Carbonated Hydroxyapatite Derived from Cerastoderma edule, Paphia undulata, and Meretrix meretrix Shells

Rosita Wati* and Yusril Yusuf

1Department of Physics, Faculty of Mathematics and Natural Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

*Corresponding Author: rositaawati@gmail.com

Abstract. Carbonated hydroxyapatite (CHAp) derived from three different shells, Cerastoderma edule, Paphia undulata, and Meretrix meretrix shells, have been synthesized by precipitation method. Calcium oxide from extracting shells, diammonium hydrogen phosphate, and ammonium bicarbonate was used to synthesis as the starting precursors. The CHAp materials were analyzed by Energy Dispersive Spectroscopy (EDS), Fourier Transform Infrared (FT-IR), and X-Ray Diffractometer (XRD). EDS analysis showed that the Ca/P ratio of CHAp powders were around 1.75-1.77 (bigger than the Ca/P ratio of HAp) and carbonate content of the powder CHAp was 3.10-4.05wt%. XRD data has produced diffraction patterns from the three different shells similar to the apatite structure. The position of the main peak (2θ) diffraction patterns detected at 31.99°, 32.23°, and 31.95°. The lattice parameter of a-axis and c-axis were obtained around 9.37 Å and 6.89 Å. Crystallite size of CHAp samples was 30-40 nm. The FTIR spectra showed that there are functional groups of OH-, PO4^3-, and CO3^2- at wave number around 3447, 571, and 873 cm^-1. Based on the results of the analysis, it shows that the CHAp characteristics that had successfully produced from Cerastoderma edule, Paphia undulata, and Meretrix meretrix shells were B-type CHAp.

1. Introduction

Hydroxyapatite (HAp) is one of calcium phosphate that widely used in biomedical applications due to its chemical composition mimicking that of bone mineral [1]. However, the composition of bone mineral differs from HAp. There are several impurity chemicals found in natural bone minerals, one of which is carbonate ions. The incorporation of carbonate ions into the HAp structure is called carbonated hydroxyapatite (CHAp). The concentration of carbonate ions in the structure of HAp in the composition of natural bone mineral is around 2-8wt%, depending on the age of the individual [2]. Carbonate ions can occupy three places in the HAp structure. A-type CHAp is formed when carbonate ions substitute hydroxyl ions; B-type CHAp is formed when carbonate ions substitute phosphate ions, while AB-type CHAp forms when carbonate ions substitute hydroxyl and phosphate ions simultaneously [3]. B-type CHAp is a substitute that is found in many natural bones [4]. B-type CHAp can reduce crystallinity and improve solubility during implantation [5].

Similar to HAp, CHAp can also be synthesized through several methods such as sol-gel method [6], mechanochemical-hydrothermal [7], nanoemulsion [1], and precipitation [8]. However, the most commonly used method is the precipitation method. The precipitation method is a simple method that can produce nanoparticles with low crystallinity [9], by-products in the form of water, and contamination from environments is low [10] and does not pollute the environment [11].

Previous studies have reported that HAp can be synthesized from biogenic materials such as cockle shells [12], green mussel shells [13], quail eggshells [14], golden apple snail shells [15], and buffalo bones [16]. This statement shows that CHAp can be synthesized through biogenic materials. The
research that has succeeded in synthesizing CHAp from the eggshell base material for drug delivery systems [17]. The results of the study stated that CaCO$_3$ content in eggshells was 94%. The carbonate content in CHAp is 6%, and there is a substitution of carbonate ions in a phosphate group which indicates the formation of type B CHAp.

The CHAp from biogenic material has a high chance of being received by organs because it has physicochemical similarities with human bones. Many biogenic materials are produced from coral, shells, and eggshells. From some examples of these biogenic materials can produce calcium carbonate. The content of calcium carbonate from biogenic shells, especially in its shell, has a big percentage (around 95-99%) so that the biogenic shell material is an excellent calcium source for synthesis CHAp. In addition, the use of biogenic sources of shells can be beneficial to the environment through recycling waste[18].

One type of shells that has quite large members is the shell from the Bivalvia class. Some members of the Bivalvia class, such as common cockle shells (*Cerastoderma edule*), batik shells (*Paphia undulata*), and milk shells (*Meretrix meretrix*), can be used for meat consumption. The Food and Agriculture Organization [19] reported that global production of quite large common cockle shells in 2014 amounted to 19,669 tons, 27,494 tons of batik shells in 2003 in the Republic of Korea and there were 7,112-47,752 milk shells produced. The abundant amount of these three types of shells can be used as a natural calcium source for CHAp synthesis and can reduce shells waste through recycling waste.

Based on these descriptions, CHAp synthesis of biogenic material from *Cerastoderma edule*, *Paphia undulata*, and *Meretrix meretrix* shells will be carried out using the precipitation method and expected to be used as a material for dental implants or bone replacement materials for bone tissue engineering.

2. Experimental Procedures

2.1 Materials

Three precursors are used to synthesis CHAp, NH$_4$HCO$_3$ as carbonate source, (NH$_4$)$_2$HPO$_4$ as phosphorus source and biogenic material from *Cerastoderma edule*, *Paphia undulata*, and *Meretrix meretrix* shells were used as calcium source (CaO). Others materials, NH$_4$OH is used to control pH of phosphorus solution and distilled water as a solvent.

2.2 Preparation of CaO

The biogenic material from *Cerastoderma edule*, *Paphia undulata*, and *Meretrix meretrix* shells were cleaned to remove dirt on the shells, washed by water, and soaked by acetone for 2x24h. The shell was dried in the sun, put in the oven at 90° C for 4h, then milled by a ball mill to obtain homogeny powdered of CaCO$_3$. The CaCO$_3$ powders were calcined in the furnace at 1000° C for 4h to decompose CaCO$_3$ to obtain CaO. The FTIR and XRD characterized CaCO$_3$. The FTIR, XRD, and EDS also examined powders of CaO. The CaO powders results were ready used to synthesis after passed through the 170 mesh sieved.

2.3. Synthesis of CHAp

The CHAp derived from *Cerastoderma edule*, *Paphia undulata*, and *Meretrix meretrix* shells were synthesized by the precipitation method. It started with dissolving 0.108 moles NH$_4$HCO$_3$ on 35 ml distilled water, as carbonate solution. Weighing 0.0648 moles (NH$_4$)$_2$HPO$_4$ was mixed by 70 ml distilled water as phosphate solution and controlled pH ≥10. Meanwhile, 0.108 moles CaO powders was added in 70 ml distilled water to form Ca(OH)$_2$ solution and kept stirring for 1h. The carbonate solution was reacted to the phosphate solution by dropwise. The mixture solution of carbonate phosphate was reacted to the calcium solution by dropwise (at ambient temperature, dropwise rate 1 ml/min, stirring rate 300 rpm) until all were complete. The mixture solution was kept stirring overnight at room temperature, then aged overnight to obtain a precipitate. The precipitate was filtered by Whatman 42 for 24h, washed by centrifuge twice for 10 minutes with speed 4000 rpm to remove unwanted ions, dried at 90° C overnight on the oven, pulverized manually with an agate mortar and pestle passed through a 170-mesh sieve, and sintered at 400°C for 2h on the furnace in the air atmosphere. The samples of CHAp derived from *Cerastoderma edule*, *Paphia undulata*, and *Meretrix meretrix* shells were called name by CHAp-1, CHAp-2, and CHAp-3 and characterized using XRD, FTIR, and EDS.
2.4 Characterization
The elemental composition of shells powder and CHAp samples were analyzed by EDS (JSM-6510LA). The sample powders were put on a semi-conductive copper tape and coated using platinum for 120 s. The data results of EDS were used to determine mass weight percentage: Ca (Calcium), P (Phosphorus), CO3 (Carbonate), and the Ca/P ratio of CHAp sample. The functional group of powdered shells was identified by FTIR (Thermo Nicolet iS10). The sample was mixed with KBr (1:100 by mass) crushing with agate and mortar and formed pellets. The spectra were collected on scanning wave number 4000-400 cm⁻¹, transmittance mode, and using 16 scans. Diffraction data were investigated by XRD (PANanalytical X'pert PRO) in a 2θ over the range 10-60°. The phase identification of shells powder and CHAp samples were compared using Joint Committee on Powder Diffraction Standard (JCPDS) card (no. 41-1475 = CaCO₃, no. 37-1479 = CaO, and no. 09-0432 = apatite structure (HAp)). The crystallography properties of CHAp samples, such as lattice parameters and crystallite size, were calculated from diffraction data of CHAp sample. The equation (1) used to determine the lattice parameter of a-axis and c-axis [20]:

\[
\frac{1}{d^2} = \frac{4}{3} \frac{h^2 + k^2 + l^2}{a^2} + \frac{l^2}{c^2}
\]

where hkl is Miller index of the plane, d is spacing between the atomic plane of the crystal lattice, a and c are lattice parameter. The equation (2) used to calculate crystallite size [21] of CHAp samples that evaluated by (300) reflections:

\[
s = \frac{k \lambda}{\beta \cos \theta}
\]

where s is crystallite size λ is the wavelength (1.54Å), k is Scherrer’s constant (0.94), β is the full width at half maximum (FWHM) of the CHAp line and θ is the diffraction angle. Degree of crystallinity all samples were determined using the equation (3):

\[
\% X_c = \left(1 - \frac{V_{112/300}}{I_{300}}\right) \times 100\%
\]

where \(X_c\) is degree of crystallinity, \(I_{300}\) is intensity of diffraction peak (300), and \(V_{112/300}\) is a hollow between diffraction peaks (112) and (300).

3. Result and Discussion

3.1 Characterization of CaCO₃ and CaO samples
FTIR spectra and the pattern of CaCO₃ sample extraction from the three different shell powder is given by Figure 1. The spectra of CaCO₃ samples showed that there were four main bands which were essentially vibration mode characteristics of the CaCO₃ calcite crystal structure. The vibration mode \(\nu_3\) asymmetric stretch is at the band location of wavenumber 1477-1481 cm⁻¹ and the transmittance band 1081 cm⁻¹ is the vibration mode \(\nu_1\) in-plane symmetric stretching [22]. The band at location 858-862 cm⁻¹ with vibration mode \(\nu_2\) out of plane bend indicates that the characteristic close to CaCO₃ and 709 cm⁻¹ (\(\nu_4\) in-plane bend) is related to the Ca-O bond [23]. Identification phase of shells powder is compared with JCPDS card no. 41-1475 and produced a diffraction pattern of pure calcite structure with a hexagonal crystal structure. The highest intensity peak of the three shells powder samples corresponds to the Miller indices (111), (012), and (221).
Figure 1. Diffraction patterns (A) and FTIR spectra (B) of CaCO$_3$ powders. (a) Cerastoderma edule shell, (b) Paphia undulata shell, and (c) Meretrix meretrix shell

The CaO extracted from shells powder has been analyzed for functional groups and their diffraction patterns given by Figure 2. From the FTIR spectra, it has been identified that there is a transmittance band located at 3642-3648 cm$^{-1}$ corresponding to the O-H bond. The transmittance band at wavenumber 1417 and 877 cm$^{-1}$ is related to the C-O bond [23]. Phase identification of diffraction data was compared with JCPDS cards no. 37-1497 and produced a diffraction pattern corresponding to the face-centered cubic (FCC) crystal structure of CaO. The highest intensity peak of the CaO powder samples corresponds to the Miller indices (111), (200), and (220). There is another phase identified Ca(OH)$_2$ with weak intensity. Transforming CaO into Ca(OH)$_2$ occurred because CaO absorbed H$_2$O in atmosphere exposure [24].

Figure 2. Diffraction patterns (A) and FTIR spectra (B) of CaO powders. (a) Cerastoderma aedule shell, (b) Paphia undulata shell, and (c) Meretrix meretrix shell

The composition contained in the three types of CaO shells from EDS data (Table 2) shows a large percentage of calcium (Ca) content of around 67-70% and a small amount of phosphorus content of about 0.2% and 0.3-5% C. Therefore, CaO from these three types of shells is a good source of calcium for CHAp synthesis.
Table 1 The CaO containing of shell powders.

| Shell powders | Ca (%) | P (%) | C (%) |
|---------------|--------|-------|-------|
| C. edule      | 70.83  | 0.24  | 0.34  |
| P undulata    | 67.45  | 0.26  | 5.04  |
| M. meretrix   | 70.23  | 0.26  | 1.15  |

3.2 Characterization of CHAp samples
Diffraction peaks of all samples have similar to the apatite structure characteristics. All samples confirmed the identification of the highest intensity peak situated 2θ angles at 31.99°, 32.23°, and 31.95° corresponding to (112) plane. The phase identification showed that the diffraction pattern formed was apatite structure characteristics. All samples have poorly crystallinity about 22-33% that indicated occurring substitution of carbonate ion to the phosphate ion in HAp structure, that implies natural bone apatite has a poorer crystallinity. The functional groups of CHAp samples were identified by FTIR spectra (Figure 3(a)). The band located at 873 cm⁻¹ related carbonate vibrations (ν₂, CO₃²⁻) that are characteristic of B-type CHAp[3]. The H₂O was absorbed was detected broadband at 3448 and 1635 cm⁻¹. The bands 1065 and 1417 cm⁻¹ were carbonated which were substituted for phosphate sites, characterized by B-type CHAp[17].

![Figure 3](image)

(a) Diffraction patterns (a) and FTIR spectra (b) of CHAp samples

The shift of diffraction peak caused the lattice parameter changed. The lattice parameter of a=b and c were calculated by equation (1) using diffraction peak (300) and (002) plane. As a result, all CHAp samples occurred the increasing of c lattice parameter and decreasing of a lattice parameter comparable to the HAp. It suggested that formed the B-type CHAp[3]. The crystallite size of CHAp samples were evaluated corresponding to (300) reflection, and the results are given by Table 3. The crystallite size was obtained 30-40 nm.

Table 2. Lattice parameter, degree of crystallinity, and crystallite size of CHAp samples.

| Sample | Lattice parameter | Degree of crystallinity (%) | Crystallite size (nm) |
|--------|-------------------|-----------------------------|-----------------------|
|        | a (Å)             | c (Å)                       |                       |
| CHAp-1 | 9.376             | 6.902                       | 33.92                 | 40.8377 |
| CHAp-2 | 9.342             | 6.885                       | 22.05                 | 30.5608 |
| CHAp-3 | 9.389             | 6.887                       | 28.00                 | 40.8327 |

3.3 Elemental composition of CHAp samples
The EDS analysis used to determine the composition of CHAp samples, given in Table 3. The concentration of carbonate ion on the CHAp samples was around 3.10-4.04 wt%, mimicking to the
composition of mineral bone. CHAp samples have trace elements close to the mineral bone. Presence of these elements in material bone graft could play a role in bone regeneration [17].

Table 3. Elemental analysis of CHAp samples.

| Sample | Ca (%) | P (%) | CO\(_3\) (%) | Ca/P Ratio |
|--------|--------|-------|--------------|------------|
| CHAp-1 | 64.72  | 28.33 | 3.95         | 1.77       |
| CHAp-2 | 64.58  | 28.52 | 3.10         | 1.75       |
| CHAp-3 | 63.66  | 28.00 | 4.05         | 1.76       |

4. Conclusion
The CHAp has successfully produced from three different shells, Cerastoderma edule, Paphia undulata, and Meretrix meretrix, by the precipitated method. The CaO from three different shells has a high calcium content around 67-70% that it is an excellent calcium source for synthesis CHAp. The FTIR spectra of CHAp samples detected functional groups of OH\(_-\), PO\(_4\)\(^{3-}\), and CO\(_3\)\(^{2-}\) that correspond to the CHAp forming. The phase identification showed that the diffraction pattern formed was apatite structure characteristics. According to XRD analysis, CHAp sample has a poor crystallinity and crystallite size around 30-40 nm. The carbonate content and ratio on the CHAp samples were 3.10-4.05 wt.% and 1.7 (bigger than HAp’s ratio). The incorporation of carbonate ion into the HAp structure due to increasing c lattice parameter and decreasing a lattice parameter (comparable to HAp) that indicated the forming CHAp is B-type CHAp characteristic.

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