

Synthesis and Characterization of Calcium Hydroxyapatite Nanoparticles using Wet Chemical and Sol-Gel Method

Anjuvan Singh¹, Navkiran Kaur², Jasmeet Kaur³ and Narasimha Rao⁴

LPU, Punjab

Abstract—Nanoparticles has great interest in almost every aspect of science and technology. Hydroxyapatite is a potential sorbent which can absorb heavy metals from our soil and water. Inorganic nanoparticles such as calcium hydroxyapatite nanoparticles has enormous utilization in biomedical sciences which is a potential bioceramic and also excellent material for removing many heavy metals from our environment such as Pb, Cd, Zi, Co and U in aqueous solution because of its excellent characteristics such as biocompatibility, lack of toxicity, biodegradability, good stability and adsorption properties. An attempt has been made to synthesize nano hydroxyapatite from wet chemical, EDTA assisted growth and sol-gel. The purpose of this research work was to compare the three methodologies for their performance to obtain pure nano-ranged hydroxyapatite. The synthesized nano-hydroxyapatite powder was further characterized by using Fourier Transform Infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning Electron Microscope (SEM). The FTIR and the XRD measurements confirmed that hydroxyapatite synthesised by different routes was pure white and crystalline in nature. SEM measurements showed that the morphology of hydroxyapatite synthesised using EDTA assisted growth and sol-gel showed rod-shaped structure with diameter in range 40 nm and 50 nm respectively. However, the particle synthesised using wet chemical synthesis method had spherical shaped morphology with diameter 0.2 μm .

1. INTRODUCTION

Nanoparticles have a wide range of characteristics which includes high surface area, sorption and ability to disperse in aqueous solution.(1) Synthetic hydroxyapatite is structurally as well as chemically similar to naturally occurring needle like HA present in our bones. Naturally occurring HA is hexagonal in structure with the chemical formula of one unit cell being $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, mostly present in association with Type 1 collagen fibre having length of 25-50nm. (2-3). For a successful synthesis of hydroxyapatite nanoparticles an aspect ratio of Ca:P ~ 1.59 is mandatory. However, the synthesis has been carried out at a varying temperature range while the pH is mostly maintained at ~ 10 – 11. It has been seen that the degree of crystallinity and morphology of synthesized Hydroxyapatite nanoparticles depends upon the calcination, temperature and aging time.

Calcium hydroxyapatite nanoparticles has enormous potential applications in protecting environment as it is a capable heavy metal remover when they are present in aqueous form. It has an excellent opportunity to remove the heavy metals such as Pb, Cd, Zi, Co and U from our surroundings which makes more healthier environment(4-7). The further research suggests that these applications of HA nanoparticles can further be implemented in filtration process of water which help to clear the heavy metals from our daily life more effectively and more efficiently. To improve the properties of hydroxyapatite nanoparticles such as increase in sorption area, they are coated with dextran for better removal of heavy metals such as lead.

Nanoparticles and biomaterials such as polymers, ceramics, and metals are widely used in bone for regenerative therapies, including in bone grafts and in tissue engineering as well as for temporary or permanent implants to stabilize fractures or replace joints. Bioceramics are a class of ceramics that are specifically developed to replace parts of living system; are biocompatible with the tissues involved. Hydroxyapatite commonly referred as HA, is the emerging bioceramic, widely used in various biomedical applications, mainly in orthopedics and dentistry due to its close similarities with inorganic mineral component of bone and teeth. It has been successfully employed in areas involving repair of hard tissues, bone repair, bone augmentation, coating of implants and acting as fillers in bone or teeth. (8)

In the research carried out, Calcium hydroxyapatite nanoparticles were synthesised with wet chemical, EDTA assisted synthesis and sol gel under careful monitoring of parameters such as pH, reaction time, temperature, and concentration of reactants was done to compare its effects on the composition and morphology of the nanoparticles synthesised via various different routes. Characterization was done by XRD analysis, FTIR Spectroscopy and SEM Microscopy.

2. MATERIALS AND METHODS

The synthesis of Calcium Hydroxyapatite nanoparticles were done by three major processes which are described below.

- Wet Chemical Synthesis of Calcium hydroxyapatite nanoparticles
- 0.5M Calcium hydroxide in 100 ml of distilled water was sonicated for about 15 minutes at room temperature. The pH of calcium hydroxide solution was adjusted and maintained 10.5 by the addition of ammonium hydroxide(NH₄OH) with continuous stirring at 1550-1600 rpm and then 0.3 M of 100 ml ortho-phosphoric acid was added drop wise at a rate of 1 drop per second to calcium hydroxide solution at temperature of 80°C. The resultant precipitate was left for overnight, washed with ammonia and resultant was collected by filtration process. The precipitate was dried in the hot air oven for about 2-3 hours at 100°C. The dried powder was then grinded with the help of pestle and mortar and was calcinated at 800°C for 2 hours.(9)
- EDTA assisted wet chemical synthesis of calcium hydroxyapatite nanoparticles
- The same wet chemical synthesis of calcium hydroxyapatite nanoparticles as described above was performed again and 0.3M EDTA was added to sonicated solution of 0.3M Calcium hydroxide. The EDTA performed as capping agent.(10)
- Sol-gel synthesis of Calcium hydroxyapatite nanoparticles
- 0.05M of Calcium nitrate tetrahydrate in distilled water was taken and pH of the solution was adjusted and maintained at 10.5 by addition of Ammonium hydroxide solution with continuous stirring at 1900 rpm and then 0.03M Di-ammonium hydrogen phosphate was added at the rate of one drop per second to the solution at temperature of 80°C. The precipitate was aged in the mother solution for 24 hours and then evaporated at 60°C for 4 hours. After evaporation a thick gel was obtained which was then filtered to obtain precipitate. The precipitate was dried in hot air oven for about 40 minutes at 100°C. The dried powder was then grinded with help of mortar and pestle and was further calcinated at 250°C for 6 hours.(11)

Characterization Methodologies

The main characterization of Calcium Hydroxyapatite nanoparticles is done by Fourier Transform Infrared Radiation (FTIR) Spectroscopy, X-Ray Diffraction (XRD) Analysis and Scanning Electron Microscopy (SEM). XRD analysis was done at Punjab University, Chandigarh and SEM was performed at Council of Scientific and Industrial Research-Institute of Genomics and Integrative Biology, New Delhi.

3. RESULTS AND DISCUSSION

In the present work, hydroxyapatite was synthesized successfully by using different chemical routes. Out of the different methods employed synthesis using EDTA gave the best results on characterization followed by synthesis employing Sol-Gel route. The FTIR and XRD results of the HA synthesized using EDTA assisted growth were approximately same to those provided by NIST. Moreover, the sample showed rod shaped morphology characteristic of HA with dimensions 40nm diameter and 200nm length. A white crystalline powder was obtained after the whole process for synthesis of Calcium Hydroxyapatite nanoparticles.

• FTIR Analysis

The calcium hydroxyapatite sample prepared via wet chemical route on FTIR analysis(Fig. 1) showed peaks at 565.16cm⁻¹, 603.74cm⁻¹ referring to assymmetric bending in PO₄³⁻ functional group (12),(530-670cm⁻¹ reference NIST value for V4 band of PO₄) and the broad peak at 1033.88cm⁻¹ referring to stretching of PO₄³⁻ functional group, (1000-1120cm⁻¹ reference NIST value for V3 band of PO₄). The peaks at 470.65cm⁻¹(468cm⁻¹ OH- value cited by 13) and 3348.54cm⁻¹(3650-3390cm⁻¹cited by 14) characteristic peaks of OH-functional groups bending and stretching vibrations respectively. The peak at 871.85cm⁻¹ refers to bending of CO₃²⁻(878cm⁻¹ CO₃²⁻ value cited by 15) while the peaks at 1471.74cm⁻¹ and 1759.14cm⁻¹ refers to the stretching of CO₃²⁻ functional group(1467-1412cm⁻¹ CO₃²⁻ cited by A.C.Tas,2000 and 1714cm⁻¹ CO₃²⁻ cited by 16).

The calcinated calcium hydroxyapatite sample prepared via wet chemical route on FTIR analysis(Fig.2) showed peaks at 570.95 cm⁻¹, 603.74 cm⁻¹ and 634.6 cm⁻¹ referring assymmetric bending in PO₄³⁻ functional group(12 cites as 530-670cm⁻¹ reference NIST value for V4 band of PO₄) and the broad peak at 1000-1200cm⁻¹ referring to stretching of PO₄³⁻ functional group(12 cited as, 1000-1120cm⁻¹ reference NIST value for V3 band of PO₄). The peaks at 472.58cm⁻¹(468cm⁻¹ OH- value cited by

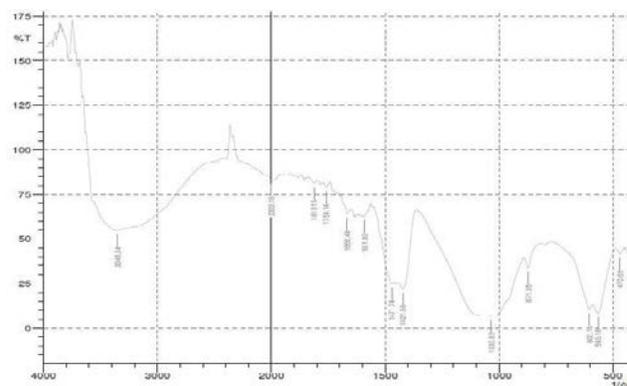


Fig. 1: FTIR Analysis of Hydroxyapatite sample prepared by Wet Chemical Synthesis method.

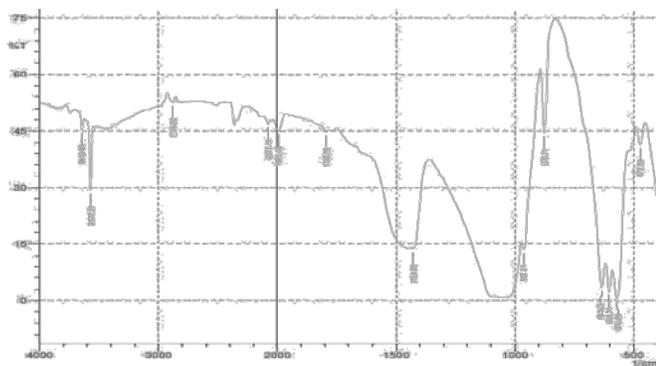


Fig. 2: FTIR sample analysis of calcinated Hydroxyapatite prepared by wet chemical synthesis

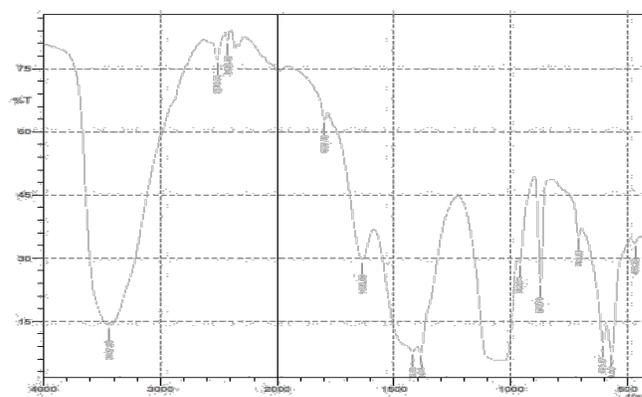


Fig. 4: FTIR Analysis of Hydroxyapatite prepared by Sol-Gel synthesis method

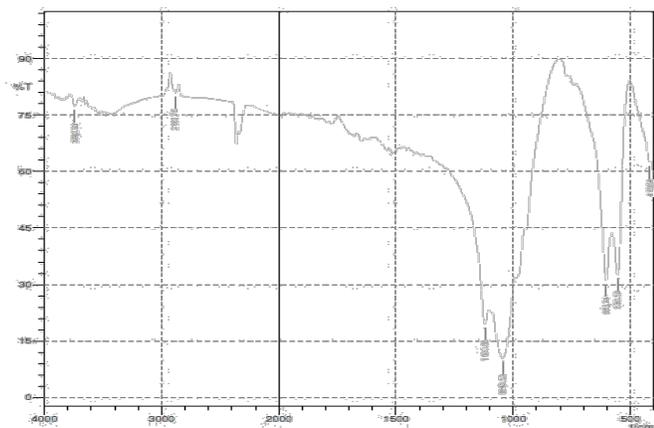


Fig. 3: FTIR Analysis of Hydroxyapatite prepared by EDTA assisted wet chemical synthesis method.

Table 1: Comparison of the phosphate and hydroxyl peaks of the hydroxyapatite synthesized through the four routes

Functional Group	Range of band	Wet chemical synthesis	Calcinated wet chemical	EDTA Assisted	Sol gel
PO ₄ ³⁻ (V4)	530 – 670 cm ⁻¹	565.15 cm ⁻¹ 603.74 cm ⁻¹	570.95 cm ⁻¹ 603.74 cm ⁻¹ 634.6 cm ⁻¹	553.59cm ⁻¹ 603.74cm ⁻¹	569.02cm ⁻¹ 605.67cm ⁻¹
PO ₄ ³⁻ (V3)	1000 – 1120 cm ⁻¹	1033.88 cm ⁻¹	1000 – 1200 cm ⁻¹	1043.5cm ⁻¹ 1120.6cm ⁻¹	1000 – 1100 cm ⁻¹
OH	3650 – 3390 cm ⁻¹	3348.54 cm ⁻¹	3572.29cm ⁻¹ 3643.65cm ⁻¹	3742.03cm ⁻¹	3437.26cm ⁻¹

13) and 3572.29cm⁻¹ and 3643.65cm⁻¹ (3650-3390cm⁻¹cited by 14) characteristic peaks of OH- functional groups bending and stretching vibrations respectively. The peak at 875.75cm⁻¹ refers to bending of CO₃²⁻(870cm⁻¹ CO₃²⁻ value cited by 17) while the peaks at 1429.30cm⁻¹ and 2879.82cm⁻¹ refers to the stretching of CO₃²⁻ functional group(1467-1412cm⁻¹ and 2368-2361cm⁻¹ CO₃²⁻ cited by 15).

The EDTA assisted calcinated calcium hydroxyapatite sample prepared via wet chemical route on FTIR analysis(Fig.3.) showed peaks at 553.59cm⁻¹ and 603.74cm⁻¹ referring asymmetric bending in PO₄³⁻ functional group(12 cites as 530-670cm⁻¹ reference NIST value for V4 band of PO₄) and the broad peak at 1043.52cm⁻¹and 1120.68cm⁻¹ referring to stretching of PO₄³⁻ functional group(12 cited as 1000-1120cm⁻¹ reference NIST value for V3 band of PO₄). The peaks at 416.64cm⁻¹(468cm⁻¹ OH- value cited by 13) and 2881.75cm⁻¹ and 3742.03cm⁻¹ (2800cm⁻¹ and 3550-3700cm⁻¹ cited by 15) characteristic peaks of OH- functional groups bending and stretching vibrations respectively.

The calcium hydroxyapatite sample prepared via sol-gel route on FTIR analysis (Fig.4.) showed peaks at 569.02cm⁻¹ and 605.67cm⁻¹ referring asymmetric bending in PO₄³⁻ functional group(12 cited as 530-670cm⁻¹ reference NIST value for V4 band of PO₄) and the broad peak at 1000-1100cm⁻¹ referring to stretching of PO₄³⁻ functional group(12 cited as 1000-1120cm⁻¹ reference NIST value for V3 band of PO₄). The peaks at 468.72cm⁻¹(468cm⁻¹ OH- value cited by 13), 1635.68cm⁻¹, 1797.72cm⁻¹ and 3437.26cm⁻¹ (1714cm⁻¹ cited by 12 and 3650-3390cm⁻¹cited by 14) characteristic peaks of OH- functional groups bending and stretching vibrations respectively. The peak at 873.76cm⁻¹ refers to bending of CO₃²⁻(870cm⁻¹ CO₃²⁻ value cited by 17) while the peaks at 1384.94cm⁻¹, 1419.66cm⁻¹, 2426.53cm⁻¹ and 2513.33cm⁻¹ refers to the stretching of CO₃²⁻ functional group(1467-1412cm⁻¹ and 2368-2361cm⁻¹ CO₃²⁻ cited by 15).

• XRD Analysis

The XRD analysis of the wet chemical route(Fig.5.) give a peak of 100% intensity at 31.87° value of theta, 72% intensity at 32.01° theta and 49% intensity at 25.85° theta. The EDTA assisted wet chemical route(Fig.6.) give give a peak of 100% intensity at 31.77° value of theta, 49% intensity at 32.19° theta and 37% intensity at 25.87° theta. The sol-gel synthesis route (Fig.7.) give a peak of 100% intensity at 31.86° value of theta, 63% intensity at 32.21° theta and 43% intensity at 25.85° theta. All the three samples showed the peak characteristic of

Hydroxyapatite cited by NIST, thus confirming the synthesis of the same.

• SEM Analysis

SEM analysis of the four samples were done, where the sample synthesized using wet chemical method had a spherical morphology with diameter size of ~200nm. The samples synthesized using EDTA assisted wet chemical method had rod shaped morphology with length ~200nm and diameter ~40nm. The sample synthesized using solution combustion method had a spherical morphology with diameter ~200nm. The sample synthesized using sol-gel synthesis method had rod-shaped morphology with length ~300nm and diameter ~50nm. It was seen that the samples synthesized using sol-gel method and EDTA assisted growth showed the rod-shaped or needle like morphology characteristic to HA particles.

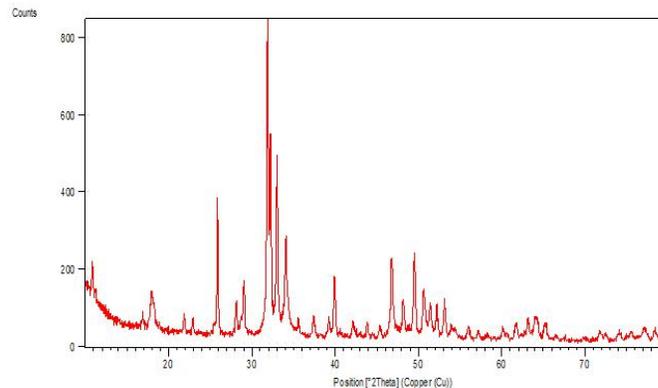


Fig. 7: Graph plot showing the XRD Analysis of sample prepared via Sol-Gel method.

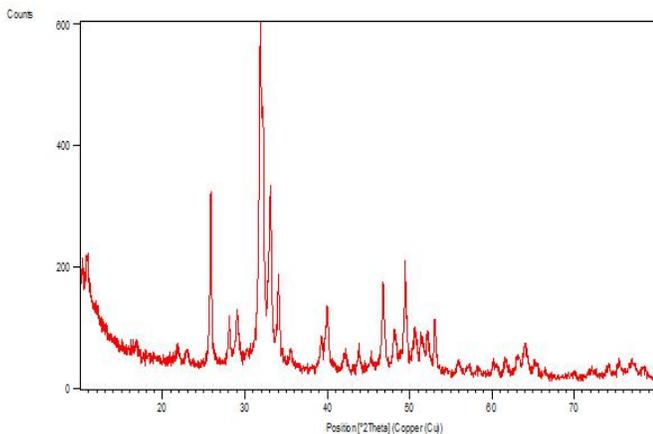


Fig. 5: Graph plot showing the XRD Analysis of sample prepared via Wet Chemical Method.

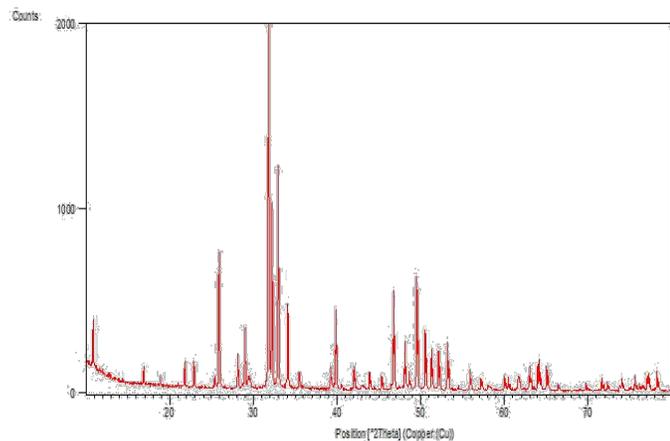


Fig. 6: Graph plot showing the XRD Analysis of sample prepared via EDTA assisted Wet Chemical Method.

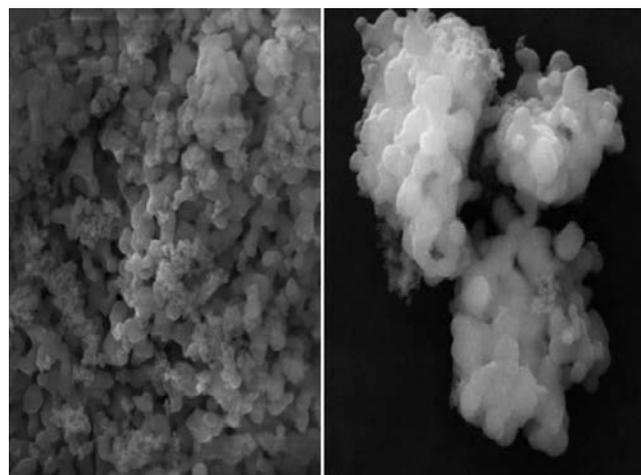


Fig. 8: SEM images of sample synthesized using Wet Chemical Synthesis Method.

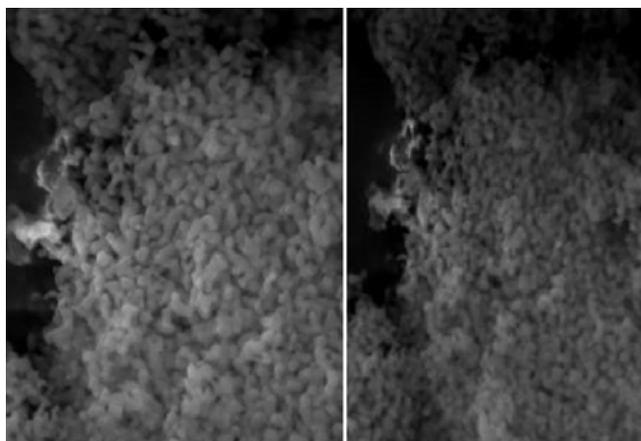


Fig. 9: SEM images of sample synthesized using EDTA assisted Wet Chemical Synthesis Method.

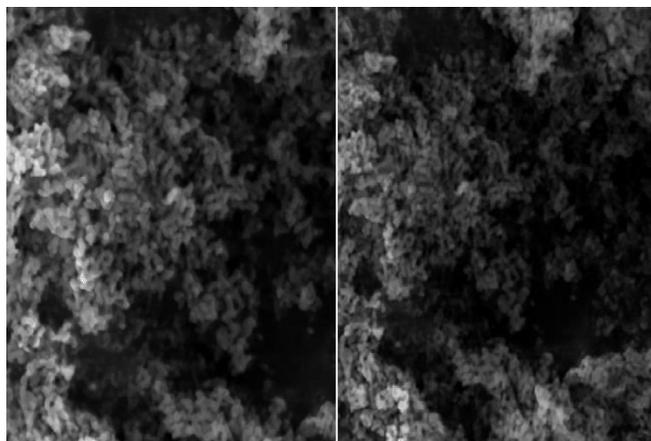


Fig. 10: SEM images of sample synthesized using Sol-Gel Method.

4. CONCLUSION

Calcium hydroxyapatite was successfully synthesized using wet chemical method. Calcium hydroxide and orthophosphoric acid were used for the synthesis and EDTA was used as a capping agent to reduce the size of particles. At the end of experiment white precipitate was obtained and after oven drying crystalline form was obtained. The samples were sent for FTIR analysis and it was seen that the water content in the calcinated sample was reduced as compared to the non calcinated sample. The sample prepared using EDTA as a capping agent was purer than the samples prepared without EDTA as it did not show the peaks associated with the carbonate group which occurs due to mixing of atmospheric CO₂ during the process. The XRD analysis of the sample prepared using EDTA as a capping agent had its values closest to that of NIST standard values. Also, the SEM analysis of the samples prepared using EDTA assisted growth and Sol-Gel showed that they had their structure in nano range with rod shaped morphology. Thus, it can be concluded that the method for synthesis using Sol-Gel and EDTA assisted growth are better than Wet Chemical synthesis methodologies.

5. ACKNOWLEDGEMENT

We would like to appreciate the support and help extended by Lovely Professional University, Phagwara for granting the funds for the above work and also national science foundation for the paper grants.

REFERENCES

[1] Adrian costescu, Ecaterina andronescu, Bogdan Ștefan vasile, Roxana trușcă, Philippe le coustumer, Emil Ștefan barna, Simona Liliana iconaru, Mikael motelica-heino, Carmen Steluța ciobanu., "Synthesis and characterisation of dextran coated hydroxyapatite for environmental applications", *U.P.B. Sci. Bull., Series B*, 2014, Vol. 76, Iss. 4

[2] Amit Kumar Nayak., "Hydroxyapatite Synthesis Methodologies: An Overview", *Int.J. ChemTech Res.*, 2010, 2(2)

[3] Alis Yovana Pataquiva Mateus et al., "Comparative study of nanohydroxyapatite microspheres for medical applications", *Journal of Biomedical Materials Research*, 2007

[4] I.Smiciklas., A.Onjia., S.Raicevic., kovic Janac., M.Mitric., "Factors influencing the removal of divalent cations by hydroxyapatite", *Journal of Hazardous Materials*, vol. 152, no.2, Apr. 2008, pp. 876–884

[5] S.Bailliez., A.Nzihou., E.Beche., G.Flamant., "Removal of Lead (Pb) by hydroxyapatite sorbent", *Process Safety and Environmental Protection*, 2004, vol. 82, no. 2, pp. 175–180

[6] R.Zhu., R.Yu., J.Yao., D.Mao., C.Xing., D.Wang., "Removal of Cd²⁺ from aqueous solutions by hydroxyapatite", *Catalysis Today*, vol. 139, 2008, pp. 94–99

[7] I.Smiciklas., S.Dimovic., I.Plecas., M.Mitric., "Removal of Co²⁺ from aqueous solutions by hydroxyapatite", *Water Research*, vol. 40, no. 12, Jul. 2006, pp. 2267–2274

[8] Hongjian Zhou., Jaebeom Lee., "Nanoscale hydroxyapatite particles for bone tissue engineering", *Acta Biomaterialia*, 2011, 7 2769–2781

[9] Aruneshan Chandrasekar., Suresh Sagadevan., Arivuoli Dakshnamoorthy., "Synthesis and characterization of nano-hydroxyapatite (n-HAP) using the wet chemical technique", *Int. J. Phys. Sci.*, 2013, 8(32), 1639-1645

[10] Kang NH., Kim SJ., Song SH., Choi Sm., Choi SY., Kim YJ., "Hydroxyapatite synthesis using EDTA", *J Craniofac Surg.* 2013; 24(3): 1042–1045.

[11] Irma Bogdanoviceneaa., Aldona Beganskienea., Kaia Tõnsuaadub., Jochen Glaserc., H.-Jürgen Meyerc., Aivaras Kareivaa., "Calcium hydroxyapatite, Ca₁₀(PO₄)₆(OH)₂ ceramics prepared by aqueous sol-gel processing", *Materials Research Bulletin*, 2006, 41 1754–1762

[12] M. Markovic., B. O. Fowler., M. S. Tung., and E. S. Lagergren., "Composition and Solubility Product of a Synthetic CalciumHydroxyapatite. Chemical and Thermal Determination of Ca/PRatio and Statistical Analysis of Chemical and Solubility Data, In: Mineral and Scale Formation", *Proc. of ACS Symposium, Z. Amjad, ed., Plenum, New York*, 1995, pp. 271–282.

[13] Erlani Pusparini et al., "Sodium-doped Hydroxyapatite Nanopowder through Sol-gel Method: Synthesis and Characterization". *Materials Science Forum*, 2011, Vol. 694 (2011) pp 128-132

[14] U. Vijayalakshmi and S. Rajeswari., "Preparation and Characterization of Microcrystalline Hydroxyapatite Using Sol Gel Method", *Trends. Biomater. Artif. Organs*, 2006, Vol 19(2) pg 57-62

[15] A.Cuneyt Tas., "Combustion synthesis of calcium phosphate bioceramic powders", *Journal of the European ceramic society*, 2000, vol 20, 2389-2394.

[16] Palanivelu. R., and A. Rubankumar., "Synthesis and Spectroscopic Characterization of Hydroxyapatite by Sol-Gel Method", *Int.J.ChemTech Res.* 2013,5(6)

[17] Iis Sopyan., Singh Ramesh., Hamdi Mohammed., "Synthesis of nano sized hydroxyapatite powder using sol-gel technique and it's conversion to dense and porous bodies", *Indian Journal Chemistry*, 2008, Vol 47A pg 1626-1631