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Research paper

Calcium phosphate nanoparticles prepared via solid-state route

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Abstract

To date, the direction of bioceramic research is focused on the improvement of the mechanical performance and biological properties of existing bioactive ceramics particularly HA. Hence, the synthesis of crystalline HA nanoparticles with expected microstructure is of primary importance because the process directly relates to the phase purity, morphology, and particle size of the final HA particles. In this work, a simple and cost-effective technique, solid state reaction method was successfully employed to synthesize highly crystalline, high purity and single phase nanostructured hydroxyapatite powder using waste eggshells (HA-Es). The process involved mixing calcined eggshell powder and dicalcium hydrogen phosphate di-hydrate followed by a heat treatment at 800 °C for 5 hours. The resultant flower-like nanostructure HA powder is composed of leaf-like flakes having 100-200 nm width and crystallite size calculated using XRD data of ~56.21 nm.

Keywords: *Calcinations; Calcium Phosphate; Eggshell; Synthesis; Solid-state Sintering.*

1. Introduction

Hydroxyapatite (HA) with the chemical composition of $Ca_{10}(PO_4)_6(OH)_2$, is the most significant among the calcium phosphate compounds as its composition is found close to the dominant inorganic component of human body hard tissues such as bone, dentin and enamel. It should be mentioned that calcium phosphate in bone is similar, but not identical to mineral HA due to the presence of significant amounts of carbonate ions and traces of other ions substituted into its structure.

Over the years, there are various techniques developed to prepare HA. In general, the raw materials used to synthesize HA originate from inorganic based precursor or from the natural organic based resources which include conversion from marine coral derivates to natural HA with acceptable porosity [1]. Nevertheless, both types of biomaterials are equally bioactive and biocompatible in nature. For the synthesis using inorganic component, the synthesis methods are divided into two types that are wet chemical methods (precipitation, sol-gel and hydrothermal) and dry processes (solid-state reactions). Literally, continuous research attempts in producing biocompatible biomaterial from natural organic waste have added a valuable aspect of its recovery. This is because some of this waste contains active compounds that have value in medical applications. Moreover, the waste is readily available and cheap as compared to the cost of the whole biomaterial production.

Literally, HA and its precursors can be converted from biological resources and these resources can be grouped into 3 types that are bone, plants and biogenic. In this case, bones present mammalian (bovine, sheep, pig), chicken and fish bones, whilst biogenic sources are eggshells, seashells and conversion from marine coral derivates to natural HA with acceptable porosity. These biological compounds that are denoted as calcium-deficient contain a wide variety of relatively small amounts of other substitute atoms or groups such as carbonate, Na, Mg and Sr ions [2-3]. It was reported that the biomolecules amount of these waste materials influenced the latter product shape, size and its crystal morphology which reflected their applications.

The nature of a bovine bone which is composed of HA nanocrystals and collagen fibers have drawn the attention to its feasible extraction of natural HA. Moreover, these mammalian bones especially the bovine bones are a rich source of ion-doped HA. It was reported that a complete conversion of bovine bone from 1.6 kg of compact bone can furnish as much as 1 kg of HA [4]. In short, process of HA extraction from natural sources (i.e. bovine bone) via various techniques (simple thermal treatment, enzymatic hydrolysis, plasma processing, subcritical water processing and alkaline hydrothermal analysis) start off with the removal process of unwanted organic matter that inherent in the bone. The animal bones are thoroughly washed, boiled with distilled water, followed by washing with NaOH or hypochlorite to remove dirt from the surface of the bones [1]. As for calcinations regime, bones are heated in a furnace at temperatures between 500 and 1400 °C to completely remove the organic materials from the bone while leaving pure HA as residue [5] and to enhance the crystallinity of HA while avoiding thermal decomposition of the final HA product [1]. However, it was reported that calcinations treatment above 850 °C destroys the pathogens and could solve the problem of probable transmission of disease from the cattle to the patient [6]. Subsequently, bones are either directly cut into small pieces or ball milled. These steps will later determine the morphology and particle size of the final product as the milling duration influence the size of the particle. For example, the Vibro milling method has been employed on deproteinized bovine bone after calcination at 800 °C for 3 hours where the longer vibro-milling time resulted in the break-down of HA needles into fine powder [7].

Another source of biogenic sources that have significant impact on the development of biomaterial are chicken eggshells. This waste eggshell has big potential in its usage as a raw material for the synthesis of HA due to its wide availability as waste product in food industries, beauty industries and personal consumption as in home cooking. These eggshells are basically discarded as waste material. However, the protein-rich membrane which adheres to the shell favours microbial growth. In addition, the expensive cost of eggshells disposal in scarce landfills space indirectly lead to environmental pollution [3]. Therefore, the recycling of waste eggshell will reduce the need for waste management and contribute to cleaner environment with improved ecosphere. Despite its wide availability, the remnants of several trace elements from the natural biological origin of the eggshells in the crystalline structure of synthesized HA make their composition similar to human bone. It was also reported that the HA synthesized from eggshell demonstrates superior sinterability and better morphology when compared with HA synthesized from other sources [1, 8]. Moreover, biocompatibility of eggshell derived HA has been well established in a few researches [6, 9-10].

Dry methods are defined by the absence of solvent in the HA preparation. This method is known to be suitable for mass production of highly crystalline HA as it does not require precise control condition. In other words, the characteristic of the synthesized HA is not highly influenced by the processing parameters [5]. Besides that, mechanochemical processing is another successful method to produce nanostructured HA in a dry state [11]. Both methods have the advantages of being straightforward and highly reproducible. However, the mechanical alloying has been greatly used by researchers compared to solid state synthesis because it offers well-defined HA structure [5]. Meanwhile, solid state method has received the least attention in the preparation of HA due to the resultant of heterogeneous HA particles with irregular shape. However, it is improper to dismiss the usage of solid-state synthesis in the synthesis of HA particles because with proper processing, nanostructured HA powder with regular shape can be achieved. By modifying the milling process to minimize contamination, the eggshell derived HA powders was fabricated using solid-state reaction.

2. Methods

Calcium carbonate ($CaCO_3$) from waste eggshell was used as a source of calcium precursor to synthesize HA. This is done via calcinations of the eggshells. Waste eggshells were thoroughly cleaned and air-dried prior to the removal of the inner membrane layer. Cleaned and dried eggshells were crushed to a fine powder consistently using a pestle and mortar. To establish an optimum calcinations temperature, various temperatures for calcination were investigated. Based on the phase purity of the $CaCO_3$ produced as well as the intensity of the inherent peaks of $CaCO_3$ obtained from the XRD diffractograms, the optimum calcination temperature is selected. In this study, the optimum calcination temperature to obtain $CaCO_3$ is found to be 700 °C.

The calcined eggshell was then added to phosphorus precursor, calcium hydrogen phosphate dehydrate, $CaHPO_{4.}2H_2O$ (DCPD) at a Ca/P ratio of 1.67 and this mixture is subjected to attrition milling with ethanol as a solvent at a speed of 400 rpm for 2 hours. The most significant effect is a modification of particle's surface charge, which depends on the nature of the solvent. The low dielectric constant and surface tension of alcohol induce a very low ionic strength of the solution and enhanced wettability of particles, which results in loosely bonded particles [12].

The obtained powder mixture was dried in the oven at 110 °C for overnight. The dried powder is sieved through the 212 µm mesh to obtain a fine powder, thus increase the number of crystallites in contact before formed into green compacts. The block was then preheat-treated at 800 °C for 5 hours to obtain a homogeneous composition.

The HA powder is obtained following the chemical reaction:

$$6CaHPO_4.2H_2O + 4CaCO_3$$

$$Ca_{10}(PO_4)_6(OH)_2 + 4\,CO_2 + 4H_2O \qquad (1)$$

The heat-treated pellets were crushed and ground to break the particle agglomerates and HA derived from eggshell was referred to as HA-Es.

3. Results and Discussion

The eggshell was first calcined to remove organic compounds in the inherent $CaCO_3$ in eggshell as it has high reactivity. Upon mixing the calcined eggshell powder and $CaHPO_4.2H_2O$ (DCPD) at 400 rpm for 2 hours, the powder mixture was subjected to thermal analysis to determine the temperature at which the chemical reaction of the HA formation has taken place. The TGA result of the mixture of calcined eggshell and DCPD is shown in Fig. 1. The TGA curve shows about ~2.68 % weight loss from room temperature to 200 °C, which was due to the evaporation of adsorbed water from the surface as well as pores [13-14]. Then the steady and continuous ~6.31 % weight loss up to ~500 °C is ascribed to the loss of adsorbed and lattice water [15]. The prominent weight loss of about ~13.28 % over the temperature range ~650 °C to 785 °C suggests that the partial reaction between $CaCO_3$ and $CaHPO_4.2H_2O$ might take place, and so this weight loss could attribute to decarboxylation of $CaCO_3$ related to a release of carbon dioxide. Subsequently, the reaction of $CaCO_3$ and DCPD might be completed at about 800 °C and as such there were only insignificant weight changes (less than 1 % weight loss) up to 1000 °C. This indicates the high thermal stability of the synthesized HA-Es within that temperature range. Therefore, the heat treatment of mixture was chosen at an initial temperature of 800 °C.

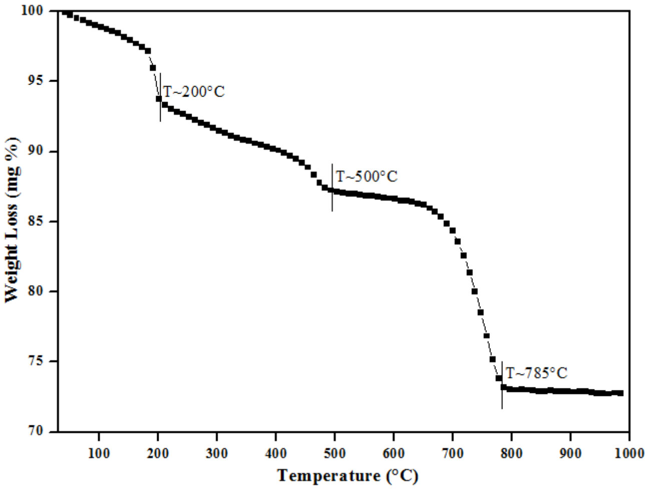

Fig. 1: TGA analysis of eggshells powder and DCPD mixture after attrition milling

The earlier observation in TGA analysis is further supported by the XRD result of the calcined eggshell and DCPD mixture to study the effect of calcination at different temperatures on HA-Es phase stability. The result shows that at 700 °C, the as-milled powder mixture has partially reacted and decomposed to biphasic calcium phosphate mixture (HA + β-TCP) with β-TCP being the dominant phase (Fig. 2 (b)). As the calcinations increases to 800 °C, HA phase was found to be more thermodynamically stable with the absence of β-TCP peak as shown in Fig. 2 (b). It was worth noting that the decomposition reaction is complete since it has transformed to phase pure HA as suggested in Equation 1. These results indicate that β-TCP was formed from the starting materials reaction meanwhile HA was then formed with an increase in heat treatment temperature. As the temperature increases to 900 °C (Fig. 2 (c)) and 1000 °C (Fig. 2 (d)), there was no decomposition to secondary phases detected. On the contrary, it was reported that heat treatment of HA derived from mixture of eggshell and DCPD, at 1000 °C with a ramp rate of 10 °C/min for

3 and 5 hours resulted in HA phase with traces of starting materials and β-TCP phase, respectively [3].

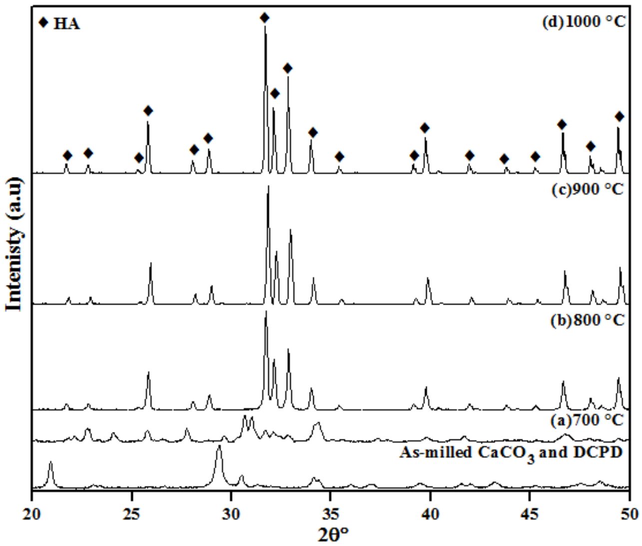

Fig. 2: XRD patterns of as-milled and calcined at (a) 700 °C, (b) 800 °C, (c) 900 °C and (d) 1000 °C, powder mixture

In this work, the XRD result in turn matched to the TGA result of insignificant weight changes observed starting from 800 °C up to 1000 °C (Fig. 1). Raynaud et al. stated that decomposition of heat treated HA ceramic at 900 °C could be the result of the non-stoichiometric composition of the starting HA powder [16]. The only difference in XRD patterns of Fig. 2(c) – (e) is in the degree of HA crystallinity with sharper and more defined peaks. In addition, it was observed that the increase in heat treatment temperature resulted in an increase in the crystallite size since larger crystallites form favorably at a higher temperature [17]. This, in turn, decreases the surface areas of the powders. The average crystallite size measured corresponding to the (211) and (112) planes is tabulated in Table 1.

Table 1: Crystallite size of calcined HA-Es powder at various temperatures

Temperature (° C)	Crystallite Size (nm)
800	56.11
900	69.52
1000	78.91

The XRD pattern of the powder mixture after heat treatment at 800 °C for 5 hours is presented in Fig. 3. Interestingly, all patterns matched with the JCPDS Card Number 09-0432 for hydroxyapatite. The highest intensity peak of (211) is observed at a 2θ angle of 31.72°. This demonstrated that single phase HA was successfully derived from eggshell. This result is encouraging as there was no appearance of β-TCP and CaO phases as the calcination temperature increases above 650 °C as reported by other work on solid state reaction [18] The average crystallite size of phase pure eggshell-derived HA (HA-Es) powder that corresponded to the (211) and (112) planes was found to be 54.60 nm whereas the specific surface area (SSA) of the HA-Es powder obtained from the BET measurement was 8.71 m²/g.

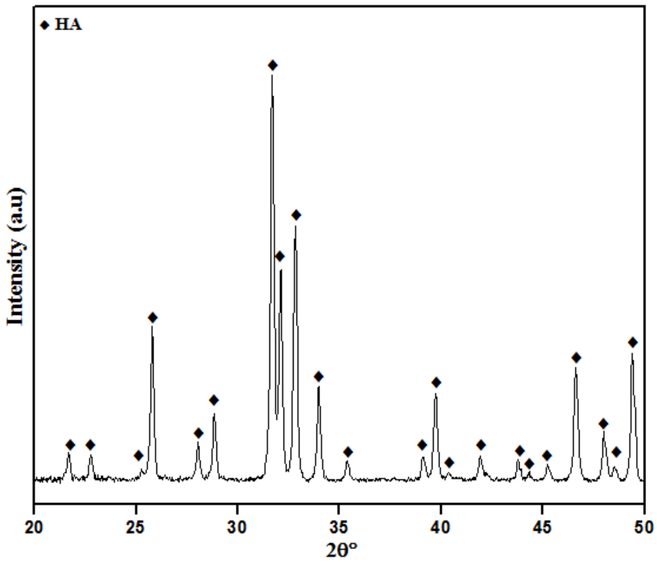

Fig. 3: XRD patterns of HA-Es powder pre-heated at 800 °C

The FTIR spectrum of the HA-Es powder is shown in Fig. 4. The characteristic peaks corresponding to PO_4^{3-} (v_3 and v_4) vibrations of HA are observed at 1038, 605 and 568 cm^{-1}. Besides that, another characteristic peak of PO_4^{3-} (v_1 and v_2) appeared at 1095, 962 and 475 cm^{-1}. A broad H_2O band observed at 3425 cm^{-1} and this is attributed to the v_3 and v_1 stretching modes of the H_2O molecules [8]. Besides that, a band observed at 3590 cm^{-1} indicates the characteristic of O-H stretching vibration of HA [19]. Meanwhile, broad band at 1643 cm^{-1} observed in calcined HA-Es powder is attributed to the v_2 bending mode of adsorbed H_2O [20]. Frequency band observed at 2365 cm^{-1} is a characteristic of free CO_2 [21]. In addition, CO_3^{2-} vibration bands are clearly observed in the calcined powder at 1463 cm^{-1}, 1421 cm^{-1} (v_3) and 875 cm^{-1} (v_2). Similar bands were also observed in previous studies of HA-Es. [8, 21-22] In this study, the presence of these carbonate bands is caused by the adsorption of non-structural carbonate on the surface of HA-Es crystals [23]. In addition, the absence of surface OH band at 3644 cm^{-1} which represents the presence of CaO in calcium phosphate has collectively supported the preceding XRD results in Fig. 3 [22].

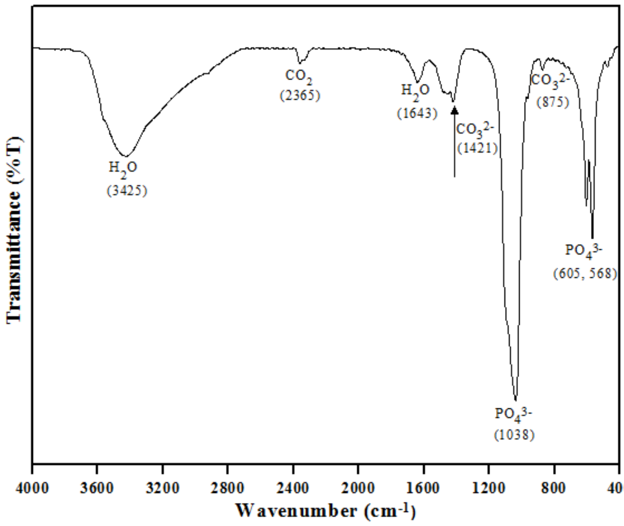

Fig. 4: FTIR spectra of HA-Es powder pre-heated at 800 °C

Fig. 5 shows the SEM microstructure of HA-Es powder after the heat treatment at 800 °C. Typical flower-like nanostructured HA was observed. The HA-Es microstructure composed of leaf-like flakes having the size of 100-200 nm in width. As mentioned, HA-Es has a low surface area of 8.71 m^2/g, hence there is less tendency to form large agglomerate due to the decreasing attraction between particles of Van der Waals interaction [3, 24-25]. In addition, the higher magnification of the particle surface that showed smooth surface of the powder particle was a typical characteristic of highly crystalline HA [26].

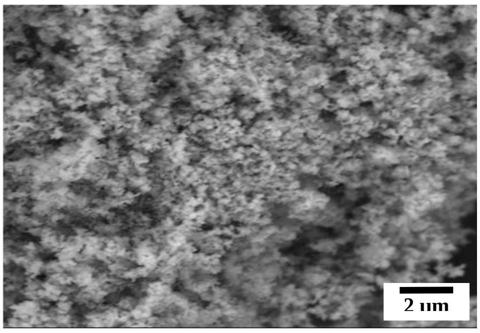

Fig. 5: SEM of HA-Es powder pre-heated at 800 °C

4. Conclusion

Phase pure hydroxyapatite using waste eggshell as calcium precursor was successfully produced through solid state reaction. The highly crystalline HA-Es powder was obtained after pre-heat treatment of calcined eggshell and DCPD at 800 °C with typical flower-like morphology, comprising of petal-like flakes having an average size of 100–200 nm in width. The crystallite size calculated using XRD data was found to be ~54.6 nm whereas the specific surface area of the HA-Es powder obtained from the BET measurement was 8.71 m^2/g. The small surface area was expected due to the calcination employed during the powder preparation which could result in hard agglomerates of particles. This flower-like HA has known to have the potential to act as effective carriers for drug delivery applications.

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