

# Characteristics of hydroxyapatite from buffalo bone waste synthesized by precipitation method

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**Abstract.** Hydroxyapatite (HA),  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , is the most widely used ceramic biomaterials. It is a mineral found in natural teeth, bone and hard tissue. In this paper, we present a synthesis and characterization for obtaining HA by wet precipitation. This study mainly focuses on the use of bio-waste materials, *i.e.*, buffalo bones to obtain HA. In order to synthesized HA from buffalo bones, we use  $\text{Ca}(\text{OH})_2$ ,  $\text{H}_2\text{O}$  and  $\text{H}_3\text{PO}_4$  as the starting precursors. The material was sintered at temperatures of 650 °C, 850 °C, and 1050 °C for 7 hours to improve its particle size distribution. Identification of functional group using FTIR shows absorption namely at wavenumber values of 3699.20, 3787.92 and 3706.91  $\text{cm}^{-1}$  which indicate hydroxyl group (OH). Phosphate band were identified by peak 1022.20 and 1118.63  $\text{cm}^{-1}$  and 1215.06 to 1459.31  $\text{cm}^{-1}$  which indicates carbonate group. Ratio of Ca/P for synthesized HA is about 1.67. XRD pattern results indicated that the synthesized buffalo bone HA closely correspond to commercial HA phase.

## 1. Introduction

Bones naturally consist of inorganic (70 %), organic compounds (20 %) and water (10 %). HA with the chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  is a main organic phase of human hard tissues with structure similar to natural teeth and bone [1]. Additionally, HA is an osteoconductive, non-toxic [2], non-inflammatory and non-immunogenic agent, has bioactive properties and due to these properties, HA becomes a promising candidate material for human hard tissues substitute, and therefore it is extensively used in biomedical applications [3, 4].

The preparation of bio-waste materials is an interesting procedure to produce HA, the advantage is not only because it uses bio-waste, but both environmental and economic benefits are obtained through waste recovery. In addition, HA generated from bio-waste materials, such as buffalo bones, may be less likely to be rejected by living organs due to its physicochemical similarity to natural human bone [5]. Comparative studies involving the synthetic and the natural bone of HA have been performed [6]. The result indicated that their chemical compositions were similar. The use of bio-waste materials to produce HA powders using various synthesis method has been widely studied using such materials as animal bone, seashells, corals and shells. Buffalo bones is one of the animal bone bio-waste in Indonesia. Studies concerning buffalo bones as a potential HA source material are scarce.

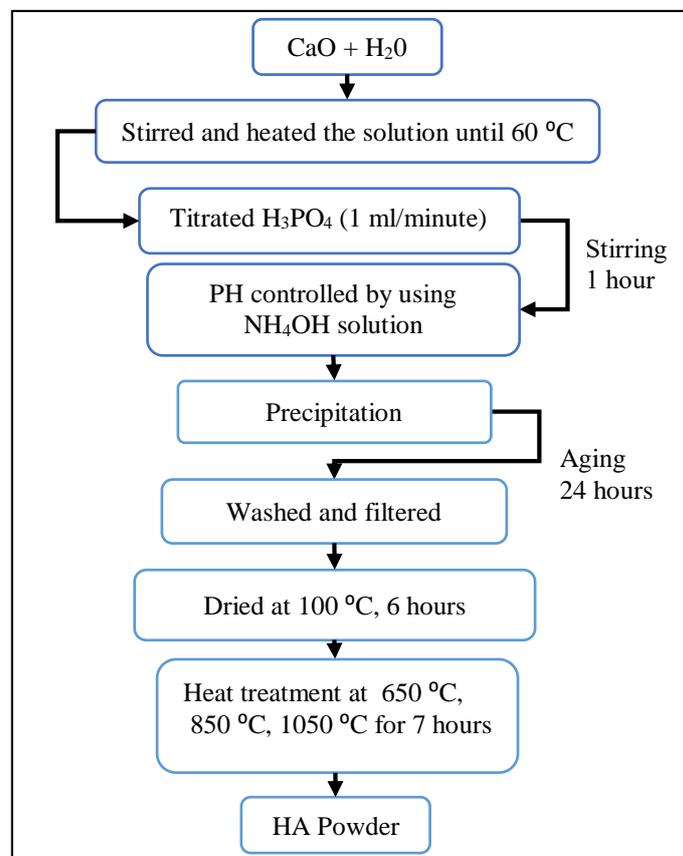
Different methods have been explored for converting materials into HA powder such as solid state reaction, sol-gel synthesis, microwave processing, hydrothermal methods and wet synthesis. The different methods are used to synthesis HA, the different morphologies, grain sizes, stoichiometries and molar Ca/P ratio can be obtained. The wet synthesis is recognized to be the most simple, cheap and suitable



method available [7]. This methods would lead to obtain the type of HA which is characterized by a high specific surface area, small size distribution including small particle agglomeration, high purity and crystallinity. The HA powders are obtained from the reaction of inorganic oxide chemical solutions.

The fabrication of bone into ceramic must be done through a sintering process, where the powder is compacted and sintered. The biological properties can be controlled by sintering at high temperatures and also the potential applications can be restricted [8]. Phases of cattle bone and bone waste sintered at different temperatures have been reported. Distinct structural changes were detected on sintering 600 °C and the formation of HA was identified after sintering as low as 600 °C [9].

The objective of this study is to obtain and to analyze the HA powders by using wet precipitation method and the phase of buffalo bones at elevated sintering temperatures of 650 °C, 850 °C, and 1050 °C which are the approximate sintering temperature ranges of synthetic HA attain.



**Figure 1.** Wet precipitation procedure for buffalo bone HA synthesis.

## 2. Experimental method

### 2.1. Preparation material

Femur from buffalo bones were first cut into pieces, then the bones were cleaned, boiled for an hour, rinsed in water and distilled water, soaked in NaOH for 24 hours, and further soaked in C<sub>3</sub>H<sub>6</sub>O for 24 hours and then dried. The bones were subsequently sintered at 1000 °C for 4 hours.

### 2.2. HA synthesis

HA was synthesized using the wet precipitation method [1]. As the starting precursors, 120 ml Ca(OH)<sub>2</sub>, 167.73 ml H<sub>2</sub>O (distilled water), 12.27 ml H<sub>3</sub>PO<sub>4</sub> were used. The chemical reaction is :



Ammonium hydroxide,  $\text{NH}_4\text{OH}$ , were used to adjust the solution to raise the pH greater than 10 in order to obtain a stoichiometric HA. The whole procedure is shown in the flow diagram in Figure 1.

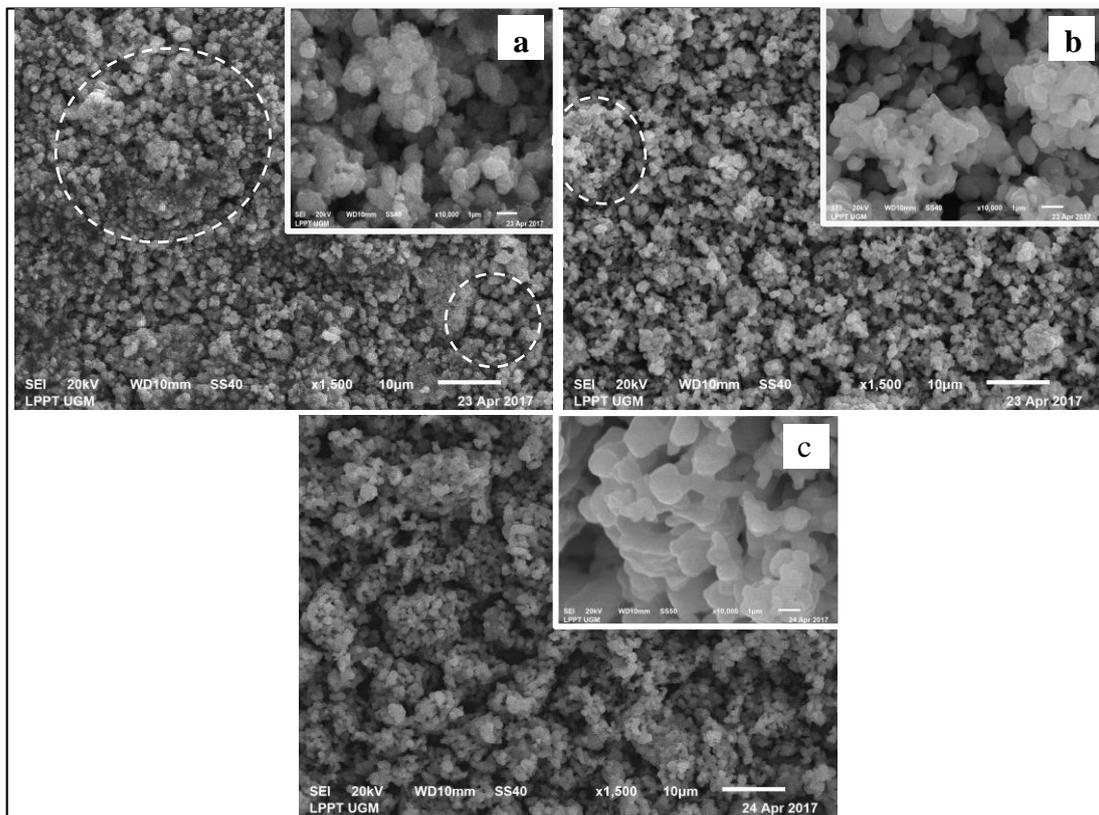
### 2.3. Characterization of HA

Microstructure and crystal size analysis of HA were performed by using Scanning Electron Microscopy (SEM). The powder samples were coated using a carbon evaporation coating unit. To identify the phases present before and after the heat treatment and to determine the particle size distribution, X-ray diffractometer was used with  $\text{CuK}\alpha$  radiation. FTIR spectroscopy was used to analysis the characteristic phase of the presence of phosphate groups and surface hydroxyl groups.

## 3. Results and discussion

### 3.1. Morphological analysis

Figure 2 shows the morphology of the HA powders sintered at 650 °C, 850 °C and 1050 °C for 7 hours. It could be seen from Figure 2(a), that at 650 °C the HA particles are partially agglomerates. This is due to HA particle surface area being quite larger. Increasing the sintering temperatures to 850 °C and 1050 °C has made the surface area of HA particle to become smaller, then the agglomeration area decreases. The uniform grain size with a narrow size distribution of HA particles could also be seen. This correspond to the crystallinity improvement of the HA, and this narrow size is clearly visible after sintering at 1050 °C for 7 hours as shown in Figure 2(a). The interconnection of HA particles has also improved by increasing the sintering temperature as shown in Figure 2(b) and 2(c).



**Figure 2.** SEM images of HA sintered at (a) 650 °C, (b) 850 °C and (c) 1050 °C for 7 hours. Insets: magnification 10.000x. Dashed line is agglomeration area.

The main chemical components of the synthesized natural buffalo bone could be expressed in term of percent mass as CaO (54.03 %) and P<sub>2</sub>O<sub>5</sub> (45.97 %). Other components of HA for different sintering temperatures are shown in Table 1.

**Table 1.** Chemical components of HA.

Elements (% mass)	Temperature (°C)		
	650	850	1050
Calcium	39.11	34.69	38.44
Posphate	18.67	16.13	19.33
Magnesium	0.63	0.62	0.64
Natrium	0.58	0.28	0.64

Chemical component of HA is dominated by calcium and phosphorus as a main component of natural bone. The existences of magnesium and natrium [10, 11] in small amounts are corresponding to the inorganic compounds in natural bone [12].

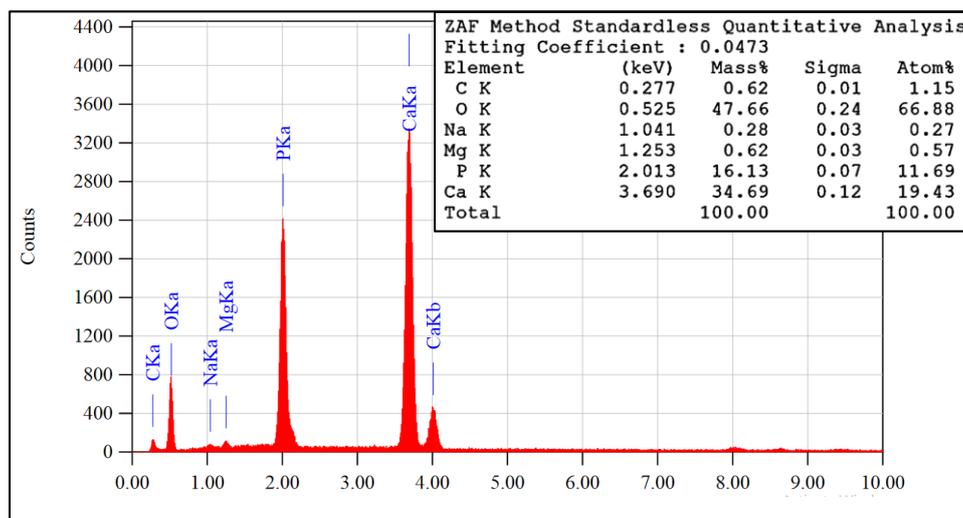
Commercial HA has a chemical formula Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub> with a calcium and phosphorus molar ratio of 1.67. Table 2 shows the molar ratio calcium/phosphorus (Ca/P) of HA based on buffalo bone.

**Table 2.** Molar ratio calcium and phosphorus at different sintering temperature of buffalo bone HA.

Temperature (°C)	Ca/P molar ratio
650	1.61
850	1.67
1050	1.52

As shown in Table 2 for sintering temperature at 650 °C, 850 °C and 1050 °C, Ca/P molar is ~1.67. At 850 °C, the Ca/P molar ratio is 1.67. Theoretically, the stoichiometric HA molar ratio is 1.667 for pure HA [12, 13]

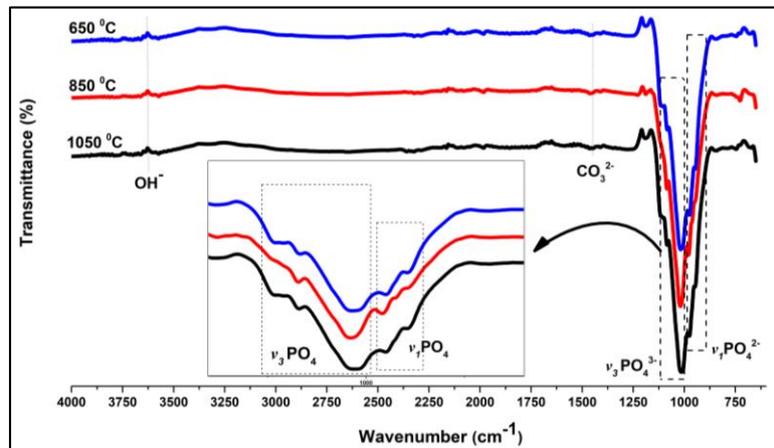
Figure 3 shows the chemical elements of buffalo bone HA with molar ratio 1.67 sintered at 850 °C. The composition consist of 34.69 % mass calcium and 16.13 % mass phosphorus.



**Figure 3.** Chemical elements of buffalo bone HA with molar ratio 1.67.

### 3.2. FTIR analysis

FTIR spectral analysis of HA sintered at 650 °C, 850 °C and 1050 °C for 7 hours is shown in Figure 4.

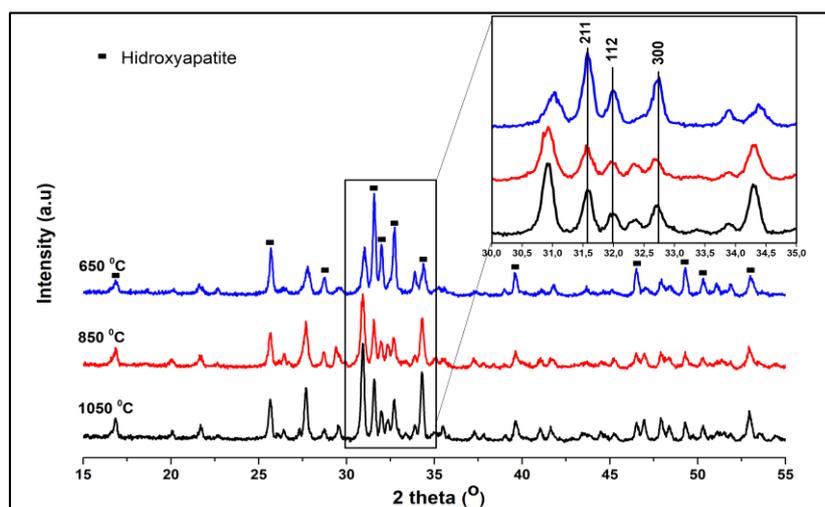


**Figure 4.** FTIR spectrum of the sintered HA at sintering temperature of 650 °C, 850 °C and 1050 °C for 7 hours.

The characteristic structural OH peaks appeared at 3572 and 633  $\text{cm}^{-1}$  [14, 15] for the sample sintered 1050 °C indicates the formation of HA. Phosphate band were identified by a peak at 1022.20  $\text{cm}^{-1}$  [15]. This band is due to the sintering process, in which increasing the temperature would also increase the crystallinity of the sample which then also increases the purity of HA, as analyzed in XRD result (Figure 5). The existence of the C-O at 1442.65  $\text{cm}^{-1}$  [16] is not surprising because human natural bones have this compound.

### 3.3. Structural analysis

Figure 5 presents an X-ray diffraction pattern of sintered HA at 650 °C, 850 °C and 1050 °C for 7 hours.



**Figure 5.** XRD Pattern of sintered at 650 °C, 850 °C and 1050 °C for 7 hours.

Characteristic of the HA powder is clearly identified by comparing the experimental XRD patterns to the Joint Committee on Powder Diffraction Standards (JCPDS) by no. #090432. There are three main peaks of HA, they are  $2\theta = 31.85^\circ$ ;  $32.31^\circ$ ;  $32.95^\circ$  respectively. The XRD pattern of the synthesized HA

at 650 °C, 850 °C and 1050 °C for 7 hours indicates the presence of the HA phase which are evidenced by the diffraction lines located at  $2\theta=31.77^\circ$ ;  $32.23^\circ$ ;  $32.94^\circ$ . This indicates the reflection corresponding to the (211), (112), and (300) *hkl* indexes. The additional peaks between 112 and 300 indicate the impurity of samples. Sintering process using conventional furnace and then Ca ions in sample is in contact with carbon dioxide to form calcium carbonate. The existence carbonate phase in the sample has been confirmed by FTIR result.

A narrow XRD peak observed at high sintering temperature is an indication of the high crystallinity of the sample [13, 18]. The structural analysis using XRD pattern showed that the HA was crystalline in all samples.

#### 4. Conclusions

Hydroxyapatite is successfully synthesized from buffalo bones by wet precipitation method and elevated sintering temperatures of 650 °C, 850 °C, and 1050 °C for 7 hours respectively. The calcium-phosphorus ratio of 1.67 is obtained at 850 °C. Uniform grain size with a narrow size distribution corresponding to the crystallinity improvement of the HA obtained after the sintered at 1050 °C for 7 hours. The presence of HA phase is evidenced by the diffraction lines located at  $2\theta=31.77^\circ$ ;  $32.23^\circ$ ;  $32.94^\circ$  indicates the reflection from 211, 112, and 300 crystal planes. All XRD peaks patterns of synthesized HA are correspond solely to commercial HA phase.

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