

CERIUM-DOPED HYDROXYAPATITE NANOPARTICLES SYNTHESIZED BY CO-PRECIPITATION METHOD

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Abstract- As the frequency of tissue-damaging or degenerative diseases has increased exponentially. To obtain an ideal treatment solution focused on the development of optimum bio-materials and to be applied for the enhancement of bio-activity and regeneration process, which are necessary to support the proper healing process of osseous tissues. The present work reports a simple adapted co-precipitation method for the synthesis of stable Ce-substituted Ca hydroxyapatite (HAp) nano-particles. The structural and morphological properties of the Ce-doped hydroxyapatite (Ce: HAp) were characterized by X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM). The optical properties of the Ce-doped hydroxyapatite were also investigated using Fourier transform infrared spectroscopy (FTIR). The Ce: HAp samples exhibited significant anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacterial strains.

Index Terms- Ce-doped Hap, Osseous tissue, FTIR, *Staphylococcus aureus*, *Escherichia coli*, Tissue damaging.

I. INTRODUCTION

Hydroxyapatite is one of the most studied biomaterial with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, (HAp) and represents the principal inorganic components of bones and teeth. Its excellent biological properties have made HAp a suitable material for the biomedical field. The crystallinity, solubility and thermal stability are also influenced by the substitution of Ca with different ions (Eu, Ag, Sm, Zn, Cu, Ce, etc.). The substitution of Ca with Ce is possible because their radius and electronegativity are very similar. It is well known that the morphology, structure and physicochemical properties of HAp powders depend on the synthesis method. Thus, various techniques are used to synthesis hydroxyapatite nano-powders, such as precipitation, sol-gel, solid-state reactions, and a mechanochemical route. It was found that Cerium (Ce) can act similarly to calcium in the human body and accumulate in bones. According to these results, Cerium-doped hydroxyapatite could improve metabolism. Cerium ions also have antimicrobial activity and unique regenerative properties. The present structural, morphological and optical studies were conducted on $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, (Ce: HAp) synthesized by the co-precipitation method. Small concentrations of Ce were incorporated in the hydroxyapatite structure in order to demonstrate that Ca was replaced by Ce in the apatite lattice. All Ce: HAp samples were characterized by X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR). This study proposes the importance of future studies concerning of rare earth materials on various ceramic materials.

II. MATERIALS AND METHODOLOGY

MATERIAL:

Cerium Nitrate Hexahydrate $[\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}]$ was purchased from ALPHA CHEMICA, MUMBAI, Calcium Nitrate Tetrahydrate $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$ was purchased from SUPER SCIENTIFIC SUPPLIES, DINDIGUL and Ammonium Phosphate $(\text{NH}_4)_2\text{HPO}_4$ was purchased from SYNTHESIS CHEMLAB, COIMBATORE. Then Ammonium Hydroxide (NH_4OH) was purchased from SUPER SCIENTIFIC SUPPLIES, DINDIGUL. All the chemicals were of analytical grade and they were used without further purification. Deionized water was used throughout the experiment. All the solution was prepared by using double distilled water purification method. Ce: HAp nano particles were prepared by using co-precipitation method with the help of PG and Research Centre of Physics, Sakthi College of Arts and Science for women, Tamilnadu, India.

METHODOLOGY:

(1) Powder Preparation

Ce-doped $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ powders with different concentration of cerium ($x_{\text{Ce}} = 9.11, 10.85$ and 15.19) were synthesized by the co-precipitation method.

(2) Preparation Of Solution A:

In order to obtain Ce: HAp powders appropriate amount of Cerium Nitrate Hexahydrate $[\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}]$ and Calcium Nitrate Tetrahydrate $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$ were dissolved in deionized water resulting 350ml of solution – [SOLUTION A].

(3) Preparation Of Solution B:

In a separate beaker, an appropriate amount of Ammonium Phosphate (NH₄)₂HPO₄ was dissolved in deionized water, resulting 350ml of solution – [SOULTION B].

(4) Preparation Of Ce: Hap Nanoparticles:

Both solutions were obtained under vigorous stirring (300rpm, 150oc, 1 hr) on the magnetic stirrer. Solution B was dropped into solution A under vigorous stirring (300rpm) at 100oc. Maintain the pH of the mixture to 10 by adding Ammonium Hydroxide (NH₄OH). The obtained precipitate was washed two or three times with deionized water and then filtered. The final powder was heated at 80°C for 1-2 hr in Hotplate.

III. CHARACTERIZATION TECHNIQUE**(1) Ultra Violet Visible Spectroscopy:**

UV spectroscopy uses ultraviolet lights to determine the absorbency of a substance. In simple terms, the technique maps the interactions between light and matter and measures. As matter absorbs light it undergoes either excitation or de excitation. This generates what is known as a spectrum. This allows scientists to measure the rate at which a beam of light weakness after passing through a substance.

(2) X-Ray Diffraction (XRD):

The powders were investigated by X-ray Diffraction using a Bruker D8 advance diffractometer, with nickel filtered radiation and a high efficiency one dimensional detector operated in the integration mode. The diffraction pat-terns were collected in the 2θ range 20–60°, with a step of 0.02° and 34 s measuring time per step. Insightful analyses of the Ce: HAp structures were performed by Rietveld refinement analysis using the MAUD code.

(3) Fourier Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. The absorbed radiation is converted into rotational and/or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm⁻¹ to 400cm⁻¹, representing a molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification. The FTIR studies of the powder were performed on a spectrum BX spectrometer using KBr pellet technique.

(4) Antimicrobial Activity:

The antibacterial activity of Ce: HAp samples were investigated against Gram-positive and Gram-negative bacterial strains that are Staphylococcus aureus and Escherichia coli. The antibacterial activity was accused using the standardized Kirby-Bauer disc diffusion method. The disc diffusion method is used to evaluate the antibacterial activity of the sample. Mueller-Hilton agar medium was poured into sterile petri dishes and inoculated with test organism. Sterile filter paper disc loaded with various concentrations of sample .The plates were incubated at 37 °C for 24 hours and the zone of inhibition was recorded in millimetre and the experiment was repeated twice.

IV. RESULTS AND DISCUSSION:**(1) UV Visible Spectroscopy:**

The UV visible light at wavelength ranging from 200-1200nm was used to evaluate the light absorption characteristics of Ce-doped nanoparticles. The UV visible spectra of Cerium doped hydroxyapatite (Ce: HAp) in an aqueous solution at room temperature. The absorption peaks obtained for the samples in the range of 282.25 nm for Ce:HAp). For the peak 0.341nm the corresponding absorption range is 11050.5U. The condition band energy is calculated from the Einstein photon energy equations,

$$E=hc/\lambda_{max}$$

Where,

λ max- maximum absorbance wavelength

H- Planks constant

C- Speed of light

The band gap energy of Cerium doped hydroxyapatite (Ce: HAp) has calculated to be - 5.0 eV

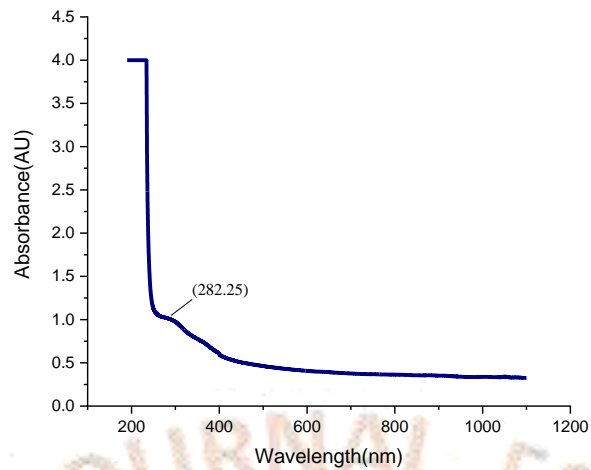


Fig1:UV spectra of Ce:HAp Nanoparticles

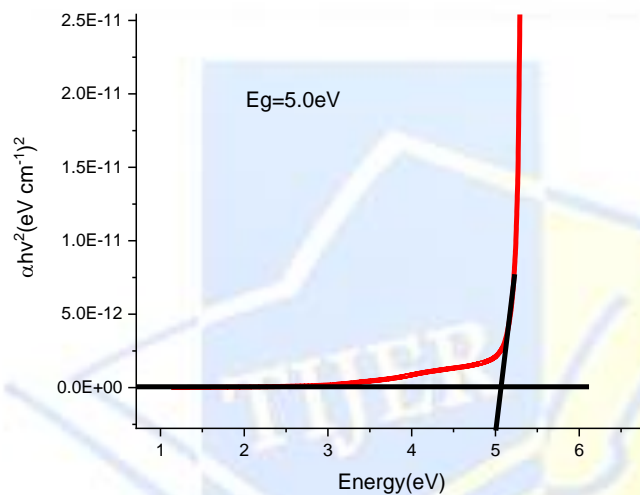


Fig2: Band Gap of UV spectra of Ce: Hap

(2) X-ray Diffraction:

The X-ray diffraction patterns of cerium doped hydroxyapatite, $\text{Ca}_{10-x}\text{Ce}_x(\text{PO}_4)_6(\text{OH})_2$, with different concentrations of cerium ($x\text{Ce} = 9.11, 10.85$ and 15.19) are depicted in (Fig. 3). The broad peaks in the X-ray diffraction patterns assigned to the characteristic (15.08), (20.72), (26.2), (30.28), (32.32), (42.92) and (50.04) planes of $\text{Ca}_{10-x}\text{Ce}_x(\text{PO}_4)_6(\text{OH})_2$ were in accordance with the expected patterns for crystalline hydroxyapatite with a hexagonal structure. Separate phases for cerium oxide or other impurity phases were not observed in these diffraction patterns. Nevertheless, when some Ca sites were replaced by Ce, there was some distortion of the lattice cell parameters. For the peaks registered at lower degrees, a slight shift was observed. This behaviour seems to be the effect of substitutions that change the crystal structure. Which according to Lim et al.²⁸ suggests a significant incorporation of the dopants into the apatite lattice. Diffraction peaks are converted to d-spacing using Bragg's law which allows the identification of materials which is specific for each material (32.32) is found as 2.76Å.

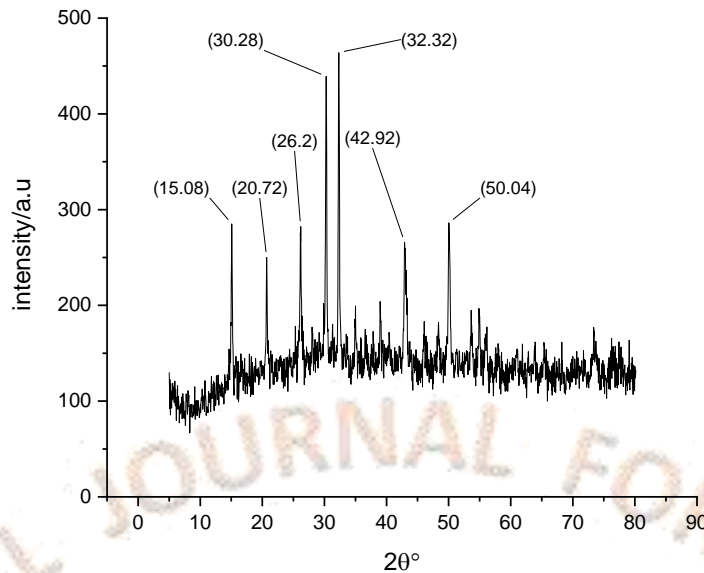


Fig.3:X-ray Diffraction patterns of Ce: HAp

(3) Fourier Transform Infrared Spectroscopy (FTIR):

In the FTIR spectra of cerium doped hydroxyapatite (Fig. 4), characteristic vibrational bands related to phosphate, hydroxyl and adsorbed water were pre-sent. Moreover, an additional band attributed to the carbonate group (1269 cm^{-1}) was also presented. The vibrational bands from the region 3100–3459 cm^{-1} were attributed to adsorbed water. The bands at 1090 and 618 cm^{-1} were assigned to the stretching and vibrational mode of the hydroxyl group (OH⁻). The presence of the band at 1090 cm^{-1} (assigned to the ν_4 bending mode of C-F⁻ groups) and 618 cm^{-1} indicate the formation of a well crystallized powder. The C-F stretching mode band was present at 1386 cm^{-1} and the C-F stretching mode band at 1500 and 1000 cm^{-1} and their intensity appearance are medium. Furthermore, the band at 1643 cm^{-1} was assigned to the ν_2 bending mode band of the C=C⁻ group. In addition, the IR wavenumber and the peak strength of the and O–H bonds decreased progressively with increasing Ce concentration.

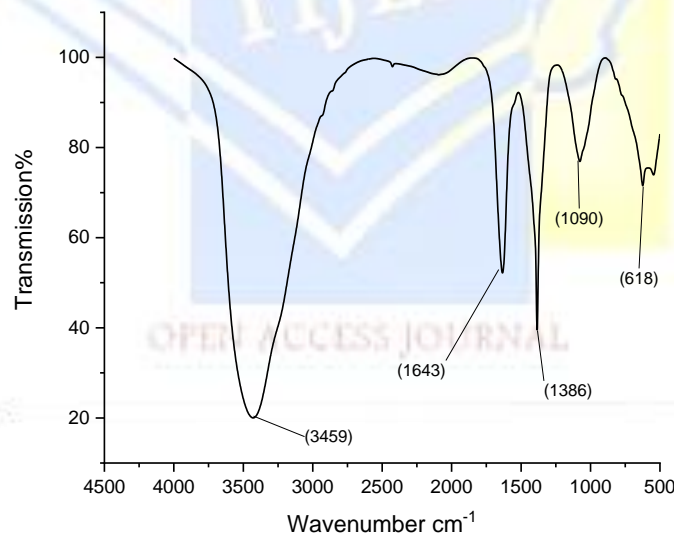


Fig4: FTIR spectra of the cerium-doped hydroxyapatite powders.

(4) Antimicrobial activity:

The antibacterial activity of sample were exhibited against one gram positive bacterial strains *Staphylococcus aureus* (MTCC 87) and one gram negative bacterial strains *Escherichia coli* (MTCC 443) were prepared as test organisms. Determination of antibacterial activity by disc diffusion method. The disc diffusion method is used to evaluate the antibacterial activity of the sample. Ten ml of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter paper disc loaded with various concentrations of v sample of 60, 80 and 100 µg/ml were placed on the top Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. The plates were incubated at 37 °C for 24 hours and the zone of inhibition was recorded in millimetre and the experiment was repeated twice.



Fig4: S.aureus (MTCC87)

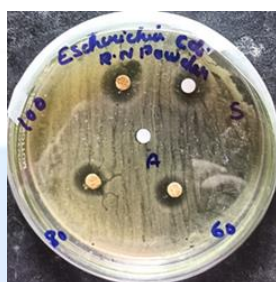


Fig5: E.coli (MTCC443)

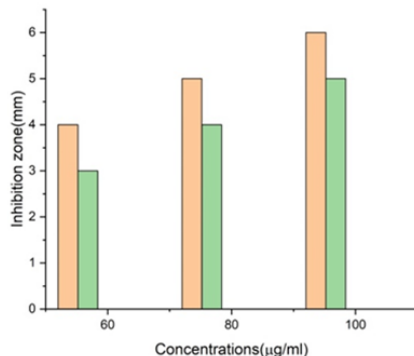


Fig5: Antibacterial activity of the sample

Anti-Fungal:

The antifungal activity of sample were exhibited against *Candida albicans* (MTCC 183), *Aspergillus flavus* (MTCC 277) were prepared as test organisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India and the fungal strains from National Chemical Laboratory (NCL), Pune, and Maharashtra, India. Disc diffusion method in order to test the antifungal activity of sample against test pathogens was carried out. In petri dishes (60 mm) filled with Sabouraud’s dextrose agar (SDA) and seeded with a 0.3 ml of test organism, a sterile filter paper disc (diameter 6 mm, whatmann paper no.3) was placed. The sterile disc was impregnated with 10 µl of samples at varying concentration of 60, 80 and 100 µg/ml respectively. The zones of growth inhibition around the disc were measured after 24 h of incubation at 37°C. while, Fluconazole was used as a positive control.

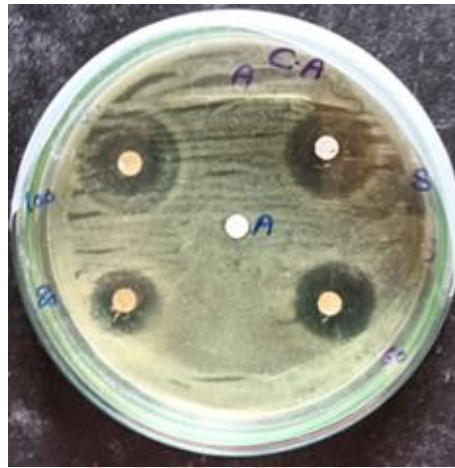


Fig6: *Candida albicans* (MTCC183)

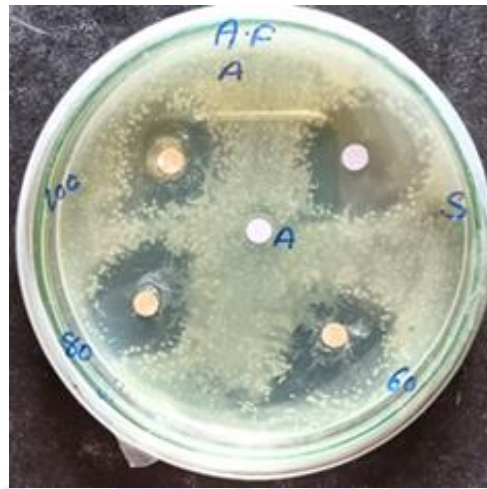


Fig7: *Aspergillus flavus* (MTCC277)

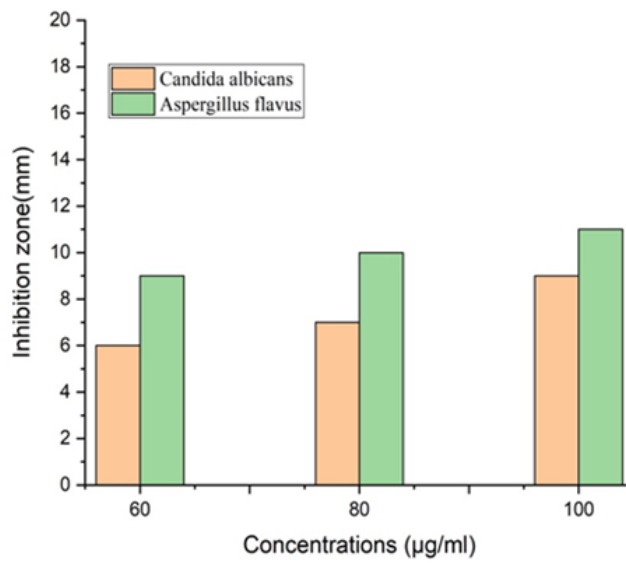


Fig8: Anti-fungal activity of the sample

V. CONCLUSION

In this article, the synthesis of hydroxyapatite doped with small concentration of cerium is reported. The lattice parameters showed that Ce substituted Ca in the apatite structure. The crystal size decreased with increasing concentration of Ce. The results obtained in the XRD studies demonstrated that the Ce: HAp powders synthesized by an adapted co-precipitation method gave hydroxyapatite with a good crystalline structure without any new phases or impurities. The nanometre dimensions of the particles were confirmed by SEM. FTIR studies confirmed the initial results obtained by XRD investigations. The deconvolution of the emission spectra of all Ce: HAp samples revealed four peaks that could be attributed to 5d–4f transitions of the Ce ions. This research demonstrated that cerium is a good candidate that can provide HAp an excellent antibacterial activity. In conclusion, the results presented in this paper encourage further studies and research on the antimicrobial properties of cerium-doped hydroxyapatite aimed at possible applications as an antimicrobial agent.

VI. REFERENCE

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