

Article

Effect of Calcium and Phosphate on Compositional Conversion from Dicalcium Hydrogen Phosphate Dihydrate Blocks to Octacalcium Phosphate Blocks

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Abstract: Octacalcium phosphate (OCP) has attracted much attention as an artificial bone substitute because of its excellent osteoconductive and bone replacement properties. Although numerous studies have investigated OCP powder fabrication, there are only a few studies on OCP block fabrication. Therefore, in this study, the feasibility of optimizing dicalcium hydrogen phosphate dihydrate (DCPD) blocks, as a precursor for OCP block fabrication, under a pH 6 adjusted acetate buffer solution at 70 °C for 2 days was investigated. When a DCPD block was immersed in acetate buffer, the block was partially converted to OCP, with a large amount of dicalcium hydrogen phosphate anhydrate (DCPA), and its macroscopic structure was maintained. When the DCPD block was immersed in a Ca-containing solution, it was converted to mainly hydroxyapatite (HAp) with DCPA. On the other hand, when the DCPD block was immersed in a PO₄-containing solution, the block was converted to OCP, and its macroscopic structure was maintained. In other words, the PO₄-induced calcium phosphate with a Ca/P molar ratio lower than 1.0 may represent an intermediate phase during the compositional transformation from a DCPD block to an OCP block through the dissolution–precipitation reaction.

Keywords: octacalcium phosphate (OCP); dicalcium hydrogen phosphate dihydrate (DCPD); dissolution-precipitation reaction; non-stoichiometric treatment; bone substitute; bioceramics

1. Introduction

Octacalcium phosphate (OCP: Ca₈(HPO₄)₂(PO₄)₄·5H₂O) has attracted much attention as an artificial bone substitute because of its excellent properties [1–4]. When a bone defect was reconstructed with OCP granules, OCP exhibited much higher osteoconductivity and bone replacing ratio than those of hydroxyapatite (HAp: Ca₁₀(PO₄)₆(OH)₂) and β-tricalcium phosphate (β-TCP: Ca₃(PO₄)₂), which are the typical artificial bone substitutes [1]. Note that OCP is replaced by the bone faster than β-TCP [3]. Although the reasons for the superior properties of OCP as an artificial bone substitute are not fully understood, being a precursor phase to the bone may be one of the reasons. Despite the usefulness of OCP, it is not used as a clinical bone substitute. One of the reasons for this is the absence of OCP blocks. In other words, OCP is only available in the powder form, at the maximum ranging sub-mm in size by simple precipitation method [5–7].

Recently, we successfully established that OCP blocks could be fabricated through a dissolution–precipitation reaction from a calcium sulphate hemihydrate (CSH: CaSO₄·1/2H₂O) block used as a precursor in a PO₄-containing solution [8,9]. However, little is known about the fabrication of OCP blocks using a precursor through a dissolution–precipitation reaction. For example, only CSH

blocks have been found to be a useful precursor. Therefore, another ceramic block is evaluated as a precursor for OCP block fabrication. However, residual SO_4 might be a serious challenge from the viewpoint of chemically pure OCP block fabrication.

The dicalcium hydrogen phosphate dihydrate (DCPD: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) block was evaluated as a precursor of OCP. DCPD contains both Ca and PO_4 , whereas CSH contains only Ca. Therefore, a feasibility study of the DCPD block can be regarded as an initial step towards the feasibility study of calcium phosphate as a precursor for OCP block fabrication. Furthermore, unlike CSH, residual ions—in this case, SO_4 —could be avoided when a DCPD block is used as the precursor.

When the CSH block is used as a precursor, it is clear that a PO_4 -containing solution should be used in the dissolution–precipitation reaction for OCP block fabrication, because CSH contains only Ca for OCP. In contrast, when the DCPD block, which contains both Ca and PO_4 , is used as a precursor for OCP block fabrication, there may be no need to supply any Ca or PO_4 from the solution, which seems to supply Ca because of stoichiometry of DCPD ($\text{Ca}/\text{P} = 1.00$).

In this study, therefore, the effect of Ca and PO_4 in the solution on the compositional transformation from the DCPD block to the OCP block through a dissolution–precipitation reaction was investigated using a Ca- and PO_4 -free weakly acidic buffer solution as the control.

2. Materials and Methods

2.1. Fabrication of Dicalcium Hydrogen Phosphate Dihydrate Blocks

DCPD blocks were fabricated by setting the reaction of α -tricalcium phosphate (α -TCP: $\text{Ca}_3(\text{PO}_4)_2$, α -TCP-B, Taihei Chemical Inc., Osaka, Japan) and H_3PO_4 (Wako Pure Chemical Co., Osaka, Japan). Approximately 0.15 g of α -TCP powder was placed in a plastic separated-type mold ($\phi 6 \text{ mm} \times 3 \text{ mm}$); onto the α -TCP powder was dropped 0.15 mL of 2.0 mol/L H_3PO_4 , and the mixture was left standing for 30 min to stimulate the following chemical Reaction (1):



Then, the reaction product was eventually dried at room temperature. The set α -TCP powder is called DCPD block for convenience (see Results).

2.2. Compositional Transformation from the Dicalcium Hydrogen Phosphate Dihydrate Block to an Octacalcium Phosphate Block through Dissolution–Precipitation

All the chemicals were purchased from Wako Pure Chemical Inc., Osaka, Japan. Three mother solutions, namely a 1.0-mol/L acetate buffer solution, a 1.0-mol/L phosphate solution, and a 1.0-mol/L Ca solution, were prepared; the pH values of these solutions were adjusted to 6.0 at 70 °C. To prepare the immersing solution for the reaction, we mixed these mother solutions according to the solution recipe.

An acetate buffer solution (1.0-mol/L acetate) with pH 6.0 at 70 °C as a mother solution was prepared from CH_3COOH and NaOH . A phosphate solution was prepared by mixing NaH_2PO_4 and Na_2HPO_4 so that the pH of the solution would be 6.0 at 70 °C. A Ca solution was made by mixing CaCl_2 and NaOH in such a way that the pH of the solution would be 6.0 at 70 °C. All phosphate and Ca solutions used for immersion contained 0.2 mol/L of acetate buffer solution for pH stabilization, with phosphate or Ca under a weak acidic condition. Therefore, the initial pH of all solutions for immersion was adjusted to 6.0 at 70 °C.

Eight DCPD blocks were immersed in 15 mL of the solutions at 70 °C for 2 days. The final pHs of the solutions were recorded with a pH electrode (LAQUA ToupH 9615S-10D with pH meter D-72, Horiba Co., Kyoto, Japan). The treated samples were washed in distilled water several times to remove the residual solutions, then placed in a dry oven at 37 °C for one day for drying.

2.3. Characterization and Mechanical Properties of Dicalcium Hydrogen Phosphate Dihydrate and Octacalcium Phosphate Blocks

For XRD analysis, the blocks were ground into a fine powder by an agate mortar and pestle. XRD patterns were recorded with a diffractometer (40 kV and 40 mA: D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany; and 40 kV and 15 mA: MiniFlex600, Rigaku Co., Tokyo, Japan) using monochromatized X-rays ($\text{CuK}\alpha$: $\lambda = 0.1542 \text{ nm}$). The diffraction angle was continuously scanned for 2θ values ranging from 3° to 70° at a scanning rate of $2^\circ/\text{min}$.

The integral strength of intensities of each peak (I_x : $x = \text{phase}$) in the obtained XRD patterns of the samples were calculated by quantitative analysis with the software PDXL2 (Rigaku Co., Tokyo, Japan). The relative rate of the yield of calcium phosphate in each sample was calculated by the $d_{(100)}$ of OCP at $\sim 4.7^\circ$, $d_{(100)}$ of hydroxyapatite [HAp: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] at $\sim 10.5^\circ$, $d_{(100)}$ of DCPD at $\sim 11.6^\circ$, and $d_{(120)}$ of dicalcium hydrogen phosphate anhydrate [DCPA: CaHPO_4] at $\sim 30.2^\circ$. The reason why the $d_{(100)}$ of HAp at $\sim 10.5^\circ$ instead of the $d_{(003)}$ of HAp at $\sim 32.7^\circ$ is adopted as the strongest peak of HAp is to avoid the overlapping of peaks of other calcium phosphate. The proportion of each phase (R_x : $x = \text{phase}$) in the samples is given by Equation (2):

$$R_x = I_x / (I_{\text{DCPD}} + I_{\text{DCPA}} + I_{\text{OCP}} + I_{\text{HAp}}) \quad (2)$$

The mechanical strength of the block was evaluated in terms of its diametral tensile strength (DTS). After drying the samples at 37°C for 24 h, the diameter and thickness of the block were measured using a micrometer (MDC-25MU, Mitutoyo Co. Ltd., Kawasaki, Japan). The samples were crushed with a universal testing machine (AGS-J, Shimadzu, Kyoto, Japan) at a constant crosshead speed of 1 mm/min . The mean DTS value for the five samples was calculated and expressed as the mean \pm standard deviation.

3. Results

Figure 1 shows the typical photographs of (a) the DCPD block, as well as the block after immersion in (b) a pH-6 acetate buffer solution, (c) a 0.5 mol/L PO_4 solution, (d) a 1.0 mol/L PO_4 solution, (e) a 0.5 mol/L Ca solution, and (f) a 1.0 mol/L Ca solution, at 70°C for two days. The DCPD blocks maintained their shapes during the solution immersion treatment, except for the case of immersion in the pH-6-adjusted 1.0 mol/L Ca solution.

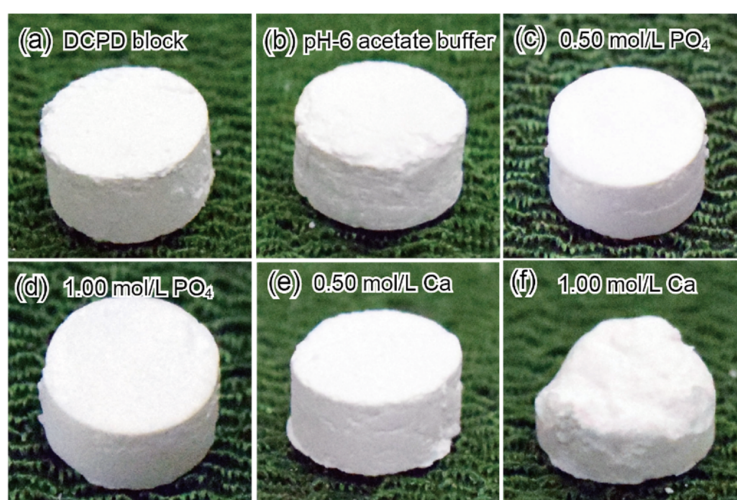


Figure 1. Photographs of the dicalcium hydrogen phosphate dihydrate (DCPD) block (a) before immersion, and after immersion in a (b) pH-6 acetate buffer, (c) 0.50 mol/L PO_4 solution, (d) 1.00 mol/L PO_4 solution, (e) 0.50 mol/L Ca solution, and (f) 1.00 mol/L Ca solution, all at 70°C for 2 days.

Figure 2 shows the typical XRD patterns of (a) the DCPD block, and (b) the block after immersion in the pH-6 acetate buffer solution, as well as immersion in the same solution containing (c) 0.25-mol/L PO_4 , (d) 0.50-mol/L PO_4 , (e) 1.00-mol/L PO_4 , (f) 0.25-mol/L Ca, (g) 0.50-mol/L Ca, and (h) 1.00-mol/L Ca. The XRD patterns of the DCPD block obtained after the setting reaction of α -TCP powder and H_3PO_4 exhibited a typical DCPD pattern without any other peaks. When the DCPD block was immersed in the pH-6 acetate buffer solution without Ca or PO_4 , its composition included a mixture of OCP and DCPA, and the block's macroscopic structure was retained. In the PO_4 -containing solutions, the DCPD block was converted to OCP, although a small amount of DCPA was formed after two days of immersion. Interestingly, in Ca-containing solutions, a small amount of OCP was formed and the composition of the DCPD block changed to HAp.

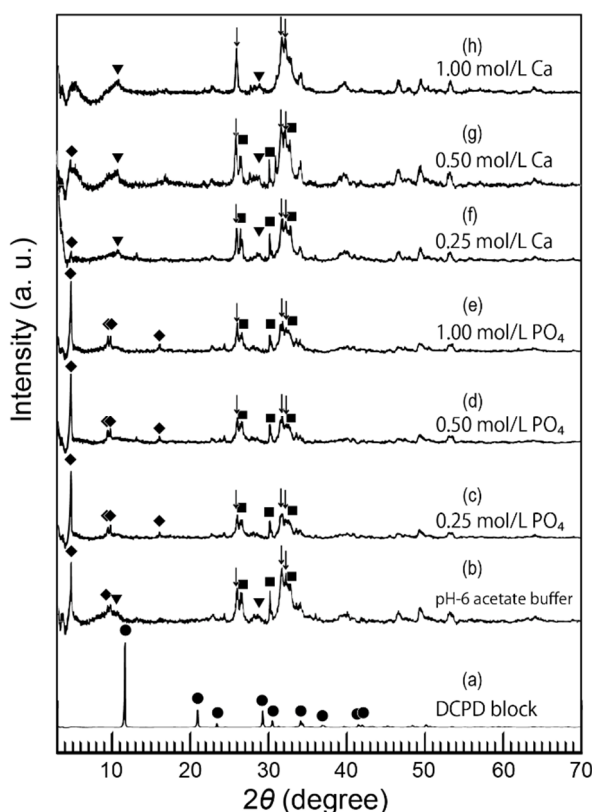


Figure 2. XRD patterns of DCPD block (a) before immersion and (b) after immersion in pH-6 acetate buffer solution, as well as after immersion in the same solution containing (c) 0.25-mol/L PO_4 , (d) 0.50-mol/L PO_4 , (e) 1.00-mol/L PO_4 , (f) 0.25-mol/L Ca, (g) 0.50-mol/L Ca, and (h) 1.00-mol/L Ca. ●: DCPD (PDF card No. 11-293), ◆: octacalcium phosphate (OCP) (PDF card No. 26-1056), ▼: hydroxyapatite (HAp) (PDF card No. 55-592), and ■: dicalcium hydrogen phosphate anhydrate (DCPA) (PDF card No. 9-80). ↓ indicated unidentified peaks (HAp or OCP).

Figure 3 shows the ratio of the yields in each sample, calculated from the intensity of each calcium phosphate peak.

Table 1 shows the final pH values of the pH-6 solution as a function of Ca or PO_4 concentration after the DCPD block was immersed in the solution at 70 °C for 2 days. When the DCPD block was immersed in a pH-6 solution without Ca or PO_4 , the pH dropped to 5. Similarly, the pH of the solution dropped to ~5 when the DCPD block was immersed in the Ca solution, regardless of its concentration. In contrast, a negligible pH change was observed when the DCPD block was immersed in the PO_4 solution.

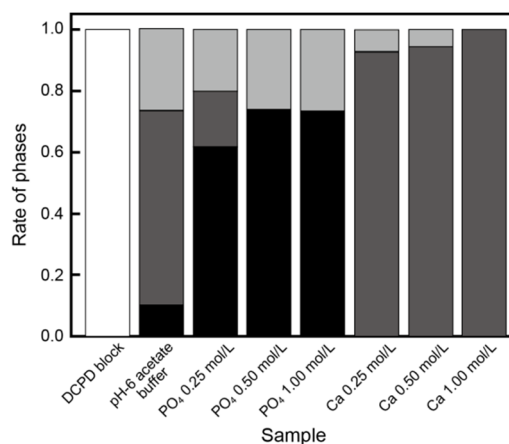


Figure 3. Phase rate of calcium phosphate yields in the samples. White: DCPD. Light grey: DCPA. Dark grey: HAp. Black: OCP.

Table 1. Final pH values of a pH-6 acetate buffer solution with PO₄ or Ca at 70 °C for 2 days.

Concentration (mol/L)	Final pH	
	PO ₄	Ca
0.00	5.03	5.03
0.25	5.96	4.94
0.50	6.06	5.02
1.00	6.09	5.17

Figure 4 shows the typical SEM images of (a, b) the DCPD block and (c, d) the DCPD block immersed in the pH-6 buffer solution, (e, f) the DCPD block immersed in 1.0 mol/L PO₄ solution, and (g, h) the DCPD block immersed in 1.0 mol/L Ca solution. The DCPD block consisted of rhombohedral plate-like crystals ~50 µm in length, ~20 µm in width, and 1–5 µm in thickness. When the DCPD block was immersed in the pH-6 buffer solution, the rhombohedral plate-like crystals disappeared, and the crystals became ~10 µm in length, 1–2 µm in width, and ~100 nm in thickness, with nanometre-sized fibre-like crystals. When the DCPD block was immersed in the PO₄ solution, however, the crystals became elongated and ribbon-like. In addition, when the DCPD block was immersed in the Ca solution, the crystals of the block become nanometre-sized and fibre-like.

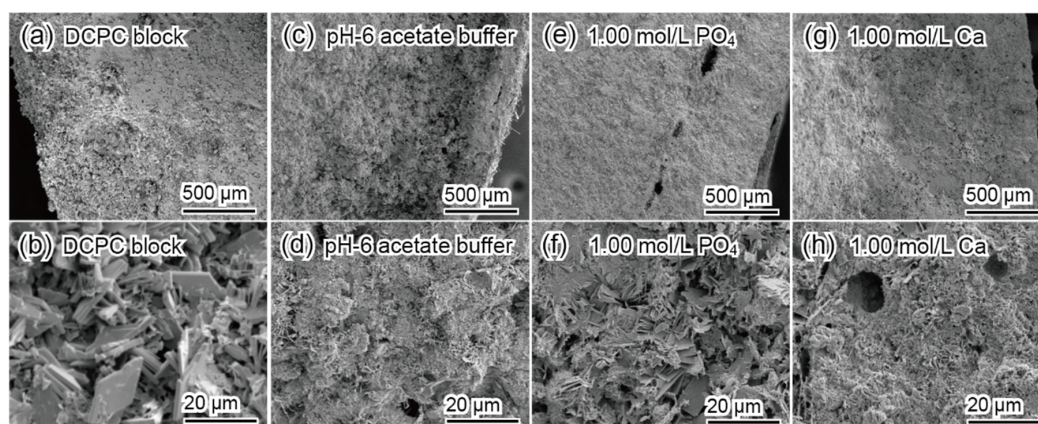


Figure 4. SEM images of the DCPD block (a,b) before immersion and after immersion in a (c,d) pH-6 adjusted solution, (e,f) pH-6 adjusted 1.00 mol/L PO₄ solution, and (g,h) pH-6 adjusted 1.00 mol/L Ca solution, each at 70 °C for 2 days.

Figure 5 shows DTS values of the DCPD block and the block after immersion in the pH-6 buffer, PO_4 , and Ca solutions at 70 °C for two days. Although after treatment the DTS values of the samples were significantly higher than those of the DCPD block, there was no significant difference among all the treated samples, except for the DCPD block immersed in the 1.0 mol/L Ca solution, which could not maintain its shape.

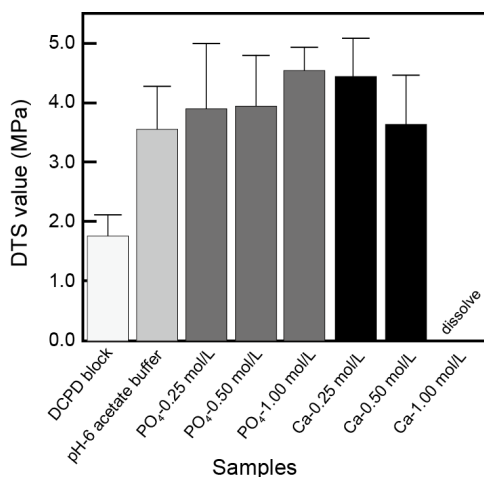


Figure 5. Diametral tensile strength (DTS) values of the DCPD block before and after immersion in a pH-6 acetate buffer solution with PO_4 or Ca at 70 °C for 2 days.

4. Discussion

The obtained results clearly indicate that the PO_4 concentrate solution has an advantage with respect to OCP formation from DCPD. On the other hand, the Ca concentrate solution has an advantage with respect to the formation of a mixture of HAp and DCPA from DCPD. The stoichiometric Ca/ PO_4 rates of DCPD and OCP are 1.00 and 1.33, respectively [10,11]. Therefore, based on the stoichiometric law, it seems consistent to add Ca for OCP fabrication from DCPD. However, the obtained results clearly indicate that supplying PO_4 is the key for converting from DCPD to OCP, even though the Ca/ PO_4 of the reaction system was significantly different from the stoichiometric chemical composition.

Many studies have been conducted on OCP formation and the dynamics of OCP in solution. OCP is not directly formed from the ions in the aqueous solution, but rather via several precursor phases, such as amorphous calcium phosphate (ACP; at least six forms) through solid–solid phase conversion instead of a dissolution–precipitation reaction [12–20]. Therefore, it is suggested that the property of the precursor, in this case the physicality of ACP, strongly affects the formation of OCP.

Habraken et al. [21] indicated that when OCP, in this case Ca-deficient OCP, was formed in weakly acidic to weakly basic solutions, ACP with a low Ca/ PO_4 ratio (0.3–0.4) (assembly of $\geq 86\%$ $\text{Ca}(\text{HPO}_4)_3^{4-}$ / $\text{Ca}(\text{H}_2\text{PO}_4)(\text{HPO}_4)_2^{3-}$ and $\geq 14\%$ CaHPO_4 / $\text{CaH}_2\text{PO}_4^+$) formed as a precursor phase, and OCP formed via ACP through structural phase transformation.

Based on the Habraken's concept, when the PO_4 concentration in the systems is increased, the amount of the precursor ACP and the final amount of OCP are increased. Therefore, DCPD was likely consumed and converted completely to OCP in solutions containing high amounts of PO_4 ions. Our foundation suspended this foundation. Furthermore, it was suggested that the DCPD blocks collapse in solutions with high Ca concentrations, as a result of the PO_4 removal process.

This study focuses on fabricating a highly pure OCP block for clinical use. For such a use, residual ions such as SO_4^{2-} might be a serious limiting factor. DCPD is a suitable precursor because the components of DCPD are essentially the same as those of OCP. Therefore, unlike calcium sulfate, it might be possible to obtain pure OCP blocks. Although the fabricated OCP blocks did not contain residual calcium phosphate, our study shows pure OCP blocks which are suitable for direct clinical use.

5. Conclusions

The effect of Ca or PO₄ on OCP block formation from the DCPD block in weakly acidic solutions was investigated. Phosphate solutions were induced in the conversion of DCPD blocks into the desired OCP blocks via the dissolution–precipitation reaction. This was unexpected given the stoichiometric ratio of Ca/P in OCP, and suggests that PO₄ is the essential ingredient for the fabrication of OCP and HAP via the dissolution–precipitation reaction.

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Conflicts of Interest: The authors declare no conflict of interests.

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