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Synthesis, structure, thermal stability, mechanical and antibacterial behaviour of lanthanum (La^{3+}) substitutions in β-tricalciumphosphate

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ABSTRACT

Five different concentrations of lanthanum (La^{3+}) substituted β-tricalcium phosphate [β-TCP, β-$Ca_3(PO_4)_2$] were formed through aqueous precipitation technique and the results were compared with stoichiometric β-TCP. All the La^{3+} substituted β-TCP powders were characterized using XRD, FT-IR, XRF, Raman spectroscopy and Rietveld refinement of the XRD data. The results from the investigation confirmed the presence of La^{3+} in rhombohedral β-TCP structure. The substitution of higher sized of La^{3+} led to the considerable enhancement in lattice parameters of β-TCP crystal structure and La^{3+} was found to have occupied the eight fold coordinated Ca (3) site of β-TCP structure. La^{3+} occupancy at the Ca (3) site resulted in the significant distortions of the associated PO_4 tetrahedra, which were supported by the Raman and FT-IR spectroscopic techniques. La^{3+} presence in the crystal lattice of β-TCP also led to the delay in allotropic phase transformation of β-TCP to α-TCP till 1300 °C, thus signifying the good thermal stability of La^{3+} substituted β-TCP powders. The antibacterial efficiency of La^{3+} substituted β-TCP powders was confirmed from the *in vitro* tests done on microbes such as *Staphylococcus aureus* and *Escheria coli*. Further, the presence of La^{3+} in the crystal lattice of β-TCP did not affect the hardness and Young's modulus values of β-TCP.

1. Introduction

Bioactive ceramics namely hydroxyapatite [HAP, $Ca_{10}(PO_4)_6OH)_2$] and β-tricalcium phosphate [β-TCP, β-$Ca_3(PO_4)_2$] are the extensively investigated materials for the bone and tooth remodelling surgical procedures owing to their salient features of chemical similarities with the biological apatite, osteogenic properties and their ability to form strong bonds with host bone tissues [1,2]. In certain applications from the biological perspective, β-TCP is preferred towards HAP because of its better dissolution behaviour leading to the fast regeneration of a new bone or tooth enamel [3,4]. Recent years are witnessing an extensive investigation on the substitution of foreign ions in the structure of HAP and β-TCP ceramics in trace levels, due to the substantial role played by these substituted ions in the biological process. The ability to form solid solution with substituted cations (either monovalent or divalent or trivalent cation) in the β-TCP structure during high temperature heat treatment conditions is found more plausible [5–7]. In case of HAP, substitution of cations and their subsequent heat treatment at higher temperatures resulted in the formation of secondary phases [8, 9]. The characteristic feature of β-TCP to accept a wide range of possible cationic substitutions in its rhombohedral crystal lattice is mainly due to the presence of five different Ca^{2+} sites displaying a wide range of sizes and coordination spheres in its structure. The crystal structure of β-TCP is classified into two different columns that run through the *c*-axis. Column A comprises Ca (4) and Ca (5) sites and have minimum coordination with the surrounding oxygen atoms [Coordination number (CN) of Ca (4) = 3 and Ca (5) = 6] thus leading to the formation of less density column. Column B comprises Ca (1), Ca (2) and Ca (3) sites and have maximum coordination with the surrounding oxygen atoms [CN of Ca (1) = 7,Ca (2) = 6 or 8 and Ca (3) = 8] thus leading to the formation of high density column [10,11].

A wide range of cations ranging from monovalent, divalent and trivalent substitutions in β-TCP structure are investigated for their essential role in biological process. In this context, the investigations on the trivalent rare earth element lanthanum (La^{3+}) substitution in β-TCP are found scarce. Recent investigations have shown that La^{3+} offers many advantages in biological activities [12,13]. Generally La^{3+} is detected as a trace element in the human bone and it is documented that the total bulk lanthanum concentration in bone remains below 10 μg/g [14]. It is also reported that lanthanum promotes the formation of osteoclast-like cells and expected to significantly increase the surface area of the resorption [15,16]. La^{3+} also exhibits beneficial functions, such as restraining the Ca^{2+} pump of the human red cells. and possesses the capability to either resist or prevent cancer cells from defusing [17, 18]. In addition, their spectroscopic properties, resulting from their unusual electronic configuration, make them a useful probe for calcium in biological systems using techniques such as nuclear magnetic resonance, luminescence or fluorescence spectroscopy [19].

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In this context, the present investigation is aimed at the synthesis and structural characterization of a series of La^{3+} doped β-TCP powders. Since β-TCP is known for its dissolution characteristics, the location of La^{3+} either in the less dense column A or high dense column B is crucial in determining the resorbable features of β-TCP. A series of five different La^{3+} substituted β-TCP were obtained through aqueous precipitation technique and the techniques involving X-ray diffraction (XRD), Raman spectra, Fourier transform infrared (FT-IR) spectroscopy, X-ray fluoresence (XRF) analysis and quantitative analysis of XRD data through Rietveld refinement were employed for the characterization of La^{3+} substituted β-TCP powders. The thermal stability of β-TCP due to the La^{3+} incorporation was also investigated. Further, the ability of La^{3+} in countering the invasion of microbes through *in vitro* study followed by the mechanical tests involving the evaluation of Young's modulus and hardness through nanoindentation technique was determined.

2. Materials and methods

2.1. Powder synthesis

The synthesis of lanthanum (La^{3+}) doped β-$Ca_3(PO_4)_2$ powders was prepared through chemical precipitation technique. For this purpose, calcium nitrate tetrahydrate [$Ca(NO_3)_2.4H_2O$, Himedia, India], di-ammonium hydrogen orthophosphate [$(NH_4)_2HPO_4$, Himedia, India], and lanthanum nitrate [$La(NO_3)_3$, Sigma-Aldrich, India] were taken as precursors for Ca^{2+}, PO_4^{3-} and La^{3+} respectively. In the present investigation, five different concentrations of the La^{3+} substitutions in β-$Ca_3(PO_4)_2$ were prepared. Along with these compositions, a stoichiometric β-$Ca_3(PO_4)_2$ [Ca/P molar ratio = 1.5] was also synthesized for the effective comparison of the results. The powder synthesis was done according to the procedure given below. A predetermined molar concentration containing the cationic mixtures of $Ca(NO_3)_2$ and $La(NO_3)_3$ solution and a predetermined molar concentration of $(NH_4)_2HPO_4$ solutions were prepared separately in a 200 ml standard measuring flask using de-ionized water. The precursor concentration of the cationic mixtures (Ca^{2+} + La^{3+}) was made in such a way that the total (Ca + La)/P molar ratios were equal to 1.5 for all the compositions. Table 1 reports the comparative experimental concentrations and elemental analysis determined from X-ray fluorescence technique after heat treatment at 1000 °C.

The prepared 200 ml stock solution containing the cationic mixtures was transferred to a 500 ml beaker and placed on a magnetic stirrer (Tarsons, India) with an operating temperature of 100 °C and a rotation speed of about 400 rpm. The separately prepared $(NH_4)_2HPO_4$ was now added to the continuously stirred stock solution containing cationic mixtures in a drop wise manner and after the completion of the addition, a white gelatinous precipitate was formed. The pH of the solution mixture was observed using a pH meter (Eutech pH Meter, Singapore) and was found to be ~4.00. Now the pH of the solution mixture was raised to 8.0 by using concentrated NH_4OH solution. After the addition of aqueous NH_4OH to the solution mixture, a dense white precipitate was noted down. The resultant precipitated solution mixture was continuously stirred under constant temperature condition for 2 h. The resultant mixture was now transferred to a stable condition at room temperature for the precipitate to settle down. The solution was decanted and the precipitate was transferred to a Hot air oven (Technico Oven, India) and dried at 120 °C overnight. The obtained dried precipitate was grounded well to fine particles and these powders were considered as prepared powders.

Table 1
Molar concentrations of the precursors used in the synthesis and elemental analysis data obtained after the heat treatment of powders at 1000 °C.

Elemental composition of La^{3+} substituted β-$Ca_3(PO_4)_2$ powder						
Sample code	Experimentally planned (in wt.%)			Elemental analysis from XRF (in wt.%))		
	Ca^{2+}	P^{5+}	La^{3+}	Ca^{2+}	P^{5+}	La^{3+}
Pure-TCP	38.783	19.966	NA	38.090	20.080	ND
*1x*La-TCP	37.294	19.591	2.636	36.170	19.870	2.980
*2x*La-TCP	35.860	19.231	5.175	34.360	19.720	5.547
*3x*La-TCP	34.479	18.883	7.622	32.870	19.390	7.965
*4x*La-TCP	33.146	18.548	9.982	31.550	18.840	10.610
*5x*La-TCP	31.860	18.225	12.260	30.380	18.370	13.000

NA—not added; ND—not determined.

2.2. Powder characterization

The as prepared powders were heat treated at different temperatures with a soaking time of 2 h in the muffle furnace (MATRI-MC 2265 A, India) and employed for further characterization. The phase purity and composition of the powders after heat treatment at different temperatures were analysed using powder X-ray diffractometer (Rigaku, Ultima IV, Japan). XRD studies for all the powders were carried out with Cu Kα radiation (λ = 1.5406 nm) produced at 40 kV and 30 mA to scan the diffraction angles (2θ) between 10° and 70° with a step size of 0.02° 2θ per second. For the quantitative analysis through Rietveld refinement, an average of three XRD scans was recorded for each powder samples with a step size of 0.01° 2θ per second. The refinement procedures were executed using GSAS-EXPGUI software package [20,21]. The initial refinement of pure β-$Ca_3(PO_4)_2$ was done with the standard CIF (crystallography Information File) from Yashima *et al.* [10]. The structural parameters taken for refinement were given as space group *R3c*, Z = 21, *a* = 10.4352 Å and *c* = 37.4029 Å, 18 independent atomic positions: five Ca positions (three in site *18b* and two in site *6a* at one-half occupancy), three P positions (two in site *18b* and one in site *6a*), and ten O positions (nine in site *18b* and one in site *6a*). The CIF file of pure β-$Ca_3(PO_4)_2$ refined from Yashima *et al.* was taken as a standard for refining the La^{3+} substitutions in β-$Ca_3(PO_4)_2$. In a first step of the refinement all the structural parameters were fixed to the literature values. Then during the successive refinement cycles numerous parameters were allowed to vary according to the relative weight amount of the observed phases. The following refinement sequences were used as a standard for all the structures: scale factor, zero shift, background as Chebyshev polynomial of fifth grade, peak profile and lattice parameters. Fittings were performed using pseudo-Voigt peak profile functions and a preferred orientation along [001] was taken into account with the Marsh model. The atomic parameters such as positional, isotropic temperature and occupancy factors were also refined during the final step.

The elemental composition for all the synthesized powders after heat treatment at 1000 °C was determined through X-ray fluorescence (XRF) spectroscopy (Bruker S4 Pioneer). For this purpose, the powder sample was mixed homogeneously with boric acid powder in the proportion of ¼ (by weight) for 15 min. The presence of Ca^{2+}, P^{5+}, La^{3+} and other elements present in trace levels was confirmed using this technique.

FT-IR spectroscopy was performed in transmission mode using a FT-IR spectrophotometer (Perkin–Elmer, USA) in the infra region (4000–400 cm^{-1}) for the functional group analysis. For FT-IR analysis, the powders were mixed well with potassium bromide (KBr) at a weight ratio of 1:100 and pressed into pellets.

The vibrational modes for La^{3+} doped β-$Ca_3(PO_4)_2$ powders were determined using Raman spectroscopy. All the vibrational modes of La^{3+} doped β-$Ca_3(PO_4)_2$ powders after heat treatment at 1000 °C were determined by using back scattering geometry of a confocal Raman microscope (Renishaw, United Kingdom). All the powder samples were excited at a wavelength of 785 nm by semiconductor diode laser (0.5% of power) with the data acquiring time of 30 s. The surface morphology of the β-$Ca_3(PO_4)_2$ powder and La^{3+} doped