THE STUDY OF FLUORINE SUBSTITUTION EFFECTS ON THE STRUCTURAL PROPERTIES AND MORPHOLOGICAL CHARACTERISTICS OF HYDROXYAPATITE

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ABSTRACT

Hydroxyapatite (HA) and fluor-hydroxyapatite (FHA) were synthesized by wet precipitation method and pH-cycling process, respectively and chemical structure, and morphological characteristics of the samples were investigated by using scanning electron microscopy (SEM), Fourier transform infrared (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), F-selective electrode, atomic absorption spectroscopy (AAS) and EDTA titration analyses. The XRD data of all samples calcite 900°C indicates well defined peaks of hydroxyapatite and fluorhydroxyapatite. The FTIR spectroscopic studies of powders confirms the incorporation of the F ion in the OH site of the HA lattice. Elemental analysis was employed for calculation of Ca/P molar ratio and measuring of fluorine content in the crystalline network of HA powder that were 1.70 and 2.64, respectively. SEM and TEM observations showed that substitution of fluoride ions instead of OH group leads in decreasing aspect ratio in comparison with that for pure HA.

Keywords: Fluorine; Fluorhydroxyapatite; Hydroxyapatite; Structural Properties; Morphological Characteristics

INTRODUCTION

Hydroxyapatite (HA; Ca$_{10}$(PO$_4$)$_6$(OH)$_2$), the main mineral component of bones in mammals (Eslami et al., 2009), has attracted much interest as an implant material over the years due to similarity of crystallography and chemical compositions to that of human hard tissues (Asefnejad et al., 2011), nevertheless, the biomaterials based on HA alone are not suitable for load bearing applications owing to lower mechanical strength of HA (Joughehdoust et al., 2008) as a result, culminating in poor thermal stability of synthetic HA by the decomposition into other phases such as tricalcium phosphate (TCP; Ca$_3$(PO$_4$)$_2$) at sintering temperatures higher than 900°C. This phase impurity increases dissolution rate in-vivo (Chen and Miao, 2005). In contrast, there exists potential that as HA forms fluorhydroxyapatite (FHA; Ca$_{10}$(PO$_4$)$_6$(OH)$_2$2F$_2$) by incorporation of F instead of hydroxyl group (OH$^-$) in order to alter dissolution properties of the material and improving the thermal characteristics (Tkalčec et al., 2014). FHA has a similar biocompatibility with HA in terms of its fixation to (Tredwin et al., 2014) bone and bone in growth and the amount of F influences cell attachment, proliferation of osteoblast cells that attributed directly to the release of the fluoride ions (Qu and Wei, 2006; Wang et al., 2009). However, it has been reported that completely replacement of OH group with F, forms FA (fluorapatite), the resulting materials is not osteo-conductive. Therefore, different methods have been developed in order to tailor the fluorine content of FHA to achieve the best biological properties (Rodríguez-Lorenzo et al., 2001). Additionally, F enhances the mineralization and crystallization of calcium phosphate compounds during the bone regeneration, as well as, FHA could facilitate joining between the implants and bone tissue due to chemical similarity to the calcium phosphate parts in the bone, and its ability to create a strong chemical bond with bone (Elliott, 1994; Miao et al., 2007). Fluorhydroxyapatite can be either produced using a solid state reaction or a wet chemical process, but later is utilized more commonly. There exist several methods to the replacement of OH$^-$ with F- such as sol-gel (Cheng et al., 2006), hydrolysis (Kurmaev, 2002), Mechanochemical (Zhang et al., 2005). The pH-cycling technique as the modified wet-chemical process was first introduced by Ramsey et al., (1973) to avoid a high temperature operation and the use of volatilized alcohol (fluorine containing reagent). FHA formed by pH-cycling approach have
been characterized using X-ray diffraction (XRD), Fourier transform infra-red (FTIR), Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In this manner, Okazaki (Okazaki et al., 1999) demonstrated that a laminated structure is formed in micrometer sized FHA precipitates produced via a multi-step fluorine supply process. In this work, we are reported synthesis of hydroxyapatite and fluoridated hydroxyapatite partially by using wet chemical process and we are focus on the changes of hydroxyapatite properties with fluorine substitution.

MATERIALS AND METHODS

To prepare hydroxyapatite, 0.09 M di-ammonium hydrogen phosphate solution [(NH₄)₂HPO₄, %99, Merck company Inc.] and 0.15 M calcium nitrate 4-hydrate solution [Ca(NO₃)₂.4H₂O, %98, Merck PROLABO] were prepared and the pH of the both solutions was brought to about 11 by adding of 1 M NaOH solution [%99, Merck company Inc.].

The phosphate solution was added drop-wise into calcium nitride solution, as a result, form HA. The precipitation of HA can be described by reaction (1) (Morales et al., 2001):

\[ 10 \text{ Ca}^{2+} + 6 \text{ HPO}_{4}^{2-} + 2 \text{ OH}^- \rightarrow \text{Ca}_{10}({\text{PO}}_{4})_{6}({\text{OH}})_2 + 6 \text{ H}^+ \]  

(1)

The precipitate was aged for overnight at room temperature on the magnetic stirrer. In the next step, the precipitated HA was centrifuged and then washed by deionized water, three times. The resulting powder was dried in a freeze-drier system for 10 h. Finally, dried powder was calcite in an electrical box furnace at 900 °C for 1 hour at the rate of 5 °C/min in air.

In order to synthesize fluorhydroxyapatite, some of prepared HA by the above method was suspended in 500 ml of 0.02 M sodium fluoride [NaF, %98.5, Merck Company Inc.] solution. This system was equilibrated to pH 7 for 24 hours. The pH of the solution was the decreased to 4 by slowly adding 1 M nitric acid [HNO₃, %68, Merck company Inc.]. After 30 min, the pH of the solution was brought back to 7 by the addition of 1M NaOH. The cycle of pH fluctuation was repeated three times. The solution was centrifuged and then washed by de-ionized water, three times.

The wet powders were dried in an oven at 90°C for 24 hours. The resulting FHA powder was calcite in an electrical box furnace at 900 °C for 1 hour at a rate of 5 °C/min in air. The synthesis of FHA can be described by reaction (2) (Eslami et al., 2009):

\[ \text{Ca}_{10}({\text{PO}}_{4})_{6}({\text{OH}})_2 + 2x\text{NaF} \rightarrow \text{Ca}_{10}({\text{PO}}_{4})_{6}({\text{OH}})_{2-2x}F_{2x}+x\text{Na}_{3}O + x\text{H}_2O \]  

(2)

After preparation of HA and FHA powders, in order to study their properties, X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FTIR), AAS and EDTA titration techniques, F-selective electrode, Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) utilized.

RESULTS AND DISCUSSION

XRD patterns of samples calcites at 900°C are presented in Figure 1. The main peaks of apatite can be seen in the range of 2θ=25-35° and indicated that both HA and FHA are well crystallized. In addition, XRD patterns displayed the formation of almost pure apatite and traces of other calcium phosphate impurities such as β-TCP were not detected by this technique that indicates the thermal stability of samples (Rameshbabu et al., 2006). It is noteworthy that in Fig 1, secondary phase CaO (calcium oxide) was observed. By calculating the ratio of the intensity of (200) lattice plan of CaO to that of (002) for HA and FHA, the amount of CaO of each sample obtained (Afshar et al., 2003) that was approximately 0.2. Although, it has been demonstrated that CaO presence does not affect the samples biocompatibility, nevertheless, the control of synthesis parameters could lead to form pure HA and FHA (Santos et al., 2004).
Fourier transform infrared spectroscopy (FT-IR) spectra are shown in Figure 2. The typical characteristic P-O bands of apatite (450-580 and 950-1100 cm\(^{-1}\)) were shown in all samples. The peaks corresponding to OH group of HA were 632 cm\(^{-1}\) and it there is its other peaks in 3555 cm\(^{-1}\). When hydroxyl groups were partially replaced with fluoride ions in hydroxyapatite, stretching mode of OH can shift to the new band that arises from OH….F bond. In FHA, OH\(^{-}\) band appeared at 3537 cm\(^{-1}\) owing to create hydrogenic bond with fluoride ions in FHA sample. Furthermore, some carbonate content also was seen (~ 744 cm\(^{-1}\)). This might have arisen from the absorption of carbon dioxide through the atmosphere (Komath and Varma, 2003). Therefore, according to these explanations, it is obvious that the synthesized powder is certainly fluorhydroxyapatite.
The molar ratio of Ca/P for samples is listed in Table 1 which was equal for both samples. The measured Ca/P ratio of synthesized samples was higher than stoichiometric ratio which can be due to local presence of carbonate apatite in which the Ca/P can be as high as 3.33 or the presence of impurities such as CaO (Eslami et al., 2009; Wei et al., 2003). In addition, the fluoride ion concentration of FHA measured by F-selective electrode was determined as 2.64%. With respect to the calculated amount of X in FHA, the formula of prepared fluorhydroxyapatite in this research is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{0.7}F_{1.3}$.

Table 1: Ca/P molar ratio and measured F- content in the synthesized hydroxyapatite and fluorhydroxyapatite

<table>
<thead>
<tr>
<th>sample</th>
<th>Ca/P molar ratio</th>
<th>Measured F- content (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>1.70</td>
<td>-</td>
</tr>
<tr>
<td>FHA</td>
<td>1.70</td>
<td>2.64</td>
</tr>
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</table>

Considering the SEM images taken from synthesized powders (Figure 3), to evaluate the morphology and particle size of samples.

Figure 3: SEM images of (a) HA and (b) FHA particles synthesized by using wet precipitation and pH-cycling methods, respectively.

There exist a distribution of small particles with a needle-like morphology and large agglomerates for HA samples. The microstructure of the fluor-hydroxyapatite particles was observed as almost like a rod with the different aspect ratio.

TEM was utilized to estimate the synthesized powders crystallites. TEM micrographs of powders are shown in Figure 4. The microstructure of the hydroxyapatite crystallite was observed as almost like a needle with a mean crystallite size of 15-20 nm in diameter and about 80 nm in length (Figure 4a). TEM micrographs of fluorhydroxyapatite are shown in Figure 4(b). The FHA particles had perfectly rod-like shape that their lengths and diameters were~ 60 nm and 20-30 nm, respectively. It is notable that the aspect ratio of FHA particles was lower than HA.
When the HA powder was suspended in the NaF solution, FHA was formed through ion-exchange between F⁻ and OH⁻ (L. M. Rodriguez-Lorenzo, K.A. Gross, 2003). It was suggested that when the pH of the fluoride solution decrease from 7 to 4, more HA dissolved into the solution (in fact, the Ca²⁺ ions dissociates from the surface of hydroxyapatite particles) and then Ca⁺² ions rapidly reacted with F⁻ ions, and formed calcium fluoride (CaF₂). The CaF₂ then reacted with the other ions in the solution and formed pre-calcination fluorhydroxyapatite as the pH increased to 7. It should be noted that, during the calcinations process, the hydroxyapatite containing fluoride (FHA) turned become homogenous. The reaction for this phenomenon is inter-diffusion of F⁻ and OH⁻ ions (Qu and Vasiliev, 2005).

**Conclusion**

1. Fluoridated hydroxyapatite was prepared through pH-cycling process by using sodium fluoride (NaF) concentration in hydroxyapatite suspension.
2. The XRD analysis and FTIR investigations displayed that synthesized fluorhydroxyapatite powder was nearly a pure fluorhydroxyapatite.
3. SEM and TEM techniques ascertained that the particles of prepared FHA powder were rod-like in comparison with needle structure of hydroxyapatite.

**REFERENCES**


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