



Structural and Morphological Analysis of Nano Sized Anions Doped Hydroxyapatite

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Abstract

Inorganic sulphate and silicate is required by all mammalian cells to function properly, it is the most abundant anion in the human plasma. Sulphate ions are the major source of sulphur which is considered an important element for sustenance of life as it is present in the essential amino and is required by cells to function properly. This paper aimed to compare the constitutional and physical property of pure hydroxyapatite (HA) doped with different kinds of anions such as sulphate ions (SO_4^{2-}) and silicate ions (SiO_4^{4-}). The pure phase of HA, SHA (SO_4^{2-} doped HA), and SiHA (SiO_4^{4-} doped HA) were successfully synthesised using microwave-assisted precipitation technique method. The acquired materials were recognized using X-ray diffractometer (XRD), Fourier transforms infrared spectroscopy (FTIR), Field emission scanning electron microscopy (FESEM), and energy-dispersive X-ray (EDX) techniques. The consequence shows that incorporation of SO_4^{2-} and SiO_4^{4-} ions revealed shrinkage along with a-axis and expansion with c-axis. FTIR shows diminished in the intensity of the OH group with an addition of anions. The particles shape significantly decreased with incorporation of SiO_4^{4-} ions and changed to rod shape with addition of SO_4^{2-} . The Ca/P ratios were in acceptable values. Based on the outcomes, demonstrated that the anions were successfully integrated in the HA crystal with no decomposition in HA phase.

Keywords: Hydroxyapatite; Sulphate ions; Silicate ions; Characterizations

1. Introduction

The hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HA) plays a versatile role in bone and hard tissues regenerations, because of its close chemical and physical resemblance to the mineral constituent of human hard tissues. In addition, it shows excellent biocompatibility, bioactivity and osteoconductive [1, 2]. Hydroxyapatite ($\text{HA}, \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is known member of the calcium phosphate family [3]. It has been prepared in the laboratory using different techniques such as wet precipitation [4], microwave-assisted wet precipitation [5-7], solid-state [8], sol-gel [9], and hydrothermal method [10, 11]. Due to its similarity with the mineral phase of human hard tissues, it has obtained several of usage that include bone tissue engineering [12], bone-implant coating [13], endodontic treatment [14] [15], drug and gene delivery [16, 17]. Established on its chemical quality, the HA has the capability to undergo ionic substitution with various ions. Cations exchange takes place in Ca site and the anions exchange take place in PO_4^{2-} and OH^- sites. However, the dopant ions perform a

significant role in physico-chemical properties of HA [18, 19].

Silicate ions (SiO_4^{4-}) is a well-known low cost, high specific surface area and cytocompatibility materials [20]. Incorporation of SiO_4^{4-} into HA crystal demonstrated good osteogenesis activity, as approved via In vivo studies conducted by Hing et al [21]. Sulphate ions (SO_4^{2-}) is a significant part of several amino acids like methionine and cysteine [22]. Furthermore, in biomedical applications, it has been used in the shape of CaSO_4 (used as bone filler) and BaSO_4 (used as radiocontrast media). This study aims to evaluate the impact of anions (SO_4^{2-} and SiO_4^{4-}) dopant on microstructure properties of HA and compare the obtained results. To the best of my knowledge, this kind of study has not been reported yet.

2. Experimental

2.1. Preparation of pure Hydroxyapatite (HA), Sulphate doped hydroxyapatite (SHA), and Silicate doped hydroxyapatite (SiHA)

Calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) and diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) were utilized to synthesis pure phase of HA, sodium

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sulphate (Na_2SO_4) (Merck, Germany) and Tetraethyl orthosilicate ($\text{SiC}_8\text{H}_{20}\text{O}_4$) (Merck, Germany) were used as SO_4^{2-} and SiO_4^{4-} exporter respectively. Ammonia solution (NH_4OH) was utilized to regulate the pH.

About 1.0 M of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ was dissolved in 200 ml of distilled water to form solution (1). A 0.6 M of $(\text{NH}_4)_2\text{HPO}_4$ was dissolved in 200 ml of distilled water to form a solution (2). Slowly add solution (2) to solution (1) under stationary stirring. The pH of the mixture was kept above 10 using few drops of NH_4OH . The precipitated mixture was irradiated for 15 min (using watch timer) during this time solution is boiling in 800W household microwave during which it was refluxed by using condenser with in-out water during the reflux the solution in round bottom [6, 23]. The precipitation was washed and collected, then heat-treated at 80°C overnight in the oven. After the dried powder was calcined in a muffle furnace at 700°C for 2 hr. SHA and SiHA materials were prepared using the same method except that the corresponding amount SO_4^{2-} (or SiO_4^{4-}) ions were added in the solution of $(\text{NH}_4)_2\text{HPO}_4$. Different molar fractions of the precursor used for the preparation of HA, SHA and SiHA are tabulated in Table 1.

Table 1. The titular synthesis of HA, SHA and SiHA samples.

Samples ID	Reactants (M)			
	Ca^{2+}	$(\text{PO}_4)^{3-}$	$(\text{SiO}_4)^{4-}$	$(\text{SO}_4)^{2-}$
HA	10.00	6.00	0.00	0.00
SO_4 -HA	10.00	5.00	0.00	1.00
SiO_4 -HA	10.00	5.00	1.00	0.00

2.2. Characterization

The synthesizes were identified using several techniques (i) the phase purity was evaluated using X-ray diffractometer (Bruker D8 Advance XRD) run at 40 kV and 30 mA utilising $\text{CuK}\alpha$ radiation. Ranging scan 20° to 80° with a step size of 0.02° and scan speeds of 1s/step. The lattice parameters, grade of crystallinity and crystallite size were using XRD software. (ii) The phosphate and hydroxyl groups were detected using Fourier transform infrared spectroscopy (FTIR, Nicolet iS50 spectrometer). Sintered samples were characterized by using a Field emission scanning electron microscopy (FESEM, Zeiss-LEO 1530). The chemical composition was investigated by Energy Dispersive X-Ray spectrometer (EDX, Swift ED 3000 from Oxford instrument operated at 20 kV).

3. Results and Discussion

XRD patterns of HA, SHA and SiHA powders sintered at 700°C were illustrated in Figure 1. The diffraction planes of pure HA were in perfect match

with a standard phase of HA (JCDPS NO 86-0740). Tricalcium phosphate phase (TCP) and calcium oxide were not detected, indicating the obtained HA presented in pure phase and the characteristic peaks of HA appeared in sharp shape indicating a high crystallinity nature of HA. With an incorporation 0.5M of SO_4^{2-} ions (SHA), serious changes have been observed on the main diffraction peaks of HA, the peaks intensity have been declined and some diffraction plane such (2 0 0) and (1 1 1) located at 21.92° and 22.96° respectively were vanished, demonstrated that the degree of crystallinity was declined (Table 2) and the full width at half maximum of the peaks was increased indicating that the crystallite size of HA was decreased with an addition SO_4^{2-} ions. However, with the addition of SiO_4^{4-} ions (SiHA) the same trend has been observed. However, the diffraction planes (2 0 0) and (1 1 1) of HA appeared again but with low intensity.

Lattice parameters, degree of crystallinity and crystallite size of HA, SHA and SiHA were calculated using XRD software and the results were concise in **Table 2**. The lattice parameters of pure HA slightly different from the standard HA could be due to different preparation methods or calcination temperature. With a substitution of SO_4^{2-} (or SiO_4^{4-}) ions revealed expansion in the crystal dimensions along with a-axis and shrinkage along with c-axis, this behaviour could be attributed to anionic exchanged that take place between the lower size of the PO_4 tetrahedron compared to the higher size of the SO_4^{2-} (or SiO_4^{4-}) tetrahedron which results in the change of HA lattice parameter [24, 25].

FTIR spectra of HA, SHA and SiHA were illustrated in **Figure 2**. Functional groups of HA appeared. The peaks located at 478, 576, 611, 964, 1045, 1097cm^{-1} were refer stretching and bending mode of PO_4^{3-} groups and the bands show at 629 and 3573cm^{-1} were belonged to bending and stretching mode of OH⁻ groups and location of the peaks were in good match with previous studies [26, 27]. No peak was detected at 867cm^{-1} confirming the prepared samples was free of HPO_4^- [24]. Furthermore, no bands were detected at $871\text{--}873\text{cm}^{-1}$ and $1416\text{--}1486\text{cm}^{-1}$, indicating the entire materials were free of carbonate groups [28]. With addition of SO_4^{2-} ions, neglected shifted was observed in the wavelength of the functional groups and a clear reduction in peaks sharpness was observed, more obvious for PO_4^{3-} groups which could be indicate to a reduction in degree of crystallinity as mentioned in the XRD paragraph (**Table 2**). Further expansion was observed in the PO_4^{3-} band at ($964\text{--}1097\text{cm}^{-1}$), could be due substitution of PO_4^{3-} with SO_4^{2-} ions. Furthermore, this expansion could describe overlapping with SO_4^{2-} spectra. In addition, the same orientation has been

spotted with an addition of SiO_4^{4-} ions. In the spectra of SHA and SiHA clear reduction was observed in the intensity of OH group, this reduction could be due to addition of anions resulting in the imbalance of overall charge.

Micrographic images of Ham SHA and SiHA materials were displayed in **Figure 3**. HA particles were presented in irregular shape closer to spheroid structure, the particle diameter was about 230 nm. However, the obtained results were in a good match with previous studies. Safarzadeh et al used a wet chemical method to synthesis pure phase of HA and the shape of particles appeared in spheroid shape [28]. With incorporation of SO_4^{2-} ions, the shape of the particles was completely changed to needle shape, as approved by previous analysis [29]. The particles in rod shape were presented in different dimensions. On the other hand, with addition of SiO_4^{4-} ions, no obvious changes in the particle shape were observed. However, a clear reduction in the particle size was detected. The SiHA particles were offered in the amplitude of 180 nm. The particles size of HA, SHA, and SiHA powders measured using SEM were quietly bigger than those calculated using XRD measurements which could be more than one crystallite agglomerated in particles shape.

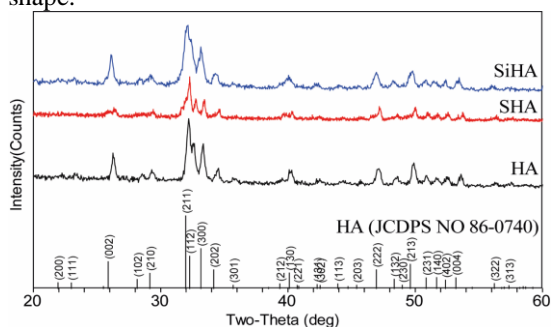


Figure 1. XRD pattern of HA, SHA and SiHA powders calcined at 700°C for 2h.

Table 2: Lattice parameters and degree of crystallinity of the studied samples.

Sample ID	Lattice parameters			Crystallinity (%)	Crystallite size (nm) by XRD		
	a (Å)	c (Å)	V (Å) ³		D(0 0 2)	D(2 0 2)	Average
JCDPS (HA)	9.352	6.882	521.30	---	---	---	---
HA	9.360	6.827	517.91	70	31	29	30.0
SHA	9.349	6.846	518.21	52	22	19	20.5
SiHA	9.357	6.860	520.16	55	31	22	26.5

Table 3: Chemical composition of HA, SHA and SiHA samples measured by EDX

Sample ID	Theoretical ratios			Measured ratios by EDX		
	Ca/P	Ca/P+S	Ca/P+Si	Ca/P	Ca/P+S	Ca/P+Si
HA	1.67	---	---	1.93	---	---
SHA	---	1.67	---	---	1.68	---
SiHA	---	---	1.67	---	---	1.54

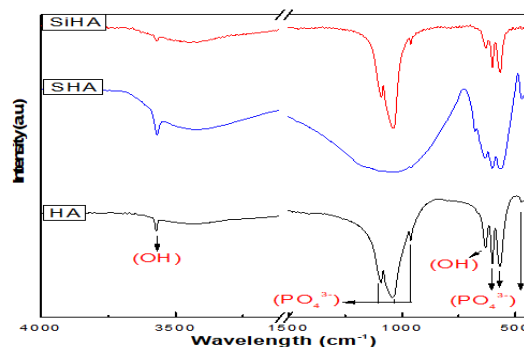


Figure 2. FTIR spectra of HA, SHA and SiHA materials calcined at 700°C for 2h

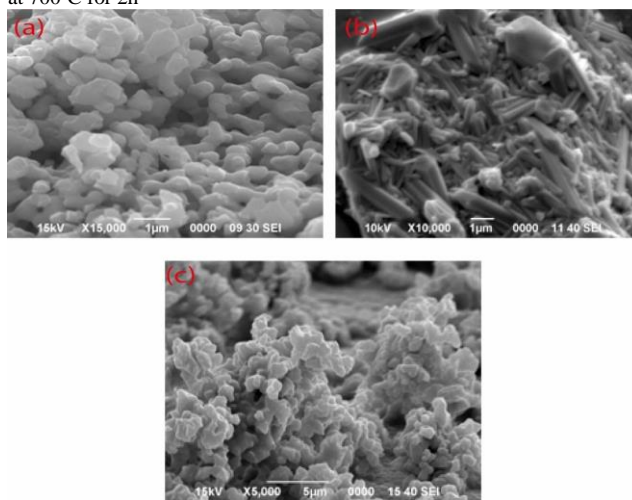


Figure 3. SEM images of HA, SHA, and SiHA materials calcined at 700°C for 2h.

The chemical structure of HA, SHA and SiHA samples measured by EDX were summarized in **Table 3**. Ca/P, Ca/P+S, and Ca/P+Si ratios were almost in good match with theoretical values, small fluctuations were detected, which could be losing some number of ions during the washing and calcination process.

4. Conclusions

In the summary, different kinds of anions were successfully incorporated into HA lattice, without decomposition of the HA phase. With addition of anions clear reduction was observed in (i) lattice parameters (a-axis), (ii) degree of crystallinity, and (iii) crystallite size of HA crystal. Addition of SO_4^{2-} ions led to change the particle shape from spheroid to rod shape, and with incorporation of SiO_4^{4-} ions reduced the particle size. In the chemical composition analysis, the dopant SO_4^{2-} and SiO_4^{4-} ions were detected and the Ca/P ratio was closer to theoretical values.

5. Conflicts of interest

“There are no conflicts to declare”.

6. Formatting of funding sources

Self

7. Acknowledgments

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