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# Analysis of pH Regulation on the Ca/P Ratio of Green Mussel Shell *Hydroxyapatite* using the Sol-Gel Method

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**Abstract.** The calcium carbonate content of green mussel shells can be used as a material. Hydroxyapatite is the main mineral that makes up bones and teeth so it has similarities to the mineral part of bones. Research was carried out on the concentration of the phosphoric acid reagent used and the pH of the reaction in obtaining the Ca/P ratio from hydroxyapatite obtained through a synthesis process using the sol-gel method. The calcium carbonate precipitation process is carried out by adding 50 ml of water to the $H_3PO4$ solution with a concentration of 1M then stirring for 3 hours using a magnetic stirrer with a stirring speed of 300 rpm, the pH is maintained at 8 – 12 (variable) using NaOH. Next, the solution is aged (aging time) at room temperature for 20 hours until a gel forms. The gel that has been formed is filtered using WhatsApp filter paper. Next, the gel was heated in an oven at 105°C for 2 hours. The resulting powder is then furnaced at a temperature of 600oC for approximately 6 hours to form dry hydroxyapatite powder. The best Ca/P ratio is 1.6708 with the use of 1M $H_3PO_4$ and a pH of 11.

***Kata kunci*:** *Hydroxyapatite*, calcium, *Precipitated Calcium Carbonate*, sol-gel

## 1. Introduction

Green mussels are an important commodity of marine aquaculture. Green mussels are also one type of shellfish biota that is prospective for its rapid growth and can be done throughout the year [1]. According to [2] green mussel shells have a high calcium carbonate composition of 95.69%. The high content of calcium carbonate composition can be utilized as a source of calcium in the synthesis of compounds containing calcium metal, for example in the synthesis of hydroxyapatite. Hydroxyapatite is the main mineral that makes up bones and teeth so that it has similarities with the mineral parts of bones and can be used as an alternative bone substitute material [3].

In Indonesia, there are various types of processed food made from green mussels. With the many enthusiasts of processed food made from green mussels, the waste produced will increase. The general public often only takes green mussel meat to be processed while the shells will come out as waste and most of the waste is disposed of directly into the environment. This is the main cause of pollution to the environment [4]. HAp applications in the field of implantology are used to help the bone healing process. The bone healing process uses a substitute material or what is called bone graft. Bone graft which is developed as an alternative choice by researchers and surgeons, uses calcium phosphate-based material. Meanwhile, the application of HAp in the field of stomatology as a bone graft material in the treatment of infraboni pockets, namely bone damage that occurs in the supporting tissues of the teeth [5].

Research conducted by Alpina [6] with the title "Hydroxyapatite Synthesis from Precipitated Calcium Carbonate (PCC) Chicken Egg Shells Through Sol-Gel Process with Variations in pH and Aging Time", in this study obtained the best hydroxyapatite results through the sol-gel method with pH 9 and aging time for 72 hours which produced a monoclinic crystal structure with a particle size of 53.89 nm with a Ca / P mole ratio of 1.52. Likewise, research conducted by Zein [6] with the title "Effect of Sintering Time on Porous Hydroxyapatite of Tenggiri Fish Bone with Sol-Gel Process", found that the sintering time affects the particle size of the hydroxyapatite formed where the longer the sintering time is carried out, it will make the hydroxyapatite particle size smaller. In addition, the smaller particle size will make the particles denser and stronger. The results of the study showed that for the variation of sintering time from 4 hours, 5 hours, and 6 hours, the most optimum results were obtained in the treatment of sintering time for 5 hours where the 5-hour time variation produced Hydroxyapatite which had a hardness level of 49.5 N. In addition, the size of Hydroxyapatite produced was 0.798 μm where the particle size was the smallest size among all variations of sintering time used.

Sol is a colloidal suspension where the dispersed phase is a solid substance that is still experiencing Brownian motion or Brownian diffusion and the dispersant is a liquid. While a gel is a substance that has semirigid pores consisting of a continuous network in three dimensions formed from polymer chains. The sol-gel method is a method used to make a solid material from nanoparticles or small molecules. The sol gel synthesis method offers the advantage of producing optimum particle size and maintaining phase purity. In general, the stages of the sol-gel process are divided into three parts, namely hydrolysis, alcohol condensation and water condensation [7].

In several studies, it was found that HAp synthesized using the sol-gel method is more efficient in improving the contact and stability of the interface between artificial bone and natural bone, both in vitro and in vivo environments. The synthesis stage in this method begins by dispersing particles in the form of colloids in a liquid. Precursor materials are mechanically mixed in a solvent with the pH adjusted to prevent precipitation. The result of this mixing will be a gel (gelation), a diphasic system consisting of a solid phase (solid) and a liquid phase (interstitial liquid). The next step is to remove the liquid phase through drying. The process of forming the apatite structure of HAp using the sol gel method is highly dependent on the chemical activity and temperature in the synthesis process by the natural chemical properties of the precursors [5].

Hydroxyapatite has a calcium-phosphate ratio or often known as the Ca / P ratio. Based on ISO 13175 2015 of 1.67. The value of the Ca / P ratio in hydroxyapatite will affect the mechanical strength of hydroxyapatite with a critical Ca / P ratio of 1.67. This is because the greater the Ca / P ratio, the stronger the hydroxyapatite produced and reaches the maximum value of the ratio of 1.67. In addition, another property of hydroxyapatite is its solubility which is related to its biocompatibility with tissues and chemical reactions with other compounds. This solubility rate will be influenced by differences in shape, porosity, crystal size, crystallinity, and crystallite size of hydroxyapatite [5].

From various literatures, it is explained that the greater the concentration of H3PO4 and the greater the reaction pH, the greater the Ca/P obtained. This study aims to determine how much influence the reaction pH has on the acquisition of the Ca / P ratio of Hydroxyapatite obtained through the synthesis of green mussel shells using the sol-gel method.

## 2. Method

The material used is green mussel shell waste obtained from the coast of Kenjeran, Surabaya which will be processed first through the PCC (Precipitated Calcium Carbonate) process. H3PO4 (phosphoric acid) is a precursor compound used in the variables run and NaOH (sodium hydroxide) is a compound used as a pH regulator. The tool used in this experiment was shown on Figure 1.

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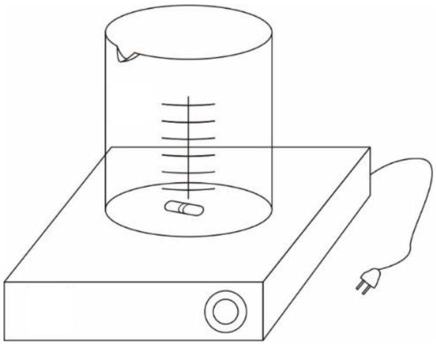

**Figure 1**. A set of *magnetic stirrer*

Preparation of PCC Material from Green Clam Shell are shown as follow10 grams of green mussel shell waste raw materials were washed to remove dirt using distilled water and alcohol. Then mashed using a mortar and then sifted using 100 mesh size. Next, the calcination process of green mussel shells is carried out using a furnace at 900oC for 2 hours. Furthermore, the results of calcination of green mussel shells were reacted by adding 100 ml of 2N HCl. Testing the pH of the mixture, with NaOH compounds until the pH becomes alkaline (according to variables). Sodium carbonate (Na2CO3) was added to the compound then stirred using a magnetic stirrer at 300 rpm for 10 minutes. Filtration of the stirring results is carried out, the precipitate (CaCO3) formed is filtered after that drying is carried out using an oven with a temperature of 100oC so that the results of Precipitated Calcium Carbonate from green mussel shell waste are obtained.

Hydroxyapatite (HAp) was synthesis by Sol-Gel CaCO3 produced from the PCC process was added with 50 ml of water and then 50 ml of 1M H3PO4 solution. The solution was stirred for 3 hours using a magnetic stirrer with a stirring speed of 300 rpm and the pH was maintained in accordance with variations using NaOH compounds. The solution was then aged at room temperature for 20 hours to form a gel. The gel formed was filtered using filter paper to separate the filtrate and precipitate. The gel was heated at 105℃ for 2 hours. The resulting powder was then furnace at 600oC for 6 hours to form Hydroxyapatite dry powder. Hydroxyapatite powder was analyzed using AAS and FTIR.

## 3. Result and Discussion

The hydroxyapatite analysis results obtained were used to determine the composition of calcium and phosphate elements contained in the product. The composition data of the two elements can then be used as the basis for calculations to determine the Ca/P ratio of the hydroxyapatite product produced. Based on the AAS analysis that has been done, the results are shown in Table 1.

**Table 1**. Data from the analysis of Ca and P levels and Ca/P ratio

| pH | Ca (%w/w) | P (%w/w) | Ca/P Ratio |
|---|---|---|---|
| 8 | 48,92 | 29,31 | 1,6691 |
| 9 | 48,89 | 29,05 | 1,6829 |
| 10 | 49,0 | 29,1 | 1,6838 |
| 11 | 48,79 | 29,20 | 1,6708 |
| 12 | 49,31 | 30,08 | 1,6393 |

Table 1 shows that in the results obtained, the Ca/P ratio of 1.6691 occurred at pH 11. Likewise, the magnitude of the error bars, both Ca and P have a small range (average) so that the Ca/P ratio obtained also does not experience significant fluctuations.

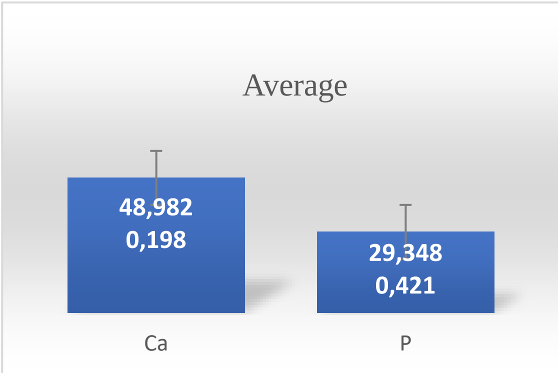


**Figure 2**. Error-bars between Ca and P

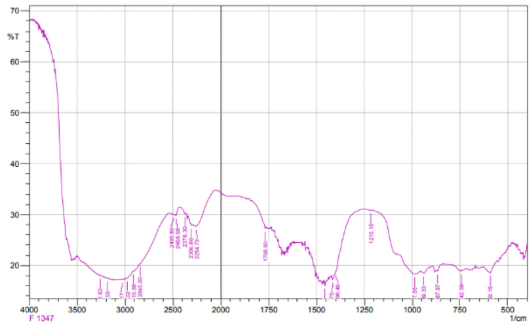


**Figure 3**. FTIR Testing Results Hydroxyapatite 1M pH 11

Based on the FTIR test results in the figure above. The best hydroxyapatite for the 1 M pH 11 sample showed the presence of PO43- group at a wavelength of 592.15 cm-1; besides that, the OH- functional group was also seen at a wavelength of 3261.63 cm-1; and for the Ca-O functional group was seen at a wavelength of 1415.75 cm-1; 1454.33 cm-1; and 1766.8 cm-1. From the results of FTIR analysis, it is known that hydroxyapatite has PO43-; OH-; and Ca-O functional groups. This is in accordance with previous researchers (Kurniawan, 2019) where the hydroxyapatite produced has PO43-, OH-, and Ca-O bonds which are the main functional groups of hydroxyapatite.

## 4. Conclusion

From the results of AAS analysis contained in the characteristics of hydroxyapatite, it shows the presence of the main hydroxyapatite functional groups in the form of PO43-; OH-; and Ca-O functional groups. The results of these characteristics were obtained in the research process using a concentration of H3PO4 of 1M and pH 11.

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