Synthesis and Characterization of Hydroxyapatite from Duck Eggshell by Wet Precipitation Process

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ABSTRACT: Hydroxyapatite (HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the most stable form of calcium phosphate and widely used in various medical applications, mainly in orthopedics and dentistry due to its close similarities with the inorganic mineral component of bone and teeth. This study aims to synthesize hydroxyapatite from duck eggshell using the precipitation method. The duck eggshell was calcined, hydrated (slaking) and underwent carbonation to form Precipitated Calcium Carbonate (PCC). Afterwards, $(\text{NH}_4)_2\text{HPO}_4$ was added to produce HAp by varying the molar ratio of Ca/P by 1.67, 1.77 and 1.87 and stirring speed by 200, 250, 300rpm under basic condition (pH 10 – 11). The best results were obtained at a molar ratio of 1.77 with 200rpm stirring speed. Furthermore, the X-ray Diffraction (XRD) analysis showed that its crystals were hexagonal with sizes of 23.062nm, in the absence of other crystalline phases. Therefore, the hydroxyapatite was obtained in the agglomerates form with a specific surface area of 55.929m²/g.

Key words: Agglomerates, calcium phosphate, duck eggshell, hydroxyapatite, precipitation.

1. INTRODUCTION

Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the main inorganic compound present in hard tissues such as human bone. Consequently, it is readily considered as a bioactive material for artificial bone and teeth substitution because of its biocompatibility, osteoconductivity, chemical and biological affinity with human bone tissues and teeth. Furthermore, it is widely used in biomedical application as filler, coating on bone and dental implants.

Hydroxyapatite is derived from natural resources rich in calcium carbonate (CaCO_3) like limestone [1], cockle shells [2], egg shells [3,4], animal bone [5] or coral [6,7]. In this study, it was synthesized from duck egg shells using precipitation method. The selection of duck egg shells as a source of calcium was due to its abundance in Indonesia. Buasri et al (2013) reported that the content of calcium in the form of calcium oxide (CaO) in duck egg shells was 98,925%.

There are quite number of methods that can be used to produce synthetic nanoparticle HAp such as : precipitation [8,9], hydrothermal [2,6], mechanochemical [10] and sol-gel [3,11]. The precipitation method was chosen in this study due to its simple and low-cost processing technique in industrial scale. This method generally produces particles in the nano-scale region and less than 100nm [12]. However, the hydroxyapatite obtained using this method contains considerable contaminant. Therefore, to solve this problem, HAp was synthesized from duck egg shells through the formation of Precipitated Calcium Carbonate (PCC) using modified carbonation method.

In our previous research, hydroxyapatite was synthesized from cockle shells and eggshells PCC's using hydrothermal and sol gel methods [2,3]. PCC is a calcium carbonate compound (CaCO_3), which is processed from natural resources containing calcium carbonate through a

series of chemical reactions. Its particles are homogenous i.e. same size with micro-scale particles and have high purity (99.8%). Azis et al, [2] obtained a highly purified hydroxyapatite from PCC cockle shells without any other crystalline phase by using hydrothermal method. Meanwhile, synthesis of hydroxyapatite from PCC duck egg shells using precipitation method has never been previously reported.

2. MATERIALS AND METHODS

2.1 Materials Preperation

The duck egg shells Fig. 1 were collected from Pekanbaru, Indonesia. Other materials used include diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$), (Merck), 2M nitric acid (HNO_3), 65% ammonium hydroxide (NH_4OH) (Merck), CO_2 and aquadest. The duck egg shell samples were thoroughly washed, cleaned and air-dried for two days. Afterwards, they were crushed and grounded to fine powder consistency using a blender.

Fig. 1. Duck Egg Shells

Received : March 26, 2021

Revised : June 14, 2021

Accepted : July 02, 2021

2.2. Process of forming precipitated calcium carbonate (PCC) from duck egg shells

The procedure for the formation of PCC from duck egg shells was carried out using the modified carbonation method, referred from Azis et al. [2]. PCC is the main material used in synthesizing hydroxyapatite via precipitation method. The reaction for the formation of PCC using modified carbonation method is represented by the following equations [13].

Calcination:

Hydration:

Precipitation:

2.3 Synthesis of hydroxyapatite from PCC duck egg shells using precipitation method

Because the solubility of PCC in water is very low, its usually dissolved in mineral acid. Therefore, in this study 5gram of PCC powder from duck egg shells were dissolved in 200ml of 0.3M HNO_3 solution. Furthermore, 360ml of $(\text{NH}_4)_2\text{HPO}_4$ solution was prepared by varying the molar ratio of Ca and P reactant by 1.67; 1.77 and 1.87. Afterwards, it was added in drops to the PCC solution at a rate of 6ml/min for 24h and stirred at 300rpm. The pH was monitored at 10-11 using 33% NH_4OH . The precipitate object was stirred by using magnetic stirrer at 200, 250 and 300rpm for 24h at room temperature (27°C) and aged for 24h. Subsequently, it was filtered with a filter paper and repeatedly washed with aquadest until the pH of solution was 7. Afterwards, the precipitates were dried at 110°C for 24h and sintered at 500°C for 1h.

The chemical equations for the reaction are shown below:

2.4 Characterization

The hydroxyapatite powder was characterized using X-ray diffraction (X'Pert Powder DY 3688) with Cu K α radiation. Meanwhile, the Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer Spectrometer Frontier) was used to analyze the bonding structure of the samples. The surface morphology was probed using scanning electron microscopy (SEM) linked to energy dispersive X-ray microanalysis (EDX) (JEOL JED 2300). Furthermore, the surface area of the hydroxyapatite was characterized using Brunauer–Emmett–Teller (BET) measurements and Surface Area Analyzer (SAA, Quantachrome NovaWin2).

3. RESULTS AND DISCUSSION

The FTIR spectrum of HAp powder (Fig. 2 a,b,c) showed the sharpen bands of PO_4^{3-} at $1025\text{--}1029\text{ cm}^{-1}$. According to Stanciu et al. [14], the sharpen peaks of PO_4^{3-}

implies that the crystallinity of the hydroxyapatite powder is good. Unfortunately, from Fig. 2 (a) and (b), it is seen that the broad bands at 3370 and 3379 cm^{-1} were ascribed to the N-H asymmetric stretching mode [15]. However, it was assumed that O-H stretching mode corresponded to H_2O at bands $3800\text{--}2500\text{ cm}^{-1}$.

Fig. 2. FTIR spectra of the HAp powder at ratio of Ca/P reactant 1.67 and stirring speed (a) 200, (b) 250, (c) 300 rpm

The variation of stirring speed in HAp synthesis did not have a significant influence on the XRD pattern of hydroxyapatite powder (Fig. 3). The XRD pattern of synthesized hydroxyapatite powder was compared to the standard hydroxyapatite ICDD 01-074-4172 (Fig. 3d). The intensity peak was observed at an angle of 2θ : 25.8° , 28.9° , 31.9° , 32.2° , 32.9° , 33.9° and 39.7° , which was very close to the standard ICDD 01-074-4172. Furthermore, the XRD pattern of the synthesized hydroxyapatite powder (Fig. 3a, b, c) did not contain any other crystalline phase.

The morphology and content of element present in the synthesized hydroxyapatite at molar ratio of Ca and P reactant : 1.67; 1.77 and 1.87 and stirring speed of 200rpm, were confirmed from the SEM micrograph data as shown in Fig. 4.

Fig. 3. XRD pattern of HAp powder at stirring speed a) 300, b) 250, c) 200 rpm, d) HAp standard ICDD 01-074-4172

Fig. 4. SEM micrograph of hydroxyapatite powder at 200 rpm and the molar ratio of Ca and P reactant: a) 1.67 b) 1.77 and c) 1.87.

The diameter of hydroxyapatite crystal was calculated using the Scherrer's equation. The value obtained at the variation of Ca and P molar ratio for 200 rpm stirring speed, is shown at Table 1.

Table 1 The diameter of hydroxyapatite crystal for 200 rpm stirring speed

No	Variable	
	Ca/P reactant	Diameter of HAp crystal (nm)
1	1.67	23.086
2	1.77	23.062
3	1.87	40.356

The best result of the synthesized hydroxyapatite at stirring speed 200rpm and molar ratio of Ca and P reactant 1.77, has crystalline size about 23.062 nm. The measurement of elemental composition (Ca, P and microelement) are summarized in Table 2.

Table 2 Ca, P and microelement content and Ca and P ratio in HAp product

Element	Measured content (wt %)	Ca and P ratio HAp product
P	17.55	1.699
Ca	29.83	
Al	0.83	

The ratio molar of Ca and P hydroxyapatite was synthesized from PCC of duck egg shells was 1.699. The surface area of hydroxyapatite powder was characterized using BET analysis and the value obtained was 55.929m²/g.

4. CONCLUSION

In this study, hydroxyapatite nanoparticle powder was successfully synthesized from the PCC of duck egg shells using precipitation method. The variation of stirring speed did not have a significant influence on the synthesized

hydroxyapatite. The best hydroxyapatite powder was synthesized at stirring speed 200rpm and molar ratio of Ca/P 1.77. The XRD pattern showed the high purity of hydroxyapatite and the nano hydroxyapatite crystalline size obtained was 23.062nm.

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ACKNOWLEDGMENTS

The authors would like to acknowledge the Kemenristekdikti and Universitas Riau for their financial support under Dana Penelitian Berbasis Kompetensi DRPM and Dana DIPA UNRI

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