ENGINEERING THE NaYF₄:Yb³⁺/Tm³⁺ UPCONVERSION NANOPARTICLE LUMINESCENCE ENERGY TRANSFER FOR A RATIOMETRIC pH INDICATOR

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Abstract

Fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) is used to develop a ratiometric pH indicator based on NaYF₄:Yb³⁺/Tm³⁺ upconversion nanoparticles (UCNPs). HPTS was functionalized onto the UCNPs surfaces via a modified co-condensation silica coating (UCNP@SiO₂-HPTS). The as-prepared UCNP@SiO₂-HPTS core-shell nanostructure was characterized with transmission electron microscopy, scanning electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy (FT-IR), and photoluminescence spectroscopy. Luminescence resonance energy transfer (LRET) from NaYF₄:Yb³⁺/Tm³⁺ UCNP to HPTS was found to exhibit pH-sensitivity simultaneously under near-infrared (NIR) irradiation (λ = 980 nm).

Keywords: Energy transfer; Luminescence resonance; NIR; Upconversion nanoparticle.
1. INTRODUCTION

Upconversion nanoparticles (UCNPs) have been extensively studied because of their enormous potential biomedical applications, such as biological labeling, imaging, and therapeutics due to the ability to manipulate the emission of colors (Arai & de Camargo, 2021; Li et al., 2019; Mahata et al., 2021). Typically, the absorption phase occurs in the infrared, while the emission phase occurs in the visible or ultraviolet regions of the electromagnetic spectrum. UCNPs exhibit anti-Stokes luminescence, which can convert near-infrared (NIR) light into visible light (Liang et al., 2020; Gao et al., 2021). Interestingly, the NIR laser, which provides a long wavelength, can effectively penetrate a substantial depth into cell tissue and reduce the interference or damage to biological targets. UCNPs convert NIR into higher energy, enabling the highest tissue permeability in all tissue types. Bio-application and therapeutic systems have used UCNPs as a photoreaction inducer (Lee & Park, 2018; Fitzmaurice et al., 2022; Zhang et al., 2021). UCNPs contain three main components: a sensitizer, an activator, and a host component. The sensitizer ensures the energy transfer to the activator and increases the absorption of the doped phosphor (Haase & Schäfer, 2011; Wang & Liu, 2009). Therefore, the activators, which are lanthanide ions, determine the optical properties of the UCNP.

In various biological and clinical applications, pH is a vigorous and crucial physiological parameter (Shangquan et al., 2016). Most cellular activities are sensitive to pH, including cell-cycle progression (Karagiannis & Young, 2001), apoptotic and necrotic cells (Cao et al., 2015), ion transport (Casey et al., 2010), and cell proliferation and activation (Loiselle & Casey, 2003). The measurement of pH provides a critical supplement for understanding the structure and functions of physiological and pathological processes (Loiselle & Casey, 2003). Several techniques have been developed to measure pH values, such as H+ permeable microelectrodes, nuclear magnetic resonance, absorbance spectroscopy, and fluorescence spectroscopy. Recently, optical methods using pH-induced fluctuations in fluorescence intensity have been widely explored for pH measurements, both intracellular and extracellular, and in environmental science (Wu et al., 2016). Several advantages of fluorescent probes are reported, such as excellent spatial and temporal resolution, rapid response, high signal-to-noise ratio, non-invasiveness, and good sensitivity. Their use remains a considerable challenge because the single intensity change is influenced by many non-pH factors, including photo-bleaching and the concentration of probe and environmental effects (Shangquan et al., 2016; Ma et al., 2015). For instance, the response and specialization of most fluorescent pH indicators using light at visible or UV wavelengths can increase the interference with tissue or cause poor optical transparency. A new pH sensor concept uses nanomaterial-based ratiometric signaling. Different ratiometric nanosensors for intracellular monitoring have been developed using nanomaterials such as carbon dots (Espina-Casado et al., 2021; Huang et al., 2020; Wang et al., 2021) and quantum dots (Pacheco-Liñán et al., 2020; Zhang et al., 2022). However, those pH nanosensing systems are generally excited by short wavelengths that also increase the interference background and cannot deeply penetrate tissues or organs. To overcome these challenges, UCNP-based pH sensing platforms have been studied and developed (Tsai et al., 2020; Ma et al., 2015; Näreoja et al., 2017). The UCNPs convert NIR light into a broad range of emissions from...
UV to NIR. In this connection, the NIR excitation source eliminates the background interference and provides deeper tissue penetration. However, most previous studies of UCNP-based pH sensing report measurements in a narrow pH range and use single output signal recording.

In this work, ratiometric pH sensing based on a core-shell nanostructure of NaYF₄:Yb³⁺/Tm³⁺ and SiO₂-HPTS was engineered and demonstrated to exhibit luminescent resonance energy transfer (LRET). Two or more wavelengths of the relative output signal were recorded, and the ratio of different emission intensities revealed the pH-induced fluctuation.

2. MATERIALS AND METHODS

2.1. Materials

Yttrium (III) chloride hexahydrate (YCl₃·6H₂O, Aldrich, 99.99%), ytterbium (III) chloride hexahydrate (YbCl₃·6H₂O, Aldrich, 99.99%), thulium (III) chloride hexahydrate (TmCl₃·6H₂O, Aldrich, 99.99%), tetraethyl orthosilicate (TEOS, Aldrich, 98%), ammonium hydroxide (Daejung, 25%–28%), 3-aminopropyl triethoxysilane (APTES, Aldrich, 98%), sodium hydroxide (Daejung, 97%), ammonium fluoride (Junsei, 97%), Cetyltrimethylammonium Bromide (CTAB), and 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, Aldrich, 97%) were used as supplied, without further purification.

2.2. Methods

Figure 1 illustrates the fabrication procedure and the application of the pH sensing system. The outer surface of NaYF₄:Yb³⁺/Tm³⁺ UCNP was deposited with 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS), a fluorescent pH indicator dye, via co-condensation silica. The attractive optical properties of HPTS, which has pH-dependent absorption at 450 nm, overlapped with the blue emission band (1D2 → 3F4) of NaYF₄:Yb³⁺/Tm³⁺. A novel pH nanosensing UCNP@HPTS-SiO₂ was constructed for luminescence resonance energy transfer. We incorporated a fluorescent dye into a silica layer for UCNP-LRET because it exhibits better optical characteristics than standard organic dyes in terms of absorption, excitation, and emission. The fluorescent dye was protected from the harsh environment by a silica layer, which also prevented photo-bleaching.

2.2.1. Preparation of Yb³⁺/Tm³⁺ codoped β-NaYF₄ nanoparticles (NaYF₄:Yb³⁺/Tm³⁺)

The NaYF₄:Yb³⁺/Tm³⁺ UCNP’s were prepared using the modified solvothermal method. Typically, a mixture of YCl₃ (0.798 mmol), YbCl₃ (0.2 mmol), and TmCl₃ (0.002 mmol) in methanol was added to a mixture of oleic acid (6 mL) and octadecene (15 mL) in a three-neck flask. The resulting solution was next heated to 140°C under vigorous stirring and nitrogen flow for an hour to remove water and oxygen. After cooling to 50°C, 5 mL of methanol solution containing NH₄F (0.15 g) and NaOH (0.1 g) was added dropwise. The mixture was slowly heated and kept at 110°C for 30 min to completely remove methanol and residual water. Then, the reaction mixture was quickly heated and kept at 305°C for 1.5 h. The product was collected by centrifugation.
(5000 rpm, 10 min). The final UCNPs were washed with toluene three times and re-dispersed in 5 mL of toluene.

Figure 1. Engineering of NaYF₄:Yb³⁺/Tm³⁺ UCNP-based LRET

Notes: (a) Fabrication process of UCNP@SiO₂-HPTS; (b) Design strategy.

2.2.2. Preparation of SiO₂ and HPTS coated UCNP (UCNP@SiO₂-HPTS)

Before the deposition of a layer of SiO₂ and HPTS on the UNCPs, the surface ligand of the as-prepared UNCPs was simply exchanged from oleic acid (OA) to Cetyltrimethylammonium Bromide (CTAB) by water-bath sonication for an hour. Then, the CTAB-UNCPs were separated by centrifugation and stored in ethanol (15 mL) for further use. Meanwhile, HPTS (30 mg) was dissolved in ethanol (1.5 mL) and mixed with APTES (5 µL) in the dark for 2 h before adding TEOS (300 µL). UCNP@SiO₂-HPTS was fabricated by the co-condensation method. The solution of CTAB-UCNP in ethanol was added to NH₄OH (400 µL) as a catalyst. Then, the mixture of TEOS and HPTS in ethanol was slowly added to the solution, which was kept under vigorous stirring at room temperature for 2 h. The final UCNP@SiO₂-HPTS product was collected by centrifugation (5000 rpm, 10 min) and washed with deionized water three times before drying in a vacuum at 60°C for 24 h.

2.2.3. Instrumentation

NaYF₄:Yb³⁺/Tm³⁺ UCNP was characterized with a Rigaku DMAX 2200 X-ray diffractometer using K-α radiation (λ = 1.506 Å) from 10° to 90°. The morphologies of the nanoparticles were checked using a JEOL JSM-7500F scanning electron microscope.
(SEM) and a Hitachi H-7100 transmission electron microscope (TEM). The photoluminescence emissions were measured with a Cary Eclipse fluorescence spectrophotometer using 980-nm continuous-wave laser excitation. FT-IR spectra were obtained with a Jasco FT/IR-4100 spectrometer and UV/vis absorption spectra were measured with a SCINCO spectrophotometer.

3. RESULTS AND DISCUSSION

![Figure 2](image_url)

**Figure 2. The characterization of the as-prepared NaYF₄:Yb³⁺/Tm³⁺ UCNP**

Notes: (a) SEM images of NaYF₄:Yb³⁺/Tm³⁺ UCNP; (b) TEM image of NaYF₄:Yb³⁺/Tm³⁺ UCNP; (c) Digital photograph showing the blue emission color of UCNP dispersed in toluene; (d) UCNP luminescent spectrum under 980 nm NIR excitation; (e) XRD pattern of NaYF₄:Yb³⁺/Tm³⁺ UCNP and the standard pattern of pure hexagonal NaYF₄ (JCPDS card no. 16-0334); (f) EDS spectrum of NaYF₄:Yb³⁺/Tm³⁺ UCNP.

Mono-dispersed NaYF₄:Yb³⁺/Tm³⁺ UCNP (79.8:20:0.2) were successfully synthesized at 305°C for 1 h. The UCNP fabrication process employed a liquid-solid two-phase strategy. At high temperatures, the nanoparticles transformed from the cubic phase to the major hexagonal phase. Figure 2 shows SEM images (Figure 2a) and a TEM image (Figure 2b) of hexagonal UCNP approximately 120 nm in size. The as-fabricated nanoparticles are hydrophobic and well-dispersed in a nonpolar organic solvent such as cyclohexane and toluene because their surfaces are capped with oleic acid as a ligand. Figure 2c presents a digital photograph of bright blue emission under NIR laser (λ = 980 nm) irradiation. The photoluminescent spectrum of prepared NaYF₄:Yb³⁺/Tm³⁺ UCNP was measured at room temperature and is displayed in Figure 2d. The Yb³⁺/Tm³⁺-doped UCNP exhibited sharp emission bands at 450, 475, 650, and 800 nm, which are related to the 1D2 → 3F4, 1G4 → 3H6, 1G4 → 3F4, and 3H4 → 3H6 energy transitions of Tm³⁺ ions, respectively (Meng et al., 2022; Liu et al., 2014; Wang et al., 2008). The X-ray diffraction (XRD) pattern of NaYF₄:Yb³⁺/Tm³⁺ UCNP is shown in Figure 2e. The
diffraction peak positions confirm the hexagonal phase of NaYF$_4$ (JCPDS standard card no. 16-0334) (Ding et al., 2015). The energy dispersive spectroscopy (EDS) result indicated the signal of elements in the components of the prepared UCNP, which are Na, F, Br, Tm, Yb, and Tm (Cui et al., 2011), as shown in Figure 2f.

![Figure 3. Characterization of UCNP@SiO$_2$-HPTS](image)

Notes: (a) SEM and TEM images of the UCNP@SiO$_2$-HPTS structure; (b) FT-IR spectra of OA-UCNP, HPTS, and UCNP@SiO$_2$-HPTS.

A ligand exchange process from OA to CTAB was carried out to turn UCNPs from hydrophobic to hydrophilic. The ligand of OA has demonstrated a significant influence on the product NaYF$_4$ nanoparticles. Here the hydrophobic UCNPs in toluene were transferred into an aqueous solution using CTAB, followed by a co-condensation reaction. The hydrophobic OA on the UCNP surface strongly interacted with the hydrophobic tails of the CTAB. Consequently, the hydrophilic head groups of CTAB rendered the UCNPs water soluble. The transfer of UCNPs into an aqueous solution using CTAB is a critical factor for depositing the silica layer and the HPTS on the outer surface of the UCNPs. The deposition of the SiO$_2$ and HPTS on the UCNPs is also confirmed by SEM and TEM images, as shown in Figure 3a. The hybrid nanoparticles, which are composed of the UCNPs and a core-shell structure of SiO$_2$ and HPTS, were successfully fabricated. The deposition of the SiO$_2$ layer and HPTS is a critical factor for the application of UCNPs in bio-imaging and in drug labeling and delivery under physiological conditions and environments. The core-shell structure with the silica shell endows the HPTS with good distribution and stability in the water phase. The FT-IR spectra of OA-UCNP, HPTS, and UCNP@SiO$_2$-HPTS are shown in Figure 3b. Typical OA-UCNP peaks were observed at 1560 and 1419 cm$^{-1}$, arising from C=C stretching and C–H scissoring vibrations, respectively (Ma et al., 2015; Tian et al., 2010). Peaks in the spectrum of UCNP@SiO$_2$-HPTS appeared at 1057, 1450, 1667, and 3340 cm$^{-1}$, which is attributed to the stretching vibration of Si–O, shear bending vibration of N–H, and stretching vibration of N–H The characteristic peaks of HPTS were recorded at 2650, 2165, 2104, and 1635 cm$^{-1}$ corresponding to the stretching vibrations of the rings. These results indicate that the SiO$_2$ and HPTS were successfully deposited on the
NaYF₄:Yb³⁺/Tm³⁺ UCNPs after the ligand exchange and co-condensation polymerization reactions.

![Figure 4](image)

**Figure 4. Direction of energy transfer between NaYF₄:Yb³⁺/Tm³⁺ UCNP and HPTS**

Notes: (a) Overlap between HPTS absorption and UCNP emission; (b) Digital photograph of the blue emission color of pure UCNP powder; (c) Red emission color of UCNP@SiO₂-HPTS powder under irradiation by a 980-nm NIR laser.

The excellent overlap between the emission bands of NaYF₄:Yb³⁺/Tm³⁺ UCNPs and the absorption band of HPTS at 450 and 475 nm indicates efficient luminescence resonance energy transfer from NaYF₄:Yb³⁺/Tm³⁺ UCNPs to HPTS (Ma et al., 2015). Figure 4A presents the close match between the luminescence of NaYF₄:Yb³⁺/Tm³⁺ UCNPs and the absorption band of HPTS. Under the excitation of an NIR laser (λ = 980 nm), the Tm³⁺-doped UCNPs show sharp Tm³⁺ emission bands at 450 nm (1D₂ → 3F₄), 475 nm (1G₄ → 3H₆), and 650 nm (1G₄ → 3F₄) (Meng et al., 2022). The blue and red colors of UCNP and UCNP@SiO₂-HPTS under 980-nm excitation by an NIR laser are shown in Figures 4B and 4C. Under NIR excitation, the blue emission of the UCNPs was absorbed by HPTS, and only red emission is exhibited. Moreover, the emission of HPTS, a fluorescent dye, has been well documented at 520 nm. Since the luminescence of UCNP@SiO₂-HPTS was also expected to appear at 520 nm, the pH monitoring can be observed from separation bands at 450, 475, and 520 nm. The luminescence energy transfer from UCNP to organic dye or another nanomaterial has been reported (Li et al., 2012; Zayakhuu et al., 2015; Sun et al., 2009), and a number of multifunctional biosensors have been developed for applications in biological sensing and labeling. Relative to that of the previous NaYF₄:Yb³⁺/Tm³⁺ UCNP and pH indicator
combination, the use of fluorescent pH indicator (HPTS) as the acceptor for the donor of NaYF₄:Yb³⁺/Tm³⁺ UCNP.

![Figure 5. The effect of pH on the as-prepared core-shell structure](image)

**Notes:**
(a) The decrease of the HPTS absorption band at 450 nm over a pH range of 4–9 in a buffer solution (0.5 mg mL⁻¹). The inset is a visualization of the pH-dependent response of HPTS;
(b) Upconversion luminescent spectra of UCNP@SiO₂-HPTS response to pH from 4 to 9 under excitation by a 980-nm NIR laser;
(c) Titration curve of ratiometric luminescent intensity I₄₅₀/I₆₅₀ over a pH range of 4–9;
(d) Titration curve of ratiometric luminescent intensity I₄₇₅/I₆₅₀ over a pH range of 4–8.

A good overlap exists between the emission bands of NaYF₄:Yb³⁺/Tm³⁺ UCNP and the absorption band of HPTS at ~450 nm (Figure 5a), which efforts efficient energy transfer from NaYF₄:Yb³⁺/Tm³⁺ UCNP to HPTS, in which the UCNP donor emits light and the HPTS acceptor absorbs it (Ma et al., 2015). Under the excitation of an NIR laser, the emission of NaYF₄:Yb³⁺/Tm³⁺ UCNP at ~450 nm (1D2 → 3F4) absorbed by the HPTS is efficient in LRET. The inset is a visualization of the pH-dependent response of HPTS. When the pH increased from 4 to 9, the absorption intensity of HPTS at 450 nm was gradually enhanced. Also, the emission band at 520 nm appeared after luminescent
resonance energy transfer to HPTS. The pH-dependence of UCNP@SiO$_2$-HPTS was investigated in a 30-mM solution of phosphate buffered saline (PBS) buffer. As shown in Figure 5b, the emission intensity of UCNP at 450 and 475 nm decreased continuously over the pH range from 4 to 9; however, the emission band at 520 nm appeared and gradually increased in response to the increased absorption of HPTS at 450 nm. When the pH value is low, the absorption band of HPTS at 450 nm is weak, resulting in high UCNP emission intensities at 450 and 475 nm. When the pH value increased, the absorption band of HPTS at 450 nm recovered gradually, resulting in a remarkable decrease in the emission intensities of UCNP at 450 and 475 nm as well as an increase at 520 nm. The change in pH value was not observed to influence the emission band at 650 nm. The ratiometric sensing of pH can be realized by the ratio of the emission bands at 520 and 475 to that at 650 nm. The luminescence intensity ratios $I_{520}/I_{650}$ and $I_{475}/I_{650}$ of UCNP@SiO$_2$-HPTS were plotted against pH values (Figures 5c and 5d). The UCL intensity ratio of $I_{520}/I_{650}$ was fit with the equation $y = 6.58009E^{-4}x^{4.00413}$ ($R^2 = 0.99154$) over the pH range of 4 to 9; while the $I_{475}/I_{650}$ ratio was fit with the linear equation $y = 0.04457x + 7.27217$ ($R^2 = 0.99084$) over the pH range of 4 to 8. The changes in luminescent emission intensity were caused by the discrepancy in energy transfer from NaYF$_4$:Yb$^{3+}$/Tm$^{3+}$ UCNP donors to HPTS acceptors. The large overlap between NaYF$_4$:Yb$^{3+}$/Tm$^{3+}$ UCNP emission and HPTS absorption bands ensured efficient luminescence resonance energy transfer.

4. CONCLUSIONS

In this work, a sensing platform consisting of a hybrid nanostructure of NaYF$_4$:Yb$^{3+}$/Tm$^{3+}$ UCNP and pH indicator fluorescent dye HPTS was engineered and fabricated. The NaYF$_4$:Yb$^{3+}$/Tm$^{3+}$ UCNPs exhibited their main emission bands at 450, 475, 650, and 800 nm. The peak at 450 nm overlapped with the HPTS absorption band, providing efficient energy transfer from NaYF$_4$:Yb$^{3+}$/Tm$^{3+}$ UCNP to HPTS. Two wavelengths of relative output signal were recorded, and the ratio of the different emission intensities revealed the pH-induced fluctuation at 520 and 475 nm over pH values from 4 to 9. The results indicate that the as-prepared UCNP@SiO$_2$-HPTS exhibits great luminescence energy transfer from NaYF$_4$:Yb$^{3+}$/Tm$^{3+}$ UCNP to HPTS under near-infrared (NIR, $\lambda = 980$ nm) irradiation. The sensing platform is suitable for developing intracellular pH monitoring, paving the way for measuring the most biologically relevant materials and for bio-imaging diagnostics.

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REFERENCES


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