THE UTILIZATION OF GREEN MUSSEL SHELL WASTE FOR THE PRODUCTION OF HYDROXYAPATITE USING SOL-GEL METHOD

Aninda Ikaputri[1*], Moh Nur Fuadzi[1], Luluk Edahwati[2], Sutiyono S[2]
[1]Chemical Engineering, Universitas Pembangunan Nasional ”Veteran” Jawa Timur,
[2]Mechanical Enginering, Universitas Pembangunan Nasional ”Veteran” Jawa Timur
Jl. Rungkut Madya No.1, Gunung Anyar, Kota Surabaya, Jawa Timur 60294, Indonesia
*e-mail: aninda.ikaputri@gmail.com

Abstrak

Hidroksiapatit merupakan salah satu jenis biomaterial keramik yang tersusun dari kalsium dan fosfat, dengan rumus kimia $Ca_{10}(PO_4)_6(OH)_2$. Kalsium yang digunakan dalam penelitian ini diperoleh dari Endapan Kalsium Karbonat Limbah Cangkang Kerang Hijau. Penelitian dilakukan dengan metode sol-gel untuk menentukan kondisi optimal sintesis hidroksiapatit dengan variabel larutan H_3PO_4 pada 0,1 M, 0,5 M, dan 1 M. Tujuan dari penelitian ini adalah untuk mengetahui kondisi terbaik hidroksiapatit yang dihasilkan, mengetahui pengaruh konsentrasi reagen asam fosfat, dan karakteristik hidroksiapatit terbaik yang dihasilkan. Hasil pada konsentrasi H_3PO_4 pada 1 M merupakan kondisi optimal. Sintesis hidroksiapatit dengan metode sol-gel dengan konsentrasi asam fosfat 1 M pH 12 berhasil dilakukan dan diketahui kondisi optimal dalam penelitian ini. Dari hasil sintesis, karakterisasi sintesis hidroksiapatit menunjukkan adanya ikatan Ca-O pada 1494,83 cm^{-1}, P-O pada 995,27 cm^{-1}, dan ikatan OH- pada 3068,75 cm^{-1} dengan bentuk kristal kebulatan menuju ke granular. Rasio Ca/P yang dihitung adalah 1,68 yang mendekati rasio standar Ca/P hidroksiapatit.

Kata kunci : *hidroksiapatit, kerang hijau, sol-gel*

Abstract

Hydroxyapatite is a type of ceramic biomaterial made up of calcium and phosphate, with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. The Calcium used in this research was obtained from Green Mussel Shell Waste Precipitated Calcium Carbonate. The research was conducted using the sol-gel method to determine the optimal conditions for hydroxyapatite synthesis with the variable of H_3PO_4 solution at 0.1 M, 0.5 M, and 1 M. The objective of this research is to determine the optimal conditions for producing hydroxyapatite, understand the influence of phosporic acid reagent concentration, and identify the characteristics of the resulting best hydroxyapatite. The result at the concentration of H_3PO_4 on 1 M turns out the optimal condition. The hydroxyapatite synthesis using the sol-gel method with a concentration of 1 M pH 12 phosphoric acid was successfully conducted and known as the optimal condition in this research. From the synthesis results, the characterization of hydroxyapatite synthesis shows there was a Ca-O bond at 1494,83 cm^{-1}, P-O bond at 995.27 cm^{-1}, and an OH^- bond at 3068.75 cm^{-1} and the crystals form is spherical going to granular. The ratio of Ca/P calculated is 1.68 which is close to the standard ratio Ca/P of hydroxyapatite.

Keywords : *green mussel, hydroxyapatite, sol-gel*

1. INTRODUCTION

Around 62% of Indonesia's area is the ocean, with abundant natural resources, one of which is green mussels. The green mussel (*Perna viridis*) is a soft animal that has a pair of bluish-green shells. Many like processed food made from green mussels by taking only green mussel meat to be processed while the skin or shells will become waste and disposed of on the coast (Sunarsih, 2014). The main component of green mussel shell waste that will be utilized as a rawa material in hydroxyapatite synthesis is calcium (Ca) (Akbar dkk., 2019). The content of calcium carbonate found in green mussel shells is relatively high at 95,69%. The high calcium carbonate content can be used as a source of calcium in synthesizing a compound (Putri, 2016). Calcium phosphate is a compound with similar crystallographic and chemical properties to limestone in vertebrates, so this compound can replace hard tissue structures such as bones and teeth in a good, economical, and harmless manner. This compound can be used as a base material for the manufacture of hydroxyapatite (Pangestu dkk., 2021).

Hydroxyapatite is a ceramic class of biomaterial compounds composed of calcium and phosphate with a molecular formula $Ca_{10}(PO_4)_6(OH)_2$. The availability of hydroxyapatite products in Indonesia still depends on imported products and reaches a value of 1,5 million for every 5 milligrams. Utilizing the abundant source of green mussel shells as a primary ingredient for the manufacture of calcium phosphate gradually, these compounds can be used to manufacture hydroxyapatite compounds which can be used in the medical field, such as healing bones filling cavities and making dentures. This will support the Indonesian economy as a producer of high-quality hydroxyapatite (Henggu dkk., 2019). Calcium carbonate in green mussel shell waste can be purified and transformed into CaO for dental materials in the form of Precipitated Calcium Carbonate (PCC). One dental material derived from CaO is hydroxyapatite. Small-sized particles, such as nano-sized ones, possess antimicrobial properties that can significantly inhibit the growth and reproduction of bacteria (Yuliatun dkk., 2023)

Hydroxyapatite possesses important chemical properties, namely biocompatible, bioactive, and bioresorbable. Biocompatible means that the material does not elicit rejection reactions from the human immune system, which recognizes it as a foreign object. Bioresorbable materials will gradually dissolve over time (regardless of the mechanisms causing material transfer) and allow newly formed tissue to grow on any surface (Afrizal dan Gunawarman, 2016). Bioactive materials are materials commonly used to heal or reconstruct human body parts. Bioactive materials can bind directly to the bone. The advantage of this material is that it is more stable as an implant material and more durable than hydroxyapatite (Suci dan Ngapa, 2020). Hydroxyapatite, as the main component of bones, is a bioactive material with excellent osseointegration properties when applied in the field of orthopedics. Osseointegration refers to the ability of a material to integrate or fuse with bone. Osseointegration is a crucial requirement for materials used in implants (Siswoyo dan Gunawan, 2018)

In addition, the application of hydroxyapatite in the field of implantology is used to assist the bone healing process. The bone healing process uses a substitute material or bone graft developed as a choice by researchers and surgeons using calcium phosphate-based materials like hydroxyapatite (Yusuf dkk., 2019). Hydroxyapatite has several characteristics such as biocompatible and bioactive so it can increase implant biocompatibility and can act as a site for the growth of new bone tissue in the medical field (Noviyanti dkk., 2017).

Numerous scientists have attempted to synthesis hydroxyapatite in their research using various technique and materials. As a result, there exist multiple methods for producing these compounds, including dry methods such as calcination and wet methods like sol-gel and wet precipitation (Akbar dkk., 2021). Oji and his colleagues (2019) conducted a study on the Optimization of Phosphoric Acid Concentration in the Production of Hydroxyapatite from the waste of Mackerel Tuna Bones (Eutinus Affinis) using the precipitation method. The variation investigated in the study was the concentration of the phosphoric acid precursor. During the research, Hydroxyapatite synthesis was carried out using raw materials of calcined powder from mackerel tuna bones with varied concentrations of phosphoric acid precursor. The results of the study revealed different masses of Hydroxyapatite based on the treatment of phosphoric acid precursor variations. The optimal result obtained from this Hydroxyapatite synthesis was at phosphoric acid concentration of 3M, producing Hydroxyapatite with a mass of 2.56 grams and Ca/P ratio of. 1.22. Additionally, SEM analysis showed that the synthesized Hydroxyapatite exhibited fine and non-uniform grain morphology (Oji dkk., 2019).

According to another study conducted by Alpina et al. in 2017, the research focused on the Synthesis of Hydroxyapatite from Precipitated Calcium Carbonate (PCC) of Chicken Eggshell through the Sol-Gel Process with Ph and Aging Time Variations. The researchers utilized the sol-gel method to produce Hydroxyapatite crystals using PCC from chicken eggshells and ammonium dihydrogen phosphate solution ($NH_4H_2PO_4$). The study found the best results for Hydroxyapatite Synthesis through the sol-gel method at pH 9 and aging time of 72 hours, resulting in a monoclinic crystal structure with particle size of 53.89 nm and a Ca/P mol ratio of 1.52 (Alpina dkk., 2017)

The present investigation focused on synthesizing hydroxyapatite from green mussel shell waste, utilizing the sol-gel method and phosphoric acid reagent. Knowing the best conditions for the synthesis of hydroxyapatite using the sol gel method and the characteristics of the hydroxyapatite produced. This particular method was preferred due to its various benefits such as precise control over the composition, high-purity of the resulting compound, utilization of low temperatures, and exceptional homogeneity (Cahyaningrum dkk., 2021). Sol in colloidal suspension where the dispersed phase consists of solid particles undergoing Brownian motion or Brownian diffusion, and the dispersing medium is a liquid. On the other hand, a gel is a substance with semi-rigid pores consisting of a three-dimensional continuous network formed by polymer chains. The sol-gel synthesis method offers the advantage of producing optimum particle sizes and maintaining the purity of the phases (Zainul, 2018). Furthermore, the sol-gel method is relatively straightforward, making it suitable for application in medium-sized industries, thereby supporting hydroxyapatite production in Indonesia. The study employed the sol-gel method to determine the optimal conditions for synthesizing hydroxyapatite.

2. RESEARCH METHODS

This research was conducted at Advanced Materials Laboratory, UPN ”Veteran” Jawa Timur. The variable that worked was the concentration of the phosphoric acid reagent (0.1 M, 0.5 M, and 1 M) while the pH was kept constant at 12. The process consists of synthesizing Precipitated Calcium Carbonate (PCC) from green mussel shells waste and synthesizing Hydroxyapatite using sol-gel method.

To prepare the raw material from green mussel shells waste, the initial step involves cleansing the green mussel shells waste using aquadest and alcohol to remove any dirt. The shells are then crushed with a mortar and sifted using a 100-mesh sieve. Next, the shells undergo a calcination process in a furnace at 900°C for 2 hours. After calcination, the resulting material is mixed with HCl and stirred using magnetic stirrer for 30 minutes, followed by filtration to remove impurities. To maintain a pH of 12, NaOH solution is added to the filtrate, and then Na_2CO_3 is added as a precipitating agent. The mixture is stirred at 400 rpm for 60 minutes, and the precipitate is separated from the liquid and dried in an oven at 100°C for 3 hours to produce $CaCO_3$ with high-purity powder.

2.1 Sol-Gel Method

In the process, $CaCO_3$ powder was dissolved in 50 mL of ethanol, and then reacted with 50 mL of H_3PO_4 solution at different concentrations (0.1 M, 0.5 M, and 1 M) for 3 hours under constant magnetic stirring at 300 rpm, while maintaining a constant pH of 12 using NaOH solution. After aging the mixture at room temperature for 20 hours, a gel was formed and filtered using filter paper. The gel was then heated in an oven at 105°C for 2 hours, and the resulting powder was further heated in a furnace at 600°C for 6 hours to produce dry hydroxyapatite powder.

2.2 Analysis Method

The analysis of hydroxyapatite in this research conclude AAS (Atomic Absorption Spectrophotometry) analysis to calculate the content of Calcium and Phosphate later to identify the ratio of Ca/P. The AAS analysis has been conducted at Surabaya Industrial Laboratory and Consulting to calculate the Ca/P ratio of hydroxyapatite. Based on International Standardization and Organization (ISO) 13175 on 2015 the Ca/P ratio of hydroxiapatite is 1.67 (Khoirudin dkk., 2015). The identified optimal condition from AAS analysis is then examined using FTIR (Fourier Transform Infra Red) analysis, with measurements taken in the wave number range 400-4000 cm^{-1} to identify the functional groups of hydroxyapatite such as OH-, PO_4^{3-}, and Ca-O bond. Finally, the crystal’s morphology of hydroxyapatite is examined using SEM (Scanning Electron Microscope) analysis.

3. RESULTS AND DISCUSSION

3.1. The Influence of Different Phosphoric Acid Concentrations on the Ca/P Ratio for Hydroxyapatite Powder

The hydroxyapatite produced in this study will be further analyzed for its calcium and phosphate content under each variable. These elemental contents can then be used to determine the calcium-phosphate ratio (Ca/P). Hydroxyapatite with Ca/P ratios close to the standard Ca/P ratio specified in ISO 13175:205 will be recognized as the optimum hydroxyapatite produced.

Table 1. The Ratio of Ca/P on Hydroxyapatite

Concentration of H_3PO_4	Ca (wt%)	P (wt%)	Ca/P
0.1 M	49.35	28.4	1.73
0.5 M	48.82	28.9	1.69
1 M	48.9	29.1	1.68

Table 1. shows the Ca/P ratio values of hydroxyapatite for variations in the treatment of phosphoric acid concentrations used, namely 0.1 M, 0.5 M, and 1 M. Hydroxyapatite with the lowest concentration of phosphoric acid at 0.1 M yielded as Ca/P ratio of 1.73, which is the highest Ca/P ratio obtained. Additionally, for hydroxyapatite with a phosphoric acid concentration of 0.5 M, the Ca/P ratio is 1.68. according to the research conducted by Oji in 2019, the increasing Ca/P ratio values in hydroxyapatite are attributed to the low concentration of phosphoric acid reagent used, potentially leaving calcium deposits that result in high Ca/P ratios. From the AAS analysis, the best hydroxyapatite was obtained with a phosphoric acid concentration of 1 M, yielding a Ca/P ratio of 1.68, which closely approaches the ISO 13175:2015 standard of 1.67. Furthermore, the hydroxyapatite with the best Ca/P ratio was analyzed using FTIR to determine the presence of hydroxyapatite functional groups and SEM analysis to examine the crystal morphology.

The functional groups present in the obtained hydroxyapatite results were identified through the use of FTIR analysis. The wavelengths that make up these peaks indicate the presence of functional groups, which show the presence of hydroxyapatite. Ca-O, PO_4^{3-}, and OH^- are functional groups of hydroxyapatite, indicating the presence of hydroxyapatite in the sample (Haruda dkk., 2016).

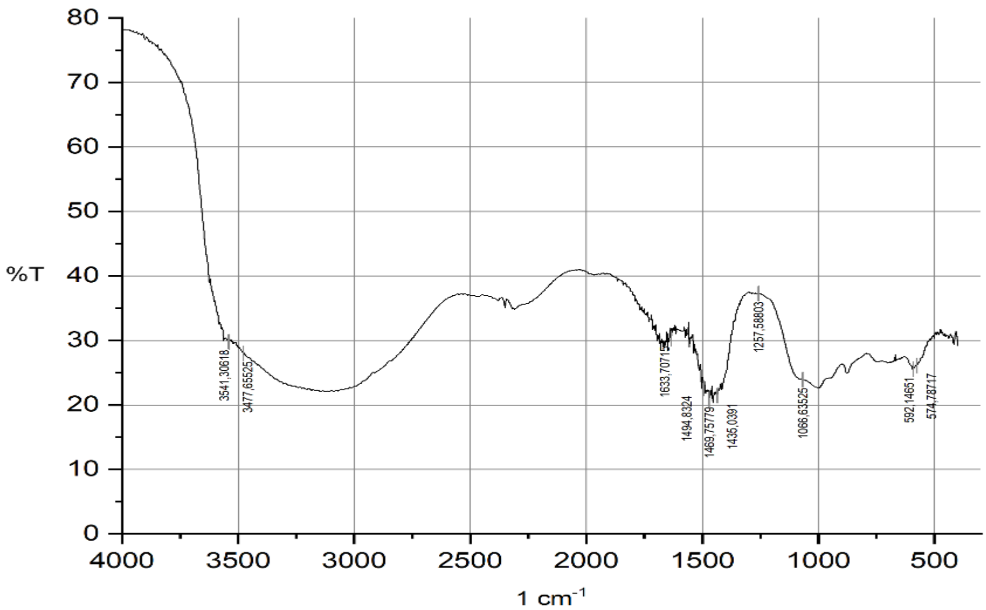

Figure 2. Hydroxyapatite FTIR spectrum at a pH 12 and a concentration of 1 M H_3PO_4.

Based on the results of the FTIR test in figure 2. Hydroxyapatite with a variable 1 M pH 12 indicates an OH^- group indicated by the O-H bond at the peak 3477,65 cm^{-1}. The presence of the P-O bond from the FTIR analysis is detected at the peak value of 1066,63 cm^{-1} and it is highly known as the PO_4^{3-} group and for the Ca-O bond is detected at the peak value of 1494,83 cm^{-1} (Kurniawan dkk., 2019). The presence of P-O and OH^- bond groups in the synthesized material confirms that it is hydroxyapatite, a compound with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, where PO_4^{3-} and OH^- are integral components of the hydroxyapatite structure.

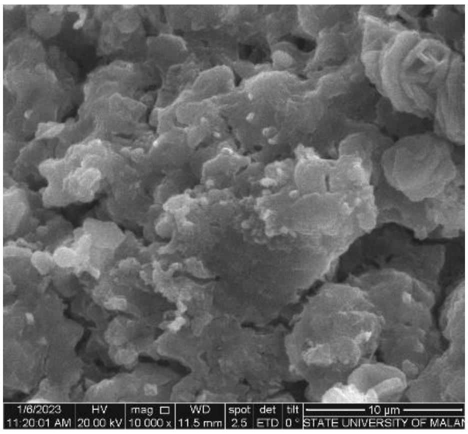

Figure 3. Results SEM Analysis of Hydroxyapatite

Source: Laboratory of Advanced Minerals and Materials (Universitas Negeri Malang)

The SEM Analysis has been conducted to identify the morphology crystal of Hydroxyapatite at pH 12 and 1 M concentration of H_3PO_4. The results indicated the form of Hydroxyapatite is spherical going to granular. The granular form is formed through the maturation process in the formation and development of calcium phosphate crystals. Typically, larger crystals will merge with smaller crystals around them. Furthermore, the uneven distribution of crystals and the formation of clumps occur due to imperfect mixing method during the synthesis process, causing the solution crystals not to be eveny dispersed (Putri, 2016).

In addition, the synthesis of hydroxyapatite has influence factors, the pH value, which will affect the purification of the resulting hydroxyapatite. Based on Haruda's research (2016) the variable pH states that the higher the pH, the higher the hydroxyapatite content (Haruda, 2016). There is also an effect of the concentration of the reagent. The higher the concentration of phosphoric acid, the more calcium will be used up to react and resulted in more hydroxyapatite produced (Kurniawan dkk., 2019).

On the other hand, the purity of the material used for synthesis will affect the purity of hydroxyapatite itself. To pretend the impurities involved in the reaction, needed to use high-purity materials used to like using Precipitated Calcium Carbonate (PCC) rather than raw Calcium Carbonate from green mussel shell waste because Precipitated Calcium Carbonate has high homogeneity and uniformity of particle shape (Maulia, 2020).

4. CONCLUSION

From the results of this research, it can be concluded that the concentration of phosphoric acid has an influence on the Ca/P ratio of the produced hydroxyapatite; as the concentration of phosphoric acid increase, the Ca/P ratio of the resulting hydroxyapatite decreases. The best Ca/P ratio obtained from this study is 1.68 for hydroxyapatite synthesized with a phosphoric acid reagent concentration of 1 M. Additionally, the characteristics observed include the presence of hydroxyapatite functional groups such as Ca-O, P-O, and OH^- bonds, with crystal morphology ranging from spherical to granular.

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