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Studying the toxicity effects of coated and uncoated NaLuF₄: Yb³⁺, Tm³⁺ upconversion nanoparticles on blood factors and histopathology for Balb/C mice’s tissue

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Abstract

In this study, NaLuF₄: Yb⁺⁺, Tm³⁺ upconversion nanoparticles were synthesized in less than 6 h under thermal decomposition conditions by changing pH and synthesis temperature. The washing process removed the hydrophobic species on the surface of nanoparticles. Then the nanoparticles were coated by calcium carbonate (CaCO₃) and were loaded with curcumin (Cur) as a drug model in one synthesis step. The effects of toxicity of uncoated and coated nanoparticles were investigated on normal cells, blood factors, and histopathology of the tissue in Balb/C mice. The most of synthesized nanoparticles had spherical shape considering the results from the SEM and TEM. The results of the DLS analysis showed that the hydrodynamic diameter of the uncoated and coated nanoparticles was 49.52 and 59.37 nm, respectively. By injecting CaCO₃@NPs nanoparticles into human blood, there was not seen a significant difference in the number of red blood cells, white blood cells, hemoglobin, and blood platelets with the treatment of the control group. Besides, cell biocompatibility studies in fibroblast cell lines showed that the toxicity of uncoated nanoparticles increased at concentrations higher than 250 µg/ml. Also, cytotoxicity increases significantly with increasing the time of 24, 48, and 72 h. The coated samples did not represent the toxicity effect. The results of cellular changes induced by the addition of uncoated and coated nanoparticles showed that the cells change from spherical to Spindle after treatment with CaCO₃@NPs, Which indicates a lack of toxicity. Histopathological examination of vital organs in mice receiving CaCO₃@NPs did not show any pathological damage compared to the control group.

1. Introduction

Currently, cancer is one of the most deadly diseases in the world that has been remarkably grown to be a public concern. The early distinguishing, detection and effective treatment of cancer have been essential matters among researchers in the last decade [1–3]. Thus, the prompt cancer identification and therapy in a specific and quick sensitive manner became the substantial importance [3, 4]. Molecular probe imaging methods such as fluorescence microscopy (FM) and magnetic resonance imaging (MRI) have been introduced as potent tools for the diagnosis of the metastasis cancer cells from normal tissues and the investigation of cancer cells dynamic properties in molecular level [3–8]. However, owing to the low resolution and sensitivity of FM and MRI techniques, the further approach is necessarily needed for attaining efficacious knowledge to assure the requirements of accurate medical imaging recognizing [9]. Thus, a model shift in the approach to cancer diagnosis and therapy is highly required. The up-conversion luminescence (UCL) imaging technique is proposed to prevail in this dilemma, owning the arising excellent optical properties in their 4f shell electrons [9–11]. The UCL molecular imaging technique is a kind of nonlinear optical phenomenon introduced by Auzel...
in the mid-1960s [12]. It is a kind of fluorescence phenomenon in which the frequency of the emission light is higher than that in stimulating light [13, 14].

Many studies have been conducted on upconversion nanoparticles (a process in which the sequential adsorption of two or more photons leads to emit light at a shorter wavelength than excitation wavelength) [15–18] due to their unique lighting properties and to find the potential applications of these materials in the field of drug delivery and medical imaging [19, 20]. With the development of nanotechnology, various methods have been provided for synthesizing these nanoparticles with particular crystalline phase, size and form [21], including co-precipitation (CPT) [22], thermal decomposition [23–27], hydro(solvo) thermal [28–30], sol-gel [31] and combustion [32], which have been reviewed in many articles. Among them, the thermal decomposition reaction was the most widely used method because of the convenient and accurate control of the shape and size of lanthanide-doped upconversion nanocrystals [28, 29]. Reaction conditions can be well-set up in thermal decomposition processes, such as reaction time, concentration, temperature, and pH, to obtain appropriate microstructural and optical properties for specific biomedical applications [33, 34].

In our latest published paper [35], the Sodium Lutetium Fluoride Luminescence nanoparticles (NaLuF$_4$) with Ytterbium ion (Yb$^{3+}$) and thulium ion (Tm$^{3+}$) was synthesized successfully by thermal decomposition and coating it with calcium carbonate. In the current study, for the first time curcumin (Cur) as a drug model was loaded in the previous synthesized NaLuF$_4$:Yb$^{3+}$, Tm$^{3+}$@CaCO$_3$ nanoparticles in order to fabricate novel multifunctional CaCO$_3$-Cur@NPS. By controlling the thermal conditions and making the thermal shock, we managed to produce the optimal amount of UCLNPs with desirable characteristics for further biomedical applications, which has been reported in detail in the current study. Then, the nanoparticles were coated by CaCO$_3$ and were loaded with Cur in one synthesis step in order to fabricate novel CaCO$_3$-Cur@NPS as a drug carrier which could act as a UCL molecular imaging agent as well. Next, the characterization of uncoated and coated nanoparticles was investigated using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), photoluminescence (PL), transmission electron microscopy (TEM), scanning electron microscope (SEM) and energy-dispersive x-ray (EDX) equipments. Due to Yb$^{3+}$ and Tm$^{3+}$ dopants, synthesized UCLNPs provide an excellent NIR-to-NIR UCL signal when excited at 980 nm. Then, the effect of uncoated and coated nanoparticles on the biocompatibility of the MTT test was used. Also, quantitative and qualitative evaluation of the biocompatibility of coated and uncoated nanoparticles was used by hemolysis test, cell change assay, CBC assay, and toxicity study on red blood cells. Besides, the pathological examinations of mice tissues were observed under inverse optical microscope.

2. Experimental

2.1. Materials and methods

For synthesizing NaLuF$_4$ nanoparticles using the methods reported in scientific sources and references, the oxide and trifluoroacetic acid precursor with various molar ratios, and deionized water and ethanol were used. Lanthanide oxides of Tm$_2$O$_3$, Lu$_2$O$_3$, Yb$_2$O$_3$ were purchased from Merck company. Reagent for oleic acid (OA), 1-Octadecene (ODE) and trifluoroacetic acid (TFA) were purchased from Dae Jung Company. Sodium hydroxide, Ammonium Fluoride, Methanol, Ethanol, Cyclohexane, Normal Hexane, Sodium Carbonate, Calcium Chloride were purchased from Merck company. Curcumin was obtained from Dine company (Iran). Distilled water was used to deploy the solution. Materials used for cell culture and cytotoxicity tests were L929 fibroblast cells, Dulbecco’s Modified Eagle’s Medium (DMEM), phosphate buffer solution (PBS), penicillin-streptomycin, fetal bovine serum (FBS) produced by Gibco BRL (Life Technologies, USA), MTT reagent (dimethyl-thiazole-2,5-Diphenyl tetrazolium bromide) produced by Sigma/Aldrich company (USA), Trypsin produced by Gibco/BRL (USA), 100% methanol, trypan blue solution of DMSO (dimethyl sulfoxide) produced by Merck Company.

NaLuF$_4$: Yb$^{3+}$, Tm$^{3+}$ nanoparticles are synthesized by the thermal decomposition process of the lanthanide oxide compounds (Lu$_2$O$_3$), including Yb$_2$O$_3$ (0.2 mmol/L), Lu$_2$O$_3$ (0.78 mmol/L) and Tm$_2$O$_3$ (0.02 mmol/L). The precursor of lanthanide oxides and trifluoroacetic acid (10 ml) are added to a mixture of 12 ml of oleic acid and 34 ml of 1-octadecene in order to synthesize NaLuF$_4$: Yb$^{3+}$, Tm$^{3+}$, and then the thermal decomposition process was performed. In this process, at first, the resulted mixture was degassed for 1 h at room temperature to perform the synthesis process without oxygen. 0.2 g of sodium hydroxide and 0.296 g of ammonium fluoride was added to 20 ml of methanol and the solution was stirred until a transparent solution was formed. Then, the solution was slowly added as drop by drop, then the solution was heated with a rate of 20 °C min$^{-1}$, at 320 °C. The reflux process was performed under a nitrogen atmosphere for 120 min After slowly cooling the mixture to room temperature, ethanol was added to create the deposition. After separating the deposition by centrifugation, it was washed by a mixture of hexane-ethanol. Then, the washed deposition was dried in an oven for 24 h at 80 °C. The temperature conditions applied in this study are as following cycles:
initially, the temperature brought up to 320 °C, which kept for 20 min, then it was cooled down to 200 °C and it kept for another 20 min. Subsequently, the temperature raised to 320 °C for 60 min, and finally, it was chilled down to the ambient temperature.

The amount of 0.5 gr synthesized nanoparticles was used to prepare coated NaLuF₄:Yb³⁺, Tm³⁺ and CUR loaded nanoparticles. Sodium carbonate (1 M) and calcium chloride (1 M) were added dropwise to NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles with or without presence of curcumin (1.35 mg/mL in ethanol). The mixture is kept at room temperature by a medium-round magnetic stirrer for 2 hours, and then the final product was washed several times with deionized water and ethanol using centrifuge (2 times); and then these nanoparticles were dried in a freeze dryer (GAMMA 1–16 LSC) for 24 h.

2.2. Characterization and toxicity study

In order to detect NPS synthesis powder and coated CaCO₃@NPs powder, X-ray diffraction (XRD) model PW1830, manufactured by the Netherlands Phillips company, was applied under a voltage of 40 kV and a current of 30 mA (Cu – k₀ radiation with a wavelength of 1.54056 angstroms). Infrared spectroscopy with FTIR Thermo Nicolet Nexus 870 model was carried out in the wavenumber of 400–4000 cm⁻¹ in the transition mode. For microstructural examinations, the determination of the size and morphology of the samples was performed using a scanning electron microscope (SEM/EDS) of the LEO company 1455VP model, as well as Transmission Electron Microscope (TEM) of Philips company CM200 model. In order to determine the hydrodynamic diameter of nanoparticles and distribute the size of particles, NPS and CaCO₃@NPs were measured by Dynamic light scattering (DLS) (Nano Zetasizer ZEN 3600 model equipped with a 633 nm laser, made by Malvern, England. The emission spectrum of NPS and CaCO₃@NPs samples was in the wavelength round of 400 to 900 nm and under radiation excitation with 980 nm wavelength by a photoluminescence (PL) machine (LS 55) made by Perkin-Elmer, the Netherlands.

In vitro MTT assay was applied to assess the percentage of cell survival after 24h incubation of samples with L929 mouse fibroblast cells obtained from the National Cell Bank of Iran. Cells were cultured in DMEM medium, 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Then, the flask was placed in an incubator with 5% CO₂ at 37 °C. The rate of proliferation and survival of L929 cells after incubation with samples was assessed directly by MTT assay. First, the cells were cultivated in 96-well plates at concentration of 3 × 10⁴ cells/mL. After 24 h, the medium was exchanged with 200 μl of synthesized NPS, CaCO₃@NPs and CaCO₃-Cur@NPs at different concentrations (50, 250, 500, 750 and 1000 μg/mL) and treated for 24 h. Next, 20 μl of tetrazolium bromide salt solution (MTT) was added to the culture medium containing the samples and cells that incubated for four hours in CO₂ incubator. Due to the addition of MTT, the insoluble crystals of fermazone created by live cells. Then, to dissolve these crystals and to change the color of the medium, 200 μl of dimethyl sulfoxide (DMSO) was replaced with the medium. Eventually, the optical absorbance was read at 570 nm by Eliza Reader (Model: Bio Rad, Model 680 instruments). The experiment was repeated three times. The relative cell viability (%) compared to the control group was obtained via Eq. (1).

\[
\text{Cell viability (\%) = } \frac{\text{OD 570(sample)}}{\text{OD 570(control)}} \times 100
\]

Animal: Experiments were performed on 12 Balb/C male mice weighing 25 to 20 grams from the Pasteur Institute of Razi and at the University Animal House. The mice were freely kept under standard maintenance conditions in a room with a temperature of 27 ± 3 °C with a cycle of 12-hour dark and 12-hour light, water and food, and observing the ethical requirements of working with laboratory animals (Code of ethics: IR.IAU. NAJAFABAAD.REC.1398.032).

CBC and hemolysis tests were performed to study the cytotoxicity of CaCO₃@NPs on the number of major blood cells. Accordingly, 100 μl of CaCO₃@NPs was injected into the mice tail veins. Direct blood sampling from the right ventricle of the heart of the mice injected with CaCO₃@NPs, and non-injected was performed after four days. The blood samples were collected inside a test tube containing heparin were delivered to the laboratory for the CBC test. For the hemolysis test, the amount of 900 μl was prepared from each sample (NPS, CaCO₃@NPs) with concentrations of 50, 100, 150 and 200 μg/mL. Then, 100 μl of RBCs suspension was added to each of the concentrations. 100 μl of RBCs suspension was added to 900 μl of saline solution, as a negative control. Incubation was performed for 2 h. After 5 min of centrifugation with 1500 rounds of the superficial solution containing lysed RBCs, its separation and absorption were examined by UV spectrophotometer (UV Cary win) at 541 nm wavelength. Triton X-100 was used as a positive control. The percentage of hemolysis was determined Using equation (2).

\[
\text{% of haemolysis} = \frac{(\text{OD sample} - \text{OD (-) control})}{\text{(OD (+) control} - \text{OD (-) control})} \times 100
\]

Where OD sample, OD (-) control, and OD (+) control refer to the absorbance of each sample control group, negative control group, and positive control group, separately.
Hard clotting and hemolysis tests were carried out to investigate the quantitative blood compatibility of coated and non-coated nanoparticles. The hard clotting test was performed in a glass slide method to determine the clot retraction power in the microscope glass slide. The samples (NPS and CaCO₃@NPS) were diluted with phosphate-buffered saline (PBS) solution (pH=7.4). The chronometer is turned on and then samples (NPS and CaCO₃@NPS) is incubated with a drop of blood on the glass slide. Then, every few seconds (every 30 seconds), once a sharp needle is placed into the bottom of the blood drop and lifted up, if the fibrin threads were observed on the needle, the chronometer was stopped and the time was recorded that considered as the hard clotting time. The qualitative blood toxicity of samples (NPS and CaCO₃@NPS) when incubated with RBCs was studied visually using optical microscopy (Leica DM IRB, Germany). To examine the toxicity of samples on RBCs, 50 µl of samples (NPS and CaCO₃@NPS) were added in 100 ml of washed RBCs and incubated at 37 °C for 10 minutes. Triton X-100 and saline were applied as positive and negative controls, respectively. To assess histopathological changes in mice’s tissues, 100 µl of PBS/CaCO₃@NPS was injected into the tail vein of mice (6 mice each group). Animal clinical symptoms, such as diarrhea, piloerection and mortality were recorded for 96 h. After four days, mice were sacrificed and then the vital organs including heart, kidney, liver, lungs, and spleen were collected. The mice’s tissues were fixed in 10% formalin solution, then, the tissue samples were processed, embedded in paraffin, and sectioned at 5 µm. The sections were mounted on glass slides stained according to the standard hematoxylin and eosin (H&E) staining method. The stained slides were observed under inverted microscope in order to determine the histopathological changes in mice’s vital organs.

3. Results and discussion

3.1. Producing and Identifying NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles by thermal decomposition method

In order to provide desirable conditions for synthesizing NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles, the temperature was set as the following cycles in this study by thermal decomposition: from 320 °C to 200 °C, which again increased to 320 °C and finally it cools to the ambient temperature. X-ray diffraction analysis was used to investigate the composition and structure of the obtained materials. The optimal pH for producing NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles is calculated in the range of 4 to 5.

3.2. X-ray diffraction spectrum (XRD)

Figure 1 is illustrated the patterns of x-ray diffraction (XRD) to determine the existing structure and compounds in NPS and CaCO₃@NPs. The pattern of X-ray diffraction with the sample code of NPS-1 has two crystalline phases of the β-hexagonal and – Cubic phase. The pattern of X-ray diffraction with the sample code of NPS – 2 has a hexagonal phase of β – NaLuF₄: Yb³⁺, Tm³⁺, which, according to the card number of 27–0726, this pattern with sharp peak represents that nanoparticles have excellent crystallinity. The β-hexagonal phase verify through existence of diffraction peaks at 2θ = 17.20 and 30.160 corresponding to (100) and (110) crystal planes respectively, while the α-cubic phase identified by three diffraction peaks at 2θ = 28.235, 46.945 and 55.687 refer to the crystal planes of (111), (220) and (311) respectively. The crystal size of fabricated NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles were calculated via the Scherrer’s equation: Particle Size (nm) = (0.89 × λ) / (FWHM cos θ)[36]

Where λ is the wavelength of X-ray (Cu – kα), FWHM is the full width at half maxima, and θ is the diffraction angle. The average grain size was calculated using Scherrer’s formula was found to be 32 nm. Since the dopant metal ions (Yb³⁺ and Tm³⁺) amount in the synthesized NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles are very low as compared to the substance, the presence of Yb³⁺ and Tm³⁺ were not detected in the XRD diffraction pattern. But the calculated value of lattice constants slightly changed that may confirm the existence of those dopant ions. The calculated alpha phase of the network parameter is a = 5.47 Å (JCPDS file no.27–0725), while the beta-phase lattice constants are a = 5.901 Å and c = 3.453 Å (JCPDS: 27–0726). The computed network constants are in excellent agreement with findings stated in the literature for the bulk phase of alpha and beta-NaLuF₄ structures that confirmed the phase purity of synthesized NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles. The ionic radius of Yb³⁺ (0.99 Å) and Tm³⁺ (1.052 Å) was larger than that of Lu³⁺ (0.98 Å); thus, Yb³⁺ and Tm³⁺ replaced Lu³⁺ in the lattice, the unit cell becomes larger, so the diffraction angle will decrease. As it can be seen by XRD of CaCO₃@NPs, CaCO₃ index peaks are seen with a rhombohedral structure (α = 90°, γ = 120° and a = b = c) which is entirely consistent with standard card data (JCPDS No.86–2339), representing the formation of calcium carbonate on NPS.

3.3. Fourier transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was performed to confirm the functional groups on the surface of the synthetic materials. As can be seen in figure (a), the synthesized precursor (Ln (OOCCH₃)₃) showed the trifluoroacetate assigned picks at 1150 and 1208 cm⁻¹ ascribed to C–F and C=Vibrations of trifluoroacetic acid. The presence of metal cations in trifluoroacetic acid is approved by the existence of bands at 1622 and
1477 cm\(^{-1}\) assignable to C=O and C–O vibration stretches, respectively. The presence of absorption peaks at 1000 to 1300 cm\(^{-1}\) ascribed to C\(_2\)F\(_4\) and C=O bonds. The evolution of 1208–1150 cm\(^{-1}\) peaks was considered as evidence for the fluorocarbon group, thus denoting the successful synthesize of lanthanide trifluoroacetate (Ln (OCCF\(_3\))\(_3\)) nanoparticles. Figures 2(b)–(d) is illustrated the FTIR spectra of the synthesized NPS, washed NPS, and CaCO\(_3\)@NPs, respectively. As it can be seen, the absorption bands at 1690, 1563.54 and 1459.99 cm\(^{-1}\) are related to the tensile peak C=O. Two additional absorption bands of 2925.08 cm\(^{-1}\) and 2854.32 cm\(^{-1}\) are related to the symmetric and asymmetric stretching vibrations of the –CH\(_2\) group, representing the confirmation of the existence of a long alkyl chain in Oleic acid. The hydrophobic species should be removed by the washing process to enhance the dispersion of particles in aqueous media. The absorption peaks of 2955 and 3434.11 cm\(^{-1}\) represent the presence of carboxylic and hydroxyl groups on the surface of NPs. CaCO\(_3\) of the absorption peaks at the range of 1797.02, 1426.67, 875.74, 711.98 cm\(^{-1}\) are related to calcium carbonate with ion (CO\(_3\)\(^{2-}\)) vibrations. The strong peak at 1426.67 cm\(^{-1}\) is related to asymmetric stretching vibrations of the C–O bond at CO\(_3\)\(^{2-}\) and weak absorption peak at 1797.02 cm\(^{-1}\) is allocated to symmetric stretching vibrations of C–O bond at CO\(_3\)\(^{2-}\). Two absorption bands at 711.98 cm\(^{-1}\) and 875.74 cm\(^{-1}\) are assigned to the bending out of plane vibrations and in plane vibrations of O–C–O at CO\(_3\)\(^{2-}\). The FTIR demonstrates coating the nanoparticles’ surface with calcium carbonate (figure 2(d)).
3.4. Microstructural Study of NPS, CaCO₃@NPs nanoparticles by Scanning Electron microscopy (SEM) and Transmission Electron microscopy (TEM)

Morphological analysis was studied with electron microscopic images. SEM images of NPS and CaCO₃@NPs are shown in figure 4. The scanning electron microscopy (SEM) images of NPS, CaCO₃@NPs indicate that calcium carbonate is coated on the surface of nanoparticles. Figure 4(a) indicates the SEM images of the synthesized NPS. According to the Figure (figures 4(a₁)–(a₃)), the synthesized particles have nanometer dimensions (figure 4(b)) and have almost uniform particle size and shape. As these images show, the particles have become strongly agglomerated. Indeed, by decreasing the particle size, the surface-to-volume ratio increases, and as a result, the ratio of their surface to volume has been increased with reducing particles’ size and, as a result, it cause increase the gravitational force increases between particles, which results in the formation of intense agglomerates that can be a reason for a small amount of synthesized particles. Figures 4(c₁)–(c₃) shows SEM images of CaCO₃@NPs. As it is clear, nanoparticles coated with calcium carbonate have a mass-like structure, and the particles’ size distribution is mostly homogeneous, and particles have nanometer dimensions. Figure 4(d) shows the X-ray energy spectrum of CaCO₃@NPs. The atoms of Na, Lu, F, and Yb represent the presence of nanoscale NaLuF₄: Yb⁺³, Tm⁺⁺. Also, the atoms of C, O and Ca confirm the presence of calcium carbonate as a coverage.

In figure 5, the grains’ shape and their size have been determined by the TEM images from coated and uncoated nanoparticles. The images indicated that the synthesized nanoparticles are almost spherical, have a uniform distribution of particle size with a mean diameter of fewer than 50 nanometers that are suitable for biological activities. TEM images confirm the results obtained from FESEM images and the estimated crystal size by the Scherrer equation from the XRD analysis. It should be noted that the crystal size obtained from the XRD analysis is about 32 nm for this sample. Also, it was found that uncoated nanoparticles have an aggregation feature.

Figure 5(b) illustrates the TEM images of the coated nanoparticle. As it can be seen in these images, the diameter of these nanoparticles is in the range of 10–50 nm. For coated NPS (CaCO₃@NPs), a core–shell structure arrangement was discernible.

3.5. Average particle size and particle size distribution (DLS analysis)

Particle size distribution for NPS and CaCO₃@NPs particles are shown in figures 6(a), and (b). As shown, the hydrodynamic diameter of NPS and CaCO₃@NPs is about \( \bar{z}_{\text{avg}} = 49.52 \) and 59.37 nm, respectively. Particle size distribution index (PDI) for the NPS and CaCO₃@NPs is 0.04528 and 0.05677, representing the
Figure 3. FTIR spectra of CaCO3@NPS and CaCO3-Cur@NPS nanoparticles.

Figure 4. The results of the SEM images from (a1 and a3) uncoated NPS nanoparticles, (b) Size distribution of NPS, (c1 and c3) coated CaCO3@NPs nanoparticles in different magnification ranges (c1) 500 nm (c2) 1 μm (c3) 2 μm, and (d) EDS spectrum of the CaCO3@NPs.
appropriate distribution of the particle size. The results from DLS are significantly larger than the values shown by the SEM images. It is due to the formation of a hydrogen bond between the carboxyl group on the adjacent surface, which can cause interaction between the particles and, as a result, increase the particle size, and thus the size of the DLS particles is larger than the SEM.

Figure 5. TEM images of uncoated NPS (a) and nanoparticles coated with CaCO₃@NPs (b). Shown scale bar is 50 nm.

Figure 6. Hydrodynamic diameter distribution of NPS (a) and CaCO₃@NPs (b) in terms of average hydrodynamic diameter versus dispersed light intensity (average particle size, $\bar{D}_{\text{average}}$, PDI).

Figure 7. Images of the intensity of converted emission by pure (NPS), coated (CaCO₃@NPs) and uncoated (NPS-1, NPS-2, and NPS-3) nanoparticles at 980 nm wavelength, (b) Schematic energy level illustration of Tm³⁺ and Yb³⁺ ions and the possible processes of the up-conversion mechanisms (The dotted, solid, and curly lines display the processes of the energy transfer, emission, and multiphonon relaxation, respectively).
3.6. Examining the Photoluminescence (PL) spectra of coated and uncoated nanoparticles

Figure 7 shows the upconversion photoluminescence spectra for bare, uncoated nanoparticles and nanoparticles coated with calcium carbonate. There is no explicit peak arising from the transition from excited states to base states for the photoluminescence spectrum for bare NaLuF$_4$ nanoparticles with the NPS code, because there is no impurity within the NaLuF$_4$ network to use its levels for absorption and diffusion of the photon. As shown in this spectrum, this sample has a very weak emission in the area of 450–500 nm. Therefore, the bare NaLuF$_4$ cannot transmit the absorbed radiation and has emission radiation. In the photoluminescence spectrum of uncoated nanoparticles (NPS-1, NPS-2, and NPS-3), three maximum radiation were detected under 980 nm laser light, including two recorded peaks at 450 and 475 nm (visible blue area) and another with a maximum of 645 nm (visible red area). Since the amount of intense blue emission is much higher than the intense red emission, the overall blue emission can be seen by the naked eye. As shown in the above spectra, the peak position is almost similar for these products, but the intensity of peak was quite different. Changing impurities has reduced the height of the photoluminescence spectrum's peaks. Also, the height of the photoluminescence spectrum's peaks decreased by coating nanoparticles. Besides, the schematic energy level diagram of Tm$^{3+}$ and Yb$^{3+}$ ions and the possible processes of the up-conversion mechanisms has displayed in figure 7(b). In other words, the blue emission centered at 476 nm is the strongest peak that originating from the transition of $^{1}G_{4}$ down to $^{3}H_{6}$ while the red emission at 651 nm emanating from the week transition of $^{1}D_{2}$ down to $^{3}F_{4}$. From the results, only the fabricated nanoparticles with $\alpha$–cubic or $\beta$–hexagonal crystal phase (NPs-3 and NPs-1) exhibited illuminating characteristics, and the nanoparticles with both $\alpha$–cubic crystal structure and $\beta$–hexagonal crystalline ($\alpha + \beta$) phases (NPs-2) have been shown to exhibit the best luminescence properties.
3.7. Cellular studies

The cells biocompatibility was investigated by MTT assay through direct incubation of samples with L929 fibroblast cell line, also to ensure that the coated nanoparticles are safe for further biomedical application the in vitro blood compatibility and the in vivo acute systemic toxicity tests were performed according to the standard methods.

3.7.1. Measuring the cytotoxicity of coated and uncoated nanoparticles on L929 fibroblast cell by MTT assay

Figure 8 illustrates the visual results of MTT assay and the cytotoxicity of coated and uncoated nanoparticles. The visual results of MTT assay cause a change in the color of the wells after adding tetrazolium and DMSO, the higher the color intensity, the greater cell viability (figure 7(a)). Figure 8 (a, c and d) presents the results of MTT assays of non-coated, coated and Cur loaded nanoparticles. The results indicated dose dependent toxicity effects of nanoparticles. The bare nanoparticles displayed great toxicity at concentration higher than 250 μg/ml. But coated nanoparticles were not toxic to the cells even at high concentration, as only about 30% of cells were died at concentration of 1000 μg/ml. However, the toxicity effects have reduced by decreasing the concentration of nanoparticles. The results of the coated sample represent the effect of calcium carbonate coating on reducing cell death and, thus, reducing the toxicity of NPS (figure 8 (c)). However, curcumin loaded nanoparticles (NaLuF₄:Yb³⁺, Tm³⁺ @CaCO₃-Cur) indicated dose dependent toxicity effects on cells.

3.7.2. Examining hemolysis test

Figure 9 illustrates a quantitative blood biocompatibility test to determine the percentage of hemolysis and the blood’s external coagulation time of NPs, CaCO₃@NPs. As shown in figure 9, the absorption rate of all samples are higher than the control, it can be seen mere absorption in samples with a higher concentration of NPs and...
CaCO$_3$@NPs, representing a higher incidence of hemolysis in them, therefore it is possible to define the direct relationship between these two variables, but there was a significant difference of hemolysis percentage between them. The maximum RBC hemolysis is observed in NPs at a concentration of 200 $\mu$g/ml, which is half in comparison with CaCO$_3$@NPs at the same concentration. Therefore, it can be concluded that CaCO$_3$@NPs reduce the toxicity of RBCs (as blood and body cell indicator) in comparison with NPs. Consequently, the toxicity was increased with enhancing concentration, but coated nanoparticles showed lower toxicity in equal concentrations. Meanwhile, it should be mentioned that CaCO$_3$@NPs had no effect on RBCs lysis, which could be a good indicator of the lack of toxicity of coating used to produce CaCO$_3$@NPs on human cells. The results of the effect of NPs on the external coagulation time of blood showed that the external coagulation time of blood has a significant change towards the control group; it reduced and this reduction was related to the concentration of nanoparticles (figure 9(c)). In figure 9(d), the coagulation test and aggregation power of CaCO$_3$@NPs clot indicated that there was not a significant change in the external coagulation time of blood between treatment and control groups.

3.7.3. Examining the CBC test

Figure 10 illustrates the effect of CaCO$_3$@NPs on blood factors (white blood cells (WBC), red blood cells (RBC), hemoglobin (HB), hematocrit (HCT), mean red cell volume (MCV), mean corpuscular of hemoglobin (MCH), mean corpuscular of hemoglobin concentration (MCHC), red blood cell distribution width (RDW), neutrophil, lymphocyte, monocyte, eosinophil, basophil, and platelet. Injecting CaCO$_3$@NPs into mice had not let to essential changes in the number of RBCs, WBCs, HB, and platelets. The results indicated that the number of red blood cells was the same in the control group. Due to the weakening of the body at high concentrations, it reduces the production of white blood cells (WBC). Increasing cell involvement in immune processes leads to reduce the number of blood cells. The reduction of the number of cells appears in severe poisoning. Blood
leukocytes index is one part of a non-specific cellular immune system that fluctuation in their number can be considered as an appropriate index concerning the mice responded to the stress. There was not a significant change in the percentage of lymphocytes and neutrophils. The low average of MCV can be considered as a positive blood parameter because shrinking the size of RBCs leads to more comfortable and faster movement in the blood vessels and prevents forming a clot.

Comparison the results from the effect of CaCO$_3$@NPs on blood platelets indicated that there was not a significant change in the group of Balb/C mice receiving CaCO$_3$@NPs and the control group (figure 11).

3.8. Blood qualitative biocompatibility test, examination of toxicity on RBCs

Figure 12 illustrates the blood qualitative biocompatibility test by injection NPS, CaCO$_3$@NPs with different concentrations. As shown in figure 12, the results of the observations indicated that by injecting NPS in RBC, various concentrations result in abundant cellular aggregation as and divisions. All RBC cells were healthy in terms of appearance using NPS at a concentration of 50 $\mu$g/ml. No morphological changes were indicating the toxicity effect of NPS (figure 12(a1)). At the concentration of 150 $\mu$g/ml, destruction-based cellular changes were observed by adding NPS to RBC (figure 12(a2)). The cellular destruction was more and more severe at a concentration of 200 $\mu$g/ml, leading to cell lysis (hemolysis) (figure 12(a3)).

3.9. Histopathologic studies

The in vivo toxicity of CaCO$_3$@NPs was tested by histology analysis. Figure 13 shows the optical microscopic images of the histopathologic examination of the heart, kidney, liver, lung, and spleen tissues of the rat treated with PBS (control group) and CaCO$_3$@NPs (treated group). It was not observed any pathologic damage in the vital organs of Balb/C mice receiving CaCO$_3$@NPs, including heart, kidney, liver, lung, and spleen (figure 13).
Also, there was not any sensible sign from structural change, inflammation, destruction, or erosion in tissues in comparison with the control tissue (figure 13). There were some changes in the kidneys that it can be due to the more aggregation of nanoparticles.

4. Conclusion

In this study, the thermal decomposition method at pH five was used to synthesize NaLuF₄: Yb³⁺, Tm³⁺ nanoparticles. Also, the application of the thermal cycles in the synthesis of nanoparticles leads to a reduction in the synthesis time, accelerating the thermal decomposition reaction, separating nanoparticles from the oil phase during the washing step and producing high purity nanoparticles. The core NPs were successfully coated with CaCO₃ and loaded with Cur as a drug model at one synthesis step that confirmed through FTIR analysis. The final synthesized nanoparticles (CaCO₃-Cur@NPS) had dimensions less than 100 nm, which were the appropriate size for further biological applications. The cells survival was dose depended manner for all synthesized samples. The blood compatibility study indicated no significant differences of blood factors in CaCO₃@NPS treated group compared with the control group. Besides, the histopathological examination in CaCO₃@NPS treated group did not indicate any pathological damage compared to the control group. To conclude, our synthesized nanoparticles have been shown adequate characteristics of newly synthesized CaCO₃-Cur@NPS for further biomedical applications such as a drug delivery and UCL molecular imaging.
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References

[1] Tse W H, Chen L, McCurdy C M, Tarapacki C M, Chronik B A and Zhang J 2019 Development of biocompatible NaGdF4:Er3+, Yb3+ upconversion nanoparticles used as contrast agents for bio-imaging Can. J. Chem. Eng. 97 2676–84
[2] Joarder B, Yani N and Kimizuka N 2018 Solid-state photon upconversion materials: structural integrity and triplet–singlet dual energy migration Chem. Soc. Rev. 38 976–89
[3] Du H, Yu J, Guo D, Yang W, Wang J and Zhang B 2016 Improving the MR imaging sensitivity of upconversion nanoparticles by an internal and external incorporation of the Gd(3+) strategy for in vivo tumor-targeted imaging Langmuir 32 1155–65
[4] Wang F and Liu X G 2009 Recent advances in the chemistry of lanthanide doped upconversion nanocrystals Chem. Soc. Rev. 38 976–89
[5] Zhou J, Liu Q, Feng W, Sun Y and Li F 2015 Upconversion luminescent materials: advances and applications Chem. Rev. 115 395–465
[6] Min Y, Li J, Liu F, Padmanabhan P, Yeow E K L and Xing D 2014 Recent advance of biological molecular imaging based on lanthanide-doped upconversion-luminescent nanomaterials Nanomaterials 4 129–54
[7] Xia A, Zhang X, Zhang J, Deng Y, Chen Q, Wu S, Huang X and Shen J 2014 Enhanced dual contrast agent, Co2+–doped NaYF4:Yb3+, Tm3+ nanorods, for near infrared–to-near infrared upconversion luminescence and magnetic resonance imaging Biomaterials 35 9167–76
[8] Shigang H, Wu X, Chen Z, Hu P, Yan H, Tang Z, Xi Z and Liu Y 2016 Uniform NaLuF4 nanoparticles with strong upconversion luminescence for background-free imaging of plant cells and ultralow power detecting of trace organic dyes Mater. Res. Bull. 73 6–13
[9] Chung J W, Gerelkhuu Z, Oh J H and Lee Y 2016 Recent advances in luminescence properties of lanthanide-doped up-conversion nanocrystals and applications for bio-imaging, drug delivery, and optosensing Appl. Spectrosc. Rev. 51 678–705
[10] Tu L, Liu X, Wang F and Zhang H 2015 Excitation energy migration dynamics in upconversion nanomaterials Chem. Soc. Rev. 44 1331–45
[11] Haase M and Schafner H 2011 Upconverting nanoparticles Angew. Chem., Int. Ed. Engl. 50 5808–29
[12] Auzel F 2004 Upconversion and anti-stokes processes with f and d ions in solids Chem. Rev. 104 139–74
[13] Chen G Y, Qiu H L, Prasad P N and Chen X Y 2014 Upconversion nanoparticles: design, nanochemistry, and applications in theranostics Chem. Rev. 114 1661–214
[14] Hu S, Wu X, Chen Z, Hu P, Yan H, Tang Z, Xi Z and Liu Y 2015 Uniform NaLuF4 nanoparticles with strong upconversion luminescence for background-free imaging of plant cells and ultralow power detecting of trace organic dyes Mater. Res. Bull. 73 6–13
[15] Shang Y, Hao S, Yang C and Chen G 2015 Enhancing solar cell efficiency using photon upconversion materials Nanomaterials (Basel) 5 1782–809
[16] Auzel F 1990 Upconversion processes in coupled ion systems J. Lumin. 45 341–5
[17] Liu D, Li AH and Sun Z 2015 Single-band red upconversion luminescence of β-NaLuF₄: Gd, Yb, Er@SiO₂ submicron particles via annealing without agglomeration Mater. Res. Express 6 086216
[18] Balabhadra S, Debasu M L, Sbrites C D, Ferreira R A S and Carlos L D 2017 Upconverting nanoparticles working as primary thermometers in different media J. Phys. Chem. C 121 13962–8
[19] Dong H, Sun L D and Yan C H 2015 Energy transfer in lanthanide upconversion studies for extended optical applications Chem. Soc. Rev. 44 1608–34
[20] Zhou J, Liu Q, Feng W, Sun Y and Li F 2015 Upconversion luminescent materials: advances and applications Chem. Rev. 115 395–65
[21] Bettinelli M, Carlos L and Liu X 2015 Lanthanide-doped upconversion nanoparticles Phys. Today 68 58–44
[22] Tian Y, Liu F, Xing M, Ran J, Fu Y, Peng Y and Luo X 2017 Upconversion luminescence properties of Y₂O₃: Eu³⁺@Y₂O₃:Yb³⁺, Tm³⁺ core–shell nanoparticles prepared via homogeneous co-precipitation Opt. Mater. 64 58–63
[23] Ostrowski A D, Chan E M, Garagas D I, Katz E M, Han G, Schuck P J, Milliron D J and Cohen B E 2012 Controlled synthesis and single-particle imaging of bright, sub-10 nm lanthanide-doped upconverting nanocrystals ACS Nano 6 2686–92
[24] Wang F, Deng R and Liu X 2014 Preparation of core–shell NaGdF₄ nanoparticles doped with luminescent lanthanide ions to be used as upconversion-based probes Nat. Protoc. 9 1634–44
[25] Ye X, Collins J E, Kang Y, Chen J, Chen D T N, Yodh A G, Murray C B and Brus L E 2010 Morphologically controlled synthesis of colloidal upconversion nanophosphors and their shape-directed self-assembly Proc. Natl. Acad. Sci. USA 107 22430–5
[26] Boyer J C, Vetrone F, Cuccia L A and Capobianco J A 2006 Synthesis of colloidal upconverting NaYF₄ nanocrystals doped with Er³⁺, Yb³⁺ and Tm³⁺ via thermal decomposition of lanthanide trifluoroacetate precursors J. Am. Chem. Soc. 128 7444–5
[27] Dong H, Sun L D, Wang Y F, Ke J, Si R, Xiao J W, Luy G M, Shi S and Yan C H 2015 Efficient tailoring of upconversion selectivity by engineering local structure of lanthanides in Na₂REF₄ₓ₋ₐ nanocrystals J. Am. Chem. Soc. 137 6569–76
[28] Wilhelm S, Kaiser M, Wurth C, Heiland J, Carrillo-Carrion C, Muhr V, Wolfbeiss O S, Parak W J, Resch–Genger U and Hirsch T 2015 Water dispersible upconverting nanoparticles: effects of surface modification on their luminescence and colloidal stability Nanoscale 7 1403–10
[29] You W, Tu D, Zheng W, Shang X, Song X, Zhou S, Liu Y, Liao R and Chen X 2018 Large-scale synthesis of uniform lanthanide-doped NaREF₄ upconversion/downshifting nanoprobes for bioapplications Nanoscale 10 11477–84
[30] Yin D, Song K, Ou Y, Wang C, Liu B and Wu M 2013 Synthesis of NaYF₄ and NaGdF₄-based upconversion nanocrystals with hydro (Solvos) thermal methods J. Nanosci. Nanotechnol. 13 4162–7
[31] Jeon Y S, Hwangbo S and Hwang K S 2019 Up-conversion photoluminescence of sol-gel derived CaY₂O₄ powders under 980 nm excitation J. Nanosci. Nanotechnol. 19 1709–13
Singh S, Kumar K and Rai S 2009 Multifunctional Er\textsuperscript{3+}/Yb\textsuperscript{3+} codoped Gd\textsubscript{2}O\textsubscript{3} nanocrystalline phosphor synthesized through optimized combustion route Appl. Phys. B\textbf{94} 165–73

DaCosta M V, Doughan S, Han Y and Krull U J 2014 Lanthanide upconversion nanoparticles and applications in bioassays and bioimaging: a review Anal. Chim. Acta\textbf{832} 1–33

Bouzigues C, Gacoin T and Alexandrou A 2011 Biological applications of rare-earth based nanoparticles ACS nano.\textbf{5} 8488–505

Asadi M, Ghahari M, Hassanzadeh-Tabrizi S A, Arabi A M and Nasiri R 2019 Synthesis, characterization, and in vitro toxicity evaluation of upconversion luminescence NaLuF\textsubscript{4}: Yb\textsuperscript{3+}/Tm\textsuperscript{3+} nanoparticles suitable for medical applications Journal of the Chinese Chemical Society (https://doi.org/10.1002/jccs.201900281)

Guo H, Hu Z, Zhao L, Wan L, Wu Y and Wang S 2017 A dual-functional NaLuF\textsubscript{4}: Yb\textsuperscript{3+}/Er\textsuperscript{3+} material for enhancing photon harvesting in dye-sensitized solar cells RSC Adv.\textbf{7} 38506–11