

EFFECT OF HYDROTHERMAL TEMPERATURE ON SYNTHESIS OF HYDROXYAPATITE FROM LIMESTONE VIA HYDROTHERMAL METHOD

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ABSTRACT

Objective: In this study, the effect of hydrothermal temperature on the synthesis of hydroxyapatite (HAp) from limestone was investigated.

Methods: Precursors of limestone (CaCO_3) and diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) with Ca/P ratio of 1.67 was mixed at pH 10 in order to synthesize hydroxyapatite by hydrothermal method. The solution was hydrothermally treated in an autoclave at various temperatures of 120, 160 and 200 °C for 24 h. Nano powder formed was then characterized.

Results: The hydroxyapatite obtained by hydrothermal method at various temperatures was characterized. The analysis of Fourier Transform Infrared (FTIR) revealed that HAp had phosphate (PO_4^{3-}) and hydroxyl (O-H) groups although carbonate (CO_3^{2-}) group remained. X-Ray Diffraction (XRD) revealed that the size of nanosize crystals was formed and then provided a large surface area. Scanning Electron Microscopy (SEM) results showed that HAp had a spherical shape. There was more agglomeration at 200 °C than at 160 °C. The ratio of the results of Energy Dispersive X-ray (EDX) analysis in 160 °C was 1.95 and at 200 °C was 1.71

Conclusion: The hydroxyapatite prepared at a temperature of 200 °C had smaller crystal size than the hydroxyapatite prepared at the temperatures of 120 and 160 °C. SEM pictures showed that the nanoparticle hydroxyapatite had a spherical shape.

Keywords: Hydroxyapatite, Limestone, Hydrothermal, Temperature, Nanoparticle

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INTRODUCTION

Hydroxyapatite (HAp) is a calcium phosphate bio-ceramic material, which has an almost identical chemical composition to mineral component of bone [1]. HAp gains considerable attraction due to its excellent osteoconductive and osteointegration properties, allowing synthetic HAp to be used widely in clinical surgery and biomedical applications [2]. HAp is not only a biocompatible, osteoconductive, non-toxic, non-inflammatory and non-immunogenic agents, but also bioactive, i.e. it has gotten the ability to form a direct chemical bond with living tissues [3].

HAp can be produced wet by the direct precipitation of calcium and phosphate ions [4]. HAp is often used in hip, knee and other implants and as a synthetic bone substitute [1]. HAp can be used as implants because they provide a good adhesion to the local tissue due to their surface chemistry and have been shown to enhance osteoblast proliferation [4]. HAp has also been studied for other non-medical applications, for example, as packing media for column chromatography, gas sensors, and catalyst [5].

HAp has a general formula in the form $\text{M}_{10}(\text{XO}_4)_6\text{Z}_2$, in which M^{2+} is a metal and species XO_4^{3-} and Z are anions; in which M is calcium (Ca^{2+}), X is phosphorus (P^{5+}) and Z is the hydroxyl radical (OH^-) [6]. Commercially available HAp particle sizes range between 10-40 μm with a Ca/P ratio range of 1.66 to 1.69 [1]. Some studies reported that the stoichiometry of HAp plays an important role in the mechanical properties; obtaining better results when the Ca/P ratio is between 1.60 and 1.67 [6]. Synthetic HAp, with the chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, includes hydroxyl groups containing impurities such as CaO, biphasic calcium phosphate (BCP, HA+ β -TCP) or β -tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$, β -TCP) [7].

HAp has been synthesized from various raw materials. Source of HAp is derived from materials with high calcium content such as bone [3] and starch [8]. There are various well-known methods of HAp synthesis namely precipitation method [9], sol-gel [10], and hydrothermal [1].

Hydrothermal methods offer good control of morphology and chemical stoichiometries [1]. Apart from the advantages that exist in

the hydrothermal synthesis and massive source of calcium ion such as limestone [11], this study aims at synthesizing HAp of limestone via a hydrothermal method with various hydrothermal temperatures. To find products that have been produced, HAp is characterized.

MATERIALS AND METHODS

Materials

Main materials used in HAp synthesis were limestone (CaCO_3), Distilled water, Nitric Acid (HNO_3), Ammonia (NH_3), Diammonium Hydrogen Phosphate ($(\text{NH}_4)_2\text{HPO}_4$). Apparatus were a thermometer, glassware, magnetic stirrer, analytical balance, Whatman Filter Paper no 42, pH meters, Fourier transform infrared spectroscopy (FTIR), Perkin Elmer 1600 series was used to provide information about the structure of HAp. The material was scanned in the spectrum between 500 and 4000 cm^{-1} . The crystals size and structure of HAp product determined with a Philips X'pert Powder, PANalytical, X-ray diffractometer (XRD) using $\text{Cu-K}\alpha$ radiation sources ($\lambda = 1.5040 \text{ nm}$). Scanning electron microscopy coupled with Energy Dispersive X-ray (SEM-EDX)-was used to determine the morphology of the HAp product by using SEM S-3400 which combined with Hitachi, EDX Emax x-act 720, Horiba to determine the element that contained in HAp.

Method

Limestone was calcinated with 900 °C for 5 h, in which limestone changed the phase of calcium carbonate (CaCO_3) into calcium oxide (CaO). Calcium oxide (CaO) 1.6 g was added to 30 ml of 2 M nitrate acid (HNO_3) stirred for 30 min at 700 rpm at 65 °C and then filtered, to change them into calcium nitrate hydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$). The filtrate was taken, then added a solution of 30 ml 0.6 M ($(\text{NH}_4)_2\text{HPO}_4$) slowly while stirring for 30 min at 700 rpm at 65 °C and at pH 10. The addition of ammonium hydroxide (NH_4OH) was for pH adjustment. The solution was hydrothermally treated in an autoclave at 120, 160 and 200 °C for 24 h. The precipitates were dried in an oven for 3 h at 110 °C. HAp was obtained after heating at 800 °C for 5 h. Nano-powder formed was then characterized.

RESULTS AND DISCUSSION

FTIR analysis

Fig. 1 shows FTIR spectrums of hydroxyapatite at (a) 120, (b) 160, and (c) 200 °C. FTIR utilized vibratory energy functional groups of constituent HAp compound, i.e. (PO₄³⁻) and (OH⁻) groups. FTIR of HAp showed adsorption band at 3443, 3571, and 3572 cm⁻¹ corresponding to (OH⁻) functional group. The absorption bands of (PO₄³⁻) functional groups were in the presence at 962, 1019, 1062, and 1087 cm⁻¹. Adsorption peak at 1653 cm⁻¹ corresponding to (CO) functional group indicated residual of carbonate (CO₃²⁻). These results are consistent with those reported [6]. FTIR Spectrum could be seen in fig. 1.

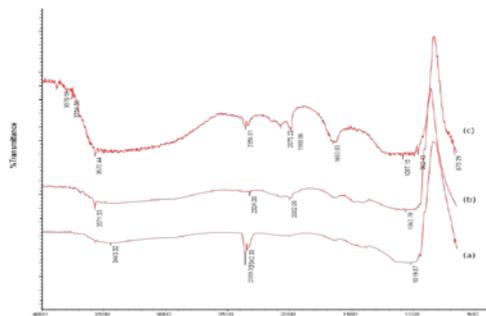


Fig. 1: FTIR spectrums of hydroxyapatite at (a) 120, (b) 160, and (c) 200 °C

XRD analysis

Fig. 2 shows XRD spectrums of hydroxyapatite at (a) 120, (b) 160, and (c) 200 °C. XRD analysis was to determine whether the HAp crystals formed as amorphous, crystalline or polycrystalline. Spectrums showed structure, orientation, and crystal size. Fig. 2 shows XRD pattern of HAp at various hydrothermal temperatures. The crystallite size (D) of the sample was calculated from the Scherrer equation [12] namely:

$$D = \frac{k\lambda}{B \cos \theta}$$

Where k was Scherrer constant (k = 0.9 assuming that particles are spherical); λ was the wavelength of the incident X-rays (λ = 1.54 Å); β was the half width of the diffracted peak, and θ was the diffracted angle of the values.

Specific surface area (S) of the HAp was determined by the formula [12] namely:

$$S = 6 \times 10^3 / d\rho$$

Where ρ was the crystallite size (nm) and d was the theoretical density of HAp (3.16 g/cm³).

Fig. 2 shows that the sharpest peak with high intensity was at 31.0809 ° angle. It suited with Inorganic Crystal Structure Database (ICSD) standards no. 009 0169. At temperature 120 °C, it was

confirmed as Calcium Phosphate compound with Miller index value (0210). The highest intensity peak at Temperature 160 °C was 31.1244 ° with Miller index value (0210) also confirmed as Calcium Phosphate. However second highest peak that's at 31.7926 ° has confirmed HAp with Miller index value (221). The highest intensity peak at 200 °C was 31.9131 ° with Miller index value (211). It confirmed that the product was HAp. XRD showed that HAp formed at 120,160, and 200 °C had crystal phase.

Through peaks in the XRD spectrum and using the Scherrer equation, the size of the HAp crystals obtained could be known. From the XRD data, using the Scherrer equation, where a sharp peak with a narrow peak width indicates a large crystal size, while the small crystal size is indicated by the peak width, can see the size of the crystals formed. It is done by entering the value of FWHM (Full Width at Maximum) Scherrer equation to the known size of hydroxyapatite crystals [13-15].

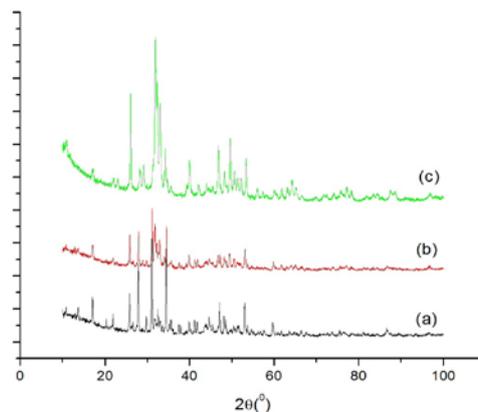


Fig. 2: XRD spectrums of hydroxyapatite at (a) 120, (b) 160, and (c) 200 °C

The size of HAp crystals at 120 °C could be seen in table 1. Table 1 shows that the size of HAp crystals had nanoparticle size in 46-53 nm range with specific surface area ±35-40 m²/g. XRD spectrum of HAp at 120 °C showed the highest absorption intensity at a 2θ=31 ° angle. Crystal size at 160 °C could be seen in table 2.

Table 2 shows that the size of Hap crystals had nanoparticle size in 32-46 nm range with specific surface area ±40-58 m²/g. XRD spectrum of HAp at 160 °C showed the highest absorption intensity at a 2θ=31 ° angle. Crystal size at 200 °C could be seen in table 3.

Table 3 shows that the size of HAp crystals had nanoparticle size in 34-60 nm ranges with specific surface area ±31-54 m²/g. XRD spectrum of HAp at 200 °C showed the highest absorption intensity at a 2θ=31 ° angle. However, the temperature at 200 °C had good purities of HAp and if it's compared to the data XRD analysis temperature at 120, 160, and 200 °C, the best condition of temperature was at 200 °C. At temperatures 120 and 160 °C, there was impurity because of Calcium Phosphate although HAp had formed indeed table 1. 2. And 3 show Hydroxyapatite Crystal Size at 120 °C, 160 °C, and 200 °C

Table 1: Hydroxyapatite crystal size at 120 °C

2Theta (°)	Line width (FWHM)	Crystal size (nm)	Specific surface area (m ² /g)
31.0809	0.1535	53.7245	35.3421
34.4373	0.1791	46.4434	40.8827
27.8659	0.1535	53.3295	35.6039

Table 2: Hydroxyapatite crystal size at 160 °C

2Theta (°)	Line width (FWHM)	Crystal size (nm)	Specific surface area (m ² /g)
31.1244	0.2047	40.2911	47.1254
31.7926	0.2558	32.2953	58.7929
34.5162	0.1791	46.4534	40.8740

Table 3: Hydroxyapatite crystal size at 200 °C

2Theta (°)	Line width (FWHM)	Crystal size (nm)	Specific surface area (m ² /g)
26.0291	0.1574	51.8087	36.6489
31.9131	0.2362	34.9857	54.2718
32.3251	0.1378	60.0303	31.6296

SEM-EDX analysis

Fig. 3 and 4 show SEM hydroxyapatite at 160 °C and 200 °C (a) 10.000x, (b) 20.000x (c) 50.000x, (d) 100.000x. SEM analysis was performed to characterize the surface morphology of the sample. In principle, the surface analysis involved surface radiation with enough energy to penetrate and cause some transitions that result in emission from beam energy surface. Fig. 3 shows SEM of HAp at 160 °C. it shows that formation of HAp was like spherical shape. HAp at 160 °C was spread and homogeneous well without agglomeration. Fig. 4 shows SEM of HAp at 200 °C. This fig. shows that Hap had a spherical shape. HAp at 200 °C was not spread and homogeneous well. In addition, particle form of HAp compound had agglomeration.

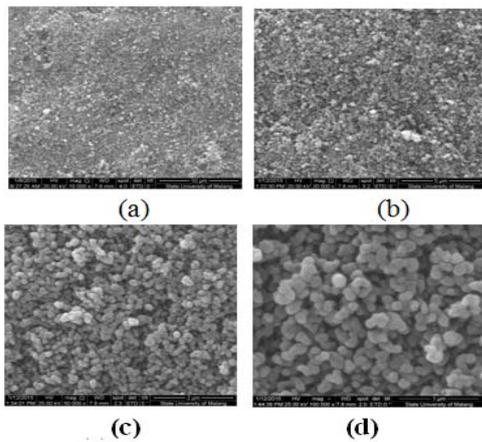


Fig. 3: SEM hydroxyapatite at 160 °C 10 (a) 10.000x, (b) 20.000x (c) 50.000x, (d) 100.000x

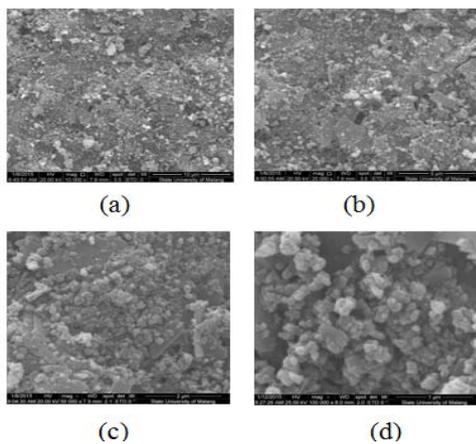


Fig. 4: SEM hydroxyapatite at 200 °C 10 (a) 10.000x, (b) 20.000x, (c) 50.000x, (d) 100.000x

Fig. 5 and 6 show EDX Spectrums of HAp at 160 °C and 160 °C. By using EDX, the composition of HAp compound was shown in fig. 5. Fig. 6 shows EDX analysis of HAp.

It indicates that the presence of other compounds such as CaO contained in the hydroxyapatite powder has been synthesized [16, 17].

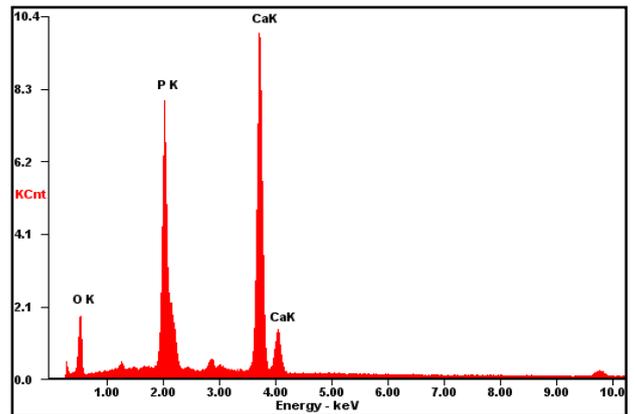


Fig. 5: EDX spectrum of hydroxyapatite at 160 °C

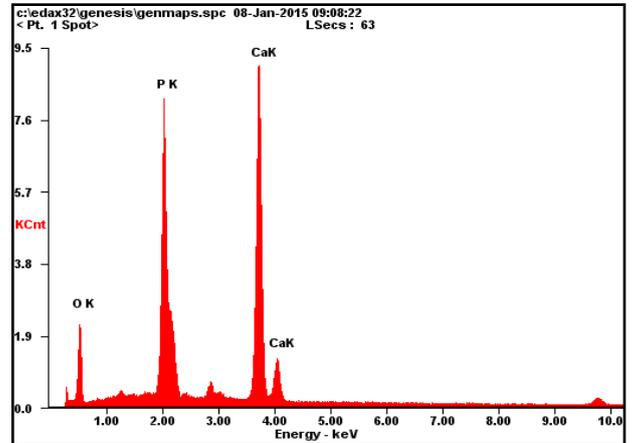


Fig. 6: EDX spectrum of hydroxyapatite at 200 °C

Table 4 and 5 show the composition of HAp from EDX Analysis at 160 °C and 200 °C. Table 4 shows that the comparison Ca/P of HAp at 160 °C was 1,95. Table 5 shows that the Ca/P comparison of HAp at 200 °C was 1, 71. It indicates that the presence of other compounds such as CaO contained in the hydroxyapatite powder has been synthesized [16, 17].

Table 4 Composition of hydroxyapatite from EDX analysis at 160 °C

Element	Wt.%	At%
OK	33.08	52.97
PK	22.64	18.72
CaK	44.29	28.31
Matrix	Correction	ZAF

Table 5: Composition of hydroxyapatite from EDX analysis at 200 °C

Element	Wt. %	At%
OK	36.85	56.88
PK	23.25	18.54
CaK	39.90	24.58
Matrix	Correction	ZAF

CONCLUSION

As a result of the hydrothermal method, a nanoparticle HAp was obtained with different hydrothermal temperatures. Analysis of microstructures revealed the structures of HAp. The analysis of FTIR revealed that HAp had phosphate (PO_4^{3-}) and hydroxyl (O-H) groups although carbonate (CO_3^{2-}) group remained. XRD revealed that the size of Nanosize crystals was formed and then provided a large surface area. SEM results showed that HAp had a spherical shape. There was more agglomeration at 200 °C than at 160 °C. The ratio of the results of EDX analysis in 160 °C was 1.95 and at 200 °C was 1.71. It indicated that pressure and temperature influenced Ca/P ratio.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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