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Synthesis and Characterization of Hydroxyapatite (HAp) from *Cerithidea Quoyii* Seashell by Chemical Method

Muhamad Ariffin Zahari[1], Muhammad Azib Adam[1], Nur Syuhada Amira Zulkarnain[1], Rozainita Rosley[1]*

[1] *Department of Science and Mathematics, Centre for Diploma Studies,*
Universiti Tun Hussein Onn Malaysia, Pagoh Education Hub, 84600 Panchor, Johor, MALAYSIA

*Corresponding Author: rozainita@uthm.edu.my
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Abstract

Seashells, which serve as protective and supporting exoskeletons for molluks, are typically made up of organic materials like proteins and chitin and over 95% calcium carbonate ($CaCO_3$). This study aims extract Calcium Oxide with the synthesis of Hydroxyapatite from *Cerithidea quoyii* seashell which is widely used in biomedical applications. The seashells were initially calcined at temperature 900 °C to convert calcium carbonate ($CaCO_3$) into calcium oxide (CaO). The calcined powder was then mixed with distilled water and phosphoric acid ranging from 0.3 M to 0.9 M before undergoing a 24-hour aging process. The resulting precipitates were centrifuged and recalcined at 800 °C. Characterization of the synthesized HAp was conducted using X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDX). The XRD analysis confirmed the formation of HAp through the successful conversion of $CaCO_3$ to CaO. XRD analysis confirmed the formation oh HAp through the successful conversion of $CaCO_3$ to CaO. FTIR spectra indicated the presence of phosphate (PO_4^{3-}), carbonate (CO_3^{2-}), and hydroxyl (OH^-) groups, further confirming the synthesis of HAp. SEM/ EDX analysis shows the best pH concentration in obtaining the smallest ratio of Ca/P is 7.0 M. EDX analysis shows the best ratio of Ca/P atomic percentage in HAp at 9.0 M concentration phosphoric acid with 5.06.

1. Introduction

The inorganic component of human bone; hydroxyapatite (HAp), is made up of calcium and phosphorus ions. Although HAp has the chemical formula $Ca_5(PO_4)_3(OH)$, it is typically expressed as $Ca_{10}(PO_4)_6(OH)_2$, indicating that the HAp unit cell is made up of two units [1]. The main benefit of employing natural sources of calcium carbonate ($CaCO_3$) for bone healing is its distinct shape [2]. These materials' large surface areas promote cellular activity and increase the implant's solubility. Inedible by-products from slaughterhouses, catering services, bakeries, and food processing industries, such as bones, seafood shells, and eggshells, are often considered waste, despite being rich in calcium [3]. This leads to the loss of valuable resources and contributes to environmental pollution [4]. To solve these problems these items can be utilized to extract HAp. Globally, different initiatives have been taken to aid waste recycling and management. Many researchers have devised effective protocols for the conversion of solid waste for the conversion of solid waste into energy, animal food and fertilizers. This novel

approach to nHAp synthesis not only offers a sustainable alternative to conventional methods but also presents a unique opportunity to develop an efficient and environmentally responsible biomaterial for bone tissue regeneration. In addition, natural sources offer desirable characteristics in the synthesized HAp, making it highly recommended for biomedical applications. The objective of this research was to extract calcium oxide (CaO) from *Cerithidea quoyii* seashell, the calcination process converts calcium carbonate to calcium oxide by releasing carbon dioxide (CO_2) as shown in equation (1).

$$CaCO_3 \rightarrow CaO + CO_2 \quad (1)$$

Then, calcium oxide was dissolved in distilled water producing calcium hydroxide as shown in equation (2).

$$CaO + H_2O \rightarrow (CaOH)_2 \quad (2)$$

Prior to, phosphoric acid, H_3PO_4 was added into the calcium hydroxide produced to form HAp from the calcium oxide, CaO extracted from *Cerithidea quoyii* seashell as shown in equation (3).

$$10(CaOH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O \quad (3)$$

Lastly, characterize the HAp with different concentration of phosphoric acid using X-ray diffraction (XRD), Fourier Transform Infrared Spectrophotometer (FTIR) and Scanning Electron Microscopy with Energy Dispersive (SEM/EDX).

2. Materials and Method

This section discussed the materials and methodology used to produce hydroxyapatite.

2.1 Materials

Seashells, obtained from a local seafood shop in Muar, Johor served as the precursor material for synthesizing and characterizing HAp. Orthophosphoric acid (H_3PO_4) from R&M Chemicals was used as a chemical reagent in the synthesis of HAp from seashell waste. Fig. 1 shows that flow chart of synthesis of hydroxyapatite (HAp).

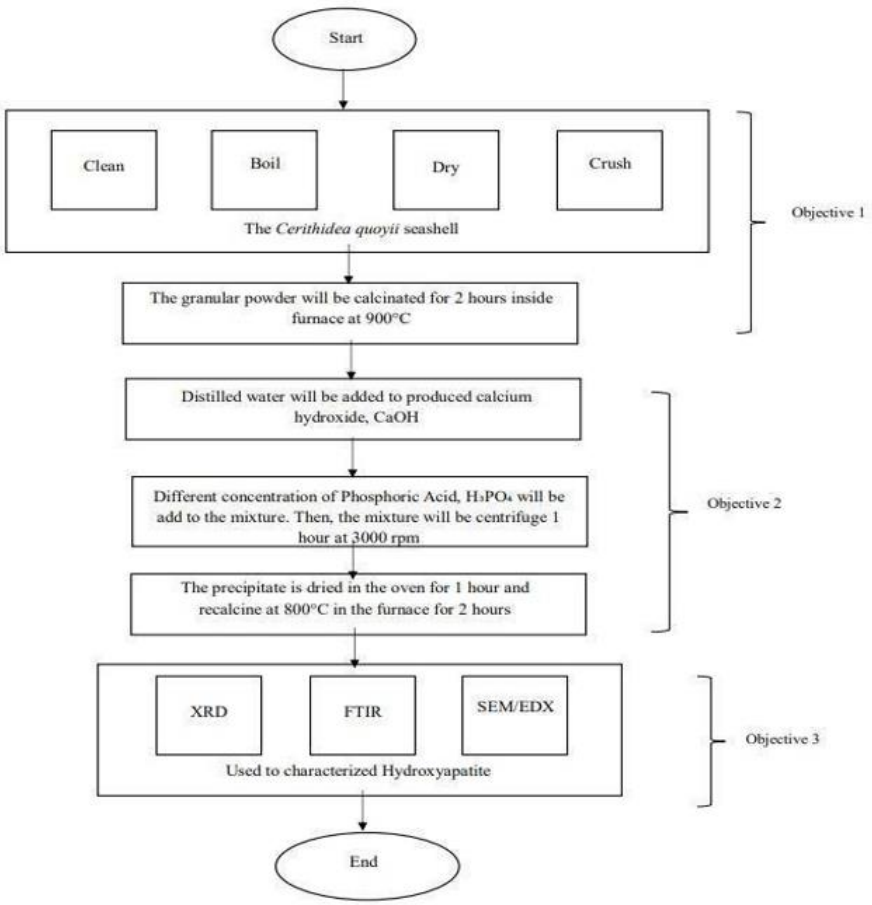

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Fig. 1 *Flow chart of synthesis of HAp*

2.2 Preparation of HAp Powder

Collected seashells were washed with soap and water to remove any impurities. Subsequently, the shells were boiled in hot water at 100 °C using a hotplate stirrer for 1 hour (refer Fig. 2). Then, the shells were dried in an oven at 110 °C for 1 hour. After drying, the shells were allowed to cool before the shell is crushed using a stone mortar until it becomes granular powder. In order to minimize oxidation during characterization, measurements were taken promptly to avoid prolonged exposure to air, which could potentially alter the observed properties.

Fig. 2 *Seashells boil at 110 °C for 1 hour*

2.3 Synthesis of HAp Powder

The 400 g shell powder was calcined at 900 °C to convert the $CaCO_3$ in the seashell into CaO. The calcination process converts $CaCO_3$ to CaO by releasing carbon dioxide as shown in equation. Then, calcium oxide was dissolved in 100 mL distilled water producing calcium hydroxide as shown in equation Prior to, phosphoric acid, H_3PO_4 was added into the calcium hydroxide produced to form HAp as shown in equation. The mixture solution was left for 24 hours to allow precipitation to occur. The process of synthesis of HAp starts with the shell powder being divided into four parts and then dissolved using distilled water to produce a calcium hydroxide solution. 3.0 M, 5.0 M, 7.0 M and 9.0 M phosphoric acid were added drop wise into each different mixture until the pH value of the solution is at 8-9 using a pH meter. After that, the mixture was centrifuged at 3000 rpm for 1 hour to separate water and precipitate. The precipitate then was rinsed and filtered with distilled water before drying in the oven at 100 °C for 1 hour. Lastly, the dried precipitate was recalcined at 800 °C in the furnace for 2 hours. 100 g of seashell powder can produce 70 g of HAp. The following calculation is used to dilute phosphoric acid for chemical methods.

2.4 Characterization of HAp

XRD, FTIR, and SEM/EDX were employed to characterize HAp after calcination. HAp produced from the previous process were characterized using a Scanning Electron Microscope with Energy Dispersive (SEM/EDX) to determine the surface morphology of the synthesized HAp, Fourier-Transform Infrared (FTIR) spectrometer was used to determine the characterization functional group of the synthesized hydroxyapatite and X-ray diffractometer (XRD) were used to analyze the crystal structure and composition of materials.

3. Result and Discussion

The results obtained from XRD, FTIR, and SEM/EDX analysis were discussed as in the following sub-topics.

3.1 X-ray Diffraction Analysis

Fig. 3 shows the X-ray diffractogram of four samples of HAp using different concentrations of phosphoric acid (3.0 M, 5.0 M, 7.0 M and 9.0 M). As the concentration increased, the intensity of $Ca(CO)_3$ peak decrease and at 9.0 M no peak of $Ca(CO)_3$ was observed. This phenomenon is due to $Ca(CO)_3$ being fully converted to CaO to produce HAp. As $Ca(CO)_3$ decomposes, the CaO peak increases. This analysis showed that as the calcination concentration

increased, the peak intensity of HAp also increased which indicates the improvement in the crystallinity of the samples. Excessive concentration, however, might encourage quick crystal growth, which could result in internal stress or flaws and compromise the stability of the HAp structure. High crystallinity with controlled crystal growth, thus exhibits the most balanced condition according to the XRD results, making it ideal for the synthesis of structurally stable and bioactive hydroxyapatite [4]. Fig. 3 illustrates the shift in peak intensities in the XRD pattern reflecting the transformation of seashells from $CaCO_3$ to CaO and HAp.

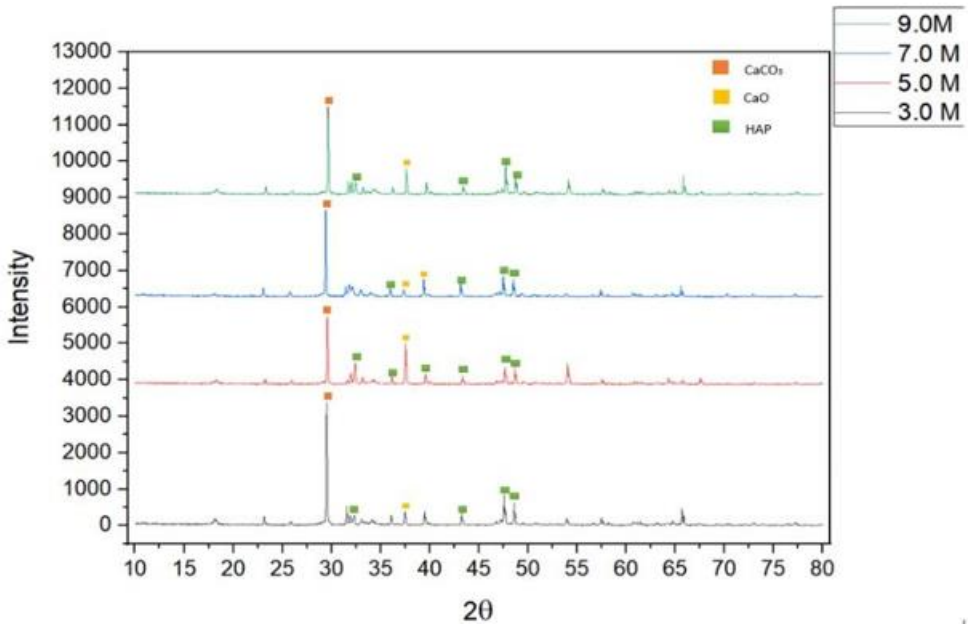

Fig. 3 *XRD pattern of calcined seashells at different concentrations of H_3PO_4*

3.2 Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Fig. 4 shows the formation of HAp was confirmed by the FTIR spectra of HAp synthesized at various molar concentrations (3.0 M, 5.0 M, 7.0 M, and 9.0 M), which showed distinct absorption bands corresponding to important functional groups. Asymmetric stretching vibrations of phosphate (PO_4^{3-}) groups, a characteristic of the HAp structure, were identified as the cause of prominent peaks in the 960–1100 cm^{-1} region [5]. Furthermore, the presence of carbonate (CO_3^{2-}) groups was indicated by absorption bands at about 870 cm^{-1} and 1410–1450 cm^{-1}. This suggests that carbonate was partially substituted into phosphate sites, which is a common observation in carbonated HAp (CHAp) formed under ambient conditions [6].

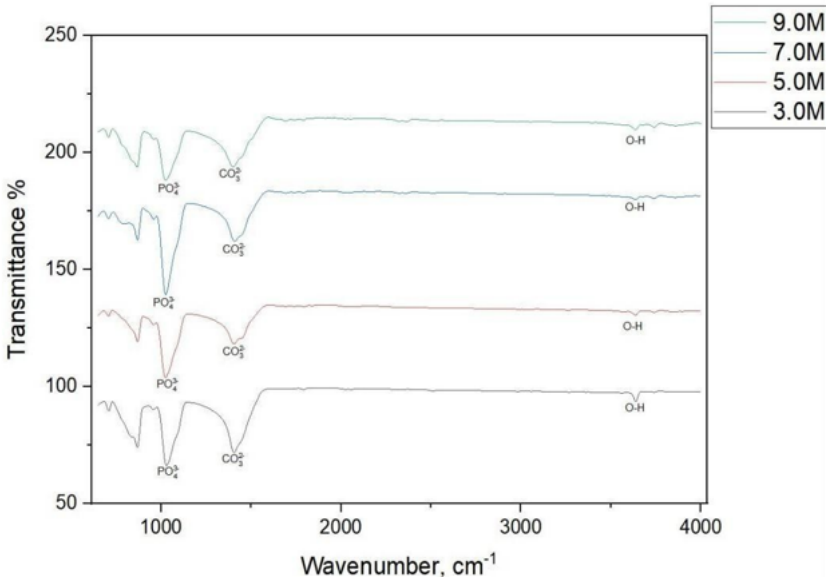

Fig. 4 *FTIR spectrum of the calcined seashells at different concentrations of H_3PO_4*

A wide O–H stretching band at about 3400 cm^{-1} provided additional evidence that hydroxyl groups were incorporated into the crystal lattice. The 9.0 M HAp showed the strongest and most distinct PO_4^{3-} and O–H peaks

out of all the samples, suggesting improved crystallinity, decreased carbonate substitution, and superior phase purity. The broader and less resolved bands seen in samples synthesized at lower molarities, on the other hand, suggested incomplete crystallization or amorphous phases. These findings unequivocally show that the ideal concentration for creating high-grade, well- crystallized hydroxyapatite with few impurities and structural flaws is 9.0 M.

3.3 Scanning Electron Microscope (SEM) Analysis

Fig. 5 shows the surface morphology, particle size, and agglomeration behavior of the HAp samples synthesized at varying precursor concentrations at 3.0 M, 5.0 M, 7.0 M, and 9.0 M varied significantly, according to the SEM analysis. Insufficient precursor availability resulted in strong agglomeration and incomplete crystal growth, as evidenced by the particles' dense aggregation at 3.0 M with irregular, poorly defined grain boundaries. A somewhat better morphology with more distinct particles and moderate porosity was observed when the concentration was increased to 5.0 M, indicating improved nucleation and crystal growth. With well- developed, plate-like and rod-like crystals, clear particle boundaries, and a uniform distribution, the 7.0 M sample had the best morphology. This structure offers a higher surface area and better bioactivity, which are critical for biomedical applications such as bone tissue engineering. However, at 9.0 M, excessive supersaturation resulted in larger, irregular crystal clusters and more uneven surfaces, indicating uncontrolled crystal growth and reduced porosity. Overall, the 9.0 M concentration produced the most favorable HAp morphology, balancing crystal growth and particle separation, which aligns with previous findings that moderate precursor concentrations facilitate optimal HAp structure and bioactivity [4].

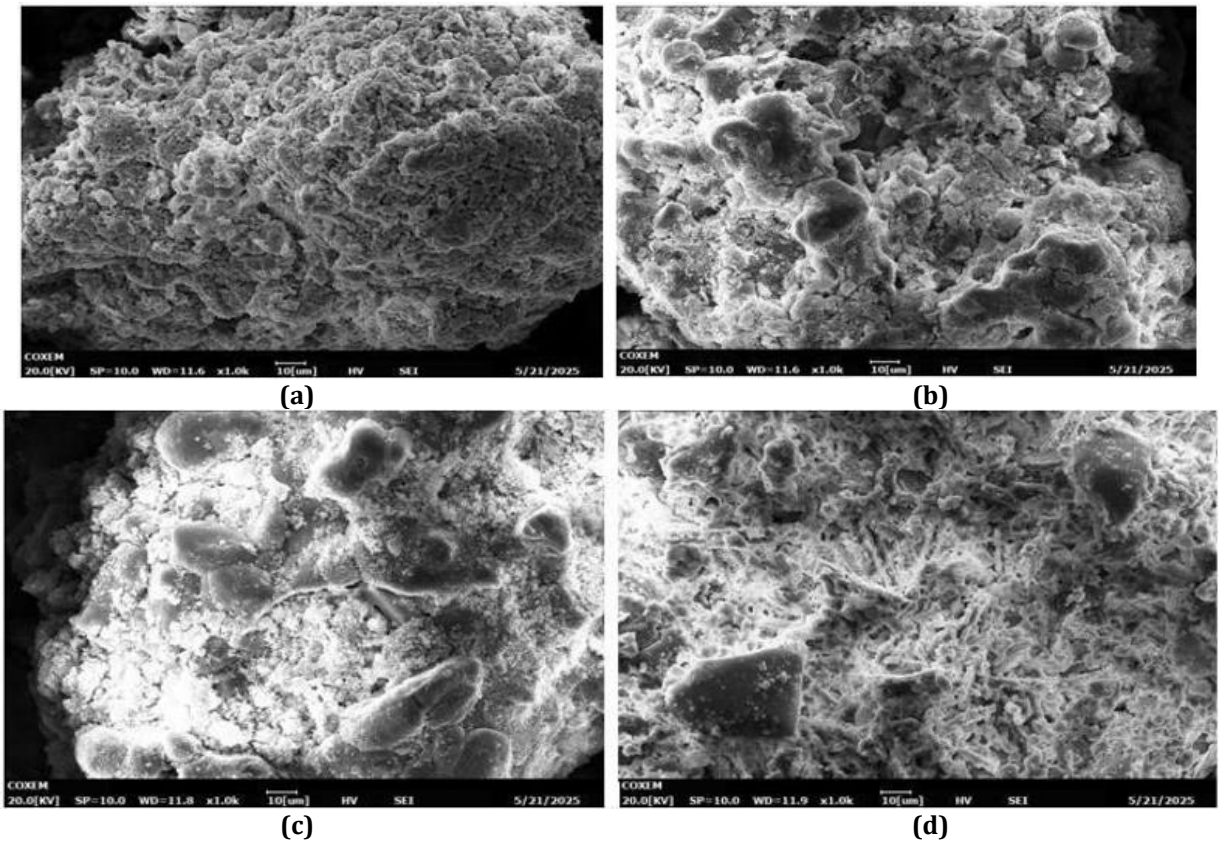

Fig. 5 *Morphology of the calcined seashells at different concentrations of H_3PO_4 (a) 3.0M; (b) 5.0M; (c) 7.0M (d) 9.0M*

3.4 Energy-dispersive X-ray spectroscopy (EDX) Analysis

All samples have Ca/P ratios higher than the optimal stoichiometric value of 1.67 for Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), according to the EDX analysis [7]. This suggests a calcium-rich composition that was probably brought on by an excess of calcium precursor or by incomplete phosphate incorporation during synthesis. The ratio of the 3.0 M is 9.34 and 5.0 M is 8.95 samples but still above the ideal. The 7.0 M concentration had balanced crystal growth, and a Ca/P ratio is 8.20. Although the 9.0 M sample has a lower ratio (5.06). This is in line with research that shows that to approach the stoichiometric Ca/P ratio and produce high-quality hydroxyapatite,

controlled synthesis conditions and optimized precursor ratios are crucial [8]. Fig. 6 shows Ca/P ratio of Energy dispersive X-ray spectroscopy (EDX) analysis of H_3PO_4.

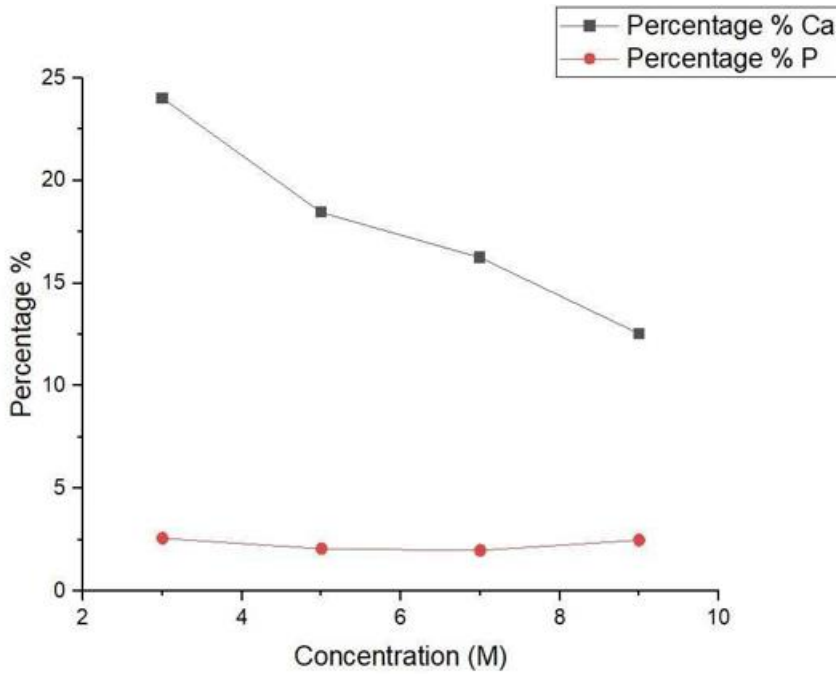

Fig. 6 *Ca/P ratio of Energy-dispersive X-ray spectroscopy (EDX) analysis of H_3PO_4*

4. Conclusion

In this study, CaO was successfully extracted from *Cerithidea quoyii* seashells, a marine biowaste material. The extraction process highlights the potential of utilizing natural calcium-rich sources as an alternative to synthetic or commercial materials, contributing to waste reduction and sustainability in material synthesis. HAp was synthesized using the extracted CaO and varying concentrations of phosphoric acid. CHAp was successfully synthesized from CaO, a precursor derived from seashell waste at concentrations of 3.0 M, 5.0 M, 7.0 M, and 9.0 M. The synthesized HAp was subsequently characterized using FTIR spectroscopy, XRD, SEM, and EDX spectroscopy. The FTIR analysis demonstrated the functional group of HAp powder which contains phosphate, carbonate, and hydroxyl groups. The particle size of HAp increases as the concentration increases as shown by SEM analysis. 7.0 M produced uniform, well-formed crystals with less agglomeration than the others, according to SEM images. All samples had Ca/P ratios above the optimal value of 1.67, according to EDX analysis. However, the 9.0 M sample had a better ratio than the others. Therefore, increasing the phosphorus concentration during the HAp synthesis process may result in higher phosphate content in the finished product. It can be concluded that seashell waste can be a sustainable alternative for HAp production. To offer financial advantages by reducing the cost of HAp production, this also serves as an excellent waste management approach to help reduce environmental issues.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design****: Muhamad Ariffin Zahari, Muhammad Azib Adam, Nur Syuhada Amira Zulkarnain, Rozainita Rosley;* ***data collection****: Muhamad Ariffin Zahari, Muhammad Azib Adam, Nur Syuhada Amira Zulkarnain, Rozainita Rosley;* ***analysis and interpretation of results****: Muhamad Ariffin Zahari, Rozainita Rosley;* ***draft manuscript preparation****: Muhamad Ariffin Zahari, Muhammad Azib Adam, Nur Syuhada Amira Zulkarnain, Rozainita Rosley. All authors reviewed the results and approved the final version of the manuscript.*

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