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Synthesis of Hydroxyapatite Based on Nano Coral Using precipitation Method For Bone Substitution

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Abstract *:* The synthesis of hydroxyapatite based nano coral by precipitation method already has been done. This method is chosen because it does not need to use organic solvents, simple, cheap and effectively. The selection of coral as a base material because the material has a fairly high calcium content. Moreover, this element is a major component of bone and availability in Indonesia is abundant. By using 3D HEM (High Energy Milling) for 20 hours milling can be formed 64.93 nm coral powder. The treatment of calcination of $CaCO_3$ at 900^0C for 3 hours on this coral powder produced CaO. Dissolution of this material in aquades can be formed Ca $(OH)_2$ and this study uses the concentration of the solution as research variables, namely (0.6, 0.85, 1.1, 1.35, 1.6) M. Hydroxyapatite can be formed by reacting calcium hydroxide solution $Ca(OH)_2$ with phosphoric acid (H_3PO_4) in a stirrer and then precipitated. Observation of the precipitate formed the occurrence of hydroxyapatite and β-TCP (β-Tricalcium Phosphate) phases. The calcium hydroxide concentration that produces the most optimal hydroxyapatite is at a concentration of 0.85 M resulting in a hydroxyapatite phase of 95.3% and a β-tricalcium phosphate phase $Ca_3(PO_4)_2$ of 4.7%. The ratio Ca/P in this concentration is 1,66, shows that almost same with an ideal hydroxyapatite 1,67, and this hydroxyapatite is a good candidate for bone substitution.

Keywords: *Hidroxyapatite, Precipitation, Coral*

1. Introduction

Bone is an important part of the human body. This material has several functions that store calcium and phosphorus, as a muscle lever to provide body movement, blood forming elements as well as storage of minerals and energy. In addition, bone as part of the skeletal system provides structural support for the whole body, as well as protecting vital organs, such as brain and heart. This material is strongly designed and durable in order to perform such functions [1] . Activity and mobility of a person will be disrupted if there is imperfection in the bone. Bone abnormalities or disorders can be caused by various factors, such as accidents, malnutrition, disease, age, and heredity.

Now, bone imperfections can be improved by various methods, such as giving filler or using graft. Grafts can be autographs, allographs, or xenographs. In use, the graft has a positive side and a negative side. The use of these three materials has immunogonic risks and their umbers are limited [2]. Another material that can be used as an implant is Hydroxyapatite. This material is biocompatible, biodegradable and has a mineral component similar to bone [3].

The hydroxyapatite used as an implant may be a powder, a porous solid, or a thin film according to need [4]. Characteristics and size of hydroxyapatite particles are very important in their application because they affect the development of new bone cells. pure hydroxyapatite is characterized by a molar ratio of Ca / P of 1.67. In these circumstances, the material will have a physical and mechanical properties similar to the original bone. High purity and crystallinity can affect the density, strength, and bioactive properties of the material [5] .

Hydroxyapatite can be obtained from natural and synthetic materials. The use of natural materials is safer compared to synthetic materials because of the risk of cross-reaction and other reactions lower [6] . In addition, the availability of natural mineral as a raw material of hydroxyapatite in Indonesia is

quite abundant. One of these natural materials is coral [7] . Coral contains high enough calcium. This mineral is one of the main components of bone. Research conducted [7] , showed that the content of $CaCO_3$ in fossilized coral was 92%. $CaCO_3$ compounds are the main ingredients of hydroxyapatite through the formation of CaO compounds [8]. The use of coral is also more economical because the material is abundant in Indonesia.

Synthesis of hydroxyapatite can be done through various methods, such as solid state, precipitation, sol-gel, and hydrothermal. The precipitation method is more effective and more economical than other methods. In addition, hydroxyapatite dissolves in water, which facilitates the precipitation process . [3]. In the precipitation process, the results obtained depend on several variables such as precipitation time, temperature, solvent and solvent concentration [9].

Research on the synthesis of hydroxyapatite by precipitation process has been done by several researchers. Vazquez, et al. [3] (2005) in the preparation of hydroxyapatite using a precipitation process through sintering (800-1400)˚C) obtained the Ca/P ratio is 2.04. Ungureanu, et al. [10] showed that at a temperature of 1200˚C, CaO compounds were formed.. Soejoko, et al. [11] found that the result of precipitation of the stirred solution at 70ºC has a relatively higher mass compared with the precipitant mass produced by the solution at 25ºC.

Other variables that influence the result of the precipitation process are solvent and soluble concentration. The solute in hydroxyapatite synthesis is calcium hydroxide ($Ca(OH)_2$). Calcium hydroxide is formed from a compound of calcium oxide (CaO) from a coral reacted with distilled water. This $CaCO_3$ compound serves to provide calcium and hydroxide groups. While acting as solvent is phosphoric acid (H_3PO_4). This phosphoric acid is responsible for the phosphate group (PO_4). Precipitation occurs through the process of nucleation and crystal growth. In the pre-nucleation process it contains one calcium ion which is in equilibrium with the triphosphate. The pre-nucleation forms a branch with a reaction-limited aggregation (RLCA) process. Reaction-limited aggregation (RLCA) is the result of repulsive interactions between particles and clusters where only part of the collision causes coagulation. The nucleation process in the formation of amorphous calcium phosphate occurs through the binding of additional calcium ions and aggregation. This process is also called post-nucleation. This post-nucleation is the basis of the crystalline structure of octacalcium phosphate (OCP). The crystalline growth process is an increase in the size of the crystals of the solute deposits in the crystal surface layer. This continues until all materials undergo consolidation and form HA crystals, and eventually form HA particles [12].

The amount of solute and solvent concentration is directly proportional to the amount of calcium phosphate in the hydroxyapatite phase [13]. In addition, the higher the concentration will multiply the molecule, so that the collision that occurs between molecules will be more. This will form calcium phosphate compounds on hydroxyapatite faster [8] (Herlinawati, 2015). Therefore, this study focused on precipitation process with variation of solute concentration to obtain pure hydroxyapatite phase. The particle size of the sample can be determined from the PSA (Particle Size Analyzer) results. And to know the composition and the amount can use XRD (X-Ray Diffraction).

2. Materials and Method

The corals used in this study were obtained from the coast of Banyuwangi, east Java, Indonesia. Other materials needed include phosphoric acid (H_3PO_4), glycerol, and aquadest. The corals are cleaned of dirt and dried in the open space. Then the coral is destroyed manually to become a smaller particle. After sufficiently fine, the sample was sieved using a 200 mesh sieve. The sieve results are then milled for 20 hours with sample ratio and ball mill is 1:20 . Furthermore, PSA test was done to find out the particle size of the sample. The obtained nanoparticles are then heated at 900 ° C to remove some unnecessary elements or compounds. In addition, this heat treatment to convert $CaCO_3$ into CaO compounds. The next step is to characterize the CaO powder using XRD to determine the phase composition. Ca $(OH)_2$ solution is prepared by reacting coral CaO with distilled water. The $Ca(OH)_2$ concentration was varied to obtain the optimum hydroxyapatite compound, i.e (0.6, 0.85, 1.1,

1.35, 1.6) M. The concentration of phosphoric acid used in this study was 1 M. The Ca $(OH)_2$ was mixed with H_3PO_4 using a stirrer for 2 hours to obtain a homogeneous solution. This mixing is done at a temperature of 70˚C to accelerate the occurrence of collisions between ions as the beginning of precipitation. Furthermore, the solution is allowed to stand for 24 hours at room temperature to form precipitate. In this precipitation process occurs ion collection (cluster aggregation) to form amorphous calcium phosphate. Then the precipitate is filtered using filter paper and washed using distilled water. To eliminate the water content, dehydration process is done at 110 ° C. After drying, the sample precipitate turns into a crystalline phase by heated at 9000C for 5 hours.

3. Results and Discussion

The coral raw material and the smoothed material with 200 mesh screen are shown in FIG. 1. This is done to separate large particles so that the milling process can be done shorter. Furthermore, coral milling using 3D HEM (High Energy Milling) for 20 hours resulted in 64.8 nm coral powder (figure 2).

(a) (b)

Figure 1. (a) Coral (b) Smoothed coral 200 mesh

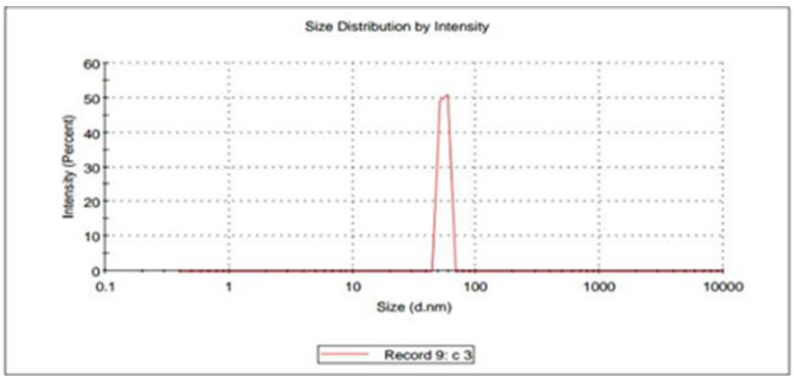


Figure 2. PSA coral with 20 hours milling

The XRD observations on coral powder measuring 58.68 nanometers were performed to confirm the constituent compounds. By using search match program , we can show that corals contain 94.4% $CaCO_3$ (Aragonite) and impurities of Ca_2SiO_5 (Dicalcium Silicate) of 5.6% (Figure 3). The next process is coral nano calcination at a temperature of 900˚C to remove some unnecessary elements or compounds and to form CaO from $CaCO_3$. The XRD data of this treatment is shown in Figure 4. The search match analysis shows that the identified phases of coral XRD yield after calcination are CaO and Ca $(OH)_2$, respectively 4,2 % and 95,8 %. Heating at 900 ° C for 5 hours to form CaO compounds. This can occur due to the breaking of the bonds present in $CaCO_3$ into CaO and CO_2 gas compounds.

The formation of Ca $(OH)_2$ is due to CaO bond with H_2O. CaO compounds are unstable compounds that make it very easy to interact with H_2O in the air.

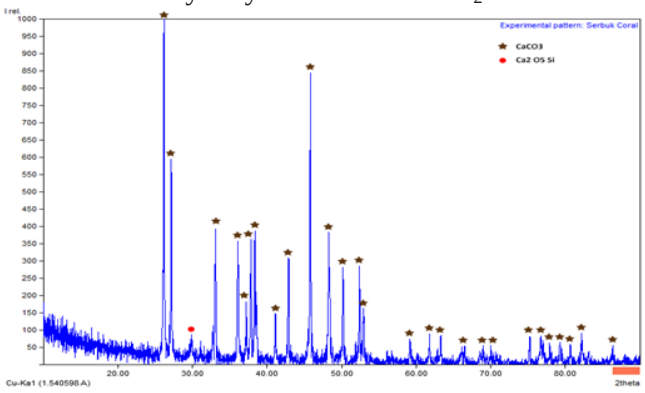


Figure 3. XRD data of corals before the calcination treatment

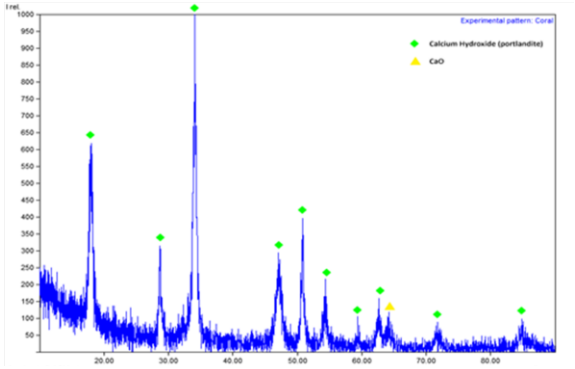


Figure 4. XRD data of corals after the calcination treatment

Hydroxyapatite synthesis was performed after identification of calcium hydroxide in calcined powder and is done by mixing calcium hydroxide calcined powder with phosphoric acid. After the process of mixing, precipitation, and filtration calcium phosphate are obtained. The compounds identified in the precipitate are brushite $CaHPO_4.2H_2O$, hydrokxyapatite $Ca_5OH(PO_4)_3$, and portlandite $Ca(OH)_2$, with volume fractions of 76%, 15.6% and 8.4% respectively (Figure 5). This phase is formed because during the settling process it is in acidic condition. Chemically the process of the formation of the phase is explained by the following reaction (equation 1, 2,3). To remove water in the precipitate is done dehydration process. This treatment was carried out at 110 ° C for 3 hours. The compounds identified in the precipitate were brushite, hydroxyapatite, and monetite $CaHPO_4$, with the fractions of volume respectively 39.8%, 30.7%, and 29.4% (Figure 6).

$$5Ca(OH)_2 + 3H_3PO_4 \rightarrow Ca_5OH(PO_4)_3 + 9H_2O \quad (1)$$

$$Ca_5OH(PO_4)_3 + 2H_3PO_4 + 9H_2O \rightarrow 5CaHPO_4 \cdot 2H_2O \quad (2)$$

$$Ca(OH)_2 + H_3PO_4 \rightarrow CaHPO_4 \cdot 2H_2O \quad (3)$$

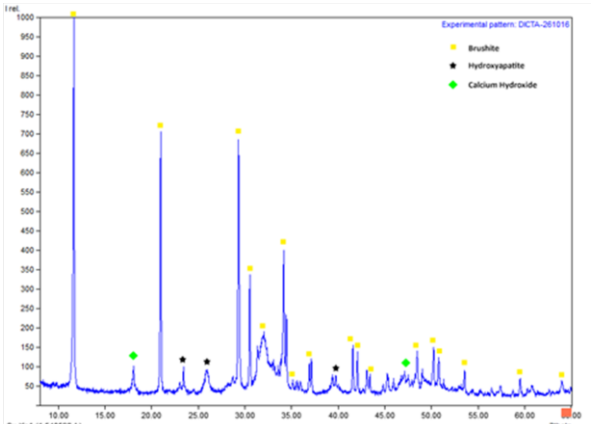


Figure 5. Diffractogram of Calcium Phosphate Deposition

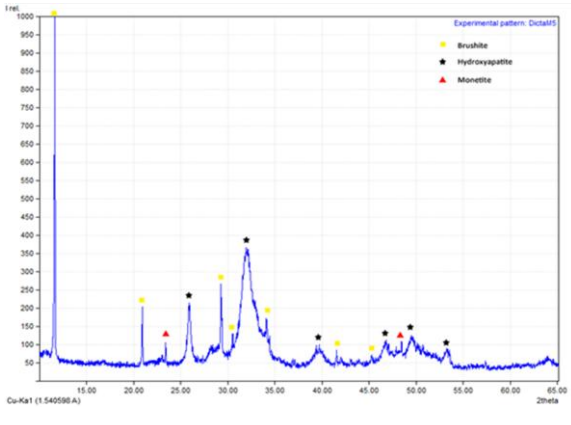
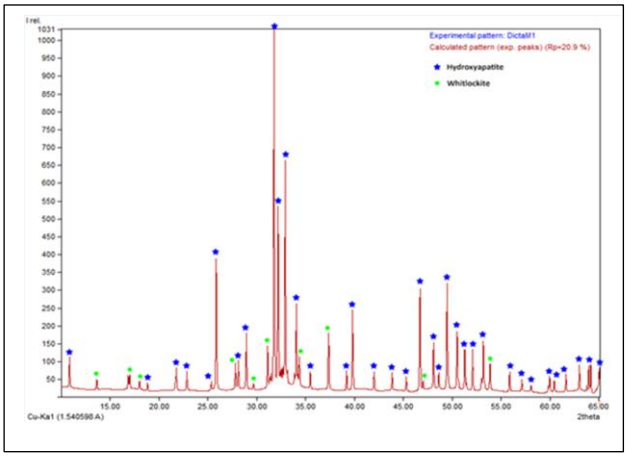
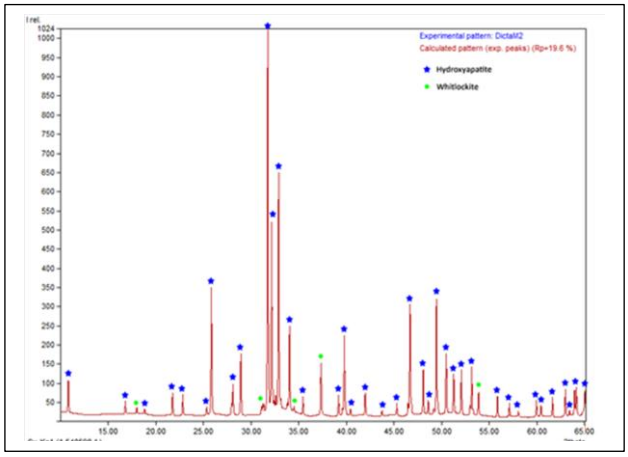


Figure 6. Diffatractogram of Hydrating precipitate

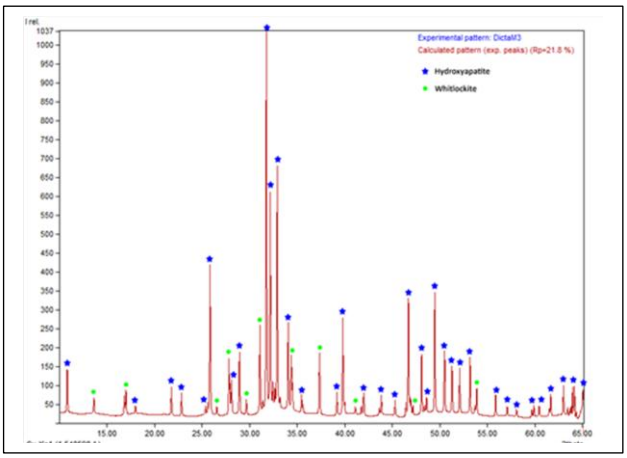
The formation of the hydroxyapatite phase was carried out by sintering precipitated dehydration at 900 ° C. for 5 hours. The phases identified in all Ca $(OH)_2$ concentrations are hydroxyapatite and β-TCP (β Tricalcium Phosphate) $Ca_3(PO_4)_2$ with volume fractions as shown in Figure 7 and Table 1.



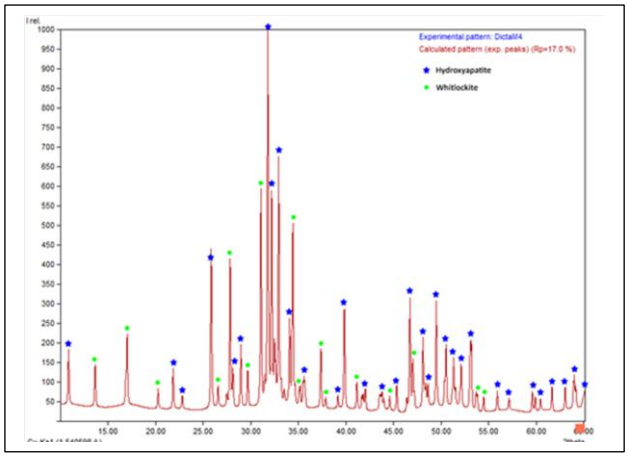
(a)



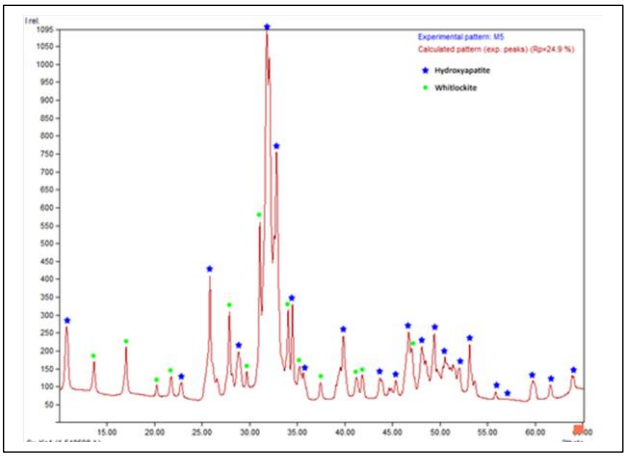
(b)



(c)



(d)



(e)

Figure 7. Diffractogram of samples after sintering at various concentration (a) 0.6 M, (b) 0.85 M, (c) 1.1 M, (d) 1.35 M, and (e) 1.6 M

Table 1. Phase indentified after sintering process

Molar (M)	Quantity (%)		Rasio of Ca/P	Crystalinity (%)
	HA	β-TCP		
0,6	86,5	13,5	1.65	77.06
0,85	95,3	4,7	1.66	79.10
1,1	82,5	17,5	1.64	78.12
1,35	59,3	40,7	1.60	76.39
1,6	49	51	1.58	76.00

Table1 shows that the highest concentration of Ca $(OH)_2$ to produce hydroxyapatite is a sample with a concentration of 0.85 M that is 95.3 %. In addition to hydroxyapatite, sintering also produces β - TCP. This compound is a decomposed hydroxyapatite due to the influence of temperature. This process occurs due to dehydroxylation (evaporation of OH groups) when sintering. The dehydroxylated hydroxyapatite forms Oksiapatite (OA) which is an unstable phase. This allows the OA to decompose into CaO, TetCP (Tetracalcium Phosphat) $Ca_4(PO_4)_2O$, or TCP (β-TCP at temperatures below 1200 ° C and α-TCP at higher temperatures). The formation of these compounds causes the Ca / P ratio to slightly deviate from 1.67, the pure hydroxyapatite value. Based on the value of the Ca / P ratio formed, this HA has good candidate as bone replacement . Based on the crystallinity of the sample varies from 76% to 79%. The result of research that has been done by Nurmawati (2007) shows that the crystallinity of human bone is in the range of 69% to 87%, so that the crystallinity of hydroxyapatite of this research is still in accordance with the original bone crystallinity. Overall, however, the samples were quite good, especially the samples with concentrations of 0,6 M and 0.85 M had candidates for bone replacement.

4. Conclusion

Some conclusions from the results of measurement and data analysis of this study are as follows. Treatment of coral milling for 20 hours has formed a powder with an average particle size of 64.93nm with a percentage of calcium hydroxide of 95.3%. The calcium hydroxide concentration that produces the most optimal hydroxyapatite is at a concentration of 0.85M . Based on the percentage of HA formation and crystallinity, the calcium hydroxide concentration sample 0.6M and 0.85M has good candidate as bone replacement.

5. References

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