



Synthesis, bioactivity and zeta potential investigations of chlorine and fluorine substituted hydroxyapatite



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ABSTRACT

Chlorine and fluorine substituted hydroxyapatites (HA-Cl-F) with different degrees of ion replacement were successfully prepared by the one step mechanochemical activation method. X-ray diffraction (XRD) and FT-IR spectra indicated that substitution of these anions in milled powders resulted in the formation of pure hydroxyapatite phase except for the small observed change in the lattice parameters and unit cell volumes of the resultant hydroxyapatite. Microscopic observations showed that the milled product had a cluster-like structure made up of polygonal and spherical particles with an average particle size of approximately ranged from 20 ± 5 to 70 ± 5 nm. The zeta potential of milled samples was performed at three different pH (5, 7.4, and 9). The obtained zeta potential values were negative for all three pH values. Negative zeta potential was described to favor osseointegration, apatite nucleation, and bone regeneration. The bioactivity of samples was investigated on sintered pellets soaked in simulated body fluid (SBF) solution and apatite crystals formed on the surface of the pellets after being incubated for 14 days. Zeta potential analysis and bioactivity experiment suggested that HA-Cl-F will lead to the formation of new apatite particles and therefore be a potential implant material.

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1. Introduction

Hydroxyapatite (HA) is a group of calcium phosphates which is the structural model for the inorganic phase of tooth and bone tissue [1]. This bioactive ceramic is broadly applied as bone fillers, bone tissue scaffolds, bioactive coatings and composites owing to their excellent biocompatibility, and osteoconductivity. They are also promising candidates for drug, protein, and gene delivery as well as fluorescence labeling, cell targeting, imaging materials [2]. The crystallinity, lattice parameters, morphology, and stability of the apatite structure can be modified by substitution of anions and cations into the HA lattice [3]. Among substituting anions, chlorine (Cl^-) and fluorine (F^-) are extensively considered as potential substituents because of their biological significance. The partial substitution of fluorine in HA structure is well known for its greater thermal and chemical stability [4]. Furthermore, the fluorine ion prevents and controls dental caries, and stimulates bone cells proliferation and differentiation [4]. It has been confirmed that fluorine substituted hydroxyapatite (FHA) implants exhibited superior integration in the bone tissue and much longer resorption time than conventional HA [5]. The presence of chlorine ions in HA structure

can also make an acidic environment on the surface of bone that stimulates osteoclasts in the bone resorption process [6]. Accordingly, this incorporation may be essential in the expansion of low pH to solubilize the alkaline salts of bone mineral and to digest the organic matrix by acid hydrolases which osteoclasts secrete [3].

Recent studies on chlorine substituted HA synthesis were mainly focused on hydrothermal treatments [7], aqueous precipitation method [8], and mechanical alloying [9]. Besides, the synthesis of FHA and fluorapatite (FA) is of great value and has been widely explored by multiple techniques, such as precipitation [10], hydrolysis [11], sol-gel [12], hydrothermal [13], and mechanochemical methods [14]. Recently, many attempts have been tried to develop ions doped hydroxyapatite by solid-state processes including mechanical activation and subsequent thermal treatment [6]. High efficiency of this process offers advantageous approach to produce nanopowders commercially.

Based on literature, Kannan et al. [3] studied HA synthesized in the presence of Cl^- and F^- from aqueous precipitation method. Their results indicated that the incorporation of substituted anions in the apatite structure and an excellent co-incidence between the initial and final element concentrations of the added elements. However, the bioactivity of their samples has not been reported. Thus, both Cl^- and F^- can be incorporated into the HA structure during the precipitation process. HA substituted with Cl^- and F^- ions is expected to be a better bioceramic for biomedical applications as compared to the individual

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pure HA. However, no specific documentation has been demonstrated on the substitution of chlorine and fluorine into the HA lattice structure using mechanochemical activation so far. Therefore, the present work aims at the synthesis of combined substitutions of chlorine and fluorine into HA structure with different degrees of ion replacement via mechanochemical activation procedure. This method refers to reactions, normally of solids, affected by the input of mechanical energy during milling [15]. The effect of subsequent annealing process on phase transformations and structural features of mechanothesized samples was also examined. The prepared specimens were characterized using XRD, Fourier Transform Infrared (FT-IR), FE-SEM, TEM, and energy dispersive X-ray spectroscopy (EDS). The zeta potential of the unsintered samples was also done at different pH values. Moreover, the bioactivity of sintered HA-Cl-F pellets was evaluated from the apatite formation on its surface in SBF solution.

2. Experimental procedures

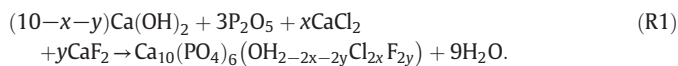
2.1. Materials and composite preparation

Calcium fluoride (≥ 99.0 wt.% CaF_2), phosphorus pentoxide (≥ 98.0 wt.% P_2O_5), calcium oxide (≥ 98.0 wt.% CaO), calcium hydroxide (≥ 98.0 wt.% $\text{Ca}(\text{OH})_2$) and calcium chloride (≥ 97.0 wt.% CaCl_2), powders were purchased from Sigma Aldrich. All the chemicals were of analytical grade and utilized without other purifications.

2.2. Powders and bulk preparation

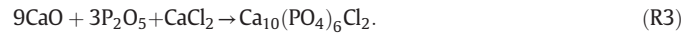
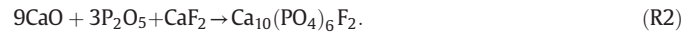
To synthesize Cl^- and F^- substituted HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x-y}\text{Cl}_x\text{F}_y$) nanopowders, the desired amounts of $\text{Ca}(\text{OH})_2$, P_2O_5 , CaF_2 and CaCl_2 were milled at different milling times (5, 15, 30, 60, 120, and 300 min) in a high energy ball mill (8000 M mixer/mill). The hardened chromium steel vials (vol. 65 ml) and two steel balls (10 cm) under air atmosphere were utilized. In all cases, the weight ratio of ball-to-powder (BPR) and clamp speed were 10:1 and 1060 cycles/min, respectively. The mole ratio of calcium to phosphorus was equal to 1.67 which is in accordance with the stoichiometric Ca/P content in the composition of HA. The various designed level of replacement of Cl^- and F^- by OH^- are denoted by the x and y values in the HA-Cl-F ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-2x-2y}\text{Cl}_{2x}\text{F}_{2y}$) formula where the x values were 0.0, 0.125, 0.25, 0.375, and the y values were 0.0, 0.375, 0.25, and 0.125, respectively.

The products were labeled as A0, A1, A2, and A3, respectively. For the mechanochemical synthesis, the amounts of each reagent required to achieve the necessary stoichiometry were milled at 60 min. Sample designation and nominal composition of synthesized powders are summarized in Table 1. The general form of the mechanochemical reaction (R1) is as follows:



Samples in which complete replacement of OH^- by Cl^- and F^- were also prepared by milling for 60 min and were labeled as A4 (fluorapatite), and A5 (chlorapatite), respectively. Accordingly, the

overall form of the mechanochemical reactions (R2) and (R3) are as follows:



To crystallize mechanothesized samples, heat treatment was done at 800 °C for 1 h at air atmosphere in an electrical furnace. The heating rate from room temperature to the desired temperature was fixed 10 °C min^{-1} . The annealed samples were also marked as A00, A11, A22, A33, A44, and A55. The appropriate quantities of unsintered powders were then pressed at 150 MPa using an uniaxial press to form disks (13 mm in diameter \times 2 mm in height) and sintered at 800 °C for 1 h. The heating and cooling rates did not exceed 10 °C min.

2.3. Characterization techniques

The phase analysis of samples was determined by X-ray diffraction (Bruker D8 Advance ECO (XRD)) equipped with $\text{Cu-K}\alpha$ radiation ($\lambda \approx 1.54$ Å), and $10^\circ \leq 2\theta \leq 80^\circ$). The XRD profiles were compared to standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS), which involved card #44-1481 for $\text{Ca}(\text{OH})_2$, #37-1497 for CaO , #24-0223 for CaCl_2 , #035-0816 for CaF_2 , #33-0271 for ClAp , #15-0876 for FAp , and #09-00432 for HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). The crystallite size and lattice strain of the products were determined by the Williamson–Hall Eq. (1) [16]:

$$B\cos\theta = 0.9\lambda/D + \eta\sin\theta \quad (1)$$

where λ is the X-ray wavelength (0.154056 nm), D is the crystallite size, η is the internal micro-strain, and θ is the Bragg angle ($^\circ$), respectively. B is the sample peak width (in radians) after subtracting the peak width owing to instrumental peak width broadening from the experimentally recorded profile. The values of η and D were obtained by plotting $B\cos\theta$ versus $\sin\theta$, where η represents the slope and $0.9\lambda/D$ the intercept of the line. In addition, the crystallinity degree (X_c) was estimated for both mechanothesized and sintered samples from the XRD data using the following Eq. (2) [17]:

$$B_{hkl}\sqrt{X_c} = K \quad (2)$$

where K and B are the fraction of crystalline phase, a constant found equal to 0.24 and full width half maximum (FWHM, $^\circ$) of selected peaks, respectively. Note that the structural features of HA powders were repeated for one group; (0 0 2), (2 1 1) and (3 0 0) Miller's planes family [1]. The average of these measurements was selected as mean fraction of crystalline phase, crystallite size and lattice strain. The lattice parameters (a, and c) can be evaluated for (211), and (300) Miller's planes from the following relation (3):

$$\frac{1}{d^2} = \frac{4h^2 + hk + k^2}{3a^2} + \frac{l^2}{c^2} \quad (3)$$

Table 1
Details of powder components and mole ratio.

Sample	Powder components	Degree of substitution [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-2x-2y}\text{Cl}_{2x}\text{F}_{2y}$]		Mole ratio
A0	$\text{Ca}(\text{OH})_2\text{-P}_2\text{O}_5$	$x = 0$	$y = 0$	10:6:2
A1	$\text{Ca}(\text{OH})_2\text{-P}_2\text{O}_5\text{-CaCl}_2\text{-CaF}_2$	$x = 0.125$	$y = 0.375$	10:6:1:0.25:0.75
A2	$\text{Ca}(\text{OH})_2\text{-P}_2\text{O}_5\text{-CaCl}_2\text{-CaF}_2$	$x = 0.25$	$y = 0.25$	10:6:1:0.5:0.5
A3	$\text{Ca}(\text{OH})_2\text{-P}_2\text{O}_5\text{-CaCl}_2\text{-CaF}_2$	$x = 0.375$	$y = 0.125$	10:6:1:0.75:0.25
A4	$\text{CaO-P}_2\text{O}_5\text{-CaF}_2$	$x = 0$	$y = 1$	10:6:2
A5	$\text{CaO-P}_2\text{O}_5\text{-CaCl}_2$	$x = 1$	$y = 0$	10:6:2

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