Synthesis of hydroxyapatite from *meti* shells (*batissa violecea* L. Von Lamark 1818) by wet precipitation method

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Abstract. Hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) has been synthesized from *Meti* shells (*Batissa violecea* L. Von Lamark 1818) by using wet precipitation method. The purposes of hydroxyapatite synthesis from *Meti* shells are to determine the effect of sintering temperature and acidity (pH) on yield and IR spectrum. The sintering temperatures used for synthesis were 800, 900, 1000, and 1100°C, while the pH of the synthesis included 8, 9, 10, and 11. The hydroxyapatite compound was characterized by using XRD and SEM. The result showed that the sintering temperature of 1100°C and pH 9 resulted in the best hydroxyapatite with a yield of 60.10%. The IR spectrum showed that utilizing of 1100°C and pH 9 was the result of the low impurity absorption of CO$_3^{2-}$ group at the wavelength of 1641.42 cm$^{-1}$ and 1415.5 cm$^{-1}$. The diffractogram of hydroxyapatite showed a high intensity at 2Θ angle of 31.74°, 39.74°, and 46.64°. Morphological analysis of hydroxyapatite showed that particles were agglomerated with a particle diameter of 0.77 μm.

1. Introduction

The *Meti* freshwater shellfish (*Batissa violacea* L. von Lamareck, 1818) is one of the animals of the Bivalvia class. This type of clam was found to be quite abundant in the Torimpio river, Petasia Timur District, Morowali Utara Regency, Central Sulawesi. This freshwater clam is used by many local people for consumption, while its shell is only thrown away and eventually becomes waste. Seashells are one source of calcium with high levels [1]. This calcium element can be the main ingredient in hydroxyapatite synthesis.

Hydroxyapatite (HAp) has the chemical formula Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, which is an apatite powder that functions as a substitute for artificial bone implanted into the human body [2]. Although the bone tissue itself shows excellent bone regeneration ability, for severe bone damage the process of handling bone graft is difficult so that porous hydroxyapatite is needed [3]. Bioceramic apatite can be synthesized through several methods, one of which is the wet precipitation method [4]. This method has the advantage of being relatively inexpensive and producing high purity of hydroxyapatite. Synthesis of hydroxyapatite is influenced by several factors, such as sintering temperature [5, 6], Ca/P ratio [7], time reaction [8], pH [9], [10], and stirring [11].

Several studies have carried out the synthesis of HAp at different temperatures. Hydroxyapatite has been synthesized from a temperature range of 800 <T <1400°C for 4 to 6 hours [8]. In the other research, HAp has been synthesized at 500-800°C for 1 hour [12]. HAp has two different crystal structures, including monoclinic and hexagonal. Monoclinic hydroxyapatite structures are obtained only under pure conditions and stoichiometric conditions of Ca:P ratio of 1.67 [10, 13]. The use of low
pH in the synthesis of HAp cause in no interaction between Ca\textsuperscript{2+} and PO\textsubscript{4}\textsuperscript{3-}, whereas when high pH causes the reaction to take place well and the interaction between Ca\textsuperscript{2+} with PO\textsubscript{4}\textsuperscript{3-} takes place high. But the purity of hydroxyapatite at pH 13 is lower than pH 7, pH 9 and pH 11 [9]. Based on this, it is necessary to study the synthesis of hydroxyapatite from Meti shells by wet precipitation method at various variations in sintering temperature and pH.

2. Experiment procedure

2.1 Materials and instrument.
The basic material that used for synthesis is an (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} solution and Meti shells obtained from Morowali, Indonesia. The instrument consisted of X-Ray Diffractometer (XRD) Rigaku Miniflex II, Fourier Transform Infrared (FTIR) Prestige 21 Shimadzu, and Scanning Electron Microscopy (SEM) HITACHI FLEXSEM 100.

2.2 Preparation of Ca(OH)\textsubscript{2} powders.
The Meti shells were cleaned, dried and pulverized. Shell powders were calcined at 900\textdegree C for 2 hours [14] and sieved with 100 mesh size as CaO powders. The CaO powders were left in contact with air for one week at room temperature to form Ca(OH)\textsubscript{2} compounds [15]. Ca(OH)\textsubscript{2} powders were analyzed by using XRD to determine the degree of crystallinity of Ca(OH)\textsubscript{2}.

2.3 Synthesis of hydroxyapatite
Hydroxyapatite was synthesized by use a wet precipitation method [5]. The Ca(OH)\textsubscript{2} suspension was reacted with an (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} solution with a mol ratio of Ca/P 1.67 at 42\textdegree C that stirred at the agitation of 300 rpm. The mixture was allowed to stand for 1 hour at 90\textdegree C, then the mixture is stirred for 2 hours. After stirring was complete, the pH of the mixture was adjusted to 8, 9, 10, and 11. The mixture was allowed to stand at room temperature for 24 hours. The precipitate was filtered, washed by using distillate water, and dried at 60\textdegree C for 24 hours. The HAp sintering process was carried out at temperatures of 800, 900, 1000, and 1100\textdegree C for 6 hours. The HAp that was obtained after heat treatment was cooled in a desiccator. The dry HAp powders were weighed and analyzed by FTIR spectrophotometer. The selected treatments were characterized by using XRD and SEM.

3. Results and Discussion
CaO was produced from Meti shells that interacted with water vapor on air to form Ca(OH)\textsubscript{2} (equation 1).

\[
\text{CaO}_{(s)} + \text{H}_2\text{O}_{(g)} \rightarrow \text{Ca(OH)}_{2(s)}
\]

Ca(OH)\textsubscript{2} was evidenced by XRD analyzing (figure 1). The diffraction of Ca(OH)\textsubscript{2} powders have strong intensity at 2\theta 28.98\textdegree, 34.37\textdegree, 47.40\textdegree, and 64.43\textdegree which is the typical diffraction pattern of Ca(OH)\textsubscript{2}. The same result has been obtained by former research that resulted in the diffraction pattern of Ca(OH)\textsubscript{2} from Bellamya javanica shells at 2\theta 17.90\textdegree, 28.64\textdegree, 34.10\textdegree, 47.06\textdegree, and 64.30\textdegree [15]. The diffractions at 2\theta 29.36\textdegree, 50.74\textdegree, 54.38\textdegree were assumed from Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6-OH}\textsubscript{2} compound.

The Ca(OH)\textsubscript{2} has been reacted with (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} to produce HAp (equation 2) by sintering temperature of 800, 900, 1000, and 1100\textdegree C.

\[
10\text{Ca(OH)}_{2} + 6(\text{NH}_4)\text{HPO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2 + 6\text{H}_2\text{O} + 12\text{NH}_3\text{OH}
\]

The yields of HAp that was resulted from using different sintering temperatures showed the highest value of 59.12\% at 1100\textdegree C, while at the temperature of 800, 900, and 1000\textdegree C have the yields of 58.73\%, 58.32\%, and 52.98\%, respectively (figure 2). But statistically, the yields of sintering temperature of 800, 900, and 1100\textdegree C was not real different (\(\alpha = 0.05\)). The crystallinity will be increase if the sintering temperature was increased [16]. The yield of HAp from material Meti shell
was higher than HAp from material cow bones. HAp that formed from material cow bone as calcium source with wet precipitation method had the yields of 48.5% [17].

![Graph showing yields vs sintering temperature](image)

**Figure 1.** X-ray diffraction pattern of Ca(OH)\(_2\) powder

![Graph showing yields vs sintering temperature](image)

**Figure 2.** The yields of HAp at each sintering temperatures

The best sintering temperature was determined by using the IR spectrum analyze. The IR spectrum of each sintering temperature shown the same stretching and bending vibration absorption of –OH groups at 3570.24 cm\(^{-1}\) and 632.65 cm\(^{-1}\), respectively. The other type of the IR spectrum of HAp was shown by stretching vibration absorption of -PO\(_4\)\(^{3-}\) groups at 1093 – 1035 cm\(^{-1}\) and bending vibration at 604 – 569 cm\(^{-1}\) (figure 3). The spectrum bands of PO\(_4\)\(^{3-}\) correspond to the vibrations of PO\(_4\)\(^{3-}\) [4]. PO\(_4\)\(^{3-}\) and OH groups are functional groups of HAp [18].

The increased intensity and sharpness of the peak of the -PO\(_4\)\(^{3-}\) group along with the temperature rise, which indicates the crystallinity of HAp increases [19]. The peak of –CO\(_3\)\(^{2-}\) groups were also found on the FTIR spectrum from the wavelength of 1643 – 1417 cm\(^{-1}\). It will be present on the HAp product as an impurity agent. Every sintering temperature resulted in different transmittance for the vibration of –CO\(_3\)\(^{2-}\), but the highest transmittance (91-98%) was obtained at 1100°C. It indicated that the purity of HAp at sintering of 1100°C are higher than others temperature.
Figure 3. FTIR spectrum of HAp after sintering at 800°C (a), 900°C (b), 1000°C (c), and 1100°C (d)

Figure 4. The yields of HAp at every acidity

The sintering temperature of 1100°C was used in the determining of pH effect. The yields of HAp at pH 8, 9, 10, and 11 were 58.93%, 60.10%, 60.31%, and 60.11%, respectively (figure 4). Statistically, the yields of HAp at pH 9, 10, and 11 are not really different (α = 0.05). But at pH 11 shows the yield of hydroxyapatite tends to decrease, this shows that if the pH is too high it will also affect the purity of hydroxyapatite. This is presumably due to the alkaline atmosphere which is too high so it forms other more basic compounds such as NH₄OH which affect the crystallinity and hydroxyapatite yield [9].

The determining of the best pH was done by FTIR spectrum analyzing of HAp. FTIR spectrum showed that the peak of –OH stretching vibration was obtained at 3570.2 cm⁻¹ and bending vibration at
632.7 cm\(^{-1}\). The spectrum of \(-\text{PO}_4^{3-}\) stretching vibration was found at 1093 -1048 cm\(^{-1}\) and bending vibration at 603 – 571 cm\(^{-1}\) (figure 5). Every pH treatment still results in a carbonate group (\(\text{CO}_3^{2-}\)) that was shown at a wavelength between 1700 cm\(^{-1}\) and 1400 cm\(^{-1}\). It was indicated that HA\(\text{p}\) is not pure yet. The presence of carbonate (\(\text{CO}_3^{2-}\)) is unavoidable if the HA\(\text{p}\) synthesis process is carried out in an open atmosphere, so there is a need for environmental inertization by inert gas [12].

\[
\text{Figure 5. FTIR spectrum of HA}\text{p after reaction at pH 8 (a), 9 (b), 10 (c), and 11 (d)}
\]

At the use of pH 9, the absorption of \(\text{CO}_3^{2-}\) has the smallest value or almost none which is shown by the FTIR spectrum with a transmittance value of almost 100%. Therefore, the using of pH 9 produces the highest level of purity HA\(\text{p}\).

The diffraction pattern of HA\(\text{p}\) after sintering temperature of 1100\(\text{o}\)C and pH 9 was illustrated in figure 6. From the XRD analysis results found peaks that indicate the presence of hydroxyapatite. The high peaks are located at 2\(\theta\) 31.74\(^{\circ}\), 39.73\(^{\circ}\), 41.91\(^{\circ}\), 44.04\(^{\circ}\), 46.64\(^{\circ}\), 50.44\(^{\circ}\), 53.15\(^{\circ}\), 61.56\(^{\circ}\), and 62.93\(^{\circ}\). Hydroxyapatite diffraction pattern based on Joint Cristal Powder Diffraction Standard (JCPDS 9-0432) is the presence of a peak at a value of 2\(\theta\) 25.88\(^{\circ}\), 28.97\(^{\circ}\), 31.77\(^{\circ}\), 32.9\(^{\circ}\), 34.05\(^{\circ}\), 39.82\(^{\circ}\), 40.45\(^{\circ}\), 44.37\(^{\circ}\), 46.71\(^{\circ}\), 50.49\(^{\circ}\), 53.14\(^{\circ}\), 61.66\(^{\circ}\), and 63.01\(^{\circ}\) [20]. The diffraction peaks that were obtained in this research were similar to JCPDS 9-0432 diffraction data.

The results of XRD analysis also showed the existence of another group namely AKA with the molecular formula Ca\(_{10}\)(PO\(_4\))\(_6\)CO\(_3\) which has the peaks at 2\(\theta\) 25.83\(^{\circ}\) and 32.85\(^{\circ}\), also there is an AKB group with the molecular formula Ca\(_{10}\)(PO\(_4\))\(_3\)(CO\(_3\))\(_3\)(OH)\(_2\) with a high peak intensity at 2\(\theta\) 28.6\(^{\circ}\) and 52.03\(^{\circ}\), and Ca(OH)\(_2\) groups at 2\(\theta\) 24.0\(^{\circ}\) and 51.21\(^{\circ}\).
Figure 6. X-ray diffraction pattern of HAp powder.

The microstructure of the HAp powder was examined by SEM, as shown in figure 7.

Figure 7. SEM micrograph of hydroxyapatite particles

The morphology of HAp observed at a magnification of 7500X shows that apatite has a distribution of small particles and large agglomerates. The average diameter of the HAp particle size is 0.77 µm. Hydroxyapatite morphology without porogenic agents tends to form granular aggregates with uneven size [21]. The same form has been reported at nanoparticles of HAp with granular aggregates size of 150-300 nm [10].

4. Conclusion
Hydroxyapatite compound can successfully be produced by wet precipitation method from Ca(OH)$_2$ of Meti shells. The hydroxyapatite that was produced under sintering temperature of 1100°C and pH 9 had a yield of 60.10% and the high purity. The diffraction pattern of HAp was shown by high peaks intensity at 2θ 31.74°, 39.73°, 41.91°, 44.04°, 46.64°, 50.44°, 53.15°, 61.56°, and 62.93°. The particles of HAp were agglomerated with a particle diameter of 0.77µm. In further studies, a porogen agent in hydroxyapatite is needed to forming the micropores.

Acknowledgments
The authors would like to acknowledge the Dean of Mathematics and Natural Science Faculty, Tadulako University who has provided research funding assistance.
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