

## Molarity optimization of calcium hydroxide in the forming of bioceramic hydroxyapatite from nano coral by precipitation method

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### Abstract

These days, hydroxyapatite as a biomaterial implant is immensely needed. The high rate of accidents, natural disasters, and bone cancer cases are the main reason for this situation. Fixing defected bones can be done by using fillers or grafts. This research aims to obtain the maximum phase of hydroxyapatite made from corals by optimizing the proses parameter of Calcium Hydroxide using Precipitation Method. Milling with High Energy Milling 3 Dimension (HEM-3D) was used so that the particles were nanometer-sized (64.93 nm) thus enhanced the reactivity of the particles. Results acquired from observation and calculations showed that sintering at 900°C consistently formed hydroxyapatite phase (HAp) and  $\beta$ -Tricalcium Phosphate ( $\beta$ -TCP) phase. The largest HAp phase was 95.3%, which occurred when the molar concentration of Calcium Hydroxide was 0.85 M. The percentage of hydroxyapatite phase formed decreases as the molar concentration of Calcium Hydroxide rises. However, the percentage of  $\beta$ -TCP phase rises simultaneously with the molar concentration of Calcium Hydroxide.

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## 1. Introduction

Bones are one of the most important parts of the human body. This material has various functions which are storing calcium and phosphor, as a lever for body movement, a blood-forming element and a storage for minerals and energy[1]. Other than that, bones as part of the skeletal system give structural support to the whole body and protect various vital organs such as the brain and heart. This material is designed to have high strength and durability in order to be able to perform these functions [2]. An individual's activity and mobility will be disturbed when there are any imperfections in the bone. Bone imperfections can now be repaired by the help numerous methods. One of the many methods that can be done is by using fillers or grafts. Grafts used can be in the form of autographs, allographs, or xenographs [3]. The usage of grafts as bone fillers have both positive and negative sides to it. Using these three materials may cause immunogenic responses in the body and is very limited [4]. Other materials that can be used as an implant is hydroxyapatite. Hydroxyapatite is biocompatible, biodegradable and possesses similar mineral components that are present bones [3] [4]. Hydroxyapatite can be obtained from natural materials and synthesis. The usage of natural materials is safer compared to synthetic materials for the reason that natural materials have a lower risk for cross-reactions and other reactions to happen[6]. Other than that, Indonesia has abundant natural resources that can be used as raw material for the production of hydroxyapatite. One of the natural materials is coral [6]. Coral has a high composition of calcium, which is one of the main components of the bone. A study conducted by Indarwati[7]. showed that sea corals contain a high level of  $\text{CaCO}_3$ , which is 92%. The compound  $\text{CaCO}_3$  is the main component to make hydroxyapatite because of the  $\text{CaO}$  contained in it [8]. The usage of corals is also very affordable because of its abundant availability in Indonesia. Hydroxyapatite synthesis can be done using various methods, such as solid-state reaction, precipitation, sol-gel and hydrothermal. The precipitation process is more effective and affordable compared to the other methods [9]. This process is often used because hydroxyapatite is soluble in water [10]. In the precipitation process, the results obtained depend on several variables, such as the settling time, temperature, solvent and solute concentration [11]. Several types of research regarding hydroxyapatite synthesis by precipitation has been done. Hydroxyapatite synthesis uses the precipitation process at sintering ( $800^\circ\text{C}$ -  $1400^\circ\text{C}$ ) resulting in a Ca / P ratio of 2.04 [12]. At  $1200^\circ\text{C}$  sintering temperature the precipitation process shows that  $\text{CaO}$  compounds have been formed [12]. Soejoko's research shows that the precipitation results of a stirred solution at  $70^\circ\text{C}$  have a relatively higher mass than the precipitated mass at  $25^\circ\text{C}$  [13]. In addition to temperature, other variables that affect the precipitation process results are solvent and solute concentrations. The solute in the formation of hydroxyapatite is calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ). Calcium hydroxide is formed from the compound calcium oxide ( $\text{CaO}$ ) obtained from coral that is reacted with distilled water. In the formation of hydroxyapatite, this compound provides calcium and hydroxide groups. The compound that acts as a solvent is phosphoric acid ( $\text{H}_3\text{PO}_4$ ). This compound provides a phosphoric group ( $\text{PO}_4$ ). Precipitation happens through the nucleation process and crystal growing. During the pre-nucleation process, one calcium ion is in equilibrium with triphosphate. Pre-nucleation forms branches with the process reaction-limited aggregation (RLCA). RLCA is the result of repulsive interactions between particles and clusters where only a part of the collision causes coagulation. The nucleation process to form amorphous calcium phosphate occurs through the binding of additional calcium ions and aggregation. This process is also known as post-nucleation. The post-nucleation aggregation is also the basis of the octacalcium phosphate (OCP) crystal structure that is formed through further absorption of calcium ions[1]. The crystal growing process is the addition in size of the crystal from the precipitate of the solute substance on the surface layer of the crystal. Afterward, crystal stabilization occurs until all materials experience consolidation and form HA crystals, then finally form HA particles. The solvent and solute concentration is directly proportional to the amount of calcium phosphate in the hydroxyapatite phase. Additionally, the higher the concentration, the higher the number of molecules which results in increased collisions between

molecules. This will accelerate the calcium phosphate compound formation of hydroxyapatite [8]. Therefore, this research will focus on the precipitation process with solute concentration variation to obtain pure hydroxyapatite phase.

## 2. Experimental

### 2.1. Materials and Chemicals

The corals used in this experiment were collected from the sea of Banyuwangi, East Java, Indonesia. Phosphoric acid ( $\text{H}_3\text{PO}_4$ ) with 99.8% purity Aldrick, distilled water, and glycerol for the forming of hydroxyapatite.

### 2.2. Instruments

The instruments used in the experiments were digital analytical balance ((FS-AR210 Fujitsu)) with a precision of 0.0001 g, beaker glass, magnetic stirrer ( Thermo Scientific Cimatec)) 1500 rpm, spin bar, graduated cylinder with precision of 1ml, mortar, filter paper, milling ball with various sizes (diameter 1 mm, 1,5 mm and 2 mm), spatula, High Energy Milling (HEM-3D) for mixing of materials, furnace using Xinyo SX2-4-1STP , Particle Size Analyser (Delsa Nano Series, Beckman Coulter), and X-Ray Diffraction (XRD) using Philips X'Pert MPD (Multi Purpose Diffractometer) with  $\text{Cu-K}\alpha$  radiation.

### 2.3. Preparation of Samples

The research was divided into a few steps, which are the preparation of raw materials, the formation of the precursor solution, and formation of hydroxyapatite compound. Sample preparation started by cleansing the corals showed in Figure 1. After the corals were completely dry, the corals were grounded into powder. Next, the powder was sifted using a shieving 200 mesh. This was done to separate big particles and shorten the milling process. The sifted powder was then milled using *High Energy Milling* (HEM) for 20 hours. The milling process produced nano-sized coral powder. This powder was then calcinated at  $900^\circ\text{C}$  for 3 hours to form  $\text{CaO}$ .



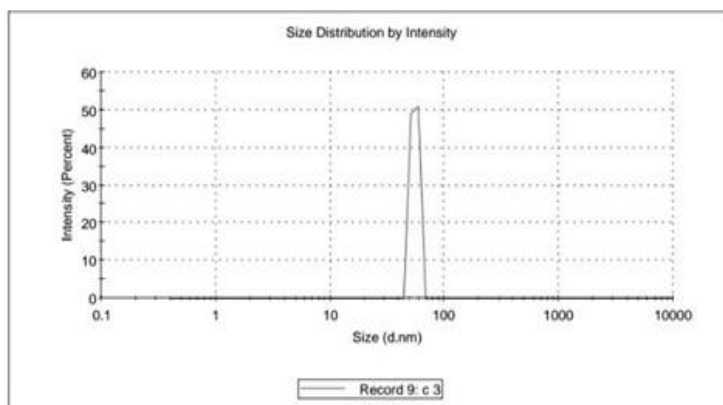
The precursor solution  $\text{Ca}(\text{OH})_2$  was formed by reacting  $\text{CaO}$  with distilled water. There were five concentration variations of the solution which were 0.60 moles; 0.85 moles ; 1.10 moles; 1.35 moles , and 1.60 moles. This was done because coral mineral has many impurities and also to obtain the optimal hydroxyapatite compound. The phosphoric acid solution concentration used was 1 M [6]. The formation of hydroxyapatite began with reaction  $\text{Ca}(\text{OH})_2$  with  $\text{H}_3\text{PO}_4$  using a stirrer for 2 hours to obtain a homogeneous solution. This reaction was done at  $70^\circ\text{C}$  to accelerate the event of ion collisions as the beginning of precipitation. Afterward, the solution was let to sit for 24 hours at room temperature with the result of the formation of a precipitate. During this process, ion aggregation (cluster aggregation) formed amorphous phosphoric acid. The precipitate was then filtered using filter paper and cleaned with distilled water. Following that step, dehydration process was done at  $110^\circ\text{C}$  to eliminate any water content. After the precipitate was dry, it was sintered at  $900^\circ\text{C}$  for 5 hours to form HA crystals.

## 3. Results and discussion

The corals used in this research were collected from Banyuwangi, East Java, Indonesia (Figure 1). The miling treatment using HEM for 20 hours with vial ratio of 1:20 formed nano-powder in the size of 64.93 nm (Figure 2).

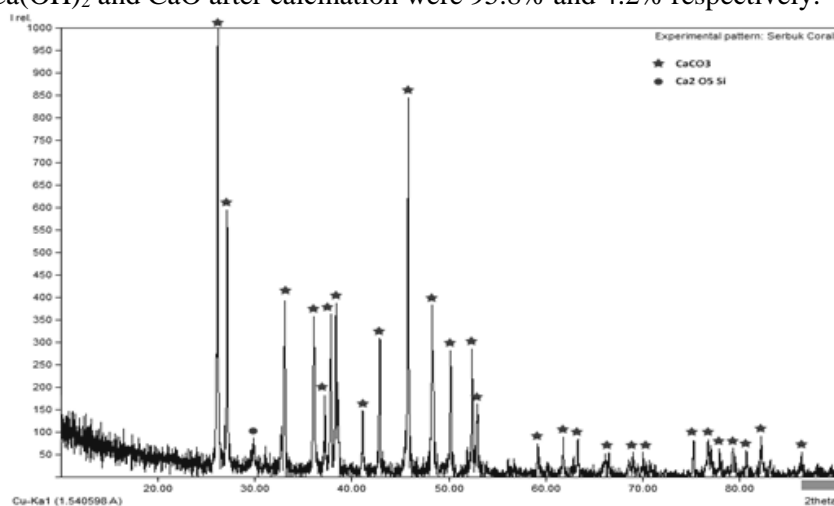


**Figure 1:** Coral raw material that has been cleaned.

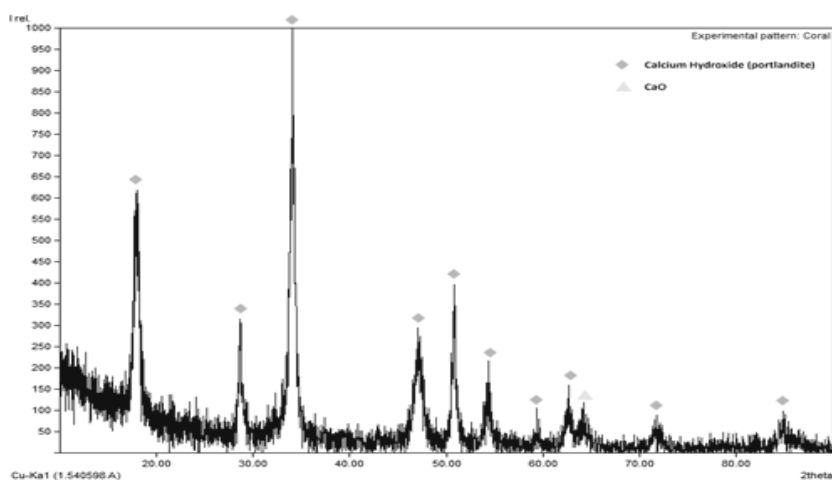


**Figure 2:** PSA data results from 20-hours milling

Identification results of XRD coral raw materials from Banyuwangi, East Java showed that the mineral consisted of the compounds  $\text{CaCO}_3$  (Aragonite) and  $\text{Ca}_2\text{SiO}_5$  (Dicalcium Silicate) with percentages of 94.4% and 5.6% respectively (Figure 3). Calcination at  $900^\circ\text{C}$  for 5 hours caused bond breakage in  $\text{CaCO}_3$  into  $\text{CaO}$  compound and  $\text{CO}_2$  gas.  $\text{CaO}$  is an unstable compound with result that it is easily bound with  $\text{H}_2\text{O}$  forming  $\text{Ca}(\text{OH})_2$ . However, the whole compound did not fully break down and react completely, there were still  $\text{CaO}$  compound remains (Figure 4). The compositions of  $\text{Ca}(\text{OH})_2$  and  $\text{CaO}$  after calcination were 95.8% and 4.2% respectively.

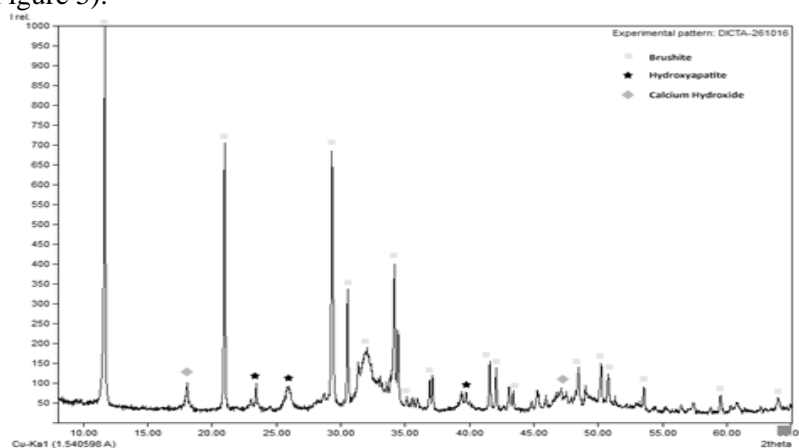


**Figure 3:** XRD spectrum of coral powder before calcination.

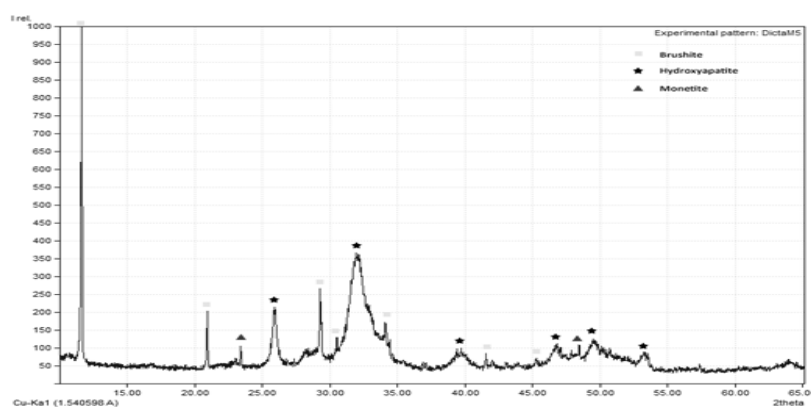


**Figure 4:** XRD spectrum of coral powder after calcination at 900°C

Hydroxyapatite synthesis was done through calcination reaction of calcium hydroxide powder (five concentration variations) with phosphorous acid. In the forming of hydroxyapatite,  $\text{Ca}(\text{OH})_2$  compound gave calcium ( $\text{Ca}^{+}$ ) and hydroxide ( $\text{OH}^{-}$ ) groups. Meanwhile, the solvent phosphorous acid ( $\text{PO}_4$ ) with the calcium group formed hydroxyapatite. Filtering the precipitate from the reaction above formed calcium phosphate. Observations of the XRD results of a sample using  $\text{Ca}(\text{OH})_2$  with 0.85 M concentration showed three phases of calcium phosphate, which are brushite ( $\text{CaHPO}_4 \cdot 2(\text{H}_2\text{O})$ ), hydroxyapatite ( $\text{Ca}_5\text{OH}(\text{PO}_4)_3$ ), and portlandite ( $\text{Ca}(\text{OH})_2$ ) with percentages of 76%, 15.6% and 8.4% respectively (Figure 5).

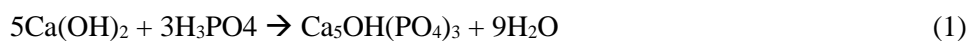


**Figure 5:** XRD diffractogram of sample precipitate



**Figure 6:** XRD diffractogram of sample precipitate after dehydration

Brushite or monocalcium phosphate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) phase has a monetite-like structure which is calcium diphosphate ( $\text{CaHPO}_4$ ) but contains crystal water. This phase was formed because of the precipitation process that was done in acidic condition. Other than brushite phase, there were also hydroxyapatite and portlandite phases in small quantities. The hydroxyapatite phase ( $\text{Ca}_5\text{OH}(\text{PO}_4)_3$ ) was formed in the precipitate because of the acidic condition explained in equation (1). The stability was caused by the incomplete dissolving of calcium hydroxide and the dissolving of the brushite phase formed that was not stable yet. The dissolving of hydroxide and brushite are directly proportional with the forming of hydroxyapatite. However, hydroxyapatite can revert to brushite when the pH increases (acidic condition) as shown in equation (2). Other than that, brushite phase can form when the calcium concentration decreases (equation 3).



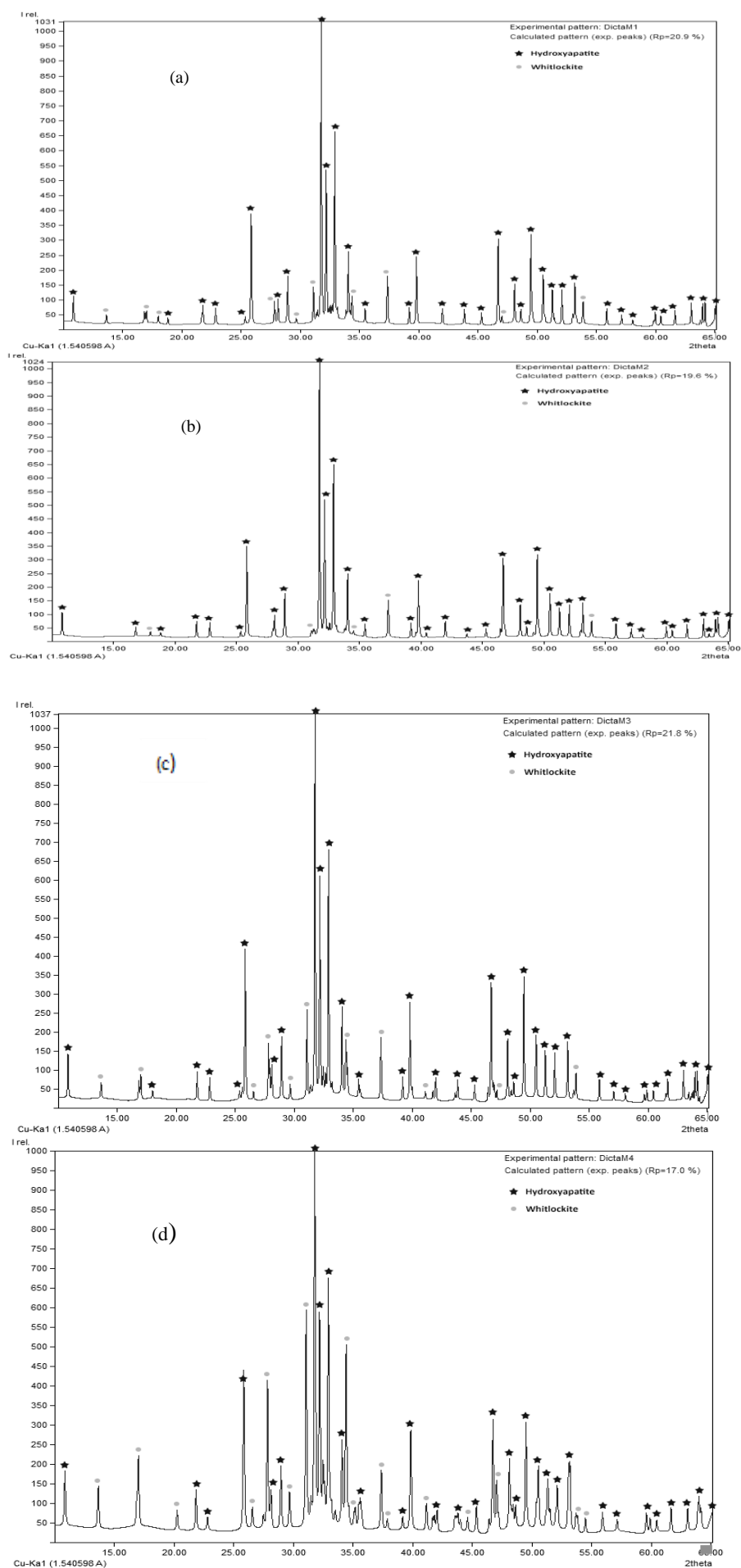
Dehydration treatment done to calcium phosphate precipitate at  $110^\circ\text{C}$  for 3 hours formed brushite, hydroxyapatite, dan monetite ( $\text{CaHPO}_4$ ) with the percentage of 39.8%, 30.7%, and 29.4% respectively (Figure 6). The forming of hydroxyapatite crystal was done with sintering at  $900^\circ\text{C}$  for 5 hours. Results of the diffraction pattern search match of the sample  $\text{Ca}(\text{OH})_2$  with molar concentrations of 0.6M; 0.85M; 1.1M; 1.35M; and 1.6M are shown in Figure 7.

Tabulation and search match results from Figure 7 are stated in Table 1. The data shows that  $\text{Ca}(\text{OH})_2$  with a concentration of 0.85M formed maximum hydroxyapatite phase with the percentage of 95.3%. When the molarity of calcium hydroxide is increased over 0.85M, the percentage of hydroxyapatite phase formed is decreased. The decrease of HAp phase effects the increase of Whitlockite phase, also known as Tricalcium phosphate ( $\beta$ -TCP). This compound is a decomposed hydroxyapatite caused by the influence of temperature. The process occurs as a result of dehydroxylation (evaporation of OH group) during sintering. Dehydroxylated hydroxyapatite forms Oxyapatite (OA) which is an unstable phase. This allows decomposed OA turn into CaO, Tetracacium Phosphate (TTCP), or TCP ( $\beta$ -TCP at the temperature under  $1200^\circ\text{C}$  and  $\alpha$ -TCP at higher temperatures) [14].

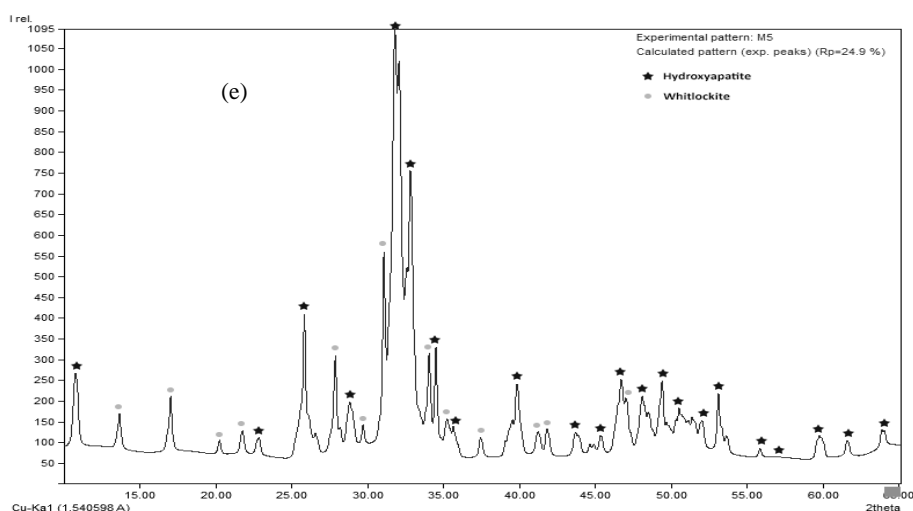
**Table 1:** Phase Quantity Samples Formed after Sintering

Molarity of $\text{Ca}(\text{OH})_2$ (M)	Quantity (%)	
	Hydroxyapatite	$\beta$ -TCP
0.6	86.5	13.5
0.85	95.3	4.7
1.1	82.5	17.5
1.35	59.3	40.7
1.6	49	51

Compared to the research conducted by Herlinawati the amount of hydroxyapatite compound formed in this research increased by 1.5% and equal with research by Jyrki Vuola [15]. The percentage increase of HAp may be a result of the smaller coral powder size used in this research (64.93nm) compared to the coral powder size used in Herlinawati's research (109.2nm). Nano-sized particles have a broader surface area which facilitates chemical reactivity during the forming of hydroxyapatite [14].







**Figure 7:** XRD samples with Na(OH)<sub>2</sub> molarities (a) 0.6M, (b) 0.85M, (c) 1.1M; (d) 1.35M; and (e) 1.6M

## 4. Conclusion

Based on the testing, observation, and results obtained from the research conducted, a few conclusions can be taken as the following. First, the PSA and XRD observation of calcium hydroxide formation shows that milling for 20 hours forms powder with an average size of 64.93nm and percentage of 95.8% of calcium hydroxide. Second, the optimum concentration of calcium hydroxide to form hydroxyapatite is 0.85M which forms 95.3% of hydroxyapatite phase and 4.7% of  $\beta$ -tricalcium phosphate phase. Third, when the molarity of calcium hydroxide is above 0.85M, hydroxyapatite phase turns into unstable  $\beta$ -tricalcium phosphate phase/

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