International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.
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https://ijpsat.org/
Vol. 46 No. 1 August 2024, pp. 298-304

Synthesis And Characterization Of Hydroxyapatite Derived From Limestone

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Abstract—Limestone has a high calcium carbonate ($CaCO_3$) content, making it an ideal source of calcium for the synthesis of hydroxyapatite. This study aims to synthesize hydroxyapatite from limestone and analyze the results by examining the phase and crystallinity. CaO was obtained through the calcination of limestone at a temperature of 1000°C for 10 hours. Hydroxyapatite synthesis was conducted by combining CaO as the calcium precursor and Diamonium hidrogen fosfat $(NH_4)_2HPO_4$ as the phosphate precursor using the precipitation method. Subsequently, the resulting samples were sintered in a furnace at a temperature of 900°C for 5 hours. After the synthesis process, the products were characterized using XRD and SEM. The synthesis results indicate that the obtained hydroxyapatite has a crystalline phase. The crystal system formed is hexagonal, with a space group of P63/m. During the synthesis of hydroxyapatite, another compound, β-tricalcium phosphate ($Ca_3(PO_4)_2$), was also formed. The resulting hydroxyapatite exhibited agglomeration properties, with individual particles tending to be spherical in shape. The size of the hydroxyapatite formed is relatively small, with particle sizes ranging from 140 to 300 nm.

Keywords— Limestone; CaO; hydroxyapatite; β-tricalcium phosphate.

I. INTRODUCTION

The increase in bone damage cases in Indonesia is caused by various factors such as age, unhealthy dietary patterns, and a rise in accidents and natural disasters. As a result, the demand for biomaterials or bone substitutes, such as hydroxyapatite, has also increased. Bone is a crucial component of the human body that serves as a passive support system. Damage to bones due to various disorders or diseases can lead to structural deformities that interfere with bodily functions. To repair these deformities, the approach used involves adding or replacing bone tissue, such as by implanting calcium phosphate-based hydroxyapatite into the bone [1] [2]. The synthesis of hydroxyapatite has become very important because, currently, Indonesia still imports hydroxyapatite used in the medical field [2].

Hydroxyapatite (HAp) is a material with a structure composed of calcium, phosphorus, and hydroxyl ions, often used as a mineral substitute in bone tissue. Hydroxyapatite is beneficial in biological applications due to its ability to facilitate optimal osteointegration, non-toxicity both locally and systemically, and lack of genotoxic activity in biological systems [3]. Most (about 65%) of the minerals found in human bones consist of hydroxyapatite. Hydroxyapatite has been widely used to repair, fill, augment, and reconstruct damaged bone and dental tissue, as well as in its application to soft tissue [4]. The calcium ions ($Ca^{2+)}$ in hydroxyapatite have the capability to bind toxic heavy metal ions and can also absorb organic chemical elements in the body. Additionally, hydroxyapatite possesses good biocompatible and bioactive properties.

HAp is an apatite mineral with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Hydroxyapatite can be synthesized by mixing calcium precursors and phosphate precursors in a Ca/P concentration ratio of 1.67. Calcium precursor in the form of $Ca(OH)_2$ can be produced through the reaction between calcium oxide and water. The synthesis of hydroxyapatite using natural materials is considered more advantageous because it can enhance bioactive, osteoconductive, and biocompatible properties [5]. The selection of natural materials in the synthesis of hydroxyapatite is based on their high calcium content. Synthetic hydroxyapatite can be made from natural materials rich in calcium, such as duck egg shells [6], Rangga clam shells [7], crab shells [8], cow bones [9], and quail egg shells [10]. In this study, limestone, which is commonly used for construction, water purification, and as an additive in paper production, will be used as a calcium precursor in the synthesis of hydroxyapatite.

Limestone is one of the abundant natural resources in Indonesia. This limestone is a solid rock that contains a significant amount of calcium carbonate [11]. Calcium carbonate is an inorganic mineral that is commercially available at an affordable price [12]. Generally, limestone contains about 95% calcium carbonate in the form of calcite, approximately 3% dolomite, and the remaining portion consists of clay minerals [13]. Therefore, limestone has great potential as a calcium precursor in the synthesis of hydroxyapatite.

II. RESEARCH METHODOLOGY

A. Preparation of Limestone Powder

The limestone used as a source of calcium in this study was sourced from Dairi Regency, North Sumatra, with a total weight of 0.5 kg. The limestone was cleaned of impurities such as crust, sand, and moss until completely clean. Next, the limestone was crushed into small chunks. After that, the crushed limestone was ground using a mortar. The resulting powder was then stored in a closed container.

B. Calcination of Limestone

A total of 10 grams of limestone powder was weighed and calcined in a furnace at a temperature of 1000°C for 10 hours to produce CaO powder. After the calcination process, it was stored in a closed container.

C. Synthesis of Hydroxyapatite

2.83 grams of CaO powder was weighed and dissolved in 100 ml of distilled water. The CaO suspension was then stirred with a stirrer for 15 minutes. Following this, 100 ml of Diamonium hidrogen fosfat $(NH_4)_2HPO_4$ solution (0.84 M) was gradually added at a rate of 5 ml/min using the wise drop method, while being stirred with a magnetic stirrer for 100 minutes at a speed of 350 rpm. After the stirring process, the suspension was allowed to settle for 24 hours, resulting in a white gel. The gel was then filtered using a Buchner funnel and washed with deionized water until neutral. Subsequently, the gel was dried in an oven at 110°C for 2 hours until it became a white powder. The resulting powder was then sintered in a furnace at a temperature of 900°C for 5 hours. After the hydroxyapatite was formed, the powder was cooled in a desiccator and weighed to calculate the efficiency of the produced hydroxyapatite. The formed hydroxyapatite was subsequently characterized using XRD and SEM.

III. RESULT AND DISCUSSION

A. Efficiency of Hydroxyapatite from Limestone

The CaO compound used in the synthesis stage was derived from limestone that was calcined for 10 hours. This synthesis process produced hydroxyapatite in powder form. The efficiency of the hydroxyapatite produced is shown in Table 1 below.

TABLE 1. Efficiency of hydroxyapatite (HAp) samples produced from CaO source.

Source of CaO	**Mass (grams)**			**Efficiency (%)**
	CaO	*Diamonium hidrogen fosfat* $(NH_4)_2HPO_4$	*HAp*	
Limestone	2.83	3.97	3.41	50,15

The mass of the hydroxyapatite (HAp) produced is significantly smaller compared to the total mass of the CaO and $(NH_4)_2HPO_4$ compounds. This is due to the mixing process of the two compounds into powder, which requires heating. This process causes evaporation, where the compounds in liquid form evaporate into gas. As a result, the mass of the HAp produced is less than the combined mass of the two compounds. Additionally, before drying, the precipitated HAp compound was first filtered. In the filtration process, some mass was lost and carried away by the distilled water. If a finer filter was used, the efficiency of the obtained hydroxyapatite might have been greater. The efficiency value of HAp obtained in this process is 50.15%, indicating that half of the mass in the synthesis could be converted into hydroxyapatite. For comparison, the mass of CaO used as the calcium source in the synthesis process was 2.83 grams, which resulted in 3.41 grams of hydroxyapatite.

B. XRD Analysis Results of Limestone

The hydroxyapatite formed from 2.83 grams of CaO derived from limestone and 3.97 grams of $(NH_4)_2HPO_4$ has a mass of 3.41 grams. Subsequently, the HAp compound was tested using XRD analysis. The measurement of X-ray diffraction patterns and the identification of the hydroxyapatite (HAp) sample obtained through the precipitation method is displayed in Figure 1. The results of the diffraction pattern measurement show diffraction peaks within the 2θ angle range of 10–65°. The phase identification of the sample was conducted by comparing its diffraction pattern against the hydroxyapatite compounds in the COD (Crystallography Open Database).

The peaks visible in Figure 1 were matched using software, and the analysis results indicated that the formed pattern corresponds to the hydroxyapatite compound listed in the COD database with entry code 00-900-2215. The COD database indicates that the produced hydroxyapatite has a crystalline phase with a hexagonal crystal system and a space group of P63/m. In the XRD data of the hydroxyapatite compound, crystal peaks were detected at the angles of 2θ: 25.81; 31.78; 32.19; 32.91; and 49.49. Additionally, there were several peaks with very high intensity that are not related to hydroxyapatite, located at the angles of 2θ: 27.802; 31.027; and 34.37. After matching these peaks using the software, it was found that the generated pattern also corresponds to β-tricalcium phosphate ($Ca_3(PO_4)_2$), or β-TCP, which is listed in the COD database with entry code 00-151-7238. The obtained information indicates that the formed β-TCP compound is a crystalline phase with a trigonal crystal system (hexagonal axes) and a space group of R3c.

Tricalcium phosphate (TCP) with the chemical formula $Ca_3(PO_4)_2$ has two different crystallographic forms, namely α-TCP and β-TCP. Both can be produced through heat treatment or sintering at temperatures above 700 °C. The β-TCP form, often referred to as whitlockite, is more widely used due to its good chemical stability, high mechanical strength, and better bioresorption compared to α-TCP.

The presence of β-TCP may be attributed to the uncontrolled pH during the synthesis process. When the pH becomes acidic ($pH < 4.2$), this can facilitate the formation of whitlockite [14]. This phase is not harmful to humans, as whitlockite is often used in pathology, especially in dental calculus. Additionally, whitlockite has higher mechanical pressure compared to hydroxyapatite, which can enhance mechanical compressive strength when applied in the field of dentistry.

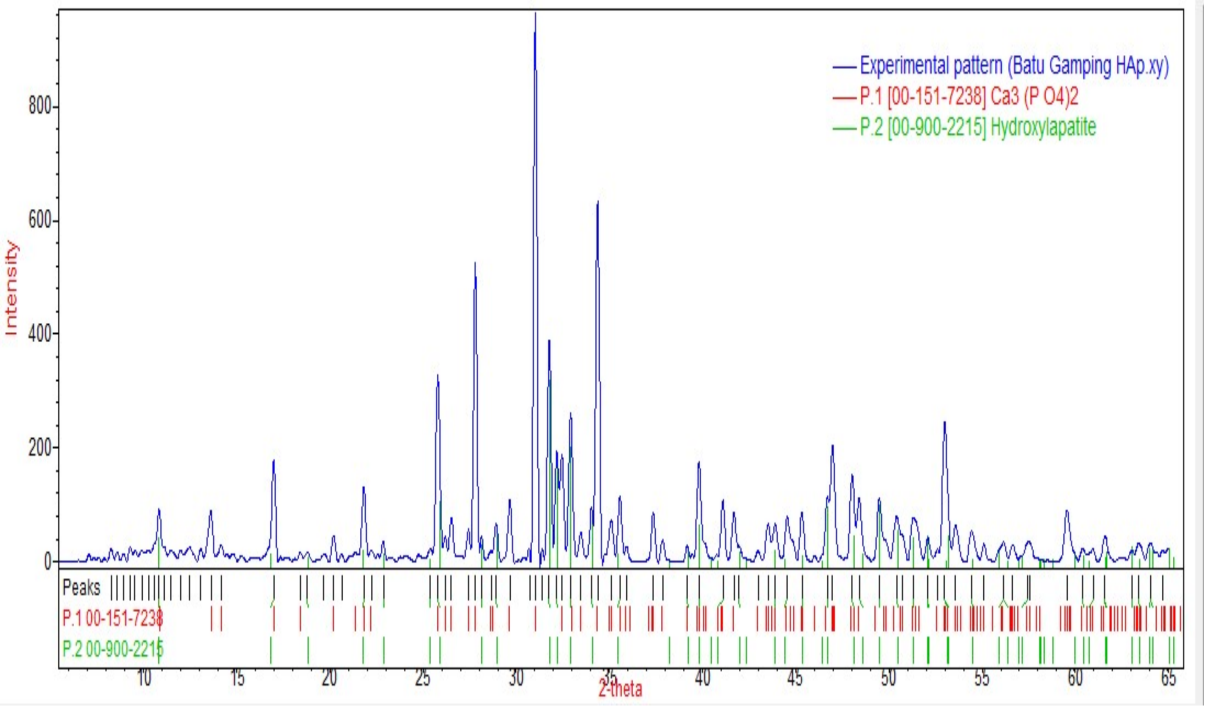

Fig. 1 shows the diffraction pattern of hydroxyapatite characterized using XRD.

C. *SEM Analysis Results of Limestone*

To study the morphology and size of the formed hydroxyapatite, characterization was performed using a Scanning Electron Microscope (SEM). The SEM characterization of the synthesized samples aimed to investigate the surface morphology of the HAp particles. The SEM instrument can identify various physical characteristics of HAp, including size, shape, structure, and morphology of the formed HAp crystals. Figures 2, 3, and 4 show the surface morphology of hydroxyapatite. From these images, it is evident that the hydroxyapatite particles experienced agglomeration or clumping.

These findings are consistent with research conducted by previous researchers, which showed that the observed particles experienced agglomeration [15]. The characteristics of the individual particles tend to be spherical in shape. The size of the individual particles is on a nanoscale, ranging from 140 to 300 nm. This indicates that the formed particles fall into the category of nanoparticles, meaning their size is small enough to facilitate applications in the field of dentistry. The largest measured size was 295 nm, while the smallest size was approximately 141 nm. The difference between the largest and smallest sizes is not significant, leading to the conclusion that the characteristics of the individual particles formed are relatively homogeneous. This result aligns with previous studies that state that HAp crystals tend to be agglomerated [16] with a size range of 210–410 nm [17].

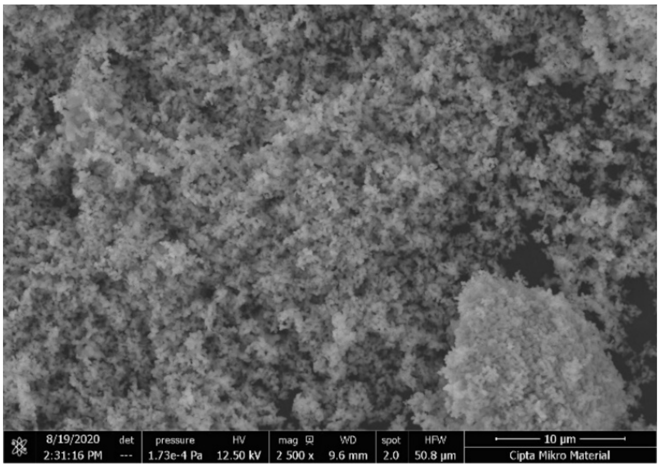

Fig. 2 SEM results of hydroxyapatite obtained from the CaO source of limestone at 2500x magnification.

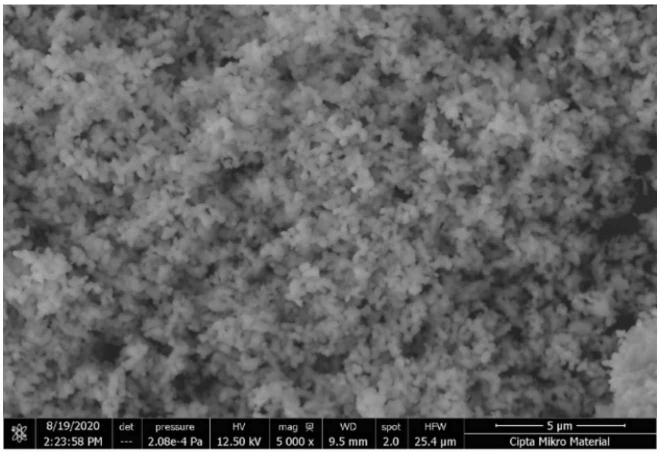

Fig. 3 SEM results of hydroxyapatite obtained from the CaO source of limestone at 5000x magnification.

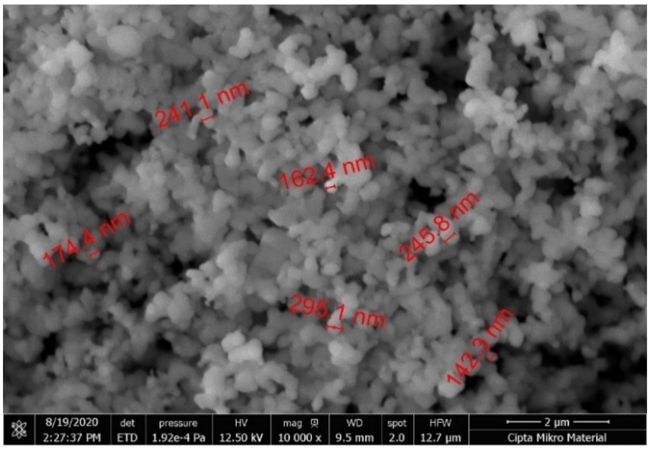

Fig.4 SEM results of hydroxyapatite obtained from the CaO source of limestone at 10000x magnification.

IV. CONCLUSION

The synthesis process of the hydroxyapatite (HAp) biomaterial using natural materials, specifically limestone, as the base material for dental applications has been successfully carried out through the wet precipitation method. The results of this synthesis indicate that the produced hydroxyapatite possesses a crystalline phase. The formed crystal system is hexagonal, with a space group of P63/m. During the synthesis of hydroxyapatite, another compound, β-tricalcium phosphate ($Ca_3(PO_4)_2$), was also formed, which has a crystalline phase. The resulting crystal system is trigonal (hexagonal axes) with a space group of R3c. The produced hydroxyapatite exhibits agglomeration, with individual particle characteristics tending to be spherical in shape. The size of the formed hydroxyapatite is relatively small, falling within the nanoscale, with particle sizes ranging from 140 to 300 nm. The maximum measured size is 295 nm, while the minimum size is approximately 141 nm.

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