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Effect of Ca:P Concentration and Calcination Temperature On Hydroxyapatite (HAp) Powders From Quail Eggshell (*Coturnix Coturnix*)

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ABSTRACT

Hydroxyapatite (HAp) that being derived from quail eggshell was successfully prepared via precipitation method with the variation of Ca:P concentration and calcination temperature. The precursors used are $Ca(OH)_2$ and H_3PO_4. This study aimed to investigate the effect of the Ca:P concentration and temperature of calcination to HAp and characterization of products with XRD, SEM-EDX and FTIR. Quail eggshells were calcined at 1000°C to produce calcium oxide (CaO). CaO and H_3PO_4 dissolved in aquabides to produce $Ca(OH)_2$ and H_3PO_4 to achieve a certain concentration, then the two materials are mixed. The result of the mixture was calcined at 650°C, 850°C, and 1050°C. The result of XRD pattern shows that Ca:P concentration at 1:0.6 and 1.67:1 have higher crystallinity than 0.5:0.3. In addition, the variation of calcination temperature shows that degree of crystallinity increases when the temperature of calcination increases. Crystallite sizes of Hap powders are in the range 13.91 to 47.09 nm. From EDX result, the obtained HAp's have a Ca:P ratio between 1.55 to 1.81. From FTIR result, the functional groups of PO_4^{3-} and OH^- indicate HAp compounds, but it also contained CO_3^{2-} group. The morphology of HAp powders show that shape of HAp becomes more uniform when temperature is higher.

Keywords: Hydroxyapatite, Quail Eggshell, Precipitation, Concentration, Calcination.

1. INTRODUCTION

Bones, the strong tissue which forms the skeleton of a human body, have some main functions as a support of the body, as the place of muscle tissue attachment, and as a protection for various vital organs in the body such as the spinal cord, brain, lungs, and heart [1]. However, the bones are known to be susceptible to fractures and degenerative diseases [2]. Implantation, a method involving the use of synthetic materials, can be used for the restoration process of it [2]. The potential synthetic material that can be used in this method is hydroxyapatite [3].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) is the basic inorganic calcium phosphate mineral component of human bones and teeth that have biocompatible, bioactive, and osteoconductive properties [4]. HAp has the same chemical content as bone, such as calcium and phosphate, so it can bind to the body tissues of living things [5].

Calcium sources that can be used for the manufacture of HAp are corals, seashells, eggshells, and also from body solutions [6]. Eggshell that has been used widely as HAp is hen's eggshell [7]. In addition to these materials, exploration needs to be done for the process of making HAp which contains high calcium. The basic materials that could be potentially used as HAp materials are quail eggshell.

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The quail eggshell (*Coturnix coturnix*) contains more calcium levels than hen and duck eggshell, ie 317.2881±13.14 mg/gr [8]. They are consumed frequently, but until now the shells are wasted and still rarely used especially for HAp material [9].

Synthesis of HAp ceramics can be done through several methods. These include precipitation, hydrothermal, mechanochemical, and microwave. The precipitation method is a method that widely used for the synthesis of HAp, because HAp can be synthesized in large quantities without using organic solvents and it is cheap enough. The crystallinity and high purity of the HAp by this method depends on several parameters, such as the synthesis temperature, the initial concentration of the reactants, stirring rate of the reactants, pH, heat treatment conditions, and acid addition rate [10]. In this study, the method to be used to obtain HAp is precipitation method with parameters to be varied is Ca:P concentration and calcination temperature.

2. METHODS

2.1 Sample Preparation

The materials used in this study were quail eggshell that functioned as a source of CaO, phosphoric acid (H_3PO_4) which functioned as a source of phosphate forming HAp, aquabides as a solvent, and ammonium hydroxide (NH_4OH) as pH controller.

CaO was obtained from a heated quail eggshells of 100°C for an hour and calcined at 1000°C. Each of CaO and H_3PO_4 were dissolved in aquabides until a certain concentration of $Ca(OH)_2$ and H_3PO_4 was obtained. Then the H_3PO_4 solution was added to $Ca(OH)_2$ solution by titration at 1 ml/min. During this titration, $Ca(OH)_2$ suspension was mixed in rapid stirring and heated at 60ºC. After titration process was completed, the mixture was then heated for an hour more at the same temperature. Then the pH of the mixture was measured by using pHmeter, if the pH was less than 9 then NH_4OH should be added. After that, the mixture was allowed to precipitate at room temperature for 24 hours. The distilled mixture was stirred for 30 minutes, then filtered using filter paper and washed with aquabides. The filtration result was dried in an oven at 100°C. After the drying process was completed, the obtained results were calcined at various temperature from 650°C to 1050°C. In this study, variations in concentrations and final temperatures were performed. The variations used and sample names can be shown in Table 1.

Table 1 Variations Ca:P concentration and temperatures of calcination

Samples	Ca:P Concentration (M)	Calcination Temperatures (°C)
HAp-1	0.5:0.3	1050
HAp-2	1:0.6	1050
HAp-3	1:0.6	850
HAp-4	1:0.6	650
HAp-5	1.67:1	1050

2.2 Characterization

Characterization of HAp used X-ray Diffractometer (XRD), Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX). XRD was used to know the crystalline phases and the crystallite size of HAp. The crystalline phases obtained compared by Joint Committee on Powder Diffraction Standards (JCPDS). The crystallite sizes (*D*) of HAp particles were determined by using the Scherer's equation as follow [11]:

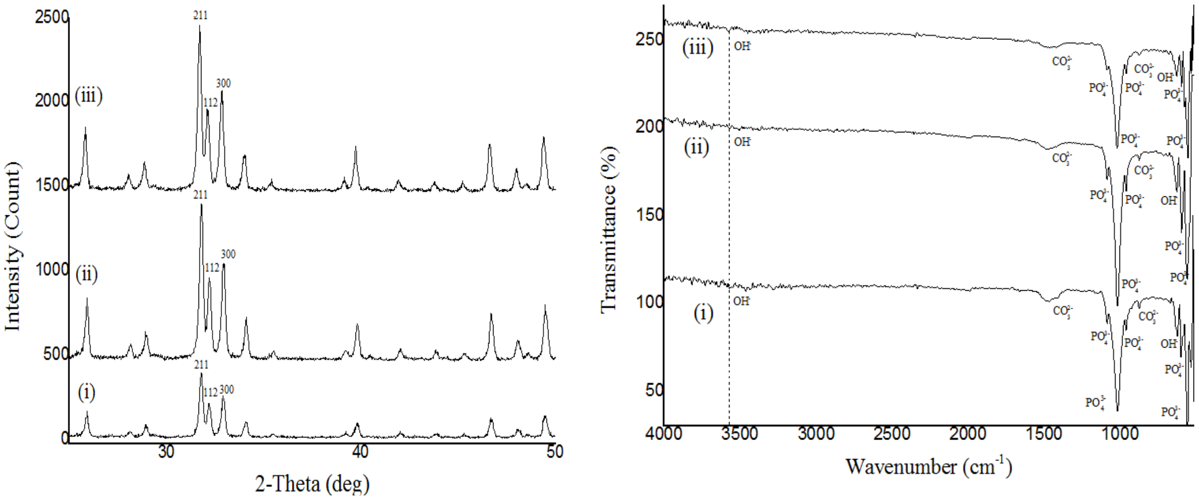
$$D = \frac{0,89\lambda}{\beta \cos\theta} \tag{1}$$

where λ is the wavelength of Cu$K\alpha$, β is the full width at half maximum of the HAp line and θ is the diffraction angle. FTIR was used to investigate the chemical bond or molecular structure of the materials. SEM was used to know the morphology of particle and EDX was used to know the mass percentage of elements in order to obtain Ca:P ratio of HAp.

3. RESULTS AND DISCUSSION

3.1 Characterization of Ca:P Concentration Variations

XRD patterns for HAp samples obtained at a different concentration at 1050°C are shown in Fig. 1a. According to the XRD patterns, it shows that all of the samples in Figure 1a have three highest peaks of 2θ spectrum at range 30° to 35°, ie 211, 112, and 300 and do not contain secondary phases in HAp. These results are agreement with JCPDS 9-432. However, HAp-1 sample shows a lower intensity peak compared to HAp-2 and HAp-5 samples. According to Suryadi [12], higher crystallinity materials will show higher intensity peaks. It can be indicated that HAp-2 and HAp-5 samples have better crystallinity than HAp-1.



(a) **(b)**

Figure 1. (a) XRD Pattern of (i) HAp-1, (ii) HAp-2, and (iii) HAp-5 at 1050°C; (b) FTIR spectra of (i) HAp-1, (ii) HAp-2, (iii) HAp-5.

The crystallite size and Ca:P ratio of HAp powders with different concentration of Ca:P are shown in Table 2. The crystallite size is calculated from XRD results using Equation 1, was taken from peak 211. It shows that all of the samples are in the nanocrystal range. However, HAp-2 sample shows the highest crystallite size values compared to other samples. This is confirmed by Solihat [13], HAp with concentration of Ca:P 1:0,6 indicates the closest crystal size of human bone. While Ca:P ratios, obtained from EDX, do not corresponding to the ratio of HAp stoichiometric, ie 1.67. According to Balgies [14], this is possible because impurities appear during the precipitation process. Balgies has explained that the Ca:P ratio depends on the amount of phosphate in the sample, the more amount of calcium added, the smaller Ca:P ratio gained. Table 2 present that the amount of calcium increases as Ca:P ratio decreases. However, HAp-2 indicates the closest value to the ideal ratio of HAp stoichiometry.

Table 2 The effect of concentration on the crystallite size and Ca:P ratio of HAp powders at 1050°C

Sample	Crystallite Size (nm)	Ca:P ratio
HAp-1	44.04	1.81
HAp-2	47.09	1.76
HAp-5	38.03	1.55

The Purpose of SEM characterization is to determine the morphology of the HAp powders. The SEM images of the HAp from quail eggshell are shown in Fig. 2. These images show that the HAp powders are in spherical shape with an average size of ~200 nm. All of the samples are uniform in size, but irregular and non-interconnect in shape. The HAp-5 indicates the presence of agglomeration. This is happened due to the presence of other phosphate compounds in the form of tricalcium phosphate (TCP) [15]. This observation is agreed as the results reported by Kalita and Verma [16].

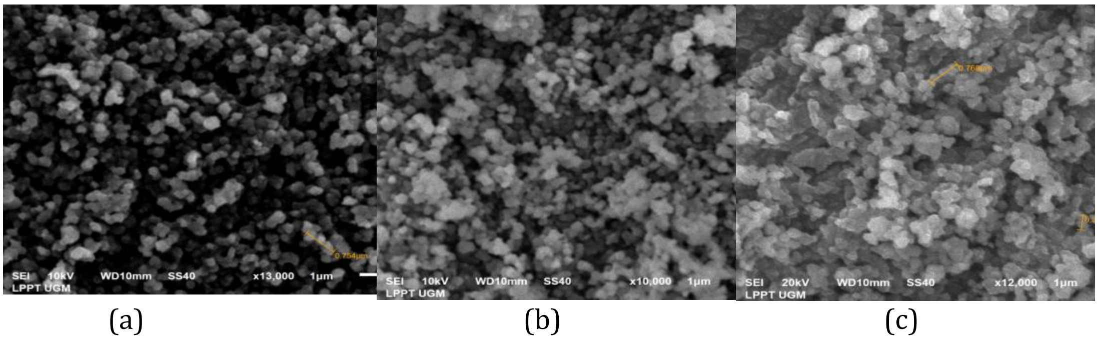
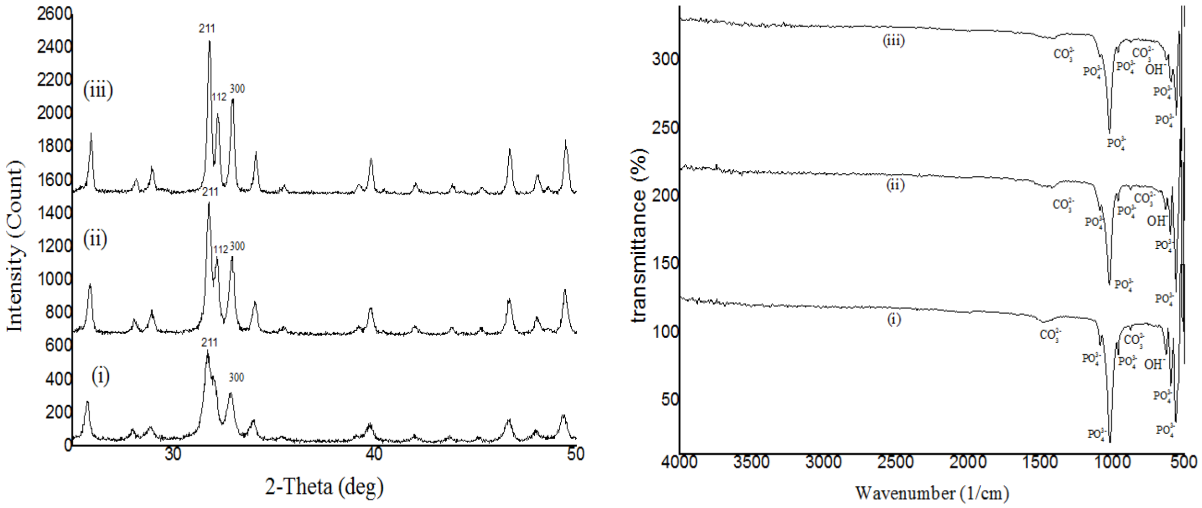


Figure 2. SEM Images of (a) HAp-1, (b) HAp-2, and (c) HAp-5.

Figure 1b shows the FTIR spectra of HAp with different concentration of Ca:P at 1050°C, respectively. All IR spectra are compared with the result reported by Goloschapov et.al [7] and Kamalanathan et.al [17]. All of the samples had PO_4^{3-}, OH^-, and CO_3^{2-} functional groups. The appearance of structural PO_4^{3-} and OH^- indicates the presence of HAp content in the samples. This is consistent with the result reported by Chaudhury et.al [18]. The advent of the CO_3^{2-} groups, which indicating the presence of PO_4^{3-} ions, is substituted by CO_3^{2-} ions that produce a B-type carbonate. Furthermore, according to Mona and Yusril [19], the appearance of the CO_3^{2-} groups was due to the interaction of CaO and CO_2 during synthetis process.

3.2 Characterization of Variations in Calcination Temperature

Figure 3a shows the XRD patterns of calcined HAp powders at different temperatures (650°C, 850°C, and 1050°C). All of the samples is not indicating any secondary phases of HAp such as TTCP, β-TCP, or α-TCP. However, the HAp-4 sample at 650°C does not show the peak at 112 as a part of three highest peak should be possessed by HAp. But the Fig. 3a shows that by the higher calcination temperatures, the peak width becomes higher and sharper. This means that the amorphous phase is reduced and the crystal phase increases as the calcination temperature increases. So it can be said that the degree of crystallinity increases as the temperature of calcination increases. Furthermore, the crystallite size also increases as the calcination temperature increases. Seen in Table 3. Similar phenomena was observed previously [11].



(a) **(b)**

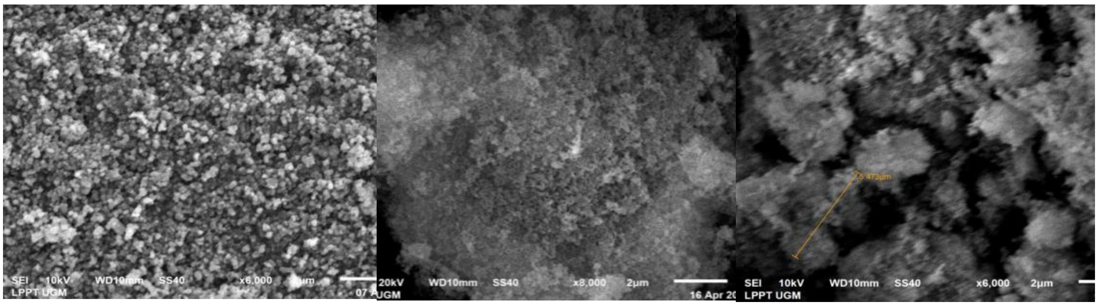
Figure 3. (a) XRD Pattern of (i) HAp-4, (ii) HAp-3, and (iii) HAp-2; (b) FTIR spectra of (i) HAp-2, (ii) HAp-3, (iii) HAp-4.

Table 3 also shows that the temperature of calcination at 850°C and 1050°C results in the ratio close to stoichiometric of HAp. According to Pattanayak [20], the synthesized powders will be apatite when Ca:P ratio is in the range 1.5-1.67. This implies that the synthesized HAp powders have not been fully in apatite phase due to the result which still indicates the formation of CaO in minor amount [11].

Table 3 The effect of calcination temperatures on the crystallite size and Ca:P ratio of HAp powders.

Sample	Crystallite Size (nm)	Ca:P ratio
HAp-2	47.09	1.76
HAp-3	32.44	1.76
HAp-4	13.91	1.80

Figure 4 shows the morphology of the synthesized Hap powders at different temperatures. The HAp powder on HAp-4 sample at 650ºC indicates a non-uniform and irregular in size. HAp-3 sample at 850ºC occurs agglomeration particle. Whereas the HAp-2 sample at 1050ºC shows uniform and spherical in shape, although irregular. This indicates that the calcinations temperature effects on the particle morphology.



(a) **(b)** **(c)**

Figure 4. SEM Images of (a) HAp-2, (b) HAp-3, dan (c) HAp-4.

Based on the previously explanation, all of IR spectra are compared with the results reported by Goloshchapov et.al [7] and Kamalanathan et.al [17]. Fig. 3b shows FTIR spectra and IR vibration of each HAp samples, respectively. It appears that all of the samples had PO_4^{3-}, OH^-, and CO_3^{2-} functional groups. PO_4^{3-} vibration of HAp samples indicates that higher temperature will represent higher transmittance. Whereas on the CO_3^{2-} vibration bands, according to Kalita & Verma [16], when the temperature increases to 1200ºC then the CO_3^{2-} peak decreases. However, CO_3^{2-} peak in this study does not change significantly when the temperature increases. It indicates that higher temperatures are required to eliminate the vibration band.

4. CONCLUSION

This work reports on the Ca:P concentration and temperature of calcinations effects on HAp powders from quail eggshell. The variation of Ca:P concentration results high crystallinity and HAp ratio closest to HAp stoichiometry at Ca:P 1:0.6 and 1.67:1. All of the samples at different concentration show uniform in size, but irregular and non-interconnect in shape. Whereas on IR spectra show PO_4^{3-}, OH^-, and CO_3^{2-} functional groups. The variation of calcination temperature shows that higher temperature affects to the peak width becomes higher and sharper. It also shows that higher temperature results to HAp ratio close to 1.67, the shape becomes more uniform and spherical, and peaks of PO_4^{3-} vibration band are higher. This indicates that the Ca:P concentration and temperature of calcination have some effects on HAp characterization from quail eggshell.

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