HYDROXYAPATITE (HAp) BIOCERAMICS MADE FROM THE Celetaiya persclupta SNAIL SHELLS FROM POSO LAKE

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ABSTRACT

Bioceramic Hydroxyapatite (HAp) was a derivated chemical synthetic compound from calcium phosphate commonly used to care for hard tissue damage. Bioceramic Hydroxyapatite can be synthesized from the compounds rich with calcium contains. This study treated the synthesis and physicochemical Characterization of the HAp made from Celetaiya persclupta snail shells obtained from Poso lake. From the characterization of the sample C. presclupta shells obtained the water contains 0.50±0.00%; ash contains 0.9913±0.0017g/g; potassium 0.0005±0.00007g/g; calcium 0.0391±0.0059g/g; phosphate 0.0221±0.0009g/g; phosphorus 0.0111±0.0005 and the ratio of Ca/P 1.7382±0.1994%. The synthesis of HAp by base precipitation method showed the yield of synthesis 85.2891±4.2496% with the level of material reduction during synthesis 18.0442±1.1684%. The result of characterization of the HAp showed that the potassium contains 0.0002±0.00003g/g; calcium 0.0121±0.0031g/g; phosphate 0.0167±0.0013g/g; phosphorus 0.0084±0.0007g/g; ratio of Ca/P 1.3724±0.2736%; porosity 9.9929±0.7626%; swelling ability 24.8416±1.4989%; and biodegradability 10.8958±1.1781%. The overall results concluded that the C. presclupta snail shells could become a source of Bioceramic Hydroxyapatite (HAp).

Keywords: bioceramics, HAp, molluscan shells

INTRODUCTION

Hydroxyapatite (HAp) was a synthetic compound derivated from calcium (Ca) have chemical name Ca10(PO4)6(OH)2 [1]; ratio of Ca/P 1.67; density 3.19g/ml [2]; room symmetric P63/m; side parameters a = 9.432Å, c = 6.875Åo and hexagonal crystal system [3]. HAp is very popular in medic, mainly because the biocompatible characteristic can replace the pen (Platina) to manage bone fracture [4]. The Chemical structure of HAp potentially uses as remineralization compounds because they have similarity with the calcium structure build the bones [5]. The HAp can be synthesized from the materials rich with calcium contains and commonly, the HAp composite apply to reparating the fractured bone and patch the tooth [5]. The chemical structure of HAp showed in Figure 1.
The hydroxyapatite was a product of the modification structure of calcium carbonate (CaCO₃), so it can be synthesized from the rich calcium contain materials [1,4,5]. Another synthesis research before in Indonesia used several kinds of calcium source as cow bones [3,8], Molluscan shells [9,10], eggshells [11,12,13,14], Ca and P ions in bacterial cellulosic membranes [15], and calcite [16].

The Molluscan shells can apply as HAp sources because they have higher calcium than eggshells [9,10]. The application of clamshells as HAp scorches conceived because of Indonesia’s large abundance was potential: 34.929 tons per year where the blood clams as the main commodity [9]. The blood clams shells containing 98.7% of calcium (Ca) and 0.002% phosphor (P) [17]. Another research before threatened the synthesis of HAp from shellfish (polymesoda erosa) reported that the stirring time would increase the synthesis result of HAp [18].

The mainly Molluscan shells used as a source of HAp were clamshells (marine or lacustrine clams) [9,17,18], but still rare for the snail shells [10], so this study treated the synthesis of HAp from Celetaiya persclupta snail shells obtained from Poso Lake, Central of Sulawesi. Those shells were chosen as HAp source because they predicted it has large calcium can be synthesized became HAp. The abundance of the empty shells is large and easy to find in large amounts along the lakeshore (on and below the sand). Those shells didn’t manage yet and free to take for now. Sometimes just take away by the tourists as merchandises. The HAp synthesized from C. persclupta shells will be conceived as a reparating compound (implant, grafting, filer) to the hard tissues.

METHODS

1. Research Locations

The C. persclupta snail shells were obtained from Poso Lake, Central of Celebes, Indonesia. The synthesis and chemical characterization of HAp from its shells were treated in the Base Chemical Laboratory of Faculty of Science and Mathematics, Satya Wacana Christian University, Salatiga Central Java.

2. The Shells Characterization

The C. persclupta shells’ chemical characterization included water contains, ash, organics, carbons, and silicon by gravimetric [19]. The Phosphor has measurements by UV-Vis Spectrophotometer and HACH photometer, calcium measured twice by titrimetric [19] and UV-Vis Spectrophotometer [16], also the potassium and sulfur by HACH photometer.

3. Synthesis of HAp from C. persclupta Shells

The synthesis of HAp from C. persclupta shells using base precipitation method [1,14].

Figure 1. The Chemical Structure of HAp [6,7]
4. The Chemical Characterization of HAp

The chemical characterization of the HAp from *C. persclupta* shells included the phosphor contains measurements by UV-Vis Spectrophotometer and HACH photometer, calcium measured twice by titrimetric [19] and UV-Vis Spectrophotometer [20], also the potassium and sulfur by HACH photometer.

5. The Biochemical Characterization of HAp

The biochemical Characterization of the HAp from *C. persclupta* shells included the Porosity, Biodegradability, and Swelling Ability measurements by gravimetric [21,22,23].

RESULTS AND DISCUSSION

The *C. persclupta* snail was one of the endemic molluscan species in Poso Lake [24], Central of Sulawesi. It's easy to find those shells in large amounts on the lakeshore, in the water, or along the Poso River. Those shells already empty then push by the rush stream or the waves to the sands on the lakeside. Those shells were containing calcium to build and strengthen the shell's structure. Genetically, the calcium synthesis in the snails' body is very high because the contains of fat and proteins in another side is used to maintain the snails' body temperature in the water. After those snails die, their shells left can be utilized if calcium contains. But in shell form, the calcium still bonds with silicon, so it can't be used immediately. The calcium from *C. persclupta* shells can be processed hydroxyapatite (HAp) bioceramics after separated from the organics and silicon. The *C. presclupta* shells showed in Figure 2.

![Figure 2. The C. persclupta Shells (Personal Documentation)](image)

The source of HAp needs to be characterized to ensure the existence of calcium and phosphor contains [5,11], so the chemical characterization treated to the *C. persclupta* shells before synthesized to HAp. The result of Characterization showed in Table 1.

| Table 1. The Result of Characterization of the *C. persclupta* Shells |
|---------------------------------|-----------|----------|----------|
| **Methods**                     | **Contains** | **Units** | **Result** |
| Gravimetric                     | Water      | %        | 0.50 ± 0.00|
|                                 | Ash        | g/g      | 0.9913 ± 0.0017|
|                                 | Organics   | g/g      | 0.0103 ± 0.0018|
|                                 | Carbon (C) | g/g      | 0.0060 ± 0.0011|
|                                 | Silicon (Si)| g/g  | 0.1186 ± 0.0054|
|                                 | Silicate (SiO)| g/g  | 0.2317 ± 0.0045|
| Titrimetric                     | Calcium (Ca)| g/g  | 0.0518 ± 0.0001|
| Spectrophotometric             | Calcium (Ca)| g/g  | 0.0391 ± 0.0059|
|                                 | Sulphate (SO₄) | g/g  | 0.0036 ± 0.0005|
|                                 | Potassium (K)| g/g  | 0.0005 ± 0.00007|
|                                 | Phosphate (PO₄) | g/g  | 0.0221 ± 0.0009|
In the molluscan shells, Calcium was not immediately in HAp form but CaCO$_3$ (Calcium Carbonate) and Ca$_2$PO$_4$ (Calcium Phosphate) [9,10,17,18], in which those compound have fragile structure and can’t be applied as bioceramics, especially to propping.

The chemical characterization treated to the C. persclupta shells showed in Table 1 strengthens the hypothesis because those shells contain the minerals needed in the HAp synthesis process. Based on Table 1 also can be predicted that the C. persclupta shells potentially produced three kinds of Apatite Bioceramics, that is Hydroxyapatite (HAp) with chemical structure Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ [1,4,5], Carbonate Apatite (Cap) with chemical structure Ca$_{10}$(PO$_4$)$_6$(CO$_3$)$_3$(OH)$_2$ (Dahlite) and Ca$_{10}$(PO$_4$)$_6$CO$_3$ (Pedolite) [25]. The study of HAp as remineralization agents to the hard tissues already exist in Indonesia used another potentially compound as HAp source. The potentiality comparison of C. persclupta shells with the references from other research showed in Table 2.

### Table 2. The Potentiality Comparison of C. persclupta Shells With the References from Another Research

| Minerals | C. persclupta Shells | References |
|----------|----------------------|------------|
|          |                      | [11] | [13] | [17] | [14] |
| Ca       | 0.0391 ± 0.0059g/g  | 0.0188±0.0007 g/g | 67.22% |
|          |                     | 0.0195±0.00 g/g  | 73.75% | 98.7% | 76.6% |
| K        | 0.0005 ± 0.0001g/g  | -              | -      | 4.20% |
| PO$_4$   | 0.0221 ± 0.0009g/g  | 0.001349 g/g    | 71.23% |
|          | 0.001355 g/g       | 78.62%         | -      | -     |
| P        | 0.0111 ± 0.0005g/g | -              | -      | 0.02% | -     |

The comparison showed in Table 3 concluded that the C. persclupta shells with those minerals have the same potentiality levels to become a HAp source. Because the potentiality of Ca and P contains then processed the synthesis of C. persclupta shells to HAp using base precipitation methods. The result of chemical and biochemical Characterization of the HAp from C. persclupta shells showed in Table 3.
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Table 3. The Result of Chemical and Biochemical Characterization to the HAp from C. persclupta Shells

| Methods              | Synthesis Parameters | Units   | Result               |
|----------------------|----------------------|---------|----------------------|
| Gravimetric          | Rendement            | %       | 18.0442 ± 1.1684     |
|                      | Yield                | %       | 85.2891 ± 4.2496     |
| **Mineral Contains** |                      |         |                      |
| Titrimetric          | Calcium (Ca)         | g/g     | 0.0152 ± 0.00005     |
|                      | Sulphate (SO₄)       | g/g     | 0.0009 ± 0.0001      |
|                      | Potassium (K)        | g/g     | 0.0002 ± 0.00003     |
|                      | Phosphate (PO₄)      | g/g     | 0.0167 ± 0.0013      |
| Spectrophotometric   | P₂O₅                 | g/g     | 0.0027 ± 0.0002      |
|                      | P₂O₅ Dissolved       | g/g     | 0.0013 ± 0.0001      |
|                      | P₂O₅ Total           | g/g     | 0.0039 ± 0.0003      |
|                      | Phosphor (P) Dissolved | g/g   | 0.0017 ± 0.0001      |
|                      | Phosphor (P) Total   | g/g     | 0.0084 ± 0.0007      |
|                      | The ratio of Ca/P (B)| %       | 1.3724 ± 0.2736      |
|                      | Phosphate (PO₄)      | g/g     | 0.0040 ± 0.0000      |
|                      | P₂O₅                 | g/g     | 0.0030 ± 0.0000      |
| HACH Photometer      | Total Phosphor (P) (490) | g/g | 0.0013 ± 0.0000 |
|                      | Total Phosphor (P) (496) | g/g | 0.0029 ± 0.00007    |
|                      | The ratio of Ca/P (O)| %       | 1.33 ± 1.32          |
| **Biochemical Parameters** |                   |         |                      |
| Gravimetric          | Porosity             | %       | 9.9929 ± 0.7626      |
|                      | Biodegradability     | %       | 10.8958 ± 1.1781     |
|                      | Swelling Ability     | %       | 24.8416 ± 1.4989     |
| **Qualitative Observation** |                   |         |                      |
| Direct Observation   | Texture              | -       | Soft                 |
|                      | Shape                | -       | Powder               |
|                      | Color                | -       | White                |

In this study, the HAp synthesis process using the base precipitation method with the incandescent temperature 900°C in 3 hours. In this incandescent temperature, confirmed the Carbon atoms and compounds (C and CO₂) then difficult to confirm the product of another kind of apatite bioceramic like Cap [25], because carbon will be incandescent at 600°C [19] so can conclude that the apatite bioceramic produced in this process was Hydroxyapatite (HAp) [1]. Another kind of bioceramic based on Potassium (K) is difficult to confirm because potassium contains very small measurements. It was 0.0005±0.00007g/g.

Along the synthesis process of HAp from molluscan shells, the chemical reaction to occurs showed in Equation 1:

\[ \text{10CaO} + 6(\text{NH}_4)_2\text{H}_2\text{PO}_4 + 2\text{H}_2\text{O} \rightarrow \text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_{2}\downarrow + 12\text{NH}_4\text{OH} \ldots \text{Eq 1} \] [18]

The HAp synthesis using base precipitation showed the level of potential for success was 85.2891±4.2496%. The reduction of mass material reduction level was 18.0442±1.1684%, which showed that the C.persclupta shells potentially to became a source of HAp. The comparison of the result of HAp synthesis from C. persclupta shells with the references showed in Table 4.
Table 4. The Comparison of the Result of HAp Synthesis from *C.persclupta* Shells with the References

| Result                          | HAp from *C. persclupta* shells | References |
|--------------------------------|---------------------------------|------------|
| % Rendement (w/w)              | 85.2891 ± 4.2496                | 73.75      |
|                                 |                                 | 53         |
|                                 |                                 | 60         |
|                                 |                                 | 71         |
| % Mass Reduction (Yield)        | 18.0442 ± 1.1684                 | -          |
|                                 |                                 | -          |
|                                 |                                 | 36         |
|                                 |                                 | 64         |

The Visibility of HAp synthesized from *C. persclupta* shells comparing with the HAp from another source showed in Figure 3. The measurement of Calcium contains to HAp need for the applicative and scientific reasons. It is necessary to report the Ca and P containing remineralization, reparations, or filler compounds for application purposes. Measurement of Ca and P content to compare the synthesis process’s efficiency and effectiveness from various HAp methods and sources. Each synthesis method causes a reduction in the mass of the source, so it is necessary to study in-depth methods with minimal mass reduction, but the Ca and P content is still in the standard range for HAp. The comparison of mineral contains in HAp showed in Table 5.

Table 5. The Comparison of Mineral Contains in HAp

| Minerals | HAp from *C. persclupta* Shells | References |
|----------|---------------------------------|------------|
|          |                                 | [14]       |
|          |                                 | [8]        |
| Ca       | 0.0121 ± 0.0031g/g              | 9.78; 10.50% (atom) | 88.88 |
| PO₄      | 0.0167 ± 0.0013g/g              | -          | -    |
| P        | 0.0084 ± 0.0007g/g              | 9.35; 8.97% (Atom) | 8.78 |

Another parameter for the Ca and P measurement contains the ratio of calcium against phosphor (Ca/P). According to the references, the common ratio Ca/P of HAp was 1.6%. The comparison of the ratio Ca/P from synthesized HAp showed in Table 6.
Table 6. The Comparison of the Ratio Ca/P from Synthesized HAp

| Parameter          | HAp from C. persclecta Shells | References |
|--------------------|--------------------------------|------------|
| Ratio Ca/P (%)     | 1.3724 ± 0.2736 (B)            | [13]       |
|                    | 1.07                           | [27]       |
|                    | 1.06                           | [2]        |
|                    | 1.33 ± 1.32 (O)                | [15]       |
|                    | 1.88                           | [9]        |
|                    | 1.78                           | [14]       |
|                    | 0.0433                         |            |
|                    | 0.0599                         |            |
|                    | 0.0423                         |            |

Table 5 concluded that HAp synthesized from C. persclecta shells was still in the range of Ca/P’s referenced ratio of Ca/P. The ratio of Ca/P connected to the particle size, the ratio of Ca/P 1.57-2.67%, can reduce the particle size to ±59nm, increasing Ca/P following by reducing the particle size [14].

The next steps threatened the biochemical Characterization of the HAp from C. persclecta shells; the Characterization included porosity, biodegradability, and swelling ability. The comparison between the HAp from C. persclecta against the references showed in Table 7.

Table 7. The Comparison Between the HAp From C.persclecta Against the References

| Parameters        | HAp from C. persclecta Shells | References |
|-------------------|--------------------------------|------------|
| Porosity (%)      | 9.9929 ± 0.7626                | [27]       |
| Biodegradability (%) | 10.8958 ± 1.1781              | [23]       |
| Sweling (%)       | 24.8416 ± 1.4989               |            |

The porosity measurements aim to predict porous in the HAp as the approach when the HAp applies to the livings. The porous important as the vessel of interactives between the mineral to the cells of hard tissues. The porous was the place where the new bone cells were produced [16,27,28]. It is easy to apply the porosity HAp to the hard tissues. A large amount of porosity can cause the structure of HAp to become fragile. But in nanoscale, the large amount of porosity didn’t give a fragile effect to the HAp [23], the Ca / P ratio of porous HAp to approximate the predicted physical strength of HAp when applied to living things. [21,22,23,26,28].

The measurements of biodegradability level aim to predict the decomposition of the HAp when it is applied. But the result is still preconceived if the degradability level was high, which means the HAp has a fragile structure [21,22,23].

The swelling ability measurement aims to predict the change of mass, volume, and density of the HAp when exposed to the body’s chemical compound, mostly water, salt, and glucose. The response reaction, especially with the liquid compounds, will cause the bioceramic’s porous binding or absorption reaction and increase its mass and volume. In the following condition, the
bioceramic structure became fragile because the liquids stuck inside the porous [21,22].

Synthetic materials derived from bone graft, bone filler, and bone-implant based on HAp due to the limited availability of bone donors but the large need [1,4,5,14]. For example, the Doktor Soetomo Surabaya hospital manages 300-400 surgical bone cases every month, then caused an increase of bone-implant materials requests [29]. On the other side, Indonesia also a country with the highest fracture sufferers in Asia [29].

CONCLUSION

The Celetaia persclupta snail shells potentiality to became a source of HAp for the medical needs mainly to reparating the hard tissues (filler, grafting, implant) based the report of the measurements of minerals contains in the shells like calcium (Ca) and phosphor (P). The mass yield from the synthesis process showed significant results. The synthesis using the alkaline precipitation method resulted in a white HAp powder indicating that the HAp from the shell of C. persclupta has the potential to be applied medically as a remineralizing agent in hard tissue.

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