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Chemical Processing of $CaHPO_4 \cdot 2H_2O$: Its Conversion to Hydroxyapatite

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The aim of this paper is to develop a robust chemical process to synthesize Na- and K-doped brushite (DCPD: dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$), a potential starting material for bone substitutes. The powders were synthesized by using sodium phosphate and potassium phosphate and aqueous solutions containing calcium chloride at room temperature, followed by drying at 37°C. DCPD powders thus formed were found to contain 460 ppm K and 945 ppm Na. On calcination in air, these powders readily transformed into monetite (DCPA: dicalcium phosphate anhydrous, $CaHPO_4$) first, and then into $Ca_2P_2O_7$ (calcium pyrophosphate). Na- and K-doped DCPD powders were shown to completely transform, in less than 1 week, into poorly crystalline carbonated apatite on immersion in an acellular simulated/synthetic body fluid (SBF) solution at 37°C. The Tris (i.e., tris(hydroxymethyl)aminomethane) buffered SBF solution used in this study had a carbonate ion concentration of 27 m*M* equal to that of human plasma. DCPD powders of this study displayed a notable apatite-inducing ability. This finding suggests the use of these DCPD powders as the starting materials for potential bone substitutes, which can be easily manufactured in aqueous solutions friendly to living tissues, at temperatures between room temperature and 37°C.

I. Introduction

POTENTIAL bone substitute materials must be actively resorbed[1] *in vivo* by the osteoclasts (cells that are able to resorb the fully mineralized bone), as they are equipped with a variety of enzymes, which lower the local pH to a range of 3.9 to 4.2. This occurs via a process called cell-mediated acidification in which the host bone can deposit new bone on those resorption sites by the osteoblasts (cells that build the extracellular matrix and regulate its mineralization). Bulk ceramics in the form of porous prismatic blocks, self-hardening cements, granules, coatings obtained by the high-temperature thermal spray techniques, injectable pastes, etc., are used as implant materials. For their successful application, they should be able to fully take part in the bone remodeling processes and must be eventually resorbed and fully replaced by the new bone within a year following the implantation.[2] Either synthetic or bovine calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) bioceramics heated at or above 1100°C during their processing do not resorb well and do not take part in the *in vivo* bone remodeling processes within the aforementioned time frame.[3,4] In general, resorbability of highly crystalline, sintered bovine-origin apatitic calcium phosphates is poor, due to the volatilization of initially present HPO_4^{2-} and CO_3^{2-} ions.

DCPD transforms (by losing its crystal water) into DCPA on heating at or above 110°C.[5] Both DCPD[6,7] and DCPA[8] phases are successfully used as starting materials in the preparation of the powder components of self-hardening apatitic calcium phosphate cements. However, recent reports[9,10] are directed toward the development of self-hardening orthopedic cements whose final product is DCPD. Such formulations have superior *in vivo* resorbability, as opposed to conventional apatitic cements. The scientific basis behind this development can be clearly explained by the dissolution data published by Tang *et al.*[11] Relying on the experimental solubility values of some of the calcium phosphate phases recently reported by Tang *et al.*,[11] it is seen that DCPD has a dissolution rate of 4.26×10^{-4} mol·m^{-2}·min^{-1} at a pH value of 5.5, and this rate is only about 3.4 times greater than that of $Ca_3(PO_4)_2$ (i.e., 1.26×10^{-4}). To compare these, the dissolution rate for carbonated apatite was reported by the same researchers[11] to be 1.42×10^{-6}.

DCPD powders can easily be synthesized in aqueous solutions at room temperature by using soluble calcium (e.g., $Ca(NO_3)_2 \cdot 4H_2O$, $CaCl_2 \cdot 2H_2O$, or $Ca(CH_3COO)_2 \cdot H_2O$) and phosphate (e.g., $NH_4H_2PO_4$, $(NH_4)_2HPO_4$, Na_2HPO_4, NaH_2PO_4, KH_2PO_4, or K_2HPO_4) salts on adjusting the Ca/P molar ratio to 1.[12–15] The use of Na- and/or K-containing starting chemicals during the synthesis of DCPD powders may also result in the production of Na- and/or K-doped DCPD.[16–19] On the other hand, pure DCPD powders can also be synthesized, for instance, by reacting a suspension of $Ca(OH)_2$ with stoichiometric amounts of H_3PO_4, as long as the solution pH is kept in the acidic range.[20,21]

Kumar *et al.*[17,18] reported previously that electrodeposited DCPD, which was doped with monovalent cations, such as potassium, might result in more rapid transformation into apatitic calcium phosphates on soaking the samples in Hanks balanced salt solution (HBSS) of pH 6.8.[22] HBSS is the historical origin of simulated body fluid (SBF) solutions of pH 7.4, which were popularized by Kokubo[23] over the last decade. The main difference between an HBSS solution and SBF lies in the value of the Ca/P molar ratios. HBSS has a Ca/P ratio of 1.823, whereas the same in SBF is 2.50. Owing to its lower Ca/P molar ratio, an HBSS solution, in contrast to SBF, needs quite long times to induce apatitic calcium phosphate formation.[24] However, to raise the Ca/P ratio of HBSS to 2.50, one only needs to add 70.5 mg of $CaCl_2 \cdot 2H_2O$ to 1 L of a commercially available HBSS solution. Therefore, soaking DCPD powders in SBF, instead of HBSS solutions, to test their apatite inducing ability would be more viable. *Tas*-SBF used in this study was a Tris-HCl buffered solution with a HCO_3^- ion concentration equal to 27 m*M*, whose preparation details were previously explained elsewhere.[25]

The main purpose of this study is to develop a robust chemical synthesis procedure for the manufacture of Na- and K-doped DCPD powders, and then study their transformation into apatitic calcium phosphates[26] by immersing them in an acellular and metastable (with respect to hydroxyapatite nucleation) SBF solution[25] over the duration of 36 h to 5 weeks. High-temperature calcination behavior of the alkali-doped DCPD powders is also reported.

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II. Experimental Procedure

The synthesis procedure used to form Na- and K-doped DCPD powders simply consisted of preparing two solutions. Solution A is prepared as follows: 4.127 g of KH_2PO_4 was dissolved in 3.5 L of deionized water, followed by the addition of 15.065 g of Na_2HPO_4, which resulted in a clear solution of pH 7.4 at room temperature (22 ± 2°C). Solution B (pH 7.3) was prepared by dissolving 20.068 g of $CaCl_2{\cdot}2H_2O$ in 250 mL of deionized water. Solution B was then rapidly added to solution A and the precipitates formed were aged for 80 min at room temperature, by continuous but moderate stirring (final solution pH 5.3). Solids recovered from their mother liquors were dried for 2 days at 37°C in an air atmosphere to obtain 16.85 g of Na- and K-doped DCPD powders.

The calcination behavior of these powders was determined by isothermal heatings over the temperature range of 300° to 1000°C, with 6 h of soak time at the peak temperatures. Simultaneous TG/DTA runs (room temperature to 1000°C, 5°C/min) were also performed, in a static air atmosphere, on the DCPD samples. Powder samples were characterized, at all stages, by XRD (XDS 2000, Scintag, Sunnyvale, CA), SEM (S-3500, Hitachi Corp., Tokyo, Japan), FTIR (Nicolet 550, Thermo-Nicolet, Woburn, MA), ICP-AES (Model 61E, Thermo Jarrell Ash, Woburn, MA), and TG/DTA (Model 851e, Mettler-Toledo, Inc., Columbus, OH) analyses.

Hydrolytic conversion of Na- and K-doped DCPD powders into poorly crystalline, apatitic calcium phosphate was studied by soaking those in a *Tas*-SBF solution at 37°C; 250 mg portions of DCPD powders were placed in 25 mL of the SBF[25] solution (2.5 m*M* Ca^{2+}, 1 m*M* HPO_4^{2-}, 27 m*M* HCO_3^-, 142 m*M* Na^+, 5 m*M* K^+, 1.5 m*M* Mg^{2+}, 0.5 m*M* SO_4^{2-}, 125 m*M* Cl^-, Tris-HCl buffered, pH 7.4), in plastic vials. During the conversion process, 15 mL aliquots of solutions were replenished with a fresh SBF solution at every 36 h. The experiment continued in 8 identical vials for solid sample recovery times of 36 h, 72 h, 1 week, 1.5 weeks, 2 weeks, 3 weeks, 4 weeks and 5 weeks. The vial contents were then filtered and washed with 400 mL of deionized water and dried at 37°C for 48 h, before characterization runs.

III. Results and Discussion

Chemically synthesized powders were characterized by using XRD, FTIR, SEM, ICP-AES, and TG/DTA to be single-phase, Na- and K-doped (460 and 945 ppm, respectively) $CaHPO_4{\cdot}2H_2O$, as shown in Figs. 1(A) (the inset is the FTIR data) through 1(C). DCPD powders were observed to have an elongated platelike morphology, with plate sizes varying over the range of 20 to 125 μm. DCPD is known to crystallize in the monoclinic space group *Cc* with the lattice parameters $a = 6.359$, $b = 15.177$, $c = 5.81$ Å, $\beta = 118.54°$.[27] As a function of increasing calcination temperature, as shown in Figs. 1(D) to (E) (XRD and FTIR data, respectively), DCPD first transformed into triclinic $CaHPO_4$ and then to $Ca_2P_2O_7$. $CaHPO_4$ has the following lattice parameters: $a = 6.910$, $b = 6.627$, $c = 6.998$ Å, $\alpha = 96.34°$, $\beta = 103.82°$, and $\gamma = 88.33°$. Its structure consists of $CaHPO_4$ chains bonded together by Ca–O bonds and three types of hydrogen bonds.[27]

The platelike morphology of transparent DCPD crystals (Fig. 1(B)) was preserved even after conversion into microporous $Ca_2P_2O_7$ by heating in air at 1000°C for 6 h (Fig. 1(E)). TG/DTA data of our DCPD powders (i.e., Fig. 1(C)) agreed perfectly with those reported by Joshi *et al.*[28] We observed that DCPD transformed to DCPA at around 180°C with a weight loss of 20.3%, and the DCPA to $Ca_2P_2O_7$ transition started above 440°C, with a further 6% weight loss. FTIR data presented in Figs. 1(A) (as-formed DCPD) and 1(F) (as a function of calcination temperature) also coincided very well with those in the literature.[28–30]

Lee *et al.*[31] recently tested commercial powders of $Ca_2P_2O_7$ as a synthetic bone graft material in comparison to hydroxyapatite. They reported a superior resorbability for $Ca_2P_2O_7$ in their canine-based proximal tibia model. The authors concluded that the calcium pyrophosphate initially functioned as an osteoconductive scaffold, which consecutively and seamlessly took part (within 3 months) in the bone remodeling process. All of the powders (i.e., DCPD, DCPA, $Ca_2P_2O_7$, and the apatitic calcium phosphates formed) of this study will need to undergo such *in vivo* tests to evaluate their biocompatibility and resorbability.

Calcination behavior of the platelike DCPD powders (Figs. 1(B) and (E)) of the current work presented a typical case for the thermally induced transformation[32] of an orthophosphate into a pyrophosphate according to the following reactions:

$$2CaHPO_4{\cdot}2H_2O(s) \rightarrow 2CaHPO_4(s) + 4H_2O(g) \quad (1)$$

$$2CaHPO_4(s) \rightarrow Ca_2P_2O_7(s) + H_2O(g) \quad (2)$$

These reactions were experimentally confirmed to take place by the TG/DTA analysis (Fig. 1(C)). β-$Ca_2P_2O_7$ phase obtained after 850° and 1000°C calcinations conformed to the ICDD PDF 9-346. On the other hand, α-$Ca_2P_2O_7$ phase (ICDD PDF 9-345) was observed in the samples calcined at 500° and 700°C (Fig. 1(D)).

DCPD is known to be a nucleation precursor, in aqueous solutions, to the apatitic calcium phosphates.[12] DCPD transforms into the thermodynamically more stable, apatitic calcium phosphate, by a dissolution–reprecipitation mechanism.[33] DCPD has a relatively low solubility in water, and thus water alone could not be sufficient to drive the reprecipitation mechanism.[34] However, if the aqueous medium of DCPD immersion contains Ca^{2+} ions, then the process will readily proceed according to the following reaction:[35]

$$6CaHPO_4{\cdot}2H_2O + (4 - x)CaCl_2 + (2 - x)H_2O \rightarrow Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} + (8 - 2x)HCl + 12H_2O \quad (3)$$

Apatite formed in reaction (3) is termed Ca-deficient hydroxyapatite (CDHA, molar Ca/P = 1.50),[36] and this formula represents the family of apatites formed at neutral pH. However, this formula is still a simplified version since it does not account for the alkali and carbonate ions incorporated into the structure.[37] Driessens *et al.*[38] have studied the synthesis of resorbable, Na- and K-containing CDHA self-setting cements. Under highly alkaline conditions (i.e., pH >10) of synthesis and precipitate aging, the formed apatitic calcium phosphates would have lesser amounts of vacancies in their OH sites.[39] If, for instance, NH_4OH were used to raise the pH value above 10 during chemical synthesis, the hydroxyapatite precipitates formed may also have some NH_4 ions incorporated into their structure.[37] Fully hydroxylated apatite samples are known to have an extremely basic surface, and on their immersion, they even cause the pH of the respective aqueous medium to rise to above 10.[40] Such high pH values on the surfaces of implant materials are not well tolerated by the live tissues, and may lead to cell necrosis.

Doping of DCPD powders with small amounts of Na and K (460 and 945 ppm, respectively) during their synthesis, as exemplified in this study, imparted a neutral surface pH (i.e., 6.90–7.10) to those, in comparison to pure, commercially available slightly acidic DCPD powders. Because of this acidity, bone substitutes made by using commercial DCPD powders until now were known to cause a certain degree of tissue inflammation during the first weeks of *in vivo* implantation.[10,41] The Na- and K-doped powders of this study, which have neutral surface pH values, are expected to circumvent this problem.

Therefore, to examine the dissolution–reprecipitation mechanism of our DCPD powders as a function of time, we selected a Tris-buffered, carbonated (27 m*M* HCO_3^-) SBF solution[25] of pH 7.4 as the immersion medium. If we were to soak the DCPD powders in pure water,[11,42] we would have mostly seen its sluggish dissolution over a period of 1 month. Ca and Cl ions present in the SBF solutions provided the driving force for reaction (3). As seen in the XRD data of Fig. 2(A) for the DCPD powders immersed in SBF solutions at 37°C, even after 72 h of soaking a significant amount of CDHA was formed as predicted by reaction

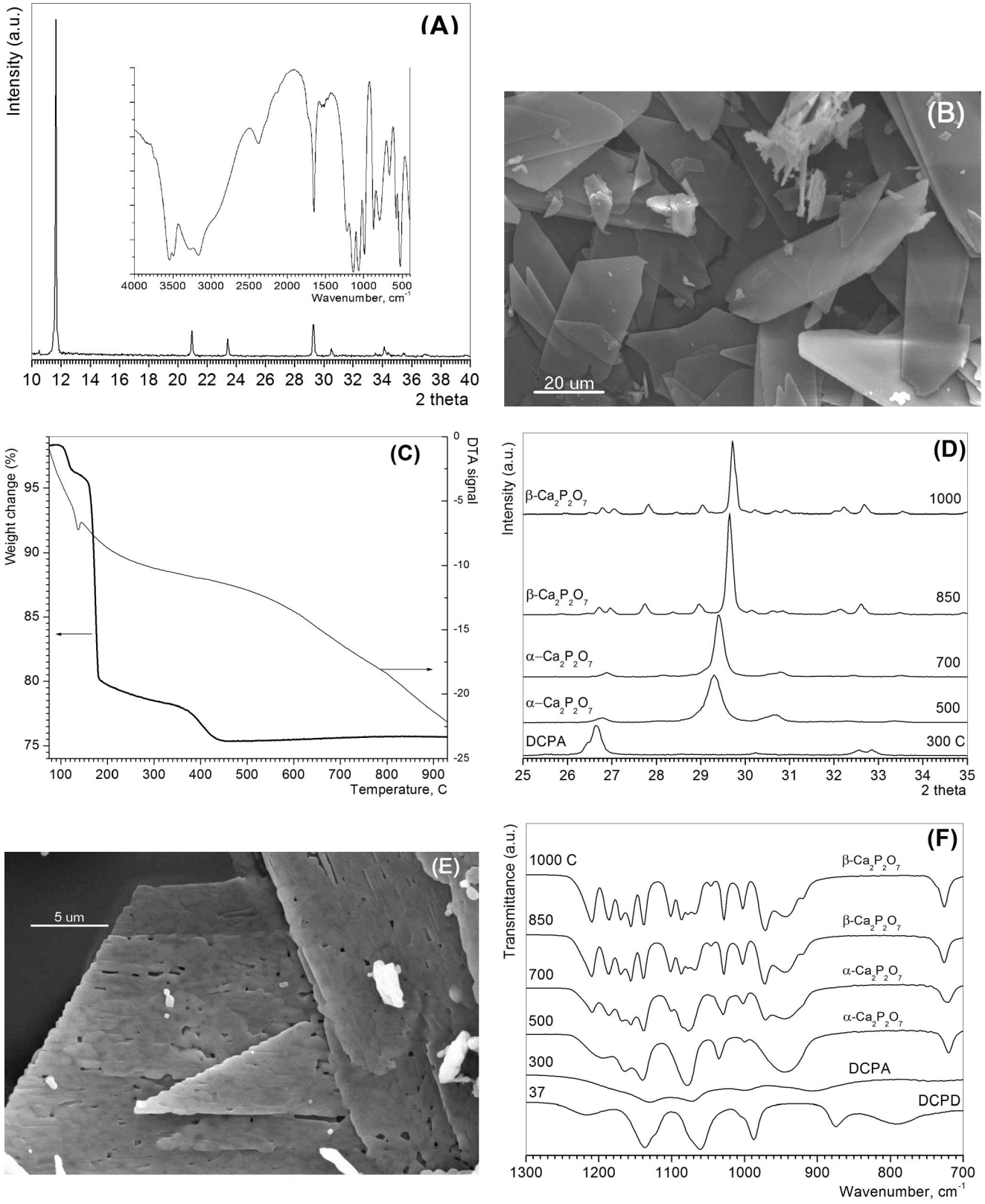

Fig. 1. (A) XRD and FTIR data of as-synthesized DCPD powders. (B) SEM photomicrograph of as-synthesized DCPD powders. (C) TG/DTA data for the as-synthesized DCPD powders. (D) XRD data of DCPD powders as a function of calcination temperature. (E) SEM photomicrograph of DCPD powders calcined at 1000°C for 6 h; resultant phase is now $Ca_2P_2O_7$. (F) FTIR data of DCPD powders as a function of calcination temperature.

(3). By the end of 1 week of soaking time, all of the crystal peaks of DCPD disappeared from the XRD patterns. This meant that the DCPD-based calcium phosphates would readily transform into bone mineral-like, apatitic calcium phosphates within less than 1 week, when soaked at 37°C and pH 7.4 in a solution simulating the human blood plasma. FTIR data given in Fig. 2(B) for those samples clearly indicated the same trend. The recognition of the characteristic bands in the FTIR spectra of apatite and DCPD phases have been unequivocally established in various references.[25–30] FTIR spectra of Fig. 2(B) confirmed that the carbonated

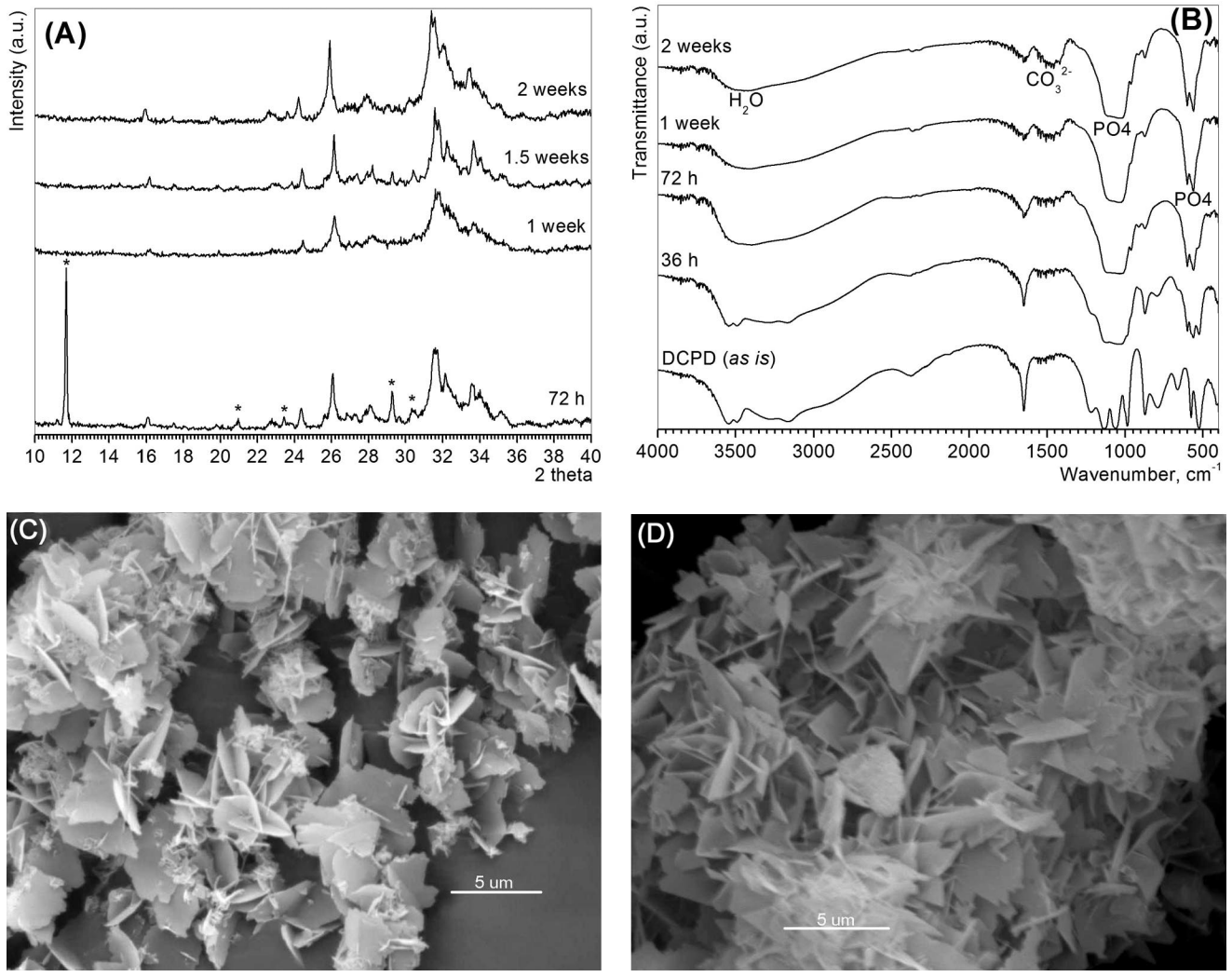

Fig. 2. (A) XRD data of DCPD powders soaked in SBF for various times (* indicates DCPD peaks). (B) FTIR data of DCPD powders soaked in SBF for various times. (C) Powders soaked in SBF at 37°C for 36 h; a phase mixture of DCPD and CDHA. (D) Powders soaked in SBF at 37°C for 1 week; single-phase CDHA.

nature of the apatitic calcium phosphate initially formed was gradually developing with an increase in soaking time. Human bones contain a significant amount of carbonate ions, i.e., 5–7 wt%. SEM photomicrographs given in Figs. 2(C) and (D), as a function of immersion time in SBF, depicted the evolution of the characteristic morphology of needlelike CDHA.

From X-ray or neutron crystallographic studies, the crystal structure of DCPD is known to contain compact sheets or bilayers parallel to the (010) plane.[27] One bilayer was found to present sheets of calcium and phosphate ions, while the other bilayer comprised water molecules. Flade *et al.*[43] reported that the hydrated bilayer was the terminating layer at the surface of the (010) face in aqueous solutions, and dissolution of DCPD must start from the ledges.

To examine the dissolution behavior of DCPD more clearly, we modified our synthesis process slightly. In that experiment, we placed a 30 mL aliquot of freshly prepared solution A (its preparation is described in the Experimental Procedure section) into a 50-mL-capacity glass beaker covered with Parafilm®. The beaker was then heated to 37°C in a constant-temperature oven. One minute after the injection (with a syringe and needle) of a 1 mL portion of solution B into that beaker, solution pH was recorded as 5.5 at 37°C, and it was rapidly cooled to 0°C by immersing it into an ice-water bath. The precipitates were separated immediately from the mother liquor by centrifugal filtration, and washed with water. The formed precipitates as shown in the optical micrograph given in Fig. 3(A) had a star- or rosette-like morphology. They were shown by XRD to be highly crystalline, single-phase DCPD. These crystals were regarded as the early-stage crystallization products of the synthesis process reported in this study.

Subsequently, to picture the dissolution behavior of those DCPD crystals, a 100 mg portion was placed into 10 mL of a freshly prepared *Tas*-SBF solution.[25] Following 3 h of immersion at 37°C in SBF, the DCPD crystals were washed with water and dried at 37°C, overnight. The XRD pattern of the samples again indicated single-phase DCPD, as shown in Fig. 1(A). The SEM photomicrograph given in Fig. 3(B) shows the dissolution of DCPD, revealing the aforementioned bilayer structure,[43] which until now could only be ascertained by using crystallographic techniques. The reprecipitation leg of this mechanism, which leads to the apatitic calcium phosphate formation, subsequently takes place at the later stages of SBF immersion, as shown above. SBF solutions of pH 7.2 to 7.4 are supersaturated with respect to apatite (i.e., Ca/P molar ratio = 2.50). Therefore, the only phase that can precipitate from such neutral pH solutions is carbonated apatitic phosphate.

The bilayers inherent in DCPD crystals are known to consist of calcium and phosphate sheets separated by another sheet of water molecules.[43] This sheet and ledge morphology has been revealed in the SEM micrograph of Fig. 3(B) after soaking DCPD crystals in an SBF solution at 37°C for 3 h. The presence of Na and K dopant ions (which help to increase the crystallographic disorder,[44] and thus, the overall solubility of the crystals) within the calcium

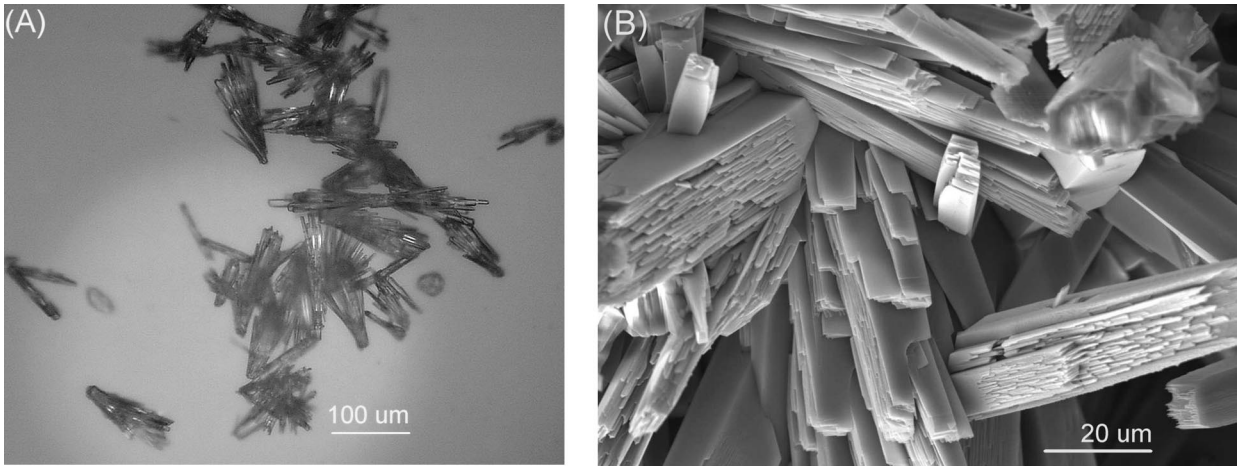

Fig. 3. (A) Early-stage crystals of DCPD formed in 1 min. (B) Dissolution of DCPD crystals in SBF at 37°C, revealing the ledge structure.

ledges is believed to enhance the hydrolysis kinetics of DCPD. The hydrolysis product is carbonated, calcium-deficient hydroxyapatite (CDHA). Calcium-rich regions in crystalline calcium phosphates are typically the first locations where the hydrolysis starts more rapidly. The formation of interlocking CDHA platelets perpendicular to the original DCPD plates suggests that these newly formed CDHA platelets are favorable to allow continuous ion transport between the SBF solution and the DCPD crystals.[36] Rapid (i.e., in just 36 h) conversion of the DCPD morphology into that of the CDHA platelets was noteworthy.

TTCP ($Ca_4(PO_4)_2O$) and α-TCP (α-$Ca_3(PO_4)_2$), which are two important calcium phosphate compounds with self-setting cement properties, generally display such hydrolytic conversion behavior as we hereby exemplified for DCPD. The hydrolysis products of both of these phases are always CDHA, with the same morphology as given in Figs. 2(C) and (D). DCPD already contains HPO_4^{2-} ions in its structure, and the hydrolytic conversion of it into CDHA is expected to be much simpler.

To summarize, conversion of $CaHPO_4 \cdot 2H_2O$ into poorly crystalline carbonated apatite becomes important when its possible uses as synthetic bone substitutes or bone defect filling materials are considered. Self-setting orthopedic cements based on the sole phase product of DCPD have already been formulated and reported for their enhanced resorbability.[20] Moreover, the platelike or needlelike morphology observed in chemically prepared DCPDs would have significant uses in improving the mechanical properties (e.g., compressive and flexural strength) of the self-setting orthopedic cements, which include DCPD as a constituent.[45]

IV. Conclusions

(1) The chemical process outlined here allowed the robust synthesis of biocompatible Na- and K-doped DCPD powders, with a unique platelike morphology, by starting with aqueous solutions at the physiologic pH (7.4) and temperature (37°C) conditions.

(2) The results showed that it was possible to preserve this particle morphology even though the powders were later converted to $Ca_2P_2O_7$ by high-temperature calcination.

(3) Finally, this study also exemplified a simple procedure for producing carbonated apatitic calcium phosphate powders, after straightforward immersion of DCPD powders in SBF solutions (pH 7.4) at 37°C. The highest temperature of processing hereby used in the manufacture of bulk, poorly crystalline apatite powders was 37°C.

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