

# Synthesis and characterization of hydroxyapatite based on green mussel shells (*perna viridis*) with the variation of stirring time using the precipitation method

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**Abstract.** Hydroxyapatite (HA) from green mussel shells (*Perna viridis*) has been successfully synthesized with a variation of stirring time using the precipitation method. The green mussel shells were calcined in furnace at temperature of 950°C for 2 h. AAS result shows that the level of Ca was 49.5757%. X-Ray diffractometer result shows that the crystallization of CaO was high because it has big crystallite size and small microstrain. The analysis of XRD shows that for HA with the stirring time of 30 min and 45 min have the same crystallization with the crystallite size of  $(74.91 \pm 4.9)$  nm and  $(74.91 \pm 4.7)$  nm. From DTA/TGA analysis, HA samples of stirring time 45 min and 15 min are more stable than HA with the stirring time of 30 min. The FTIR spectra show that HA with the stirring time of 30 min and 45 min could have lower transmittance value. From SEM result, HA with the stirring time of 15 min has small agglomerate shape and thick structure of particles. Therefore, HA with the stirring time of 15 min becomes the best synthesized from this research which it can be used as a coating material in implanted applications.

## 1. Introduction

So far, the treatment to overcome bone damage cases has used the heavy metals to replace the damaged bones in the body. However, this treatment still inflicted a problem, namely the metal stored in the body had a low biocompatibility level that caused sick and bruise in the flesh around the metal. Therefore, it taken a material that it can be used to coat the metals to be implanted into the body, so the metal attain a high biocompatibility with the body. The approach is taken using the materials that are already present in the body, constituents of bone tissues such as apatite compounds. Presently, there is an artificial bone similar to the original bone constituent, namely hydroxyapatite [1].

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , HA) is a major component of human bones and teeth [2]. It is commonly used in orthopedic, dental and maxillofacial applications [3-5]. HA has most stable crystal phase of potassium phosphate with the hexagonal structure that has the lattice parameters:  $a = 9.433\text{\AA}$   $c = 6.875\text{\AA}$  and a variable Ca/P mol ratio is 1.67 [6-7]. The advantages of hydroxyapatite are porous, bioactive, non-corrosive and wear-resistant. Hydroxyapatite has a weight of 69% of the weight of pure bone and it is the most stable compound in body fluids and dry for up to the temperature of 1200 °C [8].

A variety of synthesis techniques of hydroxyapatite have been developed such as sol-gel procedure [9], precipitation from aqueous solution [10], hydrothermal [11-13] and solid-state reactions [14]. In



this study, we use the precipitation method to synthesis HA. The selection of precipitation methods is done on several considerations such as many hydroxyapatite relatively synthesized without used the organic solvents (relatively small cost); it is simple process with large product (87%), so it is suitable for large-scale (industrial) production, it needs inexpensive reagents and Ca/P products with the phase compositions can be obtained. Although this process depends on variables such as pH, aging, and temperature, it is effective and inexpensive compared to sol-gel [15].

Hydroxyapatite made by chemical synthesis is called synthetic hydroxyapatite. Synthetic hydroxyapatite can be obtained not only through the reaction of synthetic compounds, but also it can be reacted the synthetic compound with a natural compound. HA can be synthesized from the materials that contain high calcium such as cow bones, fish bones, cuttlebones, eggshells and mussel shells [16].

Here, we use waste mussel shell in Indonesia that have not been processed in Indonesia. The production of mussel shell in Indonesia from 2002 has continued to increase [17]. The waste mussel shells have a high calcium carbonate content of 95-99% [18] so it can be used as a source of calcium in HA synthesis. The analyses of Energy Dispersive X-Ray Fluorescence (EDXRF) show that the composition of minor minerals in the green mussel shells are Ca 99.5%, Sc 0.24% and Sr 0.47% [19]. In this study, the waste green mussel shells (*Perna viridis*) were taken from the Depok beach, Yogyakarta, Indonesia.

## 2. Experimental method

### 2.1. Preparation of calcium oxide

The waste green mussel shells (*Perna viridis*) were cleaned in boiling water for 30 min and washed using distilled water to remove attached materials such as shell meat and algae. Then, they were dried using the furnace at the temperature of 100°C. It was refined using ball mill to obtaining the smaller particle size. The powder of green mussel shells was heated at the temperature of 950 °C for 2 h.

### 2.2. Synthesis of hydroxyapatite

An amount of 2 gram of calcium oxide was mixed with 50 ml distilled water. Then, (NH<sub>4</sub>)<sub>2</sub> HPO<sub>4</sub> solution (2.8285 gram in 50 ml distilled water) was slowly added dropwise at rate 1 ml/min to the CaO powder, the liquid mixture was stirred with the velocity at 300 rpm and done the variation of stirring time of 15 min, 30 min, and 45 min while heated at temperature of 70°C. The pH of the mixture was controlled above 9 by adding ammonium hydroxide (NH<sub>4</sub>OH (25 %) 3M. The mixture was stirred by magnetic stirrer for 50 min and heated at temperature of 70 °C. The solution was subjected to aging treatment for 24 h and washed using distilled water. The solution was filtered to obtain the precipitate of HA. The precipitate of HA was dried at temperature of 100 °C for 2 h. Finally, HA was calcined at temperature of 950 °C for 3 h using the furnace to obtain the pure hydroxyapatite.

### 2.3. Characterization

In the preparation step, the green mussel shells sample was characterized using the atomic absorption spectrophotometer (AAS) to determine the level of Ca in the green mussel shells. The calcium oxide was characterized using X-Ray Diffractometer (XRD) to determine crystallite size and microstrain. We use Fourier Transform Infrared Spectroscopy (FTIR) to determine the functional groups of calcium oxide. We use Scanning Electron Microscopy (SEM) to know the morphology of calcium oxide [20].

In the synthesis step, the HA samples was characterized using X-Ray Diffractometer (XRD) to determine the crystal structure of HA, Differential Thermal Analysis/Thermogravimetric Analysis (DTA/TGA) to analyse the thermal and stability properties. We use Fourier Transform Infrared Spectroscopy (FTIR) to determine the functional groups of OH<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, and CO<sub>3</sub><sup>2-</sup> in the HA samples. We use Scanning Electron Microscopy (SEM) to know the morphology of HA samples [20].

### 3. Result and discussion

#### 3.1. Calcination

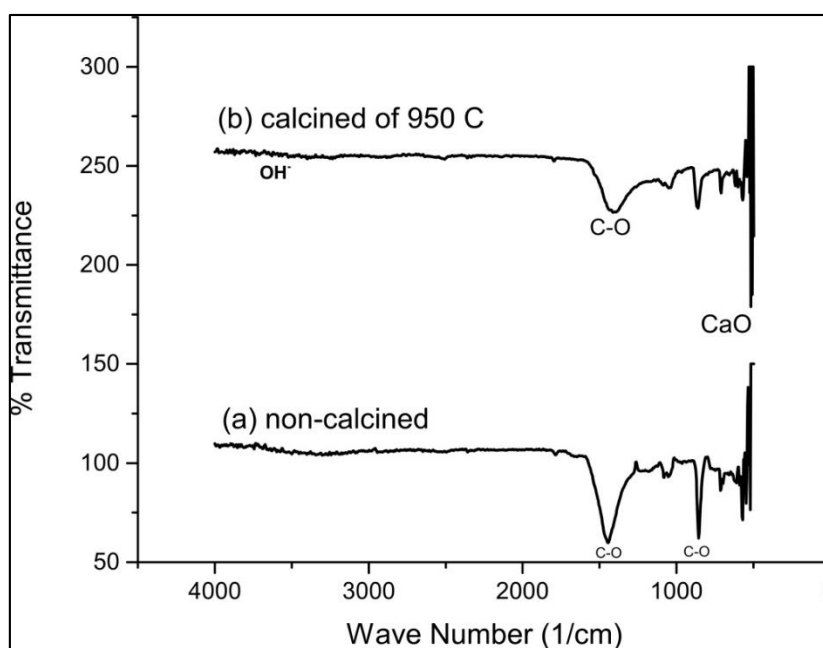
The green mussel shells need to be calcined before it used as calcium precursors (Ca). The calcination was done in order to decomposition reaction of  $\text{CaCO}_3$  to  $\text{CaO}$  occurred. In this condition, all organic components of green mussel shells will burn exhausted to  $\text{CaO}$  and  $\text{H}_2\text{O}$ . As the reaction occurring during the calcination process is as follows:



The enthalpy change for the decomposition reaction of  $\text{CaCO}_3$  was 177.7 kJ / mol [21]. Therefore, the enthalpy change for the decomposition reaction of  $\text{CaCO}_3$  was positive, so the reaction was occurred on an endothermic [21]. In this study, the calcination of the samples was done at 950 °C because the decomposition reaction of  $\text{CaCO}_3$  to  $\text{CaO}$  can be occurred at the minimum of 750 °C [22]. According to calculations using the enthalpy concept, parameter of increasing temperature was 1426 °C so that the variations of the calcination temperature can ideally be carried out at ~ 1400 °C. Giving the heat greatly helped the optimization of the decomposition reaction. When the  $\text{CaCO}_3$  compounds received heat, the moving atom would be faster and this movement broke the chemical bonds of  $\text{CaCO}_3$  into  $\text{CaO}$  and  $\text{CO}_2$ . Therefore, increasing the calcination temperature tended to make the breaking of chemical bonds  $\text{CaCO}_3$  into  $\text{CaO}$  faster. Besides that, the analysis of AAS shows that the level of Ca for the calcination temperature of 950 °C is 49.575%.

**Table 1.** Comparison the level of Ca from green mussel shells.

| Researchers | Year | Level of Ca (%)   |
|-------------|------|-------------------|
| Yulianti    | 2009 | 28.97% and 39.55% |
| Hidayat     | 2013 | 45.62%            |
| Siswanto    | 2013 | 44.53 %           |
| Mona Sari   | 2017 | 49.575 %          |



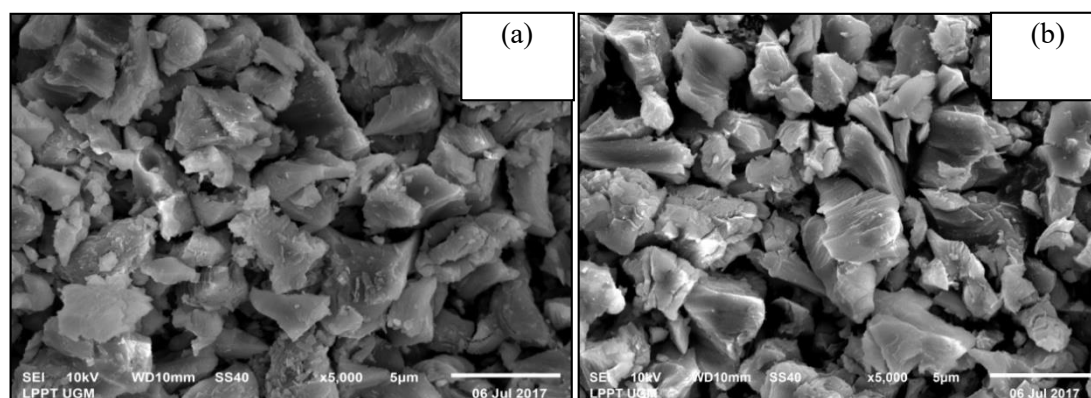
**Figure 1.** FTIR spectra of  $\text{CaO}$  samples (a) non-calcination, (b) calcination of 950°C.

According to Table 1, the level of Ca from researcher is greater than the others. Thus, the calcined green mussel shells at 950°C have become potential to precursors in production of hydroxyapatite.

The calculation of crystallite size is  $184.8 \pm 7.9$  nm and the small value of microstrain is 0.00302. This indicates that calcium oxide has a small crystal defect. Microstrain can become one of the forms of crystal imperfections due to distortion or dislocation. The small value of microstrain indicates the small defect of crystal.

FTIR spectra analysis in CaO is performed to know the functional groups of C-O, OH<sup>-</sup>, and CaO formed. According to Figure 1, the non-calcined of green mussel shells do not show the stretching mode of OH<sup>-</sup> and the functional group of CaO. The calcined of green mussel shell at temperature of 950 °C indicates the stretching mode of OH<sup>-</sup> at 3641.34 cm<sup>-1</sup>. The samples of CaO have shown the C-O functional groups of calcium carbonate contained in the green mussel shells. The functional bands of calcium carbonate indicate close to the commercial calcium carbonate functional group at 1400 cm<sup>-1</sup>, 877 cm<sup>-1</sup> and 700 cm<sup>-1</sup> [23]. The functional group of CaO for the calcination temperature of 950 °C is 871.76 cm<sup>-1</sup> even for the calcined CaO of 950 °C also shows CaO bond formed at 667.32 cm<sup>-1</sup>.

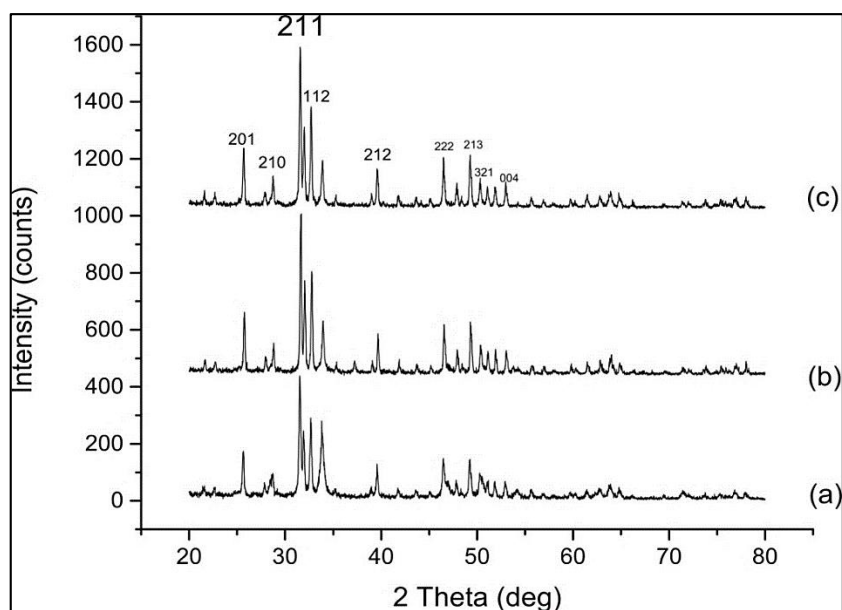
SEM characterization was done to determine the morphology of the green mussel shells and calcium oxide from the green mussel shells. In this study, the images were taken with 5000x magnification. According to Figure 2(a), the green mussel shells have a large particle shape with more heterogeneous particle distribution. The morphology of green mussel shells also shows like producing cavities. In Figures 2(b), it can be seen that there are some relief lines on the surface of the particles resulting from calcination at 950 °C.



**Figure 2.** Results of SEM Characterization for samples (a) green mussel shells, (b) calcination temperature of 950 °C.

### 3.2. XRD analysis of the HA samples

The XRD pattern of the HA from calcined green mussel shells are shown in Figure 3. From Figure 3, HA with stirring time of 15 min, 30 min and 45 min were obtained the peak of 31.54°, 31.63° and 31.57°, respectively with *hkl* index of close to 211. These agreed with the data Joint Crystal Powder Diffraction Standard (JCPDS) No.09-0432. According to the calculation using the Bragg equation, we obtained the distance between the crystal planes of HA for stirring times 15 min, 30 min and 45 min are 2.83 Å, 2.82 Å and 2.83 Å, respectively. This result is close to the crystal plane of the HA at 2.88 Å appropriate with international standards (ISO 13779-3, ISO 13175-3) for hydroxyapatite implants [24]. Therefore, the results of XRD analysis in this study are not expected to produce any other crystalline phase other than the HA crystalline phase.



**Figure 3.** XRD pattern of HA samples with the stirring time (a) 15 min, (b) 30 min, (c) 45 min.

As shown in Table 2, there is an inverse relation between the crystallite size and micro strain. Microstrain is a form of crystal imperfection that appears as a distortion or dislocation. A small microstrain value indicates a small amount of defect in the crystal [25]. The crystallite size and microstrain of HA for stirring times 30 min  $[(74.91 \pm 4.9) \text{ nm}]$  and 45 min  $[(74.91 \pm 4.7) \text{ nm}]$  are almost same. This means, they have same crystal defect.

**Table 2.** XRD results for hydroxyapatite.

| Name of Samples                     | Crystallite Size (nm) | Lattice Parameter ( $\text{\AA}$ ) | X-Ray Density ( $\text{g/cm}^3$ ) | Microstrain |
|-------------------------------------|-----------------------|------------------------------------|-----------------------------------|-------------|
| HA with the stirring time of 15 min | $66.31 \pm 4.5$       | 8.65                               | 10.31                             | 0.0077      |
| HA with the stirring time of 30 min | $74.91 \pm 4.9$       | 8.63                               | 10.38                             | 0.0067      |
| HA with the stirring time of 45 min | $74.91 \pm 4.7$       | 8.65                               | 10.31                             | 0.0068      |

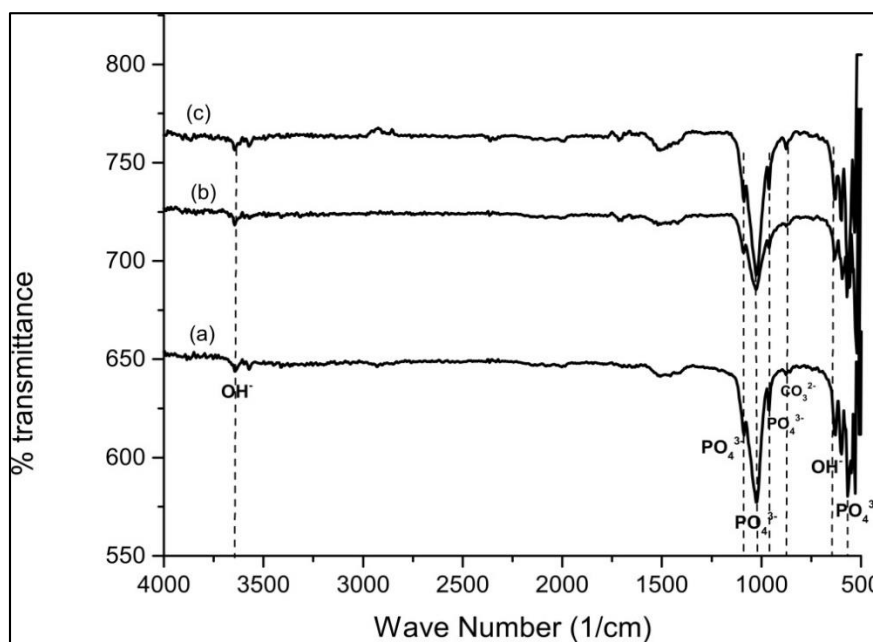
According to Table 2, the X-ray density value corresponds to the parameter lattice of samples. From samples of HA, HA with the stirring time of 30 min has the greatest X-ray density value from the others. This result indicates that the sample has larger atomic density than the others. Thus, the crystallization of HA depends on the stirring time. If the stirring time was longer than 15 min, no HA were produced [26]. This result can be seen in crystallite size, small microstrain value at HA with the stirring time of 30 min and 45 min.

### 3.3. FTIR spectra of the HA samples

According to FTIR spectra (Figure 4), all samples have HA functional group. HA with the stirring times 15 min, 30 min and 45 min show stretching mode of  $\text{OH}^-$  at  $3641.34 \text{ cm}^{-1}$ ,  $3645.19 \text{ cm}^{-1}$ ,  $3645.19 \text{ cm}^{-1}$  and  $3571.90 \text{ cm}^{-1}$ , respectively. All samples show the bending modes of  $\text{OH}^-$  at  $632.61 \text{ cm}^{-1}$  and stretching  $\nu(\text{P-O})$  modes of  $\text{PO}_4^{3-}$  at  $960.48$ ,  $1026.05$  and  $1087.77 \text{ cm}^{-1}$ . HA with the stirring



time 15 min shows the bending (P-O)  $\text{PO}_4^{3-}$  at 567.89 and 597.89  $\text{cm}^{-1}$ , however HA with the stirring times 30 min and 45 min show the bending (P-O)  $\text{PO}_4^{3-}$  at 594.03 and 563.17  $\text{cm}^{-1}$ , respectively. According to these data, HA with the stirring times 30 min and 45 min could have lower transmittance value. The spectra bands at 1419.50 and 875.62  $\text{cm}^{-1}$  represent  $\text{CO}_3^{2-}$  groups. The existence of  $\text{CO}_3^{2-}$  groups in FTIR spectra can be derived from the reaction of calcium oxide with carbon dioxide in free air during the synthesis. In addition,  $\text{CO}_3^{2-}$  groups were possibly existed in raw green mussel shells before the synthesis process. Carbon dioxide will come into contact with the distilled water that are solvent in this reaction and produce  $\text{CO}_3^{2-}$  and into the crystal lattice of HA.



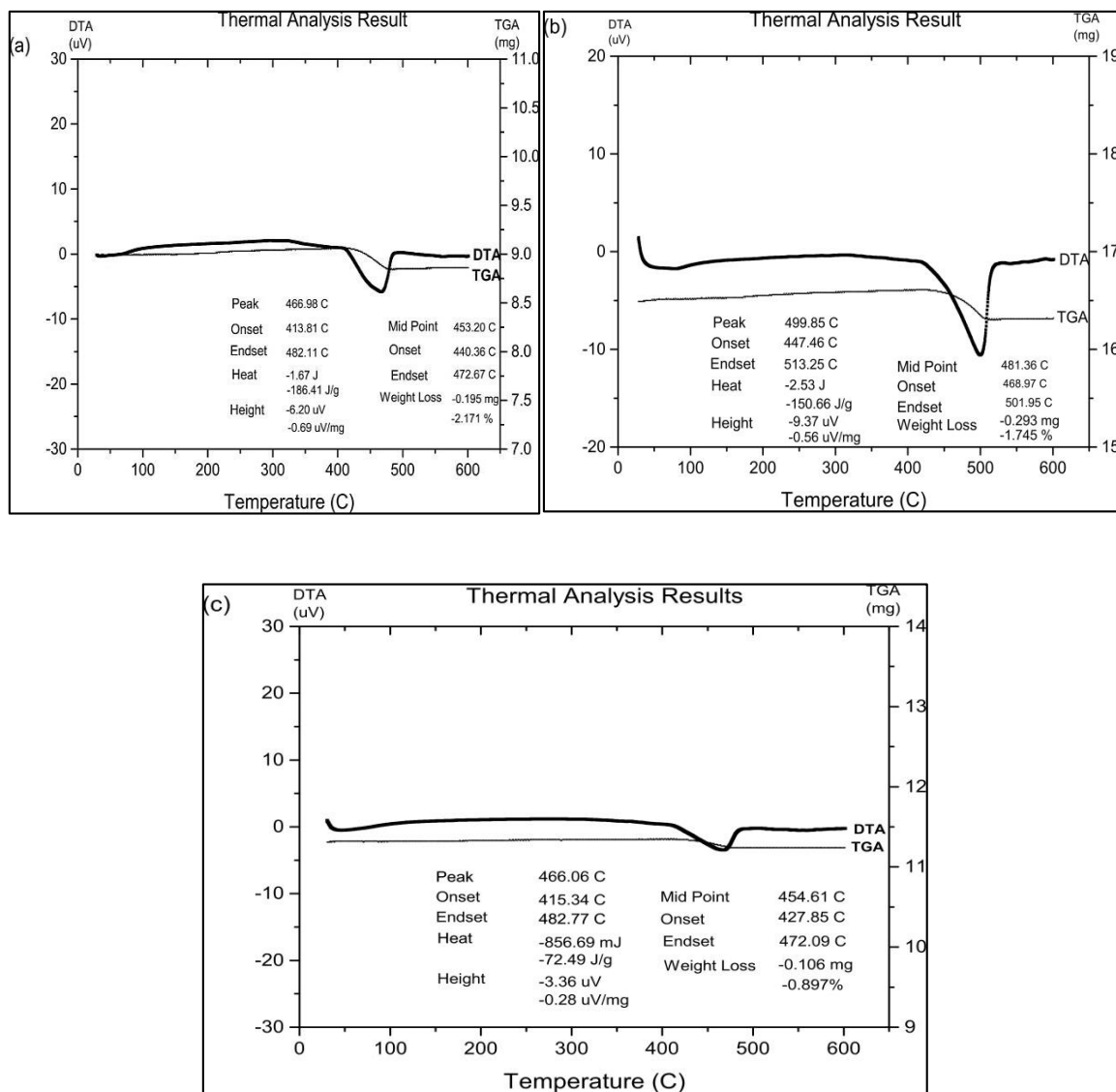
**Figure 4.** FTIR spectra of HA samples with the stirring time (a) 15 min, (b) 30 min, (c) 45 min.

The presence of carbonate in the HA structure is assessed to reduce the thermal stability to be confirmed from the DTA/TGA results. If the calcium carbonate content is high, there will be a significant decrease in the sample. The presence of  $\text{CO}_3^{2-}$  groups is common because of human bones have also them. This is the natural substitution for  $\text{PO}_4^{3-}$  according to formula of molecules  $\text{Ca}_{10}(\text{CO}_3)_x(\text{PO}_4)_{6-(2/3)x}(\text{OH})$  or called carbonated HA. The existence of carbonate is unavoidable if HA synthesis is conducted in the open air. There needs to be environmental innertization (reactor) by passing inert gas such as nitrogen ( $\text{N}_2$ ) so that the process of mixing precursor is free from outside air contaminant

### 3.4. DTA/TGA analyses of the HA samples

According to DTA result (Figure 5), the enthalpy with the exothermic process for the HA with the stirring times of 15 min, 30 min and 45 min are 186.41 J/g, 150.66 J/g, and 72.49 J/g, respectively. HA with the stirring time of 45 min removed the least energy than HA for stirring times of 15 min and 30 min. The result of TGA, HA with the stirring time of 45 min shows a faster weight loss at 427°C with a weight loss of 0.897%. For HA with the stirring time of 15 min and 30 min, the weight loss are 2.171 % and 1.745%, respectively. The weight loss was suggested due to the evaporation of moisture water as the result of  $\text{CaO}$  and  $(\text{NH}_4)_2\text{HPO}_4$  reactions. Therefore, HA with the stirring time of 15 min contains much carbonate. HA with the stirring time of 45 min shows the strong elimination of water

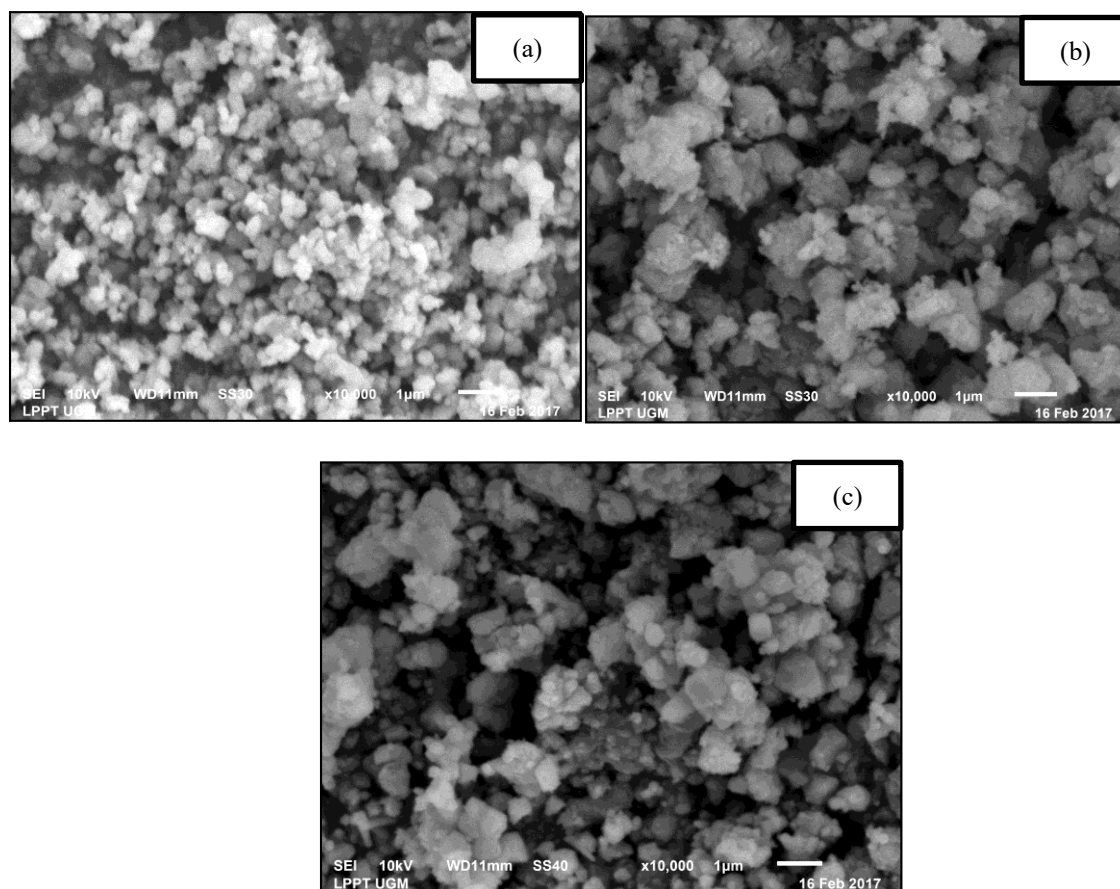
because it has the fastest weight loss at 427°C. HA samples of stirring time 45 min and 15 min are more stable than HA with the stirring time of 30 min.



**Figure 5.** Analysis of DTA/TGA for HA samples with the stirring time (a) 15 min, (b) 30 min, (c) 45 min.

### 3.5. Morphology of HA

Figure 6 shows the morphology of HA with the stirring time of 15 min, 30 min, and 45 min. HA with the stirring time of 15 min has small agglomerate shape and solid structure. The SEM results show that HA synthesized by using precipitation method is fine grain of uniform size. HA with the stirring time of 30 min and 45 min attain a form with large and uneven clumps. Therefore, by increasing the stirring times, the agglomeration will increase.



**Figure 6.** SEM images of HA sample with the stirring time of (a) 15 min, (b) 30 min, (c) 45 min.

#### 4. Conclusion

In this experiment, HA was synthesized successfully from green mussel shells through use of the precipitation method with variations of stirring time. The results of the AAS analysis showed that the Ca level at a calcination temperature of 950 °C was 49.5757%. Per the XRD results, the calcium oxide calcined at 950 °C exhibited the largest crystallite size, meaning it had high crystallinity and a shortened amorphous phase. According to the results of production of hydroxyapatite, HA with the stirring time of 30 min and 45 min have the same crystal defect because the crystallite size and microstrain of HA for stirring times 30 min are almost same. They could have lower transmittance value. HA samples of stirring time 45 min and 15 min are more stable than HA with the stirring time of 30 min. From SEM result, HA with the stirring time of 15 min has small agglomerate shape and thick structure of particles.

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## References

- [1] Mulya N Fadli A and Amri A 2016 *Jom FTEKNIK* **3** 1-7
- [2] Rujitanapanich S Kumpapan P and Wanjanoi P 2014 *Proc. 11<sup>th</sup> Eco-Energy and Mater. Sci. and Engin.* 112-7
- [3] Akram M Ahmed R Shakir I Ibrahim W and Hussain R 2014 *J. Mater. Sci.* **49** 1461-75
- [4] Gao Y Cao W-L Wang X-Y Gong Y-D Tian J-M Zhao N-M and Zhang X-F 2006 *J. Mater. Sci. Mater. Med.* **17** 815-23
- [5] Huber F-X Berger I McArthur N Huber C Kock H-P Hillmeier J and Meeder P 2008 *J. Mater. Sci. Mater. Med.* **19** 33-8
- [6] Kalita S J and Verma S 2010 *Mater. Sci. Eng. C* **30** 295-303
- [7] Mohandes F Niasari M S Fathi M H and Fereshteh Z 2014 *Mater. Sci. Eng. C* **45** 29-36
- [8] Saleha Halik M Annisa N Sudirman and Subaer 2015 *Proc. Scientific Meeting XXIX HFI Jateng & DIY Yogyakarta* 124-7
- [9] Peng H Wang J Lv S Wen J and Chen J F 2001 *Ceram. Int.* **41** 14340-9
- [10] Cox S C Jamshidi P Grover L M and Mallick K K 2014 *J. Mater. Sci. Mater. Med.* **25** 37-46
- [11] Kumar R Prakash K H Yennie K Cheang P and Khor K A 2005 *Key. Eng. Mater.* **284** 59-62
- [12] Liu F Wang F Shimizu T Igarashi K and Zhao L 2006 *Ceram. Int.* **32** 527-31
- [13] Wu S-C Tsou H K Shu H C Hsu S-K Liou S P and Ho W-F 2013 *Ceram. Int.* **39** 8183-8
- [14] Wu S-C Hsu H-C Shu H C Hsu S-K Chang Y-C and Ho W-F 2016 *J. Asian Ceram. Soc.* **4** 85-90
- [15] Suryadi 2011 *Synthesis and Characterization of Hydroxyapatite Biomaterials by Wet Chemical Precipitation Process* Thesis University of Indonesia
- [16] Sari M and Yusuf Y 2017 *Proc. The Biomaterials Int.* Fukuoka Japan
- [17] Muntamah 2011 *Synthesis and Characterization of Hydroxyapatite from the Blood Mussel Shells (Anadara granosa, sp)* Thesis Bogor Agricultural University Indonesia
- [18] Shavandi A Bekhit A E-D Ali A Sun Z and Ratnayake J T 2015 *Powder. Tech.* **273** 33-9
- [19] Siriprom W Chumnavej N Choeysuppakat A and Limsuwan P 2012 *Proc. Eng.* **32** 1123-6
- [20] Mc Mahon G 2007 *Analytical Instrumentation A Guide to Laboratory Portable and Miniaturized Instruments* edited by J. Willey and Sons Ltd England 130-70
- [21] Zhang D Lu S Gong L L Cao C-Y and Zhang H-P 2016 *Energy. Conv. Management.* **109** 94-102
- [22] Shavandi A Bekhit A E-D Ali A and Sun Z 2015 *Mater. Chem. Phys.* **149-150** 607-16
- [23] Shariffuddin J H Jones M I and Patterson D A 2013 *Chemic. Eng. Research. Design* **91** 1693-704
- [24] Gross K A Berndt C C Stephens P and Dinnebier R 1998 *J. Mater. Sci.*, **33** 3985-91
- [25] Sari M and Yusuf Y 2018 *Int. J. of Nanoelectronics and Mater.* **11**
- [26] Kalita S J and Verma S 2010 *Mater. Sci. Eng. C* **30** 295-303