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To cite this article: R M Anggraini and Yusril Yusuf 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **546** 042002

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The Effect of Stirring Time on The Characteristics of Carbonated Hydroxyapatite from Pearl Shells (*Pinctada maxima*)

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Abstract. Carbonated hydroxyapatite (CHAp) is hydroxyapatite (HAp) substituted by carbonate ions that has bioactivity because of their similarities to the natural bone. Pearl shells (*Pinctada maxima*) have been used as natural ingredients for the synthesis of B type CHAp using precipitation method with different stirring time (0 h, 2 h, and 4 h). The CHAp was characterized by using X-Ray Diffractometer (XRD), Fourier Transform Infrared (FTIR), and Scanning Electron Microscopy (SEM). The XRD results show the characteristics of B type CHAp from the position of the main peak and some diffraction plane, as well as from lattice constants a and c . The position of the main peak and plane (300) is above the position of pure HAp, plane (002) is below the position of pure HAp, and the lattice constants a are smaller than 9.418 Å and c higher than 6.884. FTIR results showed that the main peak B type CHAp found at wavenumbers 874, 1450 and 1420 cm^{-1} . According to the FTIR data, it can be concluded that stirring time variations was affecting the intensity of the functional group peaks. The smallest crystal defect found in 2 h stirring sample based on the calculation of microstrain. SEM results indicated that CHAp morphology was affecting by stirring time. The morphology of CHAp tends to agglomerate for sample with 4 h stirring time.

1. Introduction

Biomaterials are one of the materials that widely used for biomedical applications, for example in bones and teeth. There are several requirement that must be considered for the application of biomaterials in this field, such as non-toxic, not trigger inflammatory reactions, not trigger allergies, and can induce cell development or differentiation if needed [1][2]. One type of biomaterials that can be used in biomedical applications is Hydroxyapatite (HAp).

HAp is a bioceramic material and a calcium phosphate mineral phase that found in natural bone [3]. HAp is one of the apatite group which was confirmed as an artificial bone material because of its similarities to the natural bone. HAp has been synthesized and used to produce various forms of implants or as implant coatings [4]. In natural bone, HAp substituted by other ions such as carbonate ions that called as carbonated hydroxyapatite (CHAp) [5].

CHAp is considered a bioresorbable material for bone graft applications and shows higher bioactivity and smaller particle size than HAp, so that, tissue interactions with CHAp materials will be better than interactions with HAp [5][6]. Based on the location of carbonate ion substitution in the HAp lattice, CHAp consists of A, B, and AB type. A type CHAp occurs when carbonate ions replace hydroxyl ions,



B type will be formed when carbonate ions replace phosphate ions, and AB type when carbonate ions replace hydroxyl and phosphate ions [7].

The CHAp can be obtained by using the precipitation method [8]. One of the primary materials for CHAp synthesis is pearl shells (*Pinctada maxima*). Pearl shells that widely cultivated in Indonesia contained calcium carbonate (CaCO_3) as a natural source of calcium [9]. Central Beureu of Statistic noted that the trade value of pearls produced by pearl shells in Indonesia experienced an increase in the export value of 13.5% from 2012 to 2016. In 2016, Indonesia became one of the countries considered as a pearl producer with an export value of US \$ 45.9 million [10].

In this study, B type CHAp was synthesized by using precipitation method. The synthesis process was carried out by varying the duration of the stirring time which was 0 h, 2 h and 4 h. The essential source of natural calcium was originated from pearl shells (*Pinctada maxima*)

2. Experimental Procedure

2.1 Preparation of calcium oxide

Firstly, pearl shells were cleaned using water and soaked with acetone for three days. Then pearl shells were dried under direct sunlight for one day and continued to dry in the oven for 24 h at 100°C . Next, the pearl shells were crushed using a ball mill to form CaCO_3 powder. CaCO_3 powder was calcined at 1000°C for 4 h to decompose CaCO_3 into CaO powder which was the calcium source in synthesis process. CaO powder was then sieved to obtain a more homogeneous powder.

2.2 Synthesis of CHAp

The synthesis method of CHAp was precipitation method. A 0.036 moles of sodium bicarbonate (NaHCO_3) as a source of carbonate and 0.036 moles of diammonium phosphate $[(\text{NH}_4)_2\text{HPO}_4]$ as a phosphate source were dissolved using 80ml distilled water. The NaHCO_3 solution was added dropwise to $(\text{NH}_4)_2\text{HPO}_4$ solution through a titration process with a drop rate of 40 drops/minute in room temperature and stirred at 300 rpm. The pH solution was adjusted by adding NH_4OH until pH 10. After the addition of NaHCO_3 solutions, the solution was then stirred for 30 minutes. The mixture of carbonate-phosphate solution was added into a solution contained of 0.06 moles of CaO that dissolved in 80 ml of distilled water through titration process. The titration process was carried out under the same conditions as the mixing process of NaHCO_3 and $(\text{NH}_4)_2\text{HPO}_4$. After the whole solution mixed, additional stirring was carried out by varied the stirring time, which was 0 h, 2 h, and 4 h. The aging process was carried out for 24 h and followed by a filtering process. The results of this process were washed using distilled water and dried using an oven at 100°C for 24 h to produce CHAp in powder form. CHAp powder was then sintered using a furnace at 650°C for 2 h.

2.3 Characterization

The characterization of CHAp sample was carried out using Panalytical Type X'Pert Pro XRD, Thermo Nicolet iS10 FTIR, and SEM JSM-6510LA Jeol. XRD was used to determine the phase contained in the sample, position of several diffraction fields, lattice constants, crystallite size, and microstrain. Crystallite size was calculated using the Scherrer equation (equation 1), where L is the size of the crystal (nm), k is the Scherrer constant (0.94), λ is the X-ray wavelength (1.54 \AA) and B is the half-peak width (full width at half maximum = FWHM) from the peak spectrum (in radians). FTIR was used to determine the functional groups that contained in the sample, and SEM was used to determine the morphology of the sample.

$$L = \frac{k\lambda}{B \cos \theta_B} \quad (1)$$

3. Result and discussion

3.1 XRD analysis

Figure 1 shows the XRD patterns for the CHAp sample that were synthesized with stirring time variation. All samples had apatite phase without any other impurity phase. B type CHAp characteristics can be identified from the position of the main peak, the plane (300) and (002). Carbonate substitution in pure HAp will cause the main peak and plane (300) to shift towards angles higher than pure HAp, and plane (002) shifts towards a smaller angle [11]. According to ICDD PDF No. 090432, the main peak of pure HAp at $2\theta = 31.77^\circ$, (300) at 32.90° and (002) at 25.87° . In this study, all samples have a main peak at 2θ between 31.81° - 31.91° and plane (300) between 32.98° - 33.08° , where this position is above the position of pure HAp. The position of the plane (002) in the sample between 25.83° - 25.84° , where this position is below the position of HAp pure. This data shows that one of the characteristics of B type CHAp has been detected based on the peak position of the main peak and several diffraction plane.

Another characteristic of B type CHAp can be analyzed from lattice constants a and c . B type CHAp has lattice constant a smaller than pure HAp, whereas the lattice constant c is higher than pure HAp. Lattice constants a will decrease when c increase and it is proportional to the addition of carbonates substituted in HAp [12]. According to ICDD PDF No. 090432, and c lattice constants for HAp are 9.418 \AA and 6.884 \AA , respectively. All sample lattice constants a are smaller than 9.418 \AA and c are higher than 6.884 (see Table 1), which indicates that the characteristics of B type CHAp has been detected based on lattice constants a and c . The longer stirring time produces smaller lattice constant a , and higher c . This phenomena indicates that the longer stirring time, will produce the higher carbonate substituent. Based on the calculation results, the sample with 4 h stirring time has the highest carbonate content because it has the smallest a lattice constant and the largest c .

Table 1. Characteristics of CHAp synthesized with different stirring times.

| Stirring time | Lattice constant [\AA] | | Crystallite size [nm] | Microstrain |
|---------------|--------------------------------------|-------|--------------------------|-------------|
| | A | c | | |
| 0 h | 9.397 | 6.888 | 38.756 | 0.062 |
| 2 h | 9.394 | 6.888 | 37.608 | 0.016 |
| 4 h | 9.370 | 6.890 | 26.026 | 0.023 |

The crystallite size of B type CHAp samples with different stirring time were listed in Table 1. This data was obtained from calculations using equation 1 from the plane (002). The (002) plane was chosen because this peak was the highest peak. The sample stirred for 4 h have the smallest crystallite size because this samples contained the highest carbonate content. The higher carbonate content in B type CHAp, the smaller crystallite size obtain [13].

The stirring time affects the microstrain value. Microstrain describes the level of a crystal defect, where the smaller microstrain value will produce the smaller crystal defects [14]. Based on the data obtained, CHAp which was synthesized with stirring time for 2 h has the smallest microstrain value, which means it has the smallest crystal defect.

3.2 FTIR analysis

Figure 2 show the FTIR spectra of all samples. The samples appear to have B-type CHAp functional groups, i.e., OH^- , PO_4^{3-} and CO_3^{2-} . The main peak of B type CHAp found at 874 [15], 1450 and 1420 cm^{-1} [16], where this data was also supported by XRD. From XRD data, all samples can be classified as B type of CHAp. The peak of PO_4^{3-} found at 1035 - 1047 (ν_3) [7], 955 - 962 (ν_1) [16], and 472 cm^{-1} (ν_2) [17], The peak of OH^- functional group found at 3570 cm^{-1} [18].

The stirring time affects the peak intensity of the samples. The peak intensity of CO_3^{2-} at wavenumber 874 cm^{-1} looks different, where the CHAp which stirred for 4 h had the highest peak. This means that

samples had the highest carbonate content that supports previous XRD data. In the synthesis of HAp, the difference time of the stirring affects the intensity of the functional group from FTIR data [19]. In this study, the stirring time also affected the same thing as HAp.

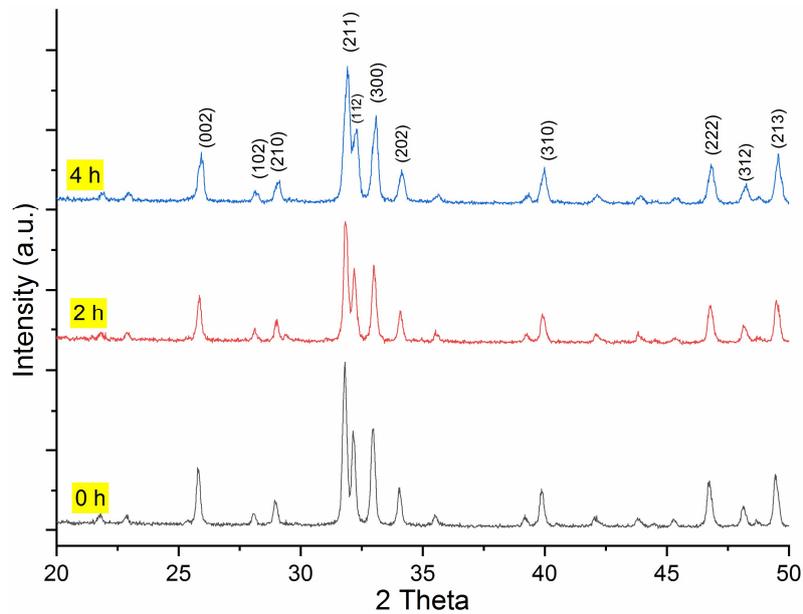


Figure 1. XRD pattern of samples with different stirring times.

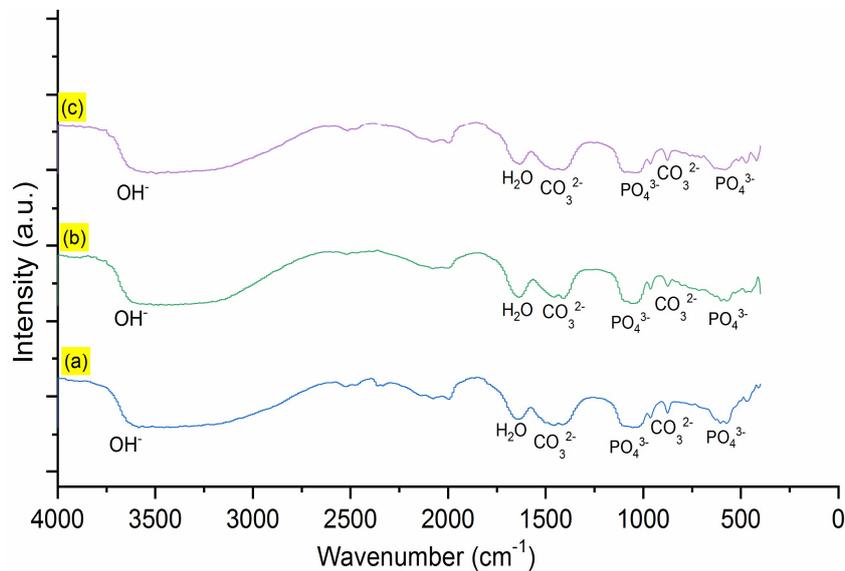


Figure 2. FTIR spectrum of CHAp synthesized with stirring time (c) 0 h; (b) 2 h; (c) 4 h

3.3 SEM analysis

SEM was used to analyze the morphology of CHAp. Figure 3 shows the morphology of B type CHAp which was synthesized with stirring time variations. The sample stirred for 4 h shows different morphology because it tends to form agglomerate. The longer stirring time can cause the higher agglomeration. It can be concluded that stirring time also affects the level of agglomeration of the sample

[19]. The shape of all particles seems irregular. The particle size cannot be determined from the image because the particles appear tight and clotted.

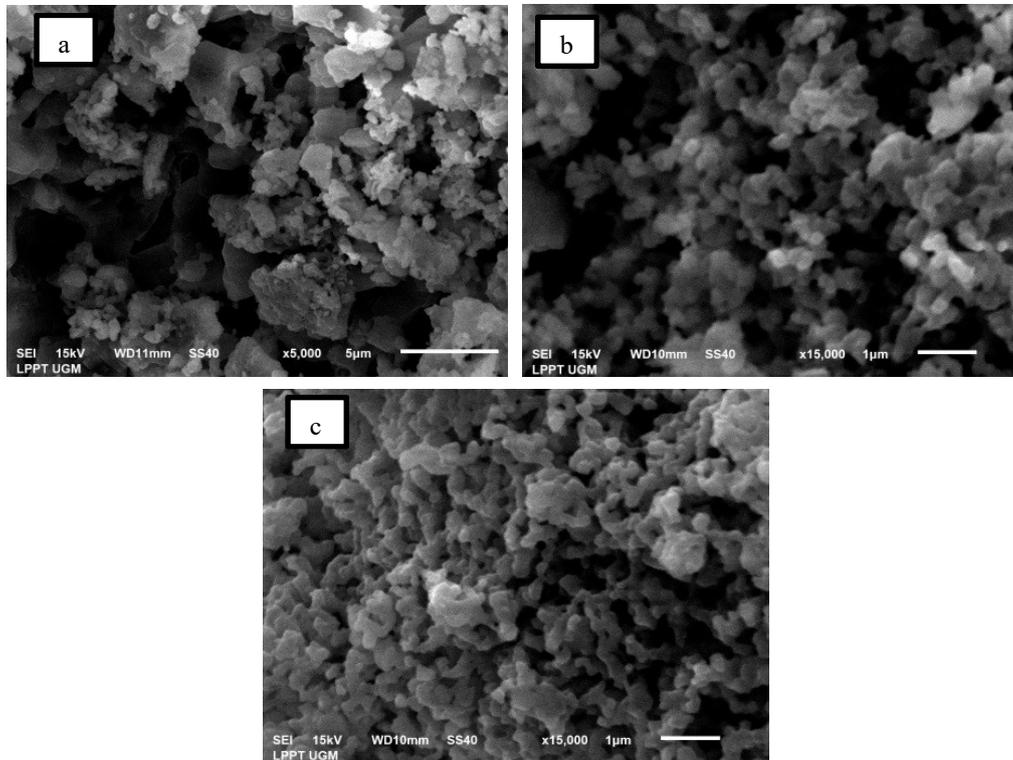


Figure 3. Morphology of CHAp samples with different stirring time (a) 0 h; (b) 2 h; (c) 4 h

4. Conclusion

The B type CHAp has been successfully synthesized from pearl shells (*Pinctada maxima*) by using precipitation method with different stirring time. Based on XRD analysis, the stirring time affects the position of the main peak, and several diffraction fields, lattice constants a and c , crystallite size and microstrain. The smallest crystallite size and highest carbonate content was obtained from sample with 4 h stirring time. This data also supported by FTIR spectra, which shown the highest peak of carbonate ion. The CHAp sample which stirred for 2 h had the smallest crystal defect that was characterized by microstrain value. The stirring time also affects the sample morphology, where the sample stirred for 4 h show more agglomerate than other samples.

Acknowledgement

The authors are immensely grateful to the Ministry of Research, Technology, and Higher Education Republic of Indonesia and Universitas Gadjah Mada (RTA 2019 Program) for financial support this research. The authors would also like to thank LPPT UGM for providing facilities and technical assistance.

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