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# Synthesis of HA-Seeded TTCP ($Ca_4(PO_4)_2O$) Powders at 1230°C from $Ca(CH_3COO)_2 \cdot H_2O$ and $NH_4H_2PO_4$

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**Tetracalcium phosphate (TTCP) $Ca_4(PO_4)_2O$ is one of the major powder components of self-setting orthopedic and dental cements. Traditionally, TTCP powders are produced by a solid-state process by soaking Ca- and P-containing precursors between 1350° and 1500°C. Such procedures require expensive high-temperature furnaces and subsequent grinding of sintered particulates. Grinding not only introduces contamination but alters the structure of TTCP, thereby reducing its bioactivity. The present paper offers a lower temperature synthesis process for TTCP with several interesting features. First, the synthesis procedure used $Ca(CH_3COO)_2 \cdot H_2O$ and $NH_4H_2PO_4$ as separate sources for Ca and P, respectively. Second, the reactants underwent multiple melting and decomposition stages, thus increasing the reactivity of the synthesis process. $NH_4H_2PO_4$ melted at 190°C and engulfed the calcium acetate particles. The Ca-acetate component decomposed into $CaCO_3$ at around 400°C while still surrounded by the molten phosphate liquid and an amorphous Ca-metaphosphate phase. Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ (HA), and β-$Ca_3(PO_4)_2$ crystallized upon heating the powder mixture to 700°C. Slightly above 1200°C, the TTCP phase was formed. This sequence of reactions led to a process temperature of 1230°C, the lowest temperature ever reported for the synthesis of TTCP. Third, the resulting powders required much less grinding, which itself is advantageous. Fourth, the resulting powders were *in situ* seeded with HA. HA-seeded TTCP powders were tested for their apatite-inducing ability by soaking them in synthetic body fluid at 37°C. TTCP powders of this study were readily covered with carbonated apatitic calcium phosphates within the first 72 h.**

## I. Introduction

In a study, Brown and Epstein[1] first showed that tetracalcium diphosphate monoxide, $Ca_4(PO_4)_2O$ (TTCP), has a structural relationship with hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ (HA). HA may be considered as the idealized form of the major inorganic phase (i.e., carbonated and substituted biological apatite) of human bones and teeth. The more usual name for $Ca_4(PO_4)_2O$ is tetracalcium phosphate, from the formula when written as $4CaO \cdot P_2O_5$. TTCP crystallizes in a monoclinic unit cell with the lattice parameters of $a = 9.462$ Å, $b = 11.965$ Å, $c = 7.023$ Å, and $\beta = 90.9°$. It has the space group $P2_1$ with $4[Ca_4(PO_4)_2O]$ per unit cell (i.e., $Z = 4$), with a density of 3.06 g/cm$^3$.[2] TTCP melts at around 1710°C.[3] The magnitudes of $a$ and $c$ parameters of the unit cells of HA (hexagonal, $P6_3/m$, $a = 9.438$ Å, and $c = 6.882$ Å) and TTCP are comparable with one another.[1] 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.[4] $Ca_4(PO_4)_2O$ is also known to display extensive twinning and suspected to have a high-temperature orthorhombic polymorph.[5] The positions of the P atoms and the Ca and oxide ions lie close to those required by an orthorhombic space group, *Pmcn*. This probably explains the appreciable twinning exhibited by $Ca_4(PO_4)_2O$.[4]

How can TTCP transform into apatite? $Ca_4(PO_4)_2O$ contains a crystallographic layer similar to an "apatitic layer" present in both $Ca_{10}(PO_4)_6(OH)_2$ and $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ (octacalcium phosphate, OCP). As a consequence of this layer, an epitaxial relationship between TTCP and HA, as well as between TTCP and OCP, may be present.[4] Epitaxy is often thought of as being governed chiefly by the metric fits of crystallographic networks based on the unit cell translations.[4] The combination of good metric and chemical fits between HA and OCP makes the epitaxy between these two compounds possible.[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 a solid solution. HA is, therefore, an unusual substance in that it has two closely related salts with which it may enter into epitaxial relationships. One salt, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, is more acidic, and the other, $Ca_4(PO_4)_2O$, is more basic than the bone mineral, HA.[4]

TTCP is the most soluble compound among all the calcium phosphates.[1] Production of $Ca_4(PO_4)_2O$ in an aqueous environment is extremely difficult, if not impossible, because of the oxygen ion in its formula. In aqueous solutions hydroxyl (OH) ions incorporate themselves into the formed precipitates. A carbonate- and/or hydroxyl ion-containing apatitic product will form readily if one attempts the synthesis of calcium phosphates in a basic aqueous solution with a Ca/P molar ratio of 2. 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 practices.[7–9] TTCP was typically synthesized by the solid-state reaction of calcium carbonate ($CaCO_3$) and dicalcium phosphate anhydrous ($CaHPO_4$) powders, mixed with one another at a Ca/P molar ratio of 2. Powders prepared by Brown and Chow,[7] Chow and Takagi,[8] and Matsuya *et al.*[9] were usually heated at 1450°–1500°C for 6–12 h, and then rapidly quenched to room temperature. Slow cooling of TTCP, instead of quenching, from high temperatures resulted in the formation of undesired secondary phases, such as HA, CaO, $CaCO_3$, and β-$Ca_3(PO_4)_2$. Greish *et al.*[10] very recently synthesized TTCP powders by mixing $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$ powders in *n*-heptane, followed by heating and quenching from 1310°C. As of now, it has been impossible to produce TTCP powders without quenching from a temperature in excess of 1300°C. Rapid cooling is essential in order to prevent the total decomposition of TTCP into HA and $CaO/Ca(OH)_2$.[11] For use as a constituent of a cement, sintered bodies of TTCP need grinding to a particle size less than 45 μm.[9] The use of higher synthesis temperatures, which further promotes sintering and grain growth, places a significant economic burden on the manufacturer in the form of costly grinding operations. Such grinding practices may also be regarded as sources of further contamination.

Sargin *et al.*[12] studied, for the first time, the influence of different starting materials on the synthesis of TTCP powders. In

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this important study, Sargin *et al.*[12] showed that by using $CaCO_3$ and $NH_4H_2PO_4$ as reactants, it was possible to decrease the temperature of synthesis of single-phase TTCP down to about 1350°C. An interesting feature reported in Sargin *et al.*[12] was that, for the first time, separate sources of Ca and P were used. In Brown and Chow,[7] Chow and Takagi,[8] Matsuya *et al.*,[9] Greish *et al.*,[10] and Bian *et al.*[13] calcium needed to form TTCP came from both $CaCO_3$ and $CaHPO_4$ or from $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$. During heating, $CaCO_3$ decomposes by evolving $CO_2$ at around 900°C, and $CaHPO_4$ first transforms into $Ca_2P_2O_7$.[14] Therefore, in Chow's solid-state approach, high temperatures (>900°C) were deemed necessary to even start the reactions between the starting materials with one another.[7–9,13]

The discovery that the basic TTCP reacts with acidic dicalcium phosphate anhydrous, $CaHPO_4$, to form pure HA led to the development of a self-setting calcium phosphate cement (CPC) by Brown and Chow.[7,8,15] TTCP-containing self-setting CPCs are now commercially available (Norian® SRS®, Skeletal Repair System, Synthes Corp., Oberdorf, Switzerland; Biopex®, Mitsubishi Materials Corp., Tokyo, Japan)[16] and have a proven track record in clinical/surgical use.

In the present study we investigated a new chemical synthesis route to prepare HA-seeded TTCP powders. One major aim was to decrease the synthesis temperature even below that reported by Sargin *et al.*[12], i.e., 1350°C. Formation of TTCP, from the starting chemicals, was monitored by using X-ray diffraction, thermogravimetric/differential thermal analysis (TG/DTA), scanning electron microscopy, and Fourier-transform infrared spectroscopy. The powder synthesis route we present here simply consisted of physical mixing of the stoichiometric amounts of calcium acetate monohydrate ($Ca(CH_3COO)_2 \cdot H_2O$) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$) powders, followed by heating at 1230°C in air in a box furnace for 12 h and quenching. This process enabled us to obtain powders at the lowest temperature ever reported for the formation of TTCP phase. Any significant decrease in the synthesis temperature of such self-setting orthopedic cement constituents is extremely important, because well-sintered TTCP samples produced by soaking between 1400° and 1500°C need to be ground into a powder prior to their use as a biomaterial. Grinding of TTCP powders would damage their stoichiometry and consequently alter their *in vivo* performance.[17] Biomimetic (i.e., with the help of an aqueous solution mimicking the ion concentrations of human blood plasma at 37°C and pH 7.4) formation of carbonated apatitic calcium phosphates on these powders was tested and confirmed by immersing these into a synthetic body fluid (SBF) from 36 to 96 h.[18]

## II. Experimental Procedure

### (1) Powder Synthesis

HA-seeded TTCP powders were synthesized in small batches. First, 0.77 g of $NH_4H_2PO_4$ (>99%, Fisher Scientific, Fairlawn, NJ) was ground into a very fine powder by using an agate mortar and pestle. 2.45 g of $Ca(CH_3COO)_2 \cdot H_2O$ (>99%, Fisher) was then added to it. Two powders were dry mixed in the mortar for about 45 min without applying too much force. The powder mixture was then heated at 300°C in air for 30 min, followed by re-mixing in a mortar for 15 min. Powders were then heated at 800°C in air for 1 h, followed by a homogenization mixing in an agate mortar. These powders had a gray/bluish tint, and they were heated (with a heating rate of 2°C/min) at 1230°C in air for 6 h. At the end of 6 h of heating at 1230°C in a box furnace, the red-hot alumina boat containing the powders was taken out and immediately placed into a desiccator. Obtained powders were only lightly ground and homogenized in an agate mortar for few minutes and re-fired at 1230°C for another 6 h, followed by similar quenching to room temperature. Powders obtained were easy to grind in the agate mortar. This firing scheme was only suitable for the synthesis of HA-seeded TTCP powders. We were careful not to over grind the resultant TTCP powders, because they are known to be prone to transform into HA even by the slightest presence of mechanochemical activation.[17]

**Table I. Preparation of SBF Solution (1 L)[20]**

| *Order* | *Reagent* | *Weight (g)* |
|---|---|---|
| 1 | NaCl | 6.547 |
| 2 | $NaHCO_3$ | 2.268 |
| 3 | KCl | 0.373 |
| 4 | $Na_2HPO_4 \cdot 2H_2O$ | 0.178 |
| 5 | $MgCl_2 \cdot 6H_2O$ | 0.305 |
| 6 | $CaCl_2 \cdot 2H_2O$ | 0.368 |
| 7 | $Na_2SO_4$ | 0.071 |
| 8 | $(CH_2OH)_3CNH_2$ | 6.057 |
| *Ion* | *Human plasma* | *SBF (mM)* |
| $Na^+$ | 142 | 142 |
| $Cl^-$ | 103 | 125 |
| $HCO_3^-$ | 27 | 27 |
| $K^+$ | 5 | 5 |
| $Mg^{2+}$ | 1.5 | 1.5 |
| $Ca^{2+}$ | 2.5 | 2.5 |
| $HPO_4^{2-}$ | 1 | 1 |
| $SO_4^{2-}$ | 0.5 | 0.5 |

SBF, synthetic body fluid.

### (2) In Vitro Testing

Apatite-inducing ability of HA-seeded TTCP powders was tested by soaking the powders in an SBF solution. The details of preparing these solutions were previously given elsewhere.[18] Briefly, the SBF solution we used was a *tris*/HCl-buffered, 27.0 mM $HCO_3^-$ ion-containing solution with the ion concentrations given in Table I.

The SBF solutions utilized in this study had the same carbonate ion concentration as that of human blood plasma. *In vitro* tests were performed in 100 mL capacity glass bottles, which contained 2 g of HA-seeded TTCP powders and 90 mL of SBF solution. Tightly sealed bottles were placed in an oven at 37° ± 1°C and kept undisturbed in that oven over the entire duration of the sampling times of 36, 72, and 96 h. At the end of the stated immersion periods, partly solidified/hardened samples were washed with an ample supply of deionized water, followed by overnight drying at 37°C, after measuring the pH values in the SBF solutions at 37°C. These SBF soaking experiments were performed in duplicate.

### (3) Characterization

Phase assemblage of all the powder samples was analyzed by using a powder X-ray diffractometer (XDS-2000, Scintag, Sunnyvale, CA), operated at 40 kV and 30 mA, equipped with a Cu-tube. A step size of 0.01° 2θ was used in collecting the XRD data. Fourier-transformed infrared reflective spectroscopy (Nicolet 550, Thermo-Nicolet, Woburn, MA) analyses were performed, again, on all the powder samples, by using a diamond ATR holder. TG/DTA (Model 851e, Mettler-Toledo, Columbus, OH) were performed in an air atmosphere only on the starting chemicals of our powder synthesis route over the range of 30°–1200°C, with a scan rate of 5°C/min. Surface morphology of the samples was studied by using a field-emission scanning electron microscope (SEM) (S-4700, Hitachi, Tokyo, Japan). Samples were coated with a thin layer of Cr or Au prior to imaging. Particle sizes of the resultant powders were deduced from the SEM micrographs. The BET surface area of the powder samples (in triplicate, $n = 3$) was determined by applying the standard Brunnauer–Emmet–Teller method to the nitrogen adsorption isotherm obtained at −196°C using a Micromeritics, ASAP 2020 instrument (Micromeritics Corp., Norcross, GA). All samples were outgassed at 25°C for 6 h prior to each adsorption experiment. The purpose of this step was to eliminate

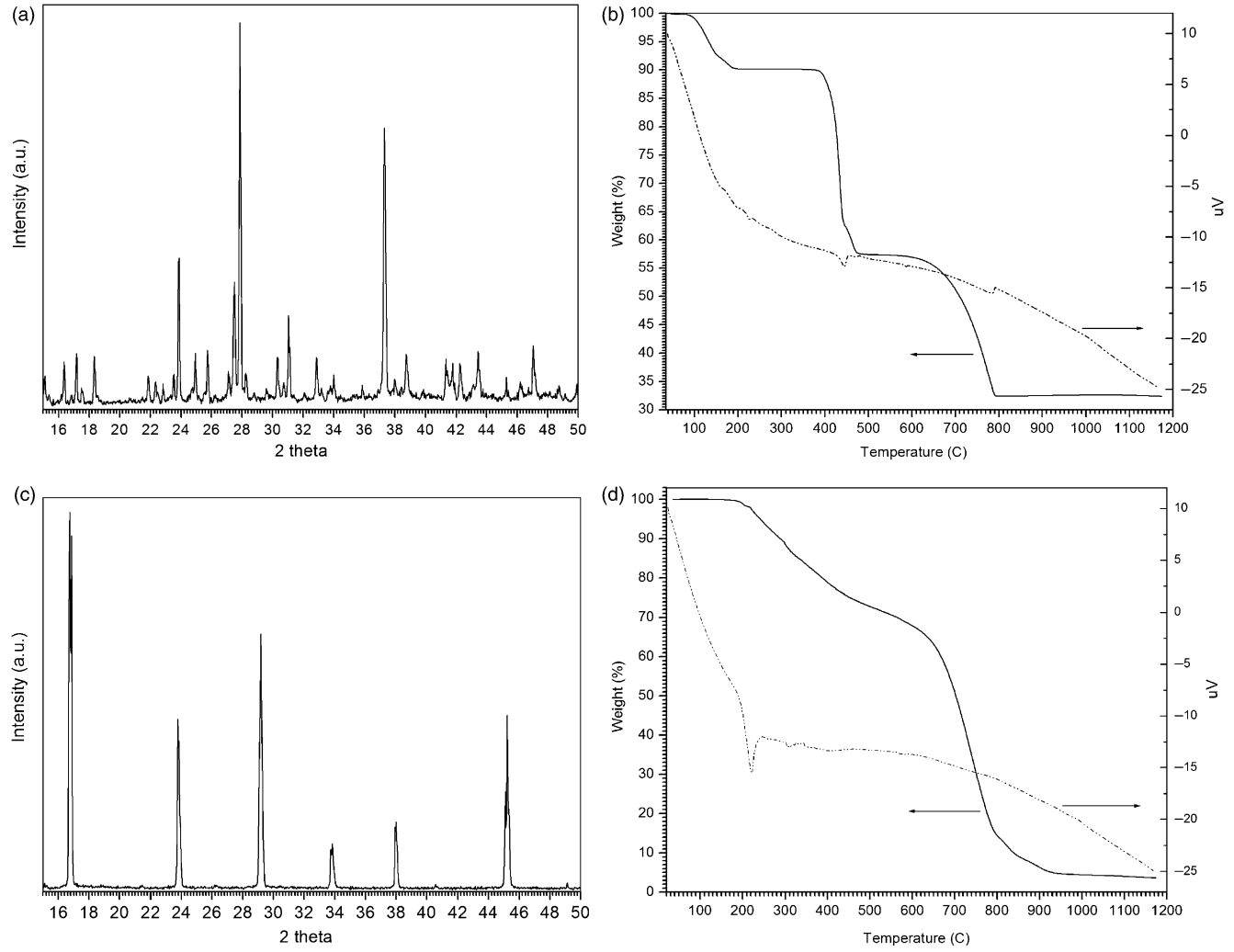


**Fig. 1.** (a) X-ray diffraction (XRD) spectrum of pure $Ca(CH_3COO)_2 \cdot H_2O$ powders. (b) Thermogravimetric and differential thermal analyses (TG/DTA) traces for pure $Ca(CH_3COO)_2 \cdot H_2O$ powders (in air). (c) XRD spectrum of pure $NH_4H_2PO_4$ powders. (d) TG/DTA traces for pure $NH_4H_2PO_4$ powders (in air).

traces of water or other adsorbed gases that may be present on the powder surfaces.

## III. Results and Discussion

XRD and TG/DTA data for the pure starting chemicals (Ca-acetate monohydrate and ammonium dihydrogen phosphate) of powder synthesis are given in Fig. 1. The FTIR traces of the starting chemicals, i.e., pure $NH_4H_2PO_4$ and $Ca(CH_3COO)_2 \cdot H_2O$, are given in Fig. 2. The FTIR trace for the unheated powder mixture, which has the stoichiometry of TTCP, is also given in Fig. 2 for comparison purposes. $NH_4H_2PO_4$ melts at around 190°C and forms an acidic phosphate liquid.[19] $NH_4H_2PO_4$ was chosen here over $(NH_4)_2HPO_4$ mainly because of (1) its lower ammonium content and (2) its definite melting point. This phosphate liquid immediately reacts with $Ca(CH_3COO)_2 \cdot H_2O$, which prior to reaching the melting point of $NH_4H_2PO_4$ (190°C) would have already lost its water of hydration and transformed into calcium acetate anhydrous. The following reactions took place until the temperature reached 200°C:

$$Ca(CH_3COO)_2 \cdot H_2O(s) \rightarrow Ca(CH_3COO)_2(s) + H_2O(g) \quad (1)$$

$$NH_4H_2PO_4(s) \rightarrow NH_4H_2PO_4(l) \quad (2)$$

Equation (1) envisages a weight loss of 10.22%. Calcium acetate anhydrous started its decomposition at around 400°C releasing volatiles, acetone in an air atmosphere, leaving behind finely particulated $CaCO_3$ according to Eq. (3*a*):[20]

$$\text{at } 400^\circ\text{C}: Ca(CH_3COO)_2(s) \rightarrow CaCO_3(s) + CH_3COCH_3(g) \quad (3a)$$

$$\text{at } 790^\circ\text{C}: CaCO_3(s) \rightarrow CaO(s) + CO_2(g) \quad (3b)$$

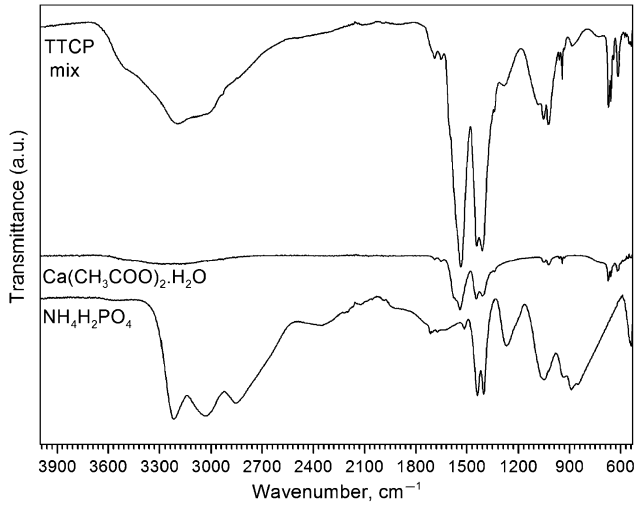


**Fig. 2.** Fourier-transform infrared spectrometry traces of starting chemicals and powder mixtures.

$CaCO_3$ completed its decomposition into CaO at around 790°C in air, as shown in Fig. 1(b). The overall decomposition reaction for calcium acetate monohydrate can, therefore, be summarized by the following reaction, with a total theoretical weight loss of 68.2%:

$$Ca(CH_3COO)_2 \cdot H_2O(s) \rightarrow CaO(s) + CH_3COCH_3(g) + CO_2(g) + H_2O(g) \quad (3c)$$

The experimentally determined weight loss for the calcium acetate monohydrate samples was found to be 67.6%, which is quite a good match. The first part of the decomposition reaction for ammonium dihydrogen phosphate, on the other hand, can be given by the following equation:

$$2\,NH_4H_2PO_4(s) \rightarrow 2\,NH_3(g) + 3\,H_2O(g) + P_2O_5(s) \quad (4)$$

Equation (4) indicated a total weight loss of 38.3%, and it explained well the weight loss observed in the pure $NH_4H_2PO_4$ samples (Fig. 1(d)) until the 680°–690°C TGA shoulder. With a further increase in temperature, the $P_2O_5$ already formed starts evaporating, bringing the total weight loss in pure $NH_4H_2PO_4$ samples to around 96%.

The main purpose in heating the powder mixtures first to 300°C followed by grinding was the homogenization of the reaction products of molten ammonium phosphate and the solid phase of partially decomposed calcium acetate. Upon continued heating to 800°C, volatilization of $NH_3$ from $NH_4H_2PO_4$, followed by its condensation to highly reactive $P_2O_5$ was completed, whereas acetone, carbon dioxide, and water vapor were the main decomposition products of Ca-acetate. At this point, the samples were ground into a fine powder, followed by heating to the final temperature of 1230°C.

Figure 3(a) showed a typical TG/DTA trace for the $Ca(CH_3COO)_2 \cdot H_2O$ and $NH_4H_2PO_4$ mixtures (i.e., TTCP mix) up to 1200°C. To summarize the thermal events seen in this TG/DTA chart, first, $NH_4H_2PO_4$ melted at around 190°C (endothermic peak) engulfing the calcium acetate particles; second, the abrupt decomposition of Ca-acetate accompanied by the drastic crystallization of $CaCO_3$ (indicated by the sharp exothermic peak in Fig. 3(a)) took place at about 400°C; and third the decarboxylation of $CaCO_3$ was observed in the vicinity of 750°C. Crystallization of $CaCO_3$ from the decomposing calcium acetate particles, which are embedded in a molten phosphate bath, occurred through an exothermic reaction. X-ray diffraction traces given in Fig. 3(b) depict the stepwise phase evolution in these mixtures, with the help of isothermal heatings (2 h at the peak temperatures, with 5°C/min heating and cooling rates) at individual temperatures indicated on this chart. Table II lists the phases assigned to the individual peaks shown in Fig. 3(b). TTCP was not detected even in samples quenched from 1000°C.

FTIR data of the TTCP powder mixtures were also useful in determining the nature of phase evolution sequence in these samples. Figure 3(c) shows the IR spectra of samples isothermally heated (2 h at peak temperatures, 5°C/min heating and cooling rates) at the temperatures indicated (RT to 1000°C) in the chart. Experimentally determined IR bands are given in Table III together with the corresponding phases.

An amorphous intermediate (consisting of long chains of condensed phosphate of the form $(PO_3)_n$) is expected to form as a result of the reactions between an acidic phosphate liquid and calcium acetate, which subsequently converts itself into partially crystalline β-$Ca(PO_3)_2$ (calcium metaphosphate) with an increase in temperature to around 250°–400°C.[21] XRD and FTIR results both confirmed this, and after an isothermal heating at 350°C, calcium metaphosphate, calcium hydrogen phos-

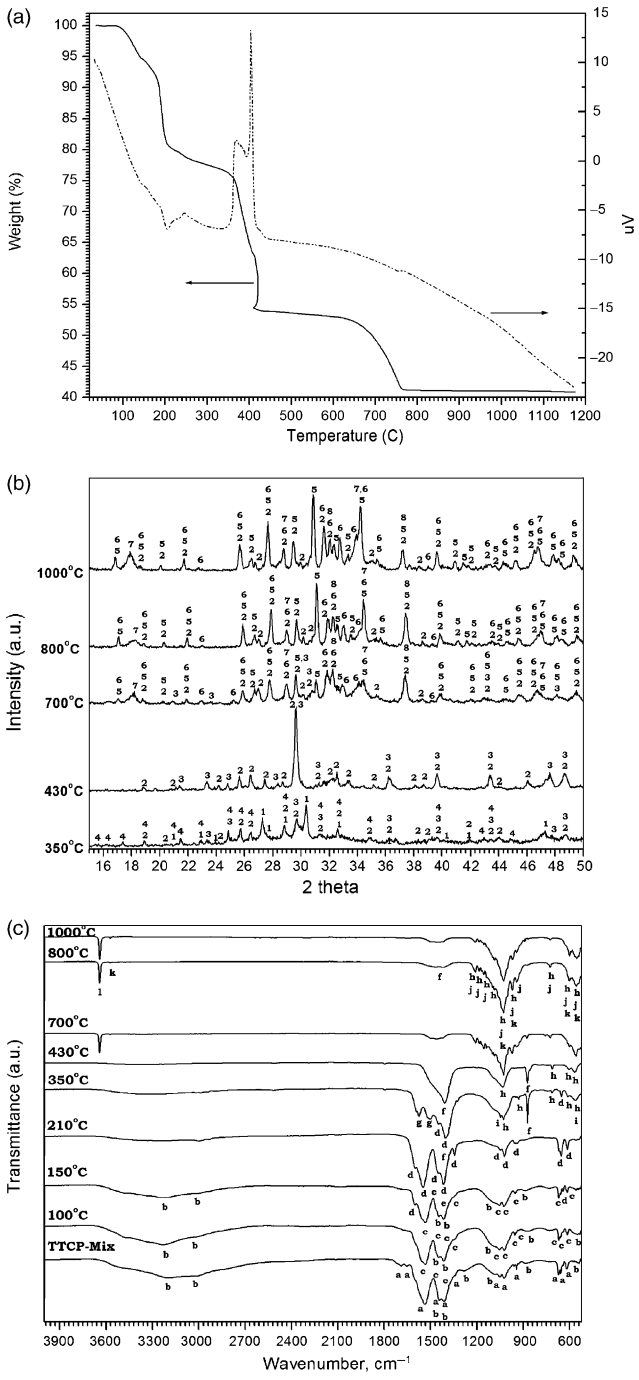


**Fig. 3.** (a) Thermogravimetric and differential thermal analyses trace for $Ca(CH_3COO)_2 \cdot H_2O$ and $NH_4H_2PO_4$ mixtures. (b) XRD traces of Ca-acetate + $NH_4H_2PO_4$ mixtures (see Table II). (c) Fourier-transform infrared spectrometry traces of Ca-acetate + $NH_4H_2PO_4$ mixtures (see Table III).

**Table II. Phases Identified (by XRD) in the Isothermal Heatings of Ca-Acetate Monohydrate+$NH_4H_2PO_4$ Mixed Powders**

| No. | Phase name | Phase formula | ICDD-PDF† |
|---|---|---|---|
| 1 | Dicalcium hydrogen phosphate | $CaHPO_4$ | 9-0080 |
| 2 | Calcium pyrophosphate | β-$Ca_2P_2O_7$ | 9-0346 |
| 3 | Calcium carbonate (*calcite*) | $CaCO_3$ | 5-0586 |
| 4 | Calcium metaphosphate | $Ca(PO_3)_2$ | 50-0584 |
| 5 | Tricalcium phosphate | β-$Ca_3(PO_4)_2$ | 9-0169 |
| 6 | Calcium hydroxyapatite | $Ca_{10}(PO_4)_6(OH)_2$ | 9-0432 |
| 7 | Calcium hydroxide | $Ca(OH)_2$ | 44-1481 |
| 8 | Calcium oxide | CaO | 37-1497 |

†ICDD–PDF, International Centre for Diffraction Data—Powder Diffraction File.

**Table III. FTIR Band Assignments**

| Label | Phase | Wavenumber ($cm^{-1}$) |
|---|---|---|
| a | $Ca(CH_3COO)_2 \cdot H_2O$ | 1689,1650, 1535, 1447,1408, 1338, 1050, 1021,945, 673, 659, 619 |
| b | $NH_4H_2PO_4$ | 3327, 3024, 1446, 1407, 1081, 869, 648 |
| c | $Ca(CH_3COO)_2$ | 1535, 1446, 1408, 1338, 1050, 1021, 955, 943, 673, 659, 619 |
| d | "Decomposing"† $Ca(CH_3COO)_2$ | 1634, 1540, 1448, 1410, 1340, 614 |
| e | "Decomposing"† $NH_4H_2PO_4$ | 1448, 1409 |
| f | $CaCO_3$ | 1403, 873 |
| g | $Ca(PO_3)_2$ | 1573, 1501 |
| h | $\beta$-$Ca_2P_2O_7$ | 1211, 1186, 1156, 1101, 1085, 1026, 970, 725, 598, 555 |
| i | $CaHPO_4$ | 1050, 555 |
| j | Â-$Ca_3(PO_4)_2$ | 1211, 1186, 1156, 1026, 970, 944, 725, 598, 555 |
| k | $Ca_{10}(PO_4)_6(OH)_2$ | 3571, 1026, 970, 598, 555 |
| l | $Ca(OH)_2$ | 3640 |

†Shifts in IR band positions because of decomposition. FTIR, Fourier-transform infrared spectrometry.

phate (Monetite, $CaHPO_4$), calcium pyrophosphate ($Ca_2P_2O_7$), and calcium carbonate phases were detected in these samples. The presence of amorphous phases, if there were any, of course, cannot be detected by XRD. Below 350°C, calcium acetate (i.e., $COO^-$ bands) and ammonium phosphate (i.e., orthophosphate and acid phosphate groups) IR bands and XRD peaks were visible. Therefore, a step of 300°C calcination (followed by grinding) was just necessary to homogenize the powder samples prior to the crystallization of any calcium phosphates. Calcium metaphosphate disappeared upon heating the powder mixtures to 430°C, and the samples consisted of a biphasic mixture of $CaCO_3$ and $Ca_2P_2O_7$ at this temperature. However, upon heating to 700°C, the presence of $CaCO_3$ significantly diminished with the simultaneous appearance of β-tricalcium phosphate, $Ca_3(PO_4)_2$ (TCP), and HA. Because of the overall stoichiometry of the initial powder mixtures (Ca/P molar ratio = 2), detection of HA or TCP in the powders should automatically call for the presence of $Ca(OH)_2$ or CaO in those. Major peaks of CaO and $Ca(OH)_2$ are at 37.36° and 34.17° 2θ, respectively. Most HA peaks overlap with those of TTCP; however, the significant peak (i.e., 002 reflection) of HA was encountered at 25.88° 2θ. The presence of $Ca(OH)_2$ was unmistakably detected by the IR spectra, with its characteristic hydroxyl stretching band observed at 3640 $cm^{-1}$ in the samples heated at 700°C and higher. Samples heated at 1000°C contained the phases of carbonated HA, TCP, $Ca(OH)_2$, and $Ca_2P_2O_7$. Complete elimination of TCP and $Ca_2P_2O_7$ and the formation of TTCP were observed at >1200°C.

The characteristic XRD diagram of TTCP powders obtained after the second step of 1230°C firing/quenching is given in Fig. 4(a). These powders contained approximately 5–6 wt% (median: 5.43, estimated standard deviation: 0.26) of HA phase, together with $Ca(OH)_2$. The amounts of HA in these powders

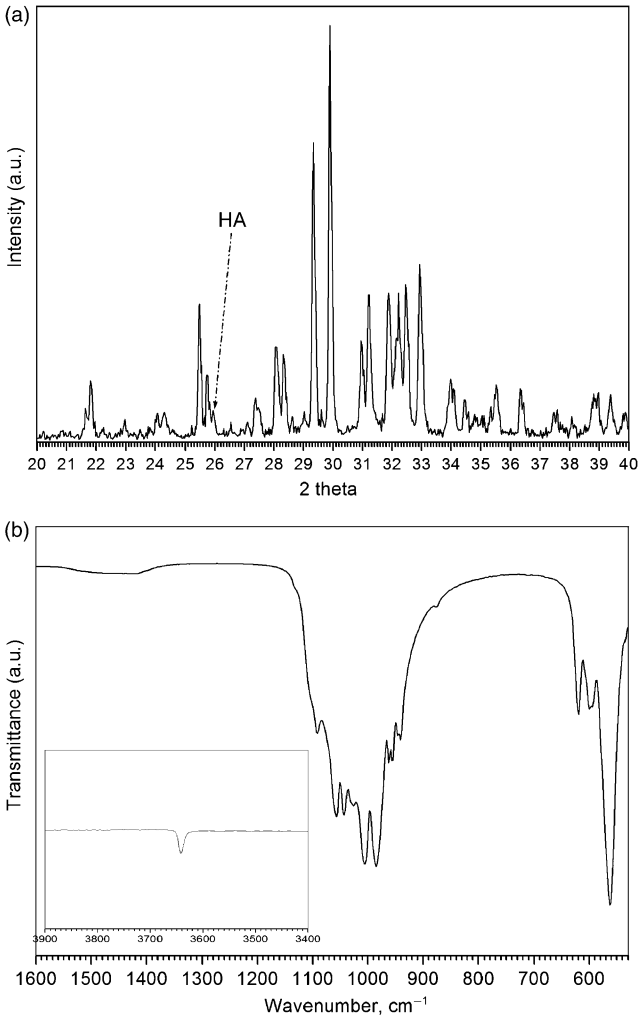


**Fig. 4.** (a) XRD trace of hydroxyapatite (HA)-seeded tetracalcium phosphate (TTCP) powders obtained at 1230°C. (b) Fourier-transform infrared spectrometry trace of HA-seeded TTCP powders.

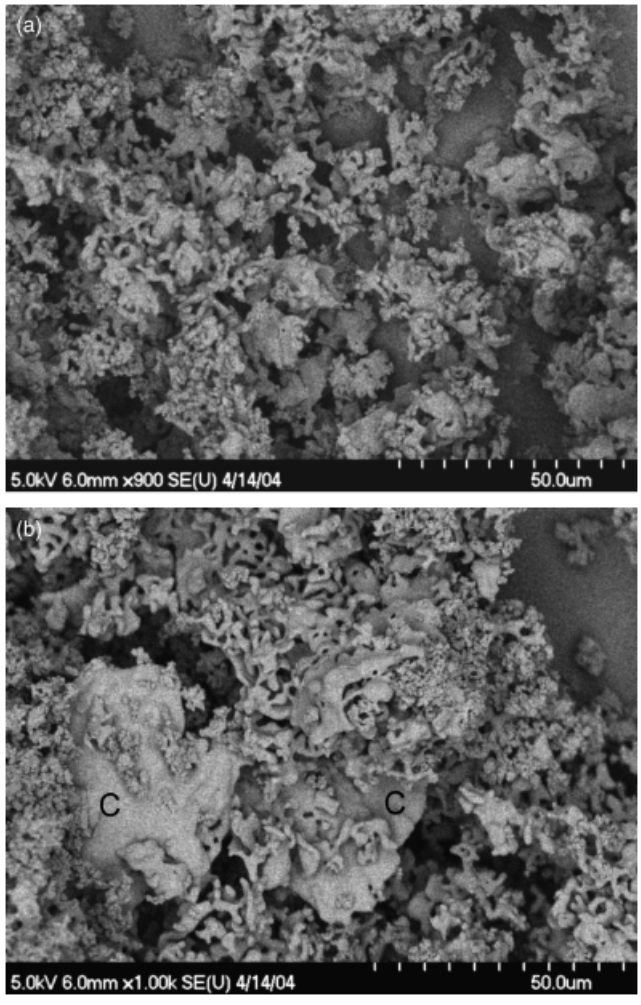


**Fig. 5.** Morphology of hydroxyapatite-seeded TTCP powders synthesized at 1230°C.

were determined, on three batches of preparation, by quantitatively comparing the peak intensities of TTCP and HA phases. The FTIR pattern of the HA-seeded TTCP powders is given in Fig. 4(b). This experimental pattern precisely matched those previously reported for tetracalcium phosphate by Sargin *et al.*[12] and Posset *et al.*,[22] respectively. Vibrational wavenumbers were assigned by the above-mentioned researchers. In the data of Fig. 4(b), threefold degenerate stretching (1105–989 $cm^{-1}$) mode ($\nu_3$), symmetric stretching (962–941 $cm^{-1}$) mode ($\nu_1$), and threefold degenerate deformational (620–571 $cm^{-1}$) mode ($\nu_4$) vibrations were all visible.[12,22] The inset in Fig. 4(b) displays the characteristic OH stretching vibration (3640 $cm^{-1}$), originating from $Ca(OH)_2$.[12] On the other hand, the weak band at 872 $cm^{-1}$ belongs to the carbonate groups, and these are considered to be associated with the carbonated HA seeds present in the samples.

The addition of HA seeds to TTCP powders has previously been tested by Hamanishi *et al.*;[23] however, they had intentionally added about 40 wt% precipitated HA into a cement mixture of TTCP–$CaHPO_4 \cdot 2H_2O$. The level of HA seeding in the present study is obviously much lower than this.

Particle morphology of the TTCP powders in this study is given in Figs. 5(a) and (b). Powders displayed a unique vermicular microstructure (less than 5 μm in size), which was indicative of the vapor- and liquid-phase reactions that took place. The remnants of the amorphous-looking chunks of Ca-doped condensed phosphates (i.e., $P_2O_7$, $(PO_3)_n$, and $P_2O_5$ in comparison with $PO_4$)[24–26] were observed in the form of larger chunks of about 40 μm. The as-quenched powder samples depicted in Figs. 5(a) and (b) had a BET surface area of $32 \pm 4$ $m^2/g$. Conventional TTCP powders prepared by 1500°C heating (for 6 h) of $CaCO_3$ and $CaHPO_4$ are first ground in ball mills to reduce their particle sizes to around 13–20 μm (coarse TTCP), and then further ground for 24 h to reduce their particle sizes to less than 5 μm (fine TTCP).[27]

SBF solutions are able[28] to induce apatitic calcium phosphate formation on metals, ceramics, or polymers (with proper surface treatments) soaked in them. 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.[29] Posner and Betts[30] proposed that the process of ACP formation in solution involved the formation of $Ca_9(PO_4)_6$ 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 $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$, where $x$ might be converging to 1.[31] Onuma and Ito[32] 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 probably trigger the hexagonal packing[32] of those nanoclusters into globules or spherulites of apatitic calcium phosphates. SEM photomicrographs given in Figs. 6(a)–(d) exhibited the rapid formation of those apatitic calcium phosphate globules after

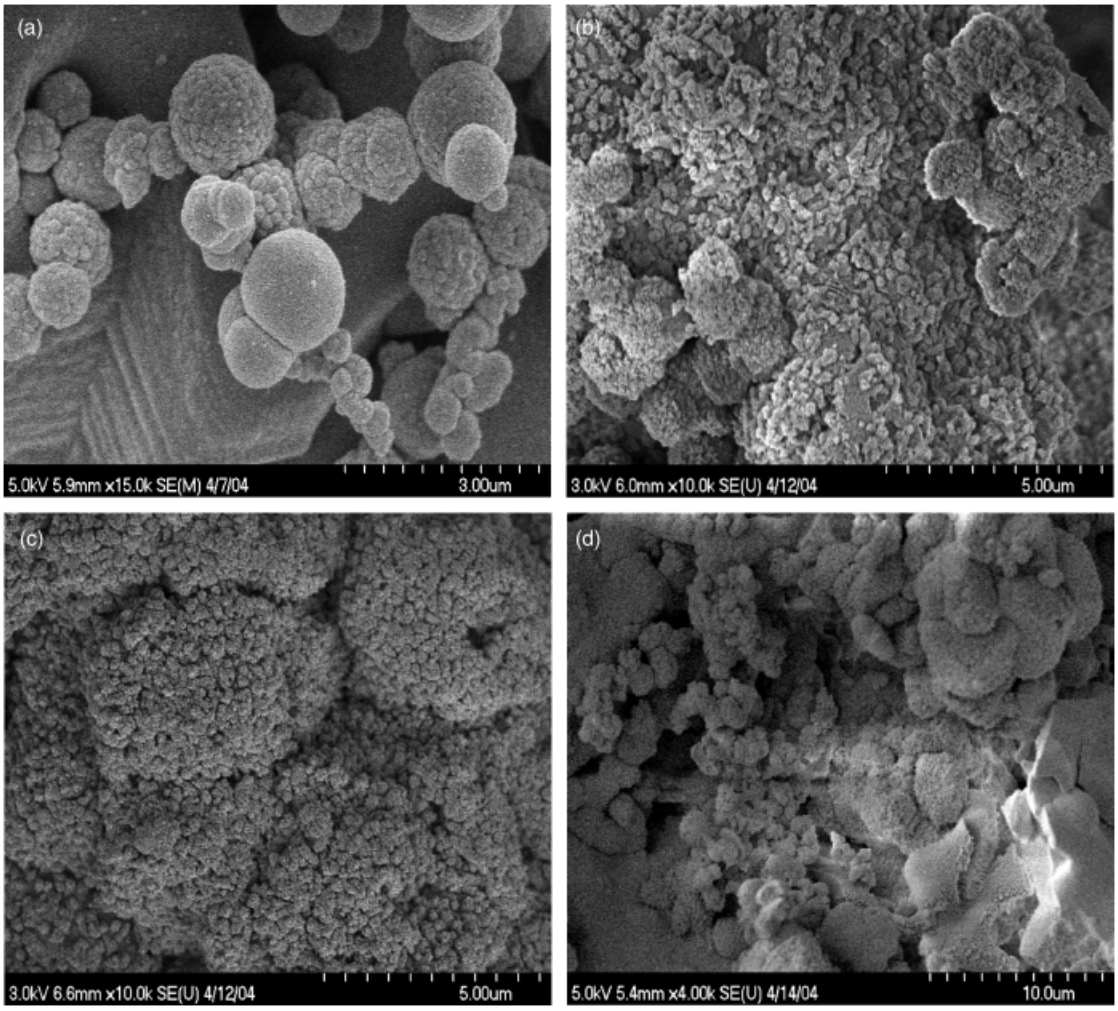


**Fig. 6.** (a) Thirty-six hours in synthetic body fluid (SBF) at 37°C (twinning; towards lower left-hand corner). (b) Seventy-two hours in SBF at 37°C. (c, d) Ninety-six hours in SBF at 37°C.

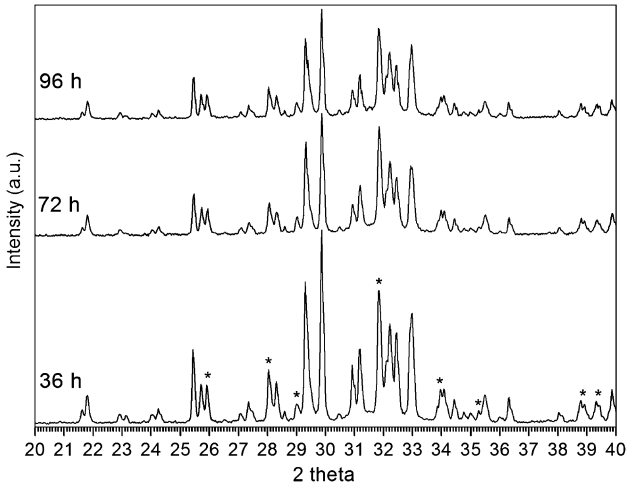


**Fig. 7.** XRD traces of SBF-soaked samples (*HA peak positions).

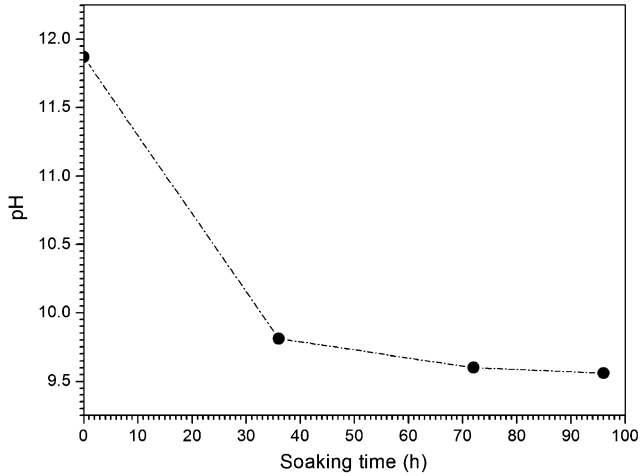


**Fig. 9.** Change in surface pH of HA-seeded TTCP powders in SBF.

soaking the HA-seeded TTCP powders in SBF for 36, 72, and 96 h at 37°C, respectively.

Figure 6(a) revealed the twinning in the TTCP grains, which was first mentioned by Dickens *et al.*[4] 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 reacted with the calcium phosphate globules (Fig. 6(a)), and within the following 36 h the external surfaces of the TTCP grains totally transformed into apatite (Fig. 6(b)). The XRD and FTIR data of these samples (as given in Figs. 7 and 8) 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 (Fig. 7). FTIR data of Fig. 8 (where * indicates carbonate groups) showed the appearance of a pronounced band at 1420 $cm^{-1}$ in the high-energy C−O region along with a well-defined band at 872 $cm^{-1}$, known to be specific for a carbonated apatite.[33] OH bands were seen at 3571 $cm^{-1}$ after soaking in SBF.

We have also noted that the surface pH values of the as-synthesized HA-seeded TTCP powders were in the vicinity of $11.8 \pm 0.05$ (measured at RT after 15 min 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, probably because of the presence of $Ca(OH)_2$, observed in the TTCP powders in this study may cause cell *necrosis*, in case they were directly implanted *in vivo*. However, as shown in Fig. 9, with an increase in soaking time in an SBF solution (at 37°C) of pH 7.4, pH started to decrease.

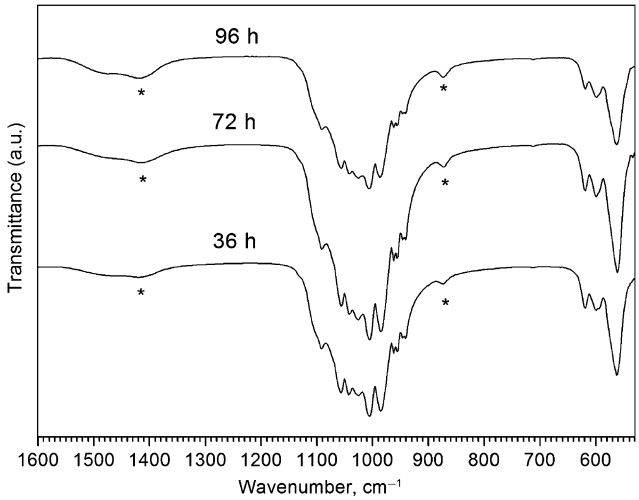


**Fig. 8.** FTIR traces of SBF-soaked TTCP samples (*carbonate bands).

This was 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 HA has a pH value of about 9.5. After 96 h of soaking in SBF, recorded pH value was $9.56 \pm 0.04$ at RT. It is reasonable to assume that this pH value would have continued to drop toward the physiological pH if we had continued the soaking beyond 4 days.[34,35] The pH diagram of TTCP powders given in Fig. 9 may very well serve the practitioner to precisely tailor the "amount of an acidic calcium phosphate (such as DCPA, $CaHPO_4$; DCPD, $CaHPO_4 \cdot 2H_2O$; MCPM, $Ca(H_2PO_4)_2 \cdot H_2O$; and MCPA, $Ca(H_2PO_4)_2$) 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 HA, i.e., the bone mineral. The presence of HA seeds in TTCP simply accelerates this *in vitro* biomineralization process. Nevertheless, it is yet to be seen if these powders can be made into useful self-setting CaP cements.

## IV. Conclusions

(1) Five to six weight percent HA-seeded TTCP ($Ca_4(PO_4)_2O$) powders were synthesized at 1230°C, by using the starting chemicals of calcium acetate monohydrate and ammonium dihydrogen phosphate, for the first time, followed by quenching from the synthesis temperature.

(2) This is the lowest temperature ever reported for the synthesis of TTCP powders, and such low synthesis temperatures can readily be achieved in inexpensive electrical resistance-heated furnaces.

(3) HA-seeded TTCP powders underwent apatitic transformation when they were brought into contact with SBF solutions of pH 7.4 at 37°C, within the first 36–96 h. The HA seeds can perhaps be seen as having a catalytic function for the TTCP transformation.

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