Development Carbonated Hydroxyapatite Powders from Oyster Shells (*Crassostrea gigas*) by Sintering Time Controlling

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Abstract. Carbonated-hydroxyapatite (CHAp) is a hydroxyapatite substituted with carbonate ions and is known to resemble the hard bone structure of humans. CHAp was synthesized from biogenic material. Oyster shells (*Crassostrea gigas*) was biogenic materials that used as a calcium source. In addition, sodium bicarbonate and ammonium hydrogen phosphate were also used as sources of carbonate and phosphate. In this research, the oyster shells used were milled, calcined, and characterized to determine the phase changes from CaCO$_3$ to CaO by decomposition process. CHAp powders which were synthesized by precipitation method, were sintered at 650 °C for 0.25, 0.5, 1, 2 and 4 hours. To find the quality of CHAp powders with different sintering time, characterization process using Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffractometer (XRD), and Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) has been successfully done. FTIR, XRD, and SEM-EDX was used to determine functional groups, to confirm the presence of carbonate groups, to analyse the crystallography, and to calculate the Ca/P molar ratio and carbonate content of CHAp powders. Based on the results of characterization process showed that the oyster shells were successfully synthesized into CHAp and the length of sintering time effects of the crystallite size and crystallinities. CHAp powders sintered for 4 h have 41.385 % and 31.499 nm degree of crystallinity and crystallite size, respectively.

1. Introduction

Carbonated hydroxyapatite (CHAp) is hydroxyapatite (HAp) substituted with carbonate ions. CHAp is divided into three types based on the location of carbonate ion substitution. The carbonate ion substituted on the hydroxyl, phosphate and both sites is called type-A, Type-B and type-AB CHAp [1]. CHAp known as materials is closely resemble with human bone in approximately 2–8 wt. % [2-4].

Substituted carbonate ions in HAp cause a decrease in crystallite size and crystallinity. Low crystals are known to affect solubility based on in vitro tests, CHAp is also more bioresorbable than HAp, can dissolve in the body's physiological pH so it is considered that CHAp is more like biological apatite [5]. Especially for B-type CHAp, the incorporation of carbonate and tetrahedral phosphate ions causes changes in lattice parameters in the CHAp hexagonal structure [2].

The precipitation method is one of the most commonly used methods to synthesize CHAp. In the synthesis process many parameters can be controlled to produce type B CHAp which can be applied to bone regeneration processes such as sintering time. In addition, to produce CHAp, biogenic material can also be used. Because of the success of some researchers synthesizing HAp from several types of shells,
so CHAp also has a great opportunity to be synthesized from shellfish as a source of natural calcium [6-9]. Oyster shells are one type of natural material that is rich in calcium carbonate.

2. Materials and Method

2.1. Materials

For synthesis, CaO was obtained from oyster shells, diammonium hydrogen phosphate (NH₄)₂HPO₄, ammonium bicarbonate NH₄HCO₃ and ammonium hydroxide (NH₄OH) were procured from Merck (Germany).

2.2. Synthesis of CHAp from Oyster Shells

The oyster shells that were collected were then cleaned and soaked using acetone, dried using an oven for 4 hours at 100 °C and milled using a ball mill to obtain CaCO₃ in powders. CaO was obtained through a decomposition process after calcination for 4 hours with decomposition reaction as following equation.

$$\text{CaCO}_3 (s) \rightarrow \text{CaO} (s) + \text{CO}_2 (g)$$  \hspace{1cm} (1)

B-type of CHAp powders were prepared by precipitation method according to the following equation:

$$\text{Ca}_{10-x/2} (\text{PO}_4)_{6-x} (\text{CO}_3)_x (\text{OH})_2$$  \hspace{1cm} (2)

Where x=1.2, by drop-wise addition of carbonate solution into phosphate solution using with pH>10 controlling. Next, the mixed solutions were drop-wised into CaO solution while stirring using a hot plate stirrer with 300 rpm at a room temperature. In addition, the CHAp solution was aged overnight, filtered to separate white precipitate and water, centrifuged and washed until the ammonia and calcium salts were removed. The last step before sintering, the CHAp gels was dried in an oven for 24 hours at 80 °C. To get low crystallinity and high phase purity sintering process carried out at 650 °C with sintering time variations i.e., 0.25, 0.5, 1, 2 and 4 hours which will be compared also with the CHAp without sintering process.

2.3. Characterization

Functional groups and the presence of carbonate ion in CHAp were determine by Infrared spectra that were recorded on a Spectrum Thermo Nicolet iS10 spectrometer using KBr pellets. Samples were examined in transmittance mode in 400-4000 cm⁻¹.

Crystallography of CHAp such as crystallinity, crystallite size, hkl plane of diffraction, phase purity and lattice parameters were analyzed by XRD Merk PANalytical Type X’Pert Pro with 1.54060 Å CuKα. The a and c-axis lattice parameters and of CHAp powders were analyzed by (002) and (300) hkl plane according with following equation (3) and (4):

$$\frac{1}{d^2} = \frac{4}{3} \left( \frac{h^2 + hk + i^2}{a^2} \right) + \frac{i^2}{c^2}$$  \hspace{1cm} (3)

Where d= distance between plane (nm) and hkl is Miller indexs. The crystallite size was calculated by XRD through Scherrer’s equation (4).

$$L = \frac{k\lambda}{\beta_{1/2} \cos \theta}$$  \hspace{1cm} (4)

Where L is crystallite size (nm), λ is wave length of Cu (1.54060 Å), β is full width at half maximum-FWHM (radian), θ is the diffraction angle (degrees), and k is the boarding constant (0.94). The hkl plane (300) is selected to calculate the crystallite size. Also, degree of crystallinity in this studi is calculated by following equations:

$$X_c = 1 - \left( \frac{\nu_{211/300}}{\nu_{300}} \right)$$  \hspace{1cm} (5)
\( X_c \) is crystallinity (%), \( I_{300} \) is intensity of (300) diffraction peak and \( V_{211/300} \) is the intensity of the hollow between (211) and (300) diffraction peaks of HAp.

Morphology of CHAp powders was examined by scanning electron microscopy (SEM, Jeol JSM-6510LA). The Ca, P, and C-content of the CHAp powders was quantified by EDX data, to obtain the ratio of Ca/P. Samples were prepared by first coating with Pt.

3. Result and Discussion

IR spectrum from samples synthesized at temperatures of 650 °C presented in Fig. 1, based on the transmittance peaks indicate that there is a group PO\(_{4}^{3-}\) in the regions of 567-568 cm\(^{-1}\) which is a type of Triply degeneration bond (O-P-O) [10]. In other regions, V\(_{4}\) also exist in 550-600 [11] and 960-962 cm\(^{-1}\) [4]. Bands in 871-873 cm\(^{-1}\) regions are characteristic of B type of carbonate group. OH\(^{-}\) group appears at the peak of transmittance with a wave number is 1020-1120 cm\(^{-1}\). So that it can be calculated that all functional groups of CHAp are detected in this study and the oyster shells was successfully synthesized into CHAp.

![Figure 1](image-url) FTIR result of CHAp powders at various of sintering time (a) without sintering (b) 0.25 h (c) 0.5 h (d) 1 h (e) 2 h (f) 4 h.

XRD analysis of CHAp powders (Fig. 2) showed the apatite phase that has been matched with ICDD Grant-in-Aid, PDF#190272. The diffraction peak formed is the identity of HAp and CHAp, it can be seen that it overlaps at several peaks, i.e., (211), (112), (300) and (202).

When the carbonate ion is substituted on the HAp structure, a lattice parameters changes. Specifically for type B CHAp, an increase in the \( c \)-axis and decrease in the \( a \)-axis are adjusted to the HAp lattice parameters [12] (\( a \)-axis= 9.418 and \( c \)-axis= 6.884 lattice parameters of HAp: ICDD-Grant-In-Aid, PDF#090432). The theory is in accordance with the results of the CHAp produced, as shown in table 1.

![Table 1](image-url) Crystallographic properties of CHAp powders.

| Sintering time (h) | Lattice parameters | Crystallinity (%) | Crystallite size (nm) | Microstrain |
|-------------------|--------------------|-------------------|-----------------------|------------|
|                   | \( a \) (Å)        | \( c \) (Å)       |                       |            |
| without sintering | 9.389              | 6.720             | 14.558                | 18.835     | 0.018 |
| 0.25              | 9.397              | 6.887             | 26.313                | 26.774     | 0.012 |
| 0.5               | 9.400              | 6.887             | 31.137                | 31.285     | 0.012 |
| 1                 | 9.401              | 6.889             | 34.117                | 31.430     | 0.011 |
| 2                 | 9.417              | 6.890             | 39.437                | 31.464     | 0.011 |
| 4                 | 9.420              | 6.888             | 41.385                | 31.499     | 0.010 |
The other crystallography is crystallite size and micro strain. By using the Scherrer’s equation (4), we obtain the crystallite size as in table 1. The results can be concluded in this study, crystal growth has increased when sintered for a longer time, the results of which are also revealed by Garskaite et al. (2014) who concluded that the crystallization process depends on the heating rate and holding temperature [11]. However, this research slightly different from their study, because in this study, no control over the heating rate was carried out.

![XRD result of CHAp powders various of sintering time](image1)

**Figure 2.** XRD result of CHAp powders various of sintering time (a) without sintering (b) 0.25 h (c) 0.5 h (d) 1 h (e) 2 h (f) 4 h

![SEM image of CHAp powders](image2)

**Figure 3.** SEM image of CHAp powders **A:** without sintering **B:** 2 h sintering time
CHAp morphology on the micrometre scale did not show any significant changes (Fig. 3). So that it can be concluded that the sintering process has no effect on the CHAp morphology synthesized from oyster shells as a source of calcium.

Elemental composition obtained from EDX data shows that CHAp prepared by following equation (2) succeeded in obtaining approximately 8 wt% carbonate content. This is in accordance with the prediction, when $x = 1.2$ it will be obtained about $\pm 8$ wt.% carbonate content in CHAp. Related to the molar ratio of Ca/P, all of CHAp powders have $<1.67$ Ca / P molar ratio. This value indicates that, when carbonate ions incorporated to the phosphate group cause a reduced phosphate content, it increases the Ca/P molar ratio.

4. Conclusions
CHAp from oyster shells has been successfully synthesized using precipitation methods with variations in sintering time. The results obtained showed that in this study, the length of sintering affected the crystallite size. The longer the sintering time, the greater the crystallite size. In addition, based on the XRD spectrum shows that the CHAp powder produced is B-type CHAp, this is also supported by the lattice parameter value which decrease on the a-axis and increase on the c-axis. For morphology on a micrometre scale, it can be seen that the length of sintering does not have a significant effect on CHAp morphology.

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