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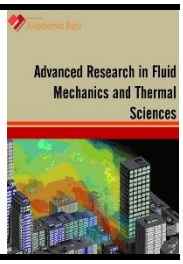

The Effect of Hydrothermal Holding Time on The Characterization of Hydroxyapatite Synthesized from Green Mussel Shells

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ABSTRACT

Hydroxyapatite is generally utilized in medical fields especially as a substitute to bone and teeth. Hydroxyapatite nanoparticles have been succesfully synthesized from green mussel shells as a source of calcium carbonate by hydrothermal method. The green mussel shells were calcined, hydrated, and undergone carbonation to form Precipitated Calcium Carbonate (PCC). The PCC of shells was then added with $(NH_4)_2HPO_4$ with the mole ratio of Ca/P = 1.67. Hydrothermal reaction was carried out at 160°C with variations of the holding time (14, 16, and 18 hrs). The formation of hydroxyapatite was characterized using XRD and SEM-EDX. The XRD patterns showed that the products were hydroxyapatite crystals. The morphology of hydroxyapatite observed using SEM showed that the crystal uniformity of hydroxyapatite. The best result was obtained at 18 hrs holding time of hydrothermal because the hydroxyapatite produced has the highest purity without any impurities phase.

1. Introduction

Hydroxyapatite (HAp) is a bioceramic compound formed from the main elements of calcium and phosphorus with the formula $Ca_{10}(PO_4)_6(OH)_2$. The development of HAp began to be applied in the medical world because of its biocompatible and bioactive nature. The use of hydroxyapatite in the

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medical world has great potential to cure various damages of human bones and teeth [1]. The development of hydroxyapatite as a bone implant material has great potential in Indonesia.

It is due to the high number of bone surgery cases. The absence of hydroxyapatite producers in the country to meet these needs has made imported hydroxyapatite increased in high value. As recorded in 2016 - 2018, Indonesia has imported more than 5.5 million tons of hydroxyapatite. The high amount of demand is directly proportional to the amount of costs that must be incurred. According to the Indonesian Central Statistics Agency, the need for bone implants reaches Rp 23 trillion in 2017 and is predicted to increase to Rp 27 trillion in 2018 [2]. Hydroxyapatite production in the future will be more economical due to the use of natural raw materials such as bovine bones, fish Scale and eggshells [2,3,4]. In addition to those three ingredients, the green mussel (P. viridis) can also be used as a raw material for making hydroxyapatite because the shell of the green mussel has a high Calcium Carbonate content [2].

Hamester *et al.,* compared the chemical composition of oyster shells, green mussel shells, and commercial $CaCO_3$. Table 1 shows the results obtained in the study. Based on Table 1, the content of calcium oxide (CaO) in commercial $CaCO_3$ is greater than the content of calcium oxide (CaO) in green mussel and oyster. The content of calcium oxide (CaO) in commercial $CaCO_3$, green mussel, and oyster are 99.1%, 98.2% and 95.7%, respectively. The content of calcium oxide (CaO) in the shells of green mussel is lowest because there were many impurities found [5].

Table 1
Comparison of the chemical composition of commercial $CaCO_3$, oyster, and green mussel [5]

Oxide Content	Green Mussel (%)	Oyster (%)	Commercial $CaCO_3$ (%)
CaO	95,7	98,2	99,1
K_2O	0,5	-	0,4
SiO_2	0,9	-	-
SrO	0,4	-	-
Fe_2O_3	0,7	-	-
SO_3	0,7	0,7	-
MgO	0,6	-	-
Al_2O_3	0,4	-	-

The use of green mussel shells as materials to make hydroxyapatite is supported by the high yield of green mussel production in Indonesia which reached 309,886 tons in 2018 [6]. The waste of green mussel shells reaches 70% of its total weight. Thus, it can be interpreted that the weight of green mussel shells waste produced is 216,902 tons. The green mussel shells is one of the natural raw materials that can be used for hydroxyapatite synthesis because of its high calcium content [2,7-12]. The use of green mussel shells waste as hydroxyapatite synthesis material can be used to reduce the potential for environmental pollution, increasing the economic value of waste, and reducing the cost of hydroxyapatite production [2].

Hydroxyapatite synthesis can be carried out by the sol-gel, mechanochemical, wet-chemical, hydrothermal, emulsion, and solid-state methods. The hydrothermal method is widely used for hydroxyapatite synthesis because it is able to produce products with high stochiometric and crystallinity [13]. Synthesis materials with high stochiometric and crystallinity can be produced by the hydrothermal method due to the control of temperature and pressure increase in the reactor. This is what causes the hydrothermal method to be more expensive than other methods of synthesis [2, 14-16]. Azis *et al.,* conducted a study about hydroxyapatite using calcium obtained from blood clam shells. The calcium obtained was purified through the precipitated calcium carbonate (PCC) method. The hydroxyapatite synthesis produced in their research was purer

because no other apatite compounds were found such as dicalcium phosphate, dibasic phosphate, tricalcium phosphate, and the amorph phase of calcium phosphate [9].

It should be noted from the above literature review, however, that limited studies are available on hydroxyapatite (HAp) synthesis from green mussle shells as Indonesian consumable activity waste and this has motivated the present study. The objective of the present study was to synthesize pure hydroxyapatite crystals from green mussle shells. The synthesis of HAp used the hydrothermal method which basic ingredient was green mussel shells that have been purified through the precipitated calcium carbonate (PCC) method.

2. Methodology

Figure 1 shows the stages of the calcium carbonate purification process from the green mussel shells using the precipitated calcium carbonate (PCC) method. The waste of green mussel shells obtained is cleaned, and then dried in the sun. The size reduction of green mussel was done by ball milling and filtered with mesh 100. This process produced green mussel shells powder which was then calcined at 900°C for 5 hours using the Carbolite (R) furnace.

The green mussel shells powder before and after calcination was characterized by SEM-EDX and XRD. In this study, PCC was made from 17 grams of calcined green mussel shells powder mixed with 300 ml of 2M HNO_3 stirred with a magnetic stirrer at 60°C for 30 minutes. Then NH_4OH was added until the pH of the solution reached 12, and the solution was filtered using Whatman 42 filter paper. The CO_2 gas was flowed to the resulting filtrate slowly to precipitate the filtrate. The resulting white milky filtrate is called a PCC product. It was then washed and filtered with distilled water to a pH value of 7 and then dried at 110°C for 2 hours [9,17]. The resulting PCC was then characterized by SEM-EDX and XRD.

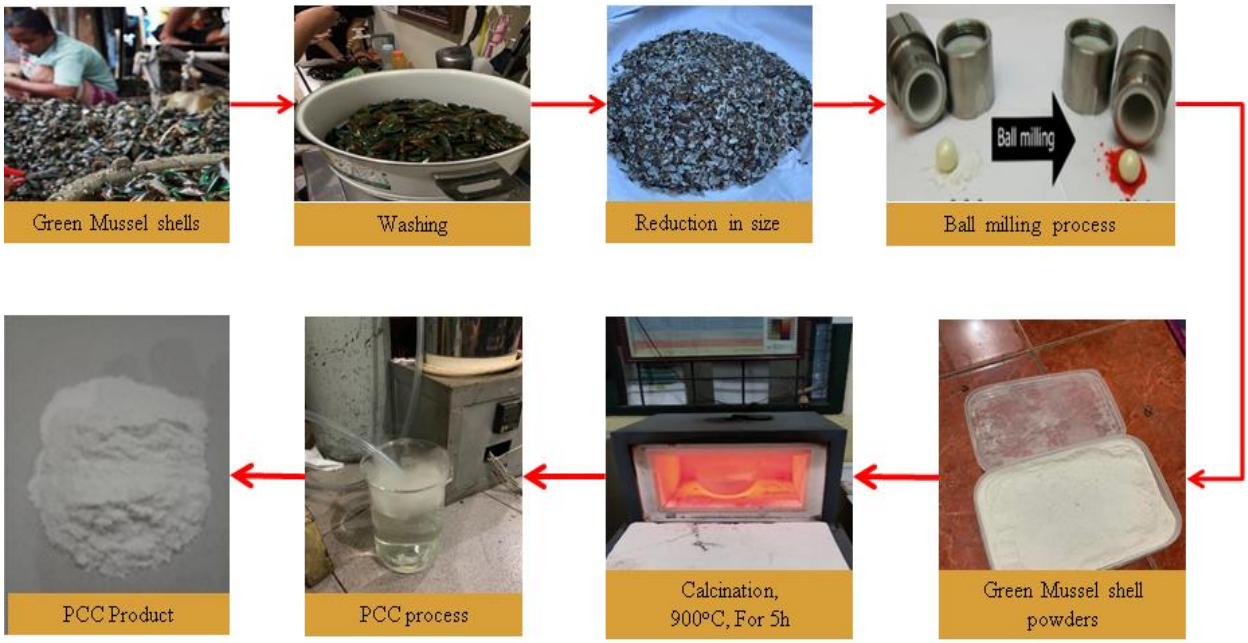


Fig. 1. The set-up experiments of green mussel shells purification with the PCC method

Figure 2 shows the process of hydroxyapatite synthesis by the hydrothermal method. The hydroxyapatite synthesis stage was carried out by mixing precipitated calcium carbonate (PCC) and $(NH_4)_2HPO_4$ with a molar ratio of Ca / P 1.67 and the pH of the mixture 12 adjusted using NH_4OH 25%. This synthesis process was carried out in an autoclave tube in a hydrothermal reactor with the

holding time variations of 14, 16 and 18 hours at a constant temperature of 160°C. Next is the filtration process used to filter the hydroxyapatite mixture from the rest of the reactants using filter paper. The precipitate obtained was then dried at 110°C for 2 hours. The hydroxyapatite produced was then characterized by SEM-EDX and XRD methods.

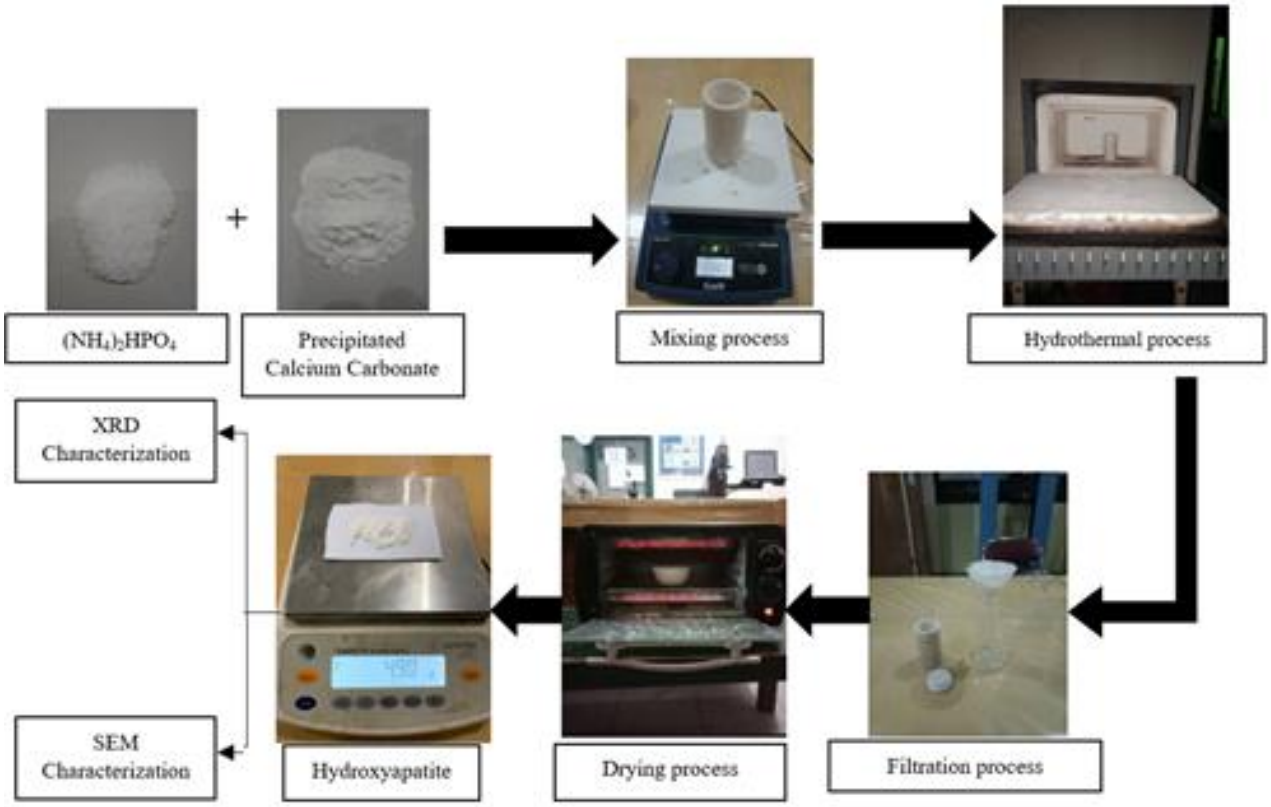


Fig. 2. The experiment set-up of the making of hydroxyapatite synthesis by the hydrothermal method

The green mussel shells powder before and after calcination, PCC product and final product synthesis (hydroxyapatite) was characterized by SEM-EDX and XRD. X-ray powder diffraction (XRD) characterization for amorphicity and crystalline analysis. The obtained materials were characterized by X-Ray diffraction using a Shimadzu X-Ray diffractometer model with CuKα radiation (λ = 1.54056 Å). The diffractograms were recorded in 2θ in the range of 25 to 70°, with step size of 0.05° and scan time of 2 s per step. Crystalline phases present in the samples were identified with the help of Joint Committee on Powder Diffraction Standards (JCPDS). The morphological structures of the synthesized products were obtained and analyzed using scanning electron micrograph (SEM). Energy Dispersive X-Ray Analysis (EDX) used to identify the elemental composition of materials [14].

3. Results

Figure 3 shows the comparison of the results of the XRD test on green mussel shells powder, calcined green mussel shells powder, and PCC product. In the green mussel shells powder, it is shown that the peak of $CaCO_3$ with the highest intensity found at an angle of 2θ respectively 33.1 °, 31.1 °, and 52.4 °. The phases formed on the green mussel shells powder were predominantly aragonite with peaks produced according to JCPDS code 05-0453 [17]. The green mussel shells powder calcined at 900 °C has the highest intensity at an angle of 2θ respectively 34.1 °, 18.1 °, and 50.8 °. The formed phase is $Ca(OH)_2$ with the peak produced according to JCPDS 04-0733 [17]. The

PCC product showing the peak of $CaCO_3$ with the highest intensity found at an angle of 2θ respectively at 27.1 °, 32.8 ° and 24.9 °. The phase formed in the PCC is dominated by the vaterite phase, with the resulting peak having an angle value of 2θ in accordance with JCPDS in code 13-0192. In addition to that, there are calcite and aragonite crystalline phases according to JCPDS in codes 05-0586 and 05-0453 [17].

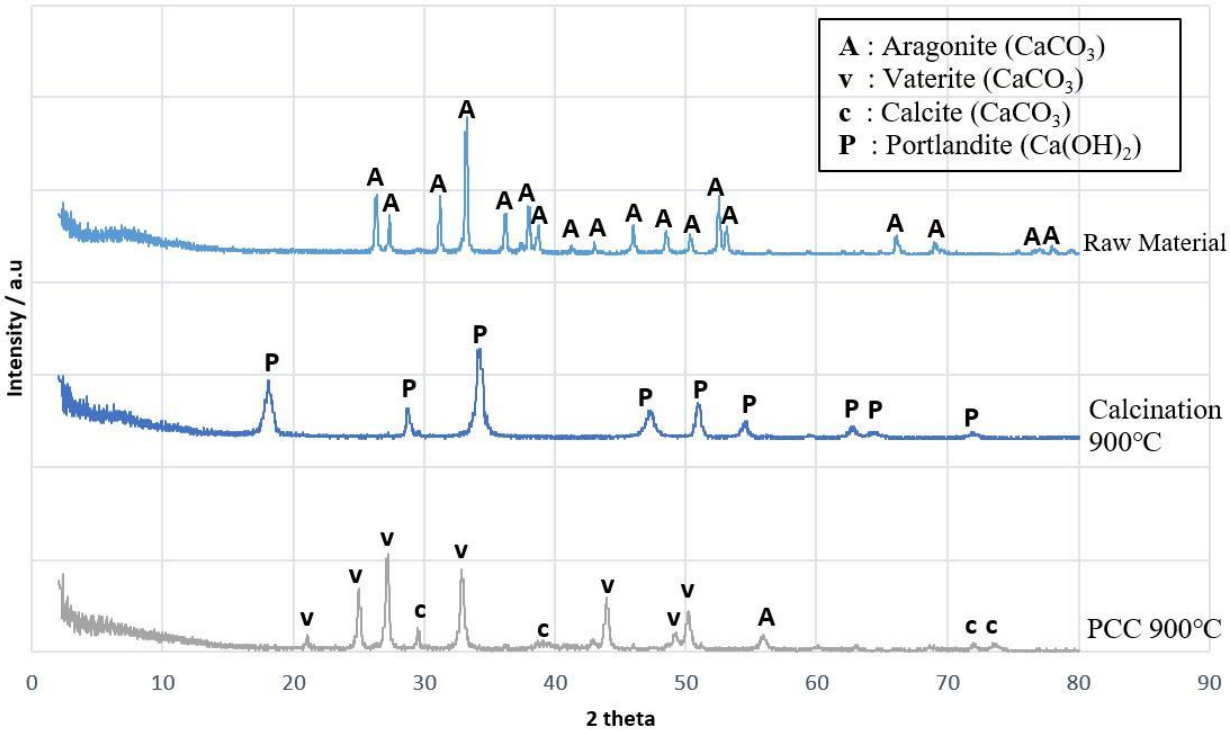


Fig. 3. The comparison of XRD charts for green mussel shells powder, calcined green mussel shells powder, and PCC product

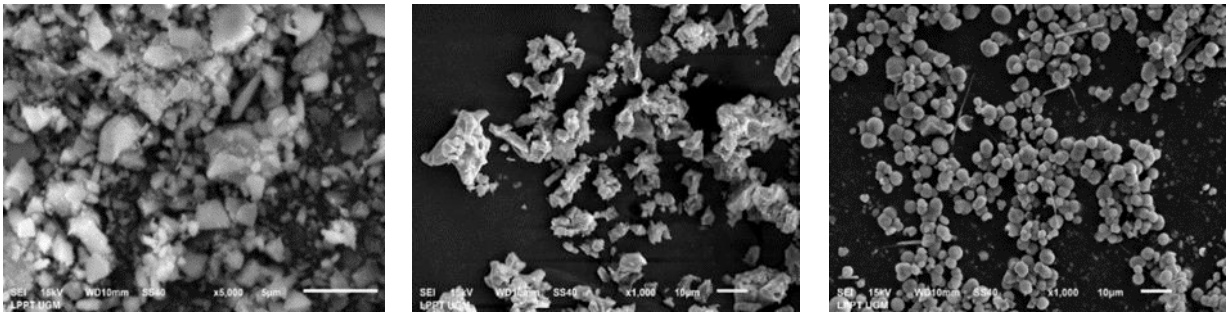
The synthesis of PCC in this study uses Ca (OH) as a base material. The synthesis was carried out at room temperature and low CO_2 gas flow rates. This resulted in the PCC product forming aragonite, calcite and vaterite phases with vaterite being the dominant phase. Synthesis of PCC carried out at room temperature and at low CO_2 gas flow rates will result in the formation of calcite and vaterite phases. In addition, at a low CO_2 gas flow rate it will reduce the supersaturation of the solution due to the lack of availability of CO_2 gas so that the supersaturation of the solution is low. At low supersaturation that causes aragonite particles to form [18-25].

The comparison of the chemical composition of the green mussel shells powder, green mussel after the calcination, and the PCC product are shown in Table 2. The chemical composition was obtained from the EDX test results. Shells of green mussels have Ca and C contents of 28.85% and 17.56%, and there are 0.5% Na impurities. After going through the calcination process, Ca content increased and C content decreased. In this process, calcium decomposition occurred, where C bound with O to form CO_2 gas, and Ca bound with O to CaO. In calcination 900 °C there was not found any impurities. PCC product has decreased the Ca content due to the carbonation process, namely the addition of CO_2 into the solution as evidenced by the increase in the content of C and O. The vaterite phase was formed along with the increase in the flow rate of CO_2 gas. It happened because the amount of CO_2 gas added will increase and improve the solubility of CO_2 gas in the solution [18].

Figure 4 shows the morphological comparison of the green mussel shells powder, green mussel shells powder after calcination, and the PCC product. The green mussel shells powder before calcination has an irregular shape and resembles a small branching stem which is characteristic of aragonite crystals (Figure 4(a)). This is in accordance with research conducted by Huang *et al.,* [19]. The crystalline phase on green mussel shells powder after calcination is non-uniform hexagonal (Figure 4(b)), which is typical of the portlandite or $Ca(OH)_2$ phase. The morphology is in accordance with the research conducted by Jiang *et al.,* [20]. Precipitated calcium carbonate (PCC) product has a uniform shape, in which there are spherical crystals that indicate the vaterite phase (Figure 4(c)). The results of the study are in accordance with the research conducted by Trushina *et al.,* [21].

Table 2
The comparison of the chemical composition of green mussel shells powder, calcined green mussel shells powder, and PCC product

	Mass (%)		
Element	Green Mussel Shells Powder	Calcined Green Mussel Shells Powder (900°C)	PCC product
C	17.56	7.13	14.11
O	53.09	51.21	52.41
Na	0.50	-	-
Ca	28.85	41.66	33.48
Total	100.00	100.00	100.00



(a) (b) (c)

Fig. 4. SEM test results (a) Green mussel shells powder; (b) Green mussel shells powder after calcination; (c) PCC product

The formation of hydroxyapatite with material with the vaterite crystalline phase is challenging given the amount in nature is not plenty [22]. The application of veterit as a calcium precursor for the synthesis of hydroxyapatite is not inferior to other calcium carbonate ($CaCO_3$) minerals, even because of the small amount, the demand for vaterite minerals is quite high. That is due to the biodegredable nature of vaterite which enable it to be used in body fluids. Vaterite can also be used in tissue engineering and regenerative medicine as an implant and scaffold material [21]. Based on the hydroxyapatite diffractogram pattern synthesis of the XRD test results shown in Figure 5, the three variations of the hydrothermal holding time give a difference to the peak of each difractrogam. Based on the JCPDS data number 09-0432, it is shown that the results of the synthesis of hydroxyapatite diffractogram in the three hydrothermal variations are dominated by hydroxyapatite crystals. In hydroxyapatite synthesis 160°C with holding time of 14 hours and 16 hours there are still impurities in the form of aragonite, whereas at a hydrothermal temperature of 160°C for 18 hours pure hydroxyapatite synthesis is produced because no other apatite compounds such as dicalcium phosphate, dibasic phosphate, tricalcium phosphate, and amorph phase of calcium phosphate were found. These results are in accordance with the research conducted by Azis *et al.,* [9].

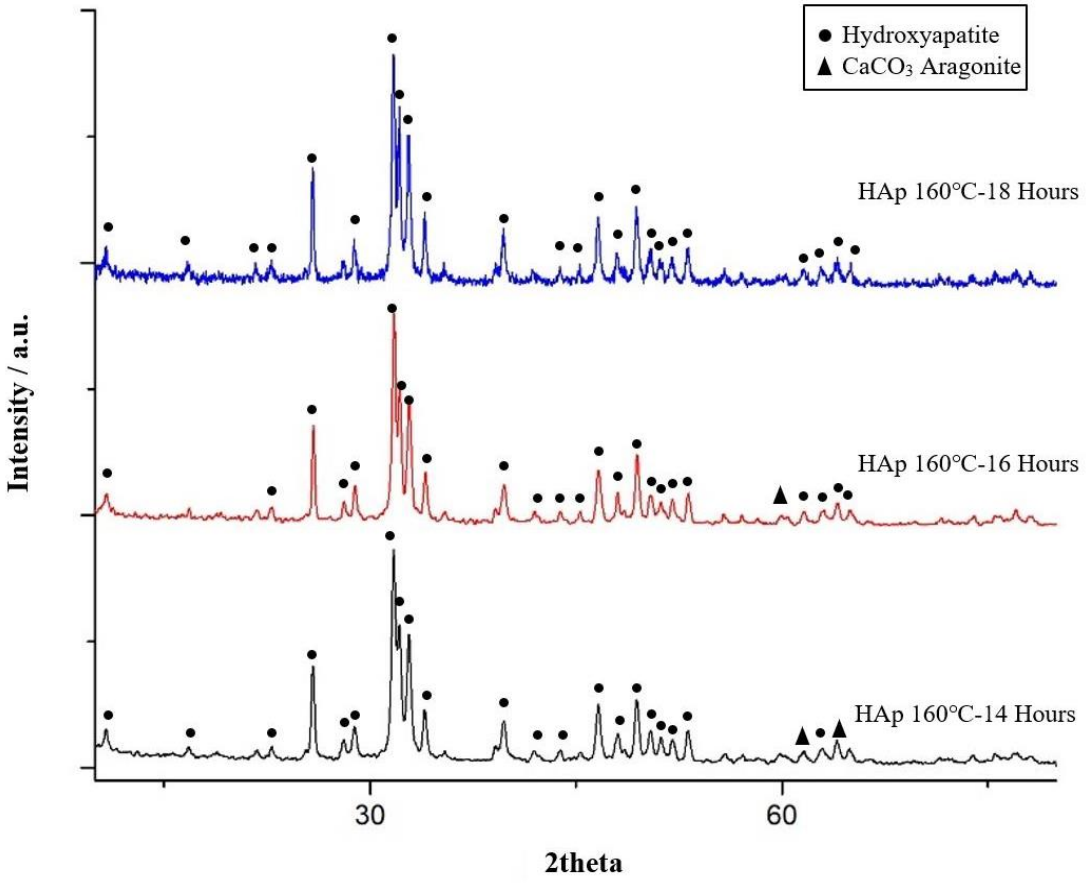


Fig. 5. The comparison of hydroxyapatite diffractogram graphic with hydrothermal holding time variations of 14, 16, and 18 hours

At the hydrothermal holding time variations of 14 and 16 hours, the dominant crystal phase of HAp was produced. However, there were still aragonite crystals that had not transformed into HAp which was marked by a peak at 62.9°. Moreover, the intensity of the aragonite at peak positions of 62,9° significantly decreased with increasing reaction time. This reveals that prolonged heating of aragonite crystal relaxes the lattice of aragonite to facilitate the formation of Hap. Then, the aragonite peaks disappeared due to the complete formation of Hap at a hydrothermal holding time of 18 hours [23]. Since hydrothermal reactions take place under constant material conditions, the precursors continue to dissolve and form a crystalline phase as the reaction progresses. Supersaturation of solutions also decreases gradually and tends to produce materials with a stable crystalline phase [24]. The increasing hydrothermal holding time is beneficial for the growth of hydroxyapatite crystals, the longer holding time will give longer time for crystal growth. The longer the reaction time, the purer the hydroxyapatite is formed [26]. Further prolong the hydrothermal holding time, the diffraction peaks become clearer and more integrated, especially on the peak peak positions of 31.7°, indicating that the crystallinity of HA is enhanced with the rise of hydrothermal holding time [26, 27].

Overall, the SEM method test results showed the morphology of the synthesis of hydroxyapatite which had clumped and shaped like a ball shown in Figure 6(a), 6(c) and 6(e). Hydroxyapatite produced has microsphere morphology. These results are consistent with research conducted by Bunaciu *et al.,* Which stated that the precursor material has an important role in morphology, size, and composition [28]. The spherical form in the resulting hydroxyapatite synthesis occurs due to the mineral form of vaterite as a spherical calcium precursor. In Figure 6(b), 6(d) and 6(f) the

observation was carried out with a magnification of 20,000x, it appeared that hydroxyapatite has a single particle shape such as a small rod or needle [29]. According to Sadat-Shojai *et al.,* Nano rod structures are formed when in an alkaline solution. At high pH, hydroxyapatite grows from spherical particles into small rods. Sadat-Shojai *et al.,* added that the hydroxyapatite morphology that is generally formed is spherical, non-uniform, and rod-like [30].

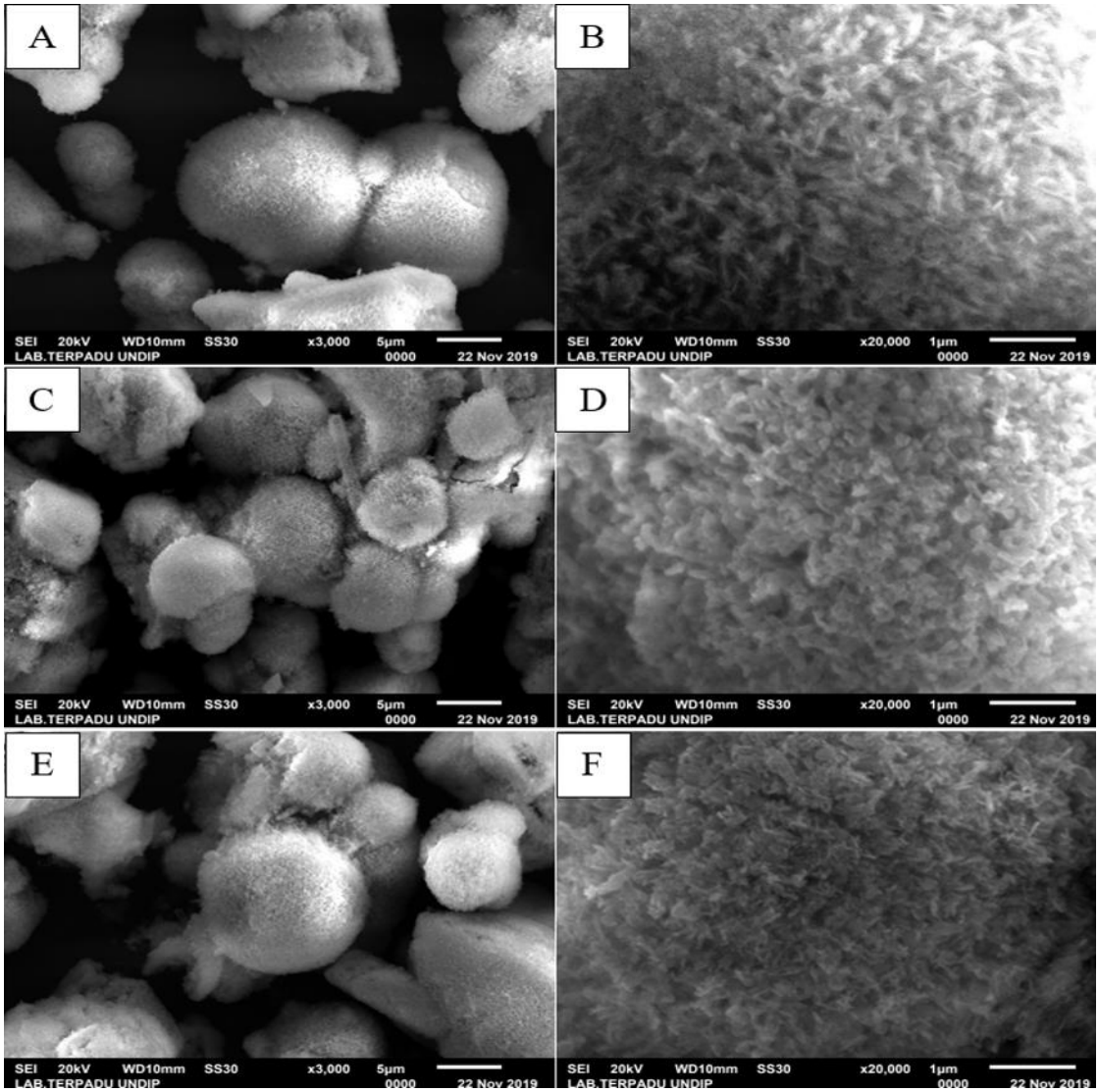


Fig. 6. The morphological comparison of SEM hydroxyapatite synthesis test results of 14 hours (a,b), 16 hours (c,d) and 18 hours (e,f)

4. Conclusions

Hydroxyapatite synthesis made from green mussel shells using the hydrothermal method has been successfully carried out. In this study, the green mussel shells purified by the precipitated calcium carbonate (PCC) method produced the crystalline phase with the dominance of the vaterite phase. The holding time of hydrothermal in hydroxyapatite synthesis made from PCC product with the dominance of the vaterite phase affected the quality of the hydroxyapatite synthesis produced. At a hydrothermal holding time of 18 hours, high purity hydroxyapatite is produced because no other apatite compounds such as dicalcium phosphate, dibasic phosphate, tricalcium phosphate,

and amorph phase from other calcium phosphates were produced. Whilst in the 14 and 16 hours holding variations, the aragonite phase was still found in the final results of the synthesis process.

Acknowledgement

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