

A facile hydrothermal synthesis of highly luminescent $\text{NaYF}_4:\text{Yb}^{3+}/\text{Er}^{3+}$ upconversion nanoparticles and their biomonitoring capability

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ABSTRACT

Using a facile hydrothermal procedure, hydrophilic $\text{NaYF}_4:\text{Yb}^{3+}/\text{Er}^{3+}$ nanoparticles (NPs) have been prepared as lanthanide-doped upconversion (UC) materials exhibiting different morphologies, crystal phases and luminescence intensity. The upconversion nanoparticles (UCNP) were characterized by means of electron microscopy and spectroscopy, X-ray diffraction (XRD) and photoluminescence analysis. The molar concentration of reactants and volumes of NaF affect the shapes and uniformity of the synthesized NPs. These parameters also have influence on crystal phase and luminescence intensity of the NPs. Adjusting hydrothermal reaction time and dopant concentration also enable the synthesis of NPs with strong UC luminescence. The as-prepared UCNP showed cellular nontoxicity to HeLa cells, and thus they are capable as promising agents for biological imaging.

1. Introduction

Upconversion (UC) utilizes non-linear process that converts low-energy excitation photons (e.g., near-infrared, NIR) into higher-energy photons (e.g. ultraviolet and visible) [1]. Trivalent lanthanide ion (Ln^{3+})-doped upconversion nanoparticles (UCNP) draw increasing attention as emerging imaging agents because of their excellent application in diagnostics, therapeutics, detection and evaluation of hazardous materials, and biomolecules [2–5]. UCNP confer their several unique properties that make them appropriate for use in multiple fields such as low autofluorescence background, deeper tissue-penetration and negligible photodamage, high resistance to photoblinking and photobleaching [6]. Among the reported host materials of Ln^{3+} -compounds, NaYF_4 crystals exhibit the highest UC luminescence efficiency due to the minimization of multiphonon-assisted relaxation in the emitting states [7,8]. Among the investigated Ln^{3+} -dopants, $\text{Yb}^{3+}/\text{Er}^{3+}$, $\text{Yb}^{3+}/\text{Tm}^{3+}$, and $\text{Yb}^{3+}/\text{Ho}^{3+}$ known to have the maximum UC luminescence efficacy up to date when excited at 980 nm. The excitation energy of the Yb^{3+} excited state ($^2\text{F}_{5/2}$) is very close to that of the neighbouring ion Er^{3+} excited state ($^4\text{I}_{11/2}$), allowing the efficient energy transfer [1,8].

In the recent years, Ostwald-ripening, thermolysis, and hydrothermal strategies are the general approaches for the synthesis of Ln^{3+} -doped UCNP [9,10]. However, the largest challenge in synthesis of

UCNP is the variation in the UC luminescence efficiency of the resulting particles. The UC luminescence efficiency of the as-prepared UCNP can fluctuate from batch to batch. To obtain the efficient UCNP, several reports have been published to get the rational conditions for synthesis of particles with high crystalline phase exhibiting high UC intensity by tuning the concentration of dopants and reactants, dopant ions, fluoride sources and reaction time and temperature [11–17]. These are critical parameters to precisely control the size and shape of nanoparticles (NPs), which affect luminescence properties of the NPs. The fine selection and tuning of experimental conditions would have a limit due to the undesired relaxation processes and absorb the enough excitation light, enabling enhancement of the UC intensity [18,19]. Therefore, the development of a facile procedure for controllable UCNP synthesis is of great interest and challenge to improve UC emission intensity for their application in biomedical related field.

Here, we synthesized UCNP using a facile hydrothermal treatment where NaYF_4 selected as a host material, and $\text{Yb}^{3+}/\text{Er}^{3+}$ as dopants. This treatment lead to the formation of hydrophilic UCNP at relatively low temperatures as compared to previously described methods which required an extensive experimental setup [1]. We examined the effect of concentration of sodium fluoride as fluoride source and reactants (Ln^{3+} -nitrates) on size and shape of UCNP. As well as the concentrations of dopants and reaction time were also adjusted to achieve tunable size and to improve UCNP hexagonal phase with enhanced visible

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luminescence intensity under 980-nm diode laser excitation. The as-prepared UCNP were further utilized for cytotoxicity studies and cell imaging analysis. This strategy is environmental benign, convenient and very useful to produce the water-soluble NPs at low temperature without yielding toxic byproducts.

2. Experimental

2.1. Reagents

All lanthanide nitrate hydrates $\text{Ln}(\text{NO}_3)_3 \cdot y\text{H}_2\text{O}$ (Ln : Y^{3+} , Yb^{3+} , Er^{3+} , purity = 99.9%) and sodium fluoride (NaF) were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium citrate was obtained from APS Biotech (Seoul, Korea). Ethyl alcohol was purchased from Emsure (Billerica, MA, USA) and nitric acid (60.0%) was provided by Samchun (Seoul, Korea). Cetyltrimethylammonium bromide (CTAB) was supplied by Daejung (Seoul, Korea). The obtained reagents were of analytical grade and deionized (DI) water (Direct-Q® Water Purification System, Millipore, Billerica, MA, USA) used for solution preparation.

2.2. General method for the synthesis of UCNP

The UCNP, $\text{NaYF}_4: \text{Yb}^{3+}/\text{Er}^{3+}$, were synthesized by a facile hydrothermal strategy with modifications [20], using $\text{Ln}(\text{NO}_3)_3 \cdot y\text{H}_2\text{O}$, and NaF as precursors, CTAB for its morphology control and chelating ability, sodium citrate as a complexing agent, and DI water, and ethanol as solvents. In the typical procedure, NaYF_4 particles doped with x% Yb^{3+} and x% Er^{3+} were synthesized by mixing xM $\text{Ln}(\text{NO}_3)_3 \cdot y\text{H}_2\text{O}$ (Ln : Y^{3+} , Yb^{3+} , Er^{3+}) with 0.1 M sodium citrate under vigorous stirring for 30 min at room temperature to form white citrate complex. Next, DI water, ethanol and 150 mg CTAB were added to the solution with magnetic stirring. Shortly thereafter, xM NaF was added dropwise and the resultant solution was maintained with strong stirring for 2 h at room temperature to form crystal nuclei. Then, 1.5 mL of nitric acid was added in the solution. The mixture solution was transferred to the 23 mL Teflon-lined autoclave and heated at 190 °C under different reaction times. The resulting nanoparticles were collected by centrifugation at $3172 \times g$ for 20 min, washed with ethanol and DI water (1:1, v/v) and dried at 60 °C in hot air oven.

In the realization of uniform and efficient synthesis of NPs, first we did a series of control experiments by using different molar proportions of precursors as ascribed in Table S1. Based on the findings from control experiments, we next synthesized the UCNP with different dopant concentrations and under different reaction times to obtain hexagonal structure, highly luminescent and uniform NPs (Table S2).

2.3. MTT assay and HeLa cell fluorescence imaging

HeLa cells were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics at 37 °C under a humidified atmosphere of 95% air and 5.0% CO_2 . For cytotoxicity study, HeLa cells were seeded with 4×10^3 cells per well onto a 96-well plate and incubated for 24 h. After that, cells culture media was replaced with different concentration of optimized UCNP ($\text{NaYF}_4: 20\% \text{Yb}^{3+}/3\% \text{Er}^{3+}$) ranging from 1 to 1000 $\mu\text{g}/\text{mL}$. The experiment was conducted in triplicate. After incubation for another 24 h at 37 °C under a humidified atmosphere of 5.0% CO_2 , 150 μL MTT reagent (1 mg/mL) was added in each well and cells were incubated for 4 h at similar conditions described above. The color development was recorded on a UV/Vis multi-mode microplate reader (BioTek Synergy H1, Winooski, VT, USA) at 540-nm detection wavelength.

For cell imaging, HeLa cells were cultured in a cover glass-bottom dish (confocal dish; SPL, Pocheon, Korea) and incubated in a humidified atmosphere of 5.0% CO_2 at 37 °C for 24 h. Afterwards, cells were washed with phosphate-buffered saline (PBS, pH 7.4) and then 3 mL of

DMEM containing 100 μg of UCNP were added to the cell culture plates and incubated for 4 h at 37 °C in 5.0% CO_2 atmosphere. After incubation, cells were washed three times with PBS to remove unbound cells and particles. Fluorescence imaging of cells was performed using microscope, JuLI Stage (NanoEntek, Seoul, Korea) under 980-nm laser excitation.

2.4. Materials characterization

The size and surface morphology of the synthesized NPs were measured by scanning electron microscope (SEM) on SIGMA instrument (Carl Zeiss, Cambridge, UK). Crystal phase information of the NPs was obtained by X-ray diffraction (XRD) analysis with Cu K α radiation source ($\lambda = 1.54056 \text{ \AA}$). The crystal structure was examined with a $0.023^\circ \text{ min}^{-1}$ scanning rate in the range of $10^\circ \leq 2\theta \leq 70^\circ$. The UC luminescence spectra were obtained with 980 nm continuous wave (CW) laser diode using Shamrock Spectrograph (Shamrock 303i, Andor Technology, Belfast, Ireland). High-angle annular dark field scanning transmission electron microscopy (HAADF STEM) and energy dispersive X-ray spectroscopy (EDS) were performed by JEM-ARM2100F (JEOL, Tokyo, Japan) at an accelerating voltage of 200 kV. Unless otherwise noted, all material characterizations were performed at room temperature.

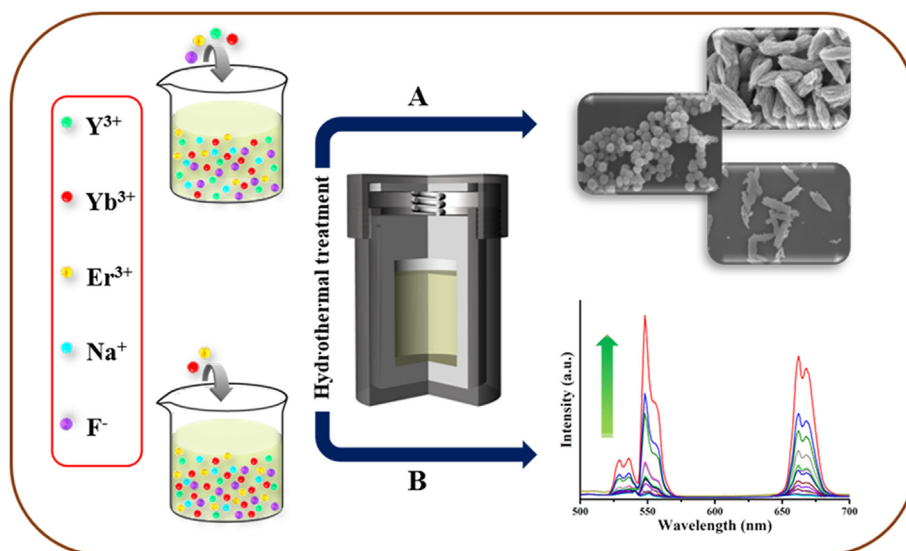
3. Results and discussion

The size, shape, crystalline phase, and luminescence intensity of UCNP were studied by optimizing the external factors, including concentrations of reactants, NaF, and dopants and reaction time as an internal factor as shown in Scheme 1.

3.1. Effect of reactants and NaF concentration

The synthetic conditions of UCNP were first optimized by changing the reactants (0.1, 0.2 M) and NaF (1.0, 0.5, 0.25 M) concentrations and by tuning the volume of NaF from 7.5 to 9.0 mL (Table S1). The molar concentration of reactants [$\text{Ln}(\text{NO}_3)_3$] represents the total quantity of Y^{3+} and doped lanthanide ions such as Yb^{3+} and Er^{3+} . SEM images show that these experimental parameters play an important role on the shape and size of UCNP (Figs. 1 and S1).

Upon same reactants concentration (0.1 M), the UCNP synthesized under different NaF contents exhibit different shapes as shown in Fig. 1A–D. The spherical-shaped NPs were obtained at 1.0 M NaF concentration (Fig. 1A and B). Although the size of the NPs was increased to 69.83–76.70 nm by changing the volume of NaF from 7.5 to 9.0 mL, the shape of the NPs was not affected. However, the aggregated-irregular-shaped NPs were formed when the concentration of NaF decreased to 0.5 M (Fig. 1C). When the volume of NaF increased to 9.0 mL, the regular spheres were synthesized with size 67.93 nm as shown in Fig. 1D [21]. This means that NaF content is not sufficient to make fine shaped particles [22,23]. Further experiments were done to investigate the effect of high concentration (0.2 M) of reactants on the size and shape of NPs. Under keeping the concentration of reactants at 0.2 M and with 1.0 M NaF concentration (7.5 mL), the regular-shaped, small UCNP were uniformly synthesized with the average size of 52.77 nm (Fig. 1E). These indicate that under high concentration of reactants, the small and homogenous spheres in nanoscale were obtained. Other NPs obtained under identical conditions, except for increasing the volume of NaF to 9.0 mL, were relatively inhomogeneous. In particular, the large UCNP were formed with size 87.97 nm (Fig. 1F). This suggests that the volume of NaF is also the key factor in controlling the size and homogeneity of NPs. The fine bitter gourd shaped NPs were obtained with the length of 606.67 nm and the width of 176.13 nm (Fig. 1G) at 7.5 mL of 0.25 M NaF (aspect ratios of 3.47; Table S1). However, a thin layer was appeared on the bitter gourd shaped NPs due to the excessive amount of NaF (9.0 mL) as shown in Fig. 1H. The as-



Scheme 1. Schematic illustration for synthesis of high luminescent and homogenous $NaYF_4:Yb^{3+}/Er^{3+}$, UCNP using different (a) reactants and NaF concentrations and, (b) dopants concentration and reaction times.

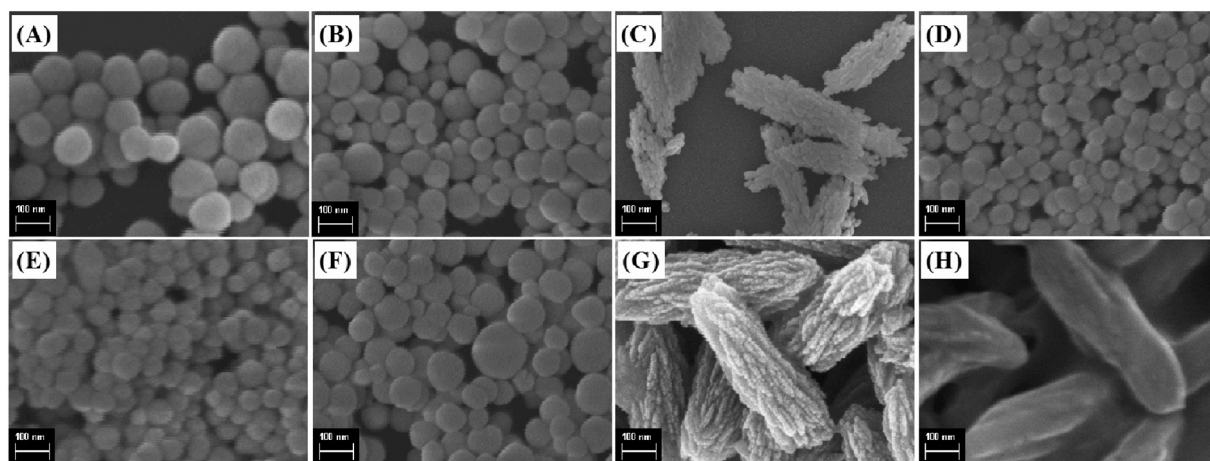


Fig. 1. (A–H) SEM images of the as-prepared particles to investigate the influence of the molar proportion of reactants and NaF with different volume as well on the particle size, shape and homogeneity. All particles are synthesized according to the conditions described in Table S1. Scale bar represents 100 nm.

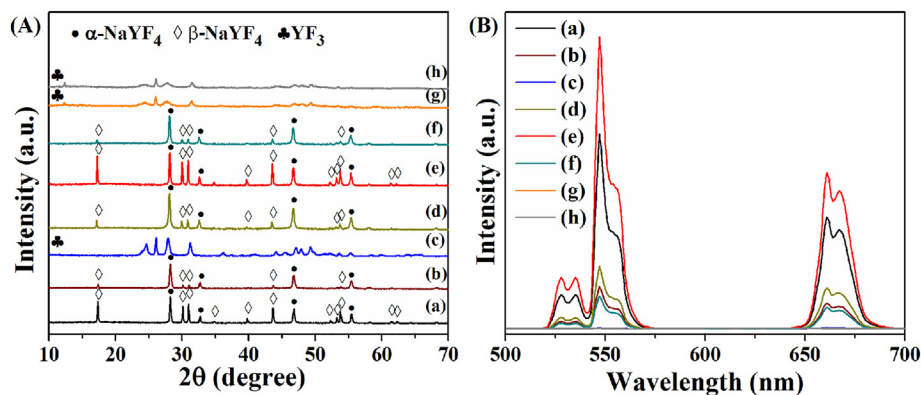


Fig. 2. (A) XRD patterns of the synthesized particles. Spherical shape particles (line; A, B, D–F) corresponding to both α - $NaYF_4$, PDF 00-027-0688 and β - $NaYF_4$, PDF 00-016-0334. Irregular (line; C) and bitter gourd shaped (line; G, H) particles corresponding to YF_3 , PDF 01-070-1935.

prepared NPs have different length and width ranged from 460 to 720 nm and 140–220 nm, respectively, with 3.35 aspect ratio (Fig. 1H and Table S1).

The possible mechanism can be suggested based on XRD patterns

(Fig. 2A). Under different contents of NaF, which is a main source of fluoride, and 0.1 M of reactants, as-prepared NPs exhibit different phases such as α - $NaYF_4$ (PDF 00-027-0688), β - $NaYF_4$ (PDF 00-016-0334) and YF_3 (PDF 01-070-1935) as shown in Fig. 2A (line; a–d). In

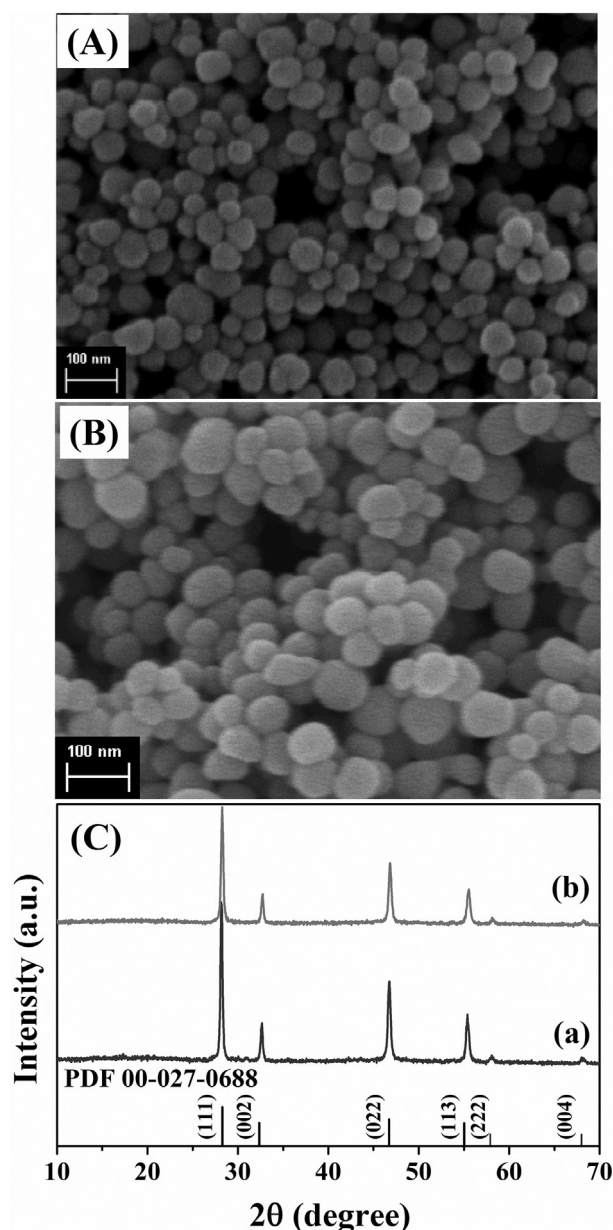


Fig. 3. SEM images (A and B) and the respective XRD spectra (C) of UCNP synthesized for 2-h hydrothermal reaction time, (A) 20% Yb^{3+} /2% Er^{3+} and (B) 30% Yb^{3+} /2% Er^{3+} . Scale bars show 100 nm. (C) XRD pattern corresponding to cubic phase of NaYF_4 , PDF 00-027-0688.

this case, the shape of NPs was also varied. The formation of irregularly-shaped NPs (Fig. 2A, line c) clearly proved by the phase transition from α - and β - NaYF_4 to YF_3 . As shown in Fig. 2, the XRD peaks of samples a, b, d, e, and f (Fig. 2A) assigned to mixed α - NaYF_4 and β - NaYF_4 , indicating less purity and crystallinity of the product. The presence of different phases and phase transformation might generate structural defects in the as-prepared UCNP and relatively less UC luminescence intensity [24], which can be overcome by further optimizing the reaction conditions [17]. However, the diffraction peak for the sample e (Fig. 2A) has a significant β - NaYF_4 intensity, indicating relatively high crystallinity and good UC luminescence intensity (Fig. 2B). Based on these results, it can be suggested that phase transition plays an important role in understanding the shape evolution and size of NPs [13,25,26]. As the unit cell structure of various shapes has different characteristics and crystallographic phases [27–29]. We also observed the shape and phase transition of as-prepared particles by keeping the

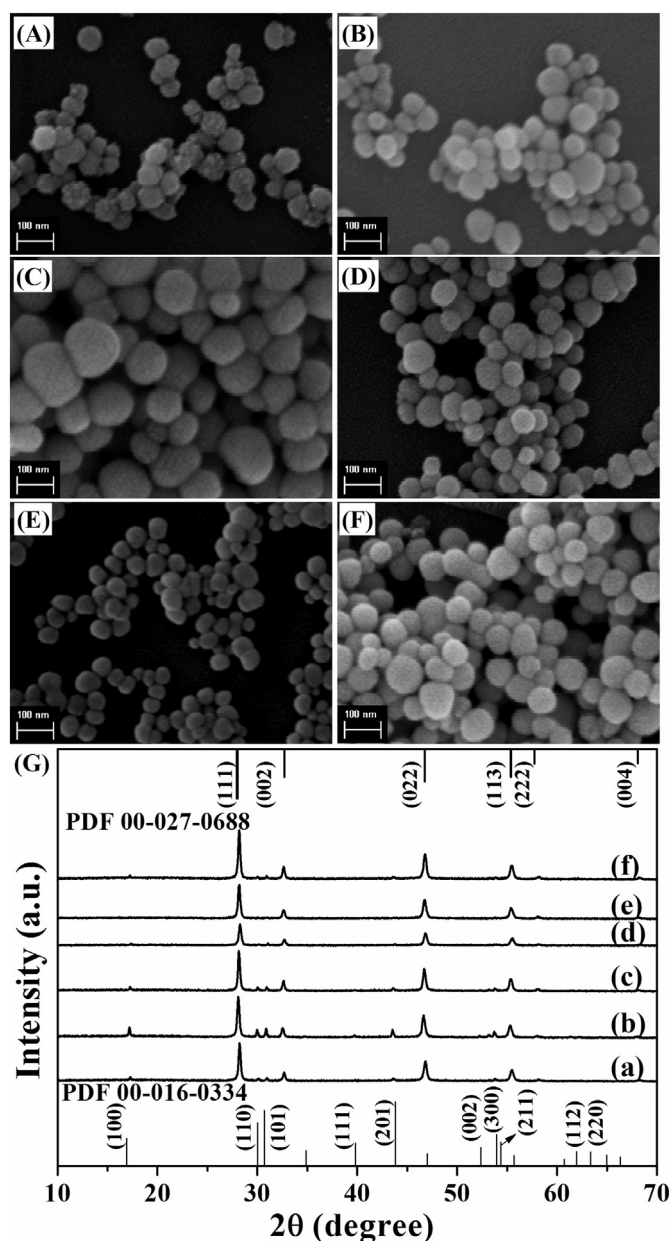


Fig. 4. SEM images (A–F) and the respective XRD spectra (G) of UCNP synthesized for 3-h hydrothermal reaction time, (A) 20% Yb^{3+} /2% Er^{3+} , (B) 20% Yb^{3+} /3% Er^{3+} , (C) 20% Yb^{3+} /4% Er^{3+} , (D) 30% Yb^{3+} /2% Er^{3+} , (E) 30% Yb^{3+} /3% Er^{3+} , (F) 30% Yb^{3+} /4% Er^{3+} . Scale bars show 100 nm. (G) XRD pattern corresponding to both α - NaYF_4 , PDF 00-027-0688 and β - NaYF_4 , PDF 00-016-0334.

same reactants concentration at 0.2 M and changing the NaF content Fig. 2A (line; g and h) and Fig. 1G and H. The NPs with different phases were synthesized under different NaF contents, indicating the contribution of fluoride source to the formation of various shapes.

To investigate the UC property of as-prepared NPs, the UC luminescence spectra of NPs were measured under 980-nm laser diode excitation (Fig. 2B). The UC luminescence was higher for the sample (Fig. 2B, line e), and it can be verified by XRD pattern (Fig. 2A, line e). The NPs with strong XRD peaks of hexagonal phase exhibited higher UC luminescence intensity [18,30]. The α - NaYF_4 contained one kind of high-symmetry cation sites irrespective of β - NaYF_4 with two kinds of relatively less symmetry cation sites which resulted in good electron cloud distortion of the cations to manage the structural change. This phenomenon favoured the formation of hexagonal phase with enhanced

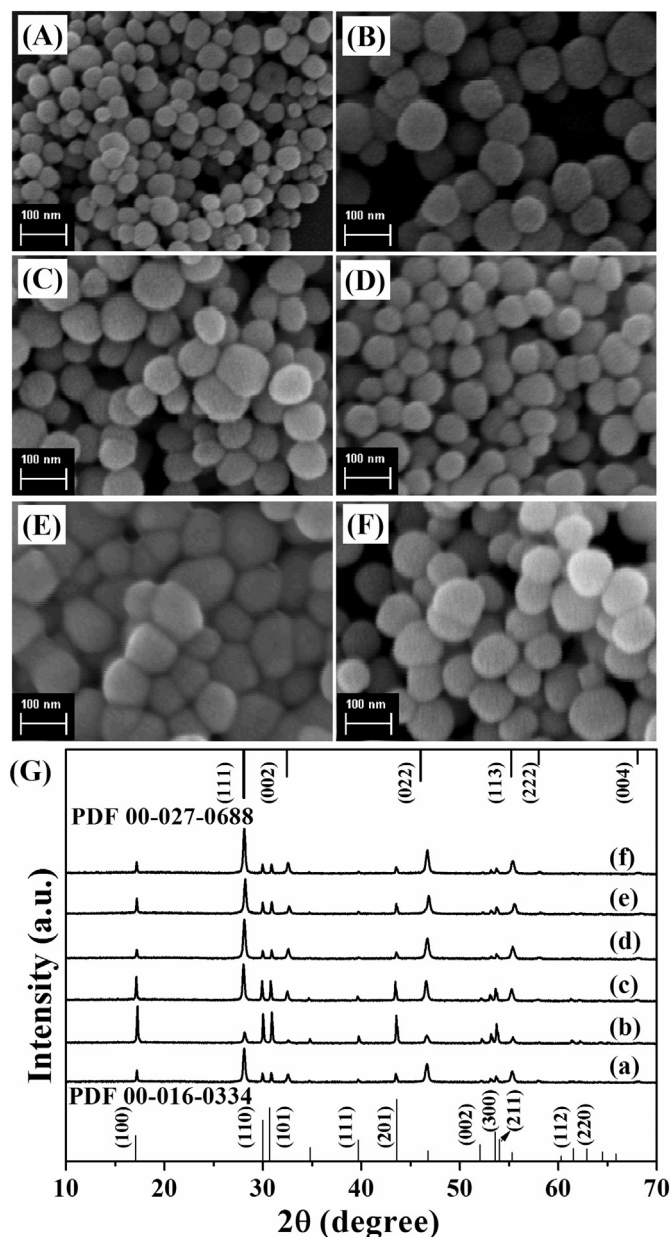


Fig. 5. SEM images (A–F) and the respective XRD spectra (G) of UCNP synthesized for 5-h hydrothermal reaction time, (A) 20% Yb^{3+} /2% Er^{3+} , (B) 20% Yb^{3+} /3% Er^{3+} , (C) 20% Yb^{3+} /4% Er^{3+} , (D) 30% Yb^{3+} /2% Er^{3+} , (E) 30% Yb^{3+} /3% Er^{3+} , (F) 30% Yb^{3+} /4% Er^{3+} . Scale bars show 100 nm. (G) XRD pattern corresponding to both $\alpha\text{-NaYF}_4$, PDF 00-027-0688 and $\beta\text{-NaYF}_4$, PDF 00-016-0334.

UC luminescence intensity [14,31]. These results indicate that by controlling the concentration of reactants and NaF content, we can obtain homogeneous hexagonal phase UCNP with strong luminescence intensity.

3.2. Effect of dopant ions and reaction time on size and crystal phase of UCNP

Based on the previously described experiments, reactants concentration and NaF concentration and volume were selected and remained constant for further experiments. We investigated the effect of hydrothermal reaction time and concentration of dopant ions such as Yb^{3+} and Er^{3+} on the size, homogeneity and crystalline phase of the UCNP as mentioned in Table S2.

Fig. 3 describes the SEM images and XRD pattern of UCNP when synthesized at 2-h hydrothermal reaction time, suggesting the formation of spherical shape particles with $\alpha\text{-NaYF}_4$, PDF 00-027-0688 phase. The respective particle size distribution spectra are shown in Fig. S2. At 3 h reaction time, the UCNP were spherical in shape and became larger in size with increasing Er^{3+} concentration as shown in Fig. 4A–F (Fig. S3). At this temperature, in addition to the $\alpha\text{-NaYF}_4$, $\beta\text{-NaYF}_4$ was appeared, suggesting the phase transition of NPs from cubic to both cubic and hexagonal phases (Fig. 4G). Among the NPs synthesized at 3 h, the NPs with 20% Yb^{3+} /3% Er^{3+} have higher intensity of hexagonal peak (Fig. 3G, line b). As shown in Fig. 5, particle size was further increased by increasing the Er^{3+} concentration and reaction time to 5 h (Fig. S4), but these factors did not alter the shape of particles. Corresponding XRD patterns shown that intensity of the hexagonal phase was increased as compared to the particles prepared for 3 h reaction time and it was higher at 20% Yb^{3+} /3% Er^{3+} as shown in Fig. 5G (line b). The crystal lattice of Ln^{3+} -doped particles can be altered with dopants having different ionic radii which may modify the crystal field and coordination environment of lanthanides [32]. This versatility may result in size and morphology variation of the synthesized UCNP. The ionic sizes of Yb^{3+} (1.125 Å) and Er^{3+} (1.144 Å) are different that may cause size and upconversion luminescence variations [33,34]. NPs synthesized for longer reaction time such as 5 h are larger in size and have good hexagonal phase intensity [15] as compared to particles prepared for 2- and 3-h reaction time.

3.3. Effect of dopant ions and reaction time on luminescence intensity of UCNP

The NIR to visible spectra of different dopant concentrations of Yb^{3+} (20 and 30%) and Er^{3+} (2, 3, 4%) under different reaction times (3 and 5 h) were attained under 980-nm diode laser excitation as shown in Fig. 6. The UC luminescence intensity was not observed at 2 h reaction time (data not shown). However, the luminescence intensity was increased with increasing reaction time and these results found to be consistent with XRD patterns, suggesting that longer reaction time required to obtain hexagonal phase. The maximum emission intensity was found at 20% Yb^{3+} and 3% Er^{3+} dopants concentration at 3-h reaction time (Fig. 6A, line b) and it can be due to the presence of good peak intensity of hexagonal phase (Fig. 4g, line b). Similar results were observed at 5-h reaction time with 20% Yb^{3+} and 3% Er^{3+} dopants concentration (Fig. 6B, line b). Fig. 6 depicts that green and red emissions were obtained at 548 and 662 nm, respectively, which are originated due to the excitation of photon from ground state of Yb^{3+} , $^2\text{F}_{7/2}$ to excited state $^2\text{F}_{5/2}$ and then transfer of photons to neighbouring Er^{3+} ion [31]. Furthermore, Er^{3+} ion excitation gives rise to the green and red emission originated from the transition $^2\text{H}_{11/2}/^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$ and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ bands in the visible region [35,36]. Insets shown the green and red emission area peaks of each spectrum. We suggest that NaYF_4 :20% Yb^{3+} /3% Er^{3+} synthesized at 5-h reaction time have high luminescence intensity (Figs. S5 and S6). Table S3 described the best obtained experimental conditions for the synthesis of UCNP with high luminescent intensity which can be further utilized for bio-application such as cell viability and live cell imaging experiments. Time-resolved UC luminescence of the optimized UCNP was also recorded (Fig. S7). The optimized UCNP were further characterized by HAADF STEM and EDS (Fig. 7). The simulated lattice image in Fig. 7C is in good agreement with XRD pattern shown in Fig. 5. In addition, the elemental mapping of as-prepared UCNP represented the well distribution of Na, F, Y, Yb and Er without any aggregation (Fig. 7D–I).

3.4. In vitro cytotoxicity and HeLa cell imaging study

For bio-application, hydrophilic UCNP, NaYF_4 :20% Yb^{3+} /3% Er^{3+} was incubated with HeLa cells at 37 °C in 5.0% CO_2 atmosphere for cytotoxicity study and *in vitro* cell imaging. The viability of HeLa cells

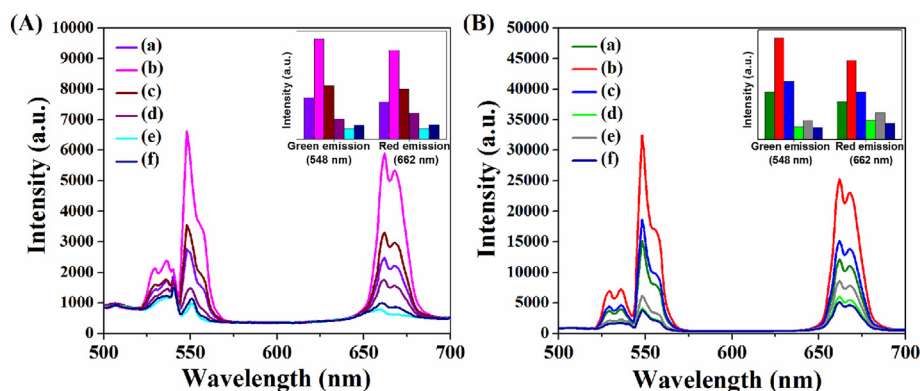


Fig. 6. NIR to visible upconversion luminescence spectra of UCNP with (A) 3-h and (B) 5-h hydrothermal reaction times and different dopant concentrations as represented in Figs. 4 and 5, respectively. Insets show the maximum green and red emission peaks observed at 548 nm and 662 nm of corresponding luminescence spectra under 980-nm laser diode excitation.

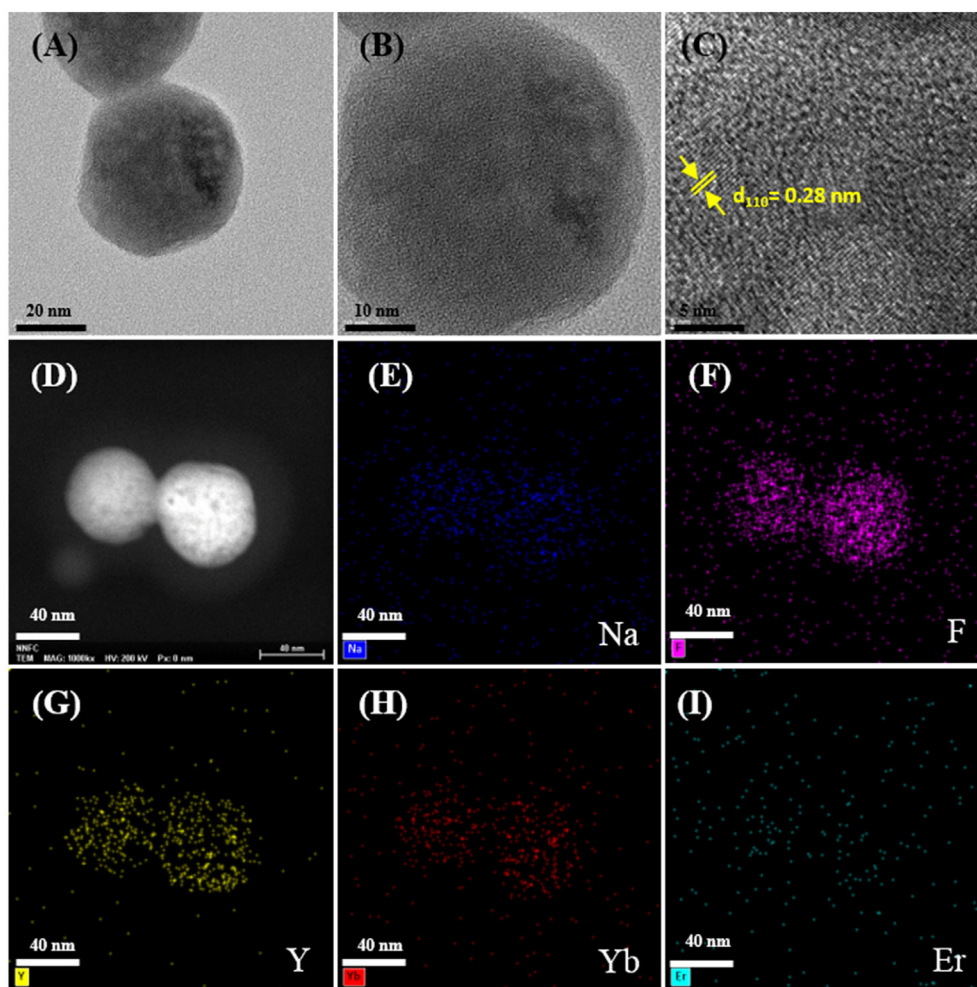


Fig. 7. (A–C) High angle annular dark field-STEM image and, (D–I) EDS analysis of $\text{NaYF}_4\text{:}20\% \text{Yb}^{3+}/3\% \text{Er}^{3+}$, UCNP.

was examined after being exposed to different concentrations of UCNP (0, 1, 3, 5, 10, 50, 100, 300, 500, and 1000 $\mu\text{g/mL}$) by a standard MTT assay (Fig. 8A). The viability of HeLa cells remained $> 80\%$ even after 24-h incubation time and at 1000 $\mu\text{g/mL}$ dosage, suggesting their low toxicity and biocompatibility.

Fig. 8B depicts the bright-field, UC luminescence, DAPI (4',6-diamidino-2-phenylindole) staining and merged image of HeLa cells. The cells nuclei were stained with DAPI, and green UC luminescence under 980-nm laser excitation was observed for the cells when treated with UCNP, indicating that NPs can be visualized and taken up into the cells successfully by endocytosis mechanism [5]. This successful internalization and uptake of UCNP make them promising imaging agents.

4. Conclusion

Herein, a facile method to synthesize hydrophilic UCNP with different sizes, shapes, crystal phases, and UC luminescence intensities was reported. We have studied different experimental conditions in detail *i.e.* reactants and NaF contents, dopant concentrations and hydrothermal reaction times. Our results suggested that high contents of reactants and NaF, appropriate dopant concentration and reaction time are responsible for shape and phase evolution, uniformity and enhanced UC luminescence intensity. Besides these, the as-prepared high luminescent UCNP exhibited negligible cytotoxicity ($> 80\%$) in HeLa cells even at 1000 $\mu\text{g/mL}$ dosage. The optimized UCNPs were also suitable

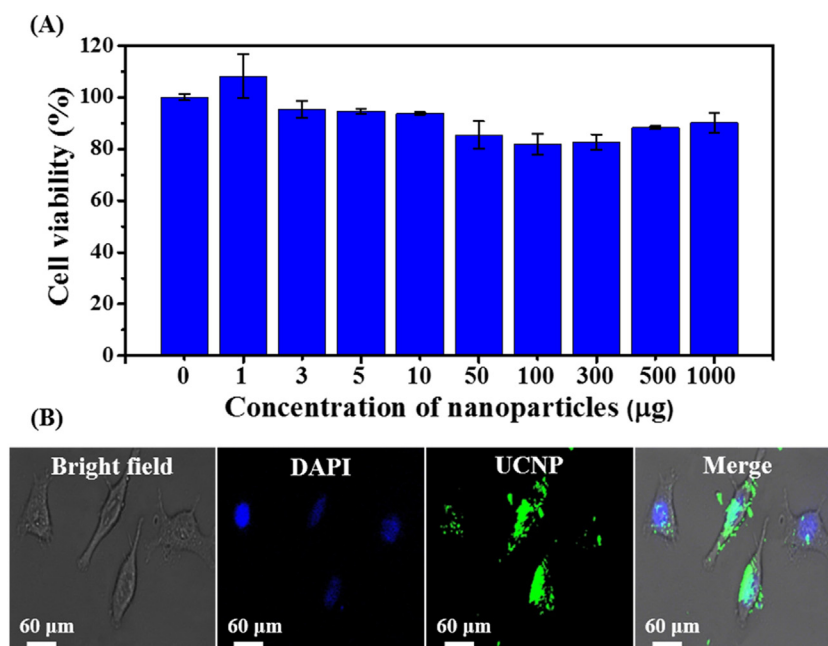


Fig. 8. (A) *In vitro* cytotoxicity of UCNPs with different concentrations in HeLa cells, (B) Fluorescence microscope images of HeLa cells incubated with 100 µg/mL UCNPs for 4 h. For UCN luminescence, the signals were obtained under 980-nm laser excitation source. All scale bars are 60 µm.

for *in vitro* live cell imaging and can be further applied for *in vivo* cell imaging and drug delivery systems.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.msec.2019.02.046>.

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