Hydrothermal synthesis of hydroxyapatite from natural source

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Dicalcium phosphate dihydrate (DCPD) and anhydrous dicalcium phosphate (DCPA) extracted from a by-product in a manufacture of gelatin from bovine bone are used as starting materials for the hydrothermal synthesis of hydroxyapatite (HA) at temperatures of 160 and 200 °C under vapor pressures of 1 and 2 MPa, respectively. The suspension of DCPA with water gives a mixture of DCPA and HA as product but after adjusting the Ca/P molar ratio to 1.50 and 1.67 by addition of $Ca(OH)_2$, a single phase of HA with needle-like crystals is obtained. DCPA whiskers are produced in the suspension of DCPD with water but, on addition of $Ca(OH)_2$, the product obtained is again, a mixture of DCPA and HA. The coexistence of DCPA and HA is observed not only in the acidic region at pH 4.6 which is close to the known quasi-invariant point but also in the basic region at pH 12.3. Without addition of $Ca(OH)_2$ to the system, the complete conversion of DCPA or DCPD to HA is not possible.
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Introduction

Calcium phosphates are recognized as a group of compounds consisting in the Ca/P molar range of 0.5 (monocalcium phosphate) to 2 (tetracalcium phosphate) [1] that play an important role in our daily lives, as an ingredient in food, medicines, biomaterials, detergents and so on. Calcium phosphates, especially hydroxyapatite (HA) have long been well known for their biocompatibility hence there have been quite a number of publications [2] on attempts to produce better calcium phosphate biomaterials for orthopedic and dental applications. However, there are very few studies on hydrothermal synthesis of these materials. The source of calcium phosphates can be either organic or inorganic.

By comparison, the purity and mechanical property of the products from the organic source are generally inferior to those from the inorganic one, i.e. calcium phosphates extracted from bone are likely to retain traces of impurities and porosity of the origin, nevertheless both types of products are equally biocompatible. In general, the high purity chemical reagents are much more expensive than bone ash, so it is interesting to prepare calcium phosphate compounds with minimal impurities from bone or its related substances. Low temperature (≤ 200 °C) hydrothermal treatment recommended by Yoshimura [3] as an attractive soft solution process (SSP) is the most appropriate means to serve the objective of this experiment since the technique is inexpensive, environmentally friendly, and produces high purity products.

Materials and method

Materials

The starting materials were dicalcium phosphate dihydrate (DCPD) and anhydrous dicalcium phosphate (DCPA) powders extracted from the by-product in the manufacture of gelatin from bovine bone, the composition of which is mostly DCPD [4]. The properties of the starting materials are shown in Table I, and their micrographs in Fig. 2. Approximately 0.250 g of DCPD or DCPA with various amounts of $Ca(OH)_2$ powder (analytical grade) added was made into a suspension with 5 ml distilled water and 0.1 ml lactic acid (density = 1.206) as chelating agent in a PTFE bottle, shaked well and ultrasonically vibrated for 5 min. The tightly capped bottles were autoclaved at 160 °C and 200 °C under a water vapor pressure of 1 MPa and 2 MPa, respectively and kept at this condition for 4 h, then they were left to cool down to room temperature, filtered, and washed with distilled water followed by acetone. The precipitate was air dried, oven dried at 40 °C, and transferred to a container. Each precipitate was characterized for phase analysis and crystal morphology by XRD (40 KV, 40 mA, CuK_α, MAC Science, Japan), FTIR

TABLE I Properties of the starting materials and by-product

Starting materials	Chemical analysis (by ICP)											
	Ca (%)	P (%)	Ca/P	Impurities					Heavy metal (ppm)				(ppb)
				Mg (%)	Fe (%)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Cd	Pb	As	Ni	Hg
DCPD	20.7	20.2	0.79	< 0.1	0.02	99	2	10	< 0.4	< 5	< 5	< 2	< 10
DCPD	25.9	21.0	0.96	0.1	0.02	105	2	9	< 0.4	< 5	< 5	< 2	< 10
By-product	23.9	17.65	1.05	0.02	0.02	120	2	10	< 0.4	< 5	< 5	< 2	< 10

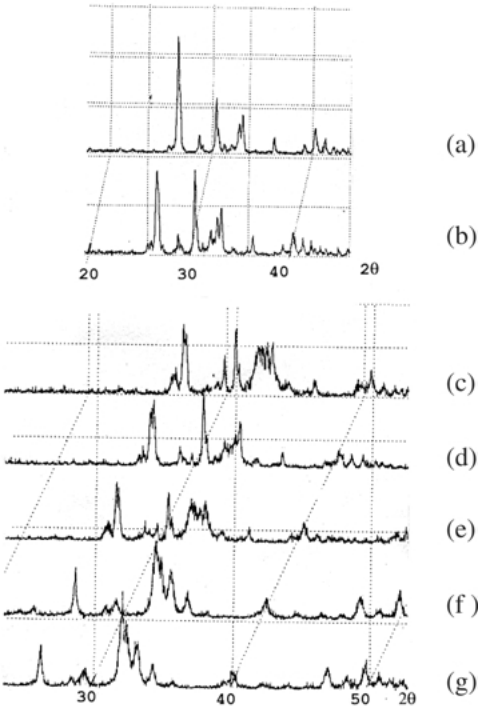

Figure 1 XRDs of hydrothermal products at 160 and 200 °C. (a) DCPD + H_2O, 200 °C; (b) DCPA + H_2O, 200 °C; (c) DCPD + $Ca(OH)_2$, Ca/P = 1.67; (d) DCPD + $Ca(OH)_2$ + L, Ca/P = 1.67; (e) DCPD + $Ca(OH)_2$, Ca/P = 1.50; (f) DCPA + $Ca(OH)_2$ + L, Ca/P = 1.67; (g) DCPA + $Ca(OH)_2$, Ca/P = 1.5.

(diffusion-reflection method, JEOL WINSPEC-100) and SEM (JSM -T200, JEOL Ltd., Japan).

Results and discussion

Characterization of starting materials

From the results of Table I, it can be seen that the trace impurity contents including that of heavy metals are well within the specified limit of ASTM F 1185–88 (''Standard specification for composition of ceramic hydroxylapatite for surgical implant''). The high Zn content is quite common in calcium phosphates which are derived from bovine bone and should not be detrimental to health since a small amount of Zn is required as nutrition and also as bone growth stimulator [5]. The SEM micrographs (Fig. 2) reveal the monoclinic, plate-like crystals of DCPD stacked together like a pack of cards, and forming bar-like particles, and the flat, triclinic crystals of DCPA. The typical size of a DCPD crystal is 9 μm in width and 30 μm in length and that of DCPA is 5 μm in width and 8 μm in length.

Characterization of hydrothermal products

The XRD results of Fig. 1 are summarized in Table II. In the DCPD set, only the suspension of DCPD with water gives DCPA whisker as product. All the remaining experiments with Ca/P ratios controlled by addition of $Ca(OH)_2$ give mixtures of DCPA and HA as product. This suggests the hydrothermal reaction be dehydration of DCPD to DCPA, followed by hydrolysis of DCPA to keep the final suspension acidic in cases when no $Ca(OH)_2$ was added. With $Ca(OH)_2$ addition, the $CaHPO_4$ obtained reacts with $Ca(OH)_2$ to form HA. The coexistence of DCPA and HA according to the solubility phase diagram for the system $Ca(OH)_2$–H_3PO_4–H_2O reported by Driessens [6] and Xie and Monroe [7] was at the quasi-invariant point of pH 4.3 and 4.8, respectively, but from the result it also appears at pH ~ 12.3. This finding suggests that the composition of the aqueous solution has reached that of another quasi-invariant point of DCPA + HA combination which is in the basic region at pH ~ 12.3. This value was also reported by [8] to be an invariant point of HA and $Ca(OH)_2$ combination. In the DCPA set, only the suspension of DCPA with water gives the mixture of DCPA and HA as product around the quasi-invariant point in the acidic region, pH ~ 4.6 and the rest gives HA in the final suspension of pH ranging from acidic to basic, consistent with the wide stability range of HA.

The possible reactions are suggested as follows:

$$CaHPO_4 \cdot 2H_2O \rightarrow CaHPO_4 + 2H_2O$$

$$CaHPO_4 + 2H_2O \rightarrow Ca(OH)_2 + 3H^+ + PO_4^{3-}$$

with $Ca(OH)_2$ addition

$$6CaHPO_4 + 4Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O$$

The presence of lactic acid as chelating agent in both sets of the experiment gives the same result which is the reduction in the aspect ratio of the product. This may be due to the high viscosity of the solution resulting from the gelation brought by the addition of the chelating agent, consistent with the report of [9] that high concentration of chelating agents tend to reduce the aspect ratio of needle-like crystals.

Figs 2(c) and (d) reveal both plate-like crystals and whiskers of DCPA, obtained by hydrothermal treatment of a DCPD suspension with water at 200 °C, 2 MPa for 4 h. The typical whiskers are 2.3 μm in length and 0.08 μm in width with an aspect ratio of 28. Some whiskers formed seem to be splitting off from the plate-like crystal. Fig. 2(f) is the micrograph of HA needles on DCPA platelet which suggests the morphological conversion of DCPA to HA [8, 10]. Fig. 2(e) shows

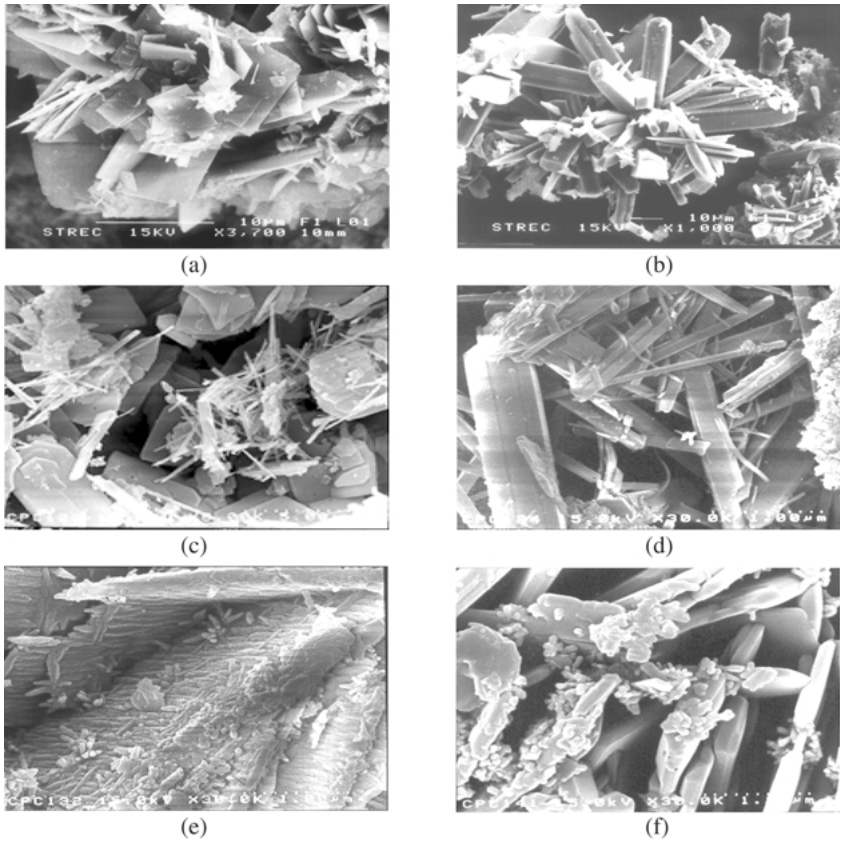

Figure 2 Electron micrographs of the starting materials and hydrothermal products. (a) DCPA (starting material); (b) DCPD (starting material); (c) and (d) DCPD + H_2O, 200 °C; (e) DCPA + $Ca(OH)_2$, Ca/P = 1.67, 200 °C; (f) DCPA + H_2O, 200 °C.

TABLE II Summarized results of the hydrothermal experiment at 160 °C, 1 MPa and 200 °C, 2 MPa

Starting materials	Ca/P	pH of suspension		Temp, Time °C, h	Phase (XRD)	Morphology of crystal
		Before	After			
DCPD + H_2O		6.2	5.3	200, 4	DCPA	Whisker + platelet
DCPD : $Ca(OH)_2$	1.5	12.0	12.1	160, 4	DCPA + HA^+	Platelet + needle
DCPD : $Ca(OH)_2$	1.67	12.3	12.3	160, 4	DCPA + HA^+	Platelet + needle
DCPD : $Ca(OH)_2$ + Lactic acid	1.67	12.3	12.3	160, 4	DCPA + HA^+	Platelet + needle
DCPA + H_2O		7.3	4.6	200, 4	DCPA + HA	Platelet + needle
DCPA : $Ca(OH)_2$	1.5	11.0	5.67	160, 4	HA	Needle
DCPA : $Ca(OH)_2$	1.67	11.2	11.5	200, 4	HA	Needle
DCPA : $Ca(OH)_2$ + Lactic acid	1.67	11.2	4.9	160, 4	HA	Needle

HA^+ = not well crystallized HA.

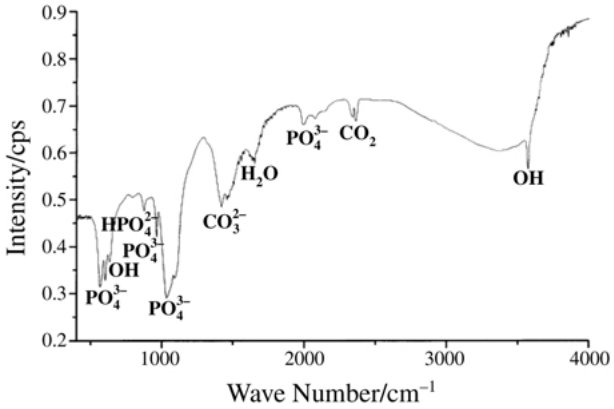

Figure 3 FT-IR spectrum of HA from the suspension of DCPA+ $Ca(OH)_2$, Ca/P = 1.67, 200 °C.

needle-shaped HA crystals growing out from the former platelet of the host, DCPA.

It appears that the HA crystals are densely packed on the platelet surface along the direction of the *c*-axis and some are splitting off as loose needles.

The FT-IR spectrum of Fig. 3 is the same as all of the hydrothermal products in that it reveals a strong absorption of CO_3^{2-} group at 1400–1570 cm^{-1} which is the lattice site of PO_4^{3-} and also the presence of HPO_4^{2-} in the lattice site of OH^- group at 870 cm^{-1}.

Conclusion

HA has been successfully synthesized by a hydrothermal treatment of its precursors, DCPA and DCPD which are extracted from bone, at temperatures of 160–200 °C

under vapor pressures of 1–2 MPa. The HA obtained is in the form of needle-like crystal with varying degree of crystallinity depending on the synthesis condition. Without the addition of $Ca(OH)_2$, the conversion of DCPA or DCPD to HA can not be completed. Single phase HA material was obtained from $DCPA + Ca(OH)_2$, $Ca/P = 1.5–1.67$, and single phase DCPA material was obtained from $DCPD + H_2O$ suspensions. The other experiments showed mixtures of DCPA and poorly crystallized HA.

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