Fabrication of calcium phosphate nanoparticles
in a continuous flow tube reactor

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Calcium phosphates (CPs) were fabricated with using a continuous flow-type tube reactor under pH 6.4 and 9, within the temperature range from 0 to 100°C. The sample fabricated at the temperature range from 0 to 60°C involved not only dicalcium hydrogenphosphate dihydrate (DCPD) as the primary phase but also octacalcium phosphate (OCP). As the relative X-ray diffraction intensity of OCP increased with increase in the synthesis temperature, particles of a sheet-like shape appeared. The samples fabricated at 80 and 100°C have a crystalline phase of hydroxyapatite (HAp) and morphology of rod-like shape. Under pH 9, in contrast, the reactor fabricated HAp crystalline, irrespective of the synthesis temperature. In particular, at 80 and 100°C, only oval or spindle-shaped HAp was yielded. It was therefore confirmed that the present tube reactor system was versatile to control the size, shape, and crystalline phase of CPs. The morphology, particle size, and specific surface area dependent on the pH and temperature of synthesis were discussed the relative stability of the relevant calcium phosphate phases and equilibrium relations among the orthophosphate ions.

Key-words : Micro-reactor, Hydroxyapatite, Calcium phosphate, Nano-particles, Morphology control

1. Introduction

Calcium phosphates (CPs) have many kinds of crystalline phases. Their relative stability in a solution is strongly dependent on pH under ambient conditions.1) In other words, it is possible to control CPs crystalline phase by adjusting pH precisely in a wet chemical procedure, where commonly mixed together solutions of a calcium salt and a phosphate one. In the acidic region,2) for example, dicalcium phosphate anhydrate CaHPO4 (DCPA, monetite; the molar ratio Ca/P = 1) and dicalcium phosphate dihydrate CaHPO4·2H2O (DCPD, brushite; Ca/P = 1) are likely to appear, while octacalcium phosphate, Ca8H2(PO4)6·5H2O (OCP; Ca/P = 1.33) is yielded in the neutral region.3) Tricalcium phosphate, Ca3(PO4)2 (TCP; Ca/P = 1.5) and Hydroxyapatite, Ca10(PO4)6(OH)2 (HAp; Ca/P = 1.67) are stable in the basic region.4) HAp has been studied frequently among those phases, because it is excellently biocompatible and it is similar in structure and bio-chemical properties to inorganic materials involved in human bone and tooth as the primary inorganic component.5) It is widely used for hard tissue repairing materials in clinics, purification of proteins and nucleic acids, or drug delivery, to name a few. HAp is a crystal with anisotropic, and indexed in either hexagonal or monoclinic system,6) and hence, the properties are dependent on the planes.7) HAp appears in a few different morphologies, such as sheet, needle, or spherical particles, depending on fabrication procedures.8) Those are two of the most significant aspects of HAp.

In the case of a large-scale batch mixing system, greater ion concentration gradients are yielded at the interface between the calcium and phosphate solutions, and hence, large heterogeneity in the calcium or phosphate ion distribution is commonly observed. Some researchers have reported that HAp was prepared with using a micro-reactor system.9–12) The micro-reactor system may suppress such homogeneity or heterogeneity within a controllable limit of primary factors such as diffusion of solution, pH, reaction temperature and aging time after the reaction. The primary factors directly affect both the phase and the morphology of the yielded CPs crystallites. The micro-reactor synthesis of CPs, particularly HAp, has been published in the literature, but they all stressed on the mixing effects on the product morphology. For example, Yang et al. have used a microporous tube-in-tube microchannel reactor (MTMCR).12) However, in the case of the HAp synthesis system, it seems necessary to control not only the mixing effects of solution but also the reaction temperature or the aging temperature of the slurry. It is true that the initial reaction temperature, together with pH, determines the phase to be precipitated, but the aging temperature is also a good factor to affect the size and morphology. The reaction and aging temperature may be more effective than concentration gradient of calcium and phosphate ions, when the molar ratio Ca/P constant.

In our previous study, Fujii et al. employed a flow-micro-reactor system equipped with a solution-switching valve to fabricate HAp with the stoichiometric molar Ca/P ratio 1.67 by mixing calcium nitrate and diammonium hydrogenphosphate solutions under a pH range from 6.4 to 10.0.13,14) Yet, the effects of the reaction and aging temperatures on the crystallite morphology and size could not be discussed, because the solution-switching valve could not
be placed in a high temperature incubator. In addition, the feeding temperature itself could not help being kept at ambient one.

In this study, a tube-type flow microreactor system was taken to control both the reaction temperature and aging temperature, which equipped with a T-letter shaped connector as a simple mixing unit. It was intended to control both morphology and crystal phase, since this system could precisely control mixed state of both calcium and phosphate solution and pH. Several CPs were prepared with a continuous flow-type tube reactor system, after the obtained samples were evaluated a morphology, and crystalline phase and surface properties.

2. Experimental

Figure 1 shows schematically the continuous flow-type tube reactor, equipped with two plunger pump, the T-letter shaped mixing unit, and the polytetrafluoroethylene (PTFE) reaction tube (2 mm in inner diameter, 10 m in length).

Two plunger pumps fed aqueous solutions of 50 mM calcium nitrate tetrahydrate and 30 mM diammonium hydrogenphosphate into 10 m PTFE tube, respectively, at a rate of 2 mL·min⁻¹, after both solutions were pre-heated at the reaction/aging temperature. Ammonium hydroxide was added to the phosphate solution, so that pH of the suspensions at the reactor spout was maintained at 6.4 and 9. Note that, without the adjustment, pH was 6.4. The chemicals were all reagent grade (nacalai tesque, Japan), and employed without any further treatment. The reactor system was placed in an incubator under the temperature range from 0 to 100°C. The residence period of the reaction suspensions within the reactor tube was about 8 min. After the completion of the reaction, the resultant suspensions were filtered with membrane filter (Pore size; 0.1 μm, ADVANTEC, Japan) and the collected products were rinsed with distilled water, before they were subjected to vacuum freeze-drying. Thus, obtained samples were denoted as n-CP (x) where n stands for the fabricating temperature in range from 0 to 100°C, and x stands for pH of the suspensions.

The crystalline phases of the products were identified by X-ray diffractometry (XRD; RIGAKU, RINT2500, Japan; Cu Kα, 40 kV·200 mA). The diffraction data were collected with step scanning from 5 to 50° in 2θ (0.02°/step and 4°/min scanning speed). The morphology of obtained sample was observed under a transmission electron microscope (TEM; JEOL, JEM2100, Japan; Acceleration voltage 200 kV). Each sample was dissolved into 0.1 M HNO₃ solution, for which the chemical composition, or the molar ratio Ca/P, was measured using induction-coupled plasma photometry (ICP; Thermo Fisher Scientific, iCAP6500).

The specific surface area (SSA) was derived by the BET N₂ adsorption method (Microtrac BEL, BELSORP-max, Japan).

3. Results

3.1 Temperature effects on the phases fabricated under pH 6.4 and 9

Figures 2(a) and 2(b) indicate the XRD profile of samples n-CP (6.4) and n-CP (9), respectively with temperature n varied from 0 to 100°C. Note that the diffraction intensity scale was kept constant. Samples n-CP (6.4) with n ranging from 0 to 60°C consisted of DCPD (JCPDF 09-0077) and OCP (JCPDF 26-1056). The relative XRD intensity of the primary phase DCPD decreased with increase in temperature, whereas, that of OCP was not strong enough to compensate for it. Only HAp (JCPDF 09-0432) was detected in the XRD profiles when the samples were fabricated at 80 and 100°C.

Figure 2(b) indicates the XRD profiles of 0-, 40- and 100-CP (9). The profile for 100-CP (6.4) was presented at the bottom for comparison. All samples n-CP (9) were single-phase products of HAp, irrespective of the synthesis temperature, since no other phases were detected in their profiles. The diffraction peaks for n-CP (9) were sharpened with increase in the temperature, that is, crystallinity was improved. It should be pointed out here that the XRD peaks for 100-CP (9) was broader than those for 100-CP (6.4).
The ratio Ca/P for samples n-CP (6.4) was plotted as a function of the synthesis temperature in Fig. 3. The ratio Ca/P ratio of the sample 0-CP (6.4) was 1.23, greater than that for the stoichiometric DCPD (Ca/P = 1.0). It was very reasonable since 1.23 was in the middle of the ratio Ca/P of 1.33 for OCP and 1.0 for DCPD. After increasing rapidly to 1.4 at 20°C, the ratio Ca/P took a plateau value around 1.44 and it started increasing again at 80°C until it reached 1.66, which was almost the same Ca/P for the stoichiometric HAp. In the plateau stage, the ratio Ca/P exceeded that of OCP, which strongly suggests that such an excess amount of calcium ions was included in the crystallites in forms that would not be detected by XRD.

3.2 Temperature effects on the particle morphology (pH 6.4 and 9)

The left and right lines of TEM photographs in Fig. 4 illustrated the morphology of the crystallites in samples n-CP (6.4) and n-CP (9), respectively. The crystallites of sample 0-CP (6.4) showed no preferred shapes, which were smaller than 200 nm, whereas samples 20-, 40- and 60-CP (6.4) appeared to consist of sheet-like particles, and the particle size was larger than 200 nm in length. Note that the crystallites of these samples were mostly rectangular but a hexagonal habit was apparently seen at edges of a few particles as indicated with arrows in Fig. 4. In contrast, those of samples 80- and 100-CP (6.4) were rod-like in shape, and particle size was 100–200 nm in length.

The particles of n-CP (9) remained small size (~100 nm) without showing any crystalline habits. Particularly, samples 20- and 40-CP (9) were heavily agglomerated. The particles of 80- and 100-CP (9) seemed oval or spindle in shape, although the particle size was much smaller (~100 nm) than those of 80- and 100-CP (6.4).

Specific surface area (SSA) data were plotted in Fig. 5, which should demonstrate such tendency of the particle size and morphology presented by the TEM photographs. SSA for n-CP (6.4) increased with increasing the synthesis temperature, reached 82 m²/g. In contrast, SSA for n-CaP (9) decreased from 135 m²/g at 0°C down to 82 m²/g at 100°C, at the expense of improved crystallinity. SSA for 100-CP (6.4) and 100-CP (9) happened to be the same.

4. Discussion

4.1 Thermodynamic stability of the CPs phases

Figure 6 shows the regions of a few CPs phases, obtained here as well as the phase that Chow described as the least soluble one
in the relative stability diagram.\(^3\) The diagram was derived for an equilibrium state calculated using the solubility data, and empirically yielded CPs phases were sometimes different from the postulation by that diagram. The present study was one of such cases that would not obey the thermodynamic stability principle. According to Reynolds number in the tube, the flow of the calcium and phosphate mixed solution was turbulent so that the two solutions would be well mixed when they met together at the mixing unit. Thus, appearance of thermochemically metastable or unstable phases is to be attributed to incomplete reactions among the calcium, phosphate and hydroxy ions. In the acidic region (pH 6.4), the presence of the hydroxy ions in an insufficient content to form HAp lattice partly contributed to precipitate DCPD and OCP, with far smaller ratio Ca/P than that of HAp. Another factor was that the precipitated CPs particles disturbed diffusion of those component ions since reaction solutions were slurries. Reaction period or aging period due to short time of residence in the reaction tube is also a factor to yield the metastable phases. Despite the low pH or other factors, the system n-CP (6.4) yielded HAp crystalline at higher temperatures, i.e., 80 and 100°C. This is attributable to vigorous diffusion of the component ions, with which the equilibrium state of the systems was reached rapidly.

### 4.2 Crystallite size, morphology and formation path

Irrespective of pH, precipitation of smaller particles at lower temperature was attributed to greater supersaturation with respect to form HAp crystalline when supersaturation was established locally at the interface of the calcium and phosphate solutions. Vigorous nucleation took place in reaction solution, while their relatively slow diffusion would suppress particle growth. Note that the reaction precipitation is different from the precipitation of pertinent crystalline phase out of homogeneous solution, which can be interpreted with homogeneous or heterogeneous nucleation and crystal growth models. It is reasonable that SSA shown in Fig. 6 for samples n-CP (9) decreased with increasing synthesis temperature because SSA is roughly inversely proportional to particle size. Above discussion would not well explain the interrelation between SSA and particle size for SSA for samples n-CP (6.4) because they had shown an inverse tendency. For interpreting the change, difference in crystallite shape should be taken into account. The crystallites of 20-, 40-, and 60-CP (6.4) appeared larger in size than those of the other samples of n-CP (6.4), but it was speculated that they were so thin and they exhibited large SSA value. In contrast, SSA of the rod-like shape for 80- and 100- CP (6.4) were larger than that of 20-, 40-, and 60-CP (6.4) such as sheet-like crystallites. It is noted here that the electron diffraction pattern of those rectangular sheet-like crystallites was not presented herein since many diffracted spots from them could not be assigned to any of OCP or DCPD single phase, while the same spots had been observed in a previous study.\(^{13}\) Such presence of not-assignable diffraction spots gave evidence that OCP and DCPD were involved together in those sheet-like crystallites.

This phenomenon is interpreted as showing those metastable phases remained without transforming to HAp crystallite that the thermodynamic stability diagram presents as the stable phase in the relevant pH range. In other words, the sheet-like crystallites in 20-, 40-, 60-CP (6.4) should be spontaneously converted to HAp crystallite if they were kept in a long reaction tube for a long time. This suggests that the rod-like HAp crystals of samples 80- and 100-CP (6.4) would be fabricated by cracking the sheet-like particles consist of OCP and DCPD. As shown in Fig. 3, the presence of the excess calcium ions in those crystallites over the ratio Ca/P 1.33 of OCP could favor this postulation. The calcium and phosphate solutions consist of the ratio Ca/P 1.67 were fed forcibly to the tube reactor under pH 6.4. As a result, DCPD or OCP dependent on pH appear in the initial nucleation stage, after then, those component ions diffused by high temperature in the particle growth stage, so that both DCPD and OCP crystallites had to transform to the HAp crystallite, which was stability in thermodynamics.

In contrast, under pH 9, the calcium and phosphate ions easily found many retained hydroxy ions to form the HAp lattice instantly when the two solutions encountered. According to the Pourbaix diagram,\(^{15}\) under pH 9, at 25°C, almost all orthophosphate ions are present as HPO$_4^{2-}$ and the presence of H$_2$PO$_4^{-}$ is mostly negligible. This means that much fewer hydroxy ions will be consumed to precipitate HAp under pH 9 than under pH 6.4, and that the larger content of hydroxy ions retained in the reaction suspension favors thermochemically the precipitation of HAp. Therefore, it was strongly suggest that HAp was formed directly without passing through any metastable phases.

### 5. Conclusions

Sheet-, rod-, and spindle-like calcium phosphate particles were wet chemically prepared from 50 mM Ca(NO$_3$)$_2$ and 30 mM (NH$_4$)$_2$HPO$_4$ solutions under pH 6.4 and 9, at the temperature ranging from 0 to 100°C with a continuous flow-type tube reactor. The residence time was about 8 min, and pH of the reaction solution was controlled at 6.4 and 9 by adding ammonium hydroxide to the phosphate solution. Under pH 6.4, samples fabricated at the low temperatures 0–60°C were consisted of OCP with the molar ratio Ca/P 1.33 and DCPD with Ca/P 1.0, while samples fabricated at the high temperature 80–100°C were consisted of only HAp crystallite, according to X-ray diffraction analysis. Transmission electron micrographs showed that morphology of samples fabricated at the low temperatures 0–60°C was sheet-like in shape, and that of samples fabricated at the high temperature 80–100°C was rod-like in shape. Under pH 9, HAp crystallites were obtained for all temperatures, morphology of particles prepared at 80°C or higher temperature were oval or spindle in shape. The effects of temperature and pH on the
precipitating phases, their shapes, and specific surface area were discussed the relative stability of CPs phases and the equilibrium correlation among the relevant orthophosphate ions (H2PO4−, HPO42−, and PO43−). It was suggested HAp would be yielded under pH 6.4 via the metastable OCP, while HAp was directly precipitated under pH 9. In conclusion, it was found that this tube reactor process was versatile to produce CPs controlled morphology or crystallite phases under ambient conditions.

References