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Synthesis of Hydroxyapatite From Biogenic Waste as Calcium Precursor Using Solid-State Reaction Method

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Abstract

Eggshells are one of the easily available organic wastes that can be used as calcium precursors in producing calcium phosphate. It has been converted to various forms such as calcium oxide, calcium hydroxide, calcium nitrate and calcium chloride but limited research has been reported on the potential of eggshell-derived calcium precursor in the form of calcium carbonate ($CaCO_3$). Hence, in this study, $CaCO_3$ from eggshell as a calcium precursor was mixed with dicalcium hydrogen phosphate dihydrate (DCPD) to synthesize a homogeneous composition of pure hydroxyapatite (HA) powder from eggshell waste via a solid-state reaction method. The mixing process was carried out for homogenization via magnetic stirring technique for 4 hours, whilst structural transition took place during heat treatment at 800°C for 5 hours. XRD results confirmed that highly crystalline eggshell-HA has been successfully synthesized with an average crystallite size of 26.35 ± 0.1 nm which is comparable to the average particle size of 0.62 ± 0.02 µm confirmed by FESEM. EDX analysis validated the elemental composition of the Ca/P ratio obtained as 1.76, which shows a close resemblance to the Ca/P ratio of 1.67 for pure HA. These findings have indirectly contributed to cost reduction in materials processing and promote the recovery of biowaste.

Discipline: Material Science

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1 Introduction

Calcium phosphate (CaP) is one of the most versatile materials for nanomedicine due to its remarkable stoichiometric versatility. The most important form of calcium phosphate substance is known as Hydroxyapatite (HA), with the chemical formula, Ca10(PO4)6(OH)2. HA constitution was found to be very near to the principal inorganic component present in the hard tissues of the human body such as bones, dentin and enamel (Elias & Metoki, 2017); hence, making them highly biocompatible (Hamidi et al., 2017).

The wet method and the solid-state reaction method are the two main methods for producing HA powder. These methods vary in terms of the precursors used and processing cost while HA powders with various morphologies, stoichiometry, and levels of crystallinity can be obtained depending on the method of synthesis (Sadat-Shojai et al., 2013,). In the production of HA via the dry technique, two precursor chemicals, which are calcium and phosphate, are combined in their dry state before being put into action (Mohd Pu'ad et al., 2020). In HA processing via solid-state reaction, the milling process has greatly influenced the particle size of calcium phosphate particles, and since most dry procedures do not require circumstances that are precisely measured and controlled, they are suitable for the manufacture of powders in large quantities (Ramesh et al., 2016).

Besides that, in producing pure HA, a variety of source of precursors can be used. Typically, the natural source of Ca for hydroxyapatite is extracted from mammalian bones (bovines, camels, and horses), marine or aquatic sources (fishbones, fish scales, plants and algae), shell sources (cockles, clams, eggshells, and seashells), and limestone (Pu'ad et al., 2020). Eggshells are one of the easily available organic wastes that can be used as calcium precursors in producing calcium phosphate. The eggshell waste consists predominantly of 94 % calcium carbonate (CaCO3) and has been used as a raw material or direct source of calcium precursor to synthesize HA. In addition, the remnants of several trace elements from the natural biological origin of the eggshells e.g., K, Na, Mg, Si and Sr, in the crystalline structure of synthesized HA make its composition similar to human bone. It has been converted to various forms such as calcium oxide calcium hydroxide, calcium nitrate and calcium chloride (Kamalanathan et al., 2014), but limited research has been reported on the potential of eggshell-derived calcium precursor in the form of calcium carbonate (CaCO3). With a proper processing regime, it can be used as a precursor material for producing HA.

A study conducted by Ahmed and Ahsan (2008) reported that when eggshell-derived HA is synthesized via the chemical precipitation method using CaCO3 as a Ca precursor, it requires an additional calcination procedure, in comparison to when using CaO as a Ca precursor where no additional calcination is involved. Besides that, it was reported that the mixing method is one of the important parameters that need to be controlled to produce a nanostructured HA (Ramesh et al., 2016). Hence, this study was conducted to synthesize a homogeneous composition of pure calcium phosphate powder from eggshell waste via a solid-state reaction method through the combination of magnetic stirring technique and heat treatment. CaCO3 from eggshell as Ca precursor was mixed with dicalcium hydrogen phosphate dihydrate (DCPD) and calcined at 800° to produce pure HA powder. Subsequently, the physical properties such as phase composition, morphology, and particle size of the synthesized HA powder were assessed. As eggshell waste was one of the many food wastes in Malaysia that are discarded predominantly; hence, the production of HA through this simple method is expected to be more economical and viable.

2 Literature Review

Hydroxyapatite (HA) is the most significant among the calcium phosphate compounds due to its structural and compositional similarity with the natural bone and teeth of vertebrates (Du et al., 2021). Nowadays, hydroxyapatite is widely used in biomedical applications, particularly in dental applications, bone substitutions filling tissue engineering, and drug delivery systems because of its biocompatibility, bioactivity and osteoconductivity (Ingole et al., 2021). However, particle size, aspect ratio, shape, crystallinity, and dispersion of HA particles have a substantial impact on the functional performance of these biomaterials.

The calcium used to synthesize HA can be either obtained from synthetic sources or natural sources. Between both sources, it is much preferable to use natural sources for their affordability and availability. Eggshell is one of the most preferred natural calcium sources as it consists of 94% of calcium carbonate (CaCO3) due to its higher reproducibility compared to other natural sources such as animal bones and corals (Abdulrahman et al., 2014). Around 250, 000 tonnes of eggshell waste were reported to be created around the world (Puad et al., 2021). However, eggshell waste is mainly disposed of in landfills, where it produces odors and microbiological growth, so it requires high management costs (Ramesh et al., 2016). Therefore, the solution to this problem is to economically transform or recycle eggshell waste to create new values, such as valuable biomaterials like HA and to obtain their benefits from these waste materials.

Up to date, various preparation methods have been explored to synthesize bioactive HA including wet chemical methods (e.g., sol-gel processes, hydrothermal), dry methods (e.g., solid state synthesis, mechanochemical method) and combination methods (e.g., hydrothermal–mechanochemical, hydrothermal–hydrolysis) (Natasha et al., 2022). Even though numerous techniques have been discovered, the development of high-quality HA remains a challenge due to the likelihood of forming harmful intermediate products during synthesis and irregularities of synthesized apatite's composition physiochemical properties. As a result, research into novel parameters for synthesizing HA continues. The wet method and the dry method are the two main methods for producing HA powder. In HA synthesis, precise control of crystal growth presents the ultimate challenge because it directly relates to the characteristics of the final particles such as grain size, material crystallinity, and geometric shape and distribution (Ramesh et al., 2016). These primary characteristics subsequently affect the mechanical properties, biocompatibility, and bioactivity of HA.

In research done via solid-state reaction using calcium carbonate (CaCO3) and di-calcium phosphate anhydrous (CaHPO4), the HA powder shows microcrystalline by XRD and when heating the powders to a high temperature, the HA powder is found to be sharper because the crystallinity of powders increases (Ebrahimi et al., 2021). Besides that, the heat treatment temperature also plays a critical role in the production of HA powder. Only a single phase of HA is seen after sintering at temperatures of 800 °C and above where the powders are made up of equiaxed crystals that aggregate into larger aggregates, resulting in median particle size (Ebrahimi et al., 2021). Fiume et al. (2016) reported that the wet method is a very sensitive technique where even minor differences in reaction conditions have a significant impact on the final product's composition and it requires approximately 20 days to obtain HA of stoichiometric composition. Thus, with a proper processing regime, the solid-state reaction method is preferable when producing high crystalline hydroxyapatite in mass due to its high reproducibility and low processing cost.

3 Method

3.1 Synthesis of HA Powder via Solid-State Reaction

Firstly, the collected raw eggshells were thoroughly cleansed by removing any sort of dirt that existed on the shell and by removing the eggshell membranes. Then, the dried eggshells were crushed by using a pestle and mortar to produce eggshell powders. The eggshell powder was sieved using a 250-mesh sieve to ensure a uniform starting eggshell powder. Subsequently, the waste eggshell was calcined at 700°C to obtain highly pure calcium carbonate as a starting Ca precursor. The calcined eggshell was then mixed with phosphorus precursor of dicalcium hydrogen phosphate dihydrate (DCPD) at a Ca/P ratio of 1.67. Then, the mixture was subsequently mixed using magnetic stirring for 4 hours stirring time of homogenization. The mixture was then dried overnight at a temperature of 110° C in the drying oven. In obtaining the fine and uniform powder, the sieving process was made by using a vibratory sieve machine after drying the mixture overnight. The powder was then subjected to a heat treatment process by using a high-temperature furnace carbolite at the temperature of 800°C with 5 °C/min of heating rate for 5 hours for the structural transition to take place.

3.2 Characterization of Synthesized Eggshell-Derived HA

Phase composition analysis was done via X-Ray Diffraction (XRD) (Rigaku Ultima IV) to identify and characterize the crystalline materials compounds based on their diffraction pattern. The sample was carried out at room temperature and operated at 40 kV and 40 mA using Cu-Kα as the radiation source. The X-ray step size was 0.02° with the scan angle (2θ) varying from 20° to 50°. In this study, the phase stability was identified by comparing the prominent XRD peaks exhibited by synthesized HA powder to the standard reference JCPDS-ICDD. The crystallite size of the synthesized HA powders was then calculated using Scherrer's equation:

$$t = 0.9\lambda / \beta\cos\theta \quad (1).$$

where t is the average crystallite size, λ is the wavelength of the radiation, β is the full width at half the maximum value of the measured diffraction peak and θ is the diffraction angle of the investigated peak.

A scanning Electron Microscope (SEM) (Hitachi SU3500) equipped with Energy Dispersive X-ray (EDX) was used to observe the surface morphology and microstructure features of the synthesized HA. The sample was coated with platinum before being set up in the SEM machine to minimize the charging effect. The average particle size of synthesized HA powder was determined via the linear intercept method using FESEM images. Meanwhile, the EDX spectra ensured the presence of the expected elements in the synthesized HA powder. The signals from this equipment reveal the information of the sample which includes external morphology (texture) and chemical composition of materials that make up the sample.

4 Result and Discussion

4.1 Phase Stability

Figure 1 demonstrates the XRD patterns of raw eggshell and calcined eggshell while Figure 2 shows the XRD peaks of synthesized HA powder. These XRD patterns are compared with those of the standard powder in the diffraction file (JCPDS). From Figure 1, the primary peaks of crushed raw eggshell powder are attributed to Calcium Carbonate (CaCO3) which has the best matches with the JCPDS Card Number 01-085-1108 of CaCO3 or known as Calcite. Calcite is the form of calcium carbonate which contains a high percentage of calcium in eggshell waste (Razak et al., 2021). Besides that, similar CaCO3 peaks but sharper and higher intensity were observed for calcined eggshells. This demonstrates that raw eggshell at 700 °C was highly pure in calcium carbonate and hence was chosen as the source for Ca precursor in the synthesis. The crystallite size of the raw eggshell and calcined eggshell was found to be 49.68 nm ± 0.1 nm and 54.74 ± 0.1 nm, respectively. Meanwhile, Figure 2 shows that a highly pure and crystalline HA phase was successfully obtained after the calcination of the powder mixture at 800 °C and the peak position was well matched with the standard data from Joint Committee on Powder Diffraction Standards (JCPDS) card number of 00-064-0738 of hydroxyapatite. These sharp main peaks of HA demonstrate that the HA powder produced was well crystalline and corresponded to the hexagonal crystal structure for HA with a crystallite size of 26.35 ± 0.1 nm.

The average HA crystallite size is comparable to the crystallite size obtained from past studies where the eggshell-derived HA crystallite size of 59.9 nm and 54.6 nm was obtained using the ball milling technique (Wu et al., 2015) and attrition milling technique (Ramesh et al., 2016), respectively. According to Puspitasari et al. (2021), the larger crystallite sizes may be caused by the agglomeration that takes place during the sintering process. Generally, calcium precursor in the form of calcium carbonate from waste eggshells has successfully been used as a starting precursor to produce HA without the need for extra calcination. Besides that, the small HA crystallite size obtained has indirectly validated the application of 4 hours of magnetic stirring technique in this work for producing homogeneous HA powder via solid-state reaction.

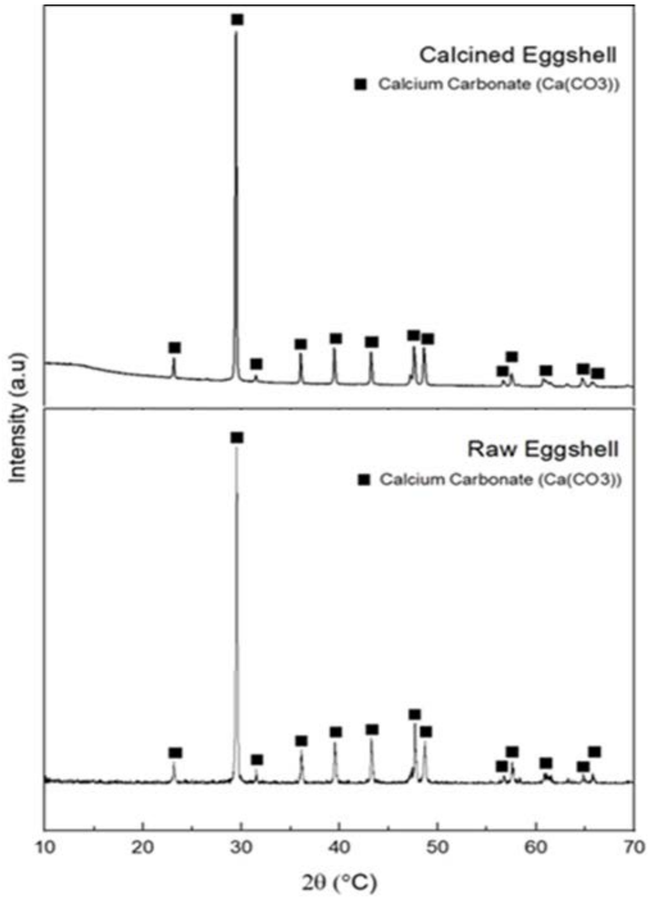

Figure 1: XRD patterns of raw eggshell and calcined eggshell powders.

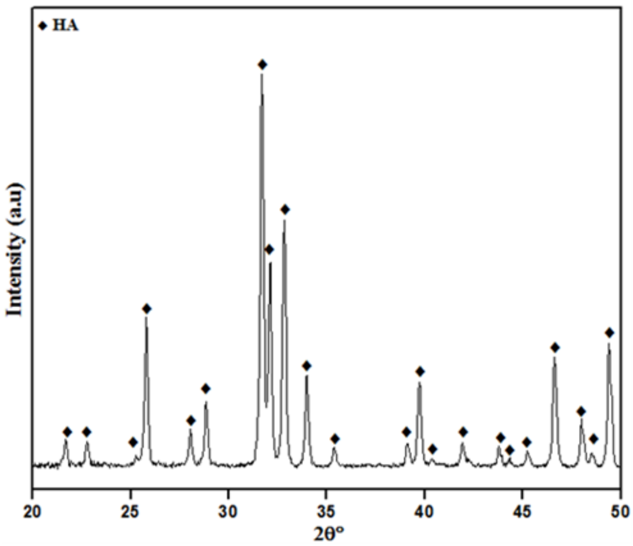

Figure 2: XRD patterns of eggshell-derived HA powder at 800 °C.

4.2 Morphological and Elemental Analysis

Figure 3 shows the FESEM image for synthesized pure HA powder samples at different magnifications. The FESEM image justifies the typical morphological pattern of HA powder which is in a globular-like shape. According to Razak et al. (2021), the pattern is formed by many agglomerations. Hamidi et al. (2017) described similar results and reported that the morphology shape of the powder tends to form agglomerates which contribute to the non-uniform size of the particles. The SEM analysis justifies that the agglomerate of shapes is nearly identical, with pores between them. Accordingly, HA powder that experiences the heating process promotes the agglomeration in part size which then forms a bigger particle due to the rise of crystallinity degree. ImageJ software has been used to calculate the average particle size of the HA powder. The average particle size observed for HA powder was 0.062 ± 0.02 μm.

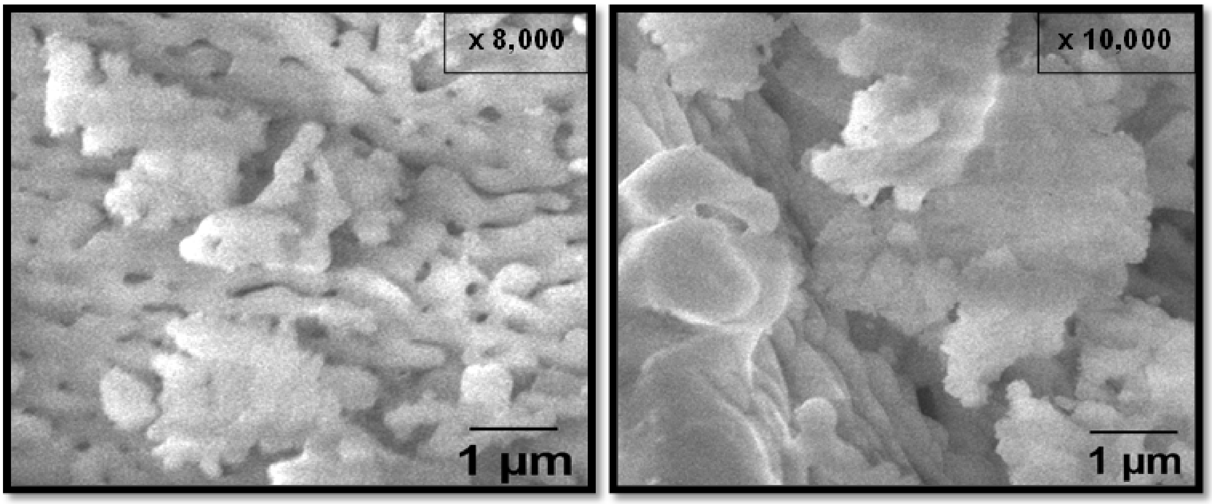

Figure 3: FESEM images of synthesized pure eggshell-derived HA powder at different magnifications.

Figure 4 illustrates the EDX results for HA powder and the evaluated atomic percentage of these elements is tabulated in Table 1. There is the presence of major constituents of HA which are calcium and phosphorus, together with a trace of carbon and oxygen elements. The same elemental composition was reported in the previous studies where the HA represents the appearance of Ca, P, O, and C (Goh et al., 2021). In Table 1, the Ca/P ratio for HA powder was found to be 1.76 and it shows a close resemblance with the HA for human bone as the Ca/P ratio for stoichiometric HA is known to be 1.67 (Mohammadi et al., 2021). A previous study reported that a high deviation in the Ca/P ratio can be due to the lack of proper heat distribution (Chen et al., 2019). Hence, in future studies, compaction into powder mixture pellets before calcination can be considered to ensure uniform heating of the samples during the structural transition process. In general, the analysis indicates that Ca and P are the predominant elements of the organic phases of HA powder.

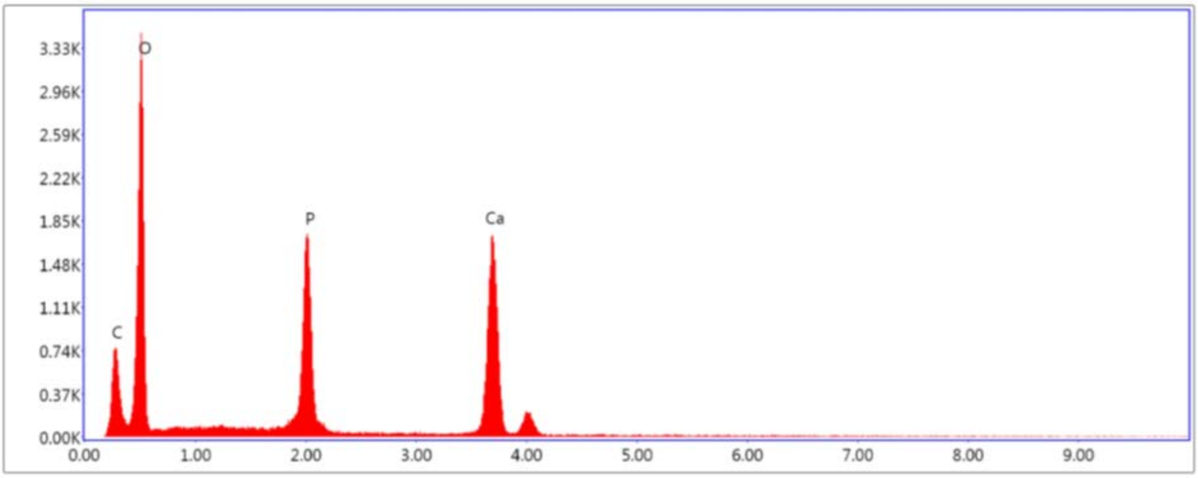

Figure 4: Elemental analysis of eggshell-derived HA powder

Table 1: Ca/P ratio for eggshell-derived HA powder

Element	Atomic %	Ca/P Ratio
C	5.6	1.76
O	62.4	
P	11.6	
Ca	20.4	
Total	100	

5 Conclusion

Pure HA was successfully produced by using calcium carbonate from waste chicken eggshells as a source of calcium precursor via a combination of magnetic stirring techniques and heat treatment. It was observed that 4 hours of magnetic stirring employed in mixing calcined eggshell with phosphorus precursor has sufficiently and successfully produced a homogeneous composition of HA with a small crystallite size after calcination. Other than that, the calcination at a temperature of 800 °C has revealed a highly crystalline pure HA phase with typical morphological globular-like shape HA particles. The average agglomerated HA particle size of 0.062 ± 0.02 μm was obtained in this work. Besides that, there is the presence of major constituents of HA which are Ca and P, with a Ca/P ratio of 1.76 which is close to the stoichiometric HA of 1.67. The results obtained via the simple solid-state reaction method could provide insight into the feasibility of using natural calcium carbonate sources from waste eggshells in the development of homegrown HA bioceramics.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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