Luminescent Hydroxyapatite Nanoparticles for Bioimaging Applications

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Abstract— Here we report the microwave synthesis of Eu^{3+} substituted HA nanoparticles and its properties towards bioimaging applications. Photoluminescence studies on Eu^{3+} substituted HA (Eu-HA) nanoparticles revealed the most intense peak around 617 nm and the fluorescence imaging of the same using confocal microscope exhibited red emission. Hence Eu-HA nanoparticles can be used as a bioprobes for fluorescence targeting.

Index Terms— Bioimaging; Hydroxyapatite; Photoluminescence.

1 Introduction

Bioimaging is a powerful tool used in biomedicine for early diagnosis and the effective treatment of disease [1]. Several luminescent materials such as fluorescent organic molecules, semiconductor quantum dots and rare earth elements have been widely investigated as contrast agents for bioimaging [2], [3], [4]. However toxicity as a result of leakage of constituent ions (e.g., cadmium, mercury, lead, etc.) of above mentioned luminescent materials restrict their wide applications. Thus number of studies has been focused on developing biocompatible luminescent materials [5], [6].

On the other hand, hydroxyapatite (HA) is one of the well known biomaterials owing to its high biocompatibility, bioactivity, osteoconductive properties, and chemical similarity with inorganic component of bone and teeth. It has been widely used as a carrier for drug delivery, a bone substitute for filling bone defects, a scaffold matrix for tissue engineering and a coating on metallic implants [7], [8]. However, HA does not show any luminescence properties whereas transition metal and rare earth ions substituted HA shows very interesting luminescent properties which makes them a biocompatible contrast agent for bioimaging [9], [10], [11]. Herein, we report microwave synthesis of Eu substituted HA and its properties towards bioimaging.

2 Experimental Procedure

Eu-HA nanoparticles were synthesized by simple microwave method. Briefly, for synthesis of Eu-HA, the phosphate precursor Na_2HPO_4 was mixed with equimolar CTAB which was then added dropwise into the calcium precursor solution which contains 0.025 M of $Eu(NO_3)_3.6H_2O$ and stirred for 1 h at room temperature. The stoichiometry ratio of Ca/P was 1.67. The pH of the mixture was maintained above 12 by using 1 M NaOH. Then, the prepared mixture was put in a microwave oven (2.45 GHz, 600 W) and irradiated with microwave for 15 min. The obtained precipitate was centrifuged and washed several times with ethanol and water. The obtained product was dried in hot air oven for 24 h at 110 °C and then crushed into powder. Pure HA was also synthesized by the above mentioned method for comparison.

The crystalline phases present in the synthesized samples were analyzed using Rigaku MiniFlex II diffractometer in the 2θ range between 20° - 60° with CuKα radiation (1.5406 Å). Scanning electron microscopy (SEM) images were taken in Zeiss Ultra Plus scanning electron microscope. Photoluminescence (PL) of the samples were studied using Horiba Jobin Yvon spectrofluorometer. The fluorescence imaging experiment was conducted on Zeiss, LSM 710 laser confocal scanning microscope. Powders were sprinkled onto conventional glass slides for confocal microscope analysis.

3 Results and Discussion

The XRD pattern of the as prepared samples is shown in Fig 1. The 2θ values of the as prepared samples are in good agreement with the standard data for HA (JCPDS No. 09-0432) which suggested that the synthesized samples constitutes HA as the unique crystalline phase. The calculated lattice parameter values for HA nanoparticles are $a = b = 9.3721$ Å, c = 6.8432 Å. The values of lattice parameters increased as $a = b = 9.40827$ Å, $c = 6.8745$ Å for Eu-HA. This is due to the reduced ionic radius of Eu^{3+} with respect to Ca^{2+} [8]. The value of average crystallite size was calculated from XRD data using the Debye - Scherrer approximation as 46.77 nm and 44.71 nm for HA and Eu-HA, respectively.

SEM images of as synthesized samples are shown in Fig 2. HA and Eu-HA consists of aggregated nanometer sized particles. However, the morphology and size of the individual particles could not be recognized precisely due to their extremely smaller size and aggregation.

Photoluminescence emission spectra of HA and Eu-HA nanoparticles are shown in Fig 3. HA does not show any luminescence properties whereas Eu-HA shows emission peaks at 592 nm, 617 nm and 654 nm. The most intense peak at

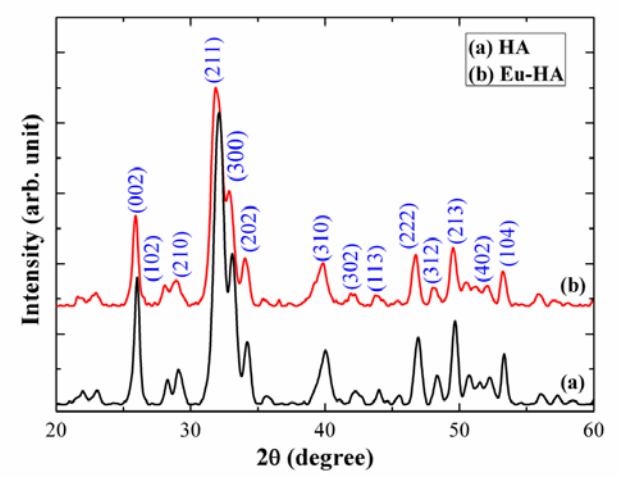

Fig. 1. XRD patterns of HA and Eu-HA samples

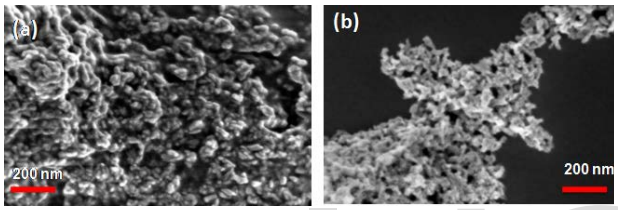

Fig. 2. SEM images of HA (a) and Eu-HA (b) nanoparticles

617 nm corresponds to the $^5D_0 \rightarrow {}^7F_2$ transition within Eu^{3+} ions, while the peaks at 590 nm and 654 nm correspond to $^5D_0 \rightarrow {}^7F_1$ and $^5D_0 \rightarrow {}^7F_3$, respectively [9]. Photoluminescence properties can provide important information about Eu^{3+} substitution in HA structure. In the structure of HA, Eu^{3+} may occupy in Ca(I) and Ca(II) sites. Due to Eu^{3+} substitution in HA structure, both Ca(II) and Ca(I) sites act as a luminescent centers and generate red emissions at 617 nm ($^5D_0 \rightarrow {}^7F_2$) and 592 nm ($^5D_0 \rightarrow {}^7F_1$), respectively. Our results showed that the peak at 617 nm has higher intensity than that from at 592 nm which indicate that Eu^{3+} ions have higher substitution percentage in Ca(II) sites than in Ca(I) sites of HAp structure. The charge compensation due to Eu substitution in HA occurs in the following form

$$Eu^{3+} + O^{2-} \leftrightarrow Ca^{2+} + OH^-$$
$$2Eu^{3+} + \square(\text{Vacancy}) \rightarrow 3Ca^{2+}$$

In the first one, the OH^- ion in HAp structure is replaced by O^{2-} ion to achieve charge compensation and resulted product formulated as $Ca_{10-x}Eu_x(PO_4)_6(OH)_{2-x}O_x$. This explanation is preferable for the Eu^{3+} substitution at Ca^{2+} (II) site because it is located closer the OH^- lattice columns than Ca^{2+} (I) site [10], [11]. The alternative is the substitution of Ca^{2+} by a vacancy, which can compensate the charge for Eu^{3+} occupying the Ca^{2+}(I) site and resulted product formulated as $Ca_{10-3x}Eu_{2x}\square_x(PO_4)_6(OH)_2$.

Confocal microscope image of Eu-HA is shown in Fig 4 which exhibits red emission under 488 nm laser. These results indicate Eu-HA nanoparticles can be considered as a potential candidate for fluorescence imaging application.

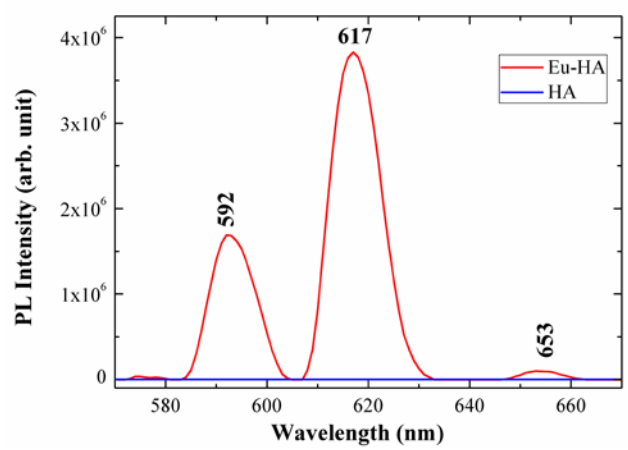

Fig. 3. Photoluminescence emission spectra of HA and Eu-HA samples

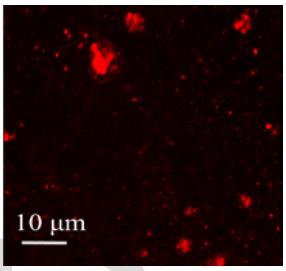

Fig. 4. Confocal microscope image of Eu-HA nanoparticles

4 Conclusion

Eu-HA nanoparticles were synthesized by microwave irradiation method. PL and confocal microscopy studies on Eu-HA nanoparticles suggest that it can be used as potential candidate for bioimaging.

Acknowledgement

This work was financially supported by University Grants Commission, India through project (Project Ref. no. 41-1013/2012 SR).

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