# Synthesis of Fibrous Hydroxyapatite through Sol-Gel Route

***Abdulsalam K. S.*** [*] ***Maria. G. R.*** [**] ***Thair L.*** [*]
***Hameed R.*** [*] ***Murtda A.Saiyah.*** [***]

Date of acceptance 16 /10 / 2008

## Abstract

The sol-gel route using an agar gel with calcium nitrate and phosphate solution as starting materials for producing hydroxyapatite (HAP). The product formed were needle like, zigzag and straight fibres. The fibrous products on sintering transformed into stoichiometric HAP with a biological Ca/P ratio of 1.67.

The influences of pH, temperature, nature of base and phosphate solution on the growth of fibrous HAP were studied. The pH of the solution was found to greatly influence the growth rate and morphology of the resultant product. The optimum gel temperature was found to be 60$^{o}$C and sintering temperature of 900$^{o}$C for 1 hour. The crystalline, thermal, functional and morphological characteristics of the fibrous HAP were investigated.

**Key words:** Bioceramic, hydroxyapatite, sol-gel, biocompatibility, agar.

## Introduction

In the field of materials science and engineering zero shrinkage manufacturing, fiber composites processing and biomimetic interface bonding are among the current topics of advanced bioceramics development. Bioactive as well as bioresorbable fibers are of particular interest for reinforcement of osteosynthetic biopolymer matrix composites and for cell carrying scaffold substrates (1).

Hydroxyapatite (HAP), chemical formula $Ca_5(PO_4)_3OH$, is considered the structural template for the mineral phase of bone, dentin and enamel because of its chemical and crystallographic similarities to the mineral constituents of bone and teeth.. Synthetic HAp is widely used in medicine and dentistry because of its biocompatibility and bioactivity properties. Biological HAP has multiple substitutions and deficiencies at all ionic sites. The close relationship between substitutions and bioactivity of synthetic substituted HAps has been demonstrated by *in vitro* and *in vivo* studies (2).

Hydroxyapatite is generally synthesized by wet chemical methods involving the addition of phosphate solution to a suspension of $Ca^{2+}$ ions which forms a precipitate which upon sintering transforms to HAP (3).

The sol- gel process has proved to be versatile and has been widely used in the preparation of organic/inorganic hybrid materials due to high product purity, homogeneous composition and comparatively low synthesis temperature (4 -6).

The development of HAP and other calcium phosphate crystals using the gel system have been reported by novel studies (4, 7) they used low concentrations of $Ca^{2+}$ (0.005M) and $PO4^{3-}$ (0.01M). According to them, when agar, silica or collagen medium was used, the HAP produced was found to coexist with dicalcium phosphate dihydrate.

Since HAP synthesized by the fibrous route technologically finds application as a material for chromatographic separations (as catalyst and as ion exchangers), apart from its orthopedics

*Directorate of Materials Science, Ministry of Science and Technology- Iraq
**Institute of Composite and Biomedical Materials – C.N.R. University of Naples Federico II- Italy
***Directorate of Chemical and Petrochemical Industries, Ministry of Science and Technology

applications, its synthesis in the purest form assumes significance (7).
The aim of the present investigation is to grow straight fibrous HAP and to study the effect of pH of the gel system on the growth rate of HAP and its characteristics.

## Material and Methods

Analytical grade $Ca(NO_3)_2.4H_2O$ (Fluka Co.) (0.4 M) and $(NH_4)_2HPO_4$ (BDH Co.) (1.0 M) were used as starting materials and dissolved in double distilled water. The growth rate of fibrous HAP was monitored by adjusting the pH 10-11 with urea- $NH_3$ aqueous mixture.
0.75g of agar (bacteriological grade, Himedia Co. India) added to 20ml of $Ca(NO_3)_2.4H_2O$ solution, boiled mixture and the hot calcium nitrate solution containing agar was then poured in to a test tube of 20 mm inner diameter and cooled to be gelled.
20 ml of $(NH_4)_2HPO_4$ adjusted to pH 11 and cautiously layering along the sides of the test tube over the gel phase. In a few minutes fibrous products emerged from the gel phase which had grown vertically along the gel system in the phosphates solution.
The system was undisturbed for 24 hour in the ambient atmosphere at room temperature. The products were carefully removed, thoroughly washed with distilled water and dried at room temperature for 24 hour, then at 110$^oC$ for 3 hour. It was sintered at 900$^oC$ in a muffle for 1 hour and left to cool inside the furnace (8, 12).

## Result and discussions

### 1 Growth of fibrous HAP

The optical micrographs of fibrous products obtained under varied experimental conditions are shown in Figure 1. Physiochemical and morphological characteristics of the products are given in table 1. The number prefix R indicates the sample identity numbers.
The products obtained were needle like (a), zigzag (b) and straight fibers (c). Calcium phosphate products form within a few minutes to a few hours after the $PO_4^{3-}$ solution is layered over the gel in different forms such as gelatinous precipitate, fibrous product (cloudy mass) and needle like fibres. The optimum growth of fibres is observed when the growth medium is in a semisolid form and negligible growth was observed on cooling the gel. Zigzag fibres moved upwards at a slow rate when compared to others with growth occurring at the tip of the fibres.
A translucent film precipitates over the solution surface being centered at the top of the fibres and occurs at a faster growth rate along the sides of the test tube.

### 2 Effect of pH

The pH of the gel system plays a crucial role in the growth of fibrous HAP and its stoichiometry. This is evident from the variations in physical appearance of the fibres, which are in zigzag, straight and needle like forms at pH 9.5, 10.0 and 11.0 respectively.
Urea hydrolysis is an excellent analytical procedure to maintain the desired pH of the system *insitue* on its hydrolysis. The concentration of urea present in the gel determines the growth rate of fibres as they are pH dependent. The optimum concentration of urea was found to be 0.75 M as observed in the Figure 2. The growth rate of the fibres gradually increases and slumps down above this concentration (9, 11).
A growth rate of 0.26 cm/min **(**Figure 3**)** was obtained with 0.75 M urea which further justifies the optimum concentration in comparison with 0.5 M and 1.0 M. the effectiveness of urea-

$NH_3$ mixture in maintaining the pH of the gel further studied.

Figure 4.a indicates that at a temperature of 60°C in calcium nitrate solution without agar, pH remains constant for about 5 minutes and slowly decreases at the optimum urea concentration whereas with 0.5 M it decreases rapidly indicating rapid loss of ammonia. The pH **(**figure 4.b**)** was maintained at 10.5 – 10.0 by uniform release of $NH_3$ for about 10 minutes at a gel temperature 60°C for 0.75 and 1.0 M concentration of urea, in the calcium nitrate solution with agar. The pH of the system decreases with the growth of fibres and is further maintained by urea hydrolysis. The pH of the system decreases gradually with time when $Ca(NO_3)_2$ was replaced with water due to the slow evaporation of ammonia. Further, on adding agar **(**figure 4.c**)** to form the gel, a decrease in pH was observed but this was effectively maintained by urea hydrolysis. This is evident from figure 4.d where, in the absence of agar, the pH of the system increases gradually with time and was maintained at 10.5. A fibre length of 7cm is obtained within 30 minutes at a urea concentration 0.75 M.

**Table 1:** Influences of the Physiochemical Parameters on fibrous HAP.

| Sample Identity no. | $Ca(NO_3)_2$ (M) | $(NH_4)_2HPO_4$ (M) | pH Gel. | Ageing Time (hr) | Morphology in agar gel system | Ca/P Molar ratio |
|---|---|---|---|---|---|---|
| R1 | 0.4 | 0.25 | 10 | 24 | Zigzag fibers | 1.08 |
| R2 | 0.4 | 0. 5 | 10 | 24 | Straight thin fibers | 1.21 |
| R3 | 0.4 | 1.0 | 10 | 24 | Thick fibers | 1.66 |

**Effect of Gel Temperature**

Among the various factors affecting the growth of fibrous HAP the reaction temperature of the gel preparation play a vital role [Pramtarova etal 2005]. The micrographs of the fibres obtained at temperatures of 40, 50 and 60°C is given in figure 1 (d, e and f) respectively. At the temperature of 60°C, dense straight fibrous products (20 μm thick and 7.0 cm length) were obtained, whereas at 50°C thin short fibres (7 μm thick and 2.7cm length) were obtained at a slow growth rate whereas at higher temperature of 70 and 80°C only a gelatinous cloudy mass was formed. At room temperature, very thin and zigzag fibrous products were obtained. This behavior explained on the basis of the fact that only at 60°C hydrolysis of urea is effective by maintaining the pH as mentioned earlier.

**3 Sintering Temperatures**

The fibres of HAP obtained were very fragile. Hence, the product will be sintered to cause densification and formation into a biologically stable form. The fibres were initially dried at 110°C in an oven to remove the adsorbed water, then heated with temperature of 240 °C for 1 hour to remove the traces of ammonium nitrate that would be formed as apart of the reaction system, followed by sintering at 900°C for 1 hour in order to get crystallizing HAP.

**4 Morphology of the Product and Growth Mechanism**

The fibrous HAP has a hallow, fragile structure with elongated ovals in a zigzag row with growth occurring at the tip of the fibres. The $Ca^{2+}$ ions present in the gel system are raised upwards through small fissures, which occur on the gelatinous surface formed just above the gel, which proceeds upward by capillary action as reported (10,13).

At the tip of the fibres, the $Ca^{2+}$ ions react with the phosphate species present in the medium, along with the incorporation of hydroxyl groups in the crystal lattice. Since the $Ca^{2+}$ ions are continuously supplied by capillary force the fibres move upward. The fibres are broken at unpredictable parts when the wall thickness becomes smaller resulting

in zigzag fibres. On the other hand, when large amounts of ACP are produced, the fibrous products are greatly elongated along the walls until it reaches a critical thickness for the breakage forming straight and hallow fibres.

Thus, the above results indicate the need to maintain the pH of the gel and phosphate solution at 10, which is effectively achieved by urea-$NH_3$ mixture and aqueous $NH_3$ respectively at an optimum urea concentration of 0.75 M. the pH of the system, was also found to greatly influence the growth rate and morphology of the fibrous hydroxyapatite.

**5 Phases Analysis**

X-ray diffraction pattern obtained for the fibrous product after sintering at 900$^{o}$C is shown in Figure 5. The position of the peaks is identical and closely correlates with the JCPDS [1981] standard reference peaks. No extraneous peaks for the presence of other calcium phosphates. The samples exhibited sharp diffraction peaks due to better crystallization at higher temperature, this also confirmed by (8).

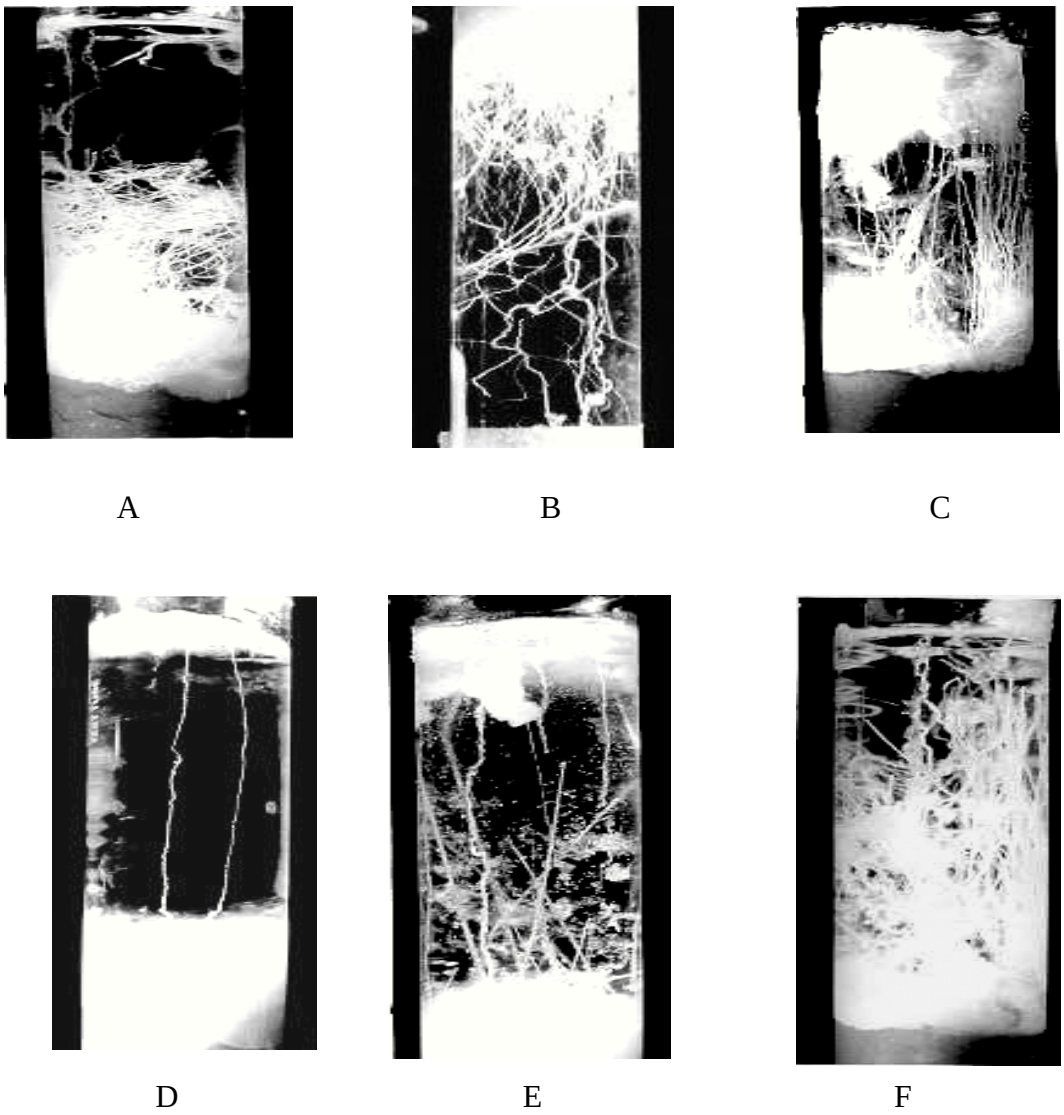


**Fig. 1:** Optical micrograph of fibrous HAP; (a) needle like (b) zigzag (c) straight fibers (d) fibers at 40 $^{o}$C (e) fibers at 50 $^{o}$C (f) fibers at 60 $^{o}$C.

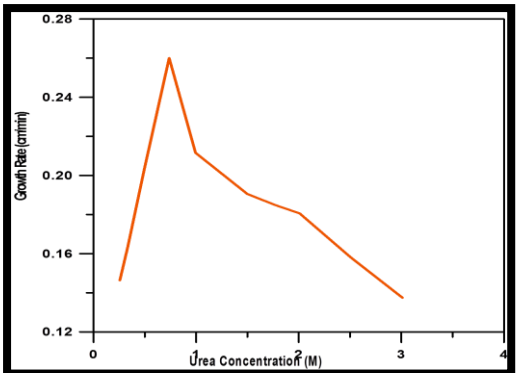


**Fig 2:** Effect of urea concentration on fibrous HAP growth rate.

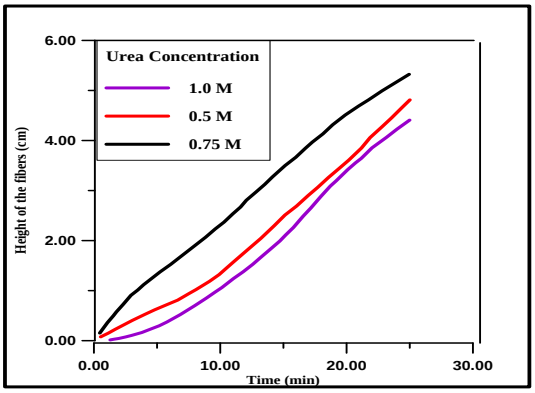


**Fig. 3:** Growth rate of fibrous HAP with time at different urea concentrations.

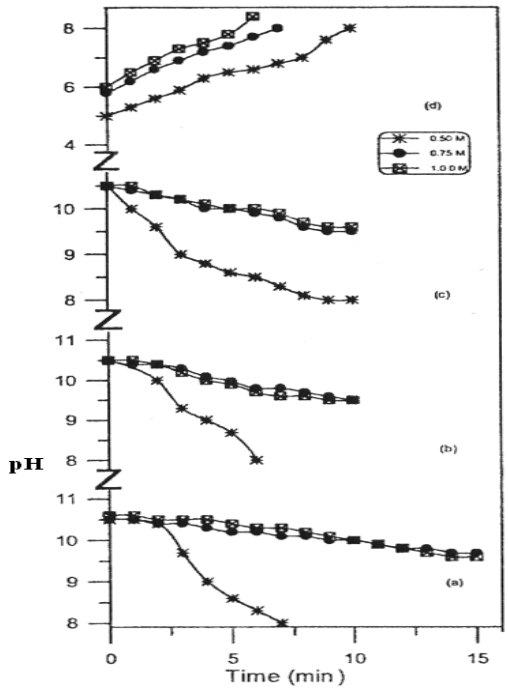


**Fig. 4:** Changes in pH with time on urea hydrolysis at 60 °C (a) in $Ca(NO_3)_2$ without agar (b) in $Ca(NO_3)_2$ with agar (c) in water with agar (d) in water without agar.

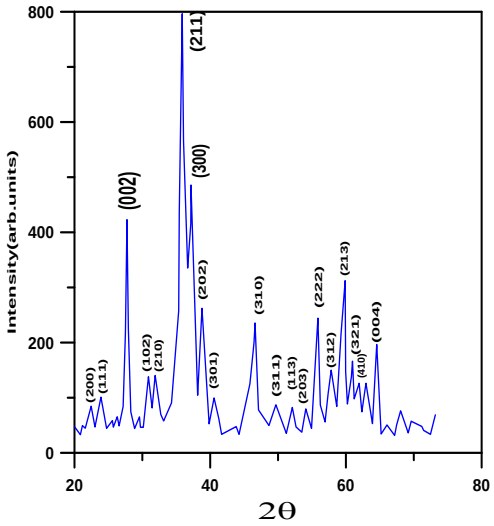


**Fig. 5:** XRD patterns of fibrous HAP

## Conclusions

A good growth rate with the stoichiometric ratio of fibrous HAP prepared was obtained by controlling the interstice factors as starting material concentrations, reacted temperature, ageing for 24 hours in mother liquor and sintering temperature resulted in crystalline HAP.

The pH of the gel system plays a crucial role in the growth of fibrous HAP and its stiochiometric. This is evident from the variations in physical appearance of the fibres, which are in zigzag, straight and needle like forms. Urea hydrolysis is an excellent analytical procedure to maintain the desired pH insitue.

## Acknowledgements

The authors are much grateful to Dr. Eman Abdulwahed of the Chemical and petrochemical research Directorate, Ministry of Science and Technology and Dr. Kadum Khudyer Aljanabi of the chemical department, college of science, Baghdad for their valuable discussions during the progress of this work.

## References


1. Greil P. and Mueller F., 2002 "Microstructure Design of Advanced Bioceramic" European Cells and Materials 3: (1) 1-2.
2. Leventouri Th. 2006 "Synthetic and biological hydroxyapatite: Crystal structure questions", J. Biomaterials 27: 3339–3342.
3. ThamaraiselviT.V, Prabakaran K. and Rajeswari S., 2006 "Synthesis of Hydroxyapatite that Mimic Bone Minerology", Trends Biomater. Artif. Organs, 19: (2) 81-83.
4. Kuriakose Tt., Kalkura N., Palanichamy M., Arivuoli D., Dieerks K., Bocelli G. andBetzel C. 2004 "Synthesis of stoichmetric nanocrystalline hydroxyapatite by ethanol based sol –gel technique at low temperature's. Crystal Growth 236: 517-523.
5. Catauro C., Raucci M. G., Gaetano F., Buri A. and Marotta A. 2003 "Sol- Gel Synthesis, Characterization and bioactivity of polycaprolactone/$SiO_2$ hybrid material" J. Mat. Sci. 3: 3097-3102.
6. Catauro C., Raucci M. G., Gaetano F., Buri A., Marotta A. and Ambrosio L. 2004 "Sol- Gel Synthesis and bioactivity of polycaprolactone/ CaO-$SiO_2$ hybrid material" J. Mat. Sci. Mat.medc., 15: 991- 995.
7. Sridhar T.M, Rajeswari S., Subbaiyan M. and Kamachi U. M., 2001 "Sintering Effects on Hydroxyapatite Coated and It's Impedance Behavior"., FAME, Bangalore, 265-269.
8. Nithyanantham T., kandassamy C., Gnanam F.D. 2002 "The Effect of Powder Processing on Densification, Microstructure and Mechanical Properties of Hydroxyapatite", J. Ceram. International.28: 355-358.
9. Hae W.K., Hyoun E.K., Vehid S. and Jonthan C.K. 2004 "Hydroxyapatite and tetania sol-gel composite coating on Ti for hard tissue implants" A. Wiely periodicals.Inc.
10. Karils G.A. & Christopher C. 2002 "Biomedical Application of Apatites", J. mineralogical Society of America, 48: 17-22.
11. Pramtarova E. P., Presker R., Pham M.T., Maitz M. F. and Stutzmann M., 2005 "Hydroxyapatite Growth Induced by Native Extraacellular Matrix Deposition on Solid Surfaces", J. Erop.Cel. Mater., 9: 19-23.
12. Prashant N.K., Charles S., Dong H.L., Ana O.D. and Daiwan C., 2005 "Nanostructure Calcium Phosphates for Biomedical Applications" Acta.Biomaterialia, 1: 65-68.
13. Robert B. H. and Richard W., 2006 "Formation and transformation of amorphous calcium phosphates on Ti alloy surfaces during atmospheric plasma spraying and their subsequent in vitro performance", J. Bio. Mater. 27: 819-823.


**تصنيع مادة الهيدروكسي ابتايت الشعيرية باستخدام طريقة السائل الهلامي**

***عبدالسلام خشان سوادي**** ***ماريا كراتسي روسي**** ***ثائر لطيف الزبيدي****

***حميد رشيد داود**** ***مرتضى عذاب صياح*****

* وزارة العلوم والتكنولوجيا / دائرة علوم المواد
** قسم هندسة المواد والانتاج / جامعة نابولي- ايطاليا
*** وزارة العلوم والتكنولوجيا / دائرة بحوث الكيمياء والصناعات البتروكيمياوية

**الخلاصة**

أن العمل المعروض في هذا البحث يتضمن طريقة جديدة لتصنيع مادة الهيدروكسي ابتايت (هاب) الشعيرية ذات الاستخدام البايولوجي. محلول نترات الكالسيوم [المخلوط بنسبة محسوبة لمادة (agar) الهلامية] وفوسفات الامونيوم تمثلان المواد الرئيسية في التحضير، والتي ينتج عنها مادة (هاب) وبالاشكال (الابري، المتعرج والمستقيم) مع الاخذ بنظر الاعتبار المحافظة على البنية الاساسية لمادة الهاب وبالنسبة المعتمدة بايولوجيا (1.67).

لاجل الحصول على تلك المادة وبالمواصفات البايولوجية الدقيقة، يجب السيطرة على المتغيرات الحاكمة وبمنتهى الدقة (الدالة الحامضية، درجة حرارة التفاعل، طبيعة المادة القاعدية وتركيز محلول الفوسفات). الفحوصات الطورية والحرارية والصور المجهرية بينت بان المادة المحضرة مطابقة للمواصفات القياسية.