



## Production of Hydroxyapatite as an Alternative Formulation of Bioresponsive Ceramic Bone Replacement via Wet Chemistry Method

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### ABSTRACT

Hydroxyapatite bioceramic powder is synthesized as an alternative formulation of a bioresponsive ceramic bone replacement as it has good biocompatibility and high ability to form strong chemical bonds with human bone.  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{P}_2\text{O}_5$  were used as precursor in this reaction with stoichiometric Ca/P ratio of 1.33. The production of HAp particles were carried out by varying the process parameter such as stirring speed from 300 rpm to 700 rpm, sintering temperature from 600 °C to 800 °C and pH value of solution from 9 to 11. Overall, FTIR spectra shows that HAp consist of OH-, CO<sub>3</sub><sup>2-</sup> and PO<sub>3</sub><sup>2-</sup> functional group. Furthermore, from XRD spectra, HAp were present at 31.8 ° which is only present for the HAp powder that have been thermally treated at 600 °C. From SEM images, HAp produced agglomerate HAp particles at range size of 7 μm to 42 μm. Other than characterize the chemical characteristics of HAp, the aim of this study was to investigate particle size of HAp based on different process parameter in order to produce smallest particle size for application as bone replacement. Thus, smallest particle size was produced by applying highest stirring rate of 700 rpm and pH of 11 but at sintering temperature of 800 °C, HAp particle produced was the largest which is caused by agglomeration and particle growth.

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## 1. Introduction

Hydroxyapatite, HAp [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}_2)$ ] is known as calcium-phosphate-based bioceramic inorganic material [1] and the combination of two words which are 'hydroxy' means it consist of hydroxyl group (OH group) and 'apatite' means group of calcium phosphate minerals. This chemical compound consists of calcium (Ca), phosphorus (P), oxygen (O) atom and hydrogen ( $\text{H}^+$ ) ions. HAp have osteoconductivity properties due to its ability to form strong chemical bonds with the host materials [2]. HAp is an inorganic mineral component that is similar to human bone and

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teeth. It is widely used in many application in the various field such as bio-medicine and prosthetics due to its good biocompatibility and non-toxicity [3]. The high quality sintered HAp that is characterized having a refined microstructure and improved mechanical properties gained significant interest worldwide as it is the most stable and least soluble of all the calcium orthophosphate in physiological condition. Therefore, HAp has been used as the bioresponsive ceramic bone replacement due to its ability to form biological response with host material. Hence, this reports a wet chemistry methods which used wet precipitation technique to manufacture HAp as the alternative bioresponsive ceramic bone replacement at different process parameter [4]. After the production of HAp, the functional group, morphologies and sizes of the sample powder are characterised by means of physico-chemical such as Fourier Transform Infrared spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM). In addition, the sinterability and properties of the sintered product derived were evaluated and compared with commercial HAp.

## 2. Materials and Methods

### 2.1 Hydroxyapatite Production

The experimental work was carried out by varying number of parameter: pH (9,10 and 11), temperature (600 °C, 700 °C and 800 °C) and agitation rate (300 rpm, 500 rpm and 700 rpm). These parameter was selected in order to determine the smallest particle size of HAp which is by using central composite design (CCD) of a Response Surface Methodology (RSM). CCD was used to make a prediction of different parameter for each experiment, so that it was easier to conduct experiment at the same time with different parameter. Calcium nitrate tetrahydrate,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and phosphorus pentoxide,  $\text{P}_2\text{O}_5$  were used as calcium and phosphorus sources. Under room temperature condition, both of the chemical sources were stirred separately in 100 ml ethanol. The important stage in this experiment chart can be respectively explained as mixing both of solution to give a Ca/P molar ratio of 10:3 at the fixed concentration. This step has been done at ambient temperature (27 °C) by adding the phosphorus solution to calcium solution dropwise at rate of 3 ml/min. Then, the mixed solution was stirred at different agiation rate which was at 300 rpm, 500 rpm and 700 rpm. The beaker was covered with aluminium foil to prevent contamination by atmospheric condition. Ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) solution was then added dropwise to control pH value of solution under different pH condition which is 9,10 and 11.

### 2.2 Functional Group Determination

Functional group that is presented in the HAp powder synthesized were examined with the Fourier Transform Infrared Spectroscopy (FTIR) by using ATR methods. Wavenumber range at 400-4000  $\text{cm}^{-1}$  of infrared spectra were recorded as the analyses were carried out with 4 scans at 2  $\text{cm}^{-1}$  resolution.

### 2.3 Phase and Chemical Stability Determination

X-Ray Diffraction (XRD) analyses has been used to determine the phase and chemical stability of HAp powder. It was carried out at scan rate of 0.02°/min and the analyses was recorded over the  $2\theta$  range of 20-80°.

## 2.4 Siza and Morphology Determination

Morphology and particle size of microstructure HAp powder synthesized were characterized with by using JEOL JSM 6460LA branded model and JFC 1600 instrument for platinum coating. The sample particle were coated with platinum for 3 minutes in order to obtain 10 nm thickness. The methods used was by coated the HAp powder synthesized with a thin layer of electron tape before being placed in the scanning electron microscopy.

## 3. Results and Discussions

### 3.1 Functional Group

The functional group of HAp can be characterized by three spectrum parameter which are the location of maximum absorption, peak width and absorption maximum of OH vibration. The overall chemical group of synthesized HAp were  $\text{OH}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{PO}_4^{3-}$  and  $\text{HPO}_4^{2-}$  that characterize the non-stoichiometric HAp. The absorption band at  $3569.34 \text{ cm}^{-1}$  shown by HAp powder was refer to the  $\text{H}_2\text{O}$  molecules bonded with hydrogen for stretching modes. The result obtained shows that all the HAp powder synthesis has this absorption bands, thus it proves the absorption of water molecules has been occurred [5]. Absorption band at  $876.56 \text{ cm}^{-1}$  was proven the presence of  $[\text{CO}_3] \nu^2$  stretching modes or presence of  $[\text{HPO}_4]$  group absorption maximum. It was difficult to detected which group is presence at the same absorption band because the  $[\text{HPO}_4]$  group band partially covers  $[\text{CO}_3] \nu^2$  [6]. Furthermore, B-type  $[\text{PO}_4]$  groups with  $[\text{CO}_3]$  groups in the HAp crystal lattice were proven presence in the as-synthesized HAp powder by the combination of absorption bands of  $[\text{CO}_3] \nu^3$  group at  $1453.07 \text{ cm}^{-1}$  and  $876.56 \text{ cm}^{-1}$ . However, all the HAp sample synthesized were absent of  $[\text{CO}_3] \nu^3$  group absorption bands. This is because the HAp products have a low crystallinity degree and not thermally treated.

### 3.2 Chemical Analysis for Stability

Chemical analysis by using XRD method provides data about HAp which was characteristics of apatite, crystal structure and phase composition. From the result of XRD spectra, the peaks of spectra with increasing temperature become narrower and sharper. This situation results to the increased size crystallinity of HAp powder synthesized [7]. The sample which is thermally treated by  $800 \text{ }^\circ\text{C}$  have higher crystallinity degree compare to  $700 \text{ }^\circ\text{C}$  and  $600 \text{ }^\circ\text{C}$ . This is because restructurization of functional  $[\text{PO}_4]$  groups have occurred. As the heat treatment increased, the XRD peaks increased which is indicated to growth of the hexagonal-dipyramidal nanocrystals inside the powder particles. This type of phase exist as the stable phase HAp at the room temperature was hexagonaldipyramidal. At  $600 \text{ }^\circ\text{C}$ , the spectra shows broad peaks which is indicates in the formation of crystalline phase. As the temperature increase from  $700 \text{ }^\circ\text{C}$  to  $800 \text{ }^\circ\text{C}$ , the peak broad of HAp samples increase which indicates that the crystallinity of HAp particles increases [8]. Besides of sintering temperature, the XRD spectra shows that the peaks become more broad as increases of pH of synthesis solution was apply. The broaded peaks shows that the carbonate content in the HAp synthesized was increased with increase in the synthesis pH value [9].

### 3.3 Shape and Morphology

The production of HAp particle at different process parameter result to the changes of topography, composition and surface morphology of materials. Therefore, it is important to

observe the effect of these process condition to the production of HAp particles by using SEM. The SEM works by interaction of electron with atoms at or near the surface of material which is the formed image of particle from the release of signal. At lower agitation rates, the HAp synthesized particles are cuboid-like while it formed elongated ellipse-like morphology at higher agitation rate [10]. The differences of particle morphology of HAp synthesized particle can be explained by the shear produced by overhead stirrer. The used of Rushton turbine impeller which is attached to the overhead stirrer gives less energy input at lower agitation rate which is result to produces low level of shear effect into the solution mixer [11]. Sintering temperature was also the factor that effect particle morphology of HAp synthesized. Ellipse-like morphology with particle size between 20 and 35 nm were formed depending on the sintering temperature [12]. At magnification of 25,000X, the microstructure of HAp powder reveals the single particles have agglomeration of micro-sized grains. The sample treated by temperature of 600 °C to the 800 °C shown aggregated spherical-like to rod-like particles. The growth of HAp microstructure which is from the nucleation process [13].

#### 4. Conclusion

Hydroxyapatite microparticle was successfully be synthesized by wet chemistry method. From this method, precipitation techniques has been demonstrated by using  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{P}_2\text{O}_5$  as the starting materials. This summarizing work can help to evaluate synthesized HAp of the functional group, particle size and morphology, phase and chemical stability which was carried out at different agitation rate, pH value and sintering temperature. Overall, HAp consist of  $\text{OH}^-$ ,  $\text{CO}_3^{2-}$  and  $\text{PO}_3^{4-}$  functional group. Furthermore, it conclude that HAp has chemical stability at temperature of 600 °C. From SEM images, different process condition produced agglomerate HAp particle with range size of 14 to 211  $\mu\text{m}$ . The smallest particle size of HAp was produced at high agitation rate which was at 700 rpm and pH 11 as high agitation rate was affected by power and Reynold number while pH value affected by zeta potential [14]. However, at high sintering temperature which was 800 °C, the particle size produced was the largest which is caused by agglomeration and growth of particle as high temperature increase the collision between molecules and formed primary nuclei [15].

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