

Influence of Aging Time on Structural Properties of Nano-Sized HAP Particles Derived From Eggshells

¹Er. Jaspal Singh, ²Er. Ravinder Pal Singh

¹Bhai Gurdas Institute of Engineering and Technology, Sangrur, Punjab, India

²Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab, India

Abstract

The most common calcium phosphate composite in implant fabrications is hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), which is most similar to the mineral component of bone and dentine. It exhibits properties such as biocompatibility, bioactivity, osteoconductivity, direct bonding to bone etc. This experimental investigation provides an environmentally beneficial and cost effective method of producing calcium phosphate bioceramic (Hydroxyapatite or Tricalcium phosphate) from eggshell waste. In this investigation effect of aging time was considered to draw the results of surface characteristics of hydroxyapatite. Synthesis process was designed for two aging time slots i.e. 24 hrs and 48 hrs and constant temperature 5°C was maintained. Wet chemical method was selected, in which eggshell powder (CaCO_3) used as a calcium source, besides this phosphoric solution (H_3PO_4) and calcium hydroxide $\text{Ca}(\text{OH})_2$ was used of analytical grade. When powder was sintered at high temperature after synthesis only hydroxyapatite and calcium oxide formed, but residual impurities exist at low sintering temperature and when aging time is 24hrs. XRD diffraction peaks for aging time 48hrs and aging temperature 5°C exhibits only HAP and CaO .

Keywords

Hydroxyapatite (HAP), Tri-calcium Phosphate (TCP), Crystal Size, Aging Time, Sintering Temperature

I. Introduction

During past few decades, considerable research efforts have been directed towards the synthesis of various bioceramics for biomedical applications. Among different classes of bioceramics, hydroxyapatite (HAP) is the most emerging bioceramic, which is widely used in various biomedical applications, mainly in orthopaedics and dentistry [1]. HAP has close similarities with inorganic mineral component of bone and teeth. It possesses exceptional biocompatibility and unique bioactivity. Naturally occurring HAP is hexagonal in structure with the chemical formula of one unit cell being $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. The hydroxyl ion (OH^-) it can be replaced by F^- , Cl^- , CO_3^{2-} , etc in the collagen fiber matrix [2].

Synthetic HAP is known to be similar to naturally occurring HAP on the basis of crystallographic and chemical studies. Because, synthetic HAP is thermodynamically stable at physiological pH 5 and osteoconductive, it has been widely used in hard tissue replacement and reconstruction applications, such as implant coatings, and bone substitutes etc [3]. Its porous character also offers high binding affinity for a variety of pharmacological substances such as antibiotics, hormones, enzymes, antibody fragments, steroids [4]. This has opened the potential for using synthetic HAP to deliver pharmacological substances in many clinical applications with sustained release capacity for the treatment of osteomyelitis, osteoporosis, osseous cancers etc in which local delivery is effective with the need to fill defects in the skeleton [5]. New economic and versatile methods for HAP synthesis are of interest due to the importance of this material for

various biomedical applications. The most popular and widely researched technique for synthesis of HAP is precipitation technique. This technique is chosen widely to synthesize HAP in contrast to other techniques, because relatively large amount of HAP can be produced by precipitation technique in absence of organic solvents at a reasonable cost [6].

II. Materials and Methods

A. Reagents and Chemicals

Different chemicals used in the present study were of analytical grade obtained from Merck. Phosphoric acid (H_3PO_4) and another one calcium hydroxide $\text{Ca}(\text{OH})_2$ were used of Merck (99.9% purity) for analysis purpose. Eggshell powder used as a calcium source and it exhibit only calcium carbonate phase CaCO_3 .

B. Sample Preparation

The pre-treated (washed and dried) eggshell wastes (CaCO_3) were grounded and sieved the powder (mesh size 30). Finest Eggshell powder 50gm, Phosphoric acid (H_3PO_4) 50gm and Double distilled aqueous solution (H_2O) 100gm were used initially. Phosphoric acid added in the eggshell powder. Double distilled aqueous solution was added simultaneously in the mixture which acts as a solvent and slows the reaction which took place instantly. After adding these constituents, mixture undergoes for vigorous stirring for 3 hours and pH values were noted, variation in pH values indicate the changes in chemical composition among these constituents at atomic level in which molecules of one compound were converted into new one, molecules of calcium carbonate and phosphate solution in the presence of water makes the new solution that can be called di-calcium phosphate CaHPO_4 solution and carbon dioxide released in gaseous form when process was undergoing and some content of water was also exist in the mixture. pH values of this mixture indicates its acidic nature due to H_3PO_4 . Following reaction took place in this process.



Now Calcium hydroxide $\text{Ca}(\text{OH})_2$ 7.4078gm is dissolved in 100 ml aqueous solution and stirrer this mixture for 2 hours at 40°C temperature and pH values of this solution was measured after every half an hour, which indicates its base nature because pH values lies between 12-13pH scale. Then CaHPO_4 (8.146gm) solution was dissolved in 100ml aqueous solution, which was extracted from eggshell powder and H_3PO_4 and this mixture was stirred for 2 hours at 40°C temperature, reaction rate increase due to slightly increase in temperature, which was more than the room temperature, the pH variation of this solution was also examined after every half an hour which indicates the changes in chemical composition of precursors. pH values shows the acidic nature of precursor, although it was dissolved in the aqueous solution. At this stage two solutions were available, one is CaHPO_4 and second is Calcium Hydroxide $\text{Ca}(\text{OH})_2$. Aqueous solution was added in these solutions to achieve the correct molar ratio of precursor. The calcium phosphorous ratio Ca/P should be 1.67 for HAP.

These two precursors were mixed together and stirred for half an hour, after that aging process starts, and aging time starts at specific temperature in the constant temperature water bath. After aging it was understood that following reactions took place in this process, because formation of HAP is possible by this reaction.



Some process parameters influence the HAP characterisation which may be crystal size, porosity, crystal sHAPe, surface morphology, strength etc. The formation of HAP is differentiating by different aging temperatures and time period at which it was kept under controlled condition. The aim of this work was to analyse the characterisation of HAP under different controlled conditioned.

C. Sample Characterization

Eggshell has three-layered structure consisting of an outer protein-rich cuticle layer, a spongy middle layer, and an inner lamellar layer. Both the middle and inner layers form a matrix constituted by protein fibers bonded to calcium carbonate crystals. The XRD diffraction peaks show that only calcium carbonate (CaCO_3) exist in raw eggshell powder shown in fig. 1.

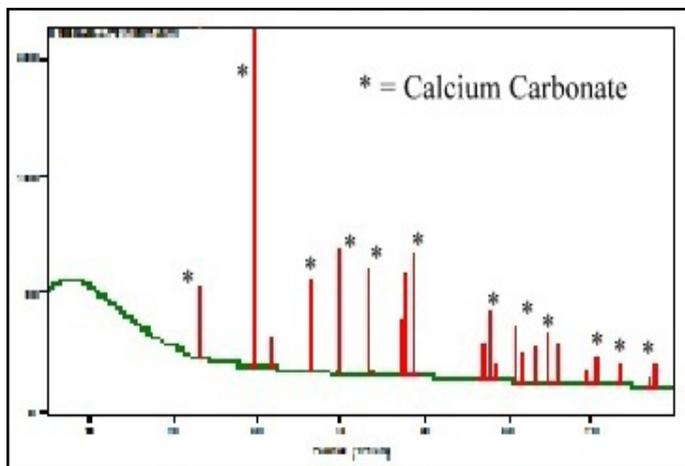


Fig. 1: XRD Pattern of Raw Eggshell Powder CaCO_3

After an initial cleaning process to prepare raw eggshell powders, the as-prepared eggshell powders were analyzed using TGA/DTA, as shown in fig. 2. The weight loss due to physically absorbed water and organic matter that had been burnt off was approximately 4% below 450°C. Also, there was a significant weight loss of about 36% from 640°C to 810°C and a sharp endothermic peak at 834°C in DTA, which may have been due to the decomposition of CaCO_3 to CaO . The results of DTA showed that there were thermal reactions, such as weight loss.

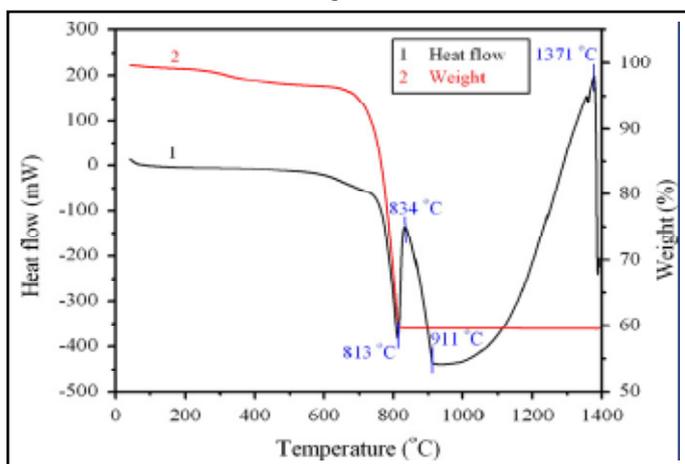


Fig. 2: TGA/DTA Analysis of Raw Eggshell Powder

III. Results and Discussion

In this experimental set up two samples were prepared to analyze the effect of aging time on HAP formation, one sample was prepared at 5°C temperature for 24hrs aging time and second was prepared at 5°C for 48hrs aging time, furthermore they were differentiated according to sintering temperature that were sample 1(a), sample 1(b), sample 1(c) for 385°C, 485°C and 760°C respectively and sample 2(a), sample 2(b), sample 2(c) same temperature for both samples. The observed sharp peaks in the XRD patterns in all the samples confirm that the samples are crystalline. Results show the presence of biphasic calcium phosphorus compound. Calcium carbonate was the main constituent in eggshell powder which converts into different phases like calcium oxide, calcium nitrate and calcium phosphorus biphasic compounds for e.g. β -TCP, α -TCP, and calcium deficient HAP etc. Sample 1(a) whose aging temperature is 5°C for 24hrs and sintering temperature 385°C has high percentage intensity of HAP shown in figure 3. Whereas sample 1(b) and sample 1(c) of this category have less residual impurities, because of high sintering temperature.

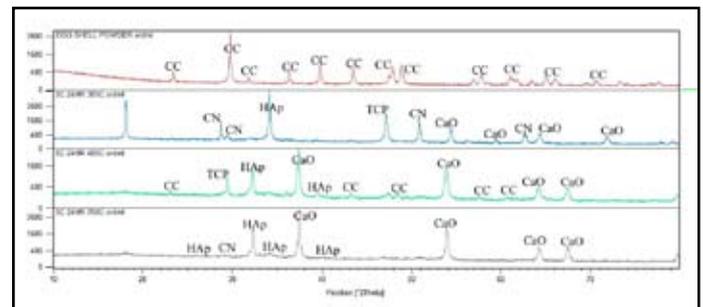


Fig. 3: XRD Patterns of Sample 5°C /24hrs Sintered at 385°C, 485°C and 760°C

Calcium oxide is a main constituent of sample 1(b) and sample 1(c). β -TCP also exists in the product but in small amount. Consequently calcium phosphorus compound and calcium oxide is a net product of this sample.

When aging temperature is same but aging time has increased 48hrs, instead of 24hrs then results slightly differ from later one, in this case we thought molecules of di-calcium phosphate (CaHPO_4) has enough time to settled down with the molecules of $\text{Ca}(\text{OH})_2$ and result will be better, in this case when product is sintered at 385°C the HAP formation is more and β -TCP also exist in more intensity, more interestingly calcium nitrate $\text{Ca}(\text{NO}_3)_2$ and calcium oxide $\text{Ca}(\text{OH})_2$ also produced in large quantity which may be the effect of atmospheric nitrogen [7], because solution gets enough time to react with the atmospheric nitrogen. When other phases and trace elements burn out due to high temperature during sintering but calcium oxide and calcium nitrate exists. Complete transform of calcium carbonate into HAP, CaO and $\text{Ca}(\text{NO}_3)_2$ gives the proof of molecular change at higher level shown in fig. 4.

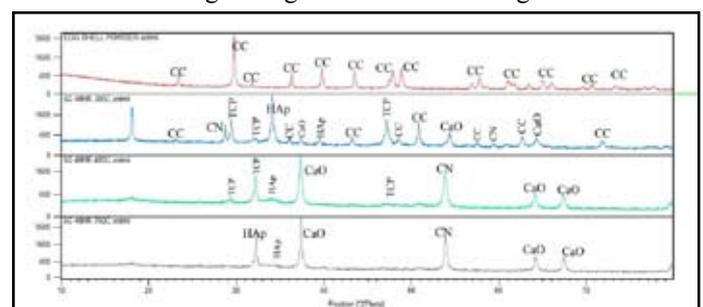


Fig. 4: XRD Pattern of Sample 5°C /48hrs Sintered at 385°C, 485°C and 760°C

The Fourier transform infrared spectra of the sintered calcium phosphorus composite are shown in fig. 5. The characteristic peaks are listed in figure. The bands that appeared at 876 cm^{-1} indicates the symmetric stretching modes ν_5 P-OH deformation of HPO_4^{2-} groups (Berry, 1961; Fowler, 1974; Fowler et al, 1996). Further, bands have been observed at 1040 and 1455 cm^{-1} because of the presence of ν_3 (PO_4) and carbonated stretching respectively (Thomas et al. 2007). The bands observed at 3644 are due to the OH stretching. The bands $631\text{--}635\text{ cm}^{-1}$ are due to the stretching vibration of the HAP hydroxyl group [8]. This may be originated from the libration bands of the OH group.

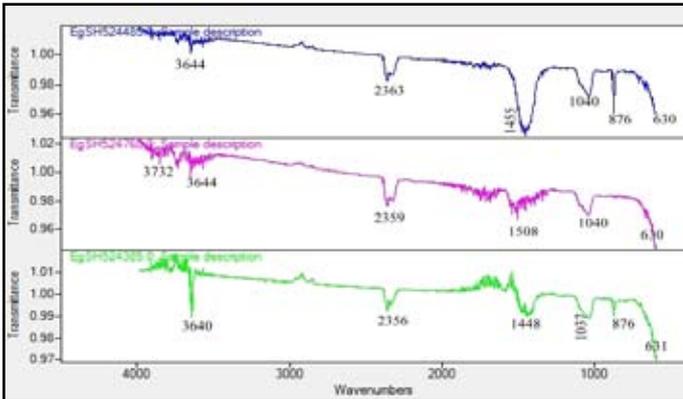


Fig. 5: FTIR Spectrums of Sample 5°C/24hrs Sintered at 385°C, 485°C and 760°C

The ν_3 P-O asymmetric stretching mode appears in the region of 1041 cm^{-1} . The peaks observed at 873 and in the range between 1380 and 1466 cm^{-1} is due to presence of carbonate ions [9] which may have arisen from the atmospheric carbon dioxide during synthesis (Wang and Li 2007, Zhang et al. 2003). Thus, the appeared peaks in the range between 874 and $1380\text{--}1466\text{ cm}^{-1}$ are due to the atmosphere carbon dioxide. The intensity of this peak decreases with an increase in sintering temperature. The observed broad peak at HAP-A3- 1040 in the region of $1041\text{--}1637\text{ cm}^{-1}$ indicates the absence of residual impurity such as carbonate group shown in fig. 6.

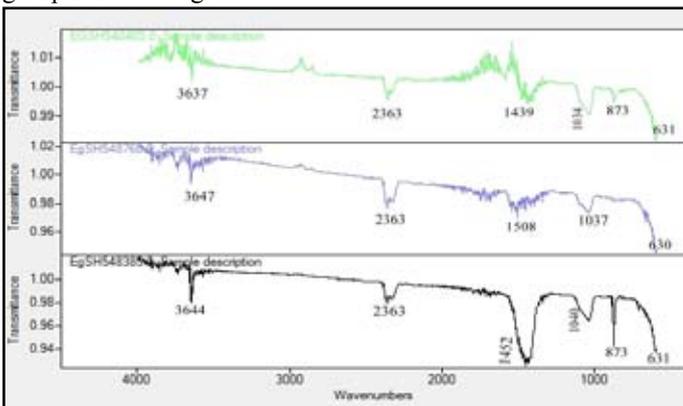


Fig. 6: FTIR Spectrums of Sample 5°C/48hrs Sintered at 385°C, 485°C and 760°C

A. Effects of Aging Time on Structural Constitution of Powders

Experiment results shows that when aging time increased then carbonate phase of calcium completely vanish from net product at high sintering temperature and intensity of HAP and TCP increased notably but more aging time pertaining with another impurity such as calcium nitrate $\text{Ca}(\text{NO}_3)_2$. Calcium phosphorus Ca/P also improves with increased in aging time, its value lies very close

to standard value i.e. 1.67. All surface characteristics improve at higher aging time such as lattice parameter (a & c), distortion ratio, TCP and HAP weight ratio etc. Crystal size increased when HAP formation prepared at higher aging time.

B. Effects of Sintering Temperatures on Structural Constitution of Powders

On the basis of experimental results, we can say that with the rise in sintering temperature weight ratio of β -TCP and HAP shows very high weight proportion of HAP. Aging temperature is low but sintering temperature is high, it means formation of HAP can be increased to increase sintering temperature. Calcium phosphorus ratio improves, when we increase the aging time but less significant for aging temperature. It is very close to standard when aging time and temperature kept at higher level. Calcium deficiency factor x is proportional to weight ratio of β -TCP and HAP. When ratio decreased, it comes closer to standard ratio. Sintering temperature also increase the crystal size shown in fig. 7. It behaves like directly proportion to sintering temperature.

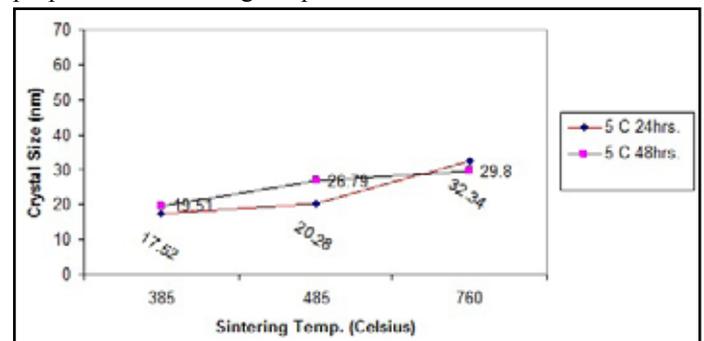


Fig. 7: Effects of Sintering Temperatures on Crystal Size

IV. Conclusion

Sample prepared at high aging time has valuable surface characteristics as well as higher formation of HAP. Calcium phosphorus ratio also improves in later case but crystal size slightly more as compare to less aging time sample. Sintering temperature also plays an important role to vanish the residual impurities and increased the crystal size, higher sintering temperature necessary to eradicate the calcium oxide whose existence remains up to 900°C .

References

- [1] Liu, D.M., "Fabrication and Characterization of Porous Hydroxyapatite Granules", *Biomaterials*, 17, pp. 1955-1957, 1995.
- [2] Braye, F., Irigaray, J.L., "Resorption kinetics of osseous substitute: natural coral and synthetic hydroxyapatite", *Biomaterials*, 17, pp. 1345-1350, 1996.
- [3] Siva Kumar, M., Sampath Kumar, T.S., "Development of Hydroxyapatite Derived from Indian Coral", *Biomaterials*, 17, pp. 1709-1714, 1996.
- [4] Xu, Y., Wang, D., Yang, L., "Hydrothermal Conversion of Coral into Hydroxyapatite", *Materials characterization*, 47, pp. 83-87, 2001.
- [5] Jinawath, S., Polchai, D., Yoshimura, M., "Low-Temp Hydrothermal Transformation of Aragonite to Hydroxyapatite", *Materials Science and Engineering*, 22, pp. 35-39, 2002.
- [6] Chua, T.M., Ortone, D., "Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures", *Biomaterials*, 23, pp. 1283-1293, 2002.

- [7] Nissan, B.B., "Morphology of sol-gel derived nano-coated coralline hydroxyapatite", *Biomaterials*, 25, pp. 4971-4975, 2004.
- [8] Murugan, R., Ramakrishna, S., "Development of nanocomposites for bones grafting", *Composites Science and Technology*, 65, pp. 2385-2406, 2005.
- [9] Pramanik, S., Agarwal, A.K., Rai, K.N., Garg, A., "Development of High Strength Hydroxyapatite by Solid State Sintering Process", *Ceramics International*, 33, pp. 419-426, 2007.



Ravinder Pal Singh is pursuing Ph.D. in Materials and Metallurgy from PEC University of Technology, Chandigarh, India in the field of Biomaterials. He has been awarded with Bharat Excellence Award in the year 2010. He is a life member of Indian Society of Technical Education and Punjab Science Congress. He is currently serving as Assistant Professor in Sri Guru Granth Sahib World University,

Fatehgarh Sahib, Punjab. His total teaching experience is more than 10 years and has published more than 35 research publications in various National and International Journals and Conferences.



Jaspal Singh is pursuing M. Tech in Production Engineering from Punjab Technical University, Jalandhar India in the field of Biomaterials. He is a life member of The Institution of Engineers (India).