

## Effects of pH and temperature on synthesis of calcium phosphate derived from calcined eggshell

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In this study, calcined eggshells were combined with phosphoric acid solutions having various concentrations and pH levels in order to examine the hydroxyapatite (HA) or  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) synthesis behavior and their powder morphology change. While mixing the calcined eggshell powder and the solution at room temperature, a vigorous exothermic reaction was observed with the formation of crystalline phases of  $\text{Ca}_3(\text{PO}_4)_2$  and tetra-calcium phosphate ( $\text{Ca}_4\text{O}(\text{PO}_4)_2$ ). In the sample prepared from a 40 wt% phosphoric acid solution, HA was successfully synthesized at 800 °C while the 60 wt% solution showed  $\beta$ -TCP. The pH of the solution influenced the microstructure of the room temperature reactants and heat treated powders. The powder morphology was changed to rod-, plate- and spherical-shaped particles according to the solution pH. The samples synthesized at pH of 0.69 showed whisker-shaped particles after being heat treated at 800 °C.

**Key words:** Biomaterials, Powder processing, Calcium Phosphate, Eggshell, Solution pH.

### Introduction

A biomaterial can be defined as any material used to make devices to replace a part or fulfill a function of the body in a safe, reliable, economic, and physiologically acceptable manner. Tri-calcium phosphate (TCP) and hydroxyapatite (HA) have been widely studied as biocompatible materials [1]. Apatite is the major mineral in the composition of hard tissues, such as bone and dentin, in the human body. The bio-mineralization of apatite is usually a self-remodeling process guided by osteoblast cells and proteins. These materials utilize apatite mineralized on their surfaces as an interface to integrate spontaneously with living tissue [2-6]. Calcium phosphate-based ceramics typically have insufficient mechanical strength. For this reason, HA is used to prepare composite materials, such as HA coatings, for placement on titanium metal. This process has recently been studied to compensate for the inadequate biocompatibility of various materials [7-9]. For such applications, the phase crystallinity and morphology of calcium phosphate powders are more important.

Calcium phosphates recently have been synthesized using natural calcium sources. Natural materials such as tuna bones, cuttlefish bones, oyster shells, cockle shells, and eggshells have been used to improve biocompatibility in previous studies. In particular, eggshell

was transformed to a CaO powder using a simple heat treatment. Calcium phosphates such as TCP and HA have been synthesized through a soft solution process using eggshell [10-15]. Liu et al. [16] investigated the influence of pH and temperature on the morphology of HA synthesized by a hydrothermal method with  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{Ca}(\text{OH})_2$  powders. By using solutions with pH 6 and pH 9 it was possible to synthesize HA with a whisker type morphology by this method. It was also reported that when the pH value of the solution is increased, the crystallinity of the synthesized HA powders is changed.

In the present study, nano-sized CaO powder through calcined and wet-milled eggshells was used as a starting powder [14, 15]. The processed powders were reacted with two concentrations of phosphoric acid solutions under various pH conditions. The possibility of synthesizing calcium phosphate and the morphological change at various heating temperatures under various pH were studied.

### Experimental Procedure

Washed raw eggshells were calcined in an air atmosphere at 900 °C for 4 hrs. The calcined eggshells were wet ball-milled to break down agglomeration. The wet milling process was performed using an isopropyl alcohol solvent (IPA, 99.5%, Daejung Chemicals & Metals CO., LTD, Korea) and zirconia ball media for 24 hrs. The volume ratio of calcined eggshell to IPA was 1 : 1. The milled powders were dried at 80 °C for 48 hrs. The main reaction for obtaining HA is induced

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**Table 1.** Mixing composition of solutions.

	40 wt% solutions				60 wt% solutions			
pH of solution	0.07	0.5	2.68	5.5	0.05	0.09	0.12	0.69
H <sub>3</sub> PO <sub>4</sub> (wt%)	40	37.95	36.10	32.89	60	58.25	56.60	53.57
NH <sub>3</sub> (wt%)	–	5.13	9.76	17.78	–	2.92	5.67	10.72
H <sub>2</sub> O (wt%)	60	56.92	54.14	49.33	40	38.83	37.73	35.71

by combining the processed powders with phosphoric acid solutions.

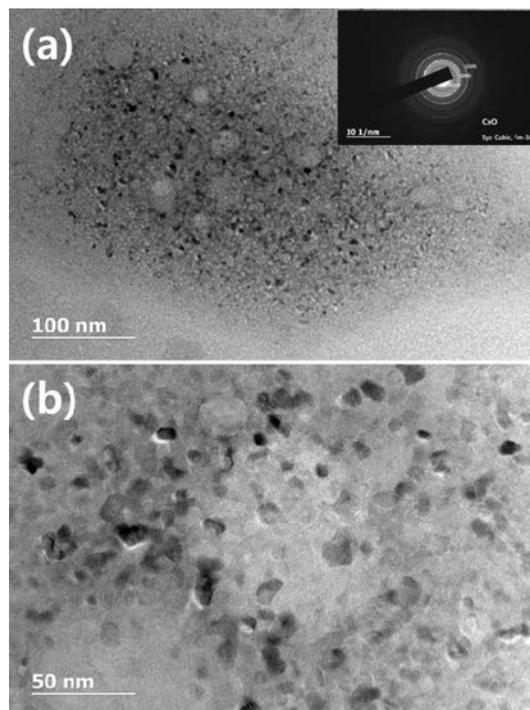
Two different acid solution concentrations were used: 40 wt% and 60 wt%. The phosphoric acid solutions were made by mixing D.I. water with a phosphoric acid reagent (85 wt% in H<sub>2</sub>O, DC Chemical Co. Ltd., Korea) and ammonia solution reagent (29% NH<sub>3</sub> in H<sub>2</sub>O, Junsei, Japan) for calibrating the pH. The solutions were calculated with consideration of the amount of water inside the reagent solutions. All of the prepared solutions and their pH values are listed in Table 1. The prepared phosphoric acid solutions were manually mixed with the processed powders at a 1 : 1.5 weight ratio. After mixing, all samples were completely dried at room temperature, and then the powders were subjected to heat treatment at temperatures ranging from 400 °C to 800 °C with a heating rate of 3 °C /min and a holding time of 4 hrs.

The development of crystalline phases in the as-synthesized powders was determined using X-ray diffraction (XRD, X'pert-pro MPD, PANalytical, Netherlands). The morphological characteristics were determined using field emission scanning electron microscopy (FE-SEM, S-4800, Hitachi, Japan) and transmission electron microscopy (TEM, JEM-2100F Jeol). The pH of the solutions was measured by a pH meter (pH Benchtop Meter, Orion 2-star, Electrode : 8157BNUMD, USA).

## Results and Discussion

TEM micrographs and the SAD pattern of the calcined, ball-milled eggshell powders are displayed in Fig. 1. The calcined ball-milled powders displayed a particle size of approximately 20 nm. The SAD pattern showed a ring pattern for the CaO, which further validates the nano-sized character of the powder. The TEM analysis showed the presence of an amorphous hydroxide around the powder. This is speculated to be the result of a reaction with the moisture in the air.

At room temperature, during mixing of the phosphoric acid solution and the processed powder, an exothermic reaction was observed, due to the high specific surface area of the powders. In the case of the solutions with the pH changed by ammonia solution calibration, the reaction did not start upon contact, but



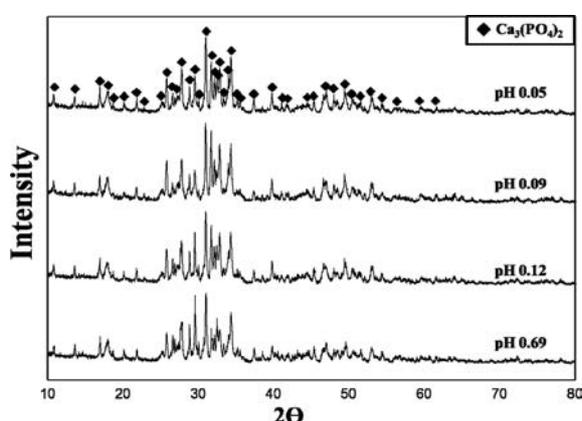
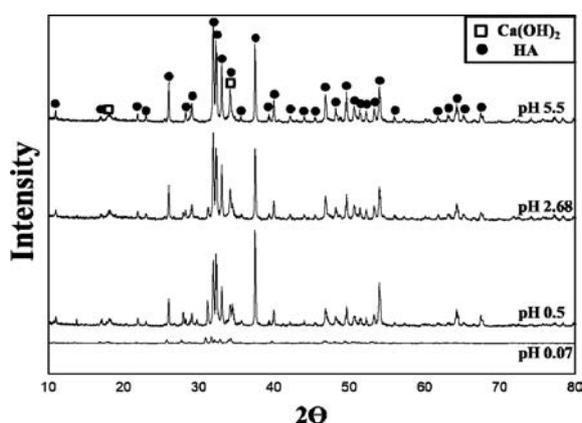
**Fig. 1.** TEM micrographs and SAD pattern for the calcined, ball-milled eggshell powders. (b) is an enlarged image of (a). CaO is indicated by the black spots in the image.

after 1 ~ 3 minutes, a much stronger exothermic reaction was observed. The exothermic reaction became stronger with the increased add ammonia. It is speculated that the ammonia ions react with the powders to generate Ca(OH)<sub>2</sub> and that the hydroxide reacts with the phosphoric ions in the solution, thus leading to a more volatile reaction in the precursors.

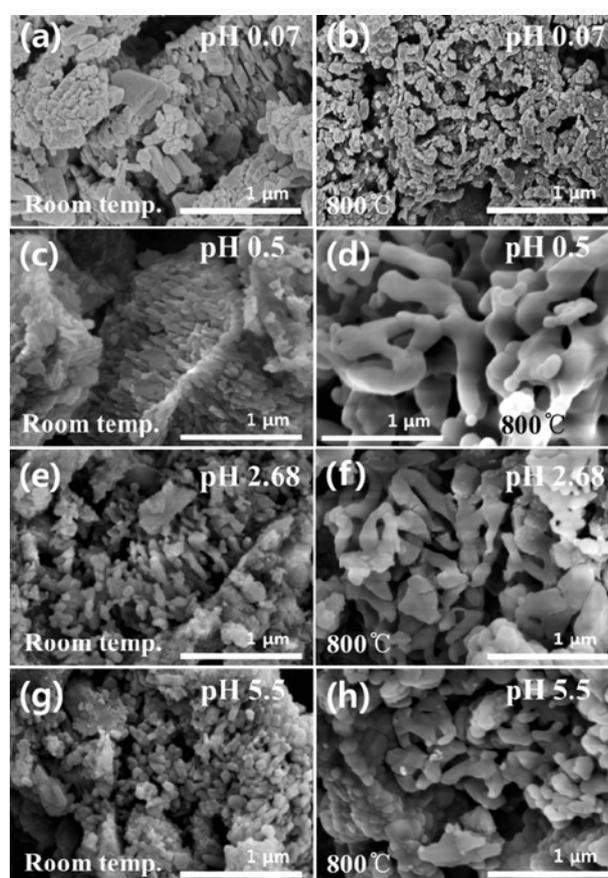
The XRD patterns of the room temperature reactants and the samples heat treated at various temperatures are shown in Table 2. The main crystal phase obtained room temperature reactants of the 40 wt% solution samples was CaHPO<sub>4</sub>, while in the case of the 60 wt% solution samples the main phase was Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> except for the sample with 0.69 pH. These differences in crystal phase are ascribed to the heat emanated during the exothermic reaction. The samples also displayed small amorphous peaks. It is speculated that in the case of the sample with the 0.69 pH phosphoric acid solution, the formation of the main crystal phase,

**Table 2.** Crystal phases detected through XRD Analysis.

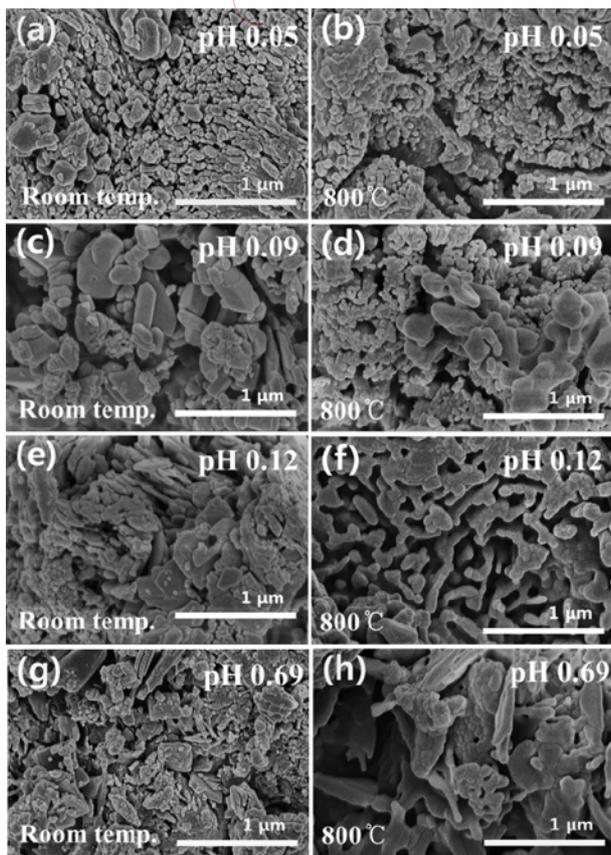
pH of the sample	40 wt% solutions				60 wt% solutions				
	0.07	0.5	2.68	5.5	0.05	0.09	0.12	0.69	
Room temperature	Main	CaHPO <sub>4</sub>	CaHPO <sub>4</sub>	CaHPO <sub>4</sub>	CaHPO <sub>4</sub>	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	Ca <sub>4</sub> O(PO <sub>4</sub> ) <sub>2</sub>
	Minor	Ca(OH) <sub>2</sub>							
400 °C	Main	Ca <sub>4</sub> O(PO <sub>4</sub> ) <sub>2</sub>							
	Minor	Ca(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>	–	–	–	–
800 °C	Main	HA	HA	HA	HA	β-TCP	β-TCP	β-TCP	β-TCP
	Minor	Ca(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>	–	–	–	–

**Fig. 2.** XRD patterns of samples reacted with 60 wt% solutions and heat treated at 800 °C.**Fig. 3.** XRD patterns of samples reacted with 40 wt% solutions and heat treated at 800 °C.

Ca<sub>4</sub>O(PO<sub>4</sub>)<sub>2</sub>, occurs because of the high temperature generated during the exothermic reaction. After heat treatment at 400 °C, all samples displayed the Ca<sub>4</sub>O(PO<sub>4</sub>)<sub>2</sub> phase. The samples synthesized using the 40 wt% solutions showed differences in crystallinity according to pH, based on the amount of ammonia solution that is added. The XRD patterns for the samples reacted with 40 wt% and 60 wt% solutions are

**Fig. 4.** SEM images of sample powders prepared from 40 wt% solution at each pH; (a), (c), (e) and (g) are the room temperature reactants; (b), (d), (f) and (h) are samples heat treated at 800 °C.

displayed in Figs. 2 and 3. By comparing the XRD patterns of the synthesized powders heat treated at 800 °C, the samples reacted with the 60 wt% solution showed similar patterns. Meanwhile, the samples reacted with the 40 wt% solution showed intensity variation of Ca(OH)<sub>2</sub> at each pH. It is speculated that in the case of the 40 wt% solution, the ammonia solution has a more intense reaction with the powder and this



**Fig. 5.** SEM images of sample powders prepared from 60 wt% solution at each pH ; (a), (c), (e) and (g) are the room temperature reactants; (b), (d), (f) and (h) are samples heat treated at 800 °C.

results in a higher amount of  $\text{Ca(OH)}_2$ , leading to higher peak intensities directly proportionate with the amount of added ammonia.

The samples reacted with 60 wt% solution displayed  $\beta$ -TCP at 800 °C. According to another study, tetracalcium phosphate ( $\text{Ca}_4\text{O(PO}_4)_2$ ) is the basic material of calcium phosphate materials and it displays a structural relationship with HA, meaning it can be transformed into HA through the acid-base reaction between other calcium phosphate system materials [17]. K. Deepak et al. [18] studied the decomposition of HA to TCP and DCP ( $\text{Ca}_2\text{P}_2\text{O}_7$ ), and they concluded that the reaction occurs above 1000 °C [18]. Theoretically, the Ca/P ratio of HA and  $\beta$ -TCP is 1.67 and 1.5, respectively. It is speculated that a lack of P ions (Ca/P ratio of samples prepared from 40 wt% solution: 3.41, 40 wt% solution: 2.28) leads to the generation of the unstable intermediate  $\beta$ -TCP crystal phase.

The powder microstructures of the samples reacted at room temperature and the samples heat treated at 800 °C are shown in Figs. 4 and 5. All the sample powders reacted at room temperature showed rod and plate type particles. The samples prepared from the 40 wt% solution showed intermediate particle forms between rod and spherical shaped particles (Fig. 4), while the samples prepared from the 60 wt% solution

displayed flake and plate type particles (Fig. 5). In particular, in the case of the samples prepared from the 40 wt% solution and heat treated at 800 °C without changing the pH value by ammonia addition (Fig. 4(b)), the particles did not show grain growth but rather a decrease in particle size. However, in the case of the samples with ammonia addition and heat treated at 800 °C, all samples displayed grain growth (Figs. 4(d-h)). The samples with ammonia addition showed a particle shape transformation into larger and irregular shaped particles according to heat treatment, and this tendency was stronger in the case of the 0.5 pH sample (Figs. 4(c, d)).

In comparison with the results obtained from the 40 wt% solution, the samples prepared from the 60 wt% solution showed less grain growth after heat treatment. The sample powder prepared under higher pH values resulted in particle transformation into whisker-type particles at high temperature (Fig. 5(h)). On the other hand, J. Liu et al. [16] obtained whisker-shaped particles by using the hydrothermal method, with heating to 140 °C for 24 hrs and varying the pH from 6 to 9. These results indicate that the amount of ammonia in the calcium phosphate synthesis has a significant influence on the morphology and the particle sizes.

## Conclusions

The nano-sized CaO powder derived from calcined eggshell was transformed to several-types of calcium phosphate crystalline phases under room temperature reaction with phosphoric acid solution at various pH conditions. After heat treatment at 400 °C, all samples displayed  $\text{Ca}_4\text{O(PO}_4)_2$  phase. The samples prepared from the 40 wt% phosphoric acid solution showed the HA phase at 800 °C, whereas in the case of the 60 wt% solution, the  $\beta$ -TCP phase was observed at 800 °C. It is speculated that this is due to the influence of a higher concentration of  $\text{Ca(OH)}_2$  in the sample prepared from the 40 wt% solution. The amount of ammonia ions influenced the powder morphology of the room temperature reactants and the heat treated samples. The grain growth with heat treatment was also affected by the ammonia amount. An increase in the concentration of phosphate ions led to particle transformation into whisker-shaped particles after heat treatment at 800 °C. It was confirmed that it is possible to change the powder morphology and size by controlling the pH levels and heating temperature.

## References

1. J. Park and R. S. Lakes, "Biomaterials: An Introduction, 3<sup>rd</sup>" (Springer, 2007) p. 371-377.
2. T. Kokubo, *J. Ceram. Soc. Jpn.* 99[10] (1991) 965-973.
3. T. Kokubo, "Bone-Bonding Materials" (Reed Healthcare Communications, 1992) p. 31-46.

4. L.L. Hench, *J. Am. Ceram. Soc.* 74[7] (1991) 1487-1510.
5. H.M. Kim, *J. Ceram. Soc. Jpn.* 109[1268] (2001) 49-57.
6. C. Ohtsuki, H. Kushitani, T. Kokubo, S. Kotani and T. Yamamuro, *J. Biomed. Mater. Res.* 25[11] (1991) 1363-1370.
7. H. Monma, T. Kamiya, M. Tsutsumi and Y.T. Hasegawa, *Gypsum & Lime* 208[3] (1987) 127-135.
8. M. Akao, N. Miura and H. Aoki, *J. Ceram. Soc. Jpn.* 92[11] (1984) 672-674.
9. M. Akao, H. Aoki and K. Kato, *J. Mater. Sci.* 16[3] (1981) 809-812.
10. I.J. Macha, L.S. Ozyegin, J. Chou, R. Samur, F.N. Oktar and B. Ben-Nissan, *J. Aust. Ceram. Soc.* 49 (2013) 122-128.
11. H. Onoda, M. Ichimura and A. Takenaka, *Phosphorus Res. Bull.* 24 (2010) 49-53.
12. H. Onoda and H. Nakanishi, *Nat. Resources* 3[2] (2012) 71.
13. M. D. Kwon, S. H. Oh and S. J. Lee, *J. Kor. Ceram. Soc.* 39[11] (2002) 1103-1107.
14. S. J. Lee, Y. S. Yoon, M. H. Lee and N. S. Oh, *Mater. Lett.* 61[6] (2007) 1279-1282.
15. S.J. Lee, Y.C. Lee and Y.S. Yoon, *J. Ceram. Proc. Res.* 8[6] (2007) 427-430.
16. J. Liu, X. Ye, H. Wang, M. Zhu, B. Wang and H. Yan, *Ceram. Int.* 29[6] (2003) 629-633.
17. K.E. Watson, K.S. Tenhuisen and P.W. Brown, *J. Mater. Sci: Mater. Med.* 10[4] (1999) 205-213.
18. D.K. Pattanayak, P. Divya, S. Upadhyay, R.C. Prasad, B.T. Rao and T.R.R. Mohan, *Trends Biomater. Artif. Organs* 18[2] (2005) 87-92.