. The decrease in PL intensity after $x = 0.12$ can be attributed to luminescence quenching due to cross-relaxation process.¹⁹

With the increase in doping concentration of europium ions in the host lattice, the distance between two Eu³⁺ ions decreases. When the Eu³⁺ concentration is 12 mol %, the two Eu³⁺ ions are far apart and each one of Eu³⁺ ions can be regarded as an isolated luminescent center, which independently emits light without any interference. On the other hand nearby

†Electronic Supplementary Information (ESI)

Eu^{3+} ions mutually interact by electric multipolar process due to the shortened distance between two Eu^{3+} ions at a high doping concentration of more than 12 mol% in the latter case, the energy transfer case of Eu^{3+} ions easily exceeds the radiative rates. Thus the observed photon energy rapidly migrates among Eu^{3+} ions in the host lattice, which can decrease the probabilities of radiative transitions of Eu^{3+} ions and even quench the fluorescence.^{18,19} Similarly, PL intensity also depends upon the growth time and temperature. The nanophosphor synthesized at temperature and time less than optimum values (800 °C, 3h) exhibit weak PL intensity due to improper diffusion/substitution of activator in the host lattice. On the other hand, at higher growth temperature and time (>800 °C or > 3h), the possibility of growth of secondary phases increases and as result, luminescence intensity quenches enormously as compared to optimum condition. Similar effects of concentration, time and growth temperature were also observed and earlier reported for other host lattices.¹⁸

Fig. 3a shows the photoluminescent excitation spectrum of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor at fixed emission wavelength $\lambda_{\text{em}} = 608 \text{ nm}$. The photoluminescent excitation spectrum reveals that a broad peak centered at 275 nm (4.5 eV) corresponding to $^8\text{S}_{7/2}\text{-}^6\text{I}_j$ transition of Gd^{3+} .¹⁹ In addition to the 275 nm excitation peak, six more peaks also appeared at wavelengths of 362, 382, 393, 403, 417, 465 nm as shown in Fig. 3a. These excitation peaks are assigned to the direct excitation of the f-f transitions of the Eu^{3+} ions. The room temperature PL emission spectrum of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor recorded at 465 nm excitation shows a sharp, intense, hypersensitive red emission centered at 608 nm corresponding to $^5\text{D}_0\text{-}^7\text{F}_2$ transition, as shown in Fig. 3b. The other four emission peaks centered at 590, 627, 653 and 705 nm are ascribed to the $^5\text{D}_0\text{-}^7\text{F}_1$, $^5\text{D}_0\text{-}^7\text{F}_2$, $^5\text{D}_0\text{-}^7\text{F}_3$ and $^5\text{D}_0\text{-}^7\text{F}_4$ radiative transition, respectively. The $^5\text{D}_0\text{-}^7\text{F}_1$ (594 nm) transition is magnetic-dipole-allowed with a selection rule, $J = 1$, and its intensity is almost independent of the local environment around Eu^{3+} ions. The $^5\text{D}_0\text{-}^7\text{F}_2$ transition is the electric-dipole transition due to an admixture of opposite parity $4f^{n-1}5d$ states by an odd parity crystal-field component with a selection rule, $J = 2$, according to Judd-Oflet theory. Therefore, it is a hypersensitive transition.^{2,16,17} The number of the $^5\text{D}_0\text{-}^7\text{F}_J$ ($J = 1, 2, 3, 4$) emission lines is governed by the selection rules, which depend on the local symmetry of the crystal fields around the sites that the Eu^{3+} ions occupy.²¹ The inset of Fig. 3b shows the estimated color coordinates of red emission: $x = 0.6478$, $y = 0.3514$. The other emission spectra at the different excitation wavelengths of 275, 362, 382, 393, 403, 417 nm are shown in Fig. S5(†ESI) and gave a maximum emission at 465 nm excitation as compared to intensity at other excitation wavelengths, which represents the best excitation wavelength for the highest emission. We have also proposed the energy level diagram of Eu^{3+} transitions in NaGdF_4 lattice as shown in Fig. 4. The decay lifetime is an important tool to investigate the quality of the material and performance for desired application.² Generally, the exciton lifetime depends upon size and shape of the nanocrystals and it can be studied using time-resolved photoluminescence spectroscopy (TRPL) technique.¹⁸ It is well established that the efficiency of radiative recombination is directly proportional to the decay time of particular transition.¹⁸ TRPL was recorded using a time-correlated single photon counting technique, with a microsecond xenon flash lamp as the source of excitation. Fig. 3c shows the luminescence decay profile of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor at room temperature.

The PL decay was recorded for the Eu^{3+} transition with 608 nm emission at 465 nm excitation wavelength recorded at room temperature. The observed lifetime data of $^5\text{D}_0 - ^7\text{F}_2$ (608 nm) transitions of Eu^{3+} in $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor were very well fitted to a double-exponential function as described in Equation (1).^{2,4,18}

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

Where τ_1 , τ_2 are the decay lifetimes of the luminescence and A_1 , A_2 are the weighting parameters. The parameters generated from the fitting are listed in Fig. 3d. The observed lifetimes are $\tau_1 = 177.4714 \mu\text{s}$ and $\tau_2 = 525.9811 \mu\text{s}$. $A_1 = 0.0825$, $A_2 = 0.9175$ $\chi^2 = 1.313$ $A = 20.633$. For double-exponential decay, the average lifetime, τ_{av} , is determined by Equation (2).^{2,4,18}

$$\tau_{av} = (A_1 \tau_1^2 + A_2 \tau_2^2) / (A_1 \tau_1 + A_2 \tau_2) \quad (2)$$

The average lifetime for this nanophosphor is estimated to be $\tau_{av} = 515.719 \mu\text{s}$, which suggests that the synthesized nanophosphor could be suitable for clinical diagnostics, biomarker and bioimaging applications. Moreover, it can also be highly useful for many optical display and sensing applications.^{2,4,18} Furthermore, we also examined the PL intensity distribution in $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor using PL mapping instrument, where 375 nm diode laser as a source of excitation. Fig. 5a exhibits the optical micrographs of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor layer drop casted using ethanol as medium, on glass slide. The nanophosphor agglomerated during layer formation on glass slide due to ultrafine size. Fig. 5b shows the PL intensity distribution on surface of layer drop casted on glass slide. The PL mapping result reveals that the PL intensity distribution throughout the surface is almost equal, which is highly desired for proposed biological application. Fig. 5c represents the PL emission spectrum of marked region in inset (red color cross circle) at excitation wavelength 375nm. To further ensure the PL intensity distribution on surface of nanophosphor, we have taken the intensity distribution across the surface from point A to B in Fig. 5d using instrument software facility of WITec. The inset of Fig. 5d clearly demonstrates that the distribution is almost equal from left to right (point A to point B).

Magnetic property of the $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor was probed by Quantum Design SQUID (superconducting quantum interference device) magnetometer (magnetic property measurement system, MPMS). The $M(H)$ curve (Fig. 6) for $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor at 300K clearly depicts a typical paramagnetic behaviour, which is attributed to inner 4f electrons in Gd^{3+} ion. It is interesting to note that the observed magnetic moment is quite high as compared to previously reported in literature for other rare-earth based paramagnetic –luminescent nanophosphors.^{2,4,10,15,16,23} The magnetic moments associated with the seven unpaired electrons in 4f shell are all non-interacting because of their screening by the outer $5s^2 5p^6$ shell electrons.^{4,15} The observed paramagnetic behaviour is

essential feature for the external magnetic tracking of the nanophosphor in the biomedical applications such as targeted drug delivery.^{2,4,15} The room-temperature $M(H)$ curve suggests that the considerable magnetic moment at high field (above 1 Tesla, T) makes it suitable for MRI due to the paramagnetic characteristics (Fig. 6). The inset of the Fig. 6 shows the α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor in glass vial with permanent magnet having strength 0.3T. Thus, the high biocompatibility of the present nanophosphor even at higher loading makes it suitable for efficient bimodal luminomagnetic nanoprobe for fluorescent imaging with external magnetic field tracking capability under permanent magnet.

The compatibility of the synthesized NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor for biological applications is investigated by the cytotoxicity analysis using MTT assay²⁴ in HeLa cells. HeLa is a cervical cancer cell line, which is one of the oldest and most commonly used cell lines in scientific research (PMID: 9892199) Fig.7 shows the effect of varying nanophosphor concentration on cell viability at different incubation periods. No considerable toxicity was observed after the incubation of NaGdF₄:Eu³⁺ nanophosphor for upto 48h. Cells incubated in growth medium without any NaGdF₄:Eu³⁺ nanophosphor was used as controls (labelled as concentration zero in Fig. 7). Cell viability after treatment with different concentrations of a well-known anticancer drug, doxorubicin was used as a positive control for MTT assay.

To investigate the feasibility of NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor for cellular imaging applications, we incubated the nanophosphor with HeLa cells. Cellular nucleus was counterstained blue by DAPI for its tracing. Fluorescence microscopic analysis suggests that NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanoparticles accumulate in the cellular cytoplasm without considerable accumulation in the nucleus region as shown in Fig.8 as well as in Fig. S6 (†ESI) and S7(†ESI). The overlap of fluorescence and phase contrast images clearly shows the specific cellular localization of NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor. The localized PL emission spectrum acquired from the cells showed the characteristic PL of Eu³⁺ centred at 608 nm (Fig. 8 f). Lack of auto-fluorescence confirms the potential capability of NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor for high-contrast PL imaging of cells *in vitro*. Altogether, the observed high luminescent and paramagnetic properties combined with high-contrast imaging capability strongly suggest that this ultrafine, biocompatible NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor could be a better bimodal nanoprobe for bioimaging applications with external magnetic tracking capabilities.

4 Conclusions

A novel strategy for the synthesis of Eu³⁺ doped α -sodium gadolinium fluoride (α -NaGd_{0.88}F₄:Eu_{0.12}³⁺) based lumino- magnetic nanophosphors having bifunctional properties is demonstrated using facile hydrothermal route. The synthesized nanophosphor exhibits luminescent and enhanced paramagnetic properties in a single host lattice, which is highly desirable for proposed bimodal applications. This highly luminescent ultrafine nanophosphor with an average particle size $\sim 5 \pm 3$ nm facilitates high-contrast fluorescent imaging capability with reduced light scattering as well as the estimated higher quantum efficiency (>83%) address the auto-fluorescence problem". *In vitro* cellular uptake in HeLa cell is shown in fluorescent microscopy that envisions the characteristic hypersensitive red emission of Eu³⁺ doped α -sodium gadolinium fluoride centered at 608 nm (⁵D₀-⁷F₂) upon

465 nm excitation wavelength. Thus, this proposed strategy for the facile synthesis of luminomagnetic α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor provides a highly-efficient bimodal nanoprobe for high-contrast *in vitro* bioimaging applications with external magnetic field tracking capability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. XRD pattern of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ (top, red) and the corresponding JCPDS card no. 27-0697 database standards for bulk $\alpha\text{-NaGdF}_4$ (bottom, blue). The right inset exhibits typical photographs of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor in circular brass holder under room light as well as 254 nm UV lamp (strong red emission of Eu^{3+} appears).

Fig. 2.

(a) SEM image of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor, (b) magnified version of the Fig. 2a, (c) Qualitative EDAX analysis (histogram) of selected spot area (marked red in Fig. 2b) on individual nanophosphor, representing the presence of F, Na, Eu and Gd elements, (d) TEM image of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor, (e) magnified version of the Fig. 2d, (f) HRTEM micrograph exhibits the fine lattice fringes of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor without lattice distortion, inset of Fig. 2f, shows the electron diffraction pattern of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor.

Fig. 3.

(a) Room temperature PLE spectrum of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor at fixed emission wavelength of 608 nm, (b) PL emission spectrum of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor recorded at 465 nm excitation showing a sharp, intense, hypersensitive red emission peak with maximum at 608 nm (⁵D₀ – ⁷F₂) at room temperature. The inset shows the color coordinates of red emission: x = 0.6478, y = 0.3514 (c) TRPL decay profile of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor recorded at room temperature while monitoring the emission at 608 nm at an excitation wavelength of 465 nm. d) Lifetime data and the parameter generated by the exponential fitting of TRPL decay profile of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor.

Fig. 4.

Energy level diagram with all possible transitions of Eu^{3+} ions in NaGdF_4 lattice.

Fig. 5.

(a) Optical micrographs of $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor drop casted layer using ethanol as medium on glass slide. (b) PL intensity distribution on surface of drop casted layer formed by $\alpha\text{-NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor powder, (c) PL emission spectrum of marked region in inset (red color cross circle) at excitation wavelength upon 375nm, (d) PL intensity distribution across the surface from point A to B.

Fig. 6. Room temperature M (H) curve of α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor. Photograph of luminomagnetic α -NaGd_{0.88}F₄:Eu_{0.12}³⁺ nanophosphor in glass vial with an external permanent magnet (0.3 T) as shown in inset.

Fig. 7. Cell viability assay with HeLa Cells, incubated with different concentrations of α - $\text{NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor.

Fig. 8.

In vitro fluorescent microscopy images of HeLa cells, cells incubated with α - $\text{NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$. Sequential images show; (a) Phase contrast picture of HeLa cells, (b) Individual nucleus stained blue with DAPI, (c) Red fluorescence staining by α - $\text{NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor, (d) Overlapped images from blue DAPI and red α - $\text{NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor images, (e) Overlap of phase contrast, blue and red from (a), (b) and (c) images respectively and (f) *In vitro* localized PL image of α - $\text{NaGd}_{0.88}\text{F}_4\text{:Eu}_{0.12}^{3+}$ nanophosphor from (e). Inset localized PL taken from level HeLa cells (red).