**Microwave assisted synthesis and characterizations of near infrared emitting Yb/Er
doped fluorapatite nanoparticles**

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Abstract

Trivalent Yb and Er doped fluoroapatite nanoparticles were prepared by a microwave assisted wet precipitation method using calcium nitrate tetrahydrate, diammonium hydrogen phosphate, ammonium fluoride, and Yb, Er nitrate tetrahydrate as precursors. Well crystallized nanoparticles of rod like morphology with length and width of about 26 ± 3 and 9 ± 2 nm was obtained. Under 980 nm excitation the nanoparticles exhibit strong near infrared emission at 1532 nm with a maximum measured internal quantum yield of 74% in 3 mol% Er and 9 mol% Yb doped sample which is 26% less than the 100% radiative quantum yield predicted by Judd-Ofelt procedure. The measured quantum yield shows a linear dependence of quantum yield on Yb concentration.

Keywords: Biomaterials; Nanoparticles; Optical properties; Quantum yield; Rare earth

1. Introduction

Fluoroapatites (FAP) have drawn a great deal of interest from researchers because of their potential use as biomedical materials, such as coating material for metallic implants, dental implants, drug delivery and deep tissue bioimaging because of their excellent biocompatibility, and similarity to the component of human bones [1-4]. FAP materials are also potential candidates for use in cell targeting, fluorescence labeling, imaging and diagnosis [5-7].

FAP crystallizes in hexagonal system with $P6_3/m$ space group. FAP contains four nonequivalent ions such as F^- , PO_4^{3-} , Ca^{2+} (I) and Ca^{2+} (II) [8] and all these ions can be doped with various ions depending on the nature of the substituting ions [9-11]. When compared to hydroxyapatite (HAP), FAP shows improved physicochemical properties such as thermal stability (melting point ~ 1650 °C), chemical stability, crystallinity and corrosion resistance [12,13]. The low vibrational energies of FAP favor for rare earth fluorescent transition that makes it suitable for biomedical imaging [14]. Moreover, FAP has shown enhanced bone cell proliferation, osteointegration and mineralization through the precipitation process that occurs with calcium and phosphate [15,16].

Recently, there has been so much interest in trivalent rare earth doped nanomaterials for several biophotonics applications such as optical imaging, therapy and drug delivery [17-19]. The ability to engineer the nanoparticles and tune the optical properties has made it possible to enhance the contrast and target the selected cells for diagnosis and treatment of diseases in an effective and noninvasive manner [20,21]. Fluorescence imaging is a powerful tool for biomedical imaging which makes use of a specific probe that is excited with a visible or near infrared (NIR) radiation and results in the visible or NIR emission [22,23]. High sensitive detection, fast feedback, ability to act as cellular and biomolecular markers are its advantages

while penetration depth is its major disadvantage. Biological optical transparency window with low absorption and scattering from biological system falls in the regions: 650 – 900 nm and 1000 – 1450 nm, termed as NIR I and NIR II windows, respectively [24]. Imaging sensitivity can be enhanced up to 10 times with emissions in the range of 1100 to 1600 nm [25-28]. Narrow emission bandwidths, less systemic toxicity, tunability of emission and stability make the inorganic rare earth doped nanoparticles extremely suitable for biomedical imaging applications [29,30]. Rare earths can be doped in a variety of materials such as phosphates, fluorides, glasses, etc. With Ytterbium (Yb^{3+}) as the sensitizer under 980 nm excitation, emission in NIR II and up to 1600 nm had been reported using different rare earth ions Ho^{3+} , Pr^{3+} , Tm^{3+} , Er^{3+} in various hosts such as phosphate and telluride glasses, fluoride and oxide materials [31-37]. Among phosphate hosts, FAP ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$) is of special interest due to its high biocompatibility. FAP is structurally and chemically similar to HAP, and known to be more chemically stable and easier to synthesize as a stoichiometric compound [38]. Due to the similarity with bone material fluorescent AP type nanoparticles can be considered as a suitable contrast agent in medical imaging applications. There are several reports on the synthesis and application of Eu^{3+} doped HAP as optical contrast agent [39,40], where the red fluorescence from Eu^{3+} can be used to image cells under UV excitation. However, the inherent limitations of the short wavelength excitation and emission in cellular imaging *viz.* tissue autofluorescence under UV excitation and low penetration depth due to scattering make this technique almost impossible to acquire intracellular details at greater depths. To circumvent these limitations, recently NIR emitting NIR nanomaterials are considered and the application of such materials shows very promising results. In this paper, we report for the first time, Yb^{3+} and Er^{3+} doped FAP with strong NIR emission

properties. We propose that under optimized conditions, the synthesized nanoparticles could be a potential optical contrast in medical imaging applications.

2. Experimental

2.1. Synthesis

Pure and Yb/Er doped FAP nanoparticles were prepared by microwave assisted wet precipitation method using calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$), ammonium fluoride (NH_4F), trisodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$), ammonia solution (NH_4OH) (all analar grade from Merck) and Erbium nitrate pentahydrate ($\text{Er}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$) and Ytterbium nitrate pentahydrate ($\text{Yb}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$) (all analar grade from Sigma Aldrich) as the starting precursors. For the preparation of FAP nanoparticles, 1 M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.6 M $(\text{NH}_4)_2\text{HPO}_4$, 0.2 M NH_4F and 0.334 M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ solutions were separately prepared and brought to pH 10 using NH_4OH . Trisodium citrate solution was first mixed with calcium solution to form a Ca-citrate complex followed by the drop-wise addition of $(\text{NH}_4)_2\text{HPO}_4$ and NH_4F solutions into the Ca-citrate complex. The solution was stirred vigorously for 1 h while maintaining the pH 10. The prepared mixture was put in a microwave oven [Samsung, 2.45 GHz, 600 W, (105±5 °C)] and irradiated with microwave for 25 min. The obtained product was centrifuged at 5000 rpm, and washed 3 times with double distilled water to remove the byproducts. The obtained precipitate was dried using freeze dryer (Boyikang Laboratory Instruments, China). The formation of FAP can be described as follows

$\text{Yb}^{3+}:\text{Er}^{3+}$ doped FAP with different molar concentration of Yb and Er were prepared by dissolving appropriate amount of $\text{Yb}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ and $\text{Er}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ in calcium solution prior to mixing with trisodium citrate. The reaction for the formation of the $\text{Yb}^{3+}:\text{Er}^{3+}$ doped FAP is expressed as below

where X and Y are Yb^{3+} and Er^{3+} molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3). All the synthesized samples were heat treated using a muffle furnace (NSW103 model) at 1300 °C for 2 h. The four sets of samples prepared will be here after referred as 1, 2, 3, 4 and FAP respectively.

2.2. Characterizations

The powder X-ray diffraction (PXRD) patterns of the as-synthesized and heat treated samples were recorded using a Rigaku MiniFlex II powder X-ray diffractometer in the 20-60° range) using Ni-filtered Cu K α radiation (1.5406 Å). Fourier transform infrared (FT-IR) spectra were recorded using a Perkin Elmer RX I FT-IR spectrometer in the region of 4000-400 cm⁻¹ with a 4 cm⁻¹ resolution by KBr pellet technique. The morphology of the samples was examined using high resolution transmission electron microscopy (HRTEM, JEOL JEM-2100, Japan). **The elemental analysis was carried out by an energy dispersive X-ray analyzer (EDX) (INCA, Oxford Instruments). The morphology of heat treated samples were examined using JEOL JSM-6060 scanning electron microscope (SEM).** Optical absorption spectra of the heat treated samples were recorded on a PerkinElmer Lambda 19 spectrophotometer. NIR fluorescence measurements were done by exciting the samples using 980 nm laser (Spectra Physics, Model 3900S) and the emission of the sample was recorded by the Quanta Master 51 spectrofluorimeter

(Photon Technology International Inc. NJ, U.S.) with an InGaAs detector (Teledyne Judson Technologies, 062-8451, U.S.). Photoluminescence decay curves of 1535 nm emission were measured on a Quanta Master 40 system (Photon Technology International Inc. NJ, U.S.) using the single shot transient digitizer technique with a Nitrogen pumped Dye Laser (Photon Technology International part GL-3300 + GL-302) as excitation source. The Nitrogen laser, with 800 ps pulse width, pumps a high resolution dye chamber to give 523 ± 0.04 nm light. The collected decay curve was analyzed by Origin 8 software (Origin lab, U.S.). For the quantum yield (QY) measurements, we employed a barium sulfate coated 203 mm diameter integrating sphere (Oriel, Model 70451) mounted on the side of the spectrofluorimeter sample chamber and excited by the 980 nm laser. More details about the QY measurements can be seen in our previous publications [41].

3. Results and discussion

3.1. Phase and morphology

Fig. 1(a) shows the XRD patterns of as-prepared samples. All the diffraction peaks can be ascribed to the FAP phase (JCPDS file No. 15-0876). The broad diffraction peak in the region 31° to 35° that correspond to the diffractions from (211), (112), (300) and (202) indicate the poor crystalline nature of the as-prepared samples. It is important to note that the position of diffraction peaks shifts to higher angle side for Yb^{3+} and Er^{3+} ion doped samples. The change in lattice parameters confirms the incorporation of Yb^{3+} and Er^{3+} ions in the FAP crystal [42,43]. The variation in the lattice parameters may be due to the smaller ionic radius of Yb^{3+} (0.86 Å) and Er^{3+} (0.89 Å) compared to Ca^{2+} (1.0 Å). Moreover, the intensity of the diffraction peaks decrease when the concentration of Yb^{3+} increases which indicates cation substitution inhibits FAP crystallization.

The powder XRD pattern of the 1300 °C heat treated sample (Fig. 1(b)) was found to be phase pure without any impurity peaks. Heat treated samples show very well resolved intense peaks from (002), (211), (300), (202), (213), (222) and (213) planes which illustrate that the crystallinity of the samples improved with heat treatment at 1300 °C. Heat treatment of the samples suppressed the crystallization inhibition effects, enabling the FAP to act as a suitable host for the doped ions [44,45].

TEM images of as-prepared samples are shown in Fig. 2. As it can be seen in the figure, FAP exhibits rod like morphology with length and width of about 26 ± 3 and 9 ± 2 nm, respectively and the particles are highly agglomerated. Yb^{3+} and Er^{3+} ions substitution in FAP reduced the length of the particles by 21 ± 2 nm. Particle size distributions (PSD) of the samples are obtained using the image analysis program ImageJ and the results are shown in Fig. 3. Based on the results the PSD of the particles are 20.49 ± 6.57 nm (FAP), 13.17 ± 5.78 (1), 14.09 ± 4.75 (2), 13.18 ± 4.82 (3) and 15.81 ± 3.19 nm (4).

The EDX spectra obtained from the elemental analysis of the synthesized samples are shown in Fig. 4. The peaks observed for FAP nanoparticles correspond to Ca, P, O and F, while Yb/Er doped FAP nanoparticles exhibited additional peaks corresponding to Yb and Er. The elemental composition acquired from EDX is given in Table 1. These results suggest that Yb and Er ions are incorporated in the FAP host. Also, it is found that the concentration of Yb increased with increase in doping concentration. The measured elemental composition of the samples is in good agreement with the expected values. The Ca/P value of FAP and $(\text{Ca} + \text{Yb} + \text{Er}) / \text{P}$ value of Yb /Er doped FAP nanoparticles is 1.67 or very close to 1.67.

SEM images of heat treated samples are shown in Fig. 5. All the heat treated samples exhibited a higher rate of grain growth and the morphology of Yb/Er doped FAP samples was obviously different from that of the FAP sample. FAP sample contained distinct grains of smaller size compared to that of Yb/Er doped FAP samples. Sample 1 is composed of large interconnected grains. Individual elongated grains of varying sizes are observed in sample 2 and 3 while irregular shaped larger and smaller grains existed in sample 4.

3.2. FTIR

FTIR spectra of as-prepared samples are shown in Fig. 6(a). The FT-IR spectrum of FAP shows the characteristic PO_4^{3-} (ν_4) vibrations of FA at 568 and 605 cm^{-1} along with other ν_1 , ν_2 and ν_3 phosphate peaks at 471, 964 and 1017 - 1109 cm^{-1} , respectively. The broad band extending from 2500 to 3600 cm^{-1} is attributed to the ν_3 and ν_1 stretching modes of the hydrogen bonded H_2O molecule absorbed in the apatite structure. [46-48]. During the preparation of the FAP with trisodium citrate, Ca^{2+} - citrate complex formation and adsorption Cit^{3-} groups on the FAP are possible owing to the strong chelating ability of Cit^{3-} groups and the adsorption effect of FAP. The existence of citrate species in the samples is revealed by the two strong bands at ~1405 and 1595 cm^{-1} assigned to the citrate anti-symmetric and symmetric stretching of carboxylate groups COO^- , respectively [43].

Fig. 6(b) shows the FTIR spectra of 1300 °C heat treated samples. On heat treatment the COO^- group disappeared due to the decomposition of citrate groups above 300 °C [43]. The broad peak around 3453 cm^{-1} attributed to the H_2O molecules disappeared with the heat treatment. The intensity of bands assigned to PO_4^{3-} increased with increasing temperature.

3.3. Radiative properties

The room temperature absorption spectra of the samples are shown in Fig. 7 with all spectral band assignments. All the absorption bands and their barycenters are found to be almost identical except with some differences in the band intensities. The absorption bands of Er^{3+} ion corresponds to transitions from the $^4\text{I}_{15/2}$ ground state to the various excited levels. By fitting the absorption spectral data with Judd-Ofelt model [49,50], we can predict the radiative quantum yield using the equation $\mu_{\text{rad}} = (\tau_{\text{rad}}/\tau_{\text{lum}})$ [51].

Here τ_{lum} is the fluorescence decay time and τ_{rad} is the radiative decay time calculated using the expression

$$\frac{1}{\tau_{\text{rad}}} = A_{\text{rad}}(J' \rightarrow J) = \frac{64\pi^4 e^2}{3h(2J'+1)\lambda^3} \left(\frac{n(n^2+2)^2}{9} S_{\text{ed}} + n^3 S_{\text{md}} \right)$$

where A_{rad} represents the radiative transition probability for transition from excited state with total angular momentum quantum number J' to ground state J . For Er^{3+} the excited state and ground state corresponding to the 1535 nm emission are $^4\text{I}_{13/2}$ and $^4\text{I}_{15/2}$ respectively. h is plank's constant, n is the refractive index, λ is the peak emission band wavelength, and e is the charge of the electron. S_{ed} and S_{md} are the electric dipole and magnetic dipole line strengths calculated using the expressions [52]

$$S_{\text{ed}}(J' \rightarrow J) = \sum_{t=2,4,6} \Omega_t \left| \left\langle (S', L') J' \right\| U^{(t)} \left\| (S, L) J \right\rangle \right|^2$$

$$S_{\text{md}} = \frac{h^2}{16\pi^2 m^2 c^2} \left| \left\langle (S', L') J' \right\| L + 2S \left\| (S, L) J \right\rangle \right|^2$$

where Ω_2 , Ω_4 , Ω_6 are the Judd-Ofelt phenomenological parameters and the $\|U^{(t)}\|$ factor represent the doubly reduced transition matrix elements of rank t ($t=2, 4, 6$). The best set of Ω parameters

are determined by a standard least square fitting of the experimental oscillator strength to the theoretical oscillator strength formula [49,50] and the values obtained are $\Omega_2 = 0.4 \times 10^{-20} \text{ cm}^2$, $\Omega_4 = 1.5 \times 10^{-20} \text{ cm}^2$, $\Omega_6 = 4.1 \times 10^{-20} \text{ cm}^2$. Following this procedure a radiative decay time of 3.3 ms was obtained and that yield a radiative quantum yield of 100%.

Fig. 8 shows the emission spectra of the heat treated samples. All the samples exhibited broad NIR emission at ~1535 nm under 980 nm excitation. No significant variation in position and shape of emission bands was observed and the 1535 nm emission band is assigned to $^4I_{13/2} \rightarrow ^4I_{15/2}$ transition. The emission intensity of 1535 nm peak shows only slight variations in the intensity with increasing of Yb concentration up to 8 mol%. None of the samples showed any upconversion emission indicating that all excitation energy has been emitted as NIR emission at 1535 nm. Fig. 9 shows the energy level diagram of the $\text{Yb}^{3+}/\text{Er}^{3+}$ system and the mechanism proposed to explain the NIR emission under 980 nm excitation. Yb^{3+} exhibits strong absorption at 980 nm which makes it an ideal candidate for use as a sensitizer for Er^{3+} emission [53,54]. This sensitization effect is based on the quasi-resonance in energy of the $^2F_{5/2}$ level of Yb^{3+} with the $^4I_{11/2}$ level of Er^{3+} . The absorption cross section of Yb^{3+} is very much higher than that of Er^{3+} under 980 nm, the pump energy can be efficiently absorbed and transferred to Er^{3+} . After the energy transfer to Er^{3+} , a fast non-radiative transition between the $^4I_{11/2}$ and the $^4I_{13/2}$ states takes place. Spectrum collected with narrow slit width and lock in amplifier setting shows 11 well resolved stark levels of Er^{3+} [55] as shown in Fig. 8 (right).

The decay curves obtained from the measurements are shown in Fig. 10. All the samples exhibit double exponential decay curves and the decay time was obtained by fitting the curve with a double exponential function of the form $I = A_1 \exp(-\frac{t}{\tau_1}) + A_2 \exp(-\frac{t}{\tau_2})$ using Origin 8 curve fitting program and the fitting parameters are summarized in Table 2. Average decay time

was calculated from the corresponding fitting parameters using the equation $\tau_{av} = \frac{A_1\tau_1^2 + A_2\tau_2^2}{A_1\tau_1 + A_2\tau_2}$.

As seen in this table, the average lifetimes are 3.34, 3.47, 3.22 and 3.38 ms for 1, 2, 3 and 4 samples, respectively.

Quantum yield is obtained as the number of emitted photons vs. number of absorbed photons. Results of quantum yield measurements of heat treated sample under 980 nm excitation are shown in Fig. 11. As seen in figure, the quantum yield is 52%, 61% and 74% for samples 1, 2 and 4, respectively. Quantum yield of the samples increased when Yb concentration increased, as energy transfer efficiency from Yb to Er improved. Though the predicted radiative quantum yield approach the maximum theoretical efficiency of 100%, experimental internal quantum yield reaches only to a maximum value of 74 %. This is 1.48 times higher than the quantum yield obtained for the β -NaYF₄: Yb, Er nanoparticles of size 110 nm [56]. The reported internal quantum yield of the Yb/Er doped FAP is comparatively higher than other reported fluoride based materials. Further, since the present material compositions shows a single emission band at 1535 nm this types of NIR emitting nanomaterials would find several technological applications such as biomedical imaging, fiber amplifiers, counterfeiting applications, *etc.* Due to the recently increasing interest of a fully biocompatible NIR sensitive contrast agent in biomedical imaging applications, we are in the process of exploring the possibilities of the proposed materials for intracellular imaging and *in vivo* imaging applications.

4. Conclusions

Microwave assisted wet precipitation method is a viable method for the synthesis of Yb/Er doped rod shaped fluorapatite nanoparticles in the size range of 13-20 nm with comparatively narrow particle size distributions. All the prepared samples are highly NIR emissive at 1535 nm with no other emission bands in the visible region indicating that there are

no excited state upconversion processes in the material. Though the predicated radiative quantum yield approach the theoretical limit of 100% the experimental internal quantum yield shows a linear tendency with Yb concentration. The comparatively higher quantum efficiency, single NIR emission at 1535 nm, smaller size as well as the excellent biocompatibility of these nanoparticles investigated are expected to enable them for various bio-applications such as biomedical imaging .

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Table Captions

Table 1

Elemental composition of the samples

Sample	Elemental composition (At.%)						(Ca+Yb+Er)/P
	O	P	F	Ca	Yb	Er	
FAP	71.73	7.43	8.38	12.47	-	-	1.67
1	64.35	12.89	1.22	19.39	1.48	0.67	1.67
2	63.11	13.39	1.16	20.09	1.63	0.62	1.66
3	60.42	14.05	2.07	20.82	1.75	0.89	1.66
4	62.80	13.12	2.14	19.03	2.27	0.64	1.67

Table 2

Decay times and fitting parameters of heat treated samples

Sample Code	A₁	A₂	τ_1 (μs)	τ_2 (ms)	τ_{av} (ms)
1	0.048	0.019	0.26	3.87	3.34
2	0.017	0.029	3.86	0.27	3.47
3	0.041	0.102	3.61	0.18	3.22
4	0.080	0.079	3.53	0.16	3.38

Figure Captions

Fig. 1. XRD patterns of the FAP nanoparticles with various Yb/Er concentrations (a) as-prepared, (b) heat treated at 1300 °C. Samples 1,2,3,4 are with Yb³⁺ and Er³⁺ molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3).

Fig. 2. TEM images of as-prepared samples for various Yb/Er concentrations. Samples 1,2,3,4 are with Yb³⁺ and Er³⁺ molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3). FAP is un-doped nanoparticle.

Fig. 3. Particle size distribution of the nanoparticles. Samples 1, 2, 3, 4 are with Yb³⁺ and Er³⁺ molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3). FAP is un-doped nanoparticle.

Fig. 4. EDX spectra of synthesized samples.

Fig. 5. SEM images of heat treated samples

Fig. 6. FTIR spectra of the (a) as-prepared and (b) 1300 °C heat treated sample indicating the various vibration spectral band assignment.

Fig. 7. Optical absorption spectra of Yb/Er doped FA nanoparticles with spectral band notations for various observed bands. Samples 1,2,3,4 are with Yb³⁺ and Er³⁺ molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3).

Fig. 8. (left) Emission spectra of the nanoparticles under 980 nm excitation. Samples 1,2,3,4 are with Yb³⁺ and Er³⁺ molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3), (Right) Emission spectrum of sample 4 collected with different slit and lock in amplifier settings.

Fig. 9. Energy level diagram of Yb³⁺/Er³⁺ system and the mechanism of NIR emission.

Fig. 10. Fluorescence decay curve of the 1535 nm emission under 980 nm excitation. Samples 1,2,3,4 are with Yb³⁺ and Er³⁺ molar concentrations viz. (0.6:0.3), (0.7:0.3), (0.8:0.4) and (0.9:0.3).

Fig. 11. Quantum yield of 1535 nm emission measured with 980 nm laser excitation.