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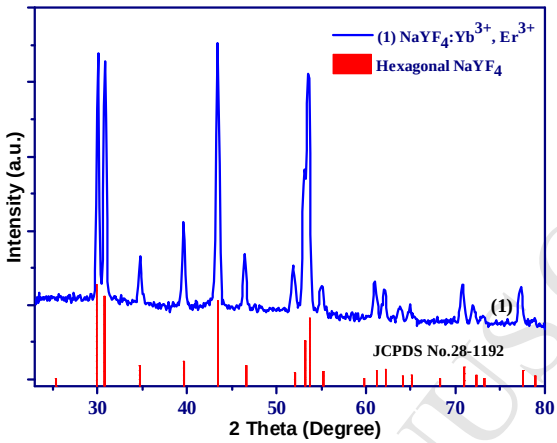
Graphic Abstract



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Synthesis and characterization of $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=folic acid nanocomplex for bioimaginable detecting MCF-7 breast cancer cells

Ha Thi Phuong[1,2,3], Tran Thu Huong[1,2*], Le Thi Vinh[4], Hoang Thi Khuyen[1,2], Do Thi Thao[2,5], Nguyen Thanh Huong[1,2], Pham Thi Lien[1,2], Le Quoc Minh[1,2,6]

[1] *Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.*

[2] *Graduate University of Science and Technology - Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.*

[3] *Department of Chemistry, Hanoi Medical University, 1-Ton That Tung, Dong Da, Hanoi, Vietnam.*

[4] *Department of Chemistry, Hanoi University of Mining and Geology, 18-Pho Vien, Bac Tu Liem, Hanoi, Vietnam.,*

[5] *Institute of Biotechnology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.*

[6] *Duy Tan University, K7/25 Quang Trung, Da Nang, Vietnam.*

Abstract: Upconversion nanophosphors are new promising nanomaterials to be used as biolabels for detection and imaging of cancer cells. These nanophosphors absorb long-wavelength excitation radiation in the infrared or near infrared region and emit shorter wavelength, higher energy radiation from ultraviolet to infrared. In this paper, we studied the hydrothermal method and optical properties of the functionalized $NaYF_4:Yb^{3+},Er^{3+}$ for biomedical application. After synthesis, these $NaYF_4:Yb^{3+},Er^{3+}$ nanophosphors were functionalized with aminosilanes and folic acid. Folic acid binds to the folate receptor on the surface of MCF-7 breast cancer cells and this binding promotes internalization of the nanophosphors via endocytosis. The sizes of the functionalized $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA (folic acid) nanophosphors can be controlled with length of the rod about 300-800 nm and diameter of the rod about 100-200 nm. Phase structure of $NaYF_4:Yb^{3+},Er^{3+}$ is in hexagonal crystal system. The photoluminescence (PL) spectra of the functionalized $NaYF_4:Yb^{3+},Er^{3+}$@silica–N=FA nanophosphors was measured. These nanophosphors emit in red color with the strongest band at 650 nm under 980 nm excitation. This result can provide $NaYF_4:Er^{3+},Yb^{3+}$@silica-N=FA complex for developing fluorescence label and image tool in cancer biology and medicine.

Keywords: Upconversion luminescence, $NaYF_4:Yb^{3+},Er^{3+}$, nanophosphors, bioimaging, cancer cell; rare earths

1. Introduction

Luminescent bio-labels are being largely investigated as a means of cancer cells detection by bio-imaging. Upconversion nanophosphors (UCNPs) are a promising option to be used as bio-labels [1-4]. In bio-medicine, upconversion nanophosphors have been successfully used for cell imaging because of their luminescence intensity, which does not fade.

Rare earth upconversion materials have many advantages such as strongest luminescent intensity, robust stability, no autofluorescence, nonblinking, bio-compatbility, low cytotoxicity in general. In particular, UCNPs based on rare earth doped ions have strong upconversion emission effect in the near infrared region promising a potential application in biomedicine [5-8]. Moreover, UCNPs use excitation near infrared laser at 980 nm that provide a high penetration depth in cells, tissues and animal bodies, therefore it makes them become more suitable for imaging, sensing and theragnostic tools in biomedicine [9-11]. To be considered as bio-labels, the UCNPs should be bio-compatible and functionalized with specific ligands on the surface that allow them to identify certain specific cells. We prepared UCNPs coated with a thin silica shell by Tetraethyl orthosilicate (TEOS);

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Corresponding author: T.T. Huong (E-mail:tthuongims@gmail.com or huongtt@ims.vast.ac.vn; **Tel.:** +48 466 599 000 or 091 2 212 004).

these were then functionalized with amine groups (NH_2), which in turn were used to enable folic acid conjugation. Silica $SiO_{2-x}(OH)_x$ had been attached to the surface of the UCNPs by the formation of a Si–O–Si bond and OH groups. 3-(Aminopropyl) trimethoxysilane (APTMS) was used to bind bio-molecules to the surface of a given nanomaterial by its amine terminal groups. The APTMS was used to promote protein adhesion and cell growth for biological implants. It can be used to join metal nanophosphors to silica because of the strong interaction between the amine group and the metal particles. The amine groups fixed to the UCNPs surface then attaches to folic acid ligands (FA). The FA binds with the folic acid receptor (FR), which is over-expressed on the surface of the MCF-7 breast cancer cells. Once the FA–FR binding occurs, the cell proceeds to internalize the UCNP-NH_2-FA by endocytosis [12-18].

In this paper, we report some results on the controllable synthesis and optical properties of the functionalized $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=folic Acid (FA) nanophosphors for development of fluorescent labelling and imaging tools with aminosilanes, folic acid, and typical MCF-7 breast cancer cells.

2. Experimental

2.1 Synthesis

2.1.1 Synthesis of $NaYF_4:Yb^{3+},Er^{3+}$ nanophosphors

$NaYF_4:Yb^{3+},Er^{3+}$ nanophosphors were prepared by hydrothermal method using $Y(NO_3)_3 \cdot 6H_2O$ (Sigma, 99.9%), $Yb(NO_3)_3 \cdot 5H_2O$ (Sigma, 99.9%), $Er(NO_3)_3 \cdot 5H_2O$ (Sigma, 99.9%), NaF (Sigma, >99%), NaOH (Merk, 99%), as starting chemicals. Firstly, the precursor solutions containing Y^{3+}, Yb^{3+} and Er^{3+} ions were mixed by using a magnetic stirrer with molar ratio of 79.5:20:0.5. Then, a solution of NaOH and polyethylene glycol - PEG (20000) was slowly added and stirred in 30 min (solution A achieved). After that, a solution of NaF was poured into the solution A and also stirred until homogeneous suspension was obtained. The reaction solution was continuously stirred for 120 min by magnetic stirrer. The final reaction solution was put into an autoclave. The autoclave was heated up at temperature around 180–200 ^oC for 10–24 h. After the hydrothermal reaction finished, the autoclave cooled down naturally to room temperature. The achieved products were collected and centrifuged at 5900 r/min. The precipitate was washed several times in water and then dried in air at 70 ^oC for 6 – 10 h.

2.1.2 Silica and aminosilane functionalization

Bio-compatible property of the UCNPs requires a core shell structure to protect the luminescent materials and the cells involved. Besides that, the biolabels need to have specific property to target a specific tumor such as its surface which has to have proper ligands that bind to the cells. Therefore, we covered the UCNPs surface with a thin silica shell by the Stöber method and then added the amine groups with the 3 aminopropyl-trimethoxylsilane, 98%, Sigma Aldrich (APTMS) [19].

In these typical syntheses, 10 mL of TEOS in absolute ethanol, acetic acid and 10 mL of as-synthesized $NaYF_4:Yb^{3+},Er^{3+}$ solutions were mixed by magnetic stirring at room temperature. The achieved products were collected, centrifuged and cleaned several times with ethanol and deionized water. After that, final products were dried at 60 ^oC in 6 – 24 h in air.

Next step, 20 mg of the $NaYF_4:Yb^{3+},Er^{3+}$ with silica shell, 1.2 mL of TEOS in absolute ethanol, 0.6 mL of APTMS, 0.5 mL of acetic acid were put in a 100 mL three-necked flask. Magnetic stirring for 60 min has been done for the mixture at room temperature. After main reaction time, the solution was gently stirred for 3 h. The achieved products were collected by three centrifugation/ dispersion steps in a water/ethanol mixture (1:1). The final products were again washed with deionized water to remove excess reactants and then dried at 60 ^oC for 24 h in air.

2.1.3 Folic acid conjugation

For bio-compatible, the preparation of amino-silane-folic acid-UCNPs isillustrated in Fig. 1. The UCNPs-NH_2 were added with folic acid ligands. The procedure was as follows: 0.5 mg FA was distilled in 10 mL of dimethyl sulfoxide (DMSO) and activated by N-hydroxysuccinimide (NHS) > 98% and N,N'-dicyclohexylcarbodiimide (DCC), 99% (molar ratio of FA/DCC/NHS:1/2/5) at room temperature, then stirred gently in the darkness for 4 h. The UCNPs-NH_2 was dissolved in 10 mL Ethanol and then added dropwise into the activated FA solution. The mixture was stirred gently again for 20 h at room temperature. The achieved products were collected and centrifuged with 5900 r/min.

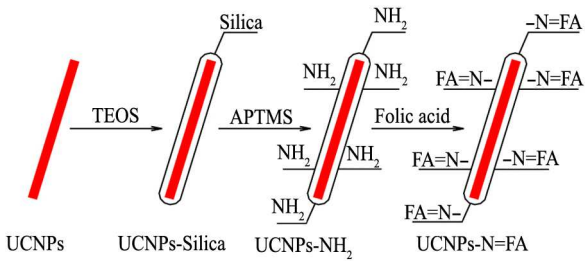

Fig. 1 Schematic illustration outlining the preparation of amino-silane-folic acid-UCNPs.

2.1.4 Cell culture and in vitro experiment

The biological materials include: MCF7 cells (the human breast carcinoma cell line from Dr. J M Pezzuto, University of Long-Island, USA); Fetal bovine serum (FBS), Gentamicin, MEME and polyethylene glycol (PEG-1500) from Sigma Chemical Co. (St. Louis, MO., USA); MEME and L-glutamine from Invitrogen (Carlsbad, CA, USA). The MCF7 cells were cultured in MEME medium supplemented with 10% in activated fetal bovine serum (FBS) and 50 µg/mL gentamicin at 37 ^{o}C and 5% CO_2 in a humidified atmosphere. The cells were seeded at a density of 5×10^4 cells/mL.

In order to study the uptake capacity of the functionalized UCNPs $NaYF_4$:Yb^{3+},Er^{3+}@silica-N=FA, the MCF7 cells at log phase were seeded into 24 well plates at the concentrations of 1×10^4 and 1×10^6 cells/mL then incubated for additional 24 h. Polyethylene glycol (PEG-1500) and $NaYF_4$:Yb^{3+},Er^{3+}@silica-N=FA were then added to the cell-seeded wells for 3 hours. The concentration of $NaYF_4$:Yb^{3+},Er^{3+}@silica-N=FA was 20 µg/mL, in incubating. After the allotted time, the medium and cells were collected into 15 mL falcon tubes. The tubes were subjected to centrifugation at 1000 r/min for 5 min to separate the cells from the cultured medium. The cells were then triply washed with sterile PBS (phosphate buffer saline) pH = 7. After being resuspended in PBS, the functionalized UCNPs $NaYF_4$:Yb^{3+},Er^{3+}@silica-N=FA uptake capacity for all treated cells was estimated. The micro-sized images were viewed using by an inverted microscope (Zeiss).

2.2 Characterization

The morphology observation and crystalline phase identification of the prepared samples were carried out by using field emission scanning electron microscope (FESEM, Hitachi, S-4800), and an X-ray diffractometer (XRD, Siemens D5000 in the range of $10^o \leq 2\theta \leq 80^o$). Infrared absorption spectra were measured by an FTIR spectrometer (IMPACT 410-Nicolet). The upconversion photoluminescence spectra were carried out on an iHR550 photoluminescence measurement system (Jobin-Yvon) under 980 nm laser excitation.

3. Results and discussion

3.1 Morphology

FE-SEM images of the nanophosphors are presented in Fig. 2 with: bare UCNPs of $NaYF_4$:Yb^{3+},Er^{3+} (Fig. 2(a)), covered UCNPs with silica $NaYF_4$:Yb^{3+},Er^{3+}@silica (Fig. 2(b)), attached amine functional group $NaYF_4$:Yb^{3+},Er^{3+}@silica-NH_2 (Fig. 2(c)) and functionalized with bio-conjunction element $NaYF_4$:Yb^{3+},Er^{3+}@silica-N=FA (Fig. 2(d)). The FE-SEM image of the $NaYF_4$:Yb^{3+},Er^{3+} indicates that the nanorods have bundles shape with the lengths of rod about 300–700 nm and diameter of rod about 100–150 nm. When the $NaYF_4$:Yb^{3+},Er^{3+} nanophosphors were covered with silica in Fig. 2(b), and attached with NH_2 group in Fig. 2(c), and functionalized with FA in Fig. 2(d), the size of rods was slightly increased with the rod length of about 300–800 nm and rod diameter of about 100–200 nm (for $NaYF_4$:Yb^{3+}, Er^{3+}@silica-N=FA samples).

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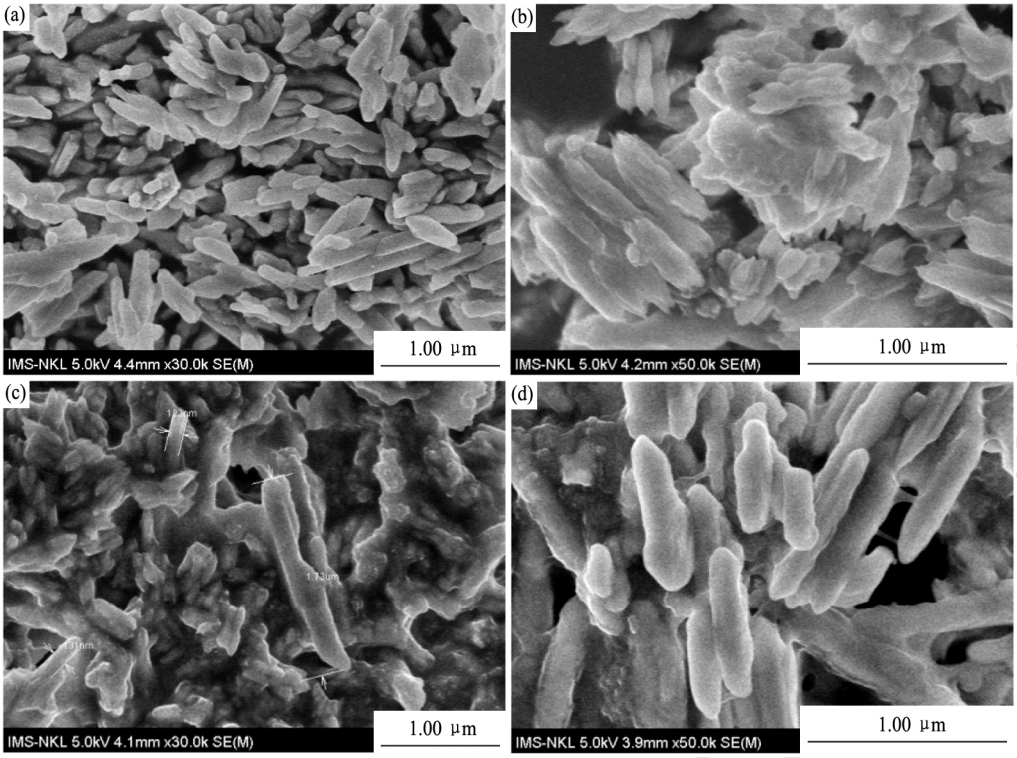

Fig. 2 FESEM images of the nanophosphors of $NaYF_4:Yb^{3+},Er^{3+}$ (a), $NaYF_4:Yb^{3+},Er^{3+}$@silica (b), $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2 (c), $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA (d)

3.2 Crystalline phase identification

The phase structures of $NaYF_4:Yb^{3+},Er^{3+}$ nanophosphors were investigated by X-ray diffraction (XRD) and the results are shown in Fig. 3. In the XRD pattern of the sample, there are diffraction peaks at 2θ: 29.5°, 30.8°, 34.7°, 39.5°, 43.5°, 53.2°, 59.8°, 61.2°, 62.2°, 68.3°, 71° and 78.95° equal to hexagonal phase of $NaYF_4$ (JCPDS card No. 00-028-1192). We found that all measured peaks are belonging to this standard pattern.

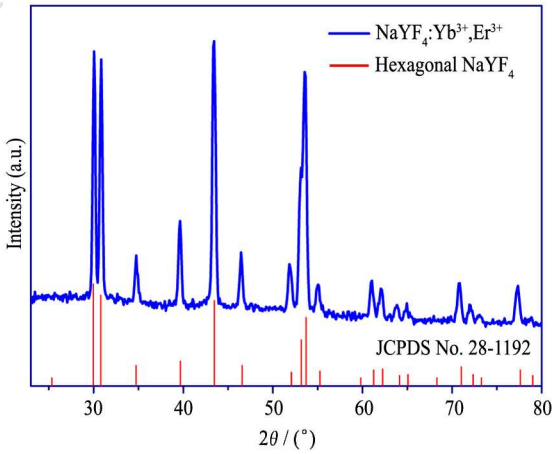

Fig. 3 XRD pattern of the nanophosphors of $NaYF_4:Yb^{3+},Er^{3+}$ at 190 °C, 24 h.

3.3. The FTIR spectra

The FTIR spectra of the nanophosphors of $NaYF_4:Yb^{3+},Er^{3+}$ (line (1)),

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$NaYF_4:Yb^{3+},Er^{3+}$@silica (line (2)), $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2 (line (3)) and $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA (line (4)) are shown in Fig. 4.

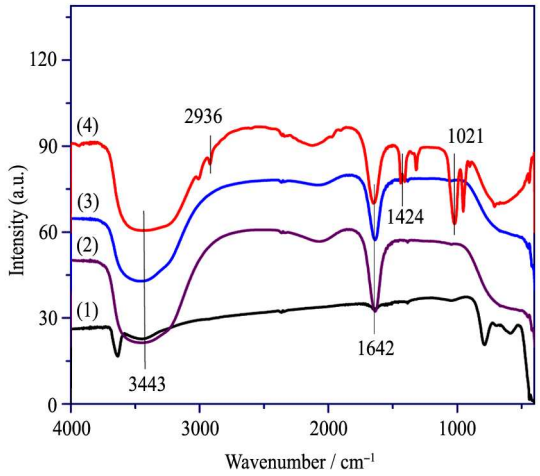

Fig. 4 FTIR spectra of the nanophosphors of $NaYF_4:Yb^{3+},Er^{3+}$ (1), $NaYF_4:Yb^{3+},Er^{3+}$@silica (2), $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2 (3) and $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA (4)

In Fig. 4 (line (3)), it is obvious that the unique absorption peaks from internal vibration of the amino bands (1642 cm^{-1}) and the strong absorption band (3443 cm^{-1}) from symmetric and asymmetric N–H stretching vibration can be observed, which demonstrated the presence of APTMS on the obtained $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2.

The band at about 1021 cm^{-1} is corresponding to C=O stretching vibration coordinating to metal cations. This suggests that the chemical bonds between PEG-20000 and inorganic components are formed. After silica-N=FA coating, we see peaks at 1424 cm^{-1} corresponding to the stretching vibration of C=O amide stretching of the a-carboxyl group from the FA molecules, as shown in Fig. 4 (line (4)). These results further confirm that FA ligands have been successfully grafted onto the $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2.

3.4. UC luminescence property

It is well known that Yb^{3+} was usually chosen as co-dopant with Er^{3+} since it possesses a large absorption cross section at 980 nm and then energy transfer occurs from Yb^{3+} to Er^{3+}. Fig. 5 shows the upconversion luminescence (UCL) spectra of the nanophosphors of $NaYF_4:Yb^{3+},Er^{3+}$, $NaYF_4:Yb^{3+},Er^{3+}$@silica $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2 and $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA under NIR laser excitation at 980 nm. The green (510 – 570 nm) and red (630 – 700 nm) emission regions were assigned to ($^2H_{11/2}$, $^4S_{3/2}$) → $^4I_{15/2}$ and $^4F_{9/2}$ → $^4I_{15/2}$ transitions of the Er^{3+} ions, respectively. When UCNPs modified with FA, the upconversion luminescent intensity of the $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA (line (4)) was changed a little but it is still strong for detection.

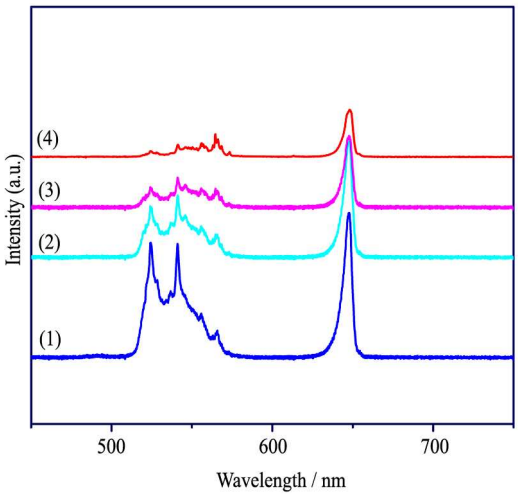

Fig. 5 The upconversion luminescence (UCL) spectra of the nanophosphors of $NaYF_4:Yb^{3+},Er^{3+}$ (1), $NaYF_4:Yb^{3+},Er^{3+}$@silica (2) $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2 (3) and $NaYF_4:Yb^{3+}$, Er^{3+}@silica-N=FA (4) under NIR laser excitation at 980 nm.

In vitro cellular imaging was performed to prove the localization of the functionalized UCNPs within the cytoplasm of breast cancer cells. The UCNPs were incubated the with MCF-7 cells for 24 h. We observed that the UCNPs have been clearly localized within the cell cytoplasm by microscope with no significant signs of cytotoxicity. The evidence confirms that the UCNPs can be potentially used as bio-labels for MCF-7 breast cancer cells. This is possibly because the high affinity via FA–FR. After the binding of the ligands, the UCNPs is internalized into the cell via invagination process.

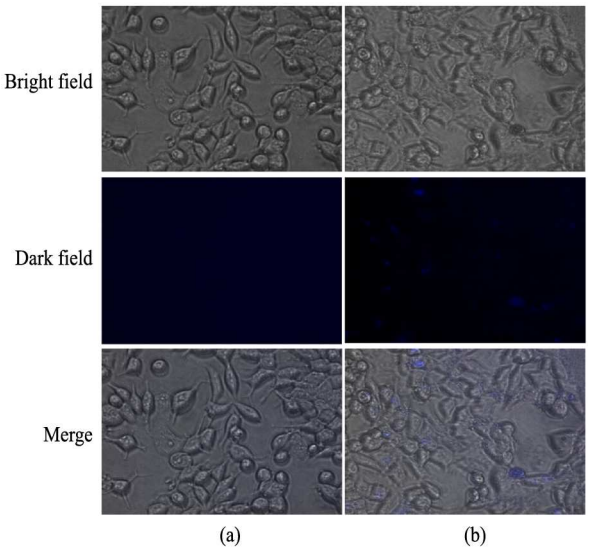

Fig. 6 Upconversion fluorescent imaging of MCF7 cells (a) and MCF7 cells after incubated with $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA (b). The complex's concentration 20 µg/mL and incubation time 3 h.

Fig. 6 demonstrates imaging results of biological test for UCNPs. In Fig. 6(a), we show images of MCF7 cells only in three cases: upper one is a bright field image, middle one is a dark field image, lower one is merged image of two above pictures. Similarly, Fig. 6(b) presents images for the case of MCF7 cells after incubated with $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA. When we use right field regime of microscope, we only see a typical MCF7 cell type, in both cases (a) and (b). For the dark field images, we do not see luminescence from bare MCF7 cells but we observe the brightness emitting dots in the MCF7 incubated with UCNPs. The merge pictures show clearly that the brightness emitting dots (represent for exitance of UCNPs) were attached around MCF7 cells. This indicated that the rich folate receptor was captured to the MCF7 cells.

4. Conclusions

In summary, the $NaYF_4:Yb^{3+},Er^{3+}$ nanophosphors were successfully synthesized by the hydrothermal method. These nanorods have sizes ranging from 200 to 700 nm in length and from 80 to 150 nm in diameter. The $NaYF_4$ hexagonal was the dominant phase for the as-prepared $NaYF_4:Yb^{3+},Er^{3+}$ nanophosphors. The functionalized $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA phosphors have bundles shape with the lengths of rod about 300–800 nm and diameter of rod about 100–200 nm.

The PL spectra of the as synthesized $NaYF_4:Yb^{3+},Er^{3+}$; $NaYF_4:Yb^{3+},Er^{3+}$@silica-NH_2 and $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA were studied. Under 980 nm laser excitation, $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA nanophosphors samples emitted green fluorescence 520 nm ($^2H_{11/2}\rightarrow{}^4I_{15/2}$), 550 nm ($^4S_{3/2}\rightarrow{}^4I_{15/2}$) and red fluorescence 650 nm ($^4F_{9/2}\rightarrow{}^4I_{15/2}$). Strong luminescent intensity indicates the great potential application of these Er nanophosphors as a fluorescence label agent in biomedical systems.

The synthesized nanoprobes have low cytotoxicity and good targeting to tumor cells with rich folate receptor expression. Therefore, the $NaYF_4:Yb^{3+},Er^{3+}$@silica-N=FA nanoprobes have a good prospect in the cancer diagnosis, especially for MCF7 breast cancer.

Our achievement is a promising result in sense of using rare earth luminescent nanomaterials for development of fluorescent labelling analysis probes and technical tools in biochemistry, molecular biology and biomedicine.

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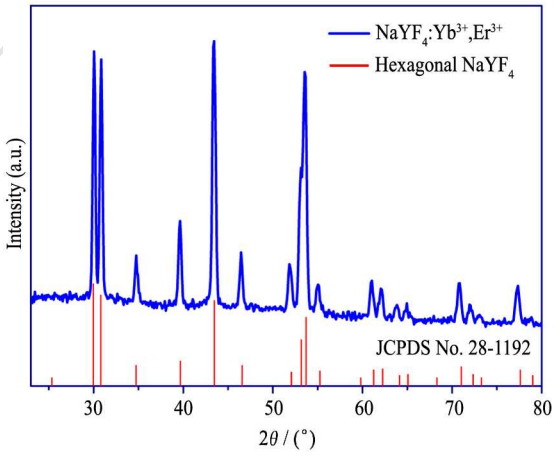
Graphic Abstract



XRD pattern of the nanophosphors $NaYF_4:Yb^{3+},Er^{3+}$ at 190 ^oC, 24 h

Synthesis and characterization of $NaYF_4:Yb^{3+}$, Er^{3+}@Silica-N=Folic Acide nanocomplex for bioimaginable detecting MCF-7 breast cancer cells

Ha Thi Phuong[1,2,3], Tran Thu Huong[1,2*], Le Thi Vinh[4], Hoang Thi Khuyen[1,2], Do Thi Thao[2,5], Nguyen Thanh Huong[1,2], Pham Thi Lien[1,2] and Le Quoc Minh[1,2,6]

[1] *Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.*

[2] *Graduate University of Science and Technology - Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.*

[3] *Department of Chemistry, Hanoi Medical University, Vietnam, 1-Ton That Tung, Dong Da, Hanoi, Vietnam.*

[4] *Department of Chemistry, Hanoi University of Mining and Geology, 18-Pho Vien, Bac Tu Liem, Hanoi, Vietnam.,*

[5] *Institute of Biotechnology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam.*

[6] *Duy Tan University, K7/25 Quang Trung, Da Nang, Vietnam.*

High lights

- ✓ The $NaYF_4$: Yb^{3+}, Er^{3+} nanophosphors were successfully synthesized by the hydrothermal method and were functionalized with aminosilanes and folic acid. The functionalized $NaYF_4$: Yb^{3+}, Er^{3+}@Silica-N=FA phosphors have bundles shape with the lengths of rod about 300-800 nm and diameter of rod about 100-200 nm. The $NaYF_4$ hexagonal was the dominant phase for the as-prepared $NaYF_4:Yb^{3+}$, Er^{3+} nanophosphors.
- ✓ Under 980 nm laser excitation, the functionalized $NaYF_4$: Yb^{3+}, Er^{3+}@Silica-N=FA nanophosphors samples emitted green fluorescence 520 nm ($^2H_{11/2}\rightarrow{}^4I_{15/2}$), 550 nm ($^4S_{3/2}\rightarrow{}^4I_{15/2}$) and red fluorescence 650 nm ($^4F_{9/2}\rightarrow{}^4I_{15/2}$). Strong luminescent intensity indicates the great potential application of these Er nanophosphors as a fluorescence label agent in biomedical systems.
- ✓ The synthesized nanoprobes have low cytotoxicity and good targeting to tumor cells with rich folate receptor expression. Therefore, the $NaYF_4:Yb^{3+}$, Er^{3+}@Silica-N=FA nanoprobes have a good prospect in the cancer diagnosis, especially for MCF7 breast cancer.