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Trends in
Biomaterials
and Artificial Organs
www.biomaterials.org.in/tibao
ISSN 0971-1198
https://doi.org/10.5281/zenodo.16792997

Original Article

Influence of Natural and Synthetic Calcium precursor on the Synthesis of pure Phase Hydroxyapatite

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Received: *16 February 2024*
Accepted: *15 March 2025*

Keywords: *biomaterials, natural waste, hydroxyapatite, co-precipitation method*

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) was proved as a remarkable material in the application of biomedical research because of its chemical composition similar to the human bone mineral. The formation of phase pure Hydroxyapatite from different sources of calcium precursors are investigated. In our study, the co-precipitation method was carried out using three different calcium precursors like eggshell powder, (white clam & scallop) seashell powder, calcium hydroxide (synthetic) by fixing phosphorus precursor as phosphoric acid (H_3PO_4) for all three reactions. Synthesizing HAP from different chemical sources in the form of calcium hydroxide ($Ca(OH)_2$) and phosphoric acid (H_3PO_4) has been achieved. The functional groups and phase formation of synthesized HAP powder were investigated by Fourier transform infrared spectroscopy (FTIR) and X-ray diffractometry (XRD). XRD results revealed the pure form of Hydroxyapatite without any other impurity peak of $Ca(OH)_2$ and secondary Phases like TCP, $Ca_2P_2O_7$ etc.,. The surface morphology was investigated by Scanning electron microscopy - Energy Dispersive X-ray Spectroscopy (SEM-EDS). The SEM results indicated that the powders had an agglomerated particles due to the presence of highly alkaline condition and poor water soluble nature of $Ca(OH)_2$, an agglomerated particles are formed when compared to natural precursors.

Introduction

Hydroxyapatite widely used in biomedical applications mainly as bone replacement, regeneration, and dental restoration materials because of its tremendous properties such as biocompatibility, bioactivity, osteoconductive, non-toxicity [1-5]. Which exhibits chemical and structural similarity with human natural bone, teeth and enamel having chemical formula as $Ca_{10}(PO_4)_6(OH)_2$. The high similarity of chemical composition with human bone minerals makes them suitable for use as orthopaedic implants (human skeleton, bones, and joints), and dental materials [6].

Hydroxyapatite can be synthesised chemically or extracted from natural sources. Prior research has reported on the various methods of synthetic and natural HAP [7-9]. The review by Sadat-Shojai et al. concluded that synthetic HAP can be produced by various methods such as dry methods (solid-state and mechanochemical), wet methods (chemical precipitation, hydrolysis, sol-gel, hydrothermal, microwave irradiation and ultrasonication), and high temperature processes (combustion and pyrolysis) etc. [10-12].

The review by A.S. Mohd Pu'ad et al. concluded that different sources of natural calcium precursor were utilised for synthesis of hydroxyapatite such as mammalian bone (e.g bovine, camel & horse), marine or quatic sources (e.g fish scale & fish bone), shells sources (e.g cockle, eggshell, clam & seashell), plant (fruit peels, leaf) and algae, minerals (e.g limestone) [13]. The natural sources primarily made up of calcium carbonate ($CaCO_3$, 94%-95%), calcium phosphate (1%) and other organic components ($MgCO_3$, 4%-5% [2,14]. which is cost effective and easy to prepare hydroxyapatite by precipitation method.

In this paper, synthesis of hydroxyapatite using three different calcium precursor sources such as two form of natural sources (chicken eggshell, white clam seashell) and another source of $Ca(OH)_2$ from SDFCL, Mumbai, India . We have successfully produced pure phase of hydroxyapatite powder by using eggshell (Biowaste), seashell (Biowaste) and pure $Ca(OH)_2$ synthetic chemicals as the raw material. Synthesized powders were characterized and the results are compared among three types of

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calcium precursors by using FT-IR, XRD, SEM with EDS analysis. This work aims to synthesize the pure phase of hydroxyapatite by utilizing various precursors, particularly the calcium source found in natural source such as eggshell and seashell to transform bio-waste into valuable products. The main aim of synthesis of HAP from natural sources was compared with the HAP synthesized from chemical precursor. Both the methods have its own advantages, whereas the one prepared from seashell natural source was found to have less particle size with more crystallinity.

Materials and Methods

The calcium precursors eggshell and white seashell (white clam shell) are collected from different places. Eggshells were collected from nearest hotel in Katpadi, Vellore. White clam seashell was collected from kovalam beach, Chennai. Calcium hydroxide, orthophosphoric acid were purchased from SDFCL, Mumbai, India and Ammonium hydroxide solution was purchased from SRL (Sisco Research Laboratories Pvt. Ltd.), Maharashtra, India.

Synthesis process

In this work, co-precipitation method was utilized to synthesize hydroxyapatite. Collected eggshell were washed and soaked in normal tap water, the inner membrane layer is removed and the outer shell was dried in sunlight. Finally, it was washed with hexane to remove the organic matrix followed by grinding and the obtained powder was heat treated at 1000°C for 3 hours by using high temperature furnace [15,16]. The product CaO was used as a calcium precursor with the addition of little amount of distilled water to convert as Ca(OH)$_2$ source. Normally CaO is not stable in room atmosphere, due to its highly hygroscopic nature it was converted as calcium hydroxide (Ca(OH)$_2$ when it is exposed in atmosphere.

To synthesize HAP, the Co-precipitation process was selected and the stoichiometric ratio of Ca/P is maintained as 1.67, orthophosphoric acid is used as phosphorus precursor in all the reactions. 1M Ca(OH)$_2$ solution was taken in beaker followed by the addition of 0.6M orthophosphoric acid solution and the solution was stirred using magnetic stirrer at 80°C for 2 hours. During the addition of phosphorus precursor on calcium source, the pH was maintained at 10 using ammonium hydroxide solution. The reaction mixture was kept for aging for 12 hours and the precipitate was filtered and washed with distilled water followed by ethanol and dried in an oven at 100°C for 12 hours. Finally, the obtained product was heat treated at 900°C for 2 hours [17-18]. The same procedure was followed for other two calcium precursors for the preparation of HAP. Figure 1 represents the steps followed for the synthesis of HAP [19,20].

Characterizations

Fourier transform infrared spectroscopy (FTIR) was used in order to detect the functional groups of synthesized powder by using instrument model Thermo Nicolet iS50 with Inbuilt ATR made by Shimadzu, Japan and the transmittance regions of 4000-400cm^{-1}. The synthesised powder crystallographic study was investigated by using X-ray diffractometry (XRD, instrument model; Bruker D8 Advance, Germany and X-ray generated from Cu-Kα with the wavelength of 1.54056 A° and operating by stepsize =0.045/deg, 2θ range of 10-90°. The morphology of HAP samples was observed by using Scanning electron microscopy (SEM, model; CARL ZEISS make by EVO 18 Research, Germany and simultaneously coupled with Energy Dispersive X-Ray spectroscopy (EDS, AZtec; Oxford instruments) for elemental analysis. Those all are characterization done in our same institute instrument facilities.

Results and Discussion

Fourier transform infrared spectroscopy analysis

FTIR spectrum was used to determine the presence of phosphate (PO$_4^{3-}$) and hydroxyl (OH$^-$) functional groups in calcium phosphate (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$). Figure 2 a,b,c shows HAP derived from eggshell CaO, seashell CaO, chemical Ca(OH)$_2$ method respectively. HAP synthesised by eggshell consists of peaks at 636 and 3568 cm^{-1} which represents the presence of bending and stretching vibrational mode of OH$^-$ group. The peaks at 961, 1019 and 1086 cm^{-1} are due to the stretching vibrational mode of phosphate ions and 466, 566 and 595 cm^{-1} indicative of bending modes of phosphate ions which represents the formation of HAP in the synthesised powder heat treated at 900°C for 2 hrs (figure 2a).

Similarly, the HAP synthesised from seashell indicates the presence of fundamental vibrational (υ_{as}, υ_s, δ_{as} and δ_s) modes for stretching and bending vibration of phosphate ions and bending and stretching mode of hydroxyl group. The peaks at 1091, 1015 cm^{-1} and at 957 cm^{-1} were responsible for the asymmetric (υ_{as}) and symmetric stretching vibration (υ_s) of phosphate group. The peaks at 561 and 600 cm^{-1} were responsible for the presence of asymmetric bending vibration (δ_{as}) and 469 cm^{-1} for symmetric bending vibration (δ_s) of phosphate group which is evident for the formation of HAP. The hydroxyl group can be confirmed by the presence of peaks at 636 and 3568 cm^{-1} respective of bending (δ) and stretching (υ) mode of OH$^-$ (figure 2b). Similar peak positions were observed for the synthetic (CaOH)$_2$ synthesised HAP (method 3) (figure 2c). All similar peak positions corresponding to the formation of HAP were identified as per the reported literatures

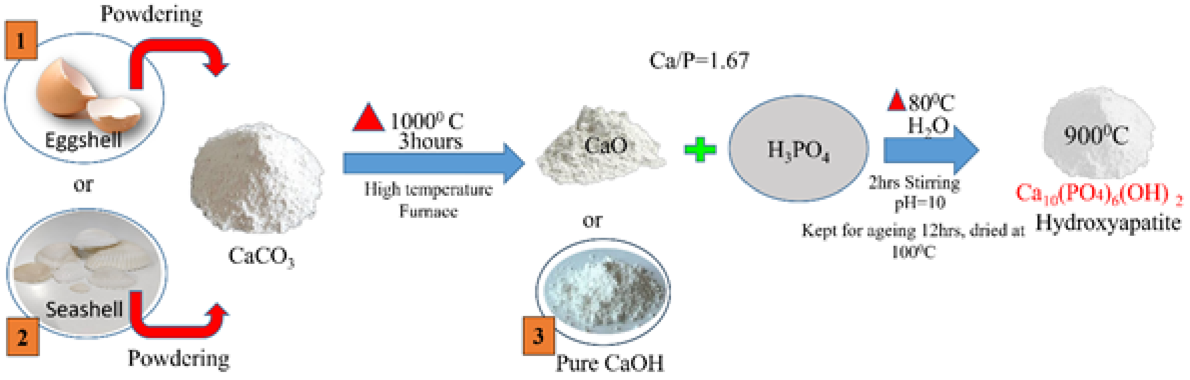


Figure 1: Schematic Procedure 1) HAP synthesis from Eggshell, 2) from Seashell, 3) from As-received Ca(OH)$_2$

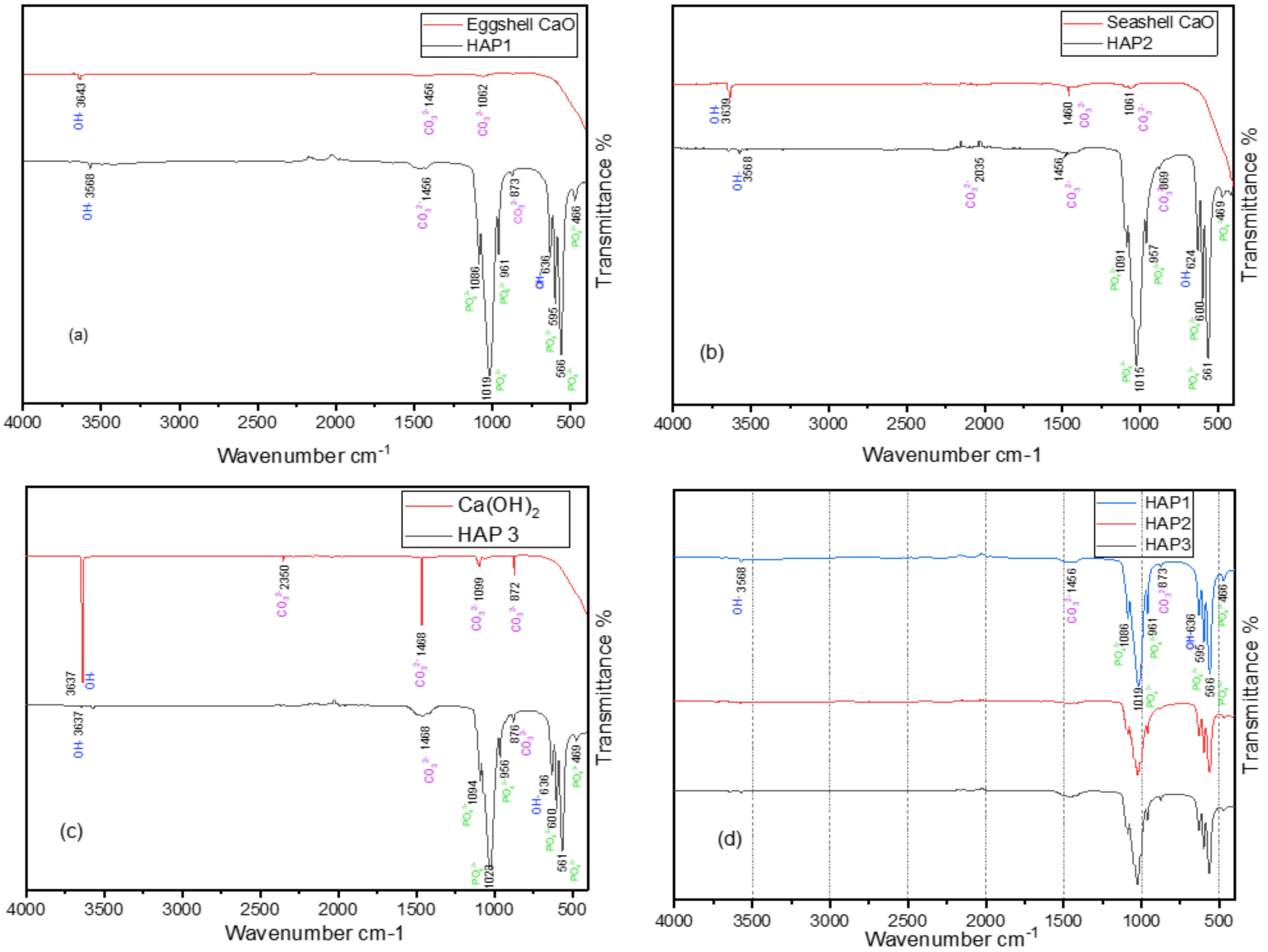


Figure 2: FTIR Result of synthesized HAP a.) Eggshell HAP b.) Seashell HAP c.) HAP by Raw Chemical $Ca(OH)_2$ and d.) Comparison spectra of HAP1, HAP2, HAP3

[21,22]. Figure 2d represents the comparison of HAP formation among the three precursors used.

The presence of carbonate (CO_3^{2-}) peaks at 872 and 1432 cm^{-1} are found to be present in the prepared HAP powder. When compared to all the other three precursors, eggshell and chemical precursor have shown carbonated apatite, whereas the HAP derived from seashell consists of less carbonated peaks. Chemical precursor derived HAP consists of more carbonated peaks when compared to natural precursor derived HAP. For natural source the complete pyrolization of residues are happened which is more evident for the presence of CaO peak, whereas the chemical precursor form $Ca(OH)_2$ due to the hygroscopic nature of the precursor at room temperature. All the peaks respective of HAP was found in the FTIR analysis which is meant to detect the functional group of compounds. Hence, the synthesised HAP is further compared by XRD spectrum.

X-ray powder diffraction (XRD) analysis

The XRD pattern of HAP synthesized from three different precursors indicates the formation of pure phase of calcium phosphate and the results are represented in figure 3 (a,b,c,d). Figure 3a shows the XRD results of HAP prepared from eggshell (HAP1), 2θ values at 26.25°, 28.34°, 29.02°, 31.94°, 32.38°, 33.11°, 34.33°, 35.64°, 39.39°, 39.97°, 42.21°, 46.83°, 49.66°, 53.45° and 64.21° and its corresponding hkl values (002), (102), (210), (211), (112), (300), (202), (301), (212), (310), (311), (222), (312), (004) and (502) are completely matched with hydroxyapatite (JCPDS- 09–0432). Similar results reported by Castro, Mário A., et al. and M. Chozhanathmisra., et al. reported the similar behaviour corresponding to HAP [23,24]. Figure 3b and 3c shows the XRD results of HAP prepared from seashell (HAP2), and from pure $Ca(OH)_2$ chemical (HAP3), all the 2θ values are exactly matched with JCPDS file no. 09-0432. At higher temperature treatment (at 900°C), all the precursors have shown pure and crystalline HAP peaks without any other secondary phases like β-TCP, α-TCP and dicalcium phosphate dihydrate (DCPD). The study reported by Matej Balá• [25], Æurkoviæ, L., et al. [26] and Rodríguez-Lugo, V., et al. [27] using hydrothermal and ball milling method of synthesis of HAP from biowastes resulted have shown mixture of phases such as β-TCP, $CaHPO_4$ along with HAP. The crystallinity and intensity of HAP peaks for the chemical precursor was found to be less when compared to the other natural precursors and are represented in figure 3d.

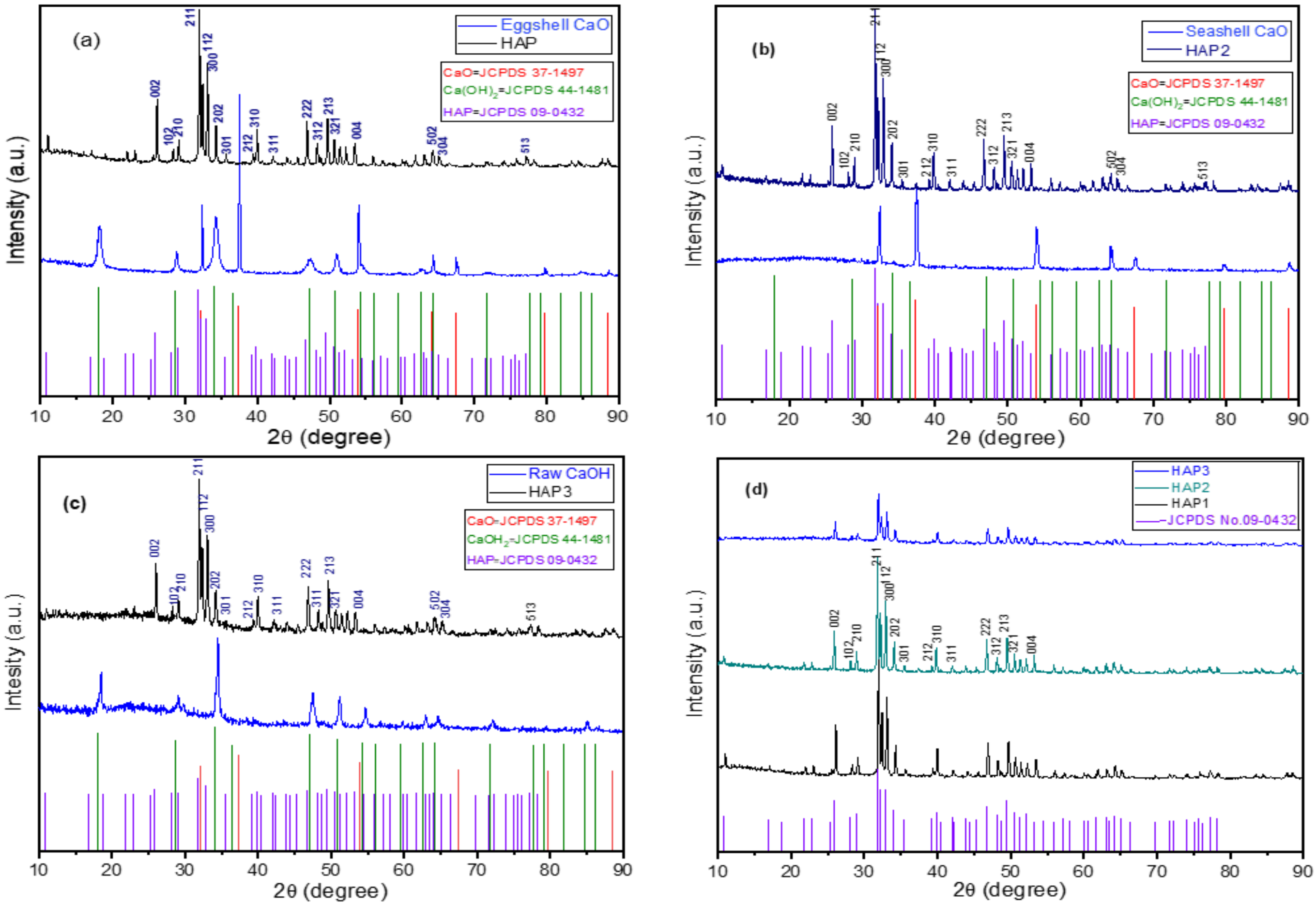


Figure 3: XRD data of synthesized HAP a) HAP1- Eggshell HAP b) HAP2-Seashell HAP c) HAP3-Raw chemical HAP d) comparison of HAP1, HAP2, HAP3

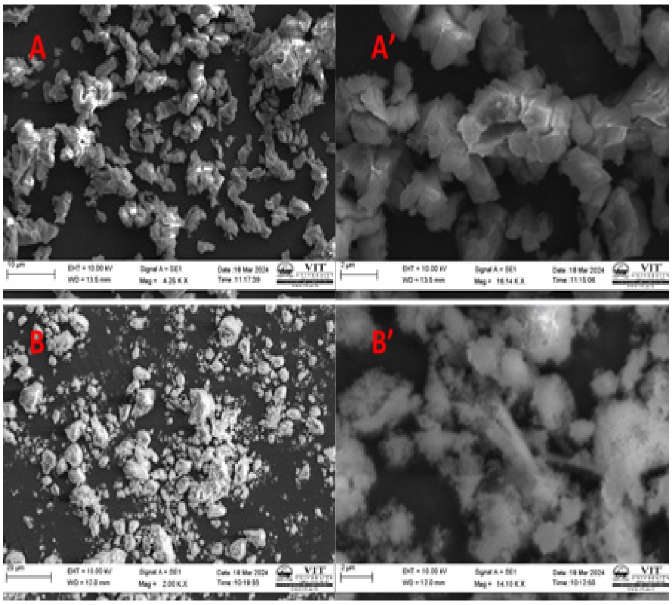


Figure 4a: SEM micrographs of CaO and HAP A,A') CaO from Eggshell powder heat treated at 1000°C at 3hrs and B,B') HAP from Eggshell CaO heat treated at 900°C at 2hrs

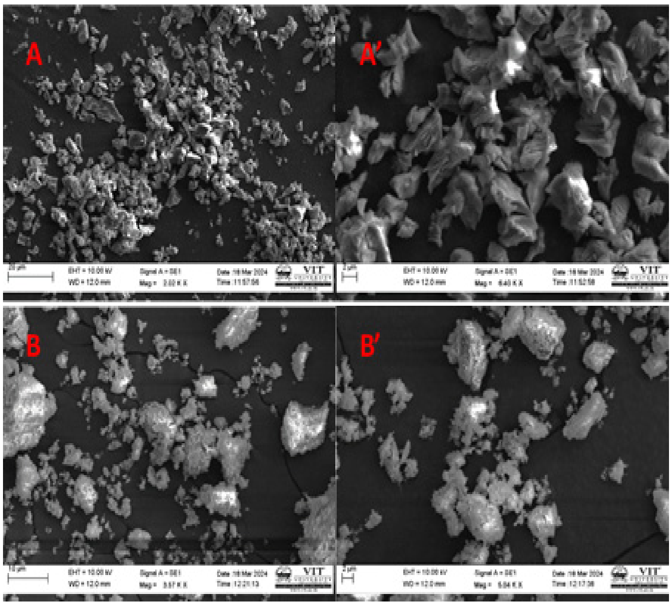


Figure 4b: SEM micrographs of CaO and HAP A,A') CaO from Seashell powder heat treated at 1000°C at 3hrs and B,B') HAP from Seashell CaO heat treated at 900°C at 2hrs

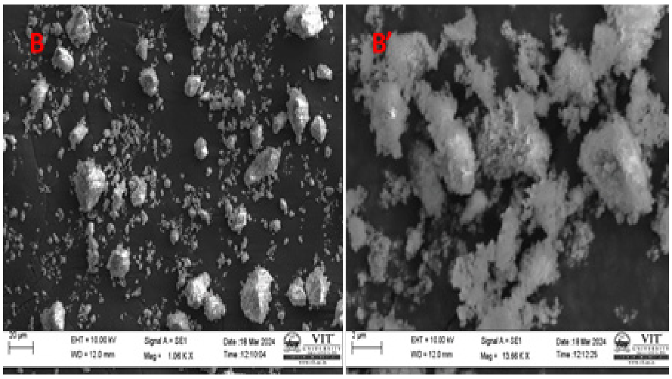
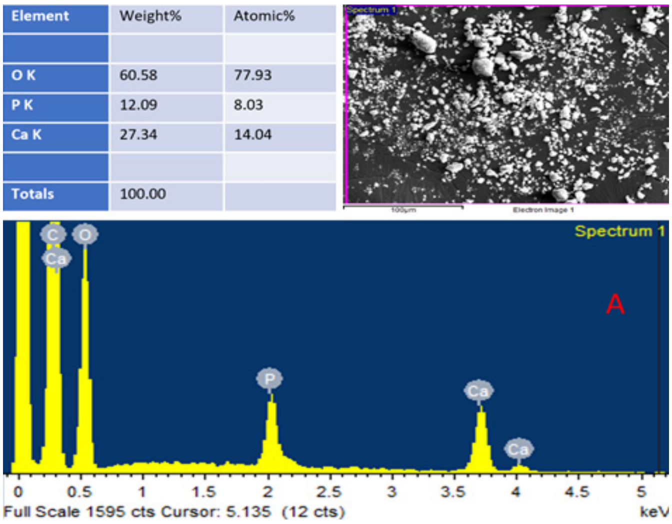
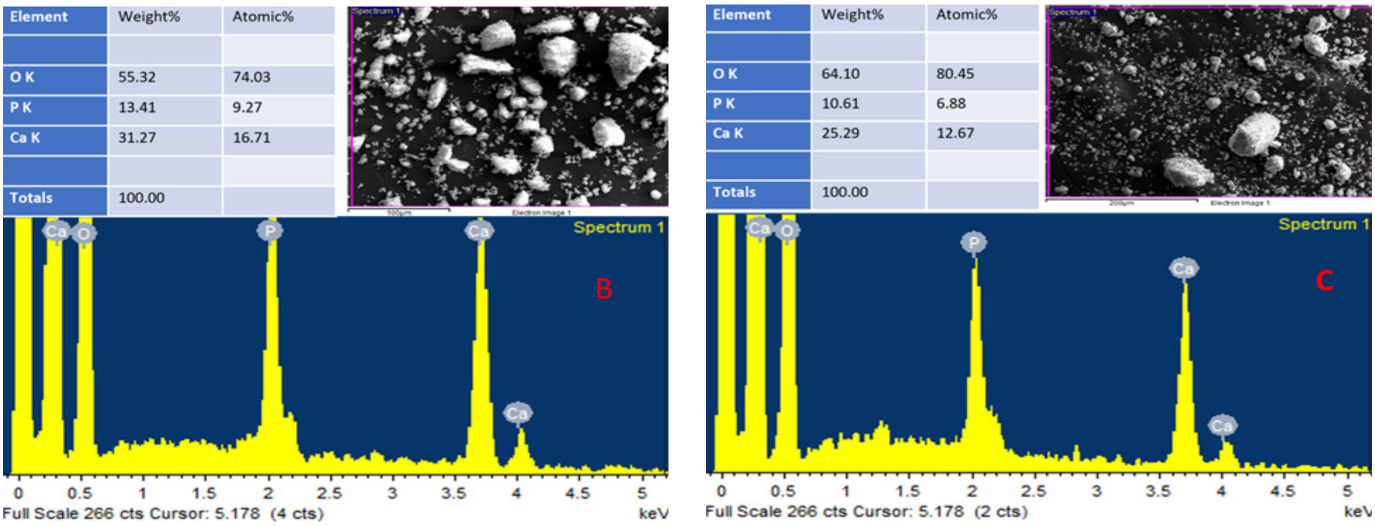


Figure 4c: SEM micrographs of HAP B,B') HAP synthesized from pure $Ca(OH)_2$ chemical heat treated at 900°C at 2hrs



Element	Weight%	Atomic%
O K	60.58	77.93
P K	12.09	8.03
Ca K	27.34	14.04
Totals	100.00	



Element	Weight%	Atomic%
O K	55.32	74.03
P K	13.41	9.27
Ca K	31.27	16.71
Totals	100.00	

Element	Weight%	Atomic%
O K	64.10	80.45
P K	10.61	6.88
Ca K	25.29	12.67
Totals	100.00	

Figure 5: Elemental analysis data of A) Eggshell HAP B) Seashell HAP C) Chemical Synthesised HAP

Scanning electron microscopy (SEM) analysis

The Surface morphology of synthesised pure hydroxyapatite was investigated by SEM analysis. Eggshell synthesised HAP showed mixture of agglomerated spherical shaped particles with more or less same size for the sample heat treated at 900°C for 2 h (figure 4a). Seashell based HAP sample have shown highly agglomerated particles with more non uniform particles for the sample heat treated at 900°C for 2 h (figure 4b) and Pure Calcium hydroxide-based HAP sample showed agglomerated particles with bigger size for the heat treated at 900°C for 2 h (figure 4c). All the three-type of calcium precursor (CaO) prepared from eggshell, seashell pyrolised by 1000°C for 3 hrs shows irregular shaped particles (figure 4) [15,26].

Elemental analysis

Energy Dispersive X-Ray spectroscopy (EDS) analysis was used to calculate the presence of elements in hydroxyapatite such us calcium, phosphorus, oxygen and the other remaining trace elements. The stoichiometric ratio of 1.67 was taken for the synthesis, comparatively an Ca/P ratio of 1.74 was obtained for the seashell HAP and a ratio of 1.80 higher than seashell Ca/P ratio was obtained for eggshell and 1.84 was obtained for $Ca(OH)_2$ based HAP. Seashell comparatively results with higher Ca/P ratio than the stoichiometric, size and crystallinity of hydroxyapatite are all affected by changing the parameters such as, calcination temperature and pH. At lower pH, the Ca/P ratio will be closer to the stoichiometric ratio of 1.67 Ca/P, while at a higher pH value the ratio of Ca/P was found to be enhanced. All the HAP derived from various calcium source have shown a Ca/P ratio more than 1.67 due to the formation of carbonated apatite. Hence, all the hydroxyapatite synthesised from various source have resulted in the formation of carbonated apatite which is close resemblance with the biomimetic HAP [27,28]. The calculated Ca/P ratio from elemental analysis for the product obtained by three methods are 1.8026, 1.7484 and 1. 8415 respectively for HAP A (eggshell), HAP B (seashell) and HAP C (chemically synthesised) respectively.

HAP synthesised from biowaste like eggshell and seashell expected to be a cheap and natural material. Francesca Cestari, et al., prepared HAP from different biogenic calcium carbonate containing biowastes like chicken eggshells (ES), cuttlefish bones (sepia officinalis) (CB) and mussel shells (mytilus galloprovincialis) (MS) and performed in vitro biological evaluation which is suitable for bone regeneration applications [29]. Youssef, Hanaa, et al., considered snail shells for the preparation of HAP and confirmed the material with good antimicrobial and biocompatibility [30]. Shahid Hussain., et al., have proved that plasma-sprayed seashell derived HAP coatings on Ti-alloy exhibits good bioactive and biocompatible [31]. In this study, we have synthesised hydroxyapatite named as HAP1, HAP2, HAP3 with close resemblance with the biomimetic HAP are planned to be considered as coating material on Ti-6Al-4V implant for further optimisation.

Conclusion

Pure phase hydroxyapatite was successfully synthesised from various natural sources like eggshell, seashell and for comparison pure calcium hydroxide was used as calcium precursor by co-precipitation method. Currently, many researchers have considered natural waste materials as one of the precursor for the synthesis of biomaterials. Waste materials like eggshell, seashell are compared with the available chemical precursor as calcium source.

From the FT-IR result, it was inferred that the carbonated apatite formation was more for the chemical precursor when compared to natural source. Among the natural source seashell precursor was found to produce less carbonated apatite than the eggshell precursor. This may be due to complete combustion of seashell compared to eggshell during the pyrolysis process. The XRD spectrum revealed that all the HAP prepared from various sources irrespective of natural and chemical precursor, have shown pure phase HAP formation. The intensity and crystallinity of the peaks are found to be less for the chemical precursor compared to natural precursors. The agglomerated non uniform particles are formed for all the prepared materials, only the Ca/P ratio was found to be high for the chemical precursor compared to the natural source.

Acknowledgement

The authors would like to thank the management of VIT, Vellore, Tamil Nadu for rendering the necessary laboratory and characterization facilities and financial support for this manuscript.

References

1. HamidiVadigh, F., Javadpour, J. The Synthesis of Hydroxyapatite Nanoparticles Using Eggshells and Two Different Phosphate Sources. European Journal of Engineering and Technology Research, 9(2), 1–4 (2024).
2. U. Anjaneyulu, Deepak, K., Pattanayak., Uthirapathy, Vijayalakshmi. Snail Shell Derived Natural Hydroxyapatite: Effects on NIH-3T3 Cells for Orthopedic Applications. Materials and Manufacturing Processes, 31, 206-216 (2016).
3. Rehman, Mujeeb Ur, Ayatullah Qureshi, & Muhammad Moazam Baloch. Extraction of Hydroxyapatite from Caprine Bones and its Anti-Bacterial Study. Mehran University Research Journal of Engineering and Technology, 40(4) 867 – 873 (2021).
4. Vijayalakshmi, U., Rajeswari, S. Preparation and characterization of microcrystalline hydroxyapatite using sol gel method. Trends in Biomaterials and Artificial Organs., 19(2), 57-62 (2006).
5. Vijayalakshmi, U., Rajeswari, S. Influence of Process Parameters on The Sol–Gel Synthesis of Nano Hydroxyapatite Using Various Phosphorus Precursors. J Sol-Gel Sci Technol., 63, 45–55 (2012).
6. Temenoff, Johnna S., and Antonios G. Mikos. Biomaterials: the intersection of biology and materials science. Upper Saddle River, NJ, USA:: Pearson/ Prentice Hall., (2008).
7. Türk, S., et al. Microwave–assisted biomimetic synthesis of hydroxyapatite using different sources of calcium. *Materials Science and Engineering: C.*, 76, 528-535 (2017).
8. Gunarajulu Renganathan., et al. Novel Bio-fabrication Modelling Approach from Marine Sources: Synthesis, Characterization and its Composite Formulations. Trends in Biomaterials and Artificial Organs., 36(S1), 59-66 (2022).
9. Lertcumfu, N., et al. Properties of calcium phosphates ceramic composites derived from natural materials. Ceramics International, 42(9), 10638-10644 (2016).
10. Mehdi, Sadat-Shojai., Mohammad, Taghi, Khorasani., Ehsan, Dinpanah-Khoshdargi., Ahmad, Jamshidi. Synthesis Methods for Nanosized Hydroxyapatite with Diverse Structures. Acta Biomaterlia, 9(8), 7591-7621 (2013).
11. T.H.M. Mysore et al. Apatite insights: From synthesis to biomedical applications, European Polymer Journal., 209, 112842 (2024).
12. Vijayalakshmi Natarajan, U., & Rajeswari, S. Influence of calcium precursors on the morphology and crystallinity of sol–gel-derived hydroxyapatite nanoparticles. Journal of Crystal Growth., 310(21), 4601–4611 (2008).
13. Mohd Pu'ad, N. A. S., Koshy, P., Abdullah, H. Z., Idris, M. I., & Lee, T. C. Syntheses of hydroxyapatite from natural sources. Heliyon, 5(5), e01588 (2019).
14. Hamideh, Faridi., Akbar, Arabhosseini. Application of eggshell wastes as valuable and utilizable products: A review. Research in Agricultural Engineering., 64, 104-114 (2018).
15. Dampang, Sarah., et al. Analysis of Optimum Temperature and Calcination Time in the Production of CaO Using Seashells Waste as CaCO3 Source. Journal of Ecological Engineering. 22(5), 221-228 (2021).
16. Goloshchapov, D., et al. Synthesis of nanocrystalline hydroxyapatite by precipitation using hen's eggshell. Ceramics International, 39(4), 4539-4549 (2013).
17. Kian Wei Goh., et al. Effect of pH on the properties of eggshell-derived hydroxyapatite bioceramic synthesized by wet chemical method assisted by microwave irradiation. Ceramics International, 47(7), 8879-8887 (2021).
18. Kamalanathan, P., et al. Synthesis and sintering of hydroxyapatite derived from eggshells as a calcium precursor. Ceramics International, 40(10), 16349-16359 (2014).
19. Saranya Kannan., et al. Waste to health- seashells and pumpkin seeds into nanohydroxyapatite, Sustainable Chemistry and Pharmacy, 37, 101438 (2024).
20. Putwi Widya Citradewi., et al. Clitorea ternatea-mediated silver nanoparticle-doped hydroxyapatite derived from cockle shell as antibacterial material, Chemical Physics Letters, 769, 138412 (2021).
21. Arunseshan, Ch., rasekar., Suresh, Sagadevan., Arivuoli, Dakshnamoorthy. Synthesis and characterization of nano-hydroxyapatite (n-HAP) using the wet chemical technique. International Journal of Physical Sciences, 8, 1639-1645 (2013).
22. Sahadat Hossain, Md., and Samina Ahmed. FTIR spectrum analysis to predict the crystalline and amorphous phases of hydroxyapatite: a comparison of vibrational motion to reflection. *RSC Advances.,* 13(21), 14625-14630 (2023).
23. Castro, Mário A., et al. Synthesis of hydroxyapatite by hydrothermal and microwave irradiation methods from biogenic calcium source varying pH and synthesis time. Boletín de la Sociedad Española de Cerámica y Vidrio., 61(1), 35-41 (2022).
24. Chozhanathmisra, Manickam, et al. Enhancement on physical, chemical, and biological properties of HNT-PVA-ALG-HAp biocomposite coating on implant substrate for biomedical application. *Ceramics International,* 48(12), 16868-16876 (2022).
25. Matej Balá•, Ball milling of eggshell waste as a green and sustainable approach: A review, Advances in Colloid and Interface Science, 256, 256-275 (2018).
26. Æurkoviæ, L., et al. From eggshells biowaste to hydroxyapatite biomaterial. Materialwissenschaft und Werkstofftechnik, 48(8), 797-802 (2017).
27. Rodríguez-Lugo, V., et al. Wet chemical synthesis of nanocrystalline hydroxyapatite flakes: effect of pH and sintering temperature on structural and morphological properties. Royal Society Open Science., 5(8), 180962 (2018).
28. Wei Xiao, B. Sonny Bal, Mohamed N. Rahaman. Preparation of resorbable carbonate-substituted hollow hydroxyapatite microspheres and their evaluation in osseous defects in vivo, Materials Science and Engineering: C, 60, 324-332 (2016).
29. Cestari, F., et al. Nano-Hydroxyapatite Derived from Biogenic and Bioinspired Calcium Carbonates: Synthesis and In Vitro Bioactivity. Nanomaterials, 11(2), 264 (2021).
30. Youssef, Hanaa., et al. Synthesis of Natural Nano-Hydroxyapatite from Snail Shells and Its Biological Activity: Antimicrobial, Antibiofilm, and Biocompatibility. Membranes, 12, 408 (2022).
31. Shahid Hussain., et al. In vitro bioactivity and biocompatibility behaviour of atmospheric plasma sprayed Indian clam seashell derived hydroxyapatite coating on Ti-alloy, Journal of Alloys and Compounds, 976, 173132 (2024).