The Influences of Sintering Process on the Characteristics of Corbiculacea (Etok) Shells Based Hydroxyapatite Powder

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Abstract. Hydroxyapatite (HA) powders were prepared via chemical solution through aqueous solution of calcium hydroxide and phosphoric acid. The calcium precursor was extracted from the calcium carbonate of Corbiculacea shells while the phosphate precursor originated from the commercially available phosphoric acid. The final product of HA powders is then manipulated through the sintering process at 500°C while the other sample was used as it is. XRD result shows significant changes in its crystallinity, crystallite size and lattice parameters after the sintering process. By sintering the HA, the crystallite size and crystallinity were increases as much as 6.25% and 5.31% respectively. SEM on the other hand showed different morphology for both sintering and non-sintering HA powders. For the sintering HA, the grains size is higher which is 5.00µm compared to non-sintering HA which is 3.91µm due to the agglomeration.

1. Introduction
Natural resources from local biodiversity have a huge potential as biomedical materials as they are abundant in nature, sustainable and inexpensive [1-5]. Hydroxyapatite (HA) with chemical formula Ca₁₀(PO₄)₆(OH)₂ is a naturally occurring mineral from calcium phosphate [6]. It have hexagonal symmetry spatial group P63/m with lattice parameters a=0.95nm and c=0.68nm [7]. HA have a wide application of areas such as biomaterials [8], adsorbents [9] and catalysis [10]. Owing to its enormous application, the demand of HA is practically high, however, the cost of production is the main issue as it requires expensive chemical [11]. Thereby, researchers had come out with the HA production based on biowaste such as eggshells [12], corals [13], fish bones [14] and seashells [15]. The shells of Malaysian sea molluscs such as Corbiculae (Etok) and Polymesoda Expansa (Lokan) which are harvested as a local seafood delicacy had been also investigated as a suitable HA resource [16-18]. Seashells composed of high calcium amount and considered as the easiest and cheapest way in synthesising the HA [19]. Synthesising HA involves several methods which are hydrothermal [20], sol
gel [21], microemulsion [22] and chemical precipitation [23], [24]. Nevertheless, the chemical precipitation method from suitable calcium and phosphorus is the most convenient and low cost in producing the HA powder [25]. The characteristics such as crystallinity, morphology and particle size of synthesised HA powder influenced its effectiveness in its application [26]. Currently, sintering technology could manipulate its bioceramics properties such as its microstructure, composition and surface chemistry [27]. In another research [28], sintering process was evaluated upon its morphology. It shows that the density of HA was increased as the sintering temperature increases. The HA’s grain also shows denser particle as the sintering temperature had increased. Generally, HA sintering is challenging, as the OH functional group is tended to decompose to other materials such as tricalcium phosphate (TCP) and anhydrous calcium phosphate at 1200°C to 1450°C. Dehydroxylation process is incorporated with heating process and resulted the released of OH group in the form of water molecules [27], [29] and consequently causing the decomposition. Dehydroxylation took place at temperature less than 800°C whereby above 800°C to 1350°C, the dehydroxylation is accelerated [27]. Hence, the objective of this work is to evaluate the role of sintering process upon the physicochemical of HA powder synthesised from Corbiculacea (Etok) shells through chemical precipitation method.

2. Materials and methods

2.1. Materials
Corbiculacea (Etok) shells were purchased from local market in Kuala Perlis, Perlis, Malaysia. Phosphoric acid (H₃PO₄) with 95%, ammonia solution (NH₃) with 25% was purchased from Merck. All the chemicals were used without further purification.

2.1.1. Method in preparation of calcium oxide (CaO)
The collected shells were cleaned in boiling water to remove any dirt and the left over flesh by using distilled water for 30 minutes. After cleaning with distilled water, the shells were dried in oven at 100°C for 1 hour. The next process is crushing the shells into powder by using grinder machine (Mill Powder Tech). The powder is in the calcium carbonate (CaCO₃) form. The powder was then heated at 1100°C for 3 hours by using furnace to transform CaCO₃ into CaO powder.

2.1.2. Method in preparation of hydroxyapatite (HA)
To synthesis the HA, chemical precipitation method was used. Generally, CaO is the calcium precursor while H₃PO₄ is the phosphate precursor. Molarity of calcium to phosphate were calculated to follow the ratio of 1.67 which is the ratio of HA’s Ca/P. After calculating the molarity of both precursor, the calcium oxide powder was mixed in distilled water by using magnetic stirrer to form the calcium hydroxide (Ca(OH)₂). After that, the solution was titrated by using H₃PO₄ solution at 5.5 ml/min. The overall pH value of the solution was monitored and maintained at pH11 by adding some drops of NH₃. After finishing all the titration process, the gelatinous solution was formed and the solution was left to age for 24 hours. The gelatinous solution was filtered by using filter paper after 24 hours ageing. The product after filtering process was washed with distilled water and dried in oven at 110°C for 6 hours to remove possible moisture completely. The HA cake formed was then powdered by using mortar and pestle. The powder was divided into two: HA powder without sintering (the control) and the sintered HA powder. The sintered HA powder was obtained by using furnace at 500°C.

2.1.3. Method of characterization
X-Ray diffraction (XRD) is the analysis to evaluate the phase and the crystallinity of the HA powder. The powder was evaluated by using Brucker D2 Phaser. To prepare the sample, an amount of the powder was compacted into the XRD holder and scanned under the range of 10° to 90° with step size 0.1° and scan rate 5°/min. The XRD pattern obtained was validated to standard powder diffraction file by using X’pert Highscore Plus V.2.2.5 software. The crystallinity degree was calculated by using equation (1):

\[ X_c = \frac{V_{112/300}}{I_{300}} \]  

(1)
where $X_c$ is the fraction of crystalline phase; $I_{300}$ is the intensity at (300) reflection; $V_{112/300}$ is the intensity of hollow between (112) and (300) diffractions. The crystallinity size, $X_s$ in direction perpendicular to the crystallographic plan was calculated by using Scherrer’s formula [30] as in equation (2);

$$\text{Crystallite size, } X_s = \frac{k\lambda}{FWHM \cos \theta}$$

where $X_s$ is crystallite size (nm); $\lambda$ is the wavelength of monochromatic X-ray beam (nm) ($\lambda=0.15406\text{nm for CuK}_\alpha$ radiation); FWHM is the full width at half maximum of the diffraction peak under consideration (rad). The diffraction peak at $2\theta=32.25^\circ$ which assign to (112) plane was chosen for calculation of crystallite size since it is sharper and isolated from others. The morphology within HA powder and sintered samples were examined on Scanning Electron Microscope model JEOL (JSM-6010LV).

3. Results and discussion

3.1. XRD analysis

The XRD spectrum of synthesized HA from both samples are shown in Figure 1. The diffraction peaks at $2\theta$ values of 32.25°, 33° and 44.40° are corresponded to (112), (300) and (210) Miller’s plane of HA respectively in PDF 01-074-0565. The XRD patterns attained are in good agreement with the standard data of HA. The diffraction peak at 25.88° corresponded to (002) was selected to calculate the crystallite size and crystallinity by using Scherrer equation due to the peak is isolated from the others. All the calculated values were presented in Table 1 including the $a$ and $c$ axises. By observing both of the XRD patterns, it can be seen that the sintered HA has the sharper peaks, for example the peak at (210) plane. In the other hand, the broad diffraction peaks were observed by the non-sintering HA sample. This suggests that the HA with sintering process having higher crystallinity compared the non-sintering one. The HA without sintering samples behave as monocrystalline. The fraction of crystalline phase is evaluated based on equation (1). The evaluated degrees for both samples as shown in Table 1. Both crystallinity and crystallite size values are higher for HA sintered at 500°C compared to HA without sintering which indicated the strong dependence of the crystallinity of prepared HA. This is agreed by [26] and in similar trend with [31]. Higher crystallinity of HA is favorable for bone tissue application as the HA is more biocompatible and could mimic the natural bone [32]–[35]. The value of lattice parameters shows that the $a$ axis is similar for both sample but for $c$ axis, the value is slightly higher for sintering HA. The increment in value is due to calcinations or sintering process [31]. Comparing the lattice parameters of both samples with the standard HA, it can be seen that the lattice parameter $a$ had decreased. This happened due to the substitution of CO$_3^{2-}$ [36]. Literally, the CO$_3^{2-}$ was present in the HA’s lattice structure due to the carbon dioxide from the environment dissolve in distilled water during the mixing process [37].
Figure 1. The XRD pattern of synthesised HA

Table 1. The crystalline parameters of synthesised HA

| Samples                  | Crystal axis a-axis (nm) | Crystal volume c-axis (nm) | Crystallinity, Xc (%) | Crystallite size, Xs (nm) |
|--------------------------|--------------------------|---------------------------|-----------------------|--------------------------|
| HA without sintering     | 9.26                     | 6.85                      | 23.26                 | 74.97                    |
| HA sintering 500°C       | 9.26                     | 6.89                      | 28.57                 | 81.22                    |

3.2. SEM analysis

The image of SEM is shown in Figure 2 and 3 for HA without sintering and HA sintering at 500°C sample respectively. From the both of images, measuring the particle size is quite difficult due to high agglomeration. Both of the samples show an irregular shape particle. By differentiating both samples, the sintered HA sample have a huge agglomeration between the particles. The average grain size of sintered HA is also bigger compared to non-sintering HA which is 5.00µm and 3.91µm respectively. During the sintering process, there’s a development of grain growth between the grain network accompanied by the movement of grain boundary [38]. Thereby, the sintering sample tended to form bigger particles size as the grains boundary were developed.
5.00µm

Figure 2. SEM images of HA without sintering process

3.91µm

Figure 3. SEM images of HA with sintering process at 500°C

Figure 2. The XRD pattern of synthesised HA

4. Conclusion
HA powders based on Corbiculacea shells were successfully synthesised via chemical precipitation. By manipulating the sintering process of HA’s sample, it influenced the phase and crystalline structure of HA. The higher crystallinity and crystallite size could be gained by sintering the HA powder. Through the sintering process, SEM analysis showed an agglomeration between the particles. The grain size also had increased through the sintering process.

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