Influence of Fluoride Addition on Hydroxyapatite Prepared for Medical Applications

*Abdulsalam Khashan Swadi** *Zuhair Wahib Jassim**
*Mohamed Ubaid Kadum** *Mutawar Redha Mohamed Ali**

Received 5, March, 2011
Accepted 1, November, 2011

Abstract:

In this study, hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) has been prepared as bioceramic material with biological specifications useful to used for orthopedic and dental implant applications. Wet chemical processing seems to form the fine grain size and uniform characteristic nanocrystalline materials by the interstice factors controlling which affected the grain size and crystallinity in order to give good mechanical and/or constituent properties similar as natural bone. Fluorinated hydroxyapatite [4-6 wt% F, (FHA, $Ca_{10}(PO_4)_6(OH)_{2-Fx}$] was developed in new method for its posses to increased strength and to give higher corrosion resistance in biofluids than pure HAP moreover reduces the risk of dental caries. The phase's and functional groups characterizations XRD & FTIR reveled the purity of the product and its free of other phases, while the morphology tests showed the compound homogeneity as fluoride interpenetrated in the compound lattice net.

Key words: hydroxyapatite, biocompatibility, bioceramic, Florien.

Introduction:

Human bone is a composite material made up of collagen and calcium phosphate mineral. The mineral phase of bone comprises 60–70% of total dry bone weight. Bone mineral is an apatitic calcium phosphate containing carbonate and small amounts of sodium, magnesium, fluorine and other trace elements [1].Artificially prepared HAP, with the same structure as that of apatitic phosphate of natural bone has good biocompatibility with the human organism. It forms chemical bond with the host hard tissue and for this reason, it is widely used in medical applications as implants, as coating on prostheses or as bone filling material [2].HAP powder can be synthesized via numerous production routes, using a range of different reactants. Some of the processing techniques include dry process, wet chemical method (precipitation), hydrolyzation of calcium phosphate, hydrothermal synthesis, spray pyrolysis, freeze-drying, gel-diffusion, sol–gel technique and electrochemical deposition. Among these methods, precipitation scores over other processes by virtue of being simple, cheap and easy application in industrial production. Moreover, HAP was prepared by precipitation also has the feature of small size, low crystallinity and high surfacial activation, which can meet different demands [3-6].Fluorine has been used to substitute some of the hydroxyl groups to form fluoridated hydroxyapatite (FHA). FHA has a more compact structure than HAP, decreased dissolution rate as well as enhanced adhesion strength between the coating and substrate, thus possesses improved stability in the

*Directorate of Materials Research-Ministry of Science and TechnologyBaghdad- Iraq
Mobile: +964 1 790 6134 935
Email: akswadi@yahoo.com

physiological environment. Fluoride has long been known to influence the activity of various enzymes *in vitro*. Observation of industrial fluorosis led to the use of fluoride in medicine as a treatment to increase bone mass in osteoporosis patients. Fluoride ingestion will harden the surface of teeth and make them less susceptible to dental caries; it seems that the effects of fluoride on bone quality are dependent on the balance between the beneficial effects and deleterious effects [7, 8]. When OH^- groups in HAP are partially substituted by F^-, fluorine substituted hydroxyapatite, [FHA, $Ca_{10}(PO_4)_6 (OH)_{2-Fx}$, $0 < x < 2$], is obtained. If the substitution is completed, fluorapatite [FA, $Ca_{10}(PO_4)_6F_2$], is formed. The presence of fluorine (F) in saliva and blood plasma is important for normal skeletal and dental development. It has been suggested that fluorine intake of 1.5–4 mg/day significantly reduces the risk of dental caries. Fluorine substitution in HAP enhances the acid resistance and stability of hydroxyapatite. Recent studies have shown that the incorporation of fluorine into HA induced better biological response [7 – 9].The bloodstream is carried to different parts of the body. Osteoblasts are responsible for the formation of new bone. They start by secreting collagen and then coat them with non-collagenous proteins that have the ability to hold minerals, mostly calcium and phosphate, from the blood stream, leading to new bone formation. Increased bone resorption is an important determinant in pathophysiology of osteoporosis and many other metabolic bone diseases [10-12].In the present study, an attempt has been made to synthesize pure and biocompatible HAP and FHA nanopowder through wet chemical method by using eggshell (a waste material) as the Ca source.

Materials and Methods:

Synthesis of HAP*:*

The egg shell consists of about 94–97% of $CaCO_3$ and the other 3% is organic matter and egg pigment. Uncrushed and washed egg shell has been calcined in an air atmosphere at 900°C/1h (Carbolite furnace, England). The amount of calcium present in the calcined egg shell has been estimated (AAS, 760 Shimadzu – Japan). A stiochiometric amount of calcined eggshell was dispersed in well-degassed distilled water. Under rigorous stirring reagent grade orthophosphoric acid solution (0.6 M) was added in drops at a controlled rate (1.0 ml/min) to the suspension at room temperature. After completion of the addition, the precipitate formed was subjected to ripening (aging) treatment for 24 h followed by 1 h refluxing. It was then stirred for another 25 min without heating and left for over 10 h. The precipitate has been filtered and thoroughly washed with double distilled water and filtered again. After drying at 80°C for 3 h, the precipitate has been calcined at various temperatures (400°C, 700°C and 900°C) for 2 h.

Synthesis of FHA:

A crystalline fluorinated hydroxyapatite with different fluorine contents have been synthesized through a wet chemical reaction. Analytical grade calcium hydroxide [$Ca(OH)_2$, Merck- Germany] **,** diammonium hydrogen phosphate [DAP, $(NH_4)_2HPO_4$, BDH-England] and ammonium fluoride [NH_4F, Merck- Germany], have been used as precursor materials for the preparation of highly crystalline FHA. A 0.25 M ammonium fluoride solution and 0.3 M DAP solutions have been prepared separately and these two solutions were added to a 0.3 M calcium hydroxide. The precipitate was thoroughly washed with distilled water

to remove (NH_4^+) ions. The product obtained after filtration was oven-dried overnight at 90°C. Ammonium fluoride was added in varying quantities (0.4-2.0 gm) to prepare powders with a chemical composition of $Ca_{10}(PO_4)_6$ $(OH)_{2-Fx}$ with (*x* = 0.5, 1.0, 1.5), these compositions correspond to a 20%, 60% and 100% fluoride substitution for OH^- groups and can be further referred to as 20 FHA, 60 FHA and FA in this paper. A small amount of powder of each composition was sintered at 900°C for 2h in a box furnace at a ramp speed of 10°C/min and furnace cooled. The apatite powders produced (HAP and FHA) have been explored to characterization studies for elemental composition analysis [(AAS, 760 Shimadzu –Japan], spectral phases (XRD diffractometer, X 6000 Shimadzu- Japan), thermal analysis (DTG–60 Shimadzu–Japan) and morphology evaluations. The crystallite sizes have been calculated using Scherrer's relationship [t = 0.9λ / B CosΘ_B (13)] $d = kl/b \cos q$, where *d* is the average diameter in Å, *k* the shape factor and *b* the broadening of the diffraction angle. The Bragg reflections at (002) and (003) planes of HAP were considered to calculate the crystallite size.

Results and Discussion:

The phase constitution and chemical homogeneity of the HAP and FHA samples have been examined by (Quantitative chemical analysis via EDTA titration, Gravimetry and Atomic absorption spectroscopy), the Ca/P molar ratio was found to be 1.67, which indicates the formation of pure apatites. Figures 1 show FTIR spectra (8400S, Shimadzu –Japan) of hydroxyapatite heated at various temperatures (80, 400, 700 and 900°C). The spectral data indicate that carbonate ion is present in the prepared HAP samples. The carbonate ion substitution is identified by characteristic doublet peak of the carbonate ions around 1420 and 1480 cm^{-1} and a singlet peak at 875 cm^{-1} which is attributed to the vibrational modes of the carbonate ions, substituted at the phosphate site For samples heated at 80°C these peaks were rather broad and it became well defined for those samples heated at 400°C and 700°C. However, peaks at 875 cm^{-1} and 1480 cm^{-1} became deficient when the samples were heated to 900°C.This confirms the elimination of CO_3^{2-}. Figure 2 shows the FTIR spectra of 20 wt% FHA, 60 wt% FHA and FA powders subjected to heat-treatment at 900°C for2 h. The characteristic bands exhibited in the 20 wt% FHA spectra are assigned two bands were OH^- bands completely disappeared in FHA, suggesting that substantial amount of fluoride has been substituted for the hydroxyl groups. A typical XRD profile of HAP powder has been shown in figure 3. The XRD phase analysis has been performed using JCPDS card number 09–0432. The samples heated at 80°C show broad peaks indicating the formation of microcrystalline phases, which increase with heating temperature. Figure 4 shows the XRD patterns of 20 FHA, 60 FHA and FA in as synthesized condition. The XRD peaks were markedly broader, which suggested that particles were nanosized. The crystal sizes of the synthesized powders have been measured by the (002) peak broadening.Crystallinity of 60 FHA and FA are higher than 20 FHA, which seems to indicate that the fluoride concentration increases the driving force for the apatite crystal growth during precipitation. The diffraction peaks of the 900°C heat-treated samples are well resolved as shown in figure 5. The presence of b-TCP phase

was observed in heat-treated samples of 20 FHA and 60 FHA and it was not detected in FA indicating its thermal stability.Figure 6 illustrates the results of thermal analysis of apatites precursor. Two exothermic peaks at 147.5°C and 489°C and two endothermic peaks at 1335°C and 1363°C have been observed in the DTA. In the investigated temperature range, there are three regions of interest in the TG curve: (i) up to 300°C, corresponding to the removal of adsorbed water, (ii) between 300 and 700°C, corresponding to HPO_4^{2-} decomposition according to the reaction: $2HPO_4^{-2}$ endothermic peaks at 1335°C and 1363°C on the DTA curve was accompanied by a 8.56% weight loss on the TG curve, which was attributed to the decomposition of HAP to *b*-TCP or dihydroxylation.
The morphology of the apatites powders Figure 7 (a&b) indicates that it is composed of spheroid and angular agglomerates with wider particle size distributions. The micrograph shows the presence of nearly spherical agglomerates of (75-85 µm) in diameter. The formation of a regular crystal structure has been observed that could be grouped in crystal colonies of different morphologies with some entanglement. The mean particle size of the prepared apatite powders which measured by using (Sald-2101, Shimadzu-Japan) were found (2.131 µm compared with 2.936 µm for standard HAP which had been produced from Merck Company (Art. 2143- Germany).

Conclusions:

The results of present work indicate that the nature of the reagents, pH of solutions, ageing time and temperature influence the Ca/P ratio of the final product, the reproducibility of the process has been achieved by close control of these parameters.

During the ageing process the amorphous phases formed are converted to crystalline apatite. Sintering of the precipitates at 900°C causes densification and increases the mechanical strength of the precipitates.

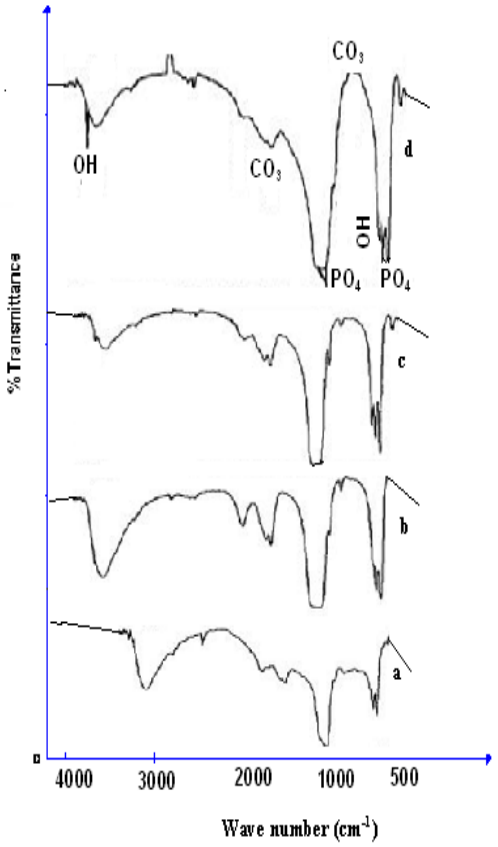


Fig. 1: FTIR spectra of HAP heated at (a) 80°C, (b) 400°C, (c) 700°C and (d) 900°C.

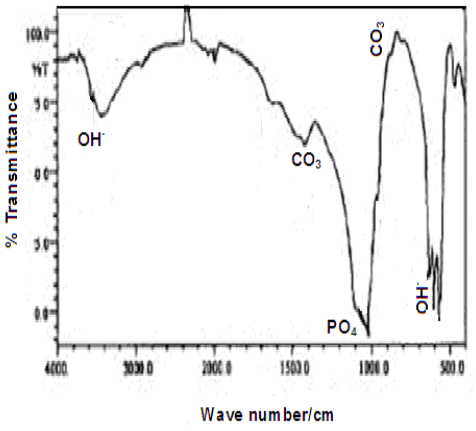


Fig 2: FTIR spectra of prepared HAP heated treated at 900°C.

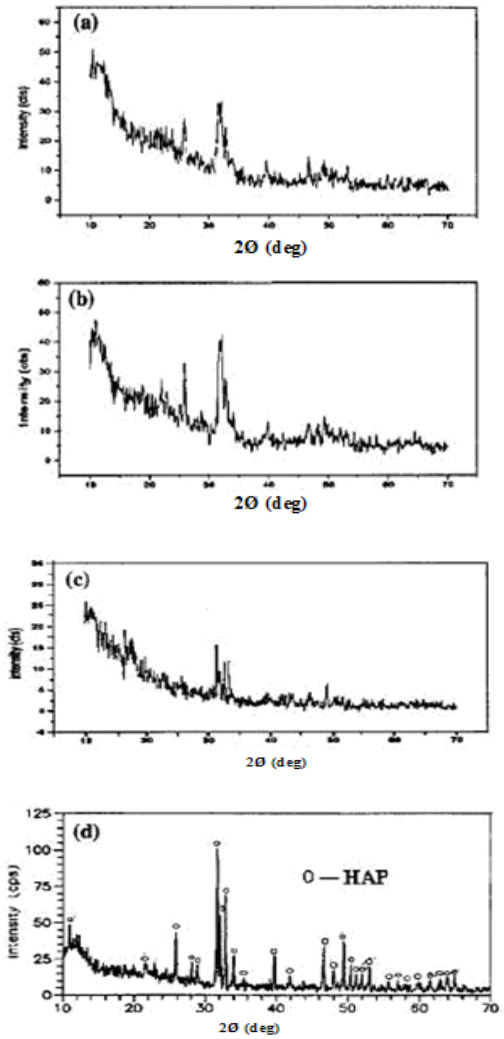


Fig. 3: XRD pattern of HAP heated at (a) 80°C, (b) 400°C, (c) 700°C and (d) 900°C.

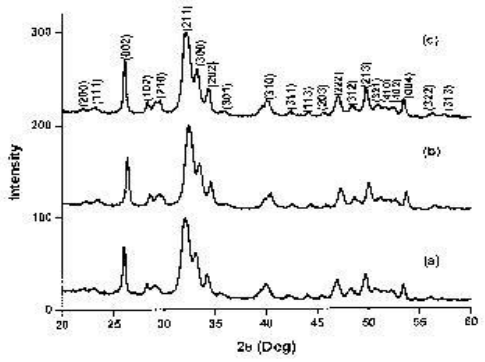


Fig. 4: The XRD patterns of (a) 20FHA, (b) 60 FHA and (c) FA samples as synthesized condition.

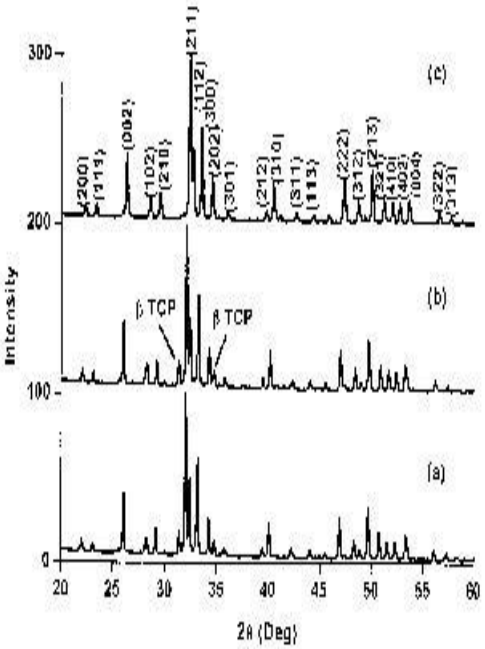


Fig. 5: The XRD patterns of (a) 20 FHA, (b) 60 FHA and (c) FA samples sintered at 900°C.

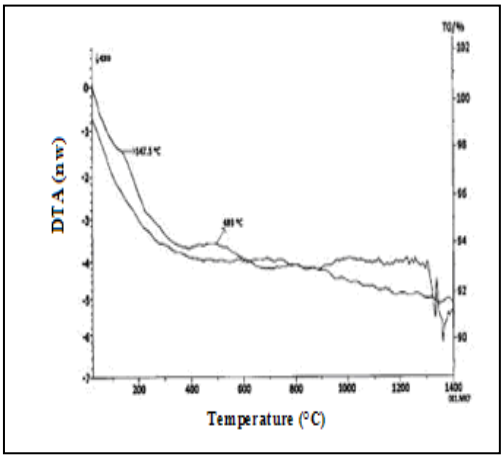
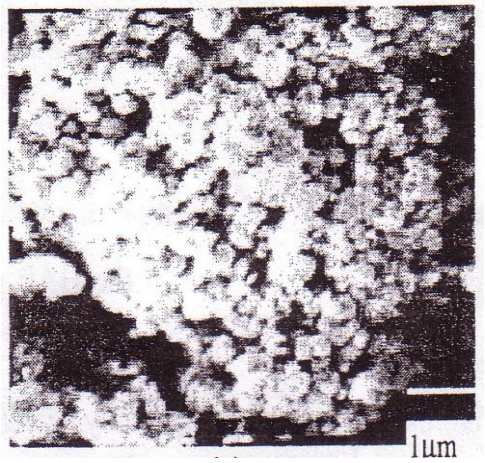
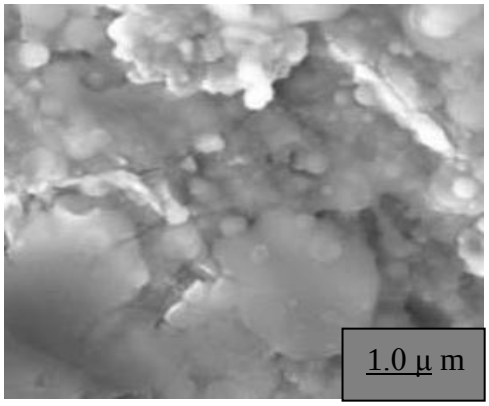


Fig. 6: Thermal analysis of HAP



(a)



(b)

Fig. 7: Optical micrographs of (a) HAP and (b) FHA.

References:

1. Amit S., Ingle A., Rmunim K., Nvaidya S., Psharma B. and Nbhisey A 2001 " Development of Calcium Phosphate Based Bioceramics" J. Bull. Mater. Sci., 24 (6) 653–657.

2. Mellatie R., Atche J., Valfredo T. and Mauros C.2001 "Synthesis of Calcium Phosphate and Chitosan Bioceramics" J. An. Acad. Bras. Cienc., 73 (4) 525–532.

3. Abdulsalam K., Thair L., Ismaeel K. and Sherin A. 2008 "Effect of Calcium Phosphate Ratio on Biotype Hydroxyapatite haracterization. Coll.Sci. Almust. Univ. 19 (1) 17- 26.

4. Nithyanantham T., kandassamy C.and Gnanam F.D. 2002 "The Effect of Powder Processing on Densification, Microstructure and Mechanical Properties of Hydroxyapatite", J. Ceram. Inter. 28 (3) 355-36.

5. Annika Park 2006 "Biomimetic Surfaces", PhD Thesis, Phys. Dept., Linkoping Univ. Sweden

6. Abdulsalam K. S. 2008"Variables Affected the Coating Development of Bioactive Hydroxyapatite By Using Electrophoretic Deposition Technique, J. Um Salama, Sci. Baghd. Univ. 5 (3) 423- 426.

7. Anna S., Jiří P. and Paul C. 2004 "Fluorine in Medicine" J. Appli. Biomed. 2; 141-150.

8. Daniela F., Sergey M., Jiulietta V., Roberto T. and Alessandro L. 2005 "Calcium Phosphate and Fluorinated Calcium Phosphate Coatings on Titanium Deposited by Nd:YAG Laser at a High Fluence" J. Biomaterials 26; 805–812.

9. Stan G. and ferreiraa J. 2007 "An Algorithm for Preparing Bioactive Fluorinated Hydroxyapatite Coatings by Sol-Gel Technique. Optoelect. Adv. mat., 9; (8) 2539 – 2542.

10. Milev A., Green D., Chai C.S. and Ben-Nissan B. 2005 "Coating of the Orthopedic Titanium Alloys with Sol-Gel Derived Hydroxyapatite" A. Dep. Chem. of mat. Science, Univ. 0f Tech., Sydney.

11. Tristan B. & Owen S. 2001 "Materials for Biomedical Engineering". J.Mater. Sci. Eng., UNSW 1-15.

12. Cullity B. D 1978 ''Elements of X-ray Diffraction" Hand book, 2nd Ed.

13. Daiwan C., Kacey G. and Prashant N. 2004 " Chemical Synthesis of Apatite/poly(e-caprolactone) Composites" Mat. Res. Bull. 39 417–432.

تأثير اضافة الفلوريد على مركب الهيدروكسي ابتايت المحضر للتطبيقات الطبية

عبدالسلام خشان سوادي* زهير وهيب جاسم* محمد عبيد كاظم *
مطور رضا محمد علي*

*وزارة العلوم والتكنلوجيا / دائرة بحوث المواد

الخلاصة:
في هذا البحث ، تم تحضير مركب الهيدروكسي ابتايت ($HAP, Ca_{10}(PO_4)_6(OH)_2$) كمركب سيراميكي وفق المواصفات القياسية المعتمدة بايولوجيا ليتلائم مع الاستخدامات الطبية لجراحة العظام والاسنان. الطريقة الكيمياوية الرطبة استخدمت في تحضير المركب بالسيطرة الدقيقة على كل المتغيرات الحاكمة التي تؤثر على بلورية وحجم الدقائق لكونها تؤثر في جعل المركب ذو مواصفات ميكانيكية وتركيبية مشابهة لمادة العظم الطبيعية . البحث يتضمن استنباط طريقة جديدة باضافة نسبة محددة من مادة الفلور (4-6%) ، حيث تؤدي هذه الاضافة الى زيادة صلادة المركب مع مقاومة عالية للتاكل بالاضافة الى فائدتها البايلوجية كونها مادة مفيدة لمنع تسوس الاسنان . لفحوصات الطورية XRD وفحوصات FTIR اظهرت نقاوة المنتج وخلوه من الاطوار الاخرى بينما اظهرت صور التراكيب المجهرية تجانس المركب بتداخل الفلور بالشبكة البلورية للمركب .