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Hydroxyapatite Preparation from Crab Shell Waste by Precipitation Method

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Abstract -- Hydroxyapatite is a biomaterial that is widely used in the medical field because of the nature and content of the same as the materials used to replace damaged tissue in the human body. Hydroxyapatite (HAp) can be obtained by chemical synthesis. In this study, hydroxyapatite synthesis method is done precipitation method. There are four stages in the research process, the first stage is the preparation and sterilization of crab shell waste. The second stage is calcination of crab shell waste to produce CaO. The third stage is the synthesis of HAp using the precipitation method. The fourth stage is the XRD and SEM characterization, and analyzes the characterization results. The result of this research is the synthesis of HAp biomaterial from crab shell waste has been successfully carried out using the precipitation method. The resulting synthesized hydroxyapatite is HAp with crystal phase. The system is hexagonal crystals formed. The space group for hydroxyapatite compounds formed is P 63 / m. There are other compounds formed during the synthesis of hydroxyapatite are compounds Mg-whitlockite ($Ca_9HMgO_{28}P_7$). The form of hydroxyapatite obtained is in the form of agglomeration. Where the single particle characteristics tend to be spherical. The size of the hydroxyapatite formed is relatively small at the nano-meter scale. The resulting hydroxyapatite has a particle size in the range between 200 - 1000 nm. The biggest size is 1028 nm and the smallest around 267 nm.

INTRODUCTION

Hydroxyapatite (HAp) is a biomaterial that has a hexagonal crystal structure and has a calcium/ phosphorus (Ca/P) ratio of 1.67. Apatite calcium contained in bones and teeth have identical characteristics with HAp [1]. HAp or can be called calcium apatite has formula $Ca_5(PO_4)_3(OH)$, but usually written $Ca_{10}(PO_4)_6(OH)_2$ this is done to make it look that the crystal unit cell contains two molecules [2]. HAp composition of human bone in about 65%. HAp is also owned by the human tooth structure, namely dentin and enamel. So that a very significant role HAp materials in the health world [3]. Synthetic hydroxyapatite (HAp) is an HAp which is produced by chemical synthesis. Chemical synthesis process can be obtained by reacting synthetic compounds and also by reacting the synthetic compounds with natural compounds [2]. Synthetic HAp is a good implant material used as a substitute for bones and teeth because it has bioactive, biocompatible, and osteoconductive properties [4]. These properties are the same as natural bone minerals, so synthetic HAp can be used as a substitute for bones and teeth. In addition, HAp, which is synthesized using natural materials, have a better osteoconductivity then the osteoconductivity properties of HAp from synthetic materials [1,5].

HAp can be made from synthetic and natural calcium sources. Synthetic calcium sources are generally used for the synthesis of HAp is CaO [6], $Ca(NO_3)_2$, $Ca(OH)_2$, and $CaCO_3$ [7]. Natural sources of calcium used for HAp synthesis generally have high levels of calcium, including Bovine bone [8], Camel bone [9], Horse bone [10], Pig bone [11], Fish

scale [12], Fish bone [13], Cockle shell [14],Clam shell [15], Sea shell [16], Egg Shell [17], Duck egg shell [18], Mussel shell [19] and Limestone [20]. HAp manufacture of natural ingredients are better than chemical synthesis. HAp from natural materials is nonstoichiometric because there are elements such as Na^{+}, Zn^{2+}, Mg^{2+}, K^{+}, Si^{2+}, Ba^{2+}, F^{-}, and CO^{3}_{2-} which makes the resulting HAp the same as that of the human body [1,5]. In this study, the source of calcium used in HAp synthesis was crab shell waste.

The crab is one of the preferred types of seafood wider community, because it tastes good and also has a high protein content. Just utilization is still limited to the food requirements by taking flesh alone while the shell is not utilized. Crab shell waste can cause air pollution and soil. The content of calcium compounds ($CaCO_3$) found in crab shell waste is relatively high, namely 53-78% of the dry shell weight [21]. This allows it to be used as a base for making HAp. With a large $CaCO_3$ content, crab shells can be used as a precursor for CaO by calcining at a temperature of 1000^{o}C. Kalsinasai aims to eliminate the carbonate ions that can interfere with the synthesis process [22].

HAp synthesis can be carried out by various methods. There are three methods of synthesis of HAp used is in between dry method (solid-state and mechanochemical), wet method (chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, and sonochemical) and the use of high temperatures (combustion and pyrolysis) [2]. Synthesis of HAp using the precipitation method has many advantages, namely the by-product is water, and the possibility of being contaminated during the synthesis process is low, so that it can produce HAp with high purity, relatively cheap synthesis costs, and the process is quite easy [23]. Moreover this method has a simple reaction, it is possible to apply diindustri large scale and does not produce waste that can pollute the environment [24]. HAp synthesized will be characterized to see the quality of the resulting HAp physics. Physical characterization using X-ray diffractometer (XRD) to see crystallinity and surface morphology using an electron microscope (SEM). Thus, the purpose of this study is a synthesis of HAp biomaterials from natural materials ie crab shell waste by using precipitation method.

METHOD

There are four stages in the research process, the first stage of preparation and sterilization of crab shell waste. The second stage is calcination of crab shell waste to produce CaO. The third stage is the synthesis of HAp using precipitation method. The fourth step is to characterize HAp using XRD and SEM [24].

A. Sample Preparation and Sterilization

The crab shell waste used is taken from the waste of a fish seller in the Kramat teak market, East Jakarta. A total of 50 grams of crab shell waste is cleaned using distilled water. Then crushed to the size of the sample becomes smaller. Furthermore, the sample is dried in the sun until the sample is dry.

B. Sample Calcination

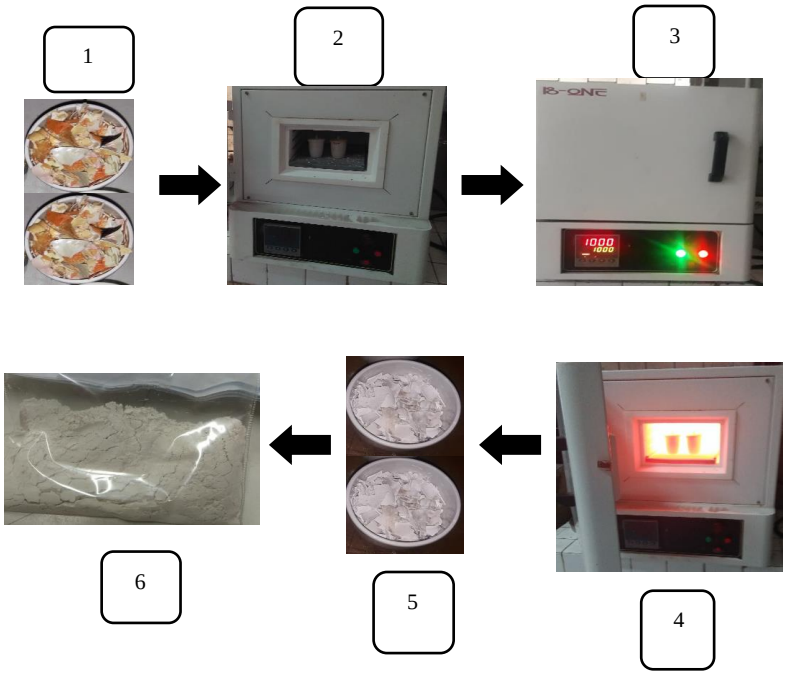
Crab shell waste prepared in the previous stage was calcined using a heating furnace at a temperature of 1000^{o}C for 10 hours. Then the sample is cooled for 10 hours. Furthermore, the results in the form of CaO calcination crushed using a mortar (Figure 1).

C. Synthesis of HAp

Place the aluminum foil on the digital balance then calibrate the digital balance that will be used. Next, weigh 2.83 grams of CaO powder and 3.97 grams of $(NH_4)_2HPO_4$ using a digital balance so that the Ca /P ratio is 1.67:1. Calcined CaO powder which has been weighed is

then dissolved with 100 mL distilled water in a beaker glass and is considered the first solution. Then $(NH_4)_2HPO_4$ which had been weighed in the previous stage was dissolved using 100 mL distilled water in a beaker glass and considered the second solution.

The precipitation method is done by dropping the second solution into the first solution. The process is to put the first solution on top of the magnetic stirrer. Drop the second solution into the first solution using a burrete and a stative. Adjust the burrute drop rate with a flow rate of 10 ml/ minute and stir the combined solution at 350 rpm for 100 minutes using a magnetic stirrer to make it homogeneous. Closed the beaker glass using aluminum foil after the two solutions have been mixed and homogeneous. Then it is deposited for one night. Furthermore, the sediment filter results using filter paper. Wash off the precipitate to remove unwanted compounds. Then the filtering results are put into crussible and dried at 110°C for 3 hours, then move the crussible into the furnace for the sintering process at 900°C for 5 hours. Furthermore, the sintering results were crushed using a mortar and characterized using XRD and SEM (Figure 2).



Information :
1. Sample: Crab shells waste
2. The sample is put in a crucible and pressed into the furnace
3. Furnace preheated up to 1000°C
4. Furnace withstands the temperature for 10 hours
5. Furnace crab shells waste samples
6. CaO powder

Figure 1. CaO Calcination Process

Information :
1. CaO and (NH4) 2 HPO4 solution
2. The CaO solution is dropped by a solution of (NH4) 2 HPO4
3. Precipitated for 1 night
4. Filtered and washed
5. Dried
6. Sintering for 5 hours

Figure 2. Hydroxyapatite Synthesis Process

D. Characteristics of HAp

2.4.1 Phase Analysis (Characteristics Using XRD). XRD characterization was carried out to see the purity of the HAp produced. The trick is to observe and identify the type of sample HAp crystal phase of the research, then the observed size of the crystals formed and the lattice parameters and the degree of crystallinity. The results of these observations were then matched with the original HAp data which is usually obtained from the JCPDS (joint committee on powder diffraction standards).

2.4.2 Morphological Analysis (Characteristics Using SEM). The morphological analysis of the samples was performed using a scanning electron microscope (SEM). The scans were performed at 2500x, 5000x, 10000x magnifications. The purpose of this analysis is to see the shape of the resulting HAp surface, the resulting particle size and the uniformity of the HAp particle shape.

RESULT AND DISCUSSION

A. Efficiency of Crab Shell Hydroxyapatite

CaO compounds used in the synthesis stage is a compound of the shells of crabs CaO calcined for 10 hours. The result of the synthesis is hydroxyapatite in powder form. Efficiency hydroxyapatite produced in the synthesis process can be seen in Table 1 below.

Table 1. Efficiency of HAp

CaO Source	Mass (gram)			Efficiency (%)
	CaO	$(NH_4)_2HPO_4$	HAp	
Crab Shell	2,83	3,97	3,36	49,42

The mass of the HAp compound is much smaller than the mass of the total compounds CaO and $(NH_4)_2HPO_4$. This is because in the process of mixing two compounds to be powder required heating using an oven, causing the evaporation process, wherein the compound in the form of liquid evaporates into a gas. So that the mass of the resulting HAp is smaller than the combined mass of the two compounds CaO and $(NH_4)_2HPO_4$. In addition, the HAp compound that has been deposited before drying will be filtered first, during the filtering process some mass is wasted by distilled water. Perhaps if the filter used when filtering sediment is changed to a filter capable of filtering a smaller size, then the hydroxyapatite efficiency obtained would have been greater. If seen the efficiency of HAp obtained by 49.42%, this value is not too bad means that half of the mass can be converted into synthesis of hydroxyapatite. When compared to the mass of CaO as a source of calcium used in the synthesis process, 2.83 grams can produce 3.36 grams of hydroxyapatite.

B. Hydroxyapatite Analysis with XRD

The results of the synthesis carried out on CaO crab shell 2.83 grams and (NH4) 2HPO4 3.97 grams resulted in HAp 3.36 grams compounds. The results obtained are relatively small. This is because the crab shell CaO compound has a smooth texture, so that when washed and filtered, some compounds are carried by distilled water. Then the HAp compound obtained was characterized using XRD. Figure 1 is the diffraction pattern of hydroxyapatite.

Measurement of the x-ray diffraction pattern and identification of the HAp phase produced by the precipitation method (Figure 1) is characterized by diffraction peaks between the 2θ angle of 10–65. HAp phase identification is done by comparing the HAp diffraction pattern with the HAp diffraction pattern in the COD database (Crystallography Open Database).

Peaks that appear in Figure 1 are matched with the help of software and found that the patterns formed in figure 1 according to the the hidoksiapatit compound on COD database (Crystallography Open Database) with the entry code 00-230-0273. From the COD database, information was obtained that the hydroxyapatite compound formed was the crystalline phase. The crystal system formed is hexagonal. The space group for hydroxyapatite compounds formed is P 63/m. The crystal peaks in the XRD data for the hydroxyapatite compound are at the 2θ angle : 25.84; 31.62; 32.64; 49.51. From the peaks that appear there are some peaks not show hydroxyapatite compound that is at an angle 2θ: 27.98; 31.16; 34.64. After matching the peaks using software and it was found that the pattern formed was a compound of Mg-whitlockite ($Ca_9HMgO_{28}P_7$) in the COD database (Crystallography Open Database) with the entry code 00-901-2415. From the COD database, information was obtained that the Mg-whitlockite compound formed was in the crystalline phase. The crystal system formed is tigonal (hexagonal axes). The space group for the Mg-whitlockite compound formed is R3c.

The peak that has the highest intensity is Mg-whitlockite, this means that the amount of Mg-whitlockite compounds produced is more dominant than hydroxyapatite compounds. While hydroxyapatite compound formed has peaks with lower intensity.

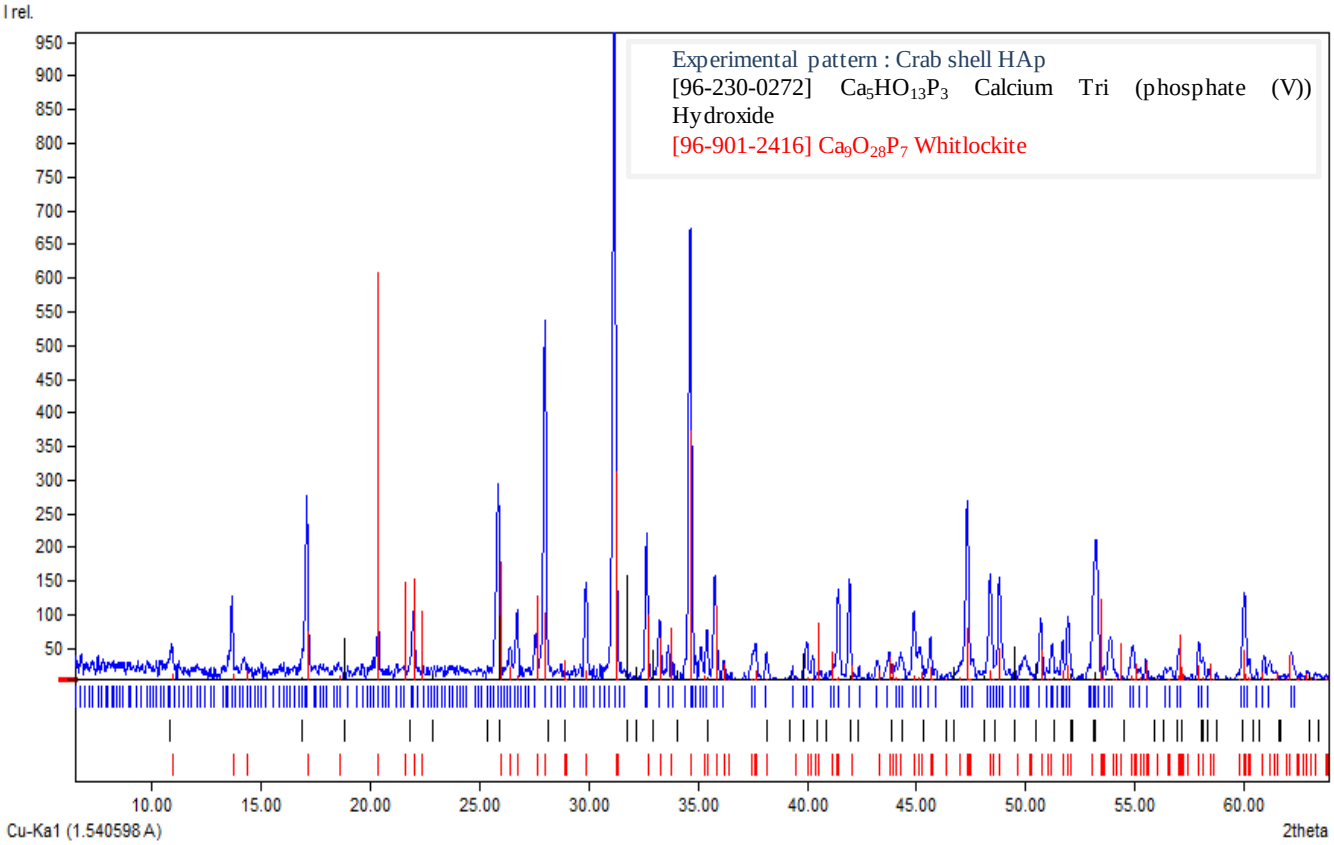


Figure 1. XRD diffraction pattern of hydroxyapatite from crab shells with a source of CaO calcination time of 10 hours.

The low hydroxyapatite formed is probably due to the fact that the CaO compound used contains MgO, even though it is very small. In addition, it can also be caused by not maintaining the pH during synthesis. If the pH became acidic (pH <4.2) will facilitate the formation of whitlockite [25]. In addition, excessive sintering temperature and stirring time will cause a change of functional groups where the position of Ca is substituted by Mg which comes from the crab shell component. Teymouri et al. (2008) also reported that a sample of Scenedesmus sp. microalgae used to synthesize HAp produced Mg-whitlockite as a byproduct other than HAp [26].

The existence of this phase is not harmful to humans, because Mg-whitlockite often used in pathology, especially in the dental calculus. In addition, whitlockite has a higher mechanical stress than hydroxyapatite, so it will maximize mechanical compressive strength when applied to dentistry.

C. Analysis by SEM

To see the morphology of the hydroxyapatite formed and its size, SEM (Scaning Electron Microscope) characterization was performed. Scanning Electron Microscopy (SEM) characterization of the synthesized samples was carried out to observe the surface morphology of the HAp particles. SEM instruments can identify the physical characteristics of the HAp, including the size, shape, structure, and morphology of the HAp crystals.

Figures 2, 3 and 4 are images of the surface morphology of hydroxyapatite. From the figure, it can be seen that the hydroxyapatite particles experience agglomeration or agglomeration. This is the same as what was reported by Hui et al (2010), where the observed

particles experienced agglomeration[27]. And its single particle characteristics tend to form spherical. The resulting single particle size is on the nanoscale, with a size between 200 - 1000 nm.

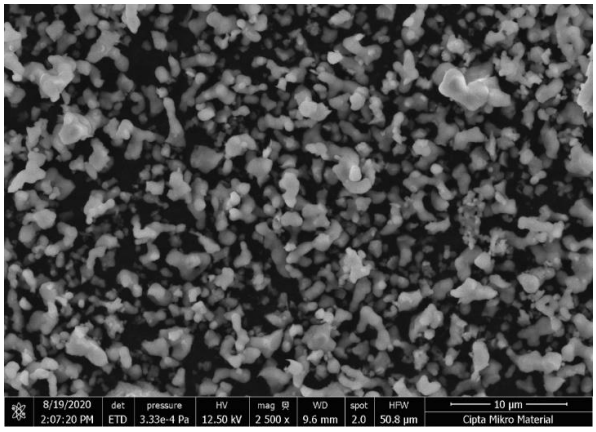


Figure 2. SEM results of hydroxyapatite with a magnification of 2500x

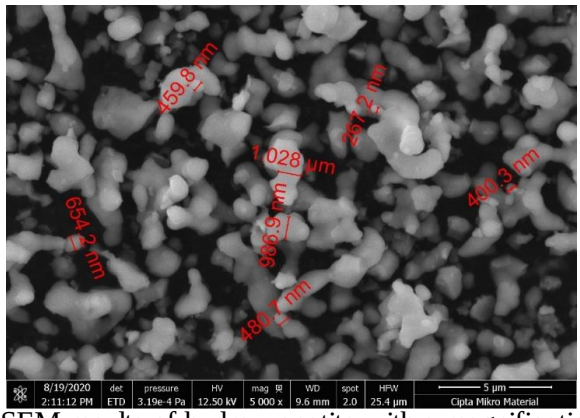


Figure 3. SEM results of hydroxyapatite with a magnification of 5000x

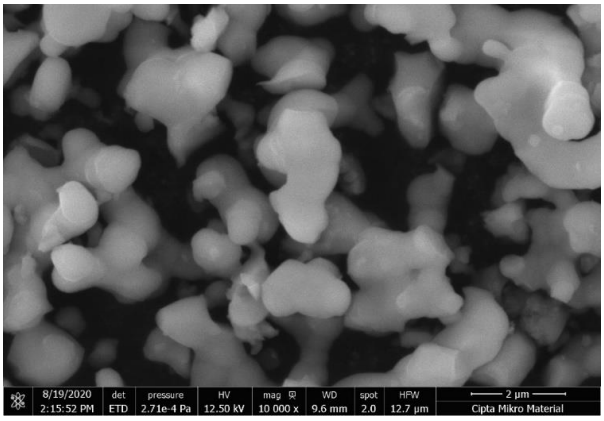


Figure 4. SEM results of hydroxyapatite with a magnification of 10000x

This means that the particles that are formed are nanoparticles, this means that the size is already small, making it easier for application in the field of dentistry. The largest size is 1028 nm and the smallest is around 267 nm. The difference between the largest and the smallest particles is quite far, apart from that the particle size varies considerably, so it can be

concluded that the characteristics of the single particles that are formed tend to be not homogeneous. These results are consistent with previously reported studies which state that HAp crystals tend to agglomerate [28]. However, the particle size is larger than that reported in previous studies with a measuring range of 210–410 nm [29]. This may be due to the presence of MgO impurities in the CaO sample, which affects the resulting particle size.

CONCLUSION

The synthesis of HAp biomaterial from natural materials, namely crab shell waste using the precipitation method has been successfully carried out. The resulting synthesized hydroxyapatite is HAp with a crystal phase. The crystal system formed is hexagonal. The space group for hydroxyapatite compounds formed is P 63 / m. There are other compounds formed during the synthesis of hydroxyapatite crab shell waste are compounds Mg-whitlockite ($Ca_9HMgO_{28}P_7$) with a crystalline phase. The crystal system formed is tigonal (hexagonal axes). The space group for the Mg-whitlockite compound formed is R3c. The form of hydroxyapatite that is formed is to form agglomeration or agglomeration. Where the characteristics of the single particle tend to be in the form of a spherical shape. The size of hydroxyapatite formed relatively small at the nanoscale. The particle size of hydroxyapatite from crab shell waste has a particle size of hydroxyapatite with between 200 - 1000 nm. The largest size is 1028 nm and the smallest is around 267 nm.

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