PREPARATION OF BRUSHITE POWDERS AND THEIR *IN VITRO* CONVERSION TO NANOAPATITES

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ABSTRACT

Brushite (DCPD: dicalcium phosphate dihydrate: $CaHPO_4 \cdot 2H_2O$) powders were chemically synthesized by using Na- and K-phosphate and calcium chloride-containing aqueous solutions at room temperature (RT), followed by drying at 37°C. DCPD powders thus formed were found to contain 460 ppm K and 945 ppm Na. Upon calcining in air these powders readily transformed into $CaHPO_4$ (monetite) first, and then into $Ca_2P_2O_7$. Na- and K-doped DCPD powders were shown to completely transform, in less than 1 week, into poorly crystalline carbonated apatite upon immersion in an acellular simulated body fluid (SBF) solution at 37°C. The tris-buffered SBF solution used in this study had a carbonate ion concentration of 27 mM, which was equal to that of human blood plasma. This finding suggests the use of these DCPD powders as potential bone-substitute materials, which can be easily manufactured in aqueous solutions friendly to living tissues at temperatures between RT and 37°C.

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 (dicalcium hydrogen phosphate dihydrate) can be synthesized in aqueous solutions at room temperature by using water-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 upon adjusting the Ca/P molar ratio to 1 [5-8]. The use of Na- and/or K-containing starting chemicals during the synthesis may also result (although it may not be necessarily and strictly so) in the production of Na- and/or K-doped DCPD powders [9-12]. 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 [13, 14]. DCPD will transform (by losing its crystal water) into $CaHPO_4$ (DCPA: monetite) upon heating at or above 110°C [15]. Both DCPD [16, 17] and DCPA [18]

phases are successfully used as starting materials in the preparation of the powder components of self-hardening apatitic calcium phosphate cements. However, recent reports [19, 20] are directed towards 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. This scientific basis behind this development can be clearly explained by the dissolution data published by Tang *et al.* [21]. Relying on the experimental solubility values of some of the calcium phosphate phases recently reported by Tang *et al.* [21], 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 [21] to be 1.42×10^{-6}.

Kumar *et al.* [10, 11] reported previously that electrodeposited DCPD, which was doped with monovalent cations, such as potassium, might result in more rapid transformation into apatitic calcium phosphates upon soaking the samples in Hanks Balanced Salt Solution (HBSS) [22]. HBSS is the historical origin of Simulated Body Fluid (SBF) solutions, 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 $CaCl_2 \cdot 2H_2O$ into 1 liter 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 [25] used in this study was a Tris-HCl buffered solution with a HCO_3^- ion concentration equal to 27 mM, whose preparation details were previously explained elsewhere.

The main purpose of this study was to develop a robust chemical synthesis procedure for the manufacture of Na- and K-doped DCPD powders, and then study their transformation into apatite [24] by immersing them in an acellular and metastable (with respect to hydroxyapatite nucleation) SBF solution [25] over the duration of 36 hours to 5 weeks. High-temperature calcination behavior of the alkali-doped DCPD powders was also studied and reported.

EXPERIMENTAL PROCEDURE

The synthesis procedure used to form Na- and K-doped DCPD powders in this study consisted of preparing two solutions. Solution-A is prepared as follows; 4.127 g KH_2PO_4 was dissolved in 3.5 L of deionized water, followed by the addition of 15.065 g Na_2HPO_4, which resulted in a solution of pH 7.4 at RT (22±2°C). Solution-B (of pH 7.3) was simply prepared by dissolving 20.068 g of $CaCl_2 \cdot 2H_2O$ in 250 mL of deionized water. Solution-B was then added at once into solution-A, and the precipitates that formed were aged for 80 minutes at RT under 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 percentage yield of the powder synthesis process was 72±1%.

Calcination behavior of these powders were 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 (RT to 1000°C) were also performed on the DCPD samples.

Powder samples were characterized, at all stages, by XRD, SEM, EDXS, FTIR, ICP-AES, and TG/DTA analysis.

Hydrolytic conversion of Na- and K-doped DCPD into poorly crystalline, carbonated, apatitic calcium phosphate powders were studied by soaking in a *Tas*-SBF solution [25] at 37°C. 250 mg portions of DCPD powders were placed in 25 mL of the *Tas*-SBF [25] solution (2.5 mM Ca^{2+}, 1 mM HPO_4^{2-}, 27 mM HCO_3^-, 142 mM Na^+, 5 mM K^+, 1.5 mM Mg^{2+}, 0.5 mM SO_4^{2-}, 125 mM Cl^-, *tris*-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 hours. 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 hours, prior to characterization runs.

RESULTS AND DISCUSSION

Chemically-precipitated powders were characterized by XRD, FTIR, SEM, ICP-AES and TG/DTA data to be single-phase, Na- and K-doped (460 and 945 ppm, respectively) $CaHPO_4{\cdot}2H_2O$, as shown in Figures 1a (the inset displays the FTIR data) through 1c. Brushite crystallizes in the monoclinic space group *Cc* with the lattice parameters, a=6.359, b=15.177, c=5.81Å, β=118.54° [26]. As a function of increasing calcination temperature, as shown in Figures 1d to 1e (XRD and FTIR data, respectively) brushite first transforms into $CaHPO_4$ (DCPA, monetite) and then to $Ca_2P_2O_7$. DCPA has the following lattice parameters; a=6.910, b=6.627, c=6.998 Å, α=96.34°, β=103.82°, and γ=88.33°. Its structure consists of $CaHPO_4$ chains bonded together by Ca-O bonds and three types of hydrogen bonds [26]. The plate-like morphology of optically transparent brushite crystals (Fig. 1b) was preserved even after conversion into microporous $Ca_2P_2O_7$ by heating in air at 1000°C for 6 hours (Fig. 1e).

Calcination behavior of plate-like DCPD powders (Figs. 1b and 1e) of this study presented a typical case for the thermally induced transformation of an orthophosphate into a pyrophosphate according to the 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. 1c). 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. 1d). TG/DTA data of DCPD powders (i.e., Fig. 1c) also agreed well with the work of Joshi *et al* [27]. We observed that the brushite transformed to monetite at around 180°C with a weight loss of 20.3%, and the monetite to $Ca_2P_2O_7$ transition was completed above 440°C, with a further 6% weight loss. FTIR data presented in Figures 1a (as-formed DCPD) and 1f (as a function of calcination temperature) also coincided very well with those in literature [27-29].

Lee *et al.* [30] have 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 scaffolding, and within three months it consecutively and seamlessly took part in the bone remodeling process.

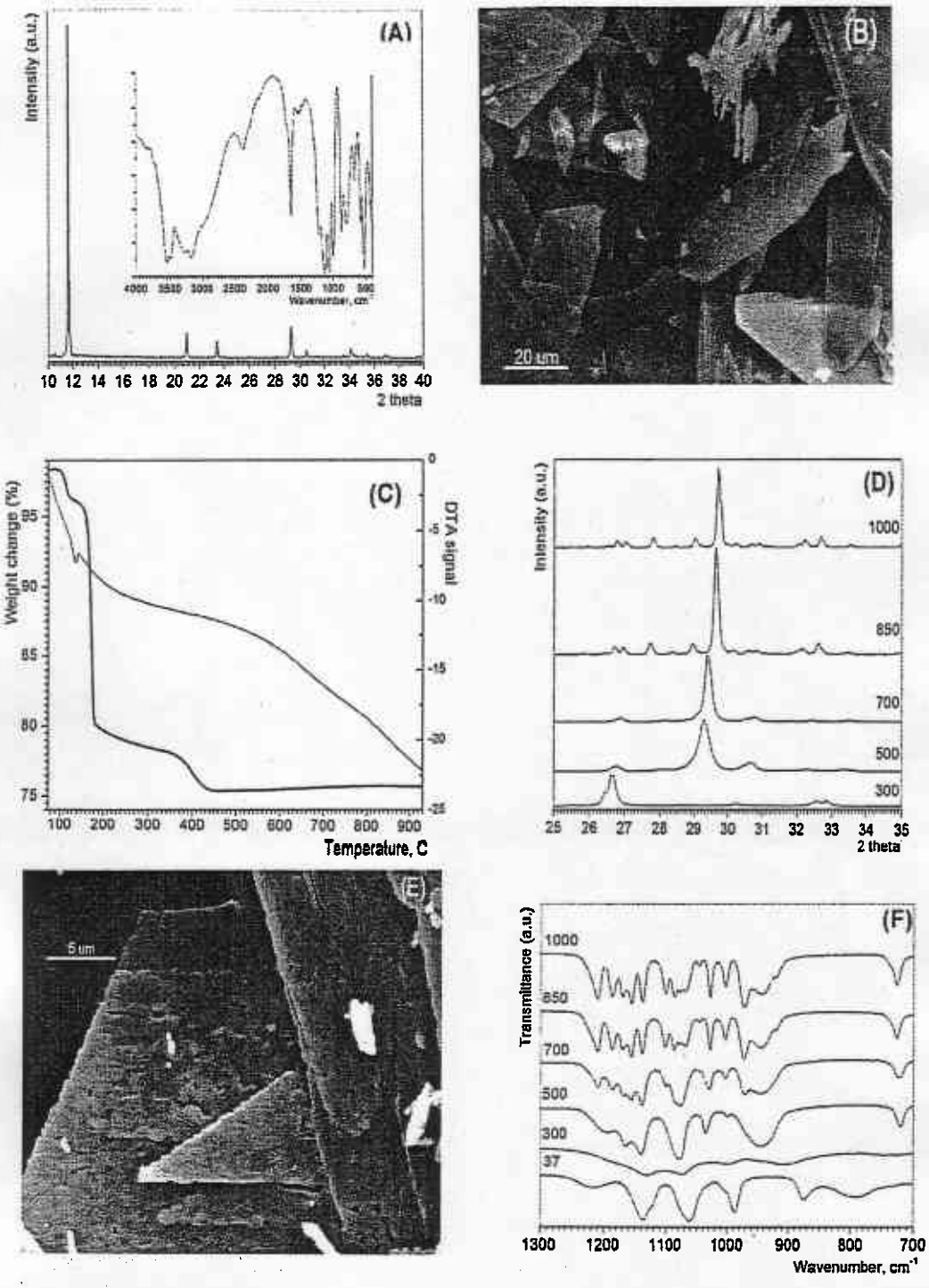


Fig. 1. *(A to C)*: XRD, FTIR, SEM, TG characterization of DCPD, *(D to F)*: Calcined DCPD

DCPD is known to be a nucleation precursor, in aqueous solutions, to the apatitic calcium phosphates [5]. DCPD transforms into the thermodynamically more stable, apatitic calcium phosphate, by a dissolution-reprecipitation mechanism [31]. DCPD has a relatively low solubility in water, and thus water alone could not be sufficient to drive the reprecipitation mechanism [32]. However, if the aqueous medium of DCPD immersion contains Ca^{2+} ions then the process will readily proceed according to the following reaction [33]:

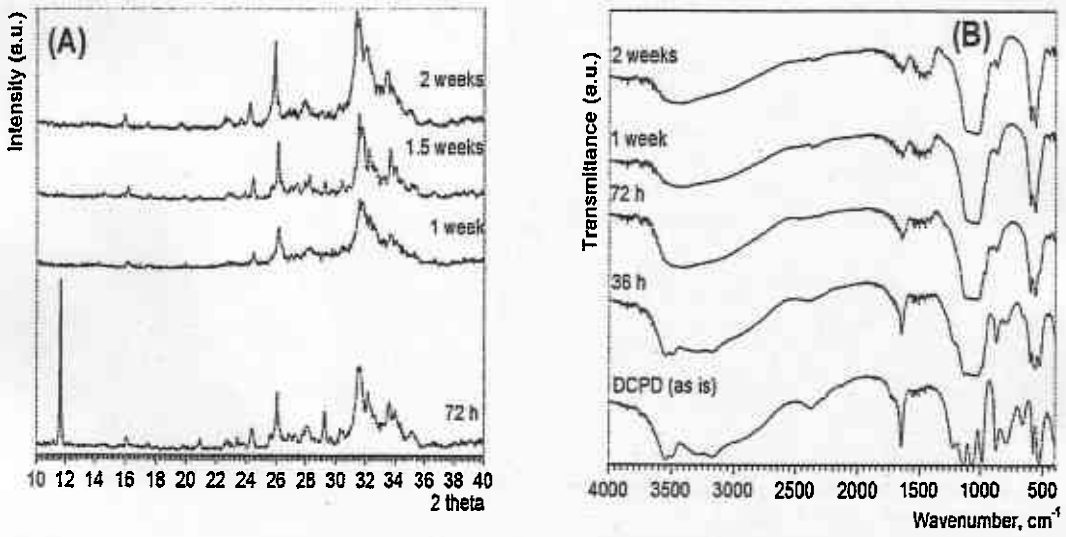
$$6CaHPO_4{\cdot}2H_2O + (4\text{-}x)CaCl_2 + (2\text{-}x)H_2O \rightarrow Ca_{10\text{-}x}(HPO_4)_x(PO_4)_{6\text{-}x}(OH)_{2\text{-}x} + (8\text{-}2x)HCl + 12H_2O \quad (3).$$

Apatite formed in reaction (3) is termed as Ca-deficient hydroxyapatite (CDHA), and this formula represents the family of apatites formed at a neutral pH. However, this formula is still a simplified version since it does not define the carbonate ions incorporated into the structure [34].

Therefore, we selected a tris-buffered, carbonated (27 mM HCO_3^-) SBF solution [25] of pH 7.4 as the immersion medium to examine the dissolution-reprecipitation mechanism of our DCPD powders as a function of time. If we were to soak the DCPD powders in pure water [11, 33], we would have mostly seen its sluggish dissolution over a period of 1 month. As seen in the XRD data of Fig. 2a for the DCPD powders immersed in SBF solutions at 37°C, even after 72 hours of soaking a significant amount of CDHA was formed as predicted by reaction (3). By the end of 1 week of soaking time, all the crystal peaks of DCPD disappeared from the XRD patterns. FTIR data given in Fig. 2b 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 [25-29]. FTIR spectra of Fig. 2b displayed that the carbonated nature of the apatitic calcium phosphate initially formed was gradually developing with an increase in soaking time. SEM photomicrographs given in Figs. 2c and 2d, as a function of immersion time in SBF, depicted the characteristic morphology of needle-like CDHA.

From X-ray or neutron crystallographic studies, crystal structure of DCPD is known to contain compact sheets or bilayers parallel to the (010) plane [26]. One bilayer was found to present sheets of calcium and phosphate ions, while the other bilayer comprised water molecules. Flade *et al.* [35] 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 explicitly, we performed a slightly modified experiment. In that experiment, we placed a 30 mL aliquot of freshly prepared Solution-A (its preparation was described in the Experimental Section) into a 50 mL-capacity glass beaker covered with Parafilm®. Beaker was then heated to 37°C in a constant temperature oven. 1 minute after the injection (with a syringe and needle) of 1 mL 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 Figure 3a 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.

To picture the dissolution behavior of those DCPD crystals, a 100 mg portion was placed in 10 mL of a freshly prepared Tas-SBF solution [25]. Following 3 hours of immersion at 37°C in SBF, the DCPD crystals were washed with water and dried at 37°C, overnight. XRD pattern of the samples again indicated single-phase DCPD, as shown in Figure 1a. SEM photomicrograph given in Figure 3b showed the dissolution of DCPD, revealing the aforementioned bilayer structure, which until now could only be ascertained by using crystallographic techniques. Reprecipitation leg of this mechanism, which leads to the apatitic calcium phosphate formation, subsequently takes place at the later stages of SBF immersion, as was 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 hydroxyapatite.



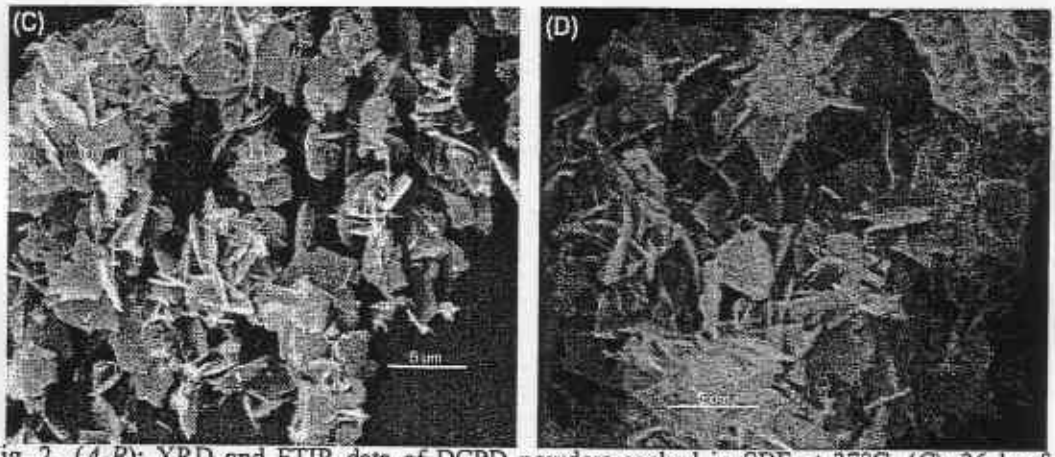


Fig. 2 (*A-B*): XRD and FTIR data of DCPD powders soaked in SBF at 37°C, (*C*): 36 h of immersion in SBF, (*D*): 1 week in SBF; apatitic needles visible in both SEM pics of (*C*) and (*D*)

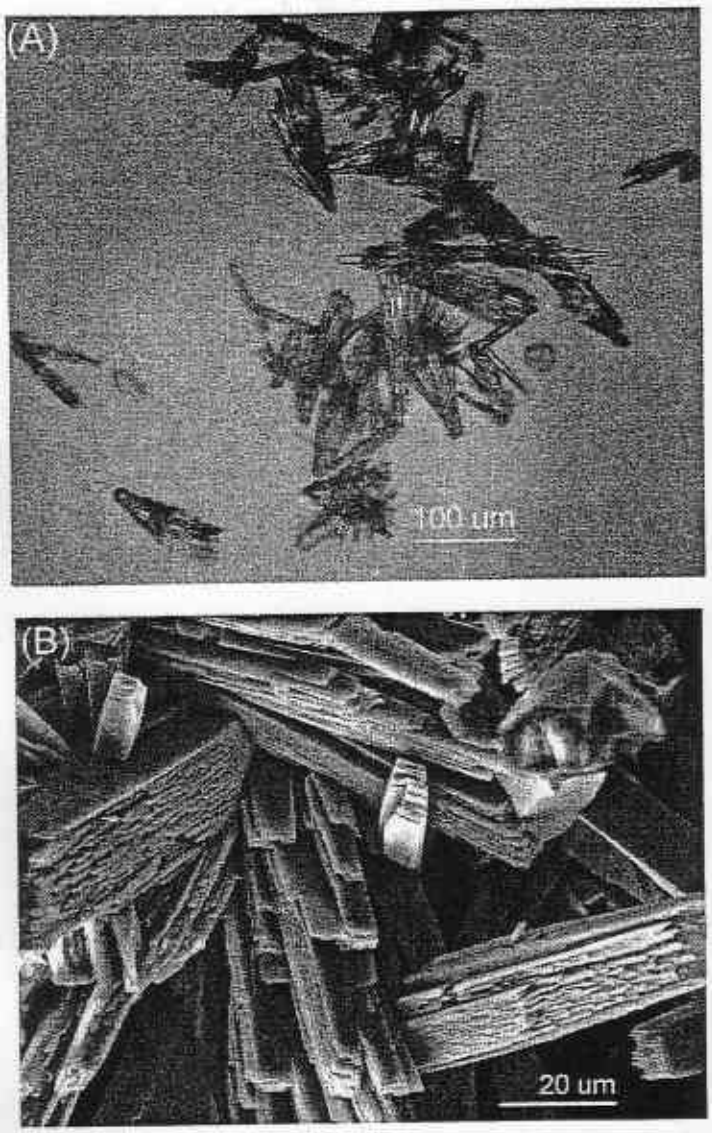


Fig. 3. (a) Early-stage crystals of DCPD formed in 1 minute, (b) dissolution of DCPD crystals in SBF at 37°C, revealing the bilayer structure

Conversion of $CaHPO_4 \cdot 2H_2O$ into poorly crystalline apatite becomes important when its possible uses as synthetic bone substitutes or bone defect filling materials are considered. Self-setting orthopedic cements based on DCPD have already been formulated and reported for their enhanced resorbability [20]. Moreover, the plate- or needle-like morphology observed in chemically prepared DCPDs might have possible uses in improving the mechanical properties (e.g., compressive and flexural strength) of the self-setting orthopedic cements, which include DCPD as a constituent [36]. Doping of DCPD powders with small amounts of Na and K 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 DCPD powders.

CONCLUSIONS

(1) The chemical process outlined here allowed the uncomplicated synthesis of biocompatible Na- and K-doped DCPD powders, with a unique plate-like morphology, by starting with aqueous solutions at the physiologic pH and temperature conditions.

(2) The results showed that it is 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 robust procedure for producing carbonated apatitic calcium phosphate powders, after simple immersion of DCPD powders in SBF solutions (pH=7.4) at 37°C. The highest temperature of processing hereby employed in the manufacture of bulk, poorly crystalline apatite powders was 37°C.

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