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Synthesis and Characterization of Hydroxyapatite from Snail Shells via Hydrothermal Method

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Abstract: *The need for the use of a biocompatible, biodegradable, non-immunogenic and cost effective materials as implants in or on the human body has led researchers to investigate the use of biomaterials from natural sources to generate hydroxyapatite. This has led to the synthesis of hydroxyapatite from snail shells through hydrothermal method. X-ray diffraction (XRD) and Fourier Transform Infra-red (FTIR) tests were carried out on the synthesized samples. XRD test showed that the phase and bond were similar to that of commercial hydroxyapatite. Diffraction measurements of raw snail shell powder showed the highest peak at 2θ =32.50^0, while sintering at 900 ^{0}C. FTIR revealed the phosphate and hydroxyl functional groups as constituent groups of hydroxyapatite. The characterization analysis shows similarities between the standard hydroxyapatite and the ones made from snail shells. The hydroxyapatite obtained from snail shells have great potentials has replacements for synthetic ones. Using these shells would also improve waste management thereby promoting a sustainable development goal (SDG) in terms of promoting a healthy living and a good environment.*

Key words: *Snail shell; Hydroxyapatite; Hydrothermal; Characterization*

Introduction

Snail meat is consumed because it is high in protein and low in fat. The high level of consumption of snail meat, has led to a high level of disposal of its shells which has become waste on land and water. Snail shells are used in animal feed because of its high calcium content and as accessories in bracelets, necklaces, and wall ornaments or aquariums (Sundalian, 2022). These biomaterials (shells) can be explored and put into more useful needs. Based on the recent studies on the development of hydroxyapatite from biomaterials, it necessary to determine the potential value of the benefits of snail shell waste in the health world. The use of biomaterials can be traced back to several thousand years of the Aztecs, Chinese and Roman were they used gold in dentistry, while wood, glass, Ivory, and some other materials were used as implants for treating long bone fracture (Oladele *et al.*, 2018).

Recently, natural materials such as corals, fish bone, eggshells, snail shell, etc., have been selected as sources for the synthesis of hydroxyapatite (Zhou *et al.*, 2016) due to their advantage of biological origin as well as recycling of biowaste. Among the several natural materials, snail shells are plentiful in nature and it is a composite material consisting of calcium carbonate and organic matter (Kumar *et al.*, 2015). The matured snail shells are mainly composed of 95–99.9 % calcium carbonate ($CaCO_3$) with some dry matter, crude protein, crude fibre, ash, nitrogen free extract and other trace or micronutrients (Nayak & Misra, 2019), (Samant *et al.*, 2017). The use of this waste as a calcium source for the synthesis of hydroxyapatite can eradicate or reduce to the bearest minimum, the amount of waste disposed and also reduce the costs from the requirement of using expensive and high purity calcium reagents to synthesize hydroxyapatite. Hydroxyapatite (HA) is a calcium phosphate ceramics compound which have been applied successfully in orthopedic, dentistry and maxillofacial surgery. Calcium compounds, calcium carbonate ($CaCO_3$) and calcium oxide (CaO), derived from natural sources, such as coral, seashell, animal bone and eggshell, can be used to synthesize HA (Sutthi *et al.*, 2017). The structure of HA which is similar to the structure of human bone is the main criteria in the creation and innovation of future synthetic bone (Zulhasif *et al.*, 2016).

Hydroxyapatite can be obtained from synthetic and natural sources. One of the basic method of producing HA synthetically is precipitation method from reagent grade chemicals (Ekren *et al.*, 2016). Hydroxyapatite of natural origin differs from the synthetic ones because it contains carbonate groups and usually small magnesium and sodium mixtures as well as traces of other minerals. The Ca/P ratio of this material is usually higher than that in the stoichiometric compound. These differences make the hydroxyapatite of natural origin more suitable for medical applications (Brzezi *et al.*, 2015). Hydroxyapatite is the major inorganic component of bone and teeth (Kumar *et al.*, 2015) and has been widely used in orthopedics and dental implants because it forms of strong bond with the hard tissue (Singh & Purohit, 2011). The inorganic phase of the bone is composed of calcium phosphates (CaP), predominantly in the form of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, corresponding to 65 %-70 %, and bone water with 5 % - 8 %. The organic phase is mainly in the form of collagen, a large fibrous protein, responsible for the elastic resistance of bone (Gomes *et al.*, 2019). A significant reason for the medical uses of hydroxyapatite is its high bioactivity. It is commonly used as a filler to replace bone amputated as a result of accidents, infections and diseases. It can be used as a substrate for artificial bone grafts (bone ingrowth). Hydroxyapatite can be used to create new bone materials that directly connect to natural bone surfaces without causing inflammation, reaction or resistance. Moreover its chemical structure is close to that of natural bones and teeth (Leelatawonchai & Laonapakul, 2014). This research work will therefore be aimed at synthesizing hydroxyapatite from snail shells via hydrothermal method.

Research Methodology

Synthesis via hydrothermal method

Hydrothermal method of hydroxyapatite synthesis is a high-pressure, high-temperature process used to transform slurries into a crystalline phase. The particle size is approximately 1mm but can be produced in needle-like form with lengths less than 200 nm. Hydroxyapatite particles are generated from the reaction of calcium carbonate and

diammonium hydrogen phosphate at recrystallization temperatures of 200–300 ^{0}C and pressures around 1–2 kbar (Fox *et al.*, 2012).

The snail shells were collected and cleaned with distilled water. They were soaked for some days to remove the impurities in the shells and sun-dried afterwards. The shells were then pounded into smaller pieces using a wooden mortar and pestle. They were then grounded into powders with a grinding machine. The snail shell powder ($CaCO_3$) was filtered using 200 mesh sieve. 200 g of $CaCO_3$ powder was dissolved in 600 mL of 2 M HNO_3 at 50 ^{0}C to convert it to $Ca(NO_3)_2$. The resulting solution was diluted with distilled water. The solution was filtered and added to diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) solution and oven-heated at 40 ^{0}C for 30 min. Precipitates were formed after 24 h and the solution was filtered to get the powder and washed with distilled water. This was oven-dried and heated to 900 ^{0}C for 1 h to obtain HA. The following reactions occurred (Zuliantoni *et al.*, 2022):

$$CaCO_3 + 2HNO_3 \rightarrow Ca(NO_3)_2 + CO_2 + H_2O \quad (1)$$

$$Ca(NO_3)_2 + 2NH_4NO_3 \rightarrow Ca(OH)_2 + 2NH_4NO_3 \quad (2)$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \quad (3)$$

The reaction between $CaCO_3$ and $(NH_4)_2HPO_4$ and water produces Hydroxyapatite (HA), $(NH_4)_2CO_3$ and H_2CO_3 (Zuliantoni *et al.*, 2022):

$$10CaCO_3 + 6(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)_2CO_3 + 4CO_2 + H_3O \quad (4)$$

Evolution of CO_2 gas occurred during the sintering process while reacting to the nitric acid (HNO_3). Carbonate apatite are formed when HA reacts with CO_2 in the air. Snail shell HA powder were characterized using XRD instrument with CuKα radiation (λ = 1,5406). The XRD scan were performed at 2θ from 10^0 to 60^0 with scan step of 2^0/minute. The XRD pattern of HA sample has many peaks in the range of 20^0-60^0. Most data required for the analysis are between this range. Peak occurred in the range of 26^0-32.50^0 to get the crystal size and the cell parameter. The XRD measurements were operated at 35 mA and 40 kV. The functional groups in the samples were identified using Fourier Transform Infra-red (FTIR) analysis and the spectra recorded in the range of 400–4000 cm^{-1}.

Results and Discussion

The XRD pattern results are presented in Fig. 1. First, the calcium carbonate of the snail shell powder was heated to 100 ^{0}C for some minutes and was left for 24 h then filtered afterwards. The $CaCO_3$ compound formed was an unstable phase polymorph, with varying width and intensity, according to literature. There were no other phases occurring in this condition. The hydroxyapatite XRD pattern with sharper peak showed carbonate values of C. This is due to the exchange of carbon and oxygen isotopes between carbonate and CO_2. The increase in the heating temperature is accompanied by the decrease in carbonate content. The XRD pattern of snail shell powder (Fig. 1), calcined at 900 ^{0}C shows the crystal phase with dense peak, higher intensity and sharp resolution. The higher and longer the temperature, the sharper the intensity (crystallinity increases) in the diffraction image. XRD pattern of snail shells calcined at 900 ^{0}C for 1 h shows the characteristics of high intensity and sharp peaks of CaO (Zuliantoni *et al.*, 2022). At a

temperature of 631–765 ^{0}C, decomposition of $CaCO_3$ occurs. At calcination temperatures above 800 ^{0}C, $CaCO_3$ turns into CaO. However, in all samples, the pattern indicated the presence of highly crystalline hydroxyapatite with major peaks dominant at 26^0, 31.5^0, 32.5^0, 33.5^0, and 47.5^0. This pattern is in good agreement with JCPDS (Joint on Powder Diffraction Standard) reference number (00-009-0432) for hydroxyapatite. No characteristic peaks of impurities, such as calcium hydroxide and calcium phosphates were observed. This implies that the HA prepared are pure.

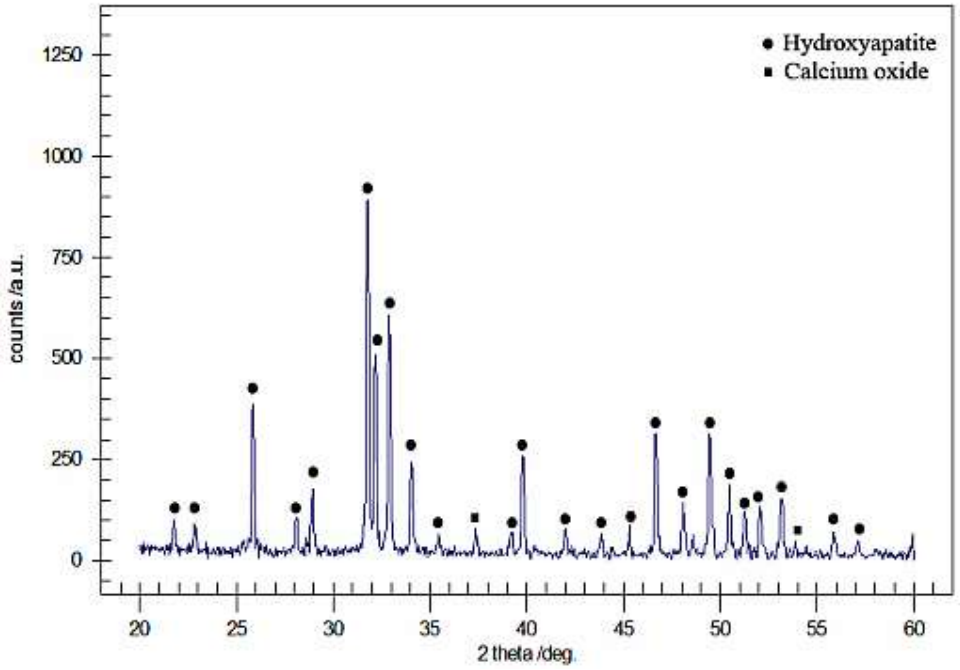

Fig. 1. The XRD pattern of snail shell at temperature of 9000C.

Figure 2 shows the FTIR test on HA powder synthesized through the hydrothermal method. From the test results, it can be seen that in Fig. 2, HA sintered at 900 ^{0}C shows O–H bonds stretching at the wave peak of the 3457 cm^{-1}. At the FTIR wave peak, the C–O and O–H bonds indicate the formation of CaO and $Ca(OH)_2$ on heated snail shells. After the chemical reaction and heating process were carried out, the C–O and O–H bonds disappeared from the sample, the peak then shifted to CO_3^{2-}. This is caused by the transformation of CaO and $Ca(OH)_2$ compounds into HA. As the temperature increases, the peaks of phosphate group becomes more apparent at 650 cm^{-1} and 1090 cm^{-1} (Kolawole *et al.*, 2020). The main evidence for HA formation is the occurrence of the phosphate wave peak at 1090 cm^{-1}. The wave peaks at 1744 and 1550 cm^{-1} are the signs of HA formation which contain the carbonate groups (CO_3^{2-}) due to the organic carbon used in the synthesis.

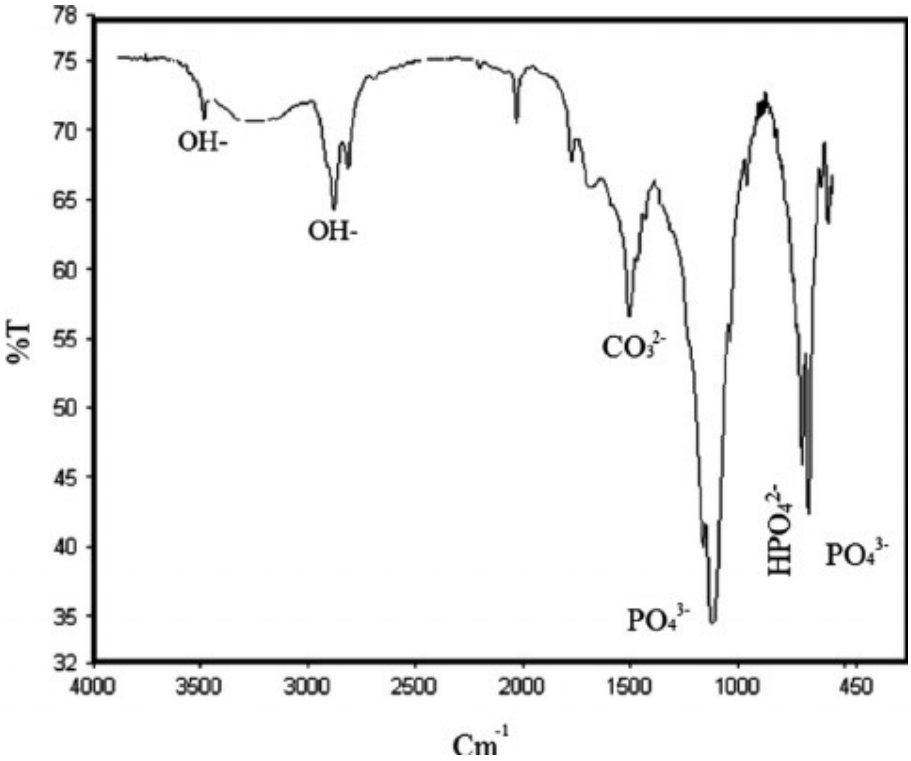

Figure 2. FTIR Spectra of synthesized Hydroxyapatite Powder.

Conclusion

The HA powder has been successfully synthesized from the reaction of calcium carbonate with phosphate hydrogen diammonium through hydrothermal reaction, which is a simple and cost effective technique. The crushed snail shells were sintered and calcined at 900 ^{0}C. The samples were characterized using the XRD and FTIR analysis, revealing the carbonate calcium in the phase while transforming to HA. These tests showed that hydroxyapatite were synthesized from the snail shells. This is revealed by the measurement of calcinated snail shell powder diffraction showing the highest peak at 2θ = 32.50^0 at 900 ^{0}C. The FTIR analysis revealed that hydroxyapatite has phosphate group (PO_4^{3-}), hydroxyl (OH^-) and carbonate group (CO_3^{2-}) left.

Hence, snail shells can be considered an alternative calcium source for the preparation of hydroxyapatite powder. The HA produced has great potential and suitability for use as biomedical materials.

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