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Calcium phosphate bioceramics synthesized from eggshell powders through a solid state reaction

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Abstract

Everyday millions of tons of eggshells are produced as biowaste around the world. Most of this waste is disposed of in landfills without any pretreatment. Eggshells in landfills produce odors and promote microbial growth as they biodegrade. The present invention provides an environmentally beneficial and cost-effective method of producing calcium phosphate bioceramics (hydroxyapatite or tricalcium phosphate) from eggshell waste. In this investigation, heat treatment produced solid state reactions between eggshell powders and dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD) or calcium pyrophosphate ($Ca_2P_2O_7$). When eggshell powders (CaO) and DCPD were heat treated at 1150 °C for 3 h, only a single hydroxyapatite (HA) phase was found; no diffraction peaks of starting materials and no β-TCP were observed. The XRD patterns of the product fabricated from raw eggshell powders ($CaCO_3$) and $Ca_2P_2O_7$ heat treated at 1100 °C for 3 h showed that almost only pure β-TCP remained with a trace amount of HA. The calcium phosphate ceramic synthesized from eggshell powders contains several important trace elements such as Na, Mg and Sr.

Keywords: D. Hydroxyapatite; Eggshell; β-tricalcium phosphate; Heat treatment; Solid state reaction

1. Introduction

Calcium phosphate bioceramics like hydroxyapatite (HA) and tricalcium phosphate (TCP) are good candidates for bone substitutes because HA and TCP have good biocompatibility and osteoconductive properties. Bioceramics have many applications, such as bone graft substitutes, sustained-release drug delivery devices, and protein purification [1]. Synthetic HA is a representative bone substitute because of its chemical similarities with the mineralized structure of natural bones [2]. Considering the numerous applications of HA in biomedical fields, development of various synthesis methods is a major issue now. Obviously, many researchers desire a simple cost-effective HA synthesis procedure.

A number of synthesis routes have been developed to prepare HA powders. Liu et al. [3] prepared HA ceramics using a sol–gel route with triethyl phosphite and calcium nitrate as phosphorus and calcium precursors, respectively. HA phase can be synthesized at 350 °C. Naruporn [4] adopted the wet-chemical precipitation by using low concentrations of the starting solution under a reflux environment to induce the formation of HA. A uniform grain size morphology with a small size distribution can be achieved by using this route, obtaining a smaller amount of as-dried powders compared to using high concentrations. Parhi et al. [5] reported the first example of a novel metathesis reaction performed in the presence of microwave irradiation. Nanocrystalline particles of HA could be synthesized by microwave treatment of calcium chloride and sodium phosphate in solid phase. Liu et al. [6] synthesized HA whiskers and crystals by hydrothermal treatment of $Ca(OH)_2$ and $CaHPO_4 \cdot 2H_2O$. They stated that

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pH value is a significant parameter variable in altering the morphology. Among the various synthesis routes, differences in the preparation routes resulted in a variety of morphology, stoichiometry, and level of crystallinity. However, lower synthesis temperature decreases the resultant HA crystallinity. Moreover, these methods might be time consuming, expensive, complicated or require pH adjustment and control.

Two major means of preparing HA powder are the wet method and the solid state reaction method. The disadvantages of the wet process are (1) the composition of the final product is greatly affected by even a slight difference in the reaction conditions and (2) about 20 days are needed to obtain HA of a stoichiometric composition, which is unacceptable for industrial-scale production. As a consequence, when producing high crystalline hydroxyapatite in mass, the dry process is more suitable than the wet process because it shows high reproducibility and is of low processing cost [7].

Recently, the processes involved in biomineralization have been greatly improved, leading to the development of biomimetic synthesis methods and the production of a new generation of biomaterials. HA powders derived from natural materials such as bovine bone [8], fish bone [9], oyster shell [10] or coral [11–13] inherit some properties of the raw materials such as pore structure and carbonate content. The hydrothermal transformation of corals into HA was first used by Roy and Linnehan in 1974 [11]. Sivakumar et al. [12] derived HA from Indian corals using a hydrothermal process. Hydrothermal conversion of Australian corals into HA was studied by Hu et al. [13]. However, corals are not available worldwide and some coral species are in danger of extinction. Therefore, exploring new alternative materials that are renewable, low-priced, and easily accessible is urgent.

Avian eggshell, with a mineral composition similar to those of corals, has been introduced as a potential bone substitute in maxillofacial and craniofacial surgery because avian eggshells can be easily acquired [14,15]. The eggshell represents 11% of the total weight of the egg and is composed of calcium carbonate (94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%) [16]. Though these are occasionally used as a fertilizer due to their high content of calcium and nitrogen, most of these are discarded as waste. Chicken eggshells contain trace elements, such as Na, Mg, and Sr, which are also found in human bone. The natural biological origin of eggshells results in HA with a crystalline structure and composition similar to that of human bone, with considerable benefits to overall physiological function following implantation [15,17]. Thus, eggshells could be a promising raw material for biomedical applications.

To the best of our knowledge, this is the first systematic study aimed at producing HA/TCP powders from raw materials derived from eggshells using a solid state reaction. In the present investigation, we developed a simple and inexpensive process with which to prepare single phase pure HA or pure TCP powders, or biphasic calcium phosphates (HA/TCP) through heat treatment in solid state. The eggshell powders were used as calcium precursors and combined with dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD) or calcium pyrophosphate ($Ca_2P_2O_7$) prior to heat treatment at various temperatures for various durations.

2. Materials and methods

The starting reactant materials were DCPD (Yakuri Chemicals Co., Ltd., Japan) or calcium pyrophosphate ($Ca_2P_2O_7$) and eggshell powders. Raw membrane-bound hen eggshell was collected from a breakfast shop on a university campus and immediately stored in a refrigerator. Its preliminary pretreatments included stripping the membrane off the eggshell, rinsing with water, drying, and then crushing and powdering using an agate mortar. The eggshell powders were sieved with a 325-mesh sieve. Additionally, calcium pyrophosphate was prepared by heat treating DCPD at 1000 °C for 3 h, and then sieved with a 325-mesh sieve before use.

The hand-ground eggshell powders and DCPD were homogeneously mixed with deionized water to form a slurry. The ratio of eggshell powders to DCPD was 4:3 (mole ratio), i.e. the stoichiometric Ca/P content in the composition of hydroxyapatite (Ca/P = 1.67). The slurry was then heat-treated at a rate of 10 °C/min and maintained at various temperatures (800, 900, 1000, 1100 or 1150 °C) for various durations (1, 3, or 5 h) in independent experiments. In one set of experiments, calcium pyrophosphate was used as a phosphate provider.

The morphological characteristics of the eggshell were examined by scanning electron microscopy (SEM; JSM-6700F, JEOL, Japan). The weight loss and thermal stability of the eggshell were also evaluated using differential thermal analysis (DTA; Q600 SDT, TA Inc., US) in a nitrogen atmosphere from room temperature to 1400 °C at a heating rate of 5 °C/min to determine the phase transition temperature. The crystalline phases of the synthesized powders were analyzed by powder X-ray diffraction with Cu Kα radiation (XRD; XRD-6000, Shimadzu, Japan). The phases were identified by comparing the experimental X-ray diffractograms to standards compiled by the Joint Committee on Powder Diffraction Standards (JCPDS). Microstructural observation was conducted using a SEM under secondary electron mode. In order to evaluate the composition of the synthesized powders and the presence of heavy metals, inductively coupled plasma-atomic emission spectrometry (ICP-AES; ICAP 9000, Jarrell-Ash Co., USA) analysis was performed.

3. Results and discussion

3.1. Characterization of eggshell samples

Eggshell has a three-layered structure consisting of an outer protein-rich cuticle layer, a spongeous middle layer, and an inner lamellar layer. Both the middle and inner layers form a matrix constituted by protein fibers bonded to calcium carbonate crystals [16,18]. The morphologies of the raw eggshell are shown in Fig. 1. A fiber-like morphology corresponding to the inner part of an eggshell was observed (Fig. 1a). The interwoven and coalescing nature of the shell membrane fibers was evident. Some intrinsic pore openings exist on the outer surface of the eggshell (Fig. 1b). After an initial cleaning process to prepare raw eggshell powders, the as-prepared eggshell powders were analyzed using TGA/DTA, as shown in Fig. 2. The weight loss due to physically absorbed water and organic matter that had been burnt off was approximately 4% below 450 °C. Also, there was a significant weight loss of about 36% from 640 °C to 810 °C and a sharp endothermic peak at 834 °C in DTA, which may have been due to the decomposition of $CaCO_3$ to CaO.

The results of DTA showed that there were thermal reactions, such as weight loss and an endothermal peak. According to these results, the eggshell powder was divided into three portions: (1) one portion (powder A) remained uncalcined; (2) the 2nd portion (powder B) was calcined at 450 °C for 2 h at a heating rate of 5 °C/min to decompose organic matter; and (3) the 3rd portion (powder C) was placed in an oven for a two-stage thermal treatment. The first stage consisted of heating the eggshell powder to 450 °C for 2 h at a heating rate of 5 °C/min, and the second stage consisted of heating the samples to 900 °C also for 2 h but with a heating rate of 2 °C/min. All the powders were sieved with a 325-mesh sieve before use.

As shown in Fig. 3, powders A and B showed $CaCO_3$ phases, and CaO was detected in powder C. In powder C, the $CaCO_3$ was completely decomposed and turned to CaO at about 900 °C [19]. The FESEM image of crushed eggshell demonstrates irregular particles with unevenly distributed particle sizes (Fig. 4a). Some particle sizes were measured in microns; other particle sizes were measured in nanometers. The morphology of the eggshell (powder B) calcined at 450 °C, prior to decomposition, was hard and agglomerated like liquid-phase sintered powder (Fig. 4b). This may have been caused by limited decomposition and the hydrophilic properties of the heated, reactive $CaCO_3$

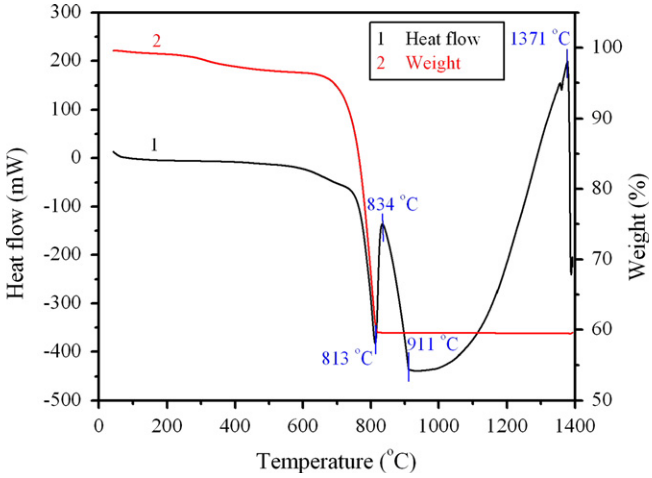

Fig. 2. TGA/DTA analysis of the eggshell powders.

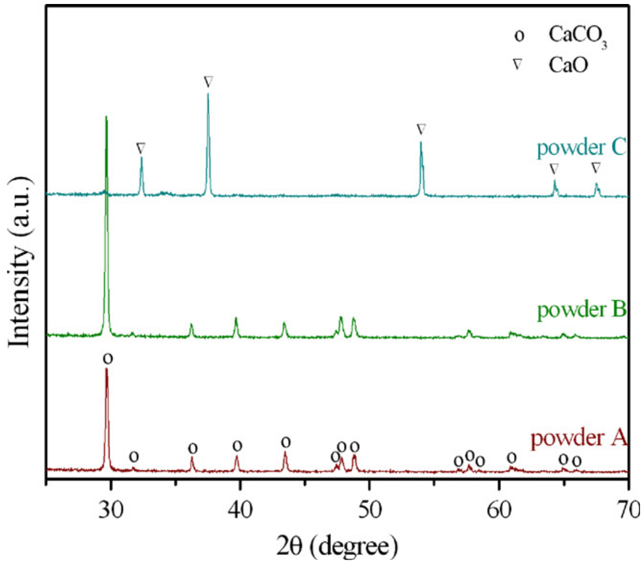

Fig. 3. XRD patterns of (a) raw eggshell powders (powder A), (b) eggshell powders calcined at 450 °C for 2 h (powder B), and (c) eggshell powders heated at 450 °C for 2 h and then heated at 900 °C for 2 h (powder C).

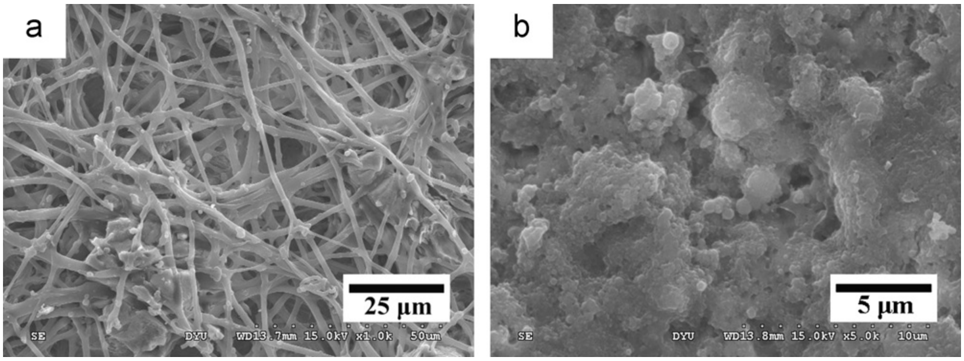

Fig. 1. SEM images of (a) the inner surface and (b) the outer surface of the eggshell.

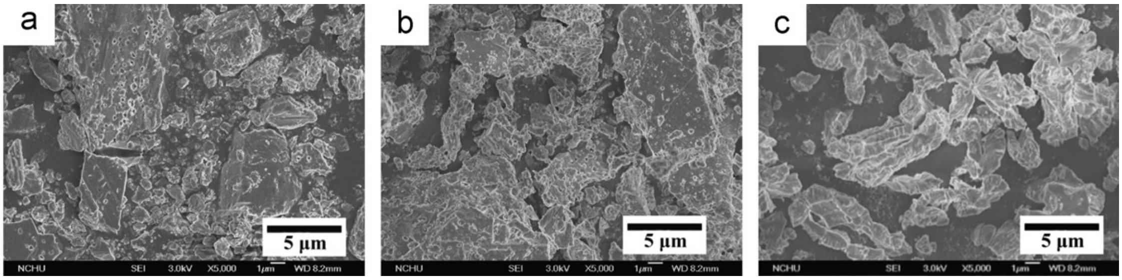

Fig. 4. SEM images of (a) raw eggshell powders (powder A), (b) eggshell powders calcined at 450 °C for 2 h (powder B), and (c) eggshell powders heated at 450 °C for 2 h and then heated at 900 °C for 2 h (powder C).

eggshell. When powder C was calcined at 900 °C, the particles were granular, like grapes, and of uniform size (Fig. 4c).

The XRD patterns of products prepared by heat treatment of DCPD and eggshell powders calcined at different temperatures (powders A–C) are shown in Fig. 5. After sintering at 1100 °C for 3 h, small peaks from starting materials (CaO and DCPD) were found in the products synthesized from powder A and DCPD (Fig. 5a), which indicated that a small amount of starting materials remained, although most starting materials reacted to produce HA and significant amounts of β-TCP. The reaction of powder B and DCPD at 1100 °C for 3 h (Fig. 5b) was more complete, although peaks from starting materials were still observed in the patterns. Additionally, the peaks of β-TCP were further reduced, indicating a gradual disappearance of β-TCP, which was replaced by HA. After powder C and DCPD were heated at 1100 °C for 3 h (Fig. 5c), the final product was mainly composed of HA crystals, but included a small quantity of β-TCP crystals; no peaks from the starting materials were found in the pattern. According to the above results, the synthesized products from powder C and DCPD powder can get calcium phosphate ceramics (HA/TCP) but without any starting materials. Accordingly, the powder C was selected as the starting reactant material for the study of the heat-treatment conditions discussed in the following sections.

Powders A–C were each combined with DCPD and heated at 1100 °C for 3 h to produce three products. SEM images of these three products are shown in Fig. 6. The particle sizes of the resultant powders appeared slightly smaller than the initial eggshell powder particle sizes. The morphologies of the synthesized powders became more rounded following heat treatment, and showed spheroidal shapes. After heat treatment, an agglomeration phenomenon was produced by high-temperature sintering.

3.2. *Effects of heat treatment temperature and time*

Fig. 7 shows the XRD patterns of products synthesized from powder C and DCPD heat-treated at different temperatures (800, 900, 1000, 1100 or 1150 °C) for 3 h. The products made at 800, 900 and 1000 °C showed significant CaO and DCPD peaks. Reactions at 1100 °C were more complete, and resulted in the formation of HA with a small quantity of β-TCP; there were almost no CaO and DCPD peaks in the pattern. The product synthesized at 1150 °C showed no diffraction peaks of β-TCP. The intensity ratio, β-TCP(0 2 10)/HA(2 1 1), also decreased with an increase in heat treatment temperature, indicating that HA phase was preferred over β-TCP. These results indicate that some quantity of β-TCP easily formed at the beginning of the reaction, but increasing quantities of HA formed from the starting materials with an increase in heat treatment temperature. The starting reactant materials were subsequently transformed into β-TCP by heat treatment because of their structural similarity [20]. However, HA is thermodynamically more stable than β-TCP [21], so after increasing the heat treatment temperature, relatively greater quantities of HA were produced.

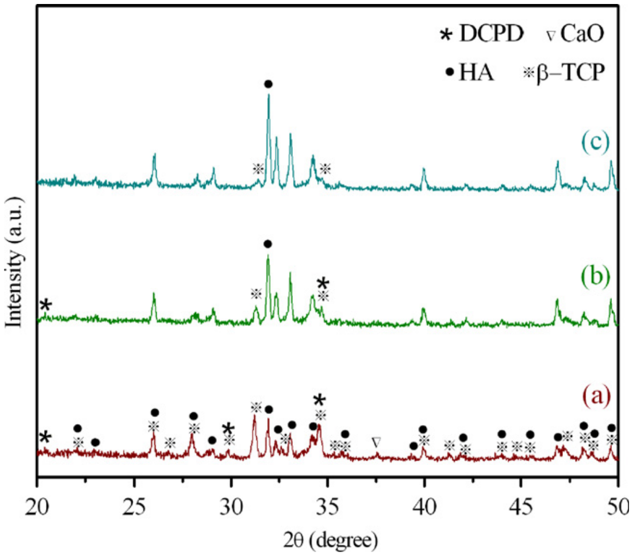

Fig. 5. XRD patterns of the products synthesized from (a) powder A and DCPD, (b) powder B and DCPD, and (c) powder C and DCPD after heat treatment at 1100 °C for 3 h.

Fig. 8 shows the XRD patterns of the products synthesized from CaO and DCPD at 1100 °C by heat treatment for various durations (1, 3, or 5 h). Following heat treatment for 1 h, β-TCP was the main phase and a small quantity of HA was observed in the pattern. Diffraction

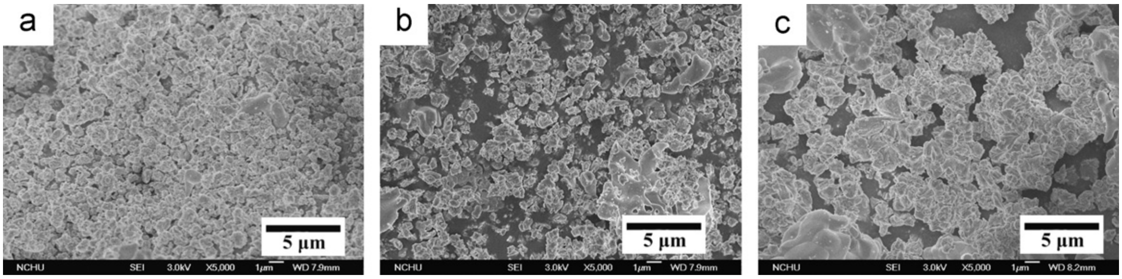

Fig. 6. SEM images of the products synthesized from (a) powder A and DCPD, (b) powder B and DCPD, and (c) powder C and DCPD after heat treatment at 1100 °C for 3 h.

lines of the starting materials were also detected at this stage. After reaction for 3 h, a large quantity of HA and a small quantity of β-TCP were observed. When the heat treatment was extended to 5 h, more HA was produced with almost no β-TCP phase. Also, the CaO and DCPD diffraction lines disappeared completely. As a result, with an increase in heat treatment duration, the quantity of starting materials decreased. The intensity of β-TCP also decreased with an increase in heat treatment duration, indicating that HA phase was preferred over β-TCP. Although we intended to produce pure HA or pure TCP from eggshell powders, some small amount of β-TCP in apatite phases would promote strong and fast bonding to natural bones by rapid resorption [22].

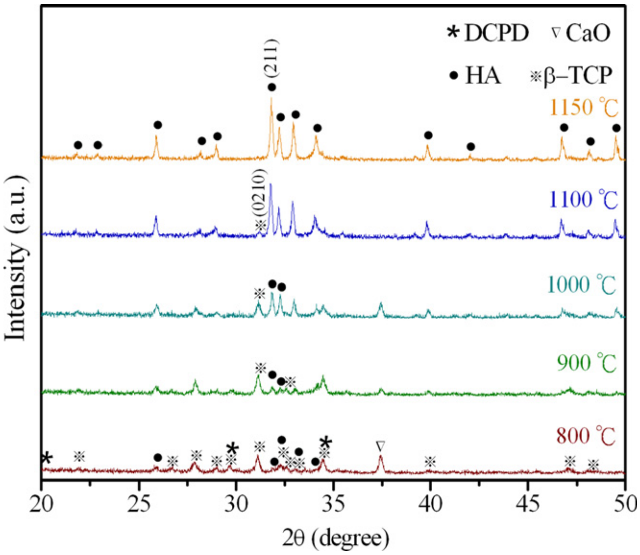

Fig. 7. XRD patterns of the products synthesized from powder C and DCPD heat-treated at various temperatures (800, 900, 1000, 1100, and 1150 °C) for 3 h.

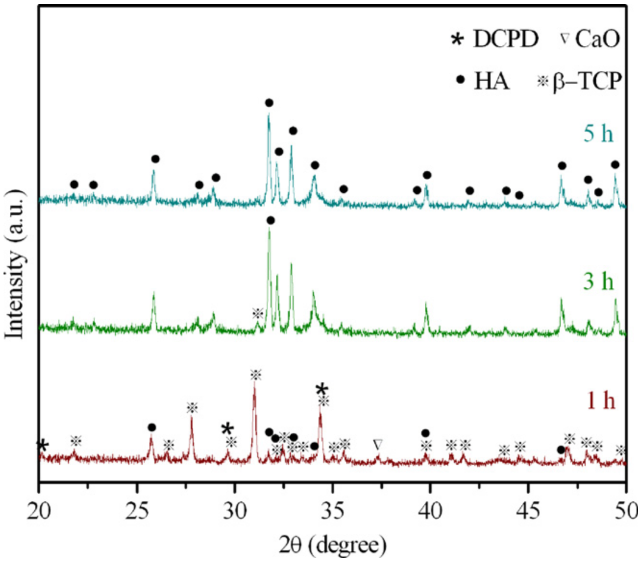

Fig. 8. XRD patterns of the products synthesized from powder C and DCPD heat-treated at 1100 °C for various durations (1, 3, and 5 h).

3.3. *Reaction of eggshell powders mixed with calcium pyrophosphate*

For comparison, we synthesized samples of eggshell powders mixed with $Ca_2P_2O_7$ by heat treatment at 1100 °C for 3 h. The XRD patterns (Fig. 9) of the product fabricated from powder A and $Ca_2P_2O_7$ showed that almost pure β-TCP remained with only a trace amount of HA. Under the same heat treatment conditions, however, the XRD patterns of the products of powder B or C mixed with $Ca_2P_2O_7$ showed a main phase of β-TCP with a significant quantity of HA.

The heat treatment of CaO from eggshells mixed with DCPD is proposed as a method to producing inexpensive pure HA powders. the following is suggested as the reaction describing the synthesis of HA [23]:

$$CaHPO_4 \cdot 2H_2O + 4CaO \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 14H_2O \quad (1)$$

Furthermore, according to Rhee [7], if there are x moles of water within the eggshell powders and $Ca_2P_2O_7$ during the heat treatment, the products of the reaction will be similar to those found in Eqs. (2) or (3), depending on the quantity of water. When $x \geq 1$ mol, the products of the reaction are only a single phase of HA, as follows:

$$Ca_2P_2O_7 + 4CaO + xH_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + (x-1)H_2O\uparrow \quad (2)$$

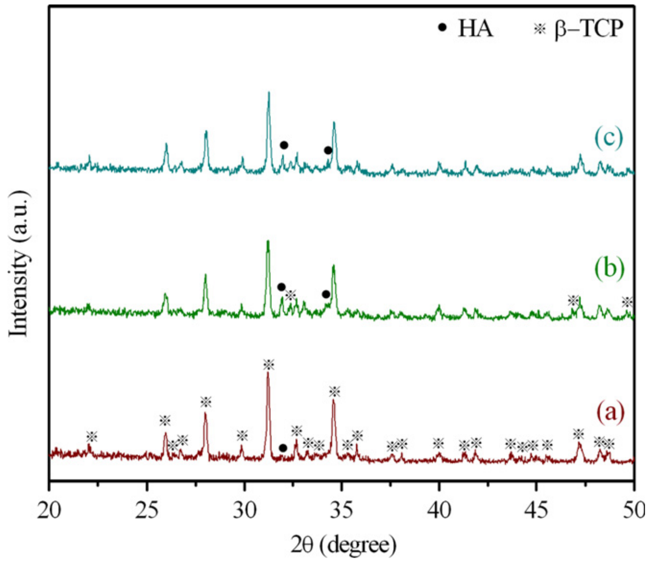

Fig. 9. XRD patterns of the products synthesized from (a) powder A and $Ca_2P_2O_7$, (b) powder B and $Ca_2P_2O_7$, and (c) powder C and $Ca_2P_2O_7$.

On the other hand, when $x < 1$ mol, the products of the reaction will be HA and TCP as follows:

$$Ca_2P_2O_7 + 4CaO + xH_2O \rightarrow xCa_{10}(PO_4)_6(OH)_2 + 3(1-x)Ca_3(PO_4)_2 \qquad (3)$$

Eq. (3) accurately describes our results for products that are synthesized from eggshell powders and $Ca_2P_2O_7$ through a solid state reaction.

Eggshell is a composite material consisting of a dominant component $CaCO_3$ (94%), together with calcium phosphate (1%), organic matter (4%) and minor quantities of other components [16]. The main component of the product obtained from powder C and DCPD after heat treatment at 1150 °C for 3 h was HA (Fig. 7). The measurements of elemental composition by the ICP-AES method showed the presence of Ca (40.2 wt%), P (14.1 wt%), Na (0.154 wt%), Mg (0.470 wt%) and Sr (0.234 wt%) in the synthesized HA, and an average Ca/P molar ratio of around 2.20. The presence of these ions is common in biological apatites. These elements play a significant role in the behavior of biological apatites, since they contribute to metabolism in the human body as well as cell adhesion. The molar Ca/P ratio of the synthesized HA was higher than the molar ratio of the stoichiometric HA; this can be attributed to the presence of carbonate ions that substituted for the phosphate, which indicates the presence of B-type carbonate HA [24]. Non-stoichiometric carbonated HA is the main mineral component of human and animal hard tissues [25]. Low CO_3^{2-} content can improve the bioactivity of HA [26].

Moreover, biological apatites have attracted particular interest because of the substitutions at the Ca^{2+}, PO_4^{3-} and OH^- sites of HA. The presence of several trace elements does not alter the basic crystallographic characteristics of HA, but can improve the overall biological performance of the implant material [27–30]. Ionic substitutions play an important role in bone formation. For example, strontium (Sr) could improve bone strength and provide benefits in the treatment of osteoporosis [31]. Sr-containing HA ceramics have exhibited mechanical properties better than those of pure HA, and have been shown to enhance the proliferation and differentiation of osteoblast cells in vitro [32]. Additionally, Na and Mg are known to be important trace elements in bone and teeth, and to play an important role in bone metabolism. Na and Mg depletion can cause bone fragility and bone loss [33,34]. Also, Mg plays a crucial role in cell proliferation and function [34].

4. Conclusions

The present study suggests the eggshell as a possible material-recycling technology for future waste management and ecology. Also, eggshell is a potential material for the synthesis of calcium phosphate ceramics for biomedical applications. Our results indicate the following important points:

1) The TGA/DTA analysis of eggshell powders shows that the weight loss due to physically absorbed water and burned organic matter was approximately 4% below 450 °C. There was a significant weight loss of about 36% from 640 °C to 810 °C and a sharp endothermic peak at 834 °C in DTA, which may have been due to the decomposition of $CaCO_3$ to CaO.
2) The product synthesized from powder C (CaO) and DCPD at 1150 °C for 3 h showed only a single HA phase. No diffraction peaks of starting materials or β-TCP were observed.
3) With an increase in heat treatment duration, the quantities of both starting materials and β-TCP decreased. When the heat treatment time was 5 h at 1100 °C, single-phase HA was produced from powder C (CaO) and DCPD, and diffraction lines of starting materials disappeared completely.
4) The XRD patterns of the product fabricated from powder A (raw eggshell powder) and $Ca_2P_2O_7$ at 1100 °C for 3 h showed that almost pure β-TCP remained with only a trace amount of HA. Under the same heat treatment conditions, however, the products of powder B (calcined eggshell powders that contained $CaCO_3$) or C (two-stage thermal treated eggshell powders that contained CaO) mixed with $Ca_2P_2O_7$ showed main phases of β-TCP with significant quantities of HA.
5) The elemental compositions evaluated by the ICP-AES method showed the presence of Ca (40.2 wt%), P (14.1 wt%), Na (0.154 wt%), Mg (0.470 wt%) and Sr (0.234 wt%) in the synthesized HA.

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