



Research Article

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Synthesis of hydroxyapatite by hydrothermal method from cockle shell (*Anadara granosa*)

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ABSTRACT

Synthesis of hydroxyapatite(HAp), $C_{A10}(PO_4)_6(OH)_2$, using cockle shell (*Anadara granosa*) as a source of calcium has been carried out using a hydrothermal method through three routes, namely: direct synthesis of powdered shells; synthesis through the process of thermal decomposition powdered shells and synthesis through the formation of Precipitated Calcium Carbonate (PCC) of the shells. HAp synthesized result has been analyzed using X-ray Diffraction (XRD), scanning electron microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The analysis result of the synthesis product has been obtained that HAp ceramic synthesis through the formation of PCC gives the best results, which is in the synthesis conditions 16 hours at a temperature of 140°C.

Keywords: cockle shell, hydroxyapatite, hydrothermal, Precipitated Calcium Carbonated

INTRODUCTION

Indonesia is a country which is rich in natural resources, both from natural materials or in the form of natural products[1]. Resource of calcium could be found from natural materials such as egg shells, corals, shells, bones, and fish scale or from the natural formations such as limestone [1-7]. Among the abundant natural resource availability is a waste of shells, such as cockle shell (Fig. 1). Cockle shell (*Anadara granosa*), is soft animals (Mollusca) of the class Bivalvia. The main composition of cockle shells (>95%) in the form of $CaCO_3$ [8,9]. Calcium from the shells could be processed into products that are more efficient in the industry or the medical field, such as Precipitated Calcium Carbonate (PCC) and hydroxyapatite (HAp) [9,10].



Fig. 1. The cockle shells

PCC is a compound of calcium carbonate (CaCO_3), which could be processed from raw material limestone [1,7,8] through a series of chemical reactions. PCC excess compared with Ground Calcium Carbonate (GCC) is more pure in particle, homogeneous and uniform with micro-scale particle size. PCC is currently widely used in industry as an additive for pharmaceuticals, food, paper, plastic, ink [8]. Jamarun [1, 2], using the PCC, which is made of by limestone as raw material in the synthesis of carbonate apatite. A high level of purity and small particle size and uniform, the PCC advantage to be transformed into apatite compounds [7-13].

In this study, waste shells would be used as a source of calcium in the manufacture of PCC. In the next stage, the PCC of shells are used as a raw material synthesis of hydroxyapatite. Hydroxyapatite compound is known very widely used in the field of biomedical for filler and coating on bone and dental implants [4-6]. Because of the porous structure, and has chemical and thermal resistance, hydroxyapatite has also been very widely used as a catalyst or adsorbent [14-16].

HAp powders can be synthesized from natural sources or chemicals through various synthesis techniques, using a range of different reactants. Some processing techniques include precipitation, hydrothermal, hydrolysis, mechano-chemical, sol-gel process, etc. [5, 7]. In this research, the Hap is prepared hydrothermally through three routes of synthesis, namely: the direct synthesis of powder shells; synthesis of CaO shells [17] and the synthesis of PCC shells, which are made through a modified carbonation method [1,2]. The hydrothermal method is a techniques that is popularly used, because its simplicity and of low-cost. The resulting commonly well crystallized and chemically homogeneous [21].

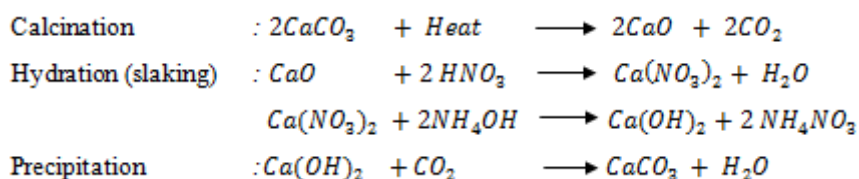
Morphology, particle size, and the purity of the resulting HAp powder, affect the properties of HAp, which would be applied in the fields of orthopedics, dental making materials, as well as a catalyst or adsorbent. Hopefully, through a hydrothermal process for PCC made from waste precursor shells, would be produced HAp which have a smooth and uniform size, so it could be used in the biomedical field as a bone filler and coating for excellent osteoconductivity.

EXPERIMENTAL SECTION

The materials were the cockle shells, which was collected from Tembilahan, Indera Giri Hulu, Riau; diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$), Merck; 2M HNO_3 solution; ammonium hydroxide (NH_4OH) 65% (Merck); CO_2 gas and aquadest. The cockle shells samples were washed and cleaned, and then sun dried for two days followed by oven dried at 110°C for two hours. Dry and clean cockle shells were then crushed and grounded with pulverizer into the cockle shells powder form.

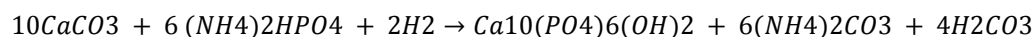
2.1.PCC formation of shells

The cockle shells powder (0,105mm) was calcinated in the furnace around 5 hours at 900°C . The calcined cockle shells was then converted into the PCC by carbonation method with the addition of nitric acid solution (HNO_3). PCC producing through carbonation method was carried out following the procedure Jamarun *et al* [1,2]. These processes could be represented by the following reactions [1,2,7,11]:



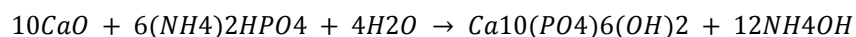
2.2 Synthesis of Hydroxyapatite by Hydrothermal Method

Hydroxyapatite producing through hydrothermal processes was carried out following the procedure Hien *et al* [17]. There were three routes of synthesis. Direct synthesis performed by varying the reaction time (16, 20, 24, 28, 32 hours) at a temperature of 180°C . A total of 5 grams of powder shells that have been refined (0.105 mm) was added into 15 ml of saturated $(\text{NH}_4)_2\text{HPO}_4$ with the mole ratio of $\text{Ca/P} = 1.67$. A small amount of high concentrated NH_4OH was added to adjust the pH into 10-11. Hydrothermal reactions were performed in sealed vessels, placed in the oven. Once the reaction is complete, the synthesized solids were washed with distilled water until neutral, then dried in an oven. Samples of pure dry and ready to be characterized. Reactions that occur on this route is [17,18]

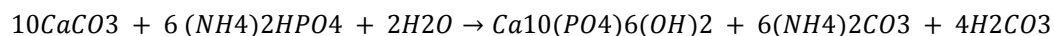


The second route was done by using CaO of shells. CaO powder was obtained from the calcination process shells for 5 hours at a temperature of 900°C . A total of 5g of CaO shells added into 15 ml of saturated $(\text{NH}_4)_2\text{HPO}_4$ the

mole ratio of Ca/P=1.67. Synthesis was done by varying the reaction time (16-32 hours) at a temperature of 180°C, follow the first route. Reactions that occur on this route [17]



Synthesis of HAp of PCC shells is the novelty of this research. Synthesis procedure follow these routes before, 5g PCC of shells added to 15 ml of a saturated solution of $(\text{NH}_4)_2\text{HPO}_4$ the mole ratio of Ca/P = 1.67. Synthesis is done by varying the reaction time (16-32 hours) at a temperature of 180°C. The reaction that occurs in the third route:[17,18]



The best route of synthesis HAp based on the analysis by XRD, would be continued by varying the reaction temperature at 140, 160 and 180°C. Furthermore, HAp synthesized tested toxic nature of the growth of *Lactobacillus cereus* bacteria in Nutrient Agar media. First of all samples were sterilized by autoclaving at 120°C, 1 atm pressure for 20 minutes. Then the sample HAp (0.1g) were placed into Nutrient Agar culture media. Observations were made every 24 hours for 3 days[6].

2.3 PCC and Hap Characterization

The mineralogy of the PCC and HAp were characterized using X-ray diffraction (X'Pert Powder DY 3688) with Cu radiation $\text{K}\lambda$. The surface morphology was probed using scanning electron microscopy (SEM) linked to energy dispersive X-ray micro analysis (EDX) (JEOL JED 2300). The Fourier Transform Infrared Spectroscopy (FT-IR, Perkin Elmer Spectrometer Frontier) was used to analysis the bonding structure in the samples.

RESULTS AND DISCUSSION

Formation of Precipitated Calcium Carbonate (PCC) was done with carbonation method modified by adding nitric acid on calcium oxide dissolution process. PCC crystals were analyzed by X-ray diffraction, the results were compared with the X-ray pattern of original shells (Fig. 2a) and standard CaCO_3 as shown in Figure 2(a-d).

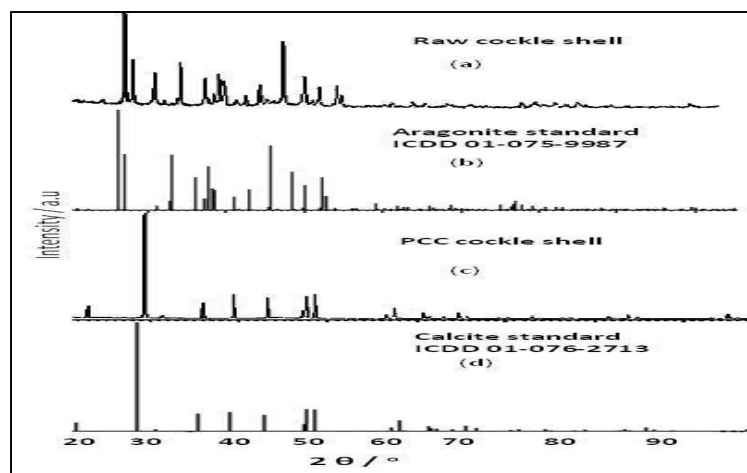


Fig.2. XRD diffractograms of product: (a) CaCO_3 of raw cockle shells, (b) Aragonite, CaCO_3 standard (ICDD 01-075-9987) (c) PCC of cockle shells (d) Calcite, CaCO_3 standard (ICDD 01-076-2713)

X-ray diffraction pattern of the PCC cockle shell (Fig.2c) were compared with data JCPDS Calcite (01-076-2713) (Fig.2d). That's found three peaks with high intensity corresponding to the X-ray pattern of a standard calcium carbonate (calcite) that was at an angle $2\theta = 29.36; 39.37; 48.46$. X-ray diffraction pattern of the PCC was different from the original shells. CaCO_3 shells have the kind of aragonite crystals, while the PCC of shells is calcite type.

SEM images for analysis of PCC morphology is shown in Figure 3 (magnification a. x100 and b. x500). PCC particles produced had the square form and uniform size (crystallite size 0.6 - 1.0 μm). PCC was used then as a base material for the manufacture of hydrothermally hydroxyapatite (the third route).

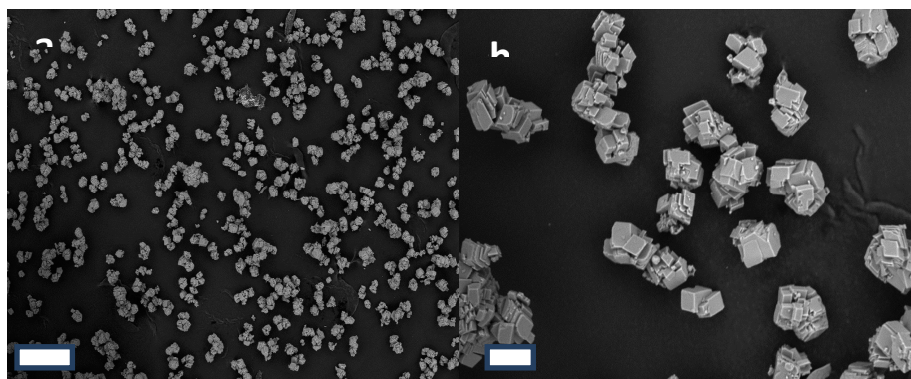


Fig. 3. SEM image of cockle shells' PCC, (magnification a. x100 and b. x500)

The results of X-ray diffraction analysis on all three synthesis routes at 180°C temperature variation synthesis time (16 to 32 hours), the synthesis of Hap was obtained through PCC gives the best results. Product synthesis through the first route was still dominant in the form of CaCO_3 (aragonite) with a bit of HAp. Results sintering at a temperature of 800°C, obtained intensity higher HAp phase while aragonite phase was converted to tricalciumbis (phosphate) ($\text{Ca}_3(\text{PO}_4)_2$, TCP). Hien *et al* [17] reported the first synthesis through the produce HAp in very small amounts, even when the reaction time reached 128 hours. In this study, HAp has been obtained in a shorter synthesis time (20 hours). This was possible, because the particle size of the shells were smooth enough (0.105 mm), while the coral Hien *et al* [17] sufficiently large particle size (about 5x5x5 mm), so that the penetration $(\text{NH}_4)_2\text{HPO}_4$ easier happen to CaCO_3 shells.

Synthesized through the second route (CaO), was found HAp phase mixed with other calcium phosphate phases such as TCP, DCP or CaCO_3 and $\text{Ca}(\text{OH})_2$. Hien *et al* [17] reported the synthesis of HAp best condition of coral at a temperature of 180°C with a reaction time of 36 hours. In this study, HAp has been produced in a reaction time of 20 hours, however still mixed with $\text{Ca}(\text{OH})_2$. Synthesis of HAp through the PCC gives the best results, which was found only one kind of calcium phosphate phase, the HAp and a little calcite of raw materials, the reaction time of 16 hours. This could be happened due to the PCC particles used were very pure and very fine size, so that the shorter reaction time (16 hours) has generated HAp. The results of XRD analysis of synthesis of HAp through different pathways at 180°C for 20 hours, is shown in Figure 4 (a-e).

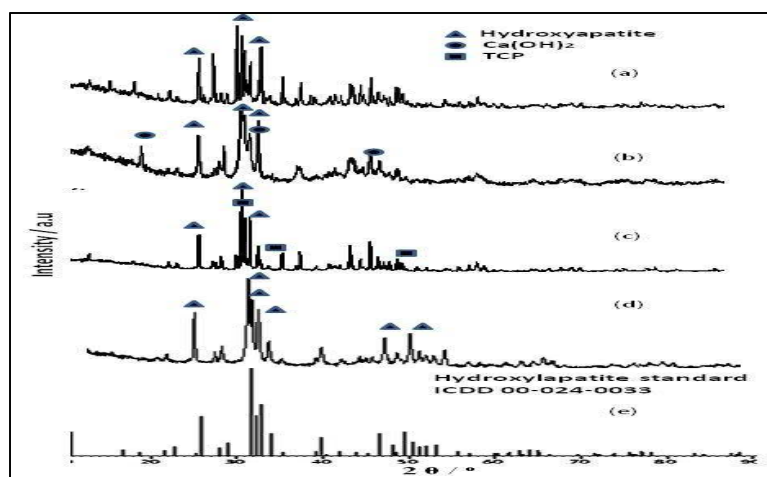


Fig. 4. XRD diffractograms for the products of synthesis using three routes at 180°C for 20h(a) direct synthesis (b) CaO cockle shells, before sintering (c) CaO cockle shells, after sintering (d) through formation PCC (e) standard of Hydroxyapatite (ICDD-PDF 00-024-0033)

Furthermore, the synthesis of HAp through formation PCC varied temperature at 140, 160 and 180°C for 16 hours. The best results were obtained in the synthesis temperature 140°C for 16 hours. Figure 5(a-d) was the result of XRD analysis of HAp of PCC at various temperatures for 16 hours. X-Ray diffraction pattern of apatite compounds synthesized gave very similar results to the standard XRD pattern hydroxyapatite (5d, JCPDS 01-074-9780). That's found some peaks with high intensity corresponding to the X-ray pattern of hydroxyapatite standard that is at 2θ angles: 25.87; 31.73; and 32.86. Based on those analyzes, it could be concluded that the synthesis result of

hydroxyapatite at 140°C during 16 h is the best product, own hexagonal crystal system and crystallites size 36-50 nm. The mean grain size of the crystallites of the HA particles (D) was calculated using the Scherrer's equation

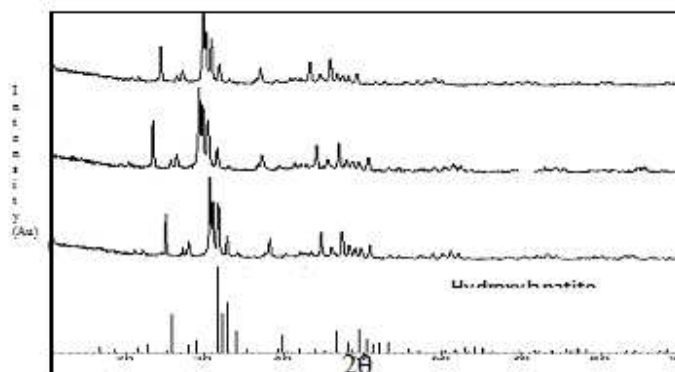


Fig. 5. XRD diffractograms of the hydroxyapatite synthesis from PCC at varied temperatur for 16 h(a) at 180°C (b) 160°C (c) 140°C (d) standard

The analysis of FTIR spectroscopy of the compounds synthesized at various temperatures and reaction time, it could be seen the specific spectrum of patterns for the PO_4 adsorption of hydroxyapatite (Fig. 6). Meanwhile, in the FTIR spectrum is found the P-O stretching asymmetric adsorption that come from PO_4^{3-} on from 1150 to 1000 cm^{-1} , the which was known as the characteristics of bands for hydroxyapatite and, a medium intensity band at about 960 cm^{-1} due to the symmetric stretching vibration were observed. The bending vibration of PO_4^{3-} was observed by bands located at 560-620 cm^{-1} . Adsorption bands at 3642 and 631 cm^{-1} corresponding to OH group and HPO_4^{2-} at 2163 cm^{-1} [3,17,21,25]. Adsorption of free H_2O band is very weak. In addition, some content is also seen carbonate (CO_3^{2-} peak around 1400 cm^{-1}), the which was an indication of the presence of carbonate or calcium carbonate apatite. This might have originated through the absorption of carbon dioxide from the atmosphere [19].

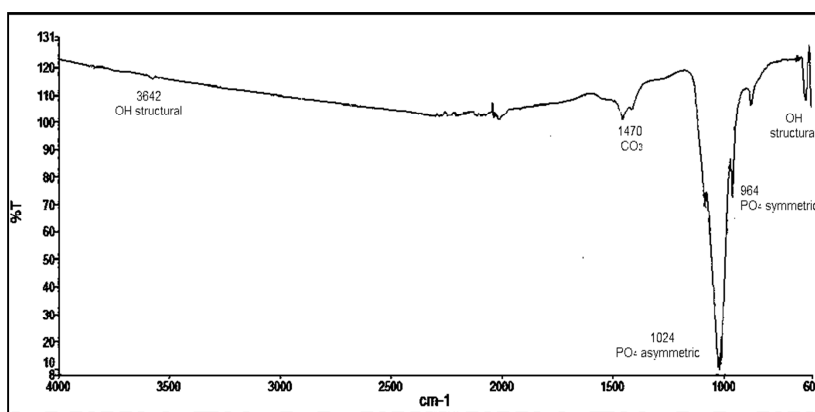


Fig. 6. FTIR spectrum hydroxyapatite of cockle shells' PCC at 140°C for 16 h

Figure 7 showed the results of SEM analysis of apatite compounds synthesized at a temperature of 140°C with a reaction time of 16 hours of PCC shells (magnification a. x100, b. x500, c. x1000 and d. x1500). From the SEM images, visible synthesized HAp had the shape and size of the particle that is almost uniform. Content of elements present in the hydroxyapatite could be confirmed from the EDX data. The Ca/P molar ratio of the hydroxyapatite that was synthesized from PCC cockle shell was about 1.78. The measured Ca/P ratio for this produced powder was higher than the stoichiometric ratio (1.67) expected for a pure HA phase. According to the EDX existence of data that shows small amounts of aluminum from phase calcite (CaCO_3), the source of matter is much more reasonable.

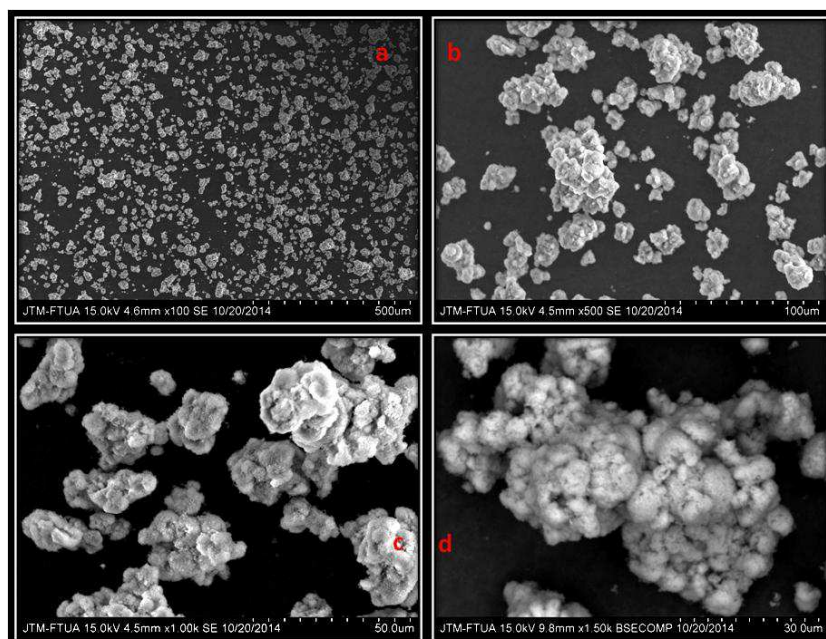


Fig. 7. SEM image of hydroxyapatite compound of cockle shells' PCC

Cytotoxicity evaluation of Hap against *Lactobacillus cereus* bacterial growth in Nutrient Agar media, was observed after incubation for 24, 48 and 72 hours. Observations showed that HAp (0.1%) did not interfere and no toxic to the growth of *L.cereus* bacteria. Fig. 8 shows HAp particles incubated for 48 and 72 hours along with NA media. It confirms that there is no toxic effect of the synthesized HAp materials were made from cockle shells waste.

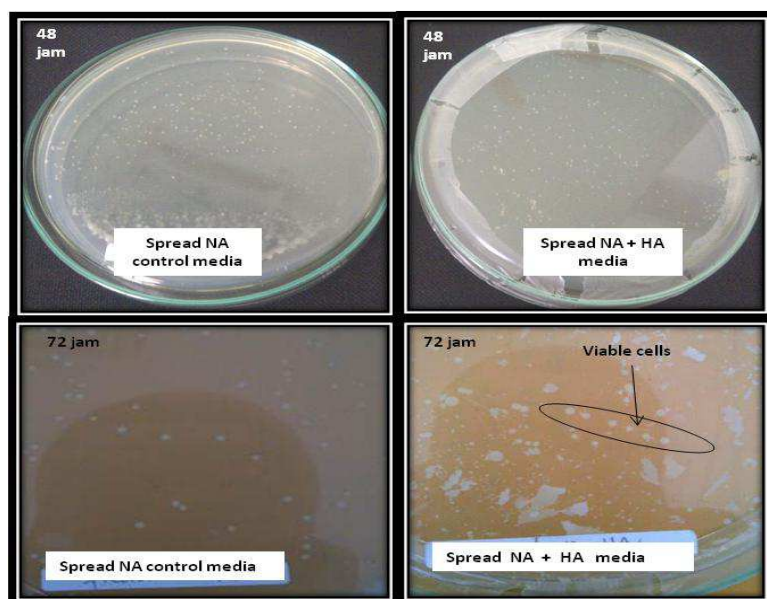


Fig. 8. Cytotoxicity evaluation of HAp against *Lactobacillus cereus* bacterial

CONCLUSION

Synthesis of hydroxyapatite of waste shells hydrothermally, using three routes of synthesis, providing the most excellent HAp results through the establishment Precipitated Calcium Carbonate (PCC). Shorter reaction time and lower temperature, an advantage of PCC formation pathway in this study. The best conditions of synthesis of HAp were obtained at a temperature of 140°C with 16 hours reaction time; produce nano-sized hydroxyapatite crystals (36nm) with Hexagonal crystal structure. HAp was obtained from cockle shells X-ray diffraction patterns and characterization FTIR compared with Hap standard was very appropriate. The synthesized HAp materials were made from cockle shells waste there is no toxic effect

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