

Effect of Temperature on Synthesis of Hydroxyapatite from Cockle Shells (*Anadara Granosa*)

Yazida Rizkayanti¹ and Yusril Yusuf^{1,2*}

¹Department of Physics, Faculty of Mathematics and Natural Science, Universitas Gadjah Mada, Yogyakarta, Indonesia.

²Nanomaterial Research Group, Universitas Gadjah Mada, Yogyakarta, Indonesia.

ABSTRACT

Hydroxyapatite (HAp) has been synthesized from biowaste cockle shells (*Anadara Granosa*) by precipitation method at various temperatures on synthesis. HAp synthesis result has been analyzed by using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and X-Ray Diffractometer (XRD). The result showed that HAp was successfully produced from cockle shells. It was found that temperature of synthesis had effects on morphology, Ca/P ratio, crystallinity and crystallite size. Temperature synthesis at 40°C was identified as the optimum temperature to produce HAp as indicated by pattern of pure HAp, the Ca/P value close to theory, well-defined morphology and the presence of functional group.

Keywords: Hydroxyapatite, Cockle Shells (*Anadara Granosa*), Temperature on Synthesis.

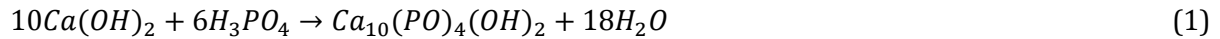
1. INTRODUCTION

Hydroxyapatite (HAp) which is represented by the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is a class of calcium phosphate-based bioceramics. HAp has been widely employed as biomaterial in the form of powders, dense or porous bodies and coatings for metallic or polymeric implants [1]. HAp in powder form is frequently used in biomedical applications such as prosthetic implants and coating implants [2]. It is widely used because bioactivity, biocompatibility, and nontoxic. Multiple techniques have been used for synthesis HAp. There are two ways for synthesis HAp powder that are wet methods and solid state reaction. The wet method can be divided into three groups: precipitate, hydrolysis and hydrothermal. Out of these methods, precipitation is the most economical, simple and high percentage of pure products for synthesis HAp. Using these techniques, HAp with different stoichiometries, morphology, grain size and crystallinity can be obtained.

In last two decades, the use of biogenic structures and materials to produce HAp using various synthesis techniques has been widely studied using such materials sea shells, egg shells corals and animal bone [2]. Biogenic material is an interesting procedure to produce HAp because economic, environmental benefits and uses biogenic resources. These biogenic materials must consist of high source of calcium as calcium precursor to produce HAp. Moreover, HAp synthesized from biogenic material has better tissue response by virtue of its porosity, chemical and structural similarity to that of the mineral phase of bone and easily bonds with natural bone [3]. Among the abundant biogenic materials are cockle shells which are one of Indonesia's main commodities. Based on data from ministry of maritime and fisheries affairs in 2010 produced the cockle shells around 34.482 ton/year. However, cockle shells is reportedly widespread Southeast Asia such as Indonesia, Malaysia and Thailand [4]. The main composition of cockle shells in the form of Calcium Carbonate (CaCO_3) was made up of 59,87% Calcium (Ca) [5]. CaCO_3

*Corresponding Author: yusril@ugm.ac.id

could be processed into Calcium oxyde (CaO) as precursor of calcium and orthophosphoric acid (H₃PO₄) as precursor of phosphate which is suitable to produce HAp following Eq. (1). Generally, these precipitation methods depend on parameter that impacts properties of HAp synthesis product such as temperature synthesis, PH of synthesis, concentration, temperature of sintering.



In this paper, the effect of temperature of synthesis of HAp was observed the best temperature conditions with cockle shells as precursor calcium. Several researchers has been studied effect of temperature of synthesis. For instance, Jamarun et al. [6] have studied effect temperature mixing when calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O) added di-ammonium hydrogen phosphate solution. They have found that optimum temperature at 90°C and the crystallite size of HAp found to be in range of 22,5-68,5 nm. Bouyer et al. [7] have studied effect of different synthesis parameters (reaction temperature and reactant addition rate) on morphology, phase and rheological properties of the colloidal HAp. They have found that a transition temperature at 60°C and size, shape, specific surface area of HAp also very sensitive to the temperature of synthesis. Wijesinghe et al. [8] have studies effect of synthesis temperature and calcination on morphology, crystallite size and crystallinity. They have found percentage crystallinity and average diameter HAp increases from 15% to 52% with increasing the preparative temperature.

2. EXPERIMENTAL PROCEDURE

The experimental procedure is divided into three parts which deals with preparation CaO from cockle shells, synthesis of HAp and characterization of HAp.

2.1 Preparation of CaO

Cockle shells cleaned with water and oven at 100°C for 3 hours to isolate contaminate. Subsequently, biowaste was ball milled for 6 minutes and furnace at 1000°C for 5 h for the calcination process. The Calcination process aims to transform biowaste consists of calcium carbonate (CaCO₃) to CaO by releasing carbon dioxide (CO₂). Chemical reactions occur at calcination process following Eq. (2) CaO was ball milled again for 6 minutes and sieved using a 170 mesh sieve to a fine powder of CaO.



2.2 Synthesis of Hap

HAp were synthesized by a wet chemical precipitation method with Ca(OH)₂ from cockle shells as calcium source and orthophosphoric acid as phosphate source. Ca(OH)₂ solution produced from mixed of CaO with destiled water and stirred about 1 h to get uniform mixing as shown in below equation.



In order to produce HAp, solution of 0,3 M H₃PO₄ was added to solution of 0,5 M Ca(OH)₂ by using titration technique at rate 1 ml/minute with continuous stirring using hot plate magnetic stirrer at 600 rpm with temperature variation room temperature, 40°C, 60°C and 80°C. The PH value of solution was controlled and maintained minimum PH 8 after titration. Ammonium hydroxide (NH₄OH) solution was added if PH value below 8. The solution formed was precipitated for 24 h. The precipitate that formed was filtered with filter paper whatman 42

and dried at 100°C for 5 h then calcinated at 1000°C for 3 hours.

2.3 Characterization of Hap

FTIR spectra were obtained using spectrophotometer (model 100 series, Perkin Elmer) over a range 4000 cm^{-1} to 500 cm^{-1} at a 1 cm^{-1} resolution averaging 32 scans. FTIR spectroscopy analysis to identify the functional groups in HAp powder. The elemental analysis of HAp and morphology of HAp were obtained using SEM instrument with energy dispersive x-ray spectroscopy (EDS) system. The phase composition of material were determined by X-Ray diffractometer (XRD).

3. RESULTS AND DISCUSSION

3.1 FTIR Analysis

FTIR spectroscopy used to identify the functional groups HAp from cockle shells. The most characteristic chemical groups in FTIR spectrum of synthesized HAp are PO_4^{3-} , OH^- , and CO_3 . The results of HAp synthesized from cockle shells (Fig. 1) showed that differentiation of the temperature synthesis affects the transmittance band shape. Phosphate groups (PO_4^{3-}) asymmetric stretching vibration synthesized was detected at wavenumber 1026 cm^{-1} which indicates that the crystals formed in the synthesis [9].

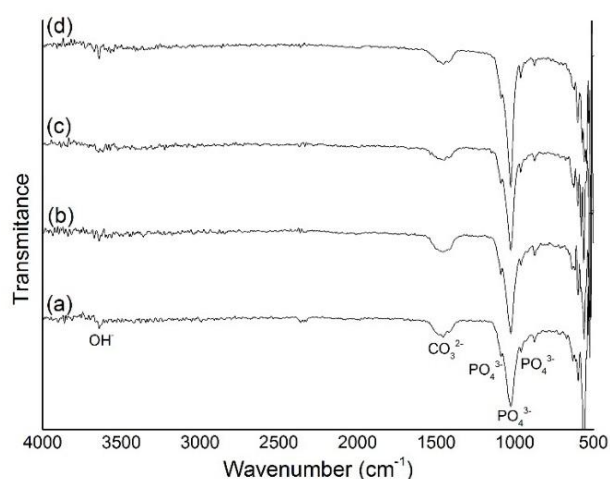


Figure 1. FTIR spectra of HAp synthesized from cockle shells at (a) room temperature, (b) 40°C, (c) 60°C, (d) 80°C [10].

PO_4^{3-} asymmetric stretching vibration also sharper because the nature of vibrating atom moves faster at higher temperature. PO_4^{3-} symmetry stretching vibration was detected at all variation temperature with wavenumber 960 cm^{-1} , 960 cm^{-1} , 964 cm^{-1} , 964 cm^{-1} which it was indicated the formation of HAp. PO_4^{3-} asymmetric bending vibration also was detected at all variation temperature. Two characteristic stretching modes of O-H bands at 474 cm^{-1} and 3572 cm^{-1} are noticed in temperature all various temperature of synthesis [11]. However, characteristic peak of CO_3 also was detected at all various temperature synthesis. The presence of CO_3 caused by HAp synthesized in the open air [12].

3.2 Study of Ca/P ratio with SEM-EDS

The result of SEM-EDS analysis to showed Ca/P ratio of HAp and to determined the element composition of HAp. Table 1 represent the EDS spectra of HAp at various temperature synthesis. The result presence of element organic cockle shells was observed and the Ca/P ratio

to compared with the theoretical ratio which is 1,67. Ca/P value of HAp at temperature of 40°C to be 1,64 which is quite close to theory. Its important because from Ca/P affect the HA phase stability, degradability, reactivity, densification and mechanical properties of sintered body [13].

Table 1 The element composition present in the HAp sample with different temperature synthesis

Weight of element composition (wt%)	Room temperature	40°	60°	80°
O	47,07	49,29	47,19	26,30
P	17,37	15,50	14,21	10,58
Ca	35,56	32,69	31,78	25,93
Zr	-	-	-	6,52
C	-	2,52	6,82	30,67
Ca/P	1,58	1,64	1,73	1,89

The result of EDS analysis showed that two elements of calcium (Ca) and phosphorous (P) main elements. These results also indicate that HAp samples contain Zirconium (Zr) as impurities from ball milled equipment which made from metal. Carbon (C) is not part of chemical composition of cockle shells because carbon is the material of choice for coating non conductive samples to allow for SEM EDS analysis.

3.3 SEM Analysis

The morphologies of the HA particles prepared at different synthesized temperature are shown in Fig. 2. The SEM result indicated different morphology forms of HAp are formed when the temperature synthesis are varied. The particles synthesized at room temperatures showed high tendency to agglomerate and irregular shape.

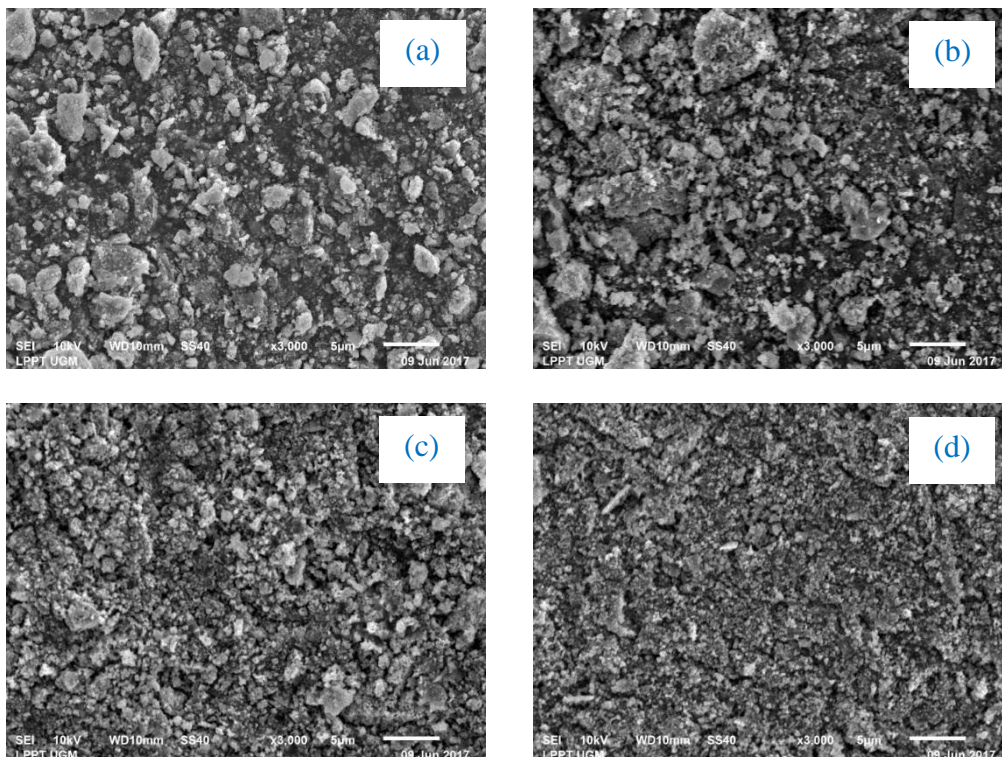


Figure 2. The SEM images of HAp at different temperature synthesis; (a) room temperature, (b) 40°C, (c) 60°C, (d) 80°C.

On the other hand, for samples prepared at temperature 80°C, the particle showed less aggregation and regular shape. HAp powder at room temperature, 40°C and 60°C have a high tendency to agglomerate and flat agglomerated structure with different size. A transition temperature morphology from irregular to regular shape of HAp is at 60°C. This result is in good agreement with the result reported by Niakan et al. [14] which explained agglomerate of HAp maybe caused by grinding effect. The change from irregular to regular particle morphology with the increase in synthetic temperature was corresponding to the increase of the crystallinity of HAp, that is, more regular shape of the particles was observed when the powders had higher crystallinity [15].

3.4 XRD Analysis

The XRD patterns of the HAp at different temperature synthesis are shown in Fig. 3. The result of XRD patterns was analyzed by comparing with standard diffraction pattern of JCPDS No. 09-0432 in range 20° - 60°. Fig. 3 shows the XRD pattern with temperature synthesis at room temperature, 40° C and 60° C has a pattern that is almost similar, where established HAp. It can be seen from three main peaks, namely, 211, 112 and 300 planes at 2θ near 31,77°, 32,19° and 32,9°, respectively.

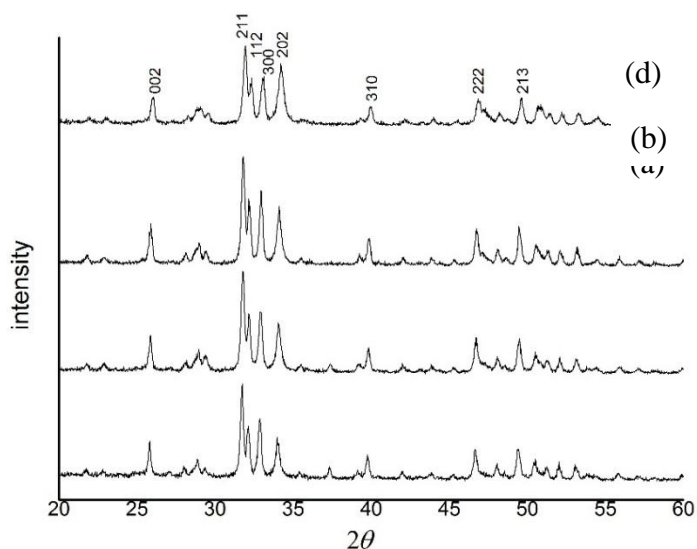


Figure 3. XRD patterns of HAp synthesized at (a) room temperature, (b) 40°C, (c) 60°C, 80°C.

It is obvious that the intensity of XRD pattern increases with increases temperature synthesis (room temperature, 40°C and 60°C). Increasing the diffraction peaks indicated increasing of crystallinity. While at 80°C has a pattern not similar with three highest peak of HAp. Its also compatible with the result of EDS which shown ratio Ca/P is 1,89. Crystallite size in a direction perpendicular to crystallographic of samples was calculated using Scherrer's equation as follows:

$$D = \frac{0,94\lambda}{B \cos \theta} \quad (4)$$

Where D is the crystallite size (nm); λ is the wavelength of monochromatic X-ray ($\lambda=0,15406$ nm for $\text{CuK}\alpha$); B is the Full Width at Half maximum (FWHM) for peak of diffraction (rad) and θ is the diffraction angle (°). The sharper diffraction peak at $2\theta=31,6$ was chosen. The result of crystallite size (Table 2) showed crystallite size decreases with increasing temperature synthesis. It also consist studies by Ahmad fadli [9] explain that the increase temperature resulted increased thermal vibration energy. Its also compatible with the result of SEM which show tenuous when temperature synthesis is lower.

Table 2 The crystallite size based on result of various temperatures synthesized

Temperature synthesis	Crystallite size (D)
Room temperature	72,76
40°C	63,73
60°C	62,2
80°C	54,19

The crystallite size of HAp found to be range 54,19-72,76 nm. The result of crystallite size showed that HAp from cockle shells has crystallite size smaller than HAp commercial (D = 151 nm). Additionally it has been reported that small crystallite's size was beneficial much higher bioactivity [13].

6. CONCLUSIONS

This study showed that cockle shells can be used as a precursor resource for production of HAp. Effect of temperature synthesis HAp from cockle shells has been presented. FTIR results showed that characteristic chemical groups HAp have been detected at all various temperature synthesis. The morphology of HAp strongly depend on the temperature of synthesis. Increasing temperature synthesis induced a change of morphology from irregular shape to regular shape. EDS results that Ca/P value varies between 1,58 and 1,89. XRD results that temperature synthesis also depend on crystallinity and crystallite size. The result of crystallite size showed crystallite size decreases with increasing temperature synthesis. According results of characterizations, HAp from cockle shells can be synthesized optimum temperature at 40°C. It can be seen from the XRD results that 40°C has a pattern of pure HAp, The SEM-EDX showed the Ca/P value of 40°C close to theory and FTIR showed the presence of functional group.

ACKNOWLEDGMENT

The authors immensely grateful to Indonesian Ministry of Research, Technology, and Higher Education through PUPT Grant (2456/UN1.P.III/DIT-LIT/LT/2017) for the financial support in this research. The authors would like to thank also to LPPT UGM for the technical assistance.

REFERENCES

- [1] D.S Gouvenia, A.H.A Bressiani, J.C Bressiani, *Trans. Tech. Publications* **531** (2006) 593.
- [2] T. Laonapakul, *KKU Engineering Journal* **42** (2015) 269.
- [3] S. Santhosh, S. B. Prabu, G. Campus, *International Journal of Nanoscience* **11** (2012) 1.
- [4] Direktorat Jenderal Perikanan Tangkap, *Statistik Perikanan Tangkap Indonesia* (2010).
- [5] A. Hamidi, *Decomposition of Calcium Carbonate in Cockle Shell* (2013).
- [6] N. Jamarun, Z. Azharman, S. Arief, T.P. Sari, *J. Chem.* **8** (2015) 133.
- [7] E. Bouyer, F. Gitzhofer, M.I. Boulos, *Journal of Materials Science: Materials in Medicine* **1** (2000) 523.
- [8] W.P.S.L. Wijesinghe, M.M.M.G.P.G Mantilaka, E.V.A Premalal, H.M.T.U Herath, S. Mahalingam, M. Ediringhe, R.P.V.J. Rajapakse, R.M.G Rajapakse, *Materials Science and Engineering C* **42** (2014) 83.
- [9] A. Fadli, F. Akbar, P. Putri, D. I. Pratiwi, I. Muhara, *The 1st Convergence on Ocean, Mechanical and Aerospace* (2014) 24.
- [10] Y. Rizkayanti, *Master Thesis, Universitas Gadjah Mada, Indonesia*, (2017).

- [11] B. Mondal, S. Mondal, A. Mondal & N. Mandal, *Materials Characterization* **121** (2016) 112.
- [12] M.Sari, Y. Yusuf, *International Journal of Nanoelectronics and Materials* **11** (2018) 357
- [13] S. Ramesh, K.L. Aw, R. Tolouei, M. Amiriyani, C.Y. Tan, M. Hamdi, *Ceramics International* **39** (2013) 111.
- [14] A. Niakan, S. Ramesh, P. Ganesan, C.Y. Tan, J. Purbolaksono, H. Chandran, S. Ramesh, W.D. Teng, *Ceramics International* **41** (2015) 3024.
- [15] Y.X. Pang, X. Bao, *Journal of the European Ceramic Society* **23** (2003) 1697.

