

# LOW-TEMPERATURE SYNTHESIS OF HA-SEEDED TTCP ( $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ) POWDERS AND THEIR *IN VITRO* APATITE-INDUCING ABILITY AT 37°C AND pH 7.4

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## ABSTRACT

This paper reports the synthesis of hydroxyapatite (HA:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ )-seeded (5 to 6 wt%) TTCP ( $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ) powders at 1230°C. This temperature is apparently much lower than the typical temperature of synthesis (i.e., 1350° to 1500°C) reported so far for TTCP powders. The starting materials used in our powder synthesis route were Ca-acetate monohydrate ( $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ ) and ammonium dihydrogen phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ). HA-seeded TTCP powders were tested for their apatite-inducing ability by soaking them in synthetic body fluid (SBF) solutions at 37°C from 36 to 96 hours. Bioactive TTCP powders of this study readily covered with carbonated apatitic calcium phosphates within the first 72 hours. Crystal structure similarity between TTCP and HA, more specifically, the presence of apatitic layers within the unit cell of TTCP causes this *in vitro* bioactivity.

## INTRODUCTION

Brown and Epstein [1] previously showed that tetracalcium diphosphate monoxide,  $\text{Ca}_4(\text{PO}_4)_2\text{O}$  (TTCP), has a structural relationship to HA;  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , which may be considered to be the idealized form of the major inorganic phase of human bone. The more commonly used name for  $\text{Ca}_4(\text{PO}_4)_2\text{O}$  is tetracalcium phosphate (from the formula  $4\text{CaO} \cdot \text{P}_2\text{O}_5$ ). TTCP crystallizes in the monoclinic unit cell  $a = 9.462$ ,  $b = 11.965$ ,  $c = 7.023$  Å,  $\beta = 90.9^\circ$  in space group  $P2_1$  with  $4[\text{Ca}_4(\text{PO}_4)_2\text{O}]$  per cell. It has a density of  $3.06 \text{ g/cm}^3$  [2]. TTCP melts at around 1710°C [3]. The magnitudes of  $a$  and  $c$  parameters of the unit cells of HA and TTCP are comparable to one another. It is also interesting to note that when one multiplies the unit cell volume of HA by a factor of 1.5, one obtains the exact value of the unit cell volume of TTCP.  $\text{Ca}_4(\text{PO}_4)_2\text{O}$  is also known to display extensive twinning, and suspected to have a high-temperature orthorhombic polymorph [4]. The positions of the P atoms and the Ca and oxide ions lie close to those required by an orthorhombic space group,  $Pm\bar{c}n$ . This is a likely explanation for the appreciable twinning exhibited by  $\text{Ca}_4(\text{PO}_4)_2\text{O}$  [5].

How can TTCP transform into apatite?  $\text{Ca}_4(\text{PO}_4)_2\text{O}$  contains a crystallographic layer similar to an "apatitic layer" present in both  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  and  $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$  (octacalcium phosphate, OCP). Because of this layer, an epitaxial relationship between TTCP and HA, as well between TTCP and OCP, may be present [5]. Epitaxy, the ordered growth of one substance on another, is often thought of as being governed chiefly by the metric fits of crystallographic networks based on the unit cell translations [5]. The combination of good metric and chemical fits between HA and OCP may cause the epitaxy between these two compounds to occur [6]. Undetected epitaxy between HA and TTCP would increase the Ca/P molar ratio of an "apparent" apatite above 1.667, and on the microscopic scale the material would resemble solid solution. HA is, therefore, an unusual substance in that it has two related salts with which it may enter into epitaxial relationships. One salt,  $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ , is more acidic, and the other,  $\text{Ca}_4(\text{PO}_4)_2\text{O}$ , is more basic [5].

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Production of  $\text{Ca}_4(\text{PO}_4)_2\text{O}$  in an aqueous environment is extremely difficult, if not impossible, because of the oxide ion. TTCP is the most soluble compound among all the calcium phosphates. In aqueous solutions hydroxyl ions incorporate themselves into the formed precipitates. A carbonate- or hydroxyl ion-containing product will form more easily. For this reason, synthesis of TTCP, which is nowadays used as one of the main components of self-setting orthopedic and dental cements, has only been limited to solid state reactive firing (SSRF) [7-9]. Tetracalcium phosphate was typically synthesized by the solid-state reaction of calcium carbonate ( $\text{CaCO}_3$ ) and dicalcium phosphate anhydrous ( $\text{CaHPO}_4$ ) powders, ball-milled with one another at a Ca/P molar ratio of 2.0. Powders prepared by Chow *et al.* [7-9] were usually heated at  $1450^\circ$  to  $1500^\circ\text{C}$  for 6 to 12 h, and then quenched to room temperature. Slow cooling of TTCP, instead of quenching, from high temperatures results in the formation of undesired secondary phases, such as HA, CaO,  $\text{CaCO}_3$ , and  $\beta\text{-Ca}_3(\text{PO}_4)_2$ . As of now, it was impossible to obtain TTCP without quenching from a temperature in excess of  $1300^\circ\text{C}$ . Rapid cooling is essential in order to prevent the formation of HA and calcium oxide, a side reaction that takes place between  $800^\circ$  and  $1250^\circ\text{C}$  in moist atmospheres [10]. Quenched, sintered body of tetracalcium phosphate was needed to be grind to a particle size less than  $45\ \mu\text{m}$ , especially if such powders were to be used in cement applications [9].

Sargin *et al.* [11] have studied the influence of different starting materials on the synthesis of TTCP powders. In this important study, Sargin *et al.* [11] have shown that if one starts with  $\text{CaCO}_3$  and  $\text{NH}_4\text{H}_2\text{PO}_4$  powder mixtures, it is possible to decrease the temperature of synthesis of single-phase TTCP down to about  $1350^\circ\text{C}$ . They also carefully analyzed the experimental X-ray and infrared data (IR) of their samples and tabulated the band assignments for TTCP for the first time. What Sargin *et al.* basically accomplished [11] was to separate Ca and P from one another in their reactants. In Chow's work [7-9, 12], in stark contrast with Sargin *et al.*, the calcium needed to form TTCP was taken from both  $\text{CaCO}_3$  and  $\text{CaHPO}_4$ . During heating,  $\text{CaCO}_3$  decomposes by evolving  $\text{CO}_2$  at around  $900^\circ\text{C}$ , and  $\text{CaHPO}_4$  first transforms into  $\text{Ca}_2\text{P}_2\text{O}_7$  [13]. Therefore, in Chow's approach, high temperatures were indeed necessary to convert the pyrophosphate ions into orthophosphates before starting to form the TTCP phase.

The discovery that the basic TTCP reacts with acid dicalcium phosphate anhydrous  $\text{CaHPO}_4$  to form pure hydroxyapatite led to the development of a novel self-setting calcium phosphate cement (CPC) by Brown and Chow in 1985 [7, 8, 14]. TTCP-containing self-setting calcium phosphate cements are now commercially available [15, 16] and have a proven track record in clinical/surgical use.

The present study investigates a new chemical synthesis route to prepare 5 to 6 wt% HA-seeded TTCP powders. One major aim is to decrease the synthesis temperature below that reported by Sargin *et al.* [11], i.e.,  $1350^\circ\text{C}$ . The powder synthesis route presented here consisted of physical mixing of stoichiometric amounts of calcium acetate monohydrate ( $\text{Ca}(\text{CH}_3\text{COO})_2\cdot\text{H}_2\text{O}$ ) and ammonium dihydrogen phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ) powders, followed by heating at  $1230^\circ\text{C}$  in air for 12 h and quenching. This process yielded finely divided powders at the lowest temperature yet reported for TTCP synthesis. Biomimetic precipitation of carbonated hydroxyapatite at  $37^\circ\text{C}$  and pH 7.4 on these powders is tested by immersing these into a synthetic body fluid (SBF) from 36 to 96 hours [17].

## EXPERIMENTAL PROCEDURE

**Powder synthesis:** HA-seeded TTCP powders were synthesized in small batches. First, 0.77 g of  $\text{NH}_4\text{H}_2\text{PO}_4$  was ground into a very fine powder by using an agate mortar and pestle. 2.45 g of  $\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$  was then added to the powder. Two powders were dry mixed in the mortar for about 45 minutes, the powder mixture was heated at  $300^\circ\text{C}$  in air for 30 minutes, followed by re-mixing in a mortar for 15 minutes. Powders were then heated at  $800^\circ\text{C}$  in air for 1 hour, followed by homogenization by further grinding. These powders had a greyish/bluish tint and were heated (with a heating rate of  $2^\circ\text{C}/\text{min}$ ) at  $1230^\circ\text{C}$  in air for 6 hours using a  $\text{MoSi}_2$  box furnace. Following firing the red-hot alumina boat containing the powders was taken out, and placed into a desiccator. Obtained powders were lightly ground and homogenized in an agate mortar for 10 minutes, and re-fired at  $1230^\circ\text{C}$  for another 6 hours, followed by quenching to RT. Powders obtained were fluffy and easily ground into a fine powder after few minutes of manual grinding in an agate mortar. This procedure is suitable for the synthesis of HA-seeded TTCP powders. Caution was taken not to overgrind the obtained TTCP powders, since they are prone to transform into HA even by a small presence of mechanochemical activation.

**In vitro testing:** Apatite-inducing ability of HA-seeded TTCP powders was tested by immersion of the powders in a synthetic body fluid (SBF) solution. The details of preparing these solutions have been previously reported [17]. Briefly, the SBF solution used was a *tris*/HCl-buffered, 27.0 mM  $\text{HCO}_3^-$  ion-containing solution with the following ion concentrations: 2.5 mM  $\text{Ca}^{2+}$ , 1 mM  $\text{HPO}_4^{2-}$ , 27 mM  $\text{HCO}_3^-$ , 142 mM  $\text{Na}^+$ , 5 mM  $\text{K}^+$ , 1.5 mM  $\text{Mg}^{2+}$ , 0.5 mM  $\text{SO}_4^{2-}$ , 125 mM  $\text{Cl}^-$ , *tris*-buffered at  $\text{pH}=7.4$ . The solutions hereby used had the same carbonate ion concentration with that of human blood plasma. In vitro tests were performed in 90 mL-capacity glass bottles, which contained 2.0 grams of HA-seeded TTCP powders and about 90 mL of SBF solution. Tightly sealed bottles were placed into an oven at  $37 \pm 1^\circ\text{C}$  and kept undisturbed in that oven over the entire sampling times of 36, 72 and 96 hours. At the end of the stated immersion periods, partly solidified samples were washed with deionized water, and dried overnight at  $37^\circ\text{C}$ .

**Characterization:** Phase assemblage of powder samples was analyzed using a powder X-ray diffractometer (XDS-2000, Scintag Corp.), operated at 40 kV and 30 mA, equipped with a Cu-tube, using a step size of  $0.01^\circ 2\theta$ . Fourier-transformed infrared reflective spectroscopy (Nicolet 550, Thermo Corp.) analyses were performed on the powder samples using a diamond ATR holder. Thermogravimetric analyses (TGA, Mettler Corp.) were performed in air on the starting chemicals of our powder synthesis over the range of 20 to  $740^\circ\text{C}$ , with a scan rate of  $5^\circ\text{C}/\text{min}$ . Surface morphology of the samples was studied using a Field-emission SEM (S-4700, Hitachi) after coating the samples for 4 to 6 minutes with Cr or Au.

## RESULTS AND DISCUSSION

Thermogravimetric data for the starting chemicals of powder synthesis are shown in Figure 1.  $\text{NH}_4\text{H}_2\text{PO}_4$  melts at around  $200^\circ\text{C}$ , and forms an acidic liquid. This acidic liquid immediately reacts with Ca-acetate, which until  $200^\circ\text{C}$  has already lost its water of hydration. The purpose of heating the powder mixture first to  $300^\circ\text{C}$  and then grinding the precursors at this stage is the homogenization of the reaction products of molten ammonium dihydrogen phosphate

and calcium acetate. Upon continued heating to 800°C, volatilization of  $\text{NH}_3$  from  $\text{NH}_4\text{H}_2\text{PO}_4$ , followed by its condensation to highly-reactive  $\text{P}_2\text{O}_5$  is completed, whereas acetone, carbon dioxide and water vapor would be the main decomposition products of Ca-acetate. An amorphous intermediate (consisting of long chains of condensed phosphate of the form  $(\text{PO}_3)_n$ ) will form as a result of these reactions, which will be converted into partially crystalline  $\beta\text{-Ca}(\text{PO}_3)_2$  with an increase in temperature [18]. At this stage, presence of small amounts of  $\text{Ca}_2\text{P}_2\text{O}_7$  may also be expected, however, this was not detected in this study. TTCP powders obtained after 2<sup>nd</sup> step of 1230°C firing/quenching has the characteristic XRD diagram given in Figure 2. These powders contained around 5 to 6 wt% of HA phase. Owing to the overall stoichiometry of the initial powder mixtures, detection of HA in the powders should automatically call for a small presence of  $\text{Ca}(\text{OH})_2$  or CaO in them. Major peaks of CaO and  $\text{Ca}(\text{OH})_2$  are around 37.36° and 34.17° 2 $\theta$ , respectively, which are probably overlapping by reflections from the TTCP phase. Most HA peaks also overlap with those of TTCP, and the only peak of HA is encountered at 25.88° 2 $\theta$ .

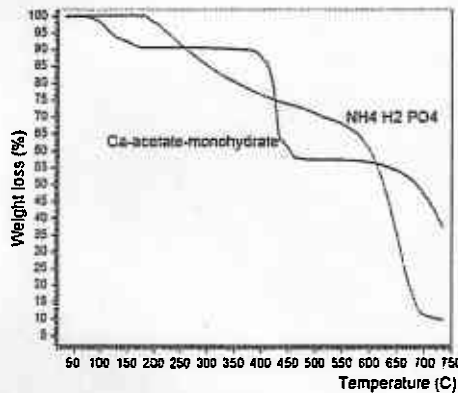


Fig. 1 TG traces of the starting chemicals

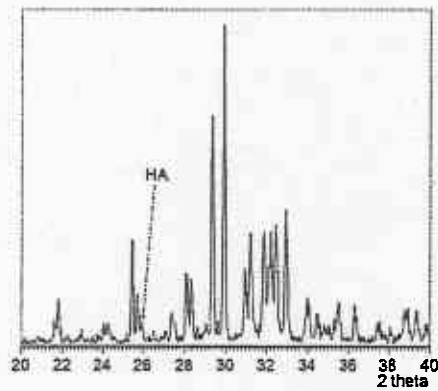
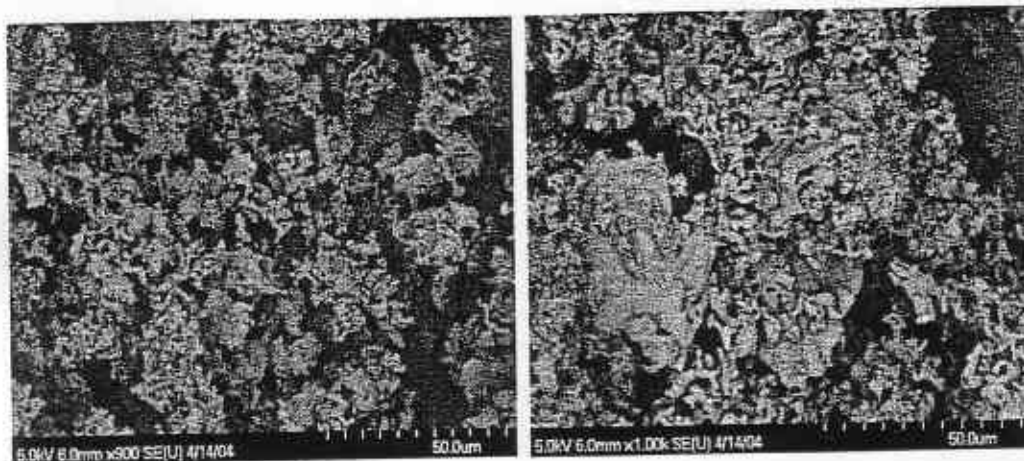


Fig. 2 XRD data of HA-seeded TTCP

The addition of HA seeds into TTCP powders have previously been tested by Hamanishi *et al.* [19], however that study intentionally added about 40 wt% precipitated HA into a cement mixture of TTCP- $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ . The level of HA-seeding in the present study is much lower than this. Particle morphology of the HA-seeded TTCP powders are given in Figures 3 and 4 below.



Figs. 3 and 4 (l-to-r) Morphology of the HA-seeded TTCP powders synthesized at 1230°C

Powders displayed a unique vermicular microstructure, which was indicative of the vapor- and liquid-phase reactions taking place. The remnants of the amorphous-looking chunks of condensed phosphates were observed in the form of isolated larger chunks. Good handling properties (between the fingers of an experienced self-setting cement practitioner) when wetted with a small amount of water ( $L/P = 0.1$  to  $0.2$  mL/g), of these powders can be ascribed to this microstructure. A decrease in the average particle size of a cement powder typically leads the way to using lesser volumes of setting starter solution, which means reaching a higher compressive strength in the studied cement system [20]. On the other hand, TTCP powders synthesized at 1450° or 1500°C, using the starting chemicals  $\text{CaCO}_3$  and  $\text{CaHPO}_4$ , do not possess such fine particle morphology, and they need grinding of the sintered ceramics to particle sizes less than 40-45  $\mu\text{m}$  [9, 21]. It has recently been reported by Gbureck *et al.* [22] that ball milling a TTCP powder synthesized at 1500°C even in pure ethanol ensures its complete transformation into HA in 24 hours. This obviously means that ball milling of TTCP strongly degrades its cement properties. The same concern is also applicable to the most versatile constituent of self-setting calcium phosphate cement formulations, i.e.,  $\alpha$ -TCP [23].

FTIR pattern of the HA-seeded TTCP powders is depicted in Figure 5. This experimental pattern precisely matches those previously reported for tetracalcium phosphate by Sargin *et al.* [11] and Posset *et al.* [24], respectively. Vibrational wavenumbers were assigned by the above-mentioned researchers. In the data of Figure 5, threefold degenerate stretching ( $1105$ - $989$   $\text{cm}^{-1}$ ) mode ( $\nu_3$ ), symmetric stretching ( $962$ - $941$   $\text{cm}^{-1}$ ) mode ( $\nu_1$ ), and threefold degenerate deformational ( $620$ - $571$   $\text{cm}^{-1}$ ) mode ( $\nu_4$ ) vibrations were all visible [11, 24]. The inset in Fig. 5 displays the characteristic OH stretching vibration ( $3650$   $\text{cm}^{-1}$ ), originating from  $\text{Ca}(\text{OH})_2$  [11]. On the other hand, the weak band at  $872$   $\text{cm}^{-1}$  belongs to carbonate groups, and those were considered to be associated with the HA present.

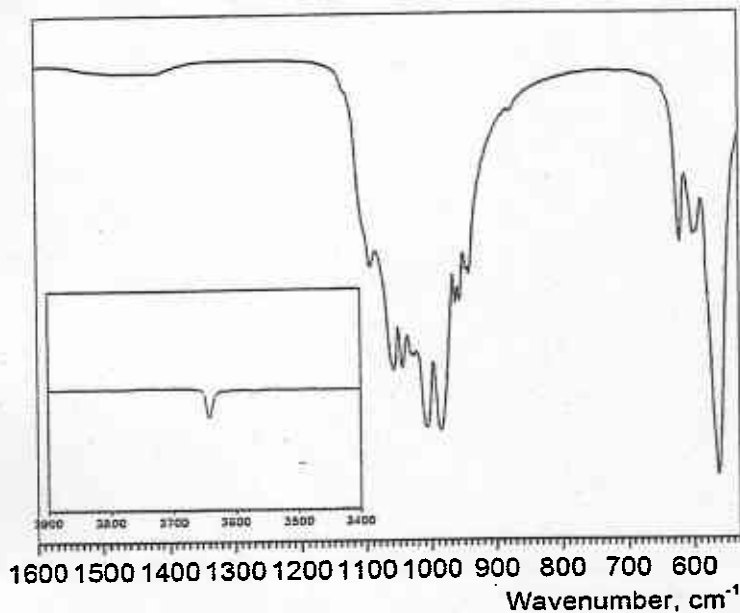


Fig. 5 FTIR trace of HA-seeded TTCP powders

SBF solutions are able [25] to induce apatitic calcium phosphate formation on metals, ceramics or polymers (with proper surface treatments). It is also known that an amorphous calcium phosphate (ACP) precursor is always present during the precipitation of apatitic calcium phosphates from highly supersaturated solutions [26]. Posner *et al.* [27] proposed that the process of ACP formation in solution involved the formation of  $\text{Ca}_9(\text{PO}_4)_6$  clusters which then aggregated randomly to produce the larger spherical particles or globules, with the intercluster space filled with water. Such clusters, we believe, are the transient solution precursors to the formation of carbonated globules with the nominal stoichiometry of  $\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_2$ , where  $x$  might be converging to 1 [28]. Onuma *et al.* [29] have demonstrated, by using dynamic light scattering, the presence of calcium phosphate clusters from 0.7 to 1.0 nm in size even in clear simulated body fluids. They reported the presence of calcium phosphate clusters in SBF even when there was no visible precipitation. Therefore, one only needs some suitable surface to be immersed into an SBF solution, which would likely trigger the hexagonal packing [29] of those nanoclusters into globules or spherulites of apatitic calcium phosphates. SEM photomicrographs given in Figures 6 through 9 below exhibit the rapid formation of those apatitic calcium phosphate globules after soaking the HA-seeded TTCP powders in SBF for 36, 72, and 96 hours at 37°C, respectively. These are bioactive powders.

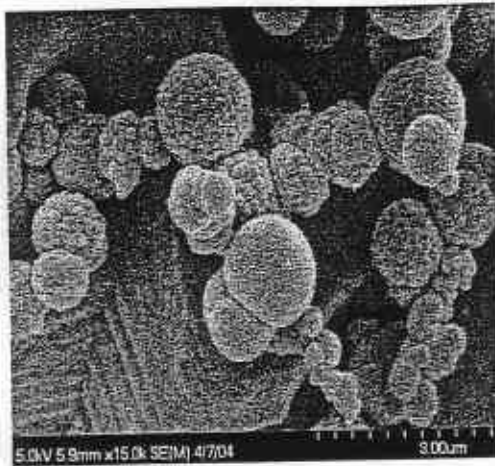


Fig. 6 36 h in SBF at 37°C

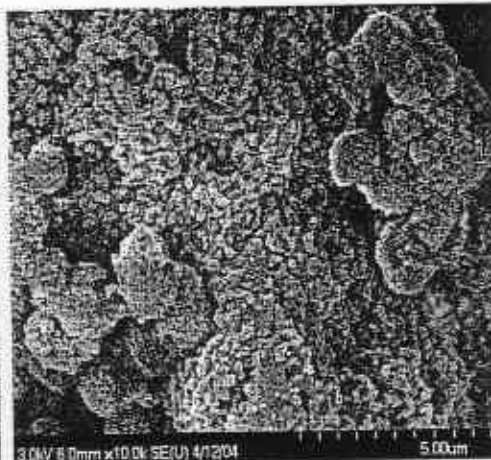


Fig. 7 72 h in SBF at 37°C

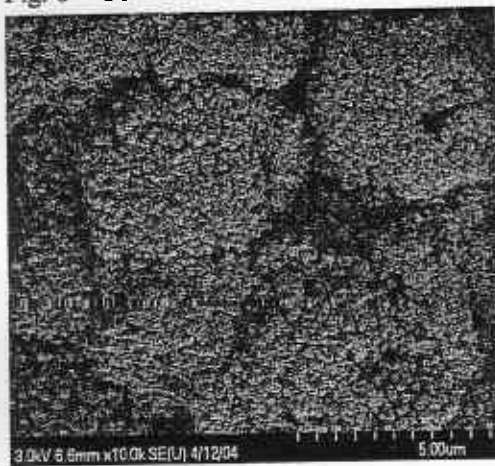


Fig. 8 96 h in SBF at 37°C (high mag)



Fig. 9 96 h in SBF at 37°C (low mag)

Figure 6 reveals the twinning in the TTCP grains, which was first mentioned by Dickens *et al.* [5] in a purely crystallographic study, more than three decades ago. In a supersaturated (with respect to apatite formation) solution, such as SBF, the apatitic layers of TTCP readily react with the calcium phosphate globules (Fig. 6), and within the subsequent 36 hours the external surfaces of the TTCP grains totally transform into apatite (Fig. 7). Why do we say “the external surfaces or skins?” Simply because the XRD and FTIR data of these samples (as given below in Figures 10 and 11) affirmed that the bulk of these samples was still TTCP. As a function of increasing soaking time in SBF, the overall XRD peak intensities were inclined to slightly decrease, with an accompanying significant increase in the intensities of the HA reflections (Fig. 10, \* indicates HA peaks). FTIR data of Figure 11 (where \* indicates carbonate groups) showed the appearance of a pronounced band at  $1420\text{ cm}^{-1}$  in the high-energy C–O region along with a well-defined band at  $872\text{ cm}^{-1}$ , known to be specific for a carbonated apatite [30]. OH bands were seen at  $3571\text{ cm}^{-1}$  after soaking in SBF.

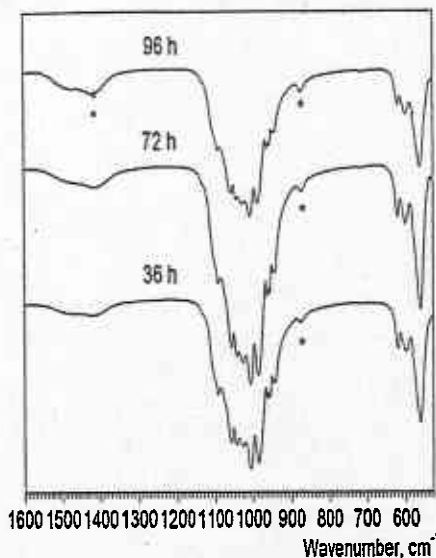
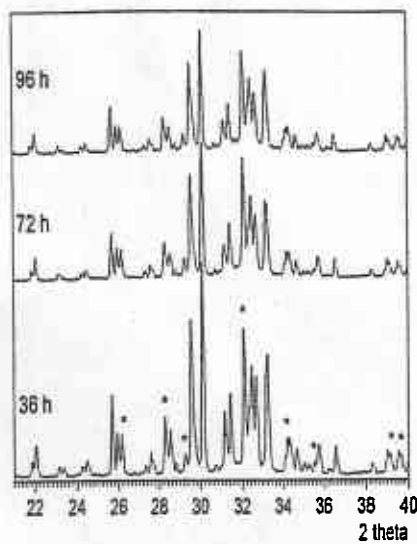


Fig. 10 XRD traces of SBF-soaked samples    Fig. 11 FTIR traces of SBF-soaked samples

We have also noted that the surface pH values of the as-synthesized HA-seeded TTCP powders were in the vicinity of 11.8 (measured after 15 minutes of adding a 50 mg portion of dry powder to 10 mL of deionized water to form a suspension, while a pH electrode is being inserted into this suspension). Such high pH values observed in such TTCP powders may cause cell *necrosis*, in case they were directly implanted *in vivo*. However, as shown in Figure 12 below, with an increase in soaking time in an SBF solution (at 37°C) of pH 7.4, pH starts decreasing. This is ascribed to the transformation of HA-seeded TTCP powders into carbonated apatitic calcium phosphates formed under biomimetic conditions provided *in vitro* by the SBF solutions. On the other hand, for comparison purposes, pure, stoichiometric, crystalline hydroxyapatite has a pH value of about 9.5. After 96 hours of soaking in SBF, recorded pH value was 9.56. It is reasonable to assume at this point that this pH value would have continued to drop toward the physiological pH, if we had continued the soaking beyond 4 days [31, 32]. The pH diagram of TTCP powders given in Figure 12 may very well serve the practitioner to precisely tailor the “amount of an acidic calcium phosphate (DCPA, DCPD, etc.) that needs to be blended” with these powders in designing a TTCP-based cement formulation having a neutral pH at the very start of the cement-forming process.

Being the calcium phosphate compound of highest solubility at the physiological pH, the hydrolysis of TTCP should be regarded as a process of initial dissolution followed by the precipitation of a less soluble phase. In an SBF environment (i.e., 37°C and pH 7.4), the precipitating phase can only be nanosize, carbonated hydroxyapatite, i.e., the bone mineral. The presence of HA seeds in TTCP simply accelerates this *in vitro* biomineralization process.



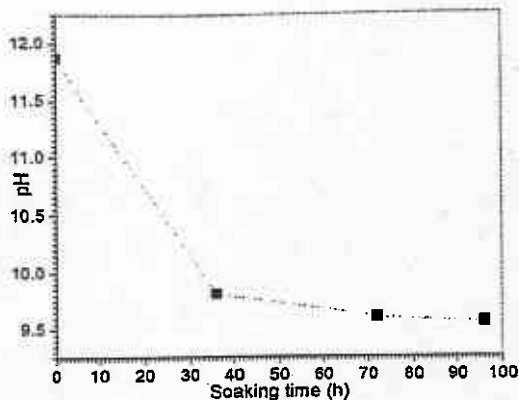


Fig. 12 Change in surface pH of HA-seeded TTCP in SBF

## CONCLUSIONS

- (1) 5 to 6 wt% HA-seeded TTCP ( $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ) powders were synthesized at  $1230^\circ\text{C}$ , by using the starting chemicals of calcium acetate monohydrate and ammonium dihydrogen phosphate, followed by quenching from the synthesis temperature.
- (2) This is the lowest temperature ever reported for the manufacture of TTCP powders.
- (3) HA-seeded TTCP powders can readily be used in the production of self-setting calcium phosphate cements, after physical blending of those with an acidic calcium phosphate salt, such as DCPA, DCPD, OCP or monocalcium phosphates.
- (4) HA-seeded TTCP powders were shown to have a very strong apatite-inducing ability when they are brought into contact with SBF solutions of pH 7.4 at  $37^\circ\text{C}$ , within the first 36 to 96 hours.

## ACKNOWLEDGMENTS

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