

## 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 confirm the incorporation of the F<sup>-</sup> ion in the OH<sup>-</sup> 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;  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), 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;  $\text{Ca}_3(\text{PO}_4)_2$ ) 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;  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-2x}\text{F}_{2x}$ ) by incorporation of F<sup>-</sup> instead of hydroxyl group (OH<sup>-</sup>) 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<sup>-</sup> 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<sup>-</sup>, 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<sup>-</sup> 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<sup>-</sup> with F<sup>-</sup> 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

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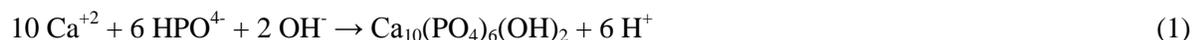
been characterized using X-ray diffraction (XRD), Fourier transform infra-red (FTIR), Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In this manner, Okazaki (Okazaki *et al.*, 1999) demonstrated that a laminated structure is formed in micrometer sized FHA precipitates 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 of hydroxyapatite, 0.09 M di-ammonium hydrogen phosphate solution [(NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, %99, Merck company Inc.] and 0.15 M calcium nitrate 4-hydrate solution [Ca(NO<sub>3</sub>)<sub>2</sub> .4H<sub>2</sub>O, %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 nitrate solution, as a result, form HA. The precipitation of HA can be described by reaction (1) (Morales *et al.*, 2001):

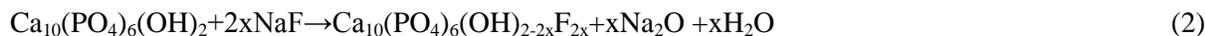


The precipitate was aged for overnight at room temperature on the magnetic stirrer. In the next step, the precipitated HA was centrifuged and then washed by deionized water, three times. The resulting powder was dried in a freeze-drier system for 10 h. Finally, dried powder was calcite in an electrical 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 [HNO<sub>3</sub>, %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.

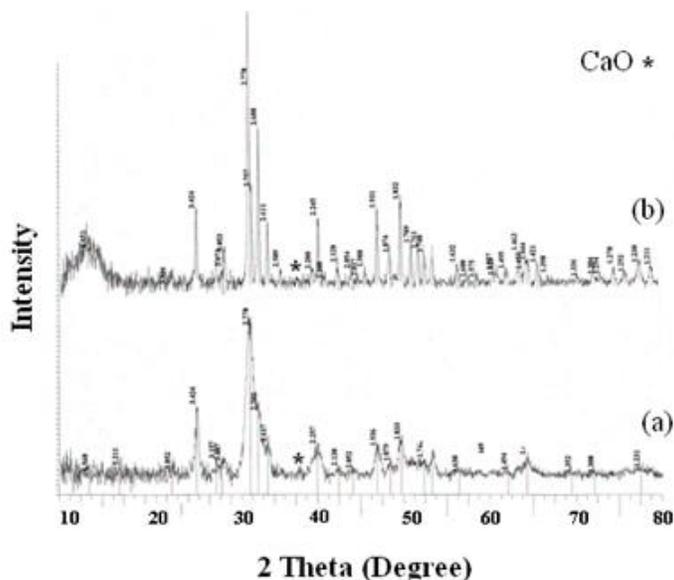
The wet powders were dried in an oven at 90°C for 24 hours. The resulting FHA powder was calcite in an electrical box furnace at 900 °C for 1 hour at a rate of 5 °C/min in air. The synthesis of FHA can be described by reaction (2) (Eslami *et al.*, 2009):



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

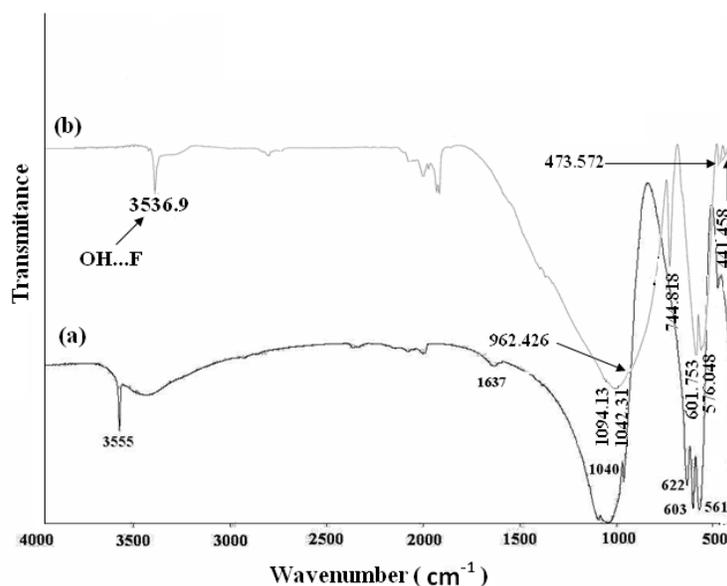
XRD patterns of samples calcites at 900°C are presented in Figure 1. The main peaks of apatite can be seen in the range of 2θ=25-35° and indicated that both HA and FHA are well crystallized. In addition, XRD patterns displayed the formation of almost pure apatite and traces of other calcium phosphate 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).



**Figure 1: X-ray diffraction diagram of the synthesized (a) HA and (b) FHA powders**

Fourier transform infrared spectroscopy (FT-IR) spectra are shown in Figure 2. The typical characteristic P-O bands of apatite ( $450\text{-}580$  and  $950\text{-}1100\text{ cm}^{-1}$ ) were shown in all samples. The peaks corresponding to OH group of HA were  $632\text{ cm}^{-1}$  and it there is its other peaks in  $3555\text{ cm}^{-1}$ . When hydroxyl groups were partially replaced with fluoride ions in hydroxyapatite, stretching mode of OH<sup>-</sup> can shift to the new band that arises from OH...F bond. In FHA, OH<sup>-</sup> band appeared at  $3537\text{ cm}^{-1}$  owing to create hydrogenic bond with fluoride ions in FHA sample. Furthermore, some carbonate content also was seen ( $\sim 744\text{ cm}^{-1}$ ). 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.



**Figure 2: FTIR spectra of the as-synthesized (a) HA and (b) FHA powders**

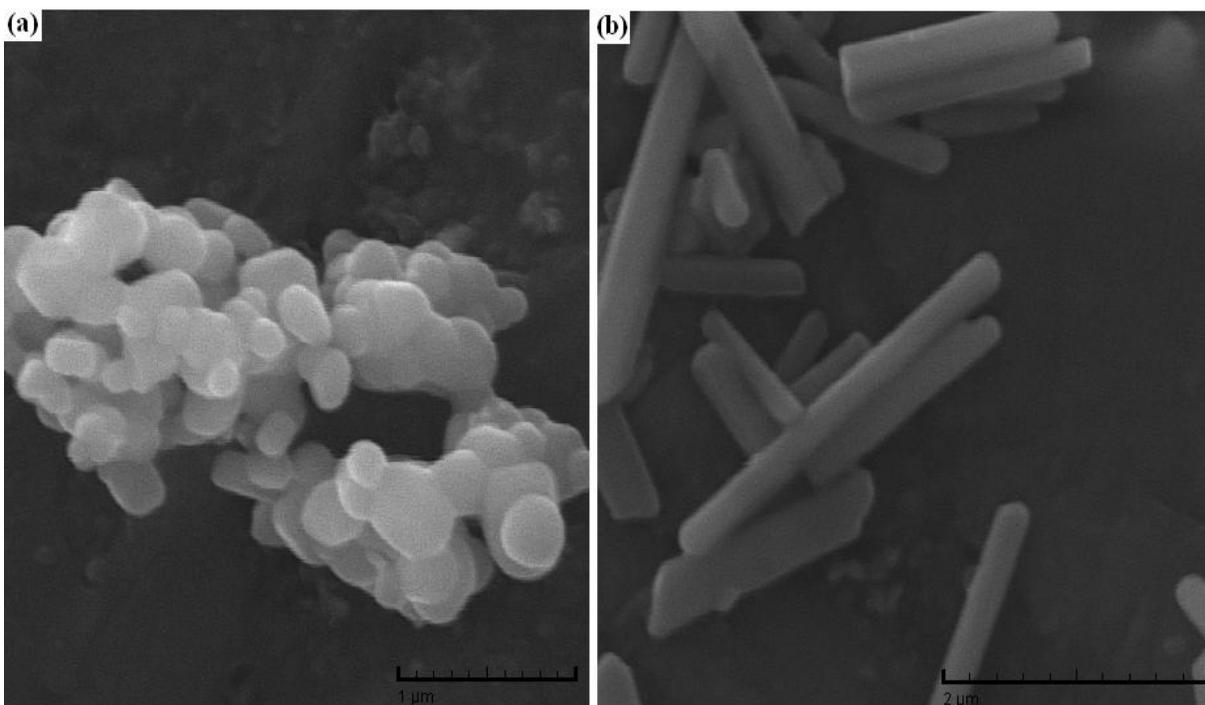
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The molar ratio of Ca/P for samples is listed in Table 1 which was equal for both samples. The measured Ca/P ratio of synthesized samples was higher than stoichiometric ratio which can be due to local presence of carbonate apatite in which the Ca/P 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  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{0.7}\text{F}_{1.3}$ .

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

	sample	Ca/P molar ratio	Measured F- content (wt%)
HA		1.70	-
FHA		1.70	2.64

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

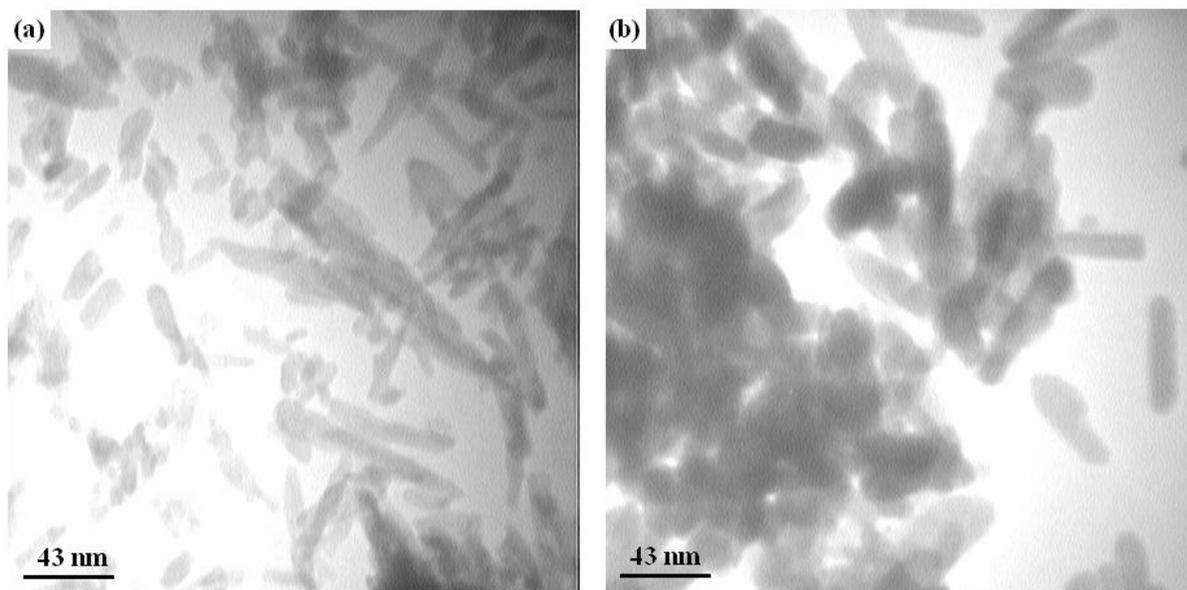


**Figure 3: SEM images of (a) HA and (b) FHA particles synthesized by using wet precipitation and pH-cycling methods, respectively**

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.

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**Figure 4: TEM images of prepared (a) HA and (b) FHA samples**

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  $Ca^{2+}$  ions dissociates from the surface of hydroxyapatite particles) and then  $Ca^{+2}$  ions rapidly reacted with  $F^-$  ions, and formed calcium fluoride ( $CaF_2$ ). The  $CaF_2$  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

- Afshar A, Ghorbani M, Ehsani N, Saeri MR and Sorrell CC (2003).** Some Important Factors in the Wet Precipitation Process of the Hydroxyapatite, *Materials & Design* **24** 197-202.
- Asefnejad A, Behnamghader A, TaghiKhorasani M and Farsadzadeh B (2011).** Polyurethane/fluorhydroxyapatite nanocomposite scaffolds for bone tissue engineering. Part I: morphological, physical, and mechanical characterization, *International Journal of Nanomedicine* **6** 93-100.
- Chen Y and Miao X (2005).** Thermal and chemical stability of fluorhydroxyapatite ceramics with different fluorine contents, *Biomaterials* **26** 1205-1210.
- Cheng K, Zhang S and Weng W (2006).** Sol-gel preparation of fluoridated hydroxyapatite in  $Ca(NO_3)_2-PO(OH)_{3-x}(Oet)_x-HPF_6$  system, *Journal of Sol-Gel Science and Technology* **38** 13-17.
- Elliott JC (1994).** Structure and chemistry of the apatite and other calcium orthophosphates, *Amsterdam, The Netherlands: Elsevier Science*.
- Eslami H, Solati-Hashjin M and Tahriri M (2009).** The comparison of powder characteristics and physicochemical, mechanical and biological properties between nanostructure ceramics of hydroxyapatite and fluoridated hydroxyapatite, *Materials Science and Engineering C* **29**.

### **Research Article**

**Joughehdoust S, Behnamghader A and Afshar A (2008).** Deposition of nano-crystalline fluor-hydroxyapatite coatings on titanium substrates via sol-gel method, *Iranian Journal of Pharmaceutical Sciences* **4** 169-176.

**Komath M and Varma HK (2003).** Development of fully injectable calcium phosphate cement for orthopedic and dental applications, *Bulletin of Materials Science* **26** 415-422.

**Kurmaev EZ, Matsuya S, Shin S, Watanabe M, Eguchi R, Ishivata Y and Takeuchi T (2002).** Observation of fluorapatite formation under hydrolysis of tetracalcium phosphate in the presence of KF by means of soft X-ray emission and absorption spectroscopy, *Journal of Materials Science: Materials in Medicine* **13** 33-36.

**Miao S, Weng W and Cheng K (2007).** In vitro bioactivity and osteoblast-like cell test of zinc containing fluoridated hydroxyapatite films, *Journal of Materials Science: Materials in Medicine* **18** 2101-2105.

**Morales JG, Burgues JT, Boix T, Fraile J and Clemente RR (2001).** Precipitation of stoichiometric hydroxyapatite by a continuous method, *Crystal Research and Technology* **36** 15-26.

**Okazaki M, Miake Y, Tohda H, Yanasisawa T, Matsumoto T and Takahashi J (1999).** Functionally graded fluoridated apatites, *Biomaterials* **20** 1421-1426.

**Qu H and Wei M (2006).** Effect of fluorine content on mechanical properties of sintered fluoridated hydroxyapatite, *Materials Science and Engineering C* **26** 46-53.

**Qu H, Vasiliev AL, Aindow M and Wei M (2005).** Incorporation of fluorine ions into hydroxyapatite by a pH cycling method, *Journal of Materials Science: Materials in Medicine* **16** 447-453.

**Rameshbabu N, Kumar TSS and Rao KP (2006).** Synthesis of nanocrystalline fluorinated hydroxyapatite by microwave processing and Its in vitro dissolution study, *Bulletin of Materials Science* **29** 611-615.

**Ramsey AC, Duff EJ, Paterson L and Stuart JL (1973).** The uptake of F<sup>-</sup> by hydroxyapatite at varying PH, *Caries Research* **7** 231-244.

**Rodriguez-Lorenzo LM and Gross KA (2003).** Encapsulation of apatite particles for improvement in bone regeneration, *Journal of Materials Science: Materials in Medicine* **14** 939-943.

**Rodríguez-Lorenzo LM, Hart JN and Gross KA (2001).** Influence of fluorine in the synthesis of apatite: Synthesis of solid solutions of hydroxy-fluorapatite, *Biomaterials* **24** 3777-3785.

**Santos MH, de Oliveira M, de Freitas Souza LP and Mansur HS (2004).** Synthesis Control and Characterization of Hydroxyapatite Prepared by Wet Precipitation Process, *Journal of Materials Research* **7** 625-630.

**Tkalčec E, Popović J, Orlić S, Milardović S and Ivanković H (2014).** Hydro-xyapatite synthesis and thermal evolution of carbonate-fluorhydroxyapatite scaffold from cuttlefish bones, *Materials Science and Engineering C* **42** 578-586.

**Tredwin CJ, Young AM, Abou Neel EA, Georgiou G and Knowles JC (2014).** Hydroxyapatite, fluor-hydroxyapatite and fluorapatite produced via the sol-gel method: dissolution behavior and biological properties after crystallisation, *Journal of Materials Science: Materials in Medicine* **25** 47-53.

**Wang C, Karlis GA, Anderson GI, Dunstan CR, Carbone A, Berger G, Ploska U and Zreiqat H (2009).** Bone growth is enhanced by novel bioceramic coatings on Ti alloy implants, *Journal of Biomedical Materials Research Part A* **90A** 419-428.

**Wei M, Evans JH, Bostrom T, GrÖndhl L and Evans JH (2003).** Synthesis and characterization of hydroxyapatite, fluoride-substituted hydroxyapatite and fluorapatite, *Journal of Materials Science: Materials in Medicine* **14** 311-320.

**Zhang HG, Zhu Q and Xie ZH (2005).** Mechanochemical-hydrothermal synthesis and characterization of fluoridated hydroxyapatite, *Materials Research Bulletin* **40** 1326-1334.