Synthesis of Calcium Phosphate Compound from Paddy Field Snail Shells (*Pila Ampullacea*) as Calcium Precursor

**Kiagus Dahlan**¹²*, Dina Yauma Asra¹, Bayu Chandra Winata¹**

¹Department of Physics, IPB University, Bogor, 16680, Indonesia
²Advanced Research Laboratory, IPB University, Bogor, 16680, Indonesia

**Abstract.** Hydroxyapatite (HA) is a biomaterial that is widely used for bone implantation because it is bioactive and biocompatible. To improve the bioactive and biocompatible properties of hydroxyapatite, natural ingredients are used as a calcium source. In this study, the material used for calcium source is from paddy field snail shells. Snail shells produced from the process of boiling, cleaning, and drying calcined at a temperature of 800°C for 3 hours to obtain the powders. Calcium phase and calcium content of the powder was identified using x-ray diffractometer (XRD) and atomic absorption spectroscopy (AAS). The results of the analysis showed that the calcium phase of the snail shell is Ca(OH)₂ with calcium content of 52.12%. Synthesis of HA was carried out by precipitation method at 75°C by dripping H₃PO₄ into calcium solution and continued with two treatments, with and without sintering process. The powders were then characterized by XRD and FTIR. The results of the analysis showed that HA formed in samples that using sintering process and carbonate apatite appears in samples without sintering process.

1. Introduction

Biomaterials are defined as inert materials implanted into living systems as replacements for the function of living tissues and organs [1]. In Indonesia, the need for biomaterials in the medical field for various purposes continues to increase. The rise of traffic accidents that occurred recently led to high demand for material that can repair damaged bones. Accidents can cause bone damage such as cracked or fractured bones, so the presence of graft is required. To deal with this case, the right material is needed for bone implantation. The selection of the right biomaterial is very necessary for the process of implantation. The selected material should be easily obtained, biocompatible, bioactive, and non-toxic [2].

Bone substitute that commonly used today is from calcium phosphate compound which is hydroxyapatite (HA). HA is a member of the apatite compound group. Commercially available HA is highly expensive due to the usage of high purity chemical reagents in the preparation [3]. So it is important that these materials are produced independently. Therefore, naturally abundant snail shell waste was selected for the preparation of HA and this is one of the advantages to minimize the cost. Hence, biological waste (bio-waste) was used as the raw material for the synthesis.

Paddy field snail shell (*Pila ampullacea*) is a type of water snail that is easily found in Asia tropic freshwaters such as in rice fields, ditches, and lakes. Within the mollusc class of animals, the snail shell has high content of calcium [4]. Snail shells are plentiful in nature and it is a composite material consisting of calcium carbonate and organic matter [5]. The soft snail’s body is protected by a hard, spiral shell. Snail is usually used by the community only part of its contents as food ingredients that have high nutritional value. The shell portion which covers about 83-85% of the total weight of the
The snail is generally discarded without being utilized [6]. The snail shell is composed of a lot of calcium carbonate [7], so it has the potential to be used as a source of calcium (Ca). Calcium of snail shell content is about 34-35% in a dry matter [8].

2. Research Methods

Snail shells and H$_3$PO$_4$ were used as starting materials for HA synthesis. Snail shells were collected and washed using distilled water. And then dried and made into small pieces. This step aims to remove macro dirt from snail shells that we used. The cleaned and dried snail shells were then calcined using the furnace at 800°C for 3 hours to remove the organic components of the sample. Snail shells which are containing CaCO$_3$ will release CO$_2$ and another organic compound and produce CaO. The result of calcination then ground to a fine powder using mortar and pestle. The powder was characterized by AAS and XRD to determine the phase and calcium contained.

Hydroxyapatite was synthesized by precipitation methods. The H$_3$PO$_4$ which has been diluted with 100 ml distilled water was then put into Ca solution using a burette at a rate of 3 ml/min and stirred using a magnetic stirrer with a speed of 300 rpm. There are 2 variations for Ca and H$_3$PO$_4$ concentration used (1M Ca solution + 0.6M H$_3$PO$_4$ and 0.5M Ca solution + 0.3M H$_3$PO$_4$). When Ca solution is added, the surface of the beaker is covered by aluminium foil so it did not react with the air. Then the precipitates are deposited for 5 hours at room temperature. The slurry is filtered using a vacuum and heated in the furnace. There are 2 temperature variations used that are 110°C for 5 hours without sintering and temperature 110°C, then sintering at 900°C. Samples were characterized using XRD and FTIR.

| Table 1 | Sample ID of molarity and sintering process |
|---------|--------------------------------------------|
| Sample ID | Molarity Ca:P | Sintering |
| A1       | 1M: 0.6M     | No        |
| A2       | 1M: 0.6M     | Yes       |
| B1       | 0.5M: 0.3M   | No        |
| B2       | 0.5M: 0.3M   | Yes       |

3. Results and Discussion

Snail Shell’s Powder Characterization

The source of calcium used in this study was snail shells. The treatments begin with boiling the snails and continue to the process of separating the meat with its shell. The snail shell that will be used as a sample is cleaned from impurities that are still attached to the shell. After that, it dried in the sun and crushed into small pieces. Small pieces of dried shells were calcined at 800°C for 3 hours using the furnace. Furthermore, the results of calcination crushed until finely ground. The mass powder is 58.31 grams from the mass of the snail flakes of 82.88 grams. The grey powders were characterized using XRD and AAS. The XRD characterization aims to determine the calcium phase that is found in the sample and can be seen in Figure 1.

The XRD results stated that the calcium contained was calcium hydroxide Ca(OH)$_2$. The XRD peaks are adjusted to the Joint Committee on Powder Diffraction Standards (JCPDS) for Ca(OH)$_2$, CaCO$_3$ and CaO. The highest peaks at Ca(OH)$_2$ are found at the angle of 18.02°, 34.07°, 50.78° and peaks of CaCO$_3$ and CaO also appear in the samples. The results of AAS analysis showed that the calcium content of paddy field snail shells were 52.12%. This result showed that snail shells have a good potential to be developed as calcium precursor for calcium phosphate synthesis.
Synthesis of Hydroxyapatite

Hydroxyapatite (HA) synthesis was carried out with a precipitation method using paddy snail shells as a calcium source \([\text{Ca(OH)}_2]\) and \(\text{H}_3\text{PO}_4\) as a phosphate source. Figure 2 (a) and (b) showed the XRD spectra of HA treated with and without sintering process. It can be analyzed by that all the peaks shown in Figure 2 (a) and (b) match with the reference XRD pattern of HA and had the same XRD peak positions. But in sample A2 appear the secondary phase that is carbonate apatite, or also known as AKA and AKB. The carbonate ion compounds are able to inhibit the crystallization process \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\) so that the results will be dominated by an amorphous phase [9].

| Sample ID | Lattice parameter | Accuracy | c (Å) | Accuracy |
|-----------|------------------|----------|-------|----------|
| HA        | 9.418            | -        | 6.884 | -        |
| A2        | 8.52             | 90.49    | 7.19  | 95.42    |
| B2        | 8.12             | 86.26    | 6.85  | 99.56    |

| Sample ID | Crystallinity (%) |
|-----------|-------------------|
| A2        | 83.13             |
| B2        | 87.78             |
The International Conference on Sciences and Technology Applications

IOP Publishing

IOP Conf. Series: Journal of Physics: Conf. Series 1485 (2020) 012021
doi:10.1088/1742-6596/1485/1/012021

Figure 2. XRD Spectra of A1 and A2 samples

The calculation results of the lattice parameters are showed in Table 2 with high accuracy almost similar to the literature. And the concentration of calcium and phosphate used didn’t show much difference because the same Ca/P ratio 1.67. The samples also showed a good crystallinity as shown in Table 3.

Furthermore, HA powders were also characterized using FTIR to make sure the presence of carbonate ions in samples without sintering process. The results of the FTIR spectrum in the figure below showed the presence of the functional groups PO$_4^{3-}$ and OH$^-$ which were HA groups. The PO$_4^{3-}$ group was detected at wavenumber 480 cm$^{-1}$ and 572 cm$^{-1}$ for the vibration group P-O, while the vibration P=O was detected at wavenumber 1045 cm$^{-1}$. The OH$^-$ group was detected at wavenumber 3451 cm$^{-1}$. And in Figure 3 (b) appear the carbonate groups which were indicated the presence of carbonate apatite. The transmittance band of the carbonate apatite group was identified at wave number 870 cm$^{-1}$ and 1420 cm$^{-1}$. This result proved that HA formed in samples with sintering treatment and carbonate apatite appears in samples without sintering process.

Figure 3. FTIR Spectra of A1 and A2 samples

4. Conclusion

Hydroxyapatite (HA) had been successfully synthesized by precipitation methods by using snail shells as calcium source. The AAS results showed that snail shells have a calcium content about 52.12% and XRD results showed that calcium phase contained in the shells is Ca(OH)$_2$. The results of the analysis showed that HA formed in samples that using sintering process and carbonate apatite appears in samples without sintering process.
References

[1]. Kalfas, Ian H, MD. 2001. Principles of bone healing. Neurosurg. Focus. Vol 10

[2]. Charlena, Irma H, Desi K. 2015. Synthesis of Hydroxyapatite from Rice Fields Snail Shell (Bellamya javanica) through Wet Method and Pore Modification Using Chitosan. Procedia Chemistry. 17 (1): 27 – 35

[3]. Raya I, Erna M, AfdaliahY, Muhammad S, Andi I. Shynthesis and Characterizations of Calcium Hydroxyapatite Derived from Crabs Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations. International Journal of Biomaterials. Pages 1-8

[4]. Fatimah, Greef S, Wellyana P, Rico N, Lusi S, Rivaldo. 2018. Microwave-synthesized hydroxyapatite from paddy field snail (Pilla ampullacea) shell for adsorption of bichromate ion. Sustainable environment research. Pages 1-8

[5]. Kumar. Utilization of snail shells to synthesize hydroxyapatite nanorods for orthopedics applications. Royal society chemistry. 5: 39544–39548.

[6]. Anjaneyulua, Deepak K, Pattanyak, Vijayalakshmia. Snail Shell derived natural Hydroxyapatite: Effects on NIH-3T3 cells for Orthopedic Applications U. Materials and manufacturing process. Cross Mark : New York (US)

[7]. Prabakaran K, Balamurugan A, Rajeswari S. 2005. Development of Calcium Phospate Based Apatite from Hen’s Eggshell. Science Engineering. 2(1) : 115-119.

[8]. Watanabe Y, Moriyoshi Y, Suetsugu Y, Ikoma T, Kasama T, Hashimoto T, Yamada H, Tanaka J. 2004. Hydrothermal Formation of Hydroxyapatite Layers on the Surface of Typa-A Zeolite. Journal of American Ceramic Society. 87 (7) : 1395-1397

[9]. Ahmad N, Wai Y, Kee C, Zaw L, Radzali O. 2014. Optimizing of new approach sintering method on carbonated hydroxyapatite bioceramics. ASEAN Engineering Journal. Vol 3 (1) : 6-21

Acknowledgments

This work is supported by Direktorat Riset dan Pengabdian Masyarakat, Direktorat Jenderal Penguatan Riset dan Pengembangan, Kementerian Riset, Teknologi, dan Pendidikan Tinggi, Republik Indonesia.