Characterization of properties in microwave irradiated-hydroxyapatite

R A Sabrina¹, Nurlely¹, Y W Sari²

¹ Department of Physics, University of Indonesia, 16424, Depok, Indonesia
² Department of Physics, Bogor Agricultural University, 16680, Bogor, Indonesia

Email: nurlely@sci.ui.ac.id

Abstract. Hydroxyapatite (HA) is a calcium phosphate with a chemical bond of Ca₁₀(PO₄)₆(OH)₂, which is similar to the inorganic substance contained in human bones. Thus makes HA convenient for further development as a biomaterial. The aim of this work is to understand the effect of variation in microwave power and time in synthesizing HA. HA was synthesized by a mixture of calcium hydroxide and di-ammonium hydrogen phosphate, maintaining the molar ratio between calcium and phosphate of 1.67. The sample was irradiated by microwave irradiation at different powers and time duration. The properties of all samples were investigated by x-ray diffraction (XRD), Fourier-transform infrared (FTIR), scanning electron microscopy and energy dispersive x-ray spectroscopy (SEM-EDX). The mass of HA samples showed a quite significant change while the crystallite size of the samples reached 34.99 nm. The FTIR results showed some carbonate peaks. It is proved that the change in irradiation power and time affects the properties of the synthesized HA. The synthesized HA powders showed a similar structure to hydroxyapatite in hexagonal P63/m space group a=b=9.418 and c=6.887.

Keywords: Biomaterial, Bones, Hydroxyapatite, Microwave

1. Introduction

Hydroxyapatite (HA) is a calcium phosphate with chemical formula Ca₁₀(PO₄)₆(OH)₂ and the ratio of calcium and phosphate around 1.67 [1]. Since HA has good biocompatibility, bioactivity, osteoconductivity, and does not pose toxicity nor induce advert immune response, synthetic HA is then further developed in medical field [2]. In synthesizing HA, some parameters such as temperature and pH environment can affect the product [1]. In previous research, it was mentioned that lower acid addition flow rate will lead to bigger crystallites [3].

There are several methods to synthesize HA. Sol-gel techniques, hydrothermal, multiple emulsion, and solid state reaction have been executed to produce HA with various morphologies. However, the wet precipitation method provides more convenience than other methods, especially under atmospheric pressure [1]. Because the required time to synthesize HA with conventional heating takes too long, more enlightenment made its way to offer advantages in preparing this material. Microwave irradiation has been proved to be the most efficient method since the duration in synthesizing HA can be reduced significantly [5].
2. Materials and methods

2.1. Synthesis of hydroxyapatite
Calcium hydroxide Ca(OH)\(_2\) (Merck KgaA Company Inc.) and di-ammonium hydrogen phosphate (NH\(_4\))\(_2\)HPO\(_4\) (Merck KgaA Company Inc.) were used as received without further purification. The process of synthesizing HA was done with precipitation between Ca(OH)\(_2\) as the calcium source and (NH\(_4\))\(_2\)HPO\(_4\) as the phosphoric source.

Each substance was mixed into the distilled water while maintaining the Ca/P molar ratio at 1.67. The (NH\(_4\))\(_2\)HPO\(_4\) was dropped into Ca(OH)\(_2\) solution at the speed of 5 mL/min approximately. The mixture was then put into a microwave oven (Sharp, R-728(W)-IN 900 W), the power and time were set at 360 W for 5 minutes. The resulting sample was filtered with Whatman No. 42 filter paper (Fisher Scientific, USA) and heated by a conventional oven. These procedures were repeated for the variations of power (450, 540, 630, and 720 W) and time (10 and 15 minutes) of the microwave oven.

On the other side, another mixture of HA was prepared without using microwave irradiation, but went through the process of aging for 24 hours instead, and proceeded to be washed repeatedly and filtered before getting dried with the conventional oven. All of the final products were in the form of white powder.

2.2. Characterization
The crystal phase and the degree of crystallinity of the samples were observed by X-ray diffractometer (XRD Panalytical X’pert Pro MPD) using 0.02 mm Ni-filtered with monochromatic Cu-K\(\alpha\) radiation at 1.540598 Å, with the tube voltage 40 kV and tube current 30 mA. The functional groups present in synthesized HA were analyzed by Fourier transform infrared spectroscopy (FTIR Bruker Tensor 27) while the morphology and the particle size of the products were studied by Scanning electron microscopy (FESEM Inspect F10, FEI) and Energy Dispersive Spectrophotometer (EDAX Apollo X).

3. Results and discussion

3.1. X-ray diffraction
The XRD spectra of the samples show similarities to the patterns of HA from the International Center for Diffraction Data PDF No. #9–432 and a good match with the hydroxyapatite XRD patterns of Wilson et al. (1999) as the lattice parameters have high resemblance [6]. XRD results of the synthesized samples which were treated under microwave irradiation at 720 W at different times were displayed on the figure below, along with the aging sample. All of the samples were found to have no traces of secondary phases and were in the form of nano-crystalline. It could be seen as they have broad diffraction peaks which decreased when the samples were irradiated under microwave irradiation. Each of the samples formed crystalline phase which belongs to the hexagonal structured HA. This shows a good agreement with the previous study [7].

Figure 1 indicated that the peaks of the samples were growing sharper and the intensity increased as the irradiation time was added. This phenomenon could be explained as the longer irradiation took place, the produced sample possessed bigger particle size and higher crystallinity than the sample irradiated at shorter time as a result of rapid energy transfer and the increment of temperature rising in a very short time causing the particle to move in fast movement and collided with each other while bearing high energy. The higher the power of irradiation that was given to the sample, the stability of H\(_2\)O was reduced and the nuclei formed by calcium and phosphate tended to grow bigger crystallite size and the product was more crystalline [8]. To determine whether the setting of microwave power and time affected the crystallinity of the synthesized HA or not, a calculation was performed and was assigned in both Table 1 and Table 2. The crystallinity index was calculated using FWHM at diffraction peak 25.879° [9]. The samples which were irradiated at 720 W produced more crystallized HA than the samples irradiated at lower power and obviously were more crystalline than the sample treated under aging method, while the sample of HA irradiated at 720 W for 5 minutes showed low crystallinity, in contrast with the HA sample irradiated at the same power within 15 minutes.
Table 1. Effect of irradiation time at 360 W on Lattice parameters, crystal size, mass, and crystallinity index of the synthesized HA.

| Irradiation Time (Min) | Lattice Parameter \(a (\text{Å})\) | Cell vol \( (\text{Å}^3)\) | Crystal Size (nm) | Mass (gram) | Crystallinity Index (CI) |
|------------------------|-----------------------------------|-------------------|------------------|-------------|---------------------|
| 5                      | 9.389                             | 523.542           | 31.45            | 9.55        | 0.793               |
| 10                     | 9.392                             | 524.325           | 31.73            | 10.04       | 0.814               |
| 15                     | 9.413                             | 527.537           | 33.49            | 9.22        | 0.957               |

Table 2. Effect of irradiation time at 720 W on Lattice parameters, crystal size, mass, and crystallinity index of the synthesized HA.

| Irradiation Time (Min) | Lattice Parameter \(a (\text{Å})\) | Cell vol \( (\text{Å}^3)\) | Crystal Size (nm) | Mass (gram) | Crystallinity Index (CI) |
|------------------------|-----------------------------------|-------------------|------------------|-------------|---------------------|
| 5                      | 9.378                             | 521.677           | 31.58            | 9.95        | 0.802               |
| 10                     | 9.382                             | 522.346           | 33.06            | 9.2         | 0.921               |
| 15                     | 9.387                             | 523.149           | 34.99            | 9.4         | 1.091               |

Figure 1. The comparative x-ray diffraction (XRD) patterns of hydroxyapatite synthesized by aging (a), and under microwave irradiation at 720 W for 5 (b), 10 (c), and 15 (d) minutes.

Figure 2. The comparative x-ray diffraction (XRD) patterns of hydroxyapatite synthesized by aging (a), and under microwave irradiation at 360 (b) and 720 (c) W for 15 minutes.

From the graphs above, it was confirmed that the enhancement of the irradiation time resulted in the displacement of the peaks moving to the left side which relates to the increase of the lattice parameters of samples. When the power was added, the lattice parameter of the samples grew more compact as a result of the evaporation of the absorbed water contained in the synthesized samples, resulting in the strain of the lattice. In Figure 2 the microwave power shows significant influence toward the lattice parameter.

As for the crystallite size, the diffraction peak at around 25.879° was chosen for the calculation as it corresponds to the Miller planes of (002). Using Scherrer formula, the result showed that the size of synthesized HA samples raised significantly as the microwave power and time increased. After the measurement of mass, it was found that the irradiated samples have bigger mass than the aging
sample which only consists of around 7 grams of HA powder. Therefore, the use of microwave irradiation did not only accelerate the reaction time but also formed more HA product with bigger crystal size and more enhancement of the crystallinity of the samples.

3.2. Fourier transform-IR
The FT-IR spectra of the synthesized HA, corresponding to samples in previous figures, exhibit clear presence of the functional groups necessary for hexagonal HA with the Ca/P ratio of 1.67 which contained the bands of phosphate group (472 cm\(^{-1}\), 564 cm\(^{-1}\), 604 cm\(^{-1}\), 1032 cm\(^{-1}\), and 1092 cm\(^{-1}\)) and hydroxyl group (630 cm\(^{-1}\) and 3564 cm\(^{-1}\)). There is also an occurrence of other band contributed to carbonate group which lays at 874 cm\(^{-1}\) \[5\]. FTIR result of the aging sample showed no occurrence of any peak at 630 cm\(^{-1}\) which may have been the result of the decrease of the hydrogen-oxygen bonds around the tetrahedral phosphate in the sample so that the librational band of OH\(^-\) cannot be detected as clear as the samples irradiated under microwave irradiation \[10\]. This peak appears most prominent when the samples were irradiated at 720 W for 10 and 15 minutes. The sample which went through the aging method also appeared to have sharper carbonate peaks due to the impurities contained in the atmosphere.

### Table 3. Assignments of infrared frequencies (cm\(^{-1}\)) of the synthesized HA.

| Functional Groups | Wavenumber (cm\(^{-1}\)) |
|-------------------|--------------------------|
|                   | Aging 5 Min | Aging 10 Min | Aging 15 Min | 360 W 5 Min | 360 W 10 Min | 360 W 15 Min | 720 W 5 Min | 720 W 10 Min | 720 W 15 Min |
| \(v_2\) PO\(_4^3^-\) | 472 | 471 | 472 | 472 | 472 | 472 | - | - | - |
| \(v_3\) PO\(_4^3^-\) | 1032 | 1033 | 1031 | 1032 | 1033 | 1032 | 1092 | 1092 | 1092 |
| \(v_4\) PO\(_4^3^-\) | 564 | 563 | 563 | 564 | 564 | 565 | 564 | 564 | 564 |
| \(v_2\) CO\(_3^2^-\) | 874 | 875 | 875 | 875 | 875 | 875 | 875 | 875 | 875 |
| OH\(^-\) | - | - | - | - | - | 630 | 629 | - | - |
| H\(_2\)O | 3564 | 3561 | 3569 | 3568 | 3566 | 3569 | 3569 | 3569 | 3569 |
|           | 3423 | 3417 | 3424 | 3423 | 3417 | 3433 | 3420 | - | - |

**Figure 3.** The FTIR spectra of hydroxyapatite synthesized by aging (a), and under microwave irradiation at 720 W for 5 (b), 10 (c), and 15 (d) minutes.

**Figure 4.** The FTIR spectra of hydroxyapatite synthesized by aging (a), and under microwave irradiation at 360 (b) and 720 (c) W for 15 minutes.
Table 4. Splitting factor of the v4 phosphate bands of the synthesized HA.

| Irradiation Time (Min) | Power (Watt) | Splitting Factor |
|------------------------|--------------|-----------------|
| 5                      | 360          | 2.94            |
|                        | 720          | 2.98            |
| 10                     | 360          | 2.99            |
|                        | 720          | 3.15            |
| 15                     | 360          | 3.27            |
|                        | 720          | 3.67            |
| Aging                  |              | 2.83            |

The splitting factor of the samples was calculated based on the FTIR data \(^{[11]}\). From the table above, it was shown that the value of splitting factor (SF) of the synthesized samples rises as the power and irradiation time was increased, indicating the increment of crystallinity degree of the samples. This pattern corresponds to the XRD results in which the crystallinity of the sample increased when it was applied to the microwave irradiation. Apparently, the peak of 3568.12 cm\(^{-1}\) seems to grow more intense as the microwave power was increased, indicating the evaporation of crystal water. Furthermore, as the irradiation was set at a higher power it also appears that the peak located at around 3420 cm\(^{-1}\) decreases which might have happened due to the loss of the absorbed water. These confirmed the XRD results of the lattice growing more compact as the time increased.

3.3. SEM & EDX

The HA samples were characterized with SEM and showed long thick plates, the size of each sample increased as the irradiation time and the microwave power were added. The range of the particle size lays between 105–133.74 nm and the most visible shape was shown by HA sample irradiated at 720 W for 15 minutes which resembles short and long columns. The qualitative results of EDX on samples chemical composition of the synthesized HA showed that the major element components were phosphorus (P) and calcium (Ca) with the ratio of calcium to phosphate at 1.62.

![SEM micrograph at 100k (a) and 200k (b) with EDX image of hydroxyapatite irradiated at 720 Watt for 15 minutes.](image)
4. Conclusions
The precipitation method combined with microwave irradiation has successfully produced nanosized HA. The XRD and FTIR results showed that the as synthesized HA has higher crystallinity and bigger crystallite size when the microwave power and the irradiation time applied to the sample were increased. When HA was exposed under the irradiation with a higher power, this caused the lattice crystal growing more compact. All of the functional groups were detected in the samples. The value of the splitting factor shows the increment of crystallinity of the samples. The particle size reaches 105–133 nm with the shapes resembling short and long columns. The HA synthesized by this method showed a similar structure to hydroxyapatite in hexagonal P63/m space group with lattice parameter corresponding to ICDD #9–432 and other previous studies.

References
[1] Wang P, Li C, Gong H, Jiang X, Wang H, Li K. (2010). Powder Technology. 203(2), 315–321.
[2] Warastuti Y, and Abbas B. (2011). Jurnal Ilmiah Aplikasi Isotop dan Radiasi. 7(2), 73–80.
[3] Berzina-Cimdina L, Borodajenko N. (2012). Infrared Spectroscopy - Materials Science, Engineering and Technology.
[4] Gomes J, Granadeiro C, Silva M, Hoyos M, Silva R, Vieira T. (2008). International Journal of Chemical Reactor Engineering. 6(1).
[5] Méndez-Lozano N, Velázquez-Castillo R, Rivera-Muñoz E, Bucio-Galindo L, Mondragón Galicia G, Manzano-Ramírez A, Ocampo M, Apátiga-Castro L. (2017). Ceramics International. 43(1), pp.451–457.
[6] Wilson R, Elliott J, Dowker S. (1999). American Mineralogist. 84(9), pp.1406–1414.
[7] Gallo R. (2011). Synthesis and characterization of substitutedapatites for biomedical applications, Tesi, Università Degli Studi di Padova.
[8] Akram M, Alshemary A, Goh Y, Wan Ibrahim W, Lintang H, Hussain R. (2015). Materials Science and Engineering: C. 56, pp.356–362.
[9] Landi E, Tampieri A. (2000). Journal of the European Ceramic Society. 20, 2377–2387.
[10] Siddharthan A, Sampath Kumar T, Seshadri S. (2009). Biomedical Materials. 4(4), p.045010.
[11] Surovell, T.A., Stiner, M.C. (2001). Journal of Archaeological Science. 28, 633–642.

Acknowledgment
This work is supported by Hibah PITTA 2018 funded by DRPM Universitas Indonesia No.5000/UN2.R3.1/HKP.05.00/2018.