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Temperature Effect on Calcium Phosphate Synthesized from Chicken Eggshells and Ammonium Phosphate

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Abstract: Calcium phosphate ceramics such as hydroxyapatite (HAp, Ca_{10} $(PO_4)_6$ $(OH)_2$) and β-tricalcium phosphate (β-TCP, Ca_3 $(PO_4)_2$) are a high potential candidate for hard tissue implants in orthopedic and dental surgeries due to their chemical and structural similarity to bone minerals. Material that shows hydroxyapatite and β-tricalcium phosphate phases is called Biphasic Calcium Phosphate (BCP). In present study, biphasic calcium phosphate was prepared and characterized for future applications on the utilization of bioactivity of HAp and resorbability of β-TCP properties. It was simply synthesized by precipitation method using eggshell as the calcium source (Ca) in the form of calcium nitrate and ammonium phosphate as the phosphate source (P) to obtain biphasic calcium phosphate. The prepared BCP powders and crystal structure were characterized by X-Ray Diffraction (XRD), Rietveld refinements and Fourier transform infrared (FT-IR) techniques. The results indicate that BCP was observed at the calcining temperature of 700 °C and above. Furthermore, the crystallinity of BCP increases with increasing temperature from 700-1200°C. The phase fractions of β-TCP in the temperature range of 700-1200°C was found to be 10, 17, 18, 19, 22 and 23 wt.%, respectively. The formation of BCP may arise from its non-stoichiometric composition of materials or a variation of synthesis parameters.

Key words: Hydroxyapatite, tricalcium phosphate, biphasic calcium phosphate, precipitation method, chicken eggshell

INTRODUCTION

Calcium Phosphate (CP) ceramics have been widely recognized as a high potential candidate for hard tissue implants in orthopedic and dental surgeries due to their excellent biocompatibility and their close chemical similarity to the inorganic component of bones and teeth (Vallet-Regi and Gonzalez-Calbet, 2004; Hench, 1991). Among these materials, particular attention has been given to hydroxyapatite (HAp, Ca_{10} $(PO_4)_6$ $(OH)_2$) because of its bioactivity and osteoconductivity and to β-tricalcium phosphate (β-TCP, Ca_3 $(PO_4)_2$) because of its resorbability in physiological medium. To enable the utilization of these dominate properties, the Biphasic Calcium Phosphate (BCP) has been developed. Therefore, the porosity generation in a dense BCP upon contact with body fluids will induce in situ formation of biological apatite, improving bone reconstruction and regeneration (Vallet-Regi and Gonzalez-Calbet, 2004; Hench, 1991).

Various techniques have been developed to prepare calcium phosphate ceramics and are categorized into two methods of preparation; solid state reactions and wet methods. The wet methods can be divided into three groups: precipitation, hydrothermal technique and hydrolysis of other calcium phosphates. Of these methods, precipitation method scores over other processes due to better control of composition and physical characteristics of powders, low cost and easily applied to industrial production.

High purity reagents for synthesizing calcium phosphate powders are very costly, thus several researches have been developed to prepare them from natural materials such as bovine bone (Ozyein *et al.*, 2004; Joschek *et al.*, 2000; Liu *et al.*, 1999), fish bone (Ozawa and Suzuki, 2002) or coral (Sivakumar *et al.*, 1996). Although, they inherit some properties of the raw materials such as the pore structure, carbonated HAp etc, problems do arise due to the variability of physical and chemical properties of raw material (Vallet-Regi and Gonzalez-Calbet, 2004). The eggshell consists of calcium carbonate ($CaCO_3$, 94%), calcium phosphate (1%), organic matter (4%) and magnesium carbonate (1%) (Rivera *et al.*, 1999). Due to its high content of calcium, eggshell wastes have been selected as calcium source in order to avoid the

cost of high quality calcium source for preparing calcium phosphate and at the same time, help promoting recycle of material (Rivera *et al.*, 1999; Lee and Oh, 2003; Prabakaran *et al.*, 2005; Sasikumar and Vijayaraghavan, 2006; Lee *et al.*, 2007; Balazsi *et al.*, 2008; Prabakaran and Rajeswari, 2009; Gergely *et al.*, 2010).

In present study, BCP powders have been synthesized from eggshells by precipitation method. The synthesized powder was characterized by powder X-Ray Diffraction (XRD), Rietveld refinements and Fourier transform infrared analysis (FT-IR) techniques. The effect of temperature on its phase transformation was investigated.

MATERIALS AND METHOD

Sample preparation: The eggs were obtained from Charoen Pokphand Foods, (CPF) public company limited, Bangkok, Thailand. The membranes of the eggs were removed and washed with distilled water to remove adhesion. The shells were dry in air for a few days. Then, these shells were ground into powder using an agate mortar. Finally, these powders were heated at the temperature range from 200 to 1300°C for 4 h with a rate of 5°C min^{-1}.

Figure 1 shows the XRD patterns of eggshells after heating in the air at the temperature range of 900-1300°C.
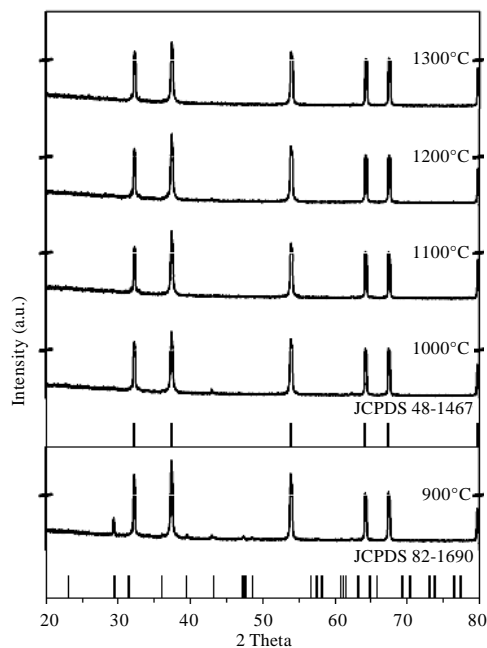

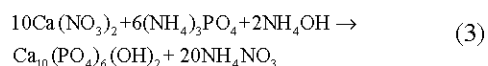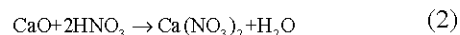Fig. 1: XRD pattern of eggshells after heating in the air at the temperature range of 900-1300°C

The crystal structure of natural eggshell was identified as $CaCO_3$ according to the JCPDS file no. 82-1690. The XRD patterns show that the eggshells were not completely transformed into calcium oxide (CaO) at the heating temperature from 700 up to 1000°C.

At the temperature of 1100°C and above, the eggshells become fragile and vary white in color. At this temperature range, the new phase of eggshell is calcium oxide (CaO) which can be indexed partially with lattice parameters of CaO (JCPDS file no. 48-1467); no other crystalline species was detected as seen in Fig. 1. It can be concluded from this fact that the eggshell ($CaCO_3$) completely transformed into CaO when it was heated at 1100°C and above for 4 h. The chemical reaction is given by an equation:

$$CaCO_3 \rightarrow CaO + CO_2 \qquad (1)$$
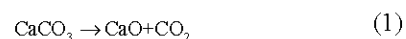
For certainty of the complete transformation of CaO, thus in present study the temperature of 1300°C for 4 h was selected in order to prepare CaO powders

Calcium phosphate preparation: Calcium Phosphate (CP) powders were prepared from eggshells by precipitation method. CaO obtained from the eggshells was mixed with HNO_3 to obtain $Ca(NO_3)_2$ solution. Then, it was precipitated with $(NH_4)_3PO_4$ solution and ammonia solution was used as agents for pH adjustment. The theoretical reaction for preparing the stoichiometric HAp is given by equations:

$$CaO + 2HNO_3 \rightarrow Ca(NO_3)_2 + H_2O \qquad (2)$$

$$10Ca(NO_3)_2 + 6(NH_4)_3PO_4 + 2NH_4OH \rightarrow$$
$$Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 \qquad (3)$$

CaO powders were completely dissolved in concentrated HNO_3 and was diluted in 300 mL of distilled water such that 1 molar $Ca(NO_3)_2$ solution was obtained. The solution was adjusted to pH 11 to 12 by the addition of NH_4OH and then diluted to 600 mL. Calcium solution, vigorously stirred for 4 h, was dropped slowly into a 0.6 molar $(NH_4)_3PO_4$ solution of in 500 mL of distilled water which similarly had been brought to pH 11 to 12 with NH_4OH and thereafter diluted to 800 mL. The reacted mixture, which was milkly, was under the pH control (pH 11-12) at the room temperature during synthesizing process. The reacted mixture was then heated at 100°C for half an hour and left overnight at room temperature to allow the precipitate to settle in the supernatant solution. The supernatant solution was separated from the precipitate by pouring and by using

the suction filter, with the application of a weak vacuum. The filtered cake was dried at 100°C for 20 h in an air oven and then broken down by grinding in a mortar. The powder was calcined at different temperatures in the ranges from 200 to 1200°C for 3 h by a rate of 5°C min^{-1}, whereas the cooling was performed as the furnace was left to cool down. Grinding again breaks down the powders if the powder agglomerated.

Calcium phosphate characterization: The crystal structures of calcined CP powders ware identified by powder X-Ray Diffractometer (XRD) (model- PW-1830). These analysis were carried out on powdered samples by a Philips diffractometer using monochromatized CuK$_\alpha$ radiation. The X-ray tube was operated at 30 kV and 25 mA. The CP powders were scanned in the 2 θ range of 10-60° with a scanning speed of 5° min^{-1}. Then the lattice parameters and atomic position were refined by the Rietveld method using FULL PROF SUITE-2000 software program. The function group of synthesized CP powders was obtained by FT–IR. All FT-IR were measured at room temperature cover the range from 400-4000 cm^{-1} using FT-IR spectrometer (Perkin Elmer model 2000) with KBr pellet technique.

RESULTS AND DISCUSSION

The identification of the crystalline phase of CP powders calcined at temperature range from 200 to 1200°C was carried out by X-ray diffraction and the diffraction patterns are shown in Fig. 2.

The major peak broadening of HAp phase of raw powder appear at (200), (111), (002), (102), (210), (211), (112), (300), (301), (212), (310), (222), (312), (213), (321), (410), (402) and (004) plans, respectively. There is no other crystalline species detected in the CP powders at temperature range from 200°C up to 600°C according to the JCPDS file no. 74-0566 for HAp which belongs to hexagonal symmetry and space group P6$_3$/m. Furthermore, it was also observed that intensity of HAp peak increases as the temperature increased. When heat-treated at 700°C, a biphasic; HAp and β-TCP nature of powder is present and the intensity of these peaks increases with increased temperature. The appearance of new peaks of β-TCP corresponds to the JCPDS file no. 70-2065 belongs to rhombohedral (R3c) symmetry. Some peaks of the β-TCP phase were corresponding to (104), (202), (300), (0210), (220) and (404) plans, respectively.

From the results above, the formation of BCP may arise from its non-stoichiometric composition of materials causing by chemical impurity, concentration of aqueous
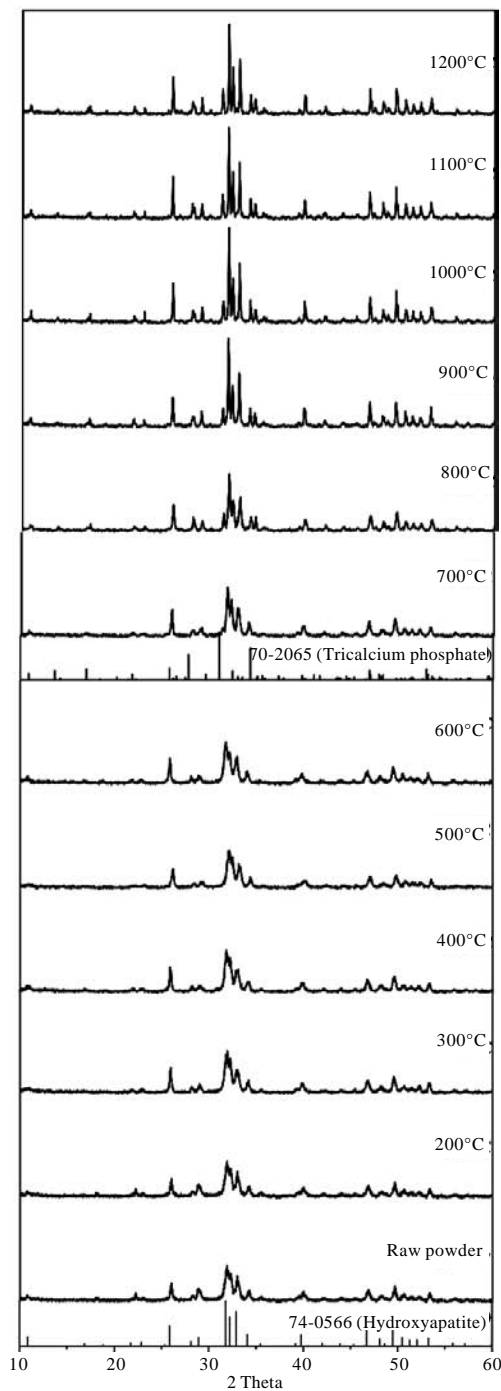

Fig. 2: X-ray diffraction patterns of CP powders heated at temperatures range from 200 to 1200°C

solutions, water adsorption or a variation of synthesis parameters such as stirring time, the pH throughout processing and the heat treatment of reacted mixture. The

Table 1: Relevant crystallographic data for CP powders, established from powder X-ray diffraction refinements

| Powder code | Phase | Phase fraction (wt.%) | Cell parameters (Å) | | | | |
			a	b	c	c/a	V(Å³)
Raw powder	HAp	100	9.4197	9.4197	6.8615	0.7284	1581.776
CP -200	HAp	100	9.4143	9.4143	6.8694	0.7297	1581.783
CP-300	HAp	100	9.4105	9.4105	6.8739	0.7305	1581.541
CP-400	HAp	100	9.4117	9.4117	6.8748	0.7304	1582.152
CP-500	HAp	100	9.407	9.4070	6.8728	0.7306	1580.112
CP-600	HAp	100	9.4076	9.4076	6.8728	0.7306	1580.314
CP-700	HAp	90	9.4146	9.4146	6.8755	0.7303	1583.288
	β-TCP	10	10.439	10.4390	37.3750	3.5832	10581.590
CP-800	HAp	83	9.4207	9.4207	6.8808	0.7304	1586.562
	β-TCP	17	10.3734	10.3734	37.2783	3.5936	10421.980
CP-900	HAp	82	9.4171	9.4171	6.8793	0.7305	1585.005
	β-TCP	18	10.3716	10.3716	37.2416	3.5907	10408.110
CP-1000	HAp	81	9.4202	9.4202	6.8806	0.7304	1586.348
	β-TCP	19	10.377	10.3770	37.3423	3.5986	10447.120
CP-1100	HAp	78	9.416	9.4160	6.8765	0.7303	1583.989
	β-TCP	22	10.3687	10.3687	37.3319	3.6004	10427.510
CP-1200	HAp	77	9.4178	9.4178	6.8796	0.7305	1585.309
	β-TCP	23	10.3786	10.3786	37.3563	3.5994	10454.260

sharper peaks as the results of heat-treated powders to higher temperatures indicate higher crystallinity of the structure.

In present study, the Rietveld refinement was used as the simplest tool to verify exact structure of CP and to quantify the phase fraction. Using this method, we analyzed the structural compositions of CP powders qualitatively and quantitatively by fitting the experimental powder XRD profiles with respect to corresponding structural parameters (i.e., lattice parameters, atomic coordinates) and instrumental parameters. A summary of the refined lattice parameters and phase fractions of CP powder upon calcination temperature range from 200 to 1200°C are reported in Table 1 corresponding to denoted powder code as CP-200, CP-300, CP-400, CP-500, CP-600, CP-700, CP-800, CP-900, CP-1000, CP-1100 and CP-1200, respectively. Figure 3 illustrates the final Rietveld refinement of powder data for the CP powders at temperature of 1200°C. The a-axis showed a large decrease at temperature up to 600°C, while the c-axis also increased at the same temperature range. As the calcination temperature was increased from 600 to 1200°C, lattice parameters showed a rather constant.

The c/a ratios of CP at the calcination temperature range from 200 to 1200°C are very similar to the standard phase (c/a = 0.7288) of HAp. It may be due to the low transformation reaction of HAp. The phase fractions of β-TCP in the temperature range of 700 - 1200 °C was found to be 10, 17, 18, 19, 22 and 23 wt.%, respectively.

Assignments of the observed vibrational frequencies and FTIR spectra of CP powders calcined at the different temperatures show phosphate, carbonate and hydroxyl vibrational bands, as summarized in Table 2 and Fig. 4, respectively. In the investigated temperature range, the formation of HAp is firstly indicated in the broad FT-IR
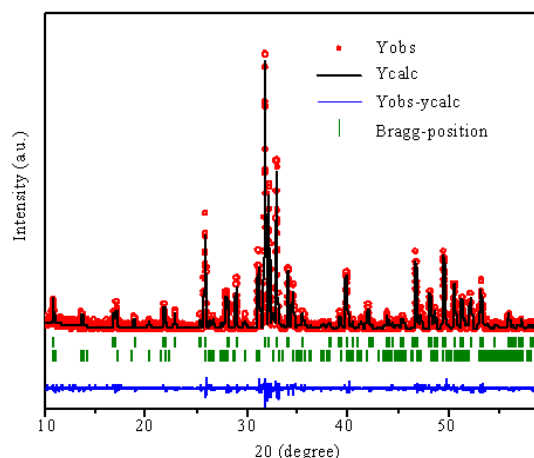

Fig. 3: X-ray powder diffraction profiles for Rietveld refinement pattern of CP at 1200°C.

band centered at about 1000-1100 cm^{-1} (Varma and Babu, 2005). The broad bands observed at about 962-963 cm^{-1} correspond to v_1 symmetric P-O stretching vibration of the phosphate ion (PO$_4^{3-}$) (Kawata *et al.*, 2004; Miyaji *et al.*, 2005). The band between 567 and 603 cm^{-1} belongs to v_4 vibration mode of PO$_4^{3-}$ group occupying two sites in the crystal lattice (Kawata *et al.*, 2004; Miyaji *et al.*, 2005). This distinguishable splitting of them indicates the low site symmetry of molecules, as two peaks confirm the presence of more than one distinctive site for phosphate group in HAp lattice (Rehman and Boneld, 1997).

For a major peak of PO$_4^{3-}$ group of HAp, the v_3 vibration peak is identified the band at 1042-1093 cm^{-1}, which is the most intensified peak among the phosphate vibration modes. The characteristic frequencies derived

Table 2: Assignments of the observed vibrational frequencies of CP heated at various temperatures

| Assignments	Observed vibrational frequencies (cm^{-1}) Calcination temperatures (°C)				
	Raw powder	600	700	800	1200
PO_4 bend v_4	571.70	567.11	571.31	572.08	571.19
PO4 bend v_4	602.67	602.83	601.73	602.14	602.02
Structural OH	632.37	632.38	631.75	632.01	630.66
CO_3^- group	878.67	878.79	878.21	878.57	861.81
PO_4 stretch v_1	962.39	962.91	962.77	963.02	962.79
PO_4 bend c_3	1042.42	1042.99	1044.21	1047.25	1047.04
PO_4 bend v^3	1092.27	1092.14	1090.21	1090.18	1089.78
CO_3- group (v_3)	1414.83	1414.77	1413.21	1421.17	1482.30
CO_3- group (v_3)	1456.62	1456.38	1455.97		
H_2O adsorbed (v_2)	1631.83	1631.65	1630.96	1631.06	1631.56
H_2O adsorbed	3434.59	3434.72	3434.70	3435.00	3441.56
Structural OH	3570.56	3571.94	3572.17	3572.31	3570.94
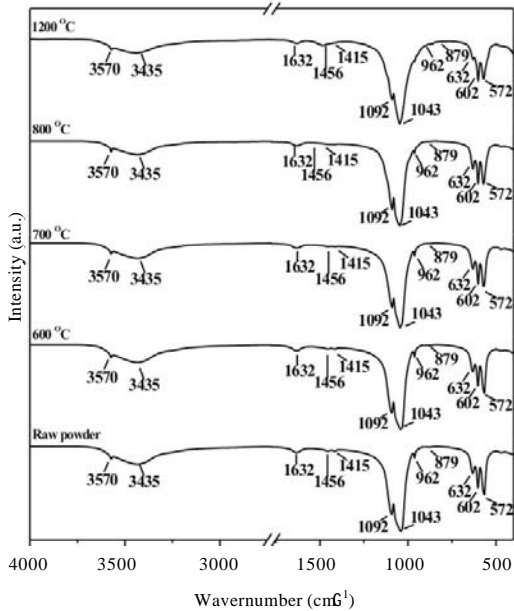
Fig. 4: FT-IR spectra of CP powders heated at various temperatures

from these PO_4^{3-} modes are better resolved with increasing of calcination temperature.

The spectral data indicates that the characteristic bands from inorganic carbonate ion (CO_4^{3-}) are present with B type carbonate substitution (Krajewski *et al.*, 2005). The peak in the region from 1413-1457 cm^{-1} is related to v_3 mode (Varma and Babu, 2005) and suggests that carbon from the organics does not pyrolyze completely and may instead dissolve into the HAp crystal, but these bands disappear at 800°C. The band at 878 cm^{-1} is the signature of v_2 of CO_3^{2-} group. At 800 and 1200°C, new peaks are present about 1421 and 1482 cm^{-1}, respectively which indicate CO_3^{2-} group of β-TCP. The broad bands observed at around 1630 and 3440 cm^{-1}

indicate water adsorption of the powders. The water band at around 3440 cm^{-1}, overlaps with the weak bands at around 3571 cm^{-1}.

Investigation results for all temperatures, show sharp and strong band assigned to the stretching modes of hydroxyl groups (-OH) at around 3572 and 632 cm^{-1} can be clearly observed in the spectra (Varma and Babu, 2005; Kawata *et al.*, 2004).

CONCLUSIONS

In present study, BCP that shows HAp and β-TCP phases can be successfully prepared by simple precipitation method using eggshell as the Ca source in the form of Ca $(NO_3)_2$ solution and $(NH_4)_3PO_4$ solution as P source and ammonia solution as agents for pH adjustment on our synthesis condition.

The results from XRD and Rietveld refinements indicate that the major phase is HAp and the formation of β-TCP is enhanced with calcination temperature of 700-1200°C. FT-IR studies showed all characteristic bands of HAp and presence of CO_3^{2-} bands of β-TCP at higher temperature. The formation of BCP may arise from its non-stoichiometric composition of materials causing by chemical impurity, concentration of aqueous solutions, water adsorption or a variation of synthesis parameters such as stirring time, the pH throughout processing and the heat treatment of reacted mixture.

Finally, eggshells are the potential material for application to synthesis of calcium phosphate ceramics. Calcium phosphates are biocompatible materials and one of the bioceramics fabricated using natural materials. Mass production may be possible in a low-cost and simple process.

ACKNOWLEDGMENTS

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