

ORIGINAL ARTICLE

SYNTHESIS OF NANO - HYDROXYAPATITE AND NANO – FLUOROAPATITE PARTICLES BY SOL-GEL METHOD

Shoaib Khan¹, Madiha Pirvani², Sadaf Humayoun³, Omair Anjum⁴, Saima Akram⁵, Muhammad Asif Nathani⁶

¹Department of Science of Dental Materials, Ziauddin College of Dentistry, Ziauddin University, Karachi; ²Department of Science of Dental Materials, Dow International Dental College, Dow University of Health Science, Karachi; ³Department of Science of Dental Materials, Rawal Institute of Health Sciences, Islamabad; ⁴Department of Science of Dental Materials, Lahore Medical and Dental College, Lahore; ⁵Department of Oral Pathology, Ziauddin College of Dentistry, Ziauddin University, Karachi; ⁶Department of Science of Dental Materials, Altamash Institute of Dental Medicine, Karachi.

ABSTRACT

Background: Hydroxyapatite is a material which resembles the composition and crystal structure of hard tissues in human body. It is being used in dentistry as a bioactive material in dental implants and is a major constituent in the bone regenerative materials. Fluoroapatite is also a bioactive material and is more stable than Hydroxyapatite. The fluoride content is anti – bacterial and is working very efficiently as a component of dental restorative materials.

Objective: The objective is to synthesize the nano Hydroxyapatite and nanoFluoroapatite powder via sol-gel method, and compare the FTIR and Raman Spectrums of synthesized material with the FTIR and Raman of nano Hydroxyapatite and Fluoroapatite.

Methods: The materials were synthesized by sol – gel method and then evaluated by the FTIR and Raman spectroscopy to confirm the chemical structure of both the materials.

Results: FTIR and Raman Spectroscopy of the synthesized Hydroxyapatite and Fluoroapatite are then evaluated and compared with market grade materials, which confirm the presence of hydroxyl, phosphate and carbonate group in the obtained samples.

Conclusion: Sol – gel is proved to be a reliable and simple method for the synthesis of nano Hydroxyapatite and Fluoroapatite particles. The obtained samples then compared with the available materials to confirm that the material synthesized is pure and chemically identical.

Keywords: Hydroxyapatite, Fluoroapatite, FTIR spectroscopy, Raman spectroscopy

Corresponding Author:

Dr. Shoaib Khan
Ziauddin University,
College of Dentistry,
4/B Shahrah-e-Ghalib, Clifton,
Karachi – 75600
Email: shoaib.khan@zu.edu.pk

INTRODUCTION

Hydroxyapatite (HA) is an excellent biocompatible material having the similar composition and crystal structure as of apatite in human dental structures and bones. It is a type of calcium phosphate, which is the major mineral component of the tooth enamel and dentine. Hydroxyapatite contain both phosphate and hydroxyl ions and chemically it is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Hydroxyapatite is able to integrate

with the bone structures which help in bonding between the bone and the implants and encouraging bone growth without decomposing^{1,2}.

Fluorine is a necessary structure for the growth of normal dental and skeletal structure. Fluorine substituted apatite are also known as Fluoroapatite (FA). The chemical formula of Fluoroapatite is very similar as hydroxyapatite in which hydroxyl ion is replaced by fluorine, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$. It is a bioactive

material and is more stable than Hydroxyapatite both chemically and thermally. FA is biocompatible and does not degrade in biological environment in contrast to HA. Chemically Fluoroapatite is less soluble than fluoride free apatites, which shows the chemical stability of the material^{3,4}.

Due to the increase in importance and application of HA, various methods have been developed for the synthesis of HA. Few techniques developed for the synthesis of hydroxyapatite are solid-state reaction, co-precipitation method, hydrothermal method, spray pyrolysis, mechano-chemical

method and sol-gel method⁵⁻⁷. The aim of this study is to synthesize nano Hydroxyapatite and Fluoroapatite via sol-gel-method and characterize the obtained material chemically using FTIR and Raman Spectroscopy.

METHODS

Materials used in this experiment were all commercial grades and were obtained from Sigma – Aldrich UK. Silorane is obtained from 3M ESPE UK. Table shows the materials used in the experiment with their product number and molecular mass.

Table 1: List of materials used in the Experiment.

Product No.	Product Name	Chemical Formula	Molecular Weight	Company
21197	Calcium Nitrate Tetra Hydrate	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	236.15	Sigma – Aldrich
215996	Di ammonium Hydrogen Phosphate	$(\text{NH}_4)_2 \text{HPO}_4$	132.06	Sigma – Aldrich
318612	Ammonium Hydroxide	NH_4OH	35.05	Sigma – Aldrich
216011	Ammonium fluoride	NH_4F	37.04	Sigma – Aldrich
227056	N,N-Dimethylformamide	$\text{HCON}(\text{CH}_3)_2$	73.09	Sigma – Aldrich
401757	Tetrahydrofuran	$\text{C}_4\text{H}_8\text{O}$	72.11	Sigma – Aldrich
320110	Acetone	CH_3COCH_3	58.08	Sigma – Aldrich
02860	Ethanol	$\text{CH}_3\text{CH}_2\text{OH}$	46.07	Sigma – Aldrich

Hydroxyapatite was synthesized by sol gel technique using the method as previously described^{1,8}. Calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$], Di - Ammonium hydrogen phosphate [$(\text{NH}_4)_2 \text{HPO}_4$], Ammonium fluoride [NH_4F], Ammonium hydroxide [NH_4OH] and Ethanol [$\text{C}_2\text{H}_5\text{OH}$] were used as obtained. Initially 50 ml of ethanol is taken into the glass beaker; 6.6 gm of Ammonium hydrogen phosphate was dissolved in ethanol to form a 0.5 mol/l solution. 50 ml of ethanol is taken into another beaker and 19.702 gm of Calcium Nitrate tetrahydrate was dissolved in it to form 1.67 mol/l solution. Figure displays the balance (Explorer OHAUS – E11140) used for the measurements. The solution of Calcium nitrate tetrahydrate was added in the Ammonium hydrogen phosphate solution using a dropping funnel at the rate of 5 ml/min. A magnetic stir bar is placed in the beaker and the beaker is then placed on a magnetic stirrer (Model - IKA C-MAG HS 7) at the temperature of 85°C. The pH of the solution was balanced at 10 with a pH meter (pHep by HANNA) by drop wise addition of Ammonium hydroxide up to the maximum amount of 5 ml. The solution was stirred continuously in oil bath to transmit the heat gradually and uniformly. Solution is stirred at the

same temperature until the white transparent gel was obtained. The gel is aged at room temperature for 4 hours and dried in hot air oven (Thermo Scientific - Heraeus Oven) at 120°C for 24 hours. The nano powder obtained was sintered in the furnace (Eurothem - Carbolite, LMF4 EER2), initially at 400°C for 2 hours and then at 800°C for further 2 hours. The powder obtained is allowed to cool and then grounded manually by mortar and pestle. Obtained sample was then analysed by FTIR to examine the purity of the material.

Fluoroapatite was synthesized by sol-gel method similar to the method used by Rehman et al¹.

Initially 6.6 gm of Ammonium hydrogen phosphate was dissolved in 50 ml of ethanol to make the molar solution of 0.5 mol/l. Then 19.702 gm of Calcium nitrate tetrahydrate was dissolved in 50 ml of ethanol to make a 1.67 M solution. Afterwards 0.62 gm of Ammonium Fluoride was added in the solution for 1.67 M solution. Calcium, Phosphorus and Fluoride were added to obtain an appropriate molar ratio of 3:5:1 (Ca: P: F = 3:5:1). The solution is then added to the initial made solution using a dropping funnel at the rate of 5 ml/min. Magnetic

stir bar was placed in the beaker and the beaker was placed on the magnetic stirrer in an oil bath at the temperature of 85°C until the white transparent gel was obtained. The gel was aged for 4 hours at room temperature and dried in the Hot air oven at 120°C for 24 hours. The nano powder obtained was then sintered, initially at 400°C for 2 hours and then at 800°C for further 2 hours. Obtained powder is then allowed to cool down and then grounded using mortar and pestle. Sample was then analyzed by FTIR to examine the purity of the material.

RESULTS

Initially the synthesized hydroxyapatite and

fluoroapatite powders have been examined using the FTIR to characterize the purity of the obtained HA and FA samples. The characteristic peaks of hydroxyl, carbonate and phosphate groups were present in the spectrum. Hydroxyl band is observed at 3570 and 636 cm^{-1} , showing the stretching and liberation mode respectively. Carbonate bands are being observed in 1420 cm^{-1} and phosphate bands can be seen at 1017, 962, 602 and 571 cm^{-1} . The band at 602 and 571 cm^{-1} are due to phosphate bending vibrations and the bands at 1017 and 962 cm^{-1} are phosphate stretching vibration

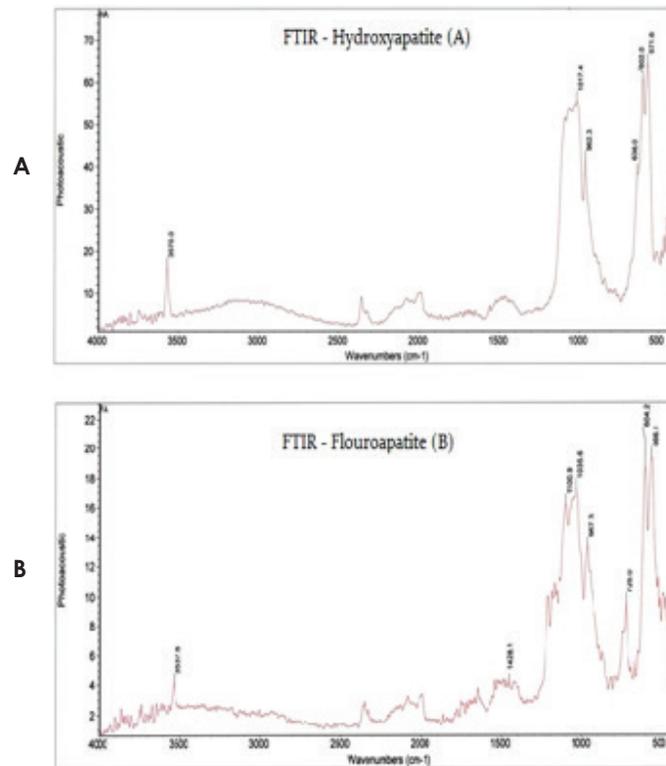


Figure 1: FTIR spectrum of (A) Hydroxyapatite and (B) Fluoroapatite

bands. The bands present in the region of 1500 – 1400 cm^{-1} are the carbonate bands, which suggest the presence of carbonate functional group. They can originate from the CO_2 present in the air and can enter in the hydroxyapatite structure. The sample of FA shows the clear reduction in the

hydroxyl peak and disappearance of phosphate peak at 636 cm^{-1} , which is the confirmation of FA formulation. The broader phosphate peak in the region of 950 – 1100 cm^{-1} shows the degree of crystallinity which is increased due to the process of sintering^{1,9}.

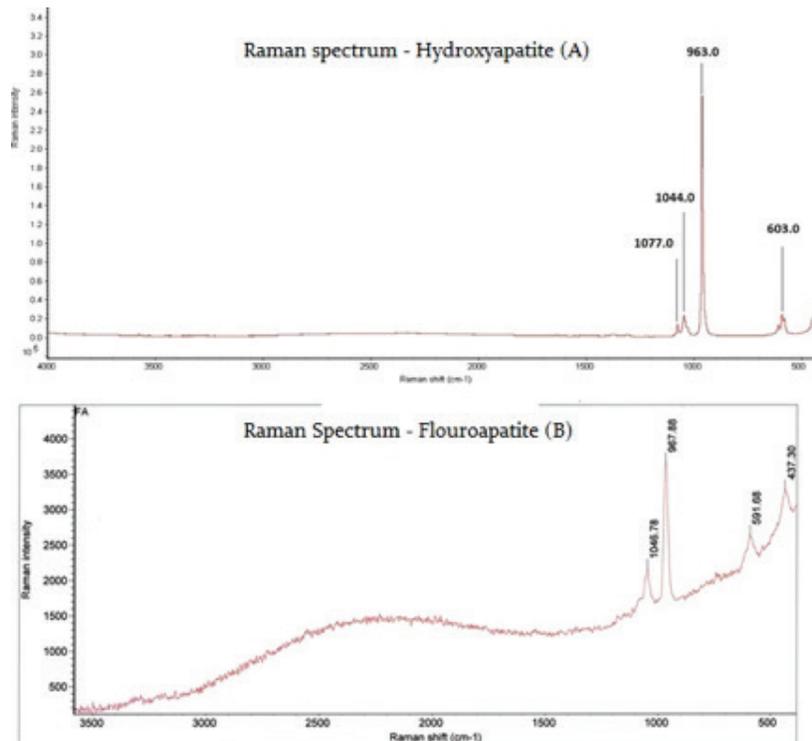


Figure 2. Raman Spectrum of (A) Hydroxyapatite and (B) Fluoroapatite

In Raman spectrum of Hydroxyapatite phosphate group can be seen at 963 cm^{-1} , which is used for quantitative analysis. Peaks at 1077 and 1044 are the ν_3 and at 963 cm^{-1} is ν_1 of P – O. The presence of peak at 603 cm^{-1} indicates the ν^4 of the O – P – ^{07,10,11}.

Figure 2 shows the Raman spectrum of fluoroapatite, in which the peaks at 1046 and 967 cm^{-1} shows the ν^3 and ν^1 stretch of phosphate respectively. The peaks at 591 and 437 cm^{-1} indicates the ν^4 and ν^2 bends respectively¹².

DISCUSSION

Several studies have been done to find out the biocompatibility of HA and none of them shows any toxicity, inflammatory response or fibrous tissue formation between the implant and the bone. HA have poor mechanical properties as all other bioactive material. That is why HA cannot be used in load bearing applications and is not suitable for bone substitute. HA is available in the form of porous, dense, granules and coatings. To improve the mechanical properties of HA, it can be used with metals such as magnesium, zinc, titanium and strontium^{4,5,13}. To improve the mechanical properties of HA, composites containing HA can be synthesized. HA is a highly bioactive material, having many clinical applications as bone replacements. It is used in dental and orthopaedic application for repairing bone defects, immediate tooth replacement, alveolar ridge augmentation and reconstruction of maxillofacial deformities⁶.

HA was first used in 1981 for the filling of periodontal lesion, since then it's been extensively used in medical field⁵. Hydroxyapatite material implants cannot be used under heavy loads, such as artificial teeth or bones as the fracture toughness of HA is very low if compared with human bone. The clinical applications of HA are restricted to small unloaded implants, powders, coatings and implants in low stress bearing areas^{14,15}.

The Fluoride in the Fluoroapatite inhibits the bacterial growth thus making it the material of choice to incorporate in dental restorative materials. Presence of fluoride results in the low solubility and good acid resistance which are the essential property of a restorative material to survive in the harsh oral environment. But the excess fluoride may result in fluorosis. Fluoride and hydroxide ions share the same charge and almost same radius and are able to replace each other in the mineral structures¹⁶. Chen and Miao have incorporated Fluoride in hydroxyapatite to form fluorohydroxyapatite and concluded that the resultant material have better thermal and chemical stability than hydroxyapatite¹⁷.

Sol-gel method is considered more advantageous as compared to others because the product obtained is pure, homogenous, highly crystalline, nano sized and requires comparatively low synthesis temperature⁵⁻⁷. The sol-gel process is a wet chemical method that does not require too high sintering temperature. Molecular mixing of calcium

and phosphorus can be achieved by this method⁸. There are six stages of sol-gel method, which are mixing, gelation, aging, drying, sintering and crushing. Calcium and Phosphorus precursors are mixed in a molar ratio of 1.67. The mixture is then heated and stirred till the white transparent gel is obtained. The obtained gel is then dried for 24 hours in an air oven. Here a dried porous material is obtained which is placed in a furnace for sintering. The powder obtained from sintering is then allowed to cool and then crushed with the help of mortar and pestle to achieve the similar size particles^{7,8}. The obtained materials are then observed under FTIR and Raman spectroscopy for confirming the chemical structure which is a reliable method of characterizing the materials chemically¹⁸.

CONCLUSION

In the present study, Hydroxyapatite and Fluoroapatite were prepared by sol gel method. Sol gel method is proved to be a reliable and simple method for the formation of nano sized Hydroxyapatite and Fluoroapatite.

ACKNOWLEDGEMENTS

The authors would like to thank to the School of Engineering and Materials, Queen Mary University of London, London, United Kingdom, for all the help during the experiment.

REFERENCES

1. Rehman I, Moshaverinia A, Ansari S, Moshaverinia M, Roohpour N, Darr JA. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into conventional glass ionomer cements (GIC). *Acta Biomater* 2008;4(2):432-40.
2. Sheikh L, Sinha S, Singhababu YN, Verma V, Tripathy S, Nayar S. Traversing the profile of biomimetically nanoengineered iron substituted hydroxyapatite: synthesis, characterization, property evaluation, and drug release modeling. *RSC Advances* 2018;8(35):19389-401.
3. Wong LH, Tio B, Miao X. Functionally graded tricalcium phosphate/fluoroapatite composites. *Mater Sci Eng C* 2002;20(1-2):111-5.
4. Aljerf L, Choukaife AE. Hydroxyapatite and Fluoroapatite Behavior with pH Change. *Int Med J* 2017;24(5):407-10.
5. Sopyan I, Singh R, Hamdi M. Synthesis of nano sized hydroxyapatite powder using sol-gel technique and its conversion to dense and porous bodies. New Delhi, INDE: India National Science Academy; 2008. 6 p.
6. Kuriakose TA, Kalkura SN, Palanichamy M, Arivuoli D, Dierks K, Bocelli G, et al. Synthesis of stoichiometric nano crystalline hydroxyapatite by ethanol-based sol-gel technique at low temperature. *J Cryst Growth* 2004;263(1-4):517-23.
7. Kala JBA, T A. Preparation and Characterization of Precipitated Nano Hydroxyapatite. *AJBAS* 2018;12(2):10-4.
8. Feng W, Mu-sen L, Yu-peng L, Yong-xin Q. A simple sol-gel technique for preparing hydroxyapatite nanopowders. *Material Letter* 2005;59(8-9):916-9.
9. Salehi S, Fathi MH. Fabrication and characterization of sol-gel derived hydroxyapatite/zirconia composite nanopowders with various yttria contents. *Ceramics Int* 2010;36(5):1659-67.
10. Kontoyannis CG, Bouropoulos NC, Koutsoukos PG. Raman spectroscopy: A tool for the quantitative analysis of mineral components of solid mixtures. The case of calcium oxalate monohydrate and hydroxyapatite. *Vib Spectrosc* 1997;15(1):53-60.
11. Rehman IU, Khan AS, Ahmed Z, Edirisinghe MJ, Wong FSL. Preparation and characterization of a novel bioactive restorative composite based on covalently coupled polyurethane-nanohydroxyapatite fibres. *Acta Biomater* 2008;4(5):1275-87.
12. Williams Q, Knittle E. Infrared and raman spectra of Ca₅(PO₄)₃F₂-fluoroapatite at high pressures: Compression-induced changes in phosphate site and Davydov splittings. *J Physic Chem Solid* 1996;57(4):417-22.
13. Mardziah CM, Sopyan I, Ramesh S. Strontium-Doped Hydroxyapatite Nanopowder via Sol-Gel Method: Effect of Strontium Concentration and Calcination Temperature on Phase Behavior. *Trends Biomaterial Artific Organ* 2009;23(2):105-13.
14. Suchanek W, Yashima M, Kakihana M, Yoshimura M. Hydroxyapatite ceramics with selected sintering additives. *Biomaterials* 1997;18(13):923-33.
15. Tavakkoli-Gilavan M, Abdizadeh H, Golobostanfard MR. Biomineralization behavior of electrophoretic-deposited hydroxyapatite-tricalcium phosphate biphasic composite. *App Surface Sci* 2018;458:988-95.
16. Kanchana P, Sekar C. Influence of sodium fluoride on the synthesis of hydroxyapatite by gel method. *J Cryst Growth* 2010;312(6):808-16.
17. Chen Y, Miao X. Thermal and chemical stability of fluorohydroxyapatite ceramics with different fluorine contents. *Biomaterials* 2005;26(11):1205-10.
18. Khan S, Pirvani M, Nathani MA. Chemical Characterization of Silorane by FTIR and Raman Spectroscopy. *Pak J Med Dent* 2015;04(03):17-9.