Characteristics of Carbonated Hydroxyapatite Based on Abalone Mussel Shells (*Halioitis asinina*) Synthesized by Precipitation Method with Aging Time Variations

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Abstract. Carbonated Hydroxyapatite (CHAp) is a substituted-hydroxyapatite (HAp) with carbonate ions that are similar to natural inorganic bone constituents. The CHAp can be synthesized from natural material that has high calcium carbonate, such as abalone shells (*Halioitis asinina*), with a calcium carbonate content of 90-95% of the total abalone shell weight. In this study, the precipitation method has succeeded in synthesizing CHAp. The effect of aging time variations (0, 24, and 48 hours) during the synthesis process on CHAp characteristics was investigated. Characterization of the samples were conducted using X-Ray Diffraction (XRD), Energy Dispersive X-Ray Spectroscopy (EDX), and Fourier Transform Infrared (FTIR). Based on XRD data analysis, the diffraction peaks at 32.02° were observed in all variations of aging time, i.e., the characteristics of B-type CHAp phase, in which the increasing of aging time reduces its crystallinity. B-type CHAp was formed when carbonate ions substitute the phosphate ions in the structure of HAp, as confirmed by EDX analysis. This means that the increasing of aging time improves its Ca/P mole ratio (1.71-1.81), which is greater than the Ca/P mole ratio of stoichiometric HAp (1.67). This is due to an increase in the number of carbonate ions that replace the position of phosphate ions as aging time increases. This is also confirmed by FTIR analysis, which showed that the increasing of aging time cause stronger absorption of carbonate ion in 872.9 cm⁻¹, characteristics to B-type CHAp. From data analysis, it can be concluded that 48 hours aging time was the optimum time to produce B-type CHAp which has similar characteristics to natural bone apatite with low crystallinity.

1. Introduction

Bioceramic materials such as calcium phosphate become a new alternative material in orthopedic applications, due to their advantages over commonly used metal materials. Calcium phosphate has excellent material properties in terms of biocompatibility as well as quality of integration with bone [1]. Bone tissue engineering has been widely developed to overcome the problem of bone defects by restoring and maintaining the natural function of bone tissues. The potential calcium phosphate that can be used in bone tissue engineering is hydroxyapatite [2].

Hydroxyapatite (*Ca₁₀(PO₄)₆(OH)₂*, HAp) is the basic inorganic calcium phosphate mineral component of human bones and teeth that have biocompatible, bioactive, and osteoconductive properties [3]. It is known that stoichiometry in natural bones is different from HAp. In natural bones, there is a carbonate mineral content that varies based on age, which ranges from 2-8%wt [4]. Because of the
presence of carbonate minerals in natural bones, now HAp substituted with carbonate minerals is a bioceramic material that has been developed to become a biomaterial in the application of bone tissue engineering [5]. HAp substituted carbonate minerals is called carbonated hydroxyapatite (CHAp) [6].

The presence of carbonate ions in HAp will increase the dissolution rate in solution, so that the solubility and bioactive properties are better than HAp [7]. In general, replacing carbonate ions in the HAp lattice structure during the synthesis process will determine the CHAp type. The carbonate ion substituted to replace the hydroxyl ion will form A-type CHAp, while the carbonate ion replaces the phosphate ion to form B-type CHAp [8]. In biomedical applications, B-type CHAp is the most widely used type because, in biological apatites, B-type CHAp can cause decreased the crystallinity and increase the solubility that to accelerate bone regeneration [9]. Some of these statements were used as the basis for this research to synthesize B-type CHAp.

Calcium sources that can be used for the manufacture of CHAp are natural ingredient or chemical ingredients, for natural ingredients can from corals, green mussel shells [10], eggshells [11], crab shells [12], etc. In addition to these materials, exploration needs to be done for the process of making CHAp which contains high calcium carbonate. The basic materials that could be potentially used as CHAp materials are abalone mussel shells. Abalone shells contain calcium in the form of calcium carbonate (CaCO₃) in its shell, which is around 90-95% [13].

The CHAp can be synthesized using various synthesis methods. Among several methods of synthesis, precipitation methods are one of the most promising methods for producing nano-sized apatite particles [14]. In the synthesis process, many synthesis parameters can be controlled to produce CHAp with the best characteristics, such as aging time, synthesis temperature, solution pH, etc. In this study, aging time was varied, which then analyzed the effect on the mol ratio of Ca/P, functional groups, crystal phase, and crystallographic properties of CHAp synthesized.

2. Experimental Procedure

2.1. Preparation of Calcium Oxide (CaO)
Abalone mussel shells (Halioitis asinina) was cleaned and scrubbed by boiling water and then washed using distilled water to remove macro impurities that are still attached, such as shellfish and algae. Then the abalone mussel shells were dried in an oven at 100°C for 4 hours. To get abalone shell powder with smaller particle sizes, a ball-milling was done. The calcination process was carried out at 1000°C for 4 hours to obtain calcium oxide (CaO) powder.

2.2. Synthesis of Carbonated Hydroxyapatite (CHAp)
(NH₄)₂HPO₄, as a phosphate source, was prepared by dissolving 8.5536 gram with 70 mL distilled water (0.0648). The pH control was carried out by adding 15 mL of ammonia solution (NH₄OH) into (NH₄)₂HPO₄ solution to form an alkaline (NH₄)₂HPO₄ solution. The (NH₄)₂HPO₄ alkaline solution was then added with NH₄HCO₃ solution (made by dissolving 5.1192 gram NH₄HCO₃ in 50 mL of distilled water) with a penetrating speed of 1 mL/minute and stirring speed of 350 rpm at room temperature. The mixture was then added with Ca(OH)₂ solution drop-wise (6.048 g CaO dissolved with 60 mL of distilled water), followed by stirring for 1 hour, 350 rpm, at room temperature to homogenize the mixture. Next, the solution was aged for 0, 24, and 48 hours and continued with filtering off the mixtures. The CHAp was then centrifuged for 20 minutes at 4000 rpm. The dry CHAp was obtained after drying it in an oven at 90°C for 24 hours and sintering at 650°C for 2 hours.

2.3. Characterization
The synthesized CHAp was characterized using XRD to identify the crystalline phase and determine its crystallographic properties which included calculation of lattice parameters, crystallite size, and microstrain. Then, FTIR analyses were done to identify functional groups that are characteristic of CHAp namely OH⁻, PO₄³⁻, and CO₃²⁻. SEM analysis was also used to characterize the morphology of
CHAp obtained from variety of aging times. The elemental content and the mol ratio of Ca/P of the CHAp was characterized using EDX.

3. Result and Discussion

3.1. XRD Analysis

The XRD patterns of the CHAp at different aging time are shown in Figure 1. The result of XRD patterns was analyzed by comparing with standard diffraction pattern of JCPDS No. 19-0272 in range of 20° - 60°. Based on the CHAp XRD spectrum pattern in Figure 1, a pattern that is characteristic to CHAp are observed, which formed three characteristic peaks at diffraction angles of 32.024°, 33.150°, and 34.122° with diffraction fields of (211), (300), and (202), respectively [4]. The lattice parameter value (a and c axes) indicated that the B-type CHAp has been formed at all of the samples, in which B-type CHAp has lower a value but higher c value than that of pure HAp.

![Figure 1. Characterization result with XRD of CHAp with aging time variations: (a) 0 hour or without aging; (b) 24 hours; and (c) 48 hours.](image)

From Figure 1, the intensity of XRD patterns were sharper and getting higher as the aging time increases. The increasing intensities of diffraction peaks indicating an increase in sample crystallinity [15]. This result is agreed with the result of lattice parameter, crystallite size and microstrain calculation, presented in Table 1. Table shows that the lattice parameter values calculated from all variations have shown the number close to the natural bone lattice parameter values, namely \(a = 9.74 \, \text{Å}\) and \(c = 6.89538 \, \text{Å}\) [16]. The crystallite size estimation was performed using Scherrer's equation, which shows that the longer the aging time improves the crystallite size. This was confirmed by XRD spectra data in Figure 1, in which, the bigger crystallite size, the higher crystallinity degree [15] with a small value of microstrain, which indicates the sample has a small crystal defect [10].
Table 1. The lattice parameter, crystallite size and microstrain of CHAp on variations of aging time.

| Samples           | Lattice parameter (Å) | Crystallite size (nm) | Microstrain |
|-------------------|-----------------------|-----------------------|-------------|
|                   | $a$                  | $c$                  |             |
| Without aging     | 9408                 | 6.895                | 21.911      | 0.005       |
| Aging 24 hours    | 9.240                | 6.892                | 26.771      | 0.004       |
| Aging 48 hours    | 9.422                | 6.889                | 48.187      | 0.002       |

3.2. FTIR Analysis

FTIR spectroscopy was used to identify the functional groups CHAp from abalone mussel shells. The most characteristic chemical groups in FTIR spectrum of synthesized CHAp are PO$_4^{3-}$, OH$^-$, and CO$_3^{2-}$. The results of CHAp synthesized from abalone mussel shells (Figure 2) showed that the aging time variations affects the intensity (%transmittance) and the band shape. Asymmetric stretching vibration of phosphate groups (PO$_4^{3-}$) was detected at 1122.5 cm$^{-1}$ which indicates that the crystals was truly formed [17].

The PO$_4^{3-}$ asymmetric stretching vibration is sharper because the of the nature of vibrating atom that moves faster. The PO$_4^{3-}$ symmetry stretching vibration was detected in all variations at 1010 and 1012.2 cm$^{-1}$ which indicate the formation of CHAp. The PO$_4^{3-}$ asymmetric bending vibration was detected in all variations at 595.4 cm$^{-1}$. The characteristic stretching modes of O-H bands at 3572 cm$^{-1}$ are also noticed in all aging time variations [18]. Characteristic peak of CO$_3^{2-}$ was also detected in all aging time variations at 1396 and 872.9 cm$^{-1}$, which indicate the formation of CHAp B-type.

![Figure 2. FTIR spectra of CHAp from all aging time variations.](image)

3.3. EDX Analysis

EDX analysis was conducted to determine the Ca/P ratio of CHAp and to determine the element composition of CHAp. Table 1 represents the EDX spectra of CHAp from all aging time variations. The longer aging time used, the lower carbonate (CO$_3^{2-}$) content resulted. The percentage of carbonate corresponds to the carbonate in natural bone, which is around 2-8wt% [4].

Regarding the molar ratio of Ca/P, the substitution of B-type carbonate ion which replaces the position of phosphate ion in HAp lattice structure, causes the amount of phosphate element decreased and become smaller, thus the Ca/P molar ratio of CHAp is higher than the Ca/P molar ratio of stoichiometric HAp. It is reveals that that the increasing aging time reduces the molar ratio of CHAp, which is around 1.71-1.81.
Table 2. The element composition present in CHAp sample with different aging time.

| Weight of element composition (wt%) | Without aging | Aging 24 hours | Aging 48 hours |
|------------------------------------|---------------|----------------|----------------|
| Ca                                 | 50.21         | 51.44          | 52.66          |
| P                                  | 21.44         | 22.85          | 23.90          |
| CO$_3^{2-}$                         | 8.00          | 7.40           | 7.15           |
| Ca/P                               | 1.81          | 1.74           | 1.71           |

3.4. SEM Analysis
The morphologies of the CHAp prepared using different aging times are shown in Figure 3. The SEM result indicated different morphology forms of CHAp are formed when the aging time are varied. The particles synthesized with 0-hour aging time showed less tendency to agglomerate and forms irregular shape. On the other hand, for samples prepared at aging times of 24 and 48 hours, the particle showed high aggregation and more regular shape. This result is in good agreement with the result reported by Niakan et al. [19] which explained agglomerate of CHAp maybe caused by the grinding effect. The change from irregular to regular particle morphology with the increasing of aging time was correspond to the increasing of HAp crystallinity, in which, more regular shape of the particles was observed when the powders had higher crystallinity [20].

![SEM images](image_url)

Figure 3. SEM images of CHAp particles obtained in various aging time; (a) without aging, (b) 24 hours, (c) 48 hours

4. Conclusion
In this study, CHAp made from abalone shells (*Halioitis asinina*) was successfully synthesized using precipitation methods with variation of aging time. Based on the characterization data using XRD, the B-type CHAp characteristic pattern were observed in all aging time variations in aging time, in which...
the longer the aging time, the better crystallinity of the sample. This is confirmed by the calculation of the crystallite size, which shows that the longer the aging time improves the size of the crystallite with a small microstrain value. The results of characterization using FTIR showed the presence of carbonate functional groups at 872.9 cm\(^{-1}\) which is characteristic to B-type CHAp and the band is getting broader along with the extension of aging time. The EDX data shows that the longer the aging time, the smaller carbonate content in the sample, in which the Ca/P molar ratio is getting smaller, which is 1.71-1.81.

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