

Peculiarities in thermal evolution of precipitated amorphous calcium phosphates with an initial Ca/P ratio of 1:1

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Abstract Thermal evolution of amorphous calcium phosphate (ACP) powder from a fast nitrate synthesis with a Ca/P ratio of 1:1 were studied in the range of 20–980 °C. The powder consisted of amorphous dicalcium phosphate anhydrate (CaHPO₄) after heating to 200 °C. CaHPO₄ gradually condensed to amorphous calcium pyrophosphate Ca₂P₂O₇ (CPP) between 200 to 620 °C. Amorphous CPP crystallized at 620–740 °C to a metastable polymorph α'-CPP of the high-temperature phase α-CPP and β-CPP. The α'-CPP/β-CPP phase ratio reached a maximum at 800 °C (60 wt% α'-CPP/40 wt% β-CPP), and α'-CPP gradually transformed to β-CPP at a higher temperature. Some β-TCP occurred at 900 °C, so that a three-phasic mixture was obtained in the powder heated to 980 °C. The occurrence of metastable α'-CPP is attributed to Ostwald's step rule, and a mechanism for β-TCP formation is proposed. The advantages of prospective biomaterials from these powders are discussed.

1 Introduction

Calcium phosphate (CP) biomaterials based on hydroxyapatite, Ca₁₀(PO₄)₆(OH)₂ (HA), tricalcium phosphate, Ca₃(PO₄)₂ (TCP, in α- and β- forms) and octacalcium

phosphate, Ca₈(HPO₄)₂(PO₄)₄·5H₂O (OCP) can be prepared from amorphous calcium phosphate precursors (ACP) [1–5].

ACP usually occurs at an early stage of precipitation in solution. ACP from the nitrate synthesis [6] has been most comprehensively studied. A number of synthesis parameters affects the characteristics of the formed ACP. These are primarily the Ca/P ratio, the salt concentration, the pH, the temperature, and the residence time of the precipitate in the mother liquor. The relationship between these parameters is well documented for ACP with a Ca/P molar ratio in the range of 1.33:1 to 1.67:1 [7, 8]. The lowest value corresponds to OCP (1.33:1), the highest value to HA (1.67:1). An intermediate value corresponds to TCP (1.5:1). However, the results are not so clear for a Ca/P ratio below 1.33:1. For instance, ACP from the nitrate synthesis at a Ca/P ratio of 1:1 and 1.25:1 in the mother liquor gave ACP with Ca/P ratios of 1:33:1 and 1.40:1, respectively [9, 10], i.e., unexpectedly higher than the ratios in the original solutions.

Calcium pyrophosphate Ca₂P₂O₇ (CPP) known in α-, β-, γ-forms [11, 12] has not yet been widely considered as a biomaterial. However, it was recently found in in vivo studies that porous β-CPP has the potential to be used as a prospective biomaterial because it was more completely incorporated into newly formed bone and more rapidly resorbed than porous HA [13].

CPP is normally prepared by calcination of crystalline dicalcium phosphate dihydrate, brushite, CaHPO₄·2H₂O (DCPD) or dicalcium phosphate anhydrate, monetite, CaHPO₄ (DCPA). γ-CPP is formed at relatively low temperatures, β-CPP at intermediate temperatures, and α-CPP is the high-temperature phase. The transformation temperatures of the polymorphs significantly depend on synthesis routes and any contaminations of the stock salts, the calcination atmosphere (e.g., air, water vapor or vacuum) and the duration of the calcination at a given temperature.

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DCPD prepared by one synthesis route (calcium sucrose was added to diluted phosphoric acid at a relatively low temperature) only transformed to β -CPP upon annealing to 900 °C, and the β -CPP transformed into α -CPP at a temperature higher than 1250 °C [14]. In another study [15], the stock salts were obtained by mixing appropriate amounts of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ and Na_2HPO_4 in the presence of NaH_2PO_4 at pH 4–5 and 25 °C for DCPD and 100 °C for DCPA. Heating samples of DCPD (4 K min^{-1}) resulted in a continuous weight loss over the temperature range of 120–460 °C. DCPD gradually lost its water molecules and transformed to DCPA at about 170 °C, and DCPA decomposed to β -CPP starting at about 270 °C. However, a mixture of β - and γ - pyrophosphates occurred after rapid heating of DCPD, while only γ -CPP was present after annealing of DCPD at 270 °C, i.e., γ -CPP was formed directly from DCPD rather than from an intermediate compound. Also, DCPA was prepared by interaction of water solutions of CaCl_2 and $\text{NH}_4\text{H}_2\text{PO}_4$. Decomposition of the washed and dried precipitate (DCPA) at 718 °C gave γ -CPP. α -CPP was formed by sintering pellets of γ -CPP at 1300 °C for approximately 20 h [11]. Thermal treatments in the described studies [14, 15] were performed in air. If the heating was performed in vacuum [16], DCPA dehydrated near 500 °C to γ -CPP, which converted to β -CPP in the range of 500–750 °C.

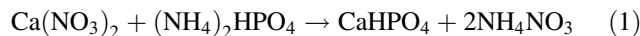
Provided that the precursors for the thermal synthesis of CPP are not crystalline as in previous studies but amorphous, a new possibility is suggested here. Results of the phase transitions during heating flame-synthesized ACPs revealed a low-temperature metastable phase crystallized at about 650 °C for the sample with $\text{Ca/P} = 1$ [17]. Chemically and structurally this phase was similar to the high-temperature polymorph α -CPP and will be referred as α' -CPP in the following. One can expect that α' -CPP, as normally a metastable low-temperature phase appearing due to the Ostwald step rule, is more defective and disperse than the original high-temperature phase. Consequently, α' -CPP may be superior in biodegradability. Unfortunately, a flame spray synthesis needs special equipment, expensive and has a low yield.

The aim of this work was to study the thermal evolution of ACP with the given $\text{Ca/P} = 1$ from the nitrate synthesis, particularly the temperature range of existence of the metastable α -CPP phase, and to shed light on a reason for formation of CP's with a higher Ca/P ratio than the given one in the mother solution.

2 Materials and methods

The preparation of the initial ACP powder was based on a fast precipitation variation of the nitrate synthesis [6, 18].

A 0.43 M aqueous solution of $(\text{NH}_4)_2\text{HPO}_4$ was rapidly added to a vessel with a 0.43 M aqueous solution of $\text{Ca}(\text{NO}_3)_2$ (molar ratio $\text{Ca/P} = 1:1$) leading to the following reaction



Besides monetite, the reaction led to the by-product ammonium nitrate. All reactants were of analytical grade (Merck, Germany), and freshly distilled water was used as a solvent. The two parent solutions were adjusted with ammonia to $\text{pH} = 10.11$ and cooled to 5 °C, i.e., the temperature and pH were chosen as in the study in which the discrepancies between the Ca/P ratios in the mother liquor and in the formed ACP phases were detected [10]. The vessel with the mixture was tightly closed, vigorously shaken and put into a freezer. The whole mixing process took about 3 min. The vessel was kept in the freezer at -25 °C for at least 24 h. The frozen mass was then taken from the freezer and slowly warmed up to 0 °C. The molten liquid was continuously removed by blotting paper. The residue in the vessel was six times washed with cold aqueous ammonia (5 °C, $\text{pH} 10\text{--}11$), and the washing solutions were decanted.

The resultant moist slurry was returned to the freezer and kept there until its weight became constant. Dry powders resulted. Before examination, the powders were kept in vacuum to avoid adsorption of atmospheric moisture and additionally to dry. The procedure without lyophilization resulted in nanocrystalline precipitates in accordance with earlier data [19].

Powder samples were examined as prepared and heated in air from 25 to 1000 °C in a muffle furnace. The samples were heated to a given temperature (5 K min^{-1}) in this range, removed from the furnace, allowed to freely cool to ambient temperature, and analyzed. They were studied by X-ray powder diffraction, XRD (DRON-2, USSR and Philips APDW 40C diffractometers; CuK_α radiation 1.54 Å), thermogravimetry and differential thermal analysis, TG-DTA (Q-Derivatograph, Hungary, 5 K min^{-1} , in air), infrared spectrometry, IR (Perkin-Elmer 1720X and Spectord 751, Germany; KBr-pellet technique). Because of the statistical background fluctuation weak bands and shoulders were considered only if they were repeatedly detected. The phase composition in the samples was determined by the Rietveld method using the program package TOPAS 4.2 from Bruker. The morphology and the sizes of particles were observed by scanning electron microscopy, SEM (ESEM Quanta 400 PEG, FEI, gold-sputtered samples).

3 Results

XRD patterns of the initial powder and after heating up to 500 °C were similar (Fig. 1a, b). They showed an

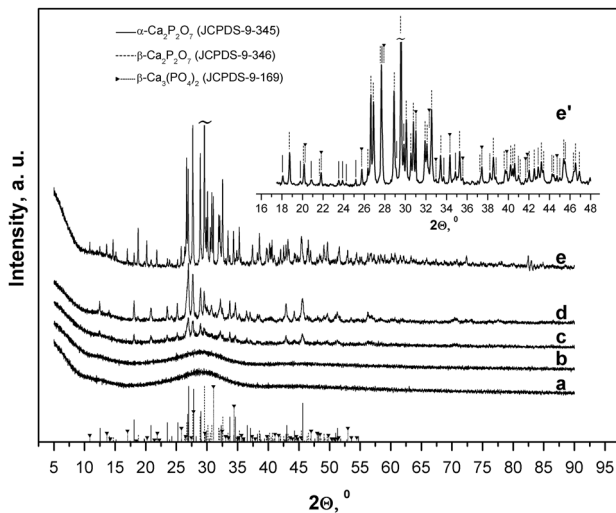


Fig. 1 XRD patterns of the powders in initial state (a) and upon heating to 500 °C (b), 640 °C (c), 800 °C (d) and 980 °C (e); e' - an extended part of pattern e to facilitate phase identification

amorphous substance with a hump at about 29° 2θ as those prepared under similar conditions in a number of earlier studies [e.g., 1, 2, 10, 19]. Despite the similarity of the diffractograms, the other methods revealed considerable differences in characteristics of the powders.

TG-DTG-DTA examination (Fig. 2) showed a rapid mass loss in the range of 75–160 °C with increasing to 120 °C and decreasing to 160 °C rates (section A–B), followed by a gradual mass loss up to 640 °C with an almost constant rate from 160 to 250 °C (section B–C) and slowly decreasing rate from 250 to 640 °C (section C–D); the range from room temperature to 75 °C was influenced by experimental noise. Two endothermic processes were detected during the heating. The first minimum (1) corresponded to the fast mass loss, and the extended second minimum (2) to the slow one. The range of the fast mass loss is typically associated with the desorption of water [1, 2, 20]. The second minimum (2) smoothly transformed to an exothermal peak (3) ended at about 760 °C. However, because of their overlapping, it is hard to detect the beginning of the exothermal process by the DTA curve.

The IR spectrum of the initial powder (Fig. 3a) showed unresolved bands at 442, 547, 875, 1006, 1072 and 2390 cm⁻¹ which are similar to those of amorphous CaHPO₄ [21]. Some foreign ions were also present. The absorption band at 1458 cm⁻¹ was in the typical range of carbonates and a weak peak at about 2348 cm⁻¹ is typical for surface adsorbed carbon dioxide [22]. However, the origin of strong and broad absorptions at about 3410, 3200, 3140 and 3015 cm⁻¹ were not clear because of their significant superimposition with absorption of water in this range.

The spectrum of the powder heated to 100 °C slightly differed by the low-frequency part from that of the initial

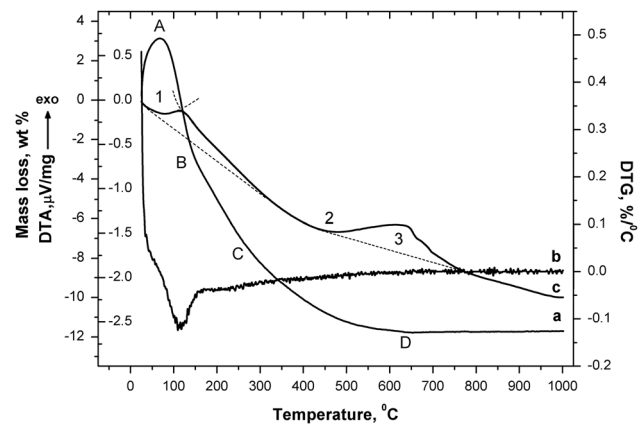


Fig. 2 TG (a), DTG (b) and DTA (c) curves for the powder

powder (spectrum not shown). However, the strong broad bands at about 3410, 3140 and 3015 cm⁻¹ and the sharp peak at about 1400 cm⁻¹ significantly decreased and then gradually disappeared in the spectra of the powders heated to 200 and 300 °C (Fig. 3b, c). Based on this, the absorptions were identified as of adsorbed water (3440 and 1645 cm⁻¹ [1, 2, 20]) and NH₄NO₃ (3410, 3140, 3030 and 1390 cm⁻¹ [23]). Three-time washing with ammoniated water (pH 10) at 20 °C was enough to remove the by-product NH₄NO₃ from ACP precipitated in a similar synthesis [20]. However, as it turned out, even six-time washing with ammoniated (pH 10) but very cold water (1–2 °C) was not enough to entirely remove the by-product. In the powder from a CP precipitate, the NH₄NO₃ by-product decomposes in the range of 170–270 °C [20]. Hence, the main mass loss with a constant rate within the B–C section (Fig. 2a, b) may be attributed to the decomposition of residual NH₄NO₃.

The spectrum of the powder heated to 200 °C (Fig. 3b) shows unresolved bands at 458, 548, 1010 and 1084 cm⁻¹ attributed to ν₂-, ν₄-, ν₁- and ν₃ modes of PO₄³⁻, respectively, as well as a band at 878 cm⁻¹ and a weak and broad absorption band near 2390 cm⁻¹ attributed to P-O(H)- and H-O(P) stretching of HPO₄²⁻ ions in amorphous CaHPO₄ [21]; also bands at 1654 and 3430 cm⁻¹ of water, 2855 and 2923 cm⁻¹ of hydrocarbons contamination [22], and of carbon impurities described above are present. Hence, the powder was amorphous monetite with some foreign ions. The spectrum of the powder heated to 300 °C (Fig. 3c) was noticeably different. The unresolved bands in the low-frequency range shifted to higher wave numbers. The HPO₄ band at 878 cm⁻¹ disappeared, and that at about 2390 cm⁻¹ decreased (the spectra in Fig. 3 are plotted with the same scale). A central well-defined band appeared at about 738 cm⁻¹. The band is typically attributed to Ca₂P₂O₇ [17, 21]. Hence, the condensation CaHPO₄ → Ca₂P₂O₇ + H₂O↑ [1, 17, 21] started in the powder. The shift was further developed, the intensity of the CPP band was progressively

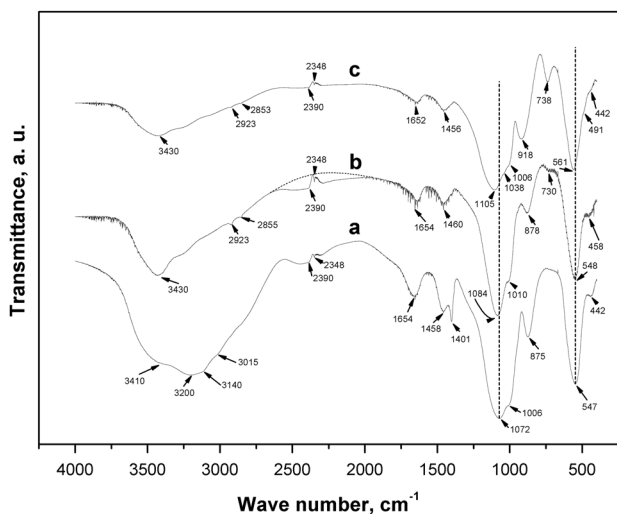


Fig. 3 IR spectra of the powder in initial state (a) and upon heating to 200 °C (b) and 300 °C (c)

increased, and that of HPO_4 was decreased as the powder was heated to a higher temperature. In the spectrum of the powder heated to 600 °C (Fig. 4a), the band at about 1460 cm^{-1} disappeared (the carbonate impurities were decomposed and desorbed [2, 24]), the broad HPO_4 band at 2390 cm^{-1} also disappeared, and the majority of the unresolved peaks started splitting, so all the appeared peaks were clearly identified for the powder heated to 640 °C (Fig. 4a, Table 1). Two pyrophosphates, α' -CPP and β -CPP crystallized from the amorphous powder. Hence, the exothermal peak was caused by the crystallization of amorphous $\text{Ca}_2\text{P}_2\text{O}_7$ started at about 600 °C (Figs. 2c and 4a).

The IR results are well consistent with XRD data showing biphasic mixtures of α' -CPP and β -CPP in the powders heated in the range of 640–800 °C (Fig. 1c, d and Fig. 5). The relative amount of α' -CPP increased as the heating temperature was increased. This may be associated with preferable crystallization of α' -CPP from amorphous CPP because the XRD pattern of the powder heated to 640 °C showed a marked amount of residual amorphous substance (the hump under principal maxima in Fig. 1c). The amorphous part (the hump) gradually decreased while the amount of α' -CPP correspondingly increased as the heating temperature was increased from 640 to 800 °C. The powder heated to 800 °C was entirely crystalline, and the relative amount of α' -CPP reached a maximum. As a result, a biphasic mixture of about 60 wt% α' -CPP/40 wt% β -CPP was obtained (Fig. 5). As noted above, the metastable pyrophosphate α' -CPP of the high-temperature polymorph α -CPP was prepared by the complicated flame spray-synthesis [17]. In our study, α' -CPP was first prepared via a simple precipitation method, and beside in combination

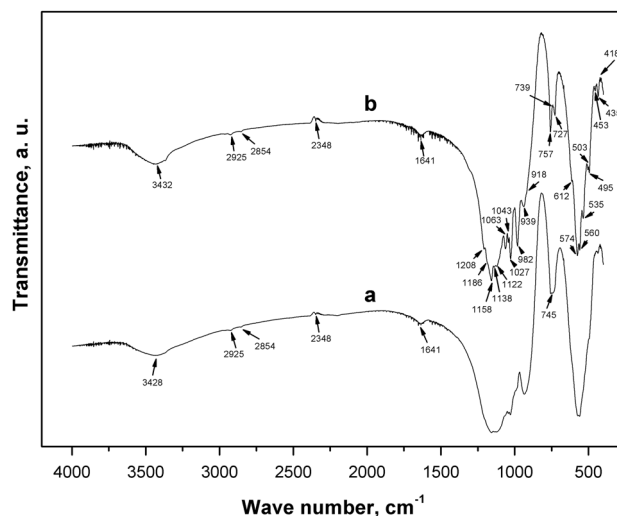


Fig. 4 IR spectra of the initial powder upon heating to 600 °C (a) and 640 °C (b)

with β -CPP which showed a potential to be used as a prospective new biomaterial [13].

Another peculiar feature in the phase evolution was revealed in the higher temperature range. Heating to a temperature above 800 °C resulted in a decrease of the α' -CPP amount, particularly above 900 °C, and an increase of the β -CPP amount, so that β -CPP became the main phase in the powder (Fig. 5). Besides, some amount of β -TCP (11.5 wt %) was detected in the powder heated to 900 °C that slightly changed in the powder heated to 980 °C (11.7 wt%; Figs. 1e and 5).

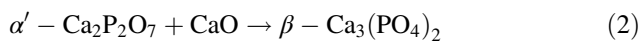
4 Discussion

Because the Ca/P ratio in the precipitate was 1:1, the occurrence of the phase with a Ca/P ratio of 1.5:1 in the high temperature range needed an additional source of calcium. Most likely, this was calcium oxide, CaO, from the thermal decomposition of calcium hydroxide and (or) carbonate. They may result from the hydration and further carbonation in air of calcium from the residual parent reagent $\text{Ca}(\text{NO}_3)_2$ as the synthesis was not completed because of short duration (not exceeding 3 min, Section 2). The residue was not completely removed by washing what was observed even for the higher soluble by-product (Fig. 3a). The solubility of NH_4NO_3 (208 g/100 g water) is almost twice as higher than of $\text{Ca}(\text{NO}_3)_2$ (121 g/100 g water) and significantly higher than of $\text{Ca}(\text{OH})_2$ (0.1 g/100 g water) or CaCO_3 ($14 \cdot 10^{-4}$ g/100 g water) at 18 °C [25]. Also, the decomposition of $\text{Ca}(\text{OH})_2$ starts at about 520 °C and of CaCO_3 at 850–900 °C [25], so, some CaO might be in the powder heated to 850–900 °C. The amount of

Table 1 Infrared absorption bands in the spectrum of the powder heated to 640 °C

Peaks (cm ⁻¹)	Reference peaks (cm ⁻¹) [12]		Peaks of residual Ca ₂ P ₂ O ₇ (cm ⁻¹ ; Fig. 3c)
	α-CPP	β-CPP	
418		422	
435	430		
453		457	
495		498	
503	500		
535	533		
560			561
574	570		
612		615	
727		728	
739			738
757	752		
918			918
939		946	
982	980		
1027	1025		
1043	1041		
1063	1060		
1122	1120		
1138		1141	
1158	1155		
1186		1190	
1208	1205		

α'-CPP decreased from 800 to 900 °C by 11.03 wt% (58.4–47.37 wt%), while the amount of β-CPP only decreased by 0.5 wt% (41.6–41.1 wt%). The amount of β-TCP was 11.54 wt% (11.03 + 0.5 wt%). Hence, mainly α'-CPP was involved into a possible reaction



(the calcium source was not present in the powder as a crystalline phase and, therefore, was not accounted the above estimation). Reaction (2) is known for a high-temperature solid state synthesis of β-TCP [26]. The amount of β-TCP decreased as the synthesis time exceeded 30 min. However, under the fast synthesis within 3–5 min, the phases formed upon crystallization of ACP were the same, and their relative amounts (as in Fig. 5) were reproducible within 10 %.

Heating an initial powder prepared without the washing step led to completion of the synthesis reaction (in the powder) which greatly accelerates at temperatures higher than a room temperature [2, 3, 18]. As a result, no unreacted Ca(NO₃)₂ was left, ACP crystallized into γ-CPP (usually with little admixture of β-CPP). γ-CPP transformed to β-CPP above 750 °C, and a single phase β-CPP was formed at 800–980 °C as a stable compound in this temperature range.

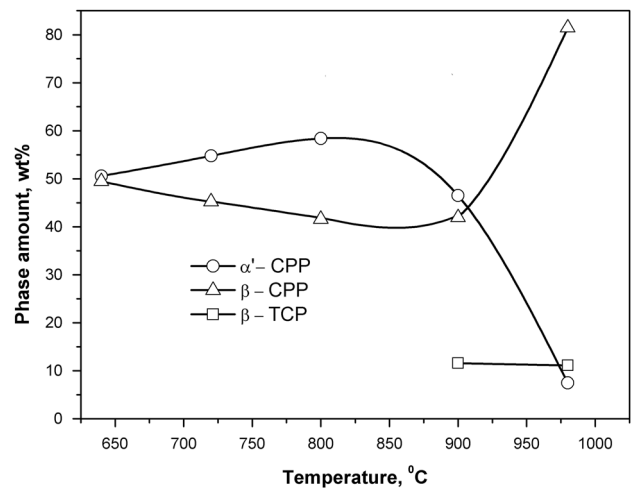


Fig. 5 Relative amounts of the crystallized phases vs. heating temperatures

No TCP appeared. This is in agreement with earlier results [16, 17].

As is indicated in Introduction, the Ca/P ratio in precipitates was found to be unexpectedly higher than the value

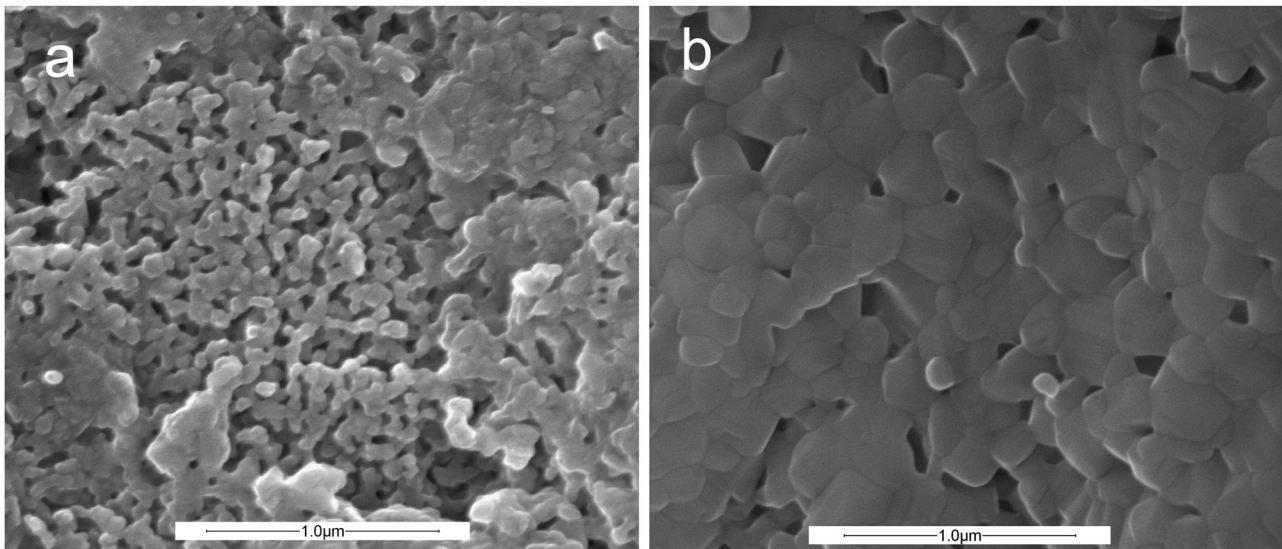


Fig. 6 SEM images of the powder heated to 720 °C (a) and 980 °C (b)

in mother solution under certain conditions in the nitrate synthesis [9, 10]. Because the synthesis variables in this study were as those in [10], the reason for the discrepancies may be the same as argued here: some unreacted calcium was left in the precipitates because of fast synthesis (relatively low synthesis temperature and short time).

The powder right after the crystallization consisted of α' -CPP and β -CPP particles with sizes of mainly 30–50 nm (Fig. 6a). Therefore, the powder is a suitable material to produce prospective biomaterials. It includes β -CPP with biological properties superior to those of HA [13]. Then, α' -CPP is expected to strengthen the biological behavior of β -CPP because metastable phases typically have enhanced solubility compared to their high-temperature original polymorphs [27, 28]. Also, the powder has a good sinterability. Bridges were formed between particles in the powder heated only to 720 °C (Fig. 6a). These reasons became stronger for the powders heated to higher temperatures. A new phase, β -TCP, occurred in the CPP mixture upon heating to 900–980 °C. β -TCP is also an excellent biomaterial [5, 29]. Additionally, the powder heated to 980 °C was significantly sintered even without typical preliminary pressing (Fig. 6b).

5 Conclusions

The thermal conversion of an ACP powder from precipitate of fast nitrate synthesis with a Ca/P ratio of 1:1 from room temperature to 980 °C was studied. The initial powder was X-ray amorphous CaHPO_4 that gradually transformed to amorphous $\text{Ca}_2\text{P}_2\text{O}_7$ between 200–600 °C.

The CPP powder crystallized at 600–760 °C and consisted of nanoparticles of a metastable α' -CPP and β -CPP. The α' -CPP/ β -CPP phasic ratio in the powder heated to a higher temperature increased and reached a maximum at 800 °C (about 60 wt% α' -CPP and 40 wt% β -CPP).

The phasic ratio decreased upon further heating, and some β -TCP (11.5 wt%) occurred at 900 °C. The β -TCP was partly formed from α' -CPP. The β -TCP amount was almost unchanged while α' -CPP progressively transformed to β -CPP from 900–980 °C.

The occurrence of metastable α' -CPP is attributed to the Ostwald step rule, and a possible mechanism for the β -TCP formation is proposed.

The reason for higher Ca/P ratio in precipitates from the nitrate synthesis than in the mother solution, observed in this and a few earlier studies, is thought to be associated with residue of (unreacted) calcium in the precipitates in case of a fast synthesis (short time, low temperature).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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