

# Production of Hydroxyapatite Recovery from Seashell Waste for Biomedical Application

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## Abstract

The extraction and purification of hydroxyapatite (HAp) from natural sources has been studied for several years to improve its quality and standard. Nowadays, it is used in various applications, especially for biomedical applications because of its bioresorbability, biocompatibility, and bioactive properties. Seashells contain a high composition of calcium carbonate which could be a promising source to extract HAp. In fact, the calcination process and wet slurry precipitation technique are the appropriate and simplest methods to produce hydroxyapatite from seashells. In this work, the effect of the reaction time of phosphate source, Potassium Dihydrogen Phosphate,  $\text{KH}_2\text{PO}_4$ , and calcium oxide, CaO on the production of hydroxyapatite was investigated. Production of HAp from seashell wastes can contribute significantly towards natural resource management, health care, waste utilization, and religious issues amongst others. Based on the results from X-ray diffraction (XRD), Fourier transform infrared spectrophotometer (FTIR), and scanning electron microscopy with energy dispersive (SEM /EDX), it is shown that HAp produced at 4 hours-reaction time is more crystalline compared to HAp produced at 2 hours and 3 hours-reaction time. It can be said that a crucial factor in the synthesis of HAp is reaction time.

## 1. Introduction

The aim of this project is to produce hydroxyapatite (HAp) recovery from seashell waste as seashell contains a high concentration of calcium carbonate which is the main compound to HAp. HAp is a calcium phosphate mineral with the chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Calcium phosphates, CaP are a group of minerals consisting of calcium ions,  $\text{Ca}^{2+}$ , orthophosphates,  $\text{PO}_4^{3-}$ , metaphosphates,  $\text{P}_2\text{O}_7^{4-}$ , hydrogen, H<sup>+</sup> and hydroxide, H<sup>-</sup> ions. HAp is one of the most versatile classes of biomaterials available today. In fact, the transformation of seashell waste into hydroxyapatite is extensively used for biomedical applications, such as pharmaceutical chemicals, bone replacement, regeneration, and dental restoration materials because of its biocompatibility, bioresorbable, and bioactive properties.

Seashell waste is used in this study due to the insane and reachable of seashell wastes worldwide. The development of HAp using seashell waste could decrease production expenses as well as the HAp price in the market. According to [1], HAp from seashells can be extracted using calcination and wet chemical precipitation methods. Thus, this study focuses on the production of HAp from seashell wastes by varying the reaction time. Specifically, the reaction times investigated are 2 hours, 3 hours, and 4 hours. The resulting samples undergo

characterization using X-ray diffraction (XRD), Fourier transform infrared spectrophotometer (FTIR), and scanning electron microscopy with energy dispersive (SEM /EDX).

## 2. Materials and Methods

### 2.1 Materials

The sample seashells were purchased online from Shopee. Potassium Dihydrogen Phosphate,  $\text{KH}_2\text{PO}_4$  were used as a phosphate source for HAp production. Distilled water in this experiment was directly obtained from the laboratory in the University Tun Hussein Onn, Malaysia.

### 2.2 Methods

#### 2.2.1 Production of HAp

The seashells were cleaned and boiled to remove impurities and organic materials that are attached to them. The cleaned seashells were dried at room temperature. Then, seashells were grinded into powder form using a ball mill.

#### 2.2.2 Calcination Process

The seashell powders were calcinated at 800 °C for 3 hours using a furnace to obtain 10 g of calcium oxide, CaO.

#### 2.2.3 Wet Slurry Precipitation Technique

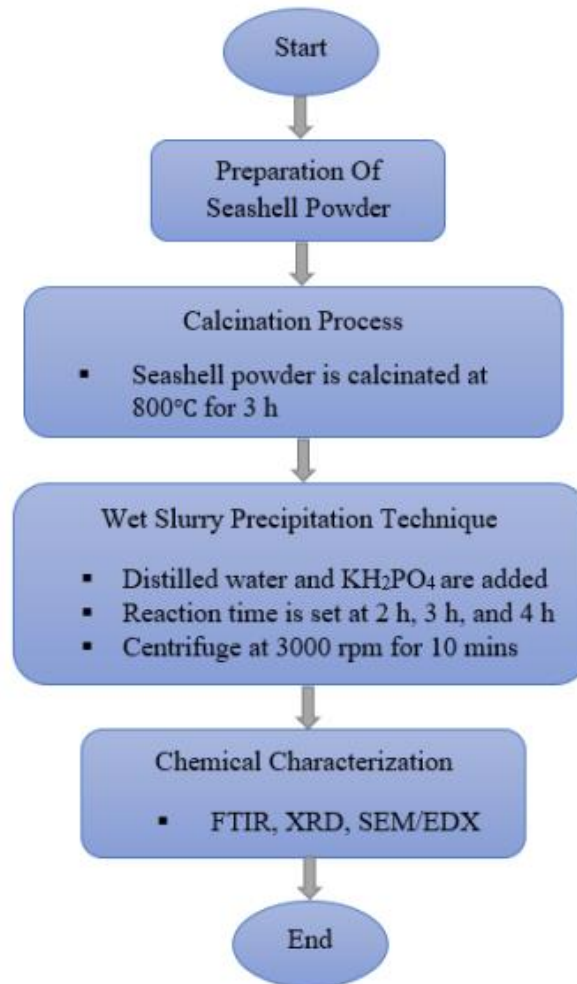
Afterward, the wet slurry precipitation technique was carried out to produce HAp. Three samples of 10 g calcium oxide, CaO were dissolved in 100 ml of distilled water to obtain  $\text{Ca}(\text{OH})_2$  solution. Then, 5 ml of potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$  were dropped into calcium hydroxide,  $\text{Ca}(\text{OH})_2$  to produce hydroxyapatite,  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$  solution. Mixtures of samples were reacted for 2, 3 and 4 hours respectively. The mixtures were then centrifuged at 3000 rpm for 10 minutes to obtain the milky white precipitate. The milky white precipitate is indicated as hydroxyapatite. Lastly, the hydroxyapatite was dried at room temperature for 24 hours.



(1) shows that the seashell powder which contains calcium carbonate,  $\text{CaCO}_3$  is calcinated to produce carbon dioxide and calcium oxide, (2) shows that calcium oxide, CaO formed were reacted with distilled water to produce calcium hydroxide,  $\text{Ca}(\text{OH})_2$  while (3) shows the calcium hydroxide,  $\text{Ca}(\text{OH})_2$  produced were reacted with the phosphate source, which is potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$  to produce hydroxyapatite,  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ .

#### 2.2.4 Chemical Characteristics

The phases of the HAp powders were analysed using XRD which provides detailed information about the phase composition and degree of crystallinity of HAp, FTIR to determine the chemical bond and the functional group present in HAp produced and SEM/EDX was used for morphological study of nanoparticles of HAp. Fig. 1 shows the overall process of the carried-out experiment starting from preparation of the seashell powder to the chemical characterization.

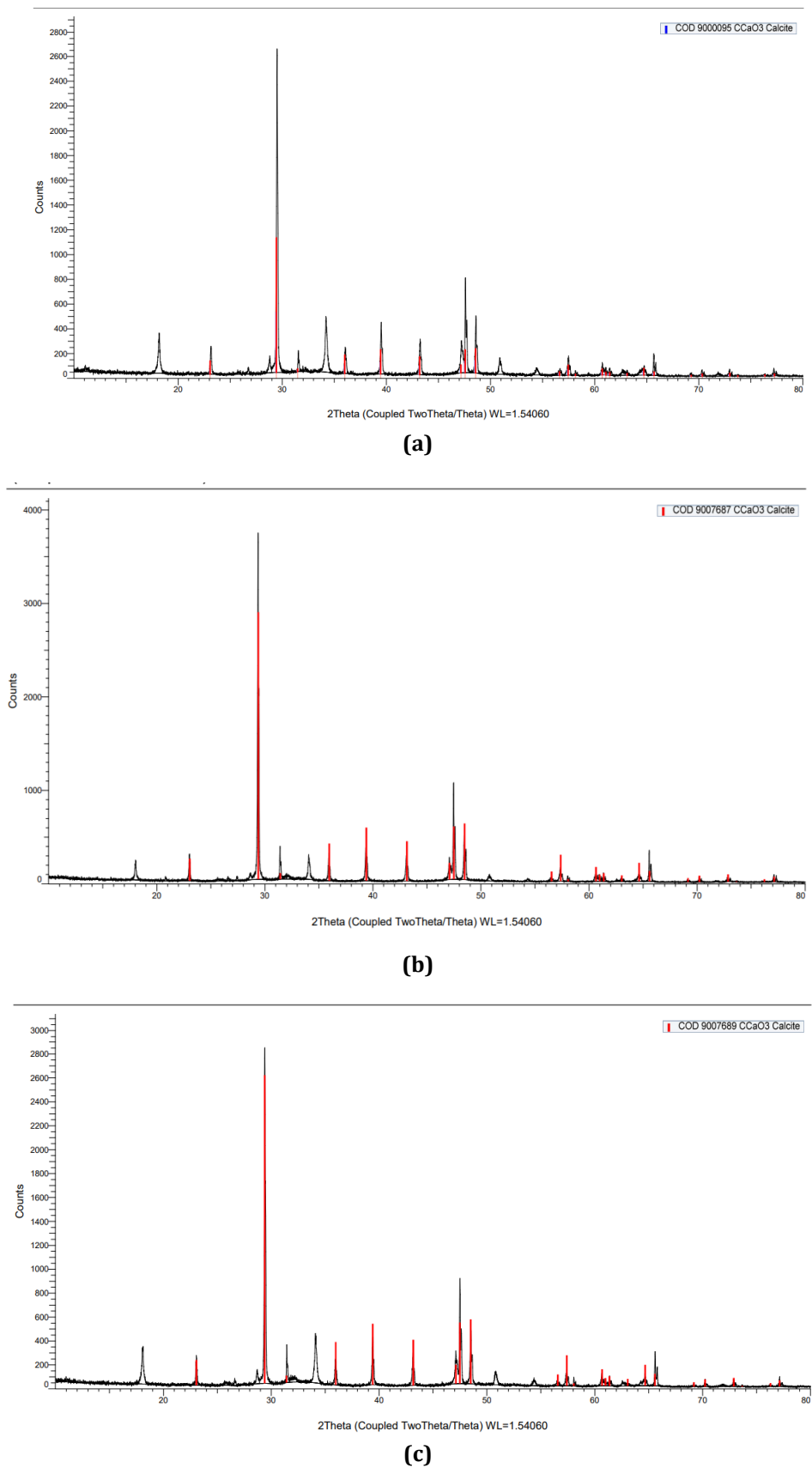


**Fig. 1** The overall process of the experiment

### 3. Results and Discussion

#### 3.1 XRD Analysis for the Production of HAp

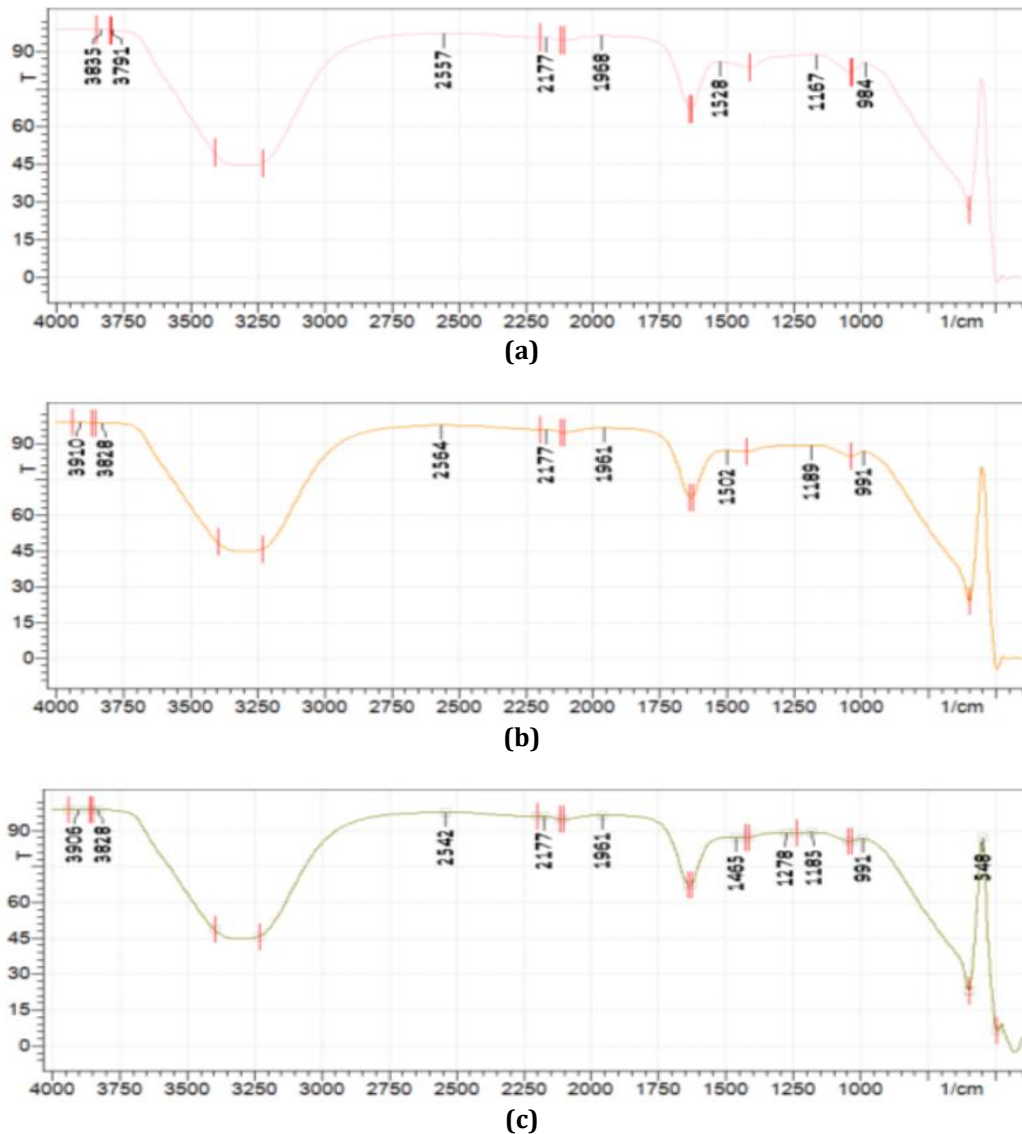
The phases of the HAp powders are analyzed using XRD which provides detailed information about the phase composition and degree of crystallinity of HAp. The XRD patterns of HAp generated at various reaction periods are shown in Fig. 2 (a)-(c). In comparison to 3 hours and 4 hours reaction times, the HAp generated at 2 hours reaction time exhibits the largest peak, according to the XRD analysis. The broad peak showed that the sample's structure is less crystalline. As opposed to HAp produced with 3 hours and 2 hours reaction times, 4 hours reaction time HAp exhibits a higher crystallinity [2]. The crystallinity of HAp has risen due to the longer reaction time. In comparison to 2 hours and 3 hours reaction times, the peak in the XRD analysis of the hydroxyapatite formed at the 4 hours reaction time is the largest. This shows that when compared to the HAp produced at the 2 hours and 3 hours reaction times, the HAp produced at the 4 hours reaction time had the highest purity [2]. The presence of a calcite peak that matches the carbonate hydroxyapatite XRD pattern is demonstrated by the samples' 2, 3, and 4-hours reaction durations.



**Fig. 2** XRD patterns of HAp with (a) 2 hours-reaction time; (b) 3 hours-reaction time; (c) 4 hours-reaction time

### 3.2 FTIR Analysis for the Production of HAp

FTIR was used to determine the chemical bond and the functional group present in HAp. Fig. 3 (a)-(c) shows the FTIR spectra of HAp produced at 2, 3- and 4-hours reaction times. From Fig. 3, the synthesis of HAp was successful based on the existence of the main functional groups of HAp which are O-H bond. These findings demonstrated that HAp was formed even at a shorter reaction period of 2 hours since FTIR analysis is intended to detect the functional group of compounds. From the FTIR spectra of the hydroxyapatite, the functional group observed is O-H at  $3240\text{ cm}^{-1}$ . In addition, the C-O bond was observed about  $1050\text{ cm}^{-1}$ . The C-O bond is observed due to the calcium carbonate which do not decompose during calcination process. These findings demonstrated that HAp had been formed even at a shorter reaction period of 3 hours. Since common bands of carbonated HAp were found, both reaction times essentially demonstrate that it has a carbonated ingredient. We can infer from the FTIR study that carbon and oxygen are present in hydroxyapatite [3].



**Fig. 3** FTIR spectra of HAp with (a) 2 hours-reaction time; (b) 3 hours-reaction time; (c) 4 hours-reaction time

### 3.3 SEM and EDX Analysis for the Production of HAp

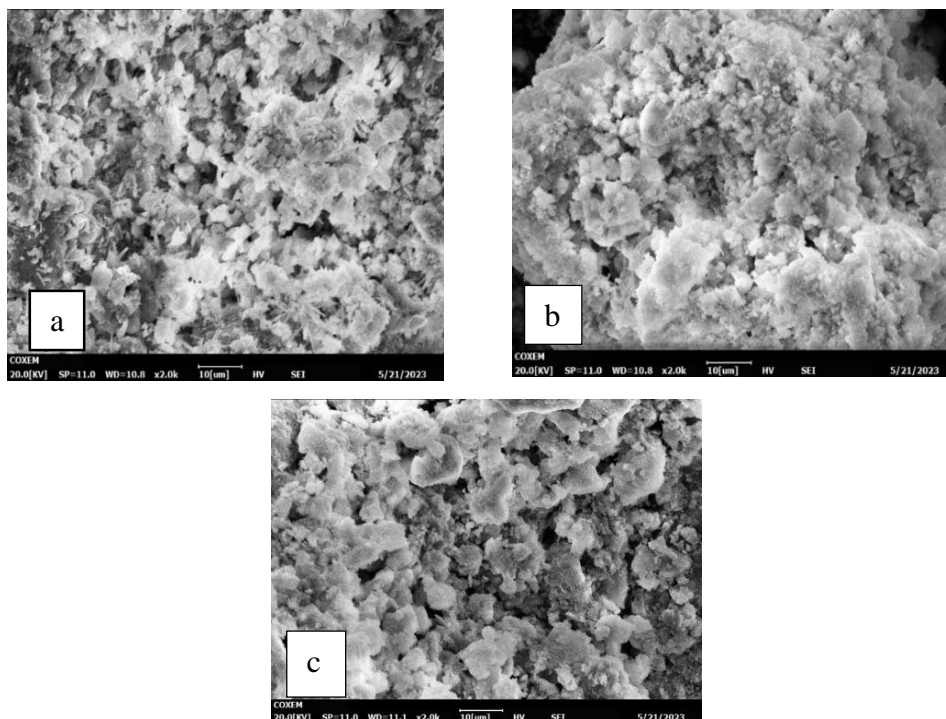
SEM /EDX was used for morphological study of nanoparticles of HAp. The surface morphologies of HAp are shown in Fig. 4 (a)-(c) through SEM analysis. By lengthening the reaction time, more areas become available for HAp particle development, creating more surfaces on which nucleate particles can adhere. HAp crystals from both settings were erratic in shape and varied in size, as shown in the SEM micrographs. Due to the uneven surface of HAp and the creation of plate-like shapes that overlapped with one another, all the synthesized HAp collected in bulky size. These HAp morphologies concurred with those found in studies by [4][5], which found that the

produced HAp has an atypical form. Compared to the HAp formed at 4 hours reaction time, 2 hours HAp contains a smaller particle. The particle size of HAp increased due to a longer reaction time. According to [6], agglomeration caused by a longer reaction time resulted in larger HAp particles. The agglomeration process is the accumulation of fine material into cohesive units, such as pellets or granules, during the processing of powders [7][8].

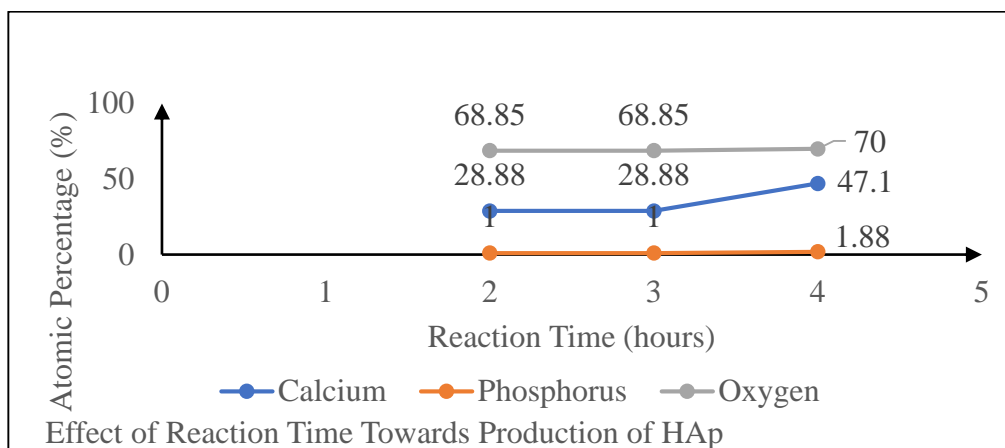
According to the results of the EDX analysis, the composition of HAp powder includes calcium, oxygen, and phosphorus. These three substances are consistent with the FTIR data shown in Fig. 4 (a)-(c) which shows the presence of phosphate groups in the powder. Potassium bands were also discovered in addition to these three principal elements. The phosphate source, potassium dihydrogen phosphate solution, is where potassium originates from. In every EDX examination, potassium has a lower atomic percentage than calcium, oxygen, and phosphorus. This happens due to enough deionized water is used to rinse out potassium content. Because of the coating applied during the SEM analysis, the aurum peak was picked up in the EDX analysis [9].

The graph demonstrating the impact of reaction time on HAp production can be seen in Fig. 5. The atomic percentage (%) also rises as time passes by hours. The calcium atomic percentage is 68.85% at 2 and 3 hours, which does not change. The calcium atomic percentage increases to 47.1% in 4 hours. At a reaction period between 2 and 3 hours, the atomic percentage of phosphorus is one. It does not change. The atomic percentage rises to 1.88% in 4 hours. The graph in Fig. 5 displays that oxygen's atomic percentage is 68.85% and stays constant. The oxygen atomic percentage rises to 70.00% after 4 hours. This data leads us to the conclusion that the atomic proportion increased as the time reaction increased. The particle size of the hydroxyapatite will similarly increase as the atomic size does [10].

Last but not least, the sample is frequently essential to coat with a thin layer of gold or gold-palladium alloy to avoid charging of the surface. Not only that, it is also used to encourage the emission of secondary electrons so that the specimen conducts evenly, and to create a homogeneous surface for analysis and imaging.



**Fig. 4** Morphology of HAp with (a) 2 hours-reaction time; (b) 3 hours-reaction time; (c) 4 hours-reaction time



**Fig. 5** The effect of reaction time on the production of HAp

#### 4. Conclusion

Overall, calcination process and wet slurry precipitation is an appropriate and suitable technique to produce hydroxyapatite, HAp from seashells waste. The calcination process can be considered as a purification process of carbonate compounds from the seashell. The precipitation method involves mixing calcium salt and phosphate solution in the appropriate molar ratio, stirring continuously, and generating colloid HAp precipitation from calcium and phosphate ions under alkaline conditions. The precursor precipitate is then dried and to produce HAP with well-crystallized and ultrafine particles. It is the simplest way to make HAP. Moreover, according to this study's XRD, FTIR, and SEM/XRD results, seashells were used to produce HAp powders for the samples with different reaction time, which is 2 hours, 3 hours, and 4 hours. It is proven by the results of SEM/EDX, XRD and FTIR analysis that 4 hours- reaction time produces more crystalline HAP than 2 hours or 3 hours-reaction time. It can be said that a crucial factor in the synthesis of HAP is reaction time.

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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:** Kang Siew Yi, Janani K Yokesvaran, Rozainita Rosley; **data collection:** Kang Siew Yi, Janani K Yokesvaran, Rozainita Rosley; **analysis and interpretation of results:** Kang Siew Yi, Janani K Yokesvaran, Rozainita Rosley; **draft manuscript preparation:** Kang Siew Yi, Janani K Yokesvaran, Rozainita Rosley. All authors reviewed the results and approved the final version of the manuscript.

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