

Granules of Brushite and Octacalcium Phosphate from Marble

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Brushite (DCPD, dicalcium phosphate dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and octacalcium phosphate (OCP, $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$) granules with millimeter sizes were prepared by using marble (calcium carbonate) granules as the starting material. The method of this study simply comprised of soaking the marble granules in aqueous solutions containing phosphate and/or calcium ions at temperatures between 20° and 37°C. This process did not cause any size change between the initial marble and final brushite or octacalcium phosphate granules. Such DCPD and OCP granules could be useful in maxillofacial, dental, and orthopedic void/bone defect filling and grafting applications. Samples were characterized by X-ray diffraction, inductively coupled plasma atomic emission spectroscopy, and scanning electron microscopy.

I. Introduction

It is easy to synthesize powders of brushite (DCPD, dicalcium phosphate dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$),^{1–5} and octacalcium phosphate (OCP, $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$).^{6–10} The log K_{SP} (solubility product) values for DCPD, OCP, and hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) are known to be –6.6, –72.5, and –117.1, at 25°C, respectively.¹¹ Therefore, one may expect solely on the basis of the above solubility values that both DCPD and OCP shall exhibit *in vivo* percentages of biodegradation and bony replacement higher than that of HA. DCPD is an acidic calcium phosphate and when implanted in the form of a cement paste containing some β -TCP (β - $\text{Ca}_3(\text{PO}_4)_2$) granules, the material was initially surrounded by macrophages and fibrous tissue due to the initial pH drop, but still showed a higher biodegradation rate than that of a control HA cement over the long run.^{12,13} Unfortunately, *in vitro* and *in vivo* data on pure DCPD powders are lacking to the best of our knowledge. On the other hand, to the advantage of OCP, when it was implanted as a dry-sieved powder (sieving apparently causing a certain degree of aggregation of the particulates) such fibrous tissue encapsulation was not observed, and OCP biodegraded well.¹⁴

The difficulty arises if one wants to produce granules or bodies of DCPD or OCP, instead of powders, which should have at least the strength to withstand the handling by the surgeon during implantation. DCPD or OCP cannot be sintered, in stark contrast to HA or β -TCP, mainly because at temperatures above 60°C they start to transform into CaHPO_4 (DCPA, monetite, dicalcium phosphate anhydrous) and HA, respectively. In the literature, there were only scarce attempts to prepare granules of monetite.^{15–17} The starting materials in these studies were either brushite cement paste

containing β -TCP^{15,16} or pure β -TCP.¹⁷ The so-called brushite cement pastes are prepared by reacting orthophosphoric or sulfuric acid (cement setting solution) with β -TCP (69.74 wt%), $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (30 wt%), and $\text{Na}_2\text{P}_2\text{O}_7$ (<0.26 wt%), therefore, the pastes contain a significant amount of unreacted β -TCP in their cores.^{16,18} Conversion of CaCO_3 , on the other hand, into HA has been also previously studied by us,^{19,20} and the literature cited in these publications^{19,20} summarizes the historical attempts to produce HA from CaCO_3 . The experimental procedure of the current study was so planned that we would not produce HA granules.

The down-to-earth approach taken in this study was to use granules of natural marble (calcite, CaCO_3) as the starting material and transforming them firstly into DCPD and secondly to OCP by soaking in specially prepared aqueous solutions in glass media bottles (without stirring) at temperatures between 20° (room temperature, RT) and 37°C. The sizes of the obtained DCPD and OCP granules resembled the initial sizes of the marble granules used.

II. Experimental Procedure

Marble granules were purchased from Merck KGaA, Darmstadt, Germany (Cat. No: 105986). The granules were 0.9–2 mm in size. Marble granules were used as-received, without any further purification or chemical treatment, only after dry sieving manually by using a sieve with 0.9 mm openings to remove very small amounts of marble dust that could be present.

For the preparation of DCPD granules, 10 g of $\text{NH}_4\text{H}_2\text{PO}_4$ (Cat. No: 101126, Merck) was dissolved in 50 mL of doubly distilled water. The pH of the obtained solution was 4.1 ± 0.1 at RT. One milliliter of this solution had 1.739×10^{-3} mole P. The solutions were prepared in 100 mL-capacity glass media bottles. In some experiments, that 10 g of $\text{NH}_4\text{H}_2\text{PO}_4$ was replaced with 13.557 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (Cat. No: 106342; Merck) or 11.826 g of KH_2PO_4 (Cat. No: 104877; Merck) to check whether the transformation from marble (CaCO_3) to DCPD would also proceed in Na- or K-phosphate solutions or not. Two grams of marble granules was then placed into the phosphate solution (Ca/P molar = 0.23). The granules were kept in sealed glass bottles at RT for about 20 h without stirring. Granules were separated from the solution by using a small sieve with 0.9 mm openings, followed by washing with 1.5 L of distilled water and overnight drying at 37°C.

An alternative phosphate solution was also prepared by slowly adding 120 mL of concentrated (85%) H_3PO_4 (Cat. No: 100573; Merck) to 730 mL of doubly distilled water, followed by the drop-wise addition of 137 mL of concentrated (28%–30%) NH_4OH (Cat. No: 105423; Merck); the resultant solution again had a pH value equal to 4.1 ± 0.1 at RT. One milliliter of this solution had 1.889×10^{-3} mole P. In using this alternative solution to form the DCPD granules, 3 g of marble granules were placed into 69 mL of the above solution (Ca/P molar = 0.23) and were kept unstirred for 20 h at RT.

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In synthesizing the OCP granules, DCPD granules as produced above (all four solutions) were used as the starting material. The OCP synthesis solution was prepared by adding the following chemicals at RT, one by one, to 1420 mL of double distilled water under vigorous stirring; 12.448 g of NaCl (Cat. No: 106404; Merck), 0.559 g KCl (Cat. No: 104933; Merck), 0.426 g Na_2HPO_4 (Cat. No: 106586; Merck) and 0.735 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Cat. No: 102382; Merck). 8.5 g of Tris (Cat. No: 108382; Merck) was then added to this slightly turbid solution, followed by titrating the solution to $\text{pH } 7.4 \pm 0.2$ by adding 60–67 mL of 1 mol/L HCl solution (Cat. No: 109057; Merck). To produce OCP granules, 500 mL of this transparent solution (with a Ca/P molar ratio of 1.667) was placed into a 500 mL-capacity glass media bottle and 2.2 g of DCPD granules was added into the bottle. The bottle was kept undisturbed at 37°C in a microprocessor-controlled oven for 1 week, however, the solution in the bottle was fully replenished at the end of the first 3 days and at the end of 6 days, as well. This solution was inspired from the one described by Wen *et al.*²¹ Granules were finally separated from the solution by using a small sieve with 0.9 mm openings, followed by washing with 1.5 L of distilled water and overnight drying at 37°C .

All granules were characterized by using an X-ray diffractometer (XRD; Advance D8, Bruker AG, Karlsruhe, Germany) after either gently mounting the granules in the sample holder on modeling clay (Play-Doh[®]) or after grinding them into a powder by using an agate mortar and pestle. XRD was operated with a Cu tube at 40 kV and 40 mA equipped with a monochromator. Samples were scanned with a step size of 0.02° and a preset time of 5 s.

Scanning electron microscopy (SEM; EVO 40, Zeiss, Dresden, Germany) was used to evaluate the morphology of the granules, after sputter-coating the granules with a 25-nm thick gold layer to impart electrical conductivity to the specimen surfaces.

Chemical analyses of granules were performed by using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Model 61E, Thermo Electron, Madison, WI). For the ICP-AES analyses, roughly 100 mg portions of granules was dissolved in 7.5 mL of concentrated (69%) HNO_3 (Cat. No: 101799; Merck) solution.

III. Results and Discussion

The chemical analyses of the initial marble granules were performed by using ICP-AES and the granules were found to consist of 55.5 wt% CaO, 2100 ppm MgO, 960 ppm SiO_2 , 430 ppm Al_2O_3 , and <200 ppm Fe_2O_3 , indicating that the granules were not pure. The values given above were the average of three measurements. No other impurities were detected. X-ray diffraction (XRD) analyses of the marble granules showed that they were consisting of single-phase calcite of high crystallinity, conforming very well with the standard powder diffraction file (PDF) of 5-0586 of International Centre for Diffraction Data (ICDD). The (104) reflection of the pristine marble granules, observed at $29.4^\circ 2\theta$, was having an X-ray diffraction intensity higher than 15 000 counts-per-second. Figure 1 depicted the SEM morphology of the initial white marble granules, with the inset showing an optical macrograph of the same including a millimetric scale to allow direct estimation of the granule sizes.

Upon soaking the marble granules in the pure ammonium dihydrogen phosphate solution for 20 h at RT, the granules were covered with characteristic DCPD crystals (Fig. 2). The SEM morphology did not exhibit any noticeable change when the marble granules were similarly soaked in the alternative $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, KH_2PO_4 or $\text{H}_3\text{PO}_4\text{-NH}_4\text{OH}$ solutions (as described in Chapter 2) and all four liquid media were able to transform the marble granules into DCPD. The solution pH increased to the range of 5.1–5.3 at the end of 20 h of aging. Such a pH increase is expected (by the forma-

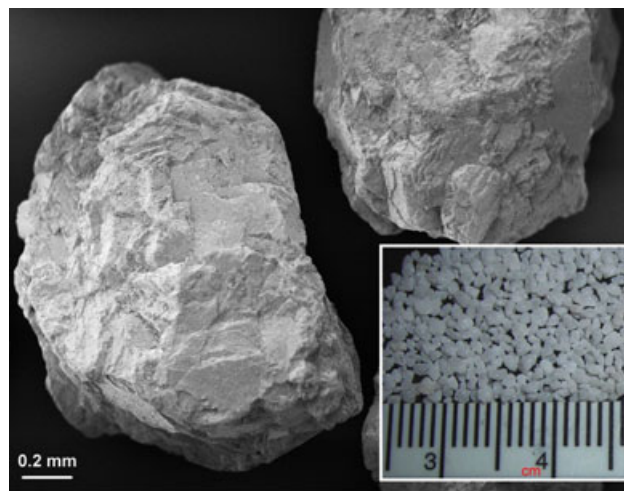


Fig. 1. SEM micrograph of the starting marble granules (inset: optical macrograph of the same).

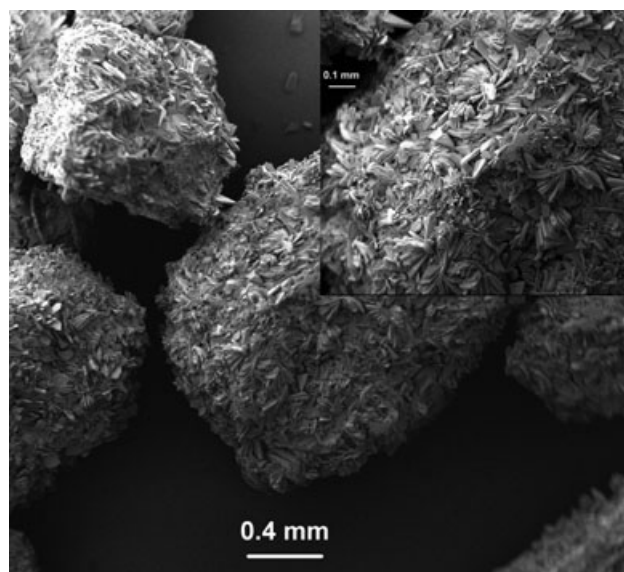
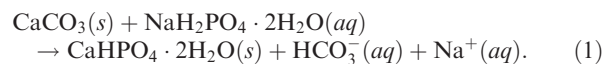


Fig. 2. SEM micrographs of DCPD granules (inset: higher magnification).

tion of bicarbonate ions and its buffering action with dissolved CO_2 (g) which may be present in the solutions) as shown in Equation 1



The XRD pattern of the DCPD granules (for the $\text{NH}_4\text{H}_2\text{PO}_4$ solution) was given in Fig. 3, all the peaks belonged to the DCPD phase (peak positions matching perfectly well with those given in ICDD PDF 09-0077). DCPD granules obtained by using $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, KH_2PO_4 , and $\text{H}_3\text{PO}_4\text{-NH}_4\text{OH}$ solutions also had the same XRD data (not shown). The XRD data of Fig. 3 were collected from the DCPD granules without grinding them into a powder (after mounting the granules directly in the sample holder). This was the reason why no CaCO_3 peak was observed in this figure. The high intensity (020) reflection located at $11.68^\circ 2\theta$ was characteristic of DCPD. One distinctive feature of these DCPD granules was that they were shining especially under the direct sun light due to the brushite crystals as shown in Fig. 2. It was actually impossible to obtain any calcium phosphate phase other than DCPD when immersing, at RT,

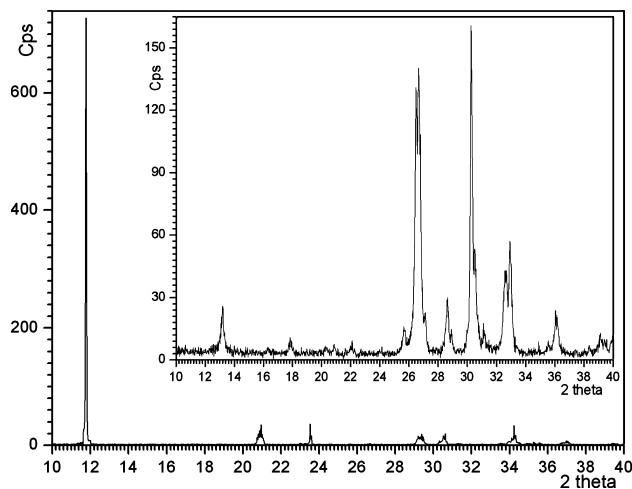


Fig. 3. XRD chart of DCPD granules obtained by using the $\text{NH}_4\text{H}_2\text{PO}_4$ solution (inset: XRD of DCPA powders obtained after stirring the marble granules in the $\text{NH}_4\text{H}_2\text{PO}_4$ solution for 20 h at 65°C).

such a reactive calcium carbonate/marble material in a calcium-free phosphate solution having a pH value of 4.^{2,8} The ICP-AES analysis of the DCPD granules showed that the Na and K amounts were less than 100 ppm, even when we used $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ or KH_2PO_4 solutions. If one added, for instance, precipitated chalk (i.e., calcite) powder into the same solution and stirred it vigorously at RT for 20 h, one would have obtained brushite powder. Similarly, when we added the same amount of marble granules into the same volume of the ammonium phosphate solution in a sealed glass bottle and stirred the solution at 65°C (at 400 rpm) constantly for 20 h, we obtained only a powder (no granules left) consisting of single-phase monetite (CaHPO_4), as shown in the XRD data of the inset of Fig. 3 where peak positions and intensities matching with those of ICDD PDF 09-0080. Increased temperature and vigorous stirring both forced (i) the DCPD to transform into DCPA and (ii) the marble granules to crumble into a powder.

It would be very difficult, if not impossible, to obtain OCP (octacalcium phosphate) directly from marble in an unstirred hydrothermal aging solution of pH 7.4. At that pH, apatitic CaP is the phase to form. This was why we preferred DCPD granules as the starting material in producing the OCP granules. On the other hand, it would have been very easy to convert DCPD into apatitic CaP by soaking them in a Ca^{2+} -containing solution with pH on the basic side (e.g., 8.5–10), but our intention here was not to produce low solubility HA.^{22,23} The solution developed²¹ for the conversion of DCPD to OCP had a Ca/P molar ratio of 1.667 and the mildly acidic DCPD granules (with Ca/P ratios roughly equal to 1, depending on the amount of CaCO_3 remaining still unreacted in the cores of those granules) would help to slowly lower both the nominal Ca/P ratio in the aging bottles to around 1.33 (i.e., to that of OCP) and the solution pH to around 6.5–6.8 (stability range of OCP).⁹ The DCPD-to-OCP transformation was a surface reaction.

Figure 4 depicted both the XRD data and the SEM micrographs of the OCP granules obtained by using the DCPD granules obtained in $\text{NH}_4\text{H}_2\text{PO}_4$ solution. OCP granules produced by using the DCPD granules obtained in the other solutions of this study also showed identical XRD and SEM data. The peak positions and intensities of the XRD pattern of the OCP (without grinding the granules into a powder, Fig. 4) granules were in good match with those of ICDD PDF 26-1056. The intermingling nanocrystals seen in the inset of Fig. 4 also depicted the characteristic habit of OCP crystals.¹⁰ The OCP granule synthesis process described herein is a benign one since it starts with a solution of

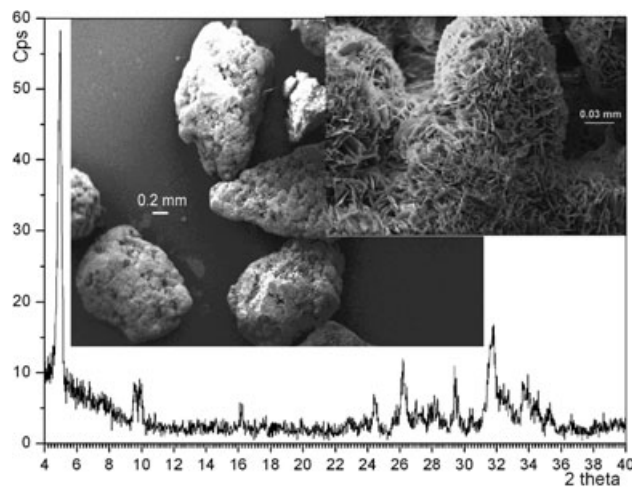
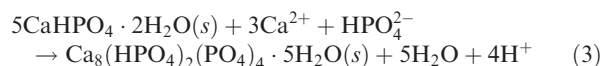
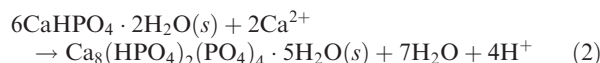


Fig. 4. XRD chart of OCP granules (inset-1: low magnification SEM photo, inset-2: high mag SEM photo showing nanocrystalline surface texture).

neutral pH and proceeds at the human body temperature. The solution pH values were measured over the range of 6.5–6.8 at the end of aging periods.

The overall conversion of brushite to OCP can be visualized by the reactions given below. Reactions help to explain the effect of Ca^{2+} and HPO_4^{2-} ions present in the aging solutions, as well as the pH drops observed.



Mg^{2+} ions are known²⁴ to significantly inhibit the nucleation and growth of cryptocrystalline apatitic calcium phosphate in aqueous media, and for this reason the presence of magnesium in the original marble granules were deemed to be simply advantageous for the synthesis of OCP. We did not assess the influence of other elements, such as Si, Al, and Fe, present in smaller amounts in the initial marble granules.

Upon grinding (in an agate mortar) both the so-called DCPD and OCP granules of this study into a fine powder and then collecting their XRD data, the small amounts of CaCO_3 remaining in the cores of the granules became visible, as shown in Fig. 5a. The strongest (104) reflections of calcite located at $29.4^\circ 2\theta$ in both DCPD and OCP samples were still much lower than the $>15\,000$ Cps intensity of the initial marble granules. The percentage decrease in the XRD intensity of the (104) reflection of calcite (from the initial marble granules ($>15\,000$ Cps) to the final DCPD or OCP granules (2300 Cps)) corresponded to about 85%. Therefore, the process reported herein was starting at the surfaces of the non-stirred marble granules, proceeding inwards and then stopping at one point where the formed DCPD or OCP layers were obstructing the reach of the solution to the remnant calcitic marble cores at RT and 37°C .

What would happen if only the temperature and aging time were increased, without stirring, in preparing the DCPD granules? The bottom trace of Fig. 5b showed the XRD pattern of granules heated at 50°C for 96 h without stirring, whereas the top trace showed the granules heated at 70°C for 96 h again without stirring. In both cases, the end products were intact granules, not powders; in contrast to the stirred sample of Fig. 3. It must be noted that while the intensity of the (104) reflection of the calcite phase was decreasing from 2285 Cps (RT, 20 h) to 1942 Cps (50°C ,

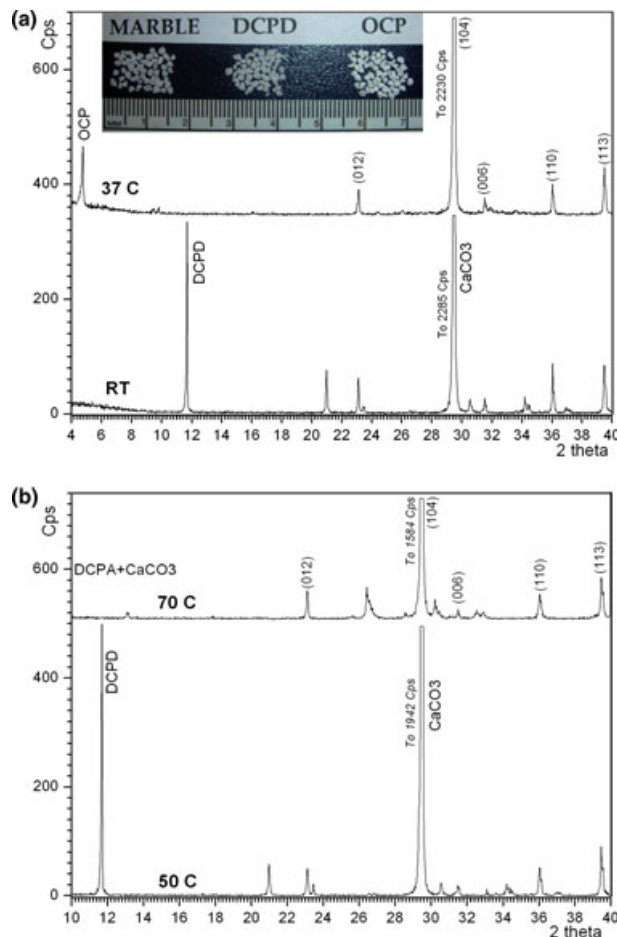


Fig. 5. (a) XRD charts of DCPD (bottom) and OCP (top) granules (after grinding into a fine powder); reflections of calcite are indicated by their crystallographic planes (inset shows the size resemblance between marble, DCPD, and OCP granules). (b) XRD charts of marble granules heated at 50°C for 96 h (bottom) and 70°C for 96 h (top) in the $\text{NH}_4\text{H}_2\text{PO}_4$ solution without stirring (after grinding the granules into a fine powder).

96 h), upon increasing the solution aging temperature to 70°C, DCPD phase completely decomposed to DCPA with a further decrease in the intensity of the calcite peak (Fig. 5b, top trace). DCPD is a low-temperature phase. The reason behind the decrease in the XRD intensity of the remnant calcite in the cores of the granules was most probably the reduced surface tension of the aqueous aging solution (in going from RT to 50° and 70°C), and thus the solution was able to penetrate more inwards through the DCPD layer and reaching a bit more of the non-reacted marble at the granule cores. Increasing the aging time also helped this transformation.

We further pursued this high-temperature transformation of marble granules by adding 2.35 g of marble granules to 140 mL of the ammonium phosphate solution (prepared by mixing concentrated solutions of H_3PO_4 and NH_4OH , Chapter 2) in a non-stirred pressure vessel (Model 4760; Parr Instrument Company, Moline, IL) with a Teflon[®] cup inside. The pressure vessel was heated in a microprocessor-controlled oven. By this way, we were able to reach 200°C (and stay there for 24 h) without any risk of evaporating the solution. This pressure vessel was not equipped with a pressure gauge. The XRD traces of marble granule samples heated at temperatures between 90° (96 h) and 200°C (24 h) are given in Fig. 6. None of these samples disintegrated into a powder, they were all recovered as strong granules. Starting from somewhere between 90° and 110°C, a new phase was appearing: β -TCP. Heating runs at 150° and 200°C, on the other hand, also resulted in another phase: HA. Therefore, as

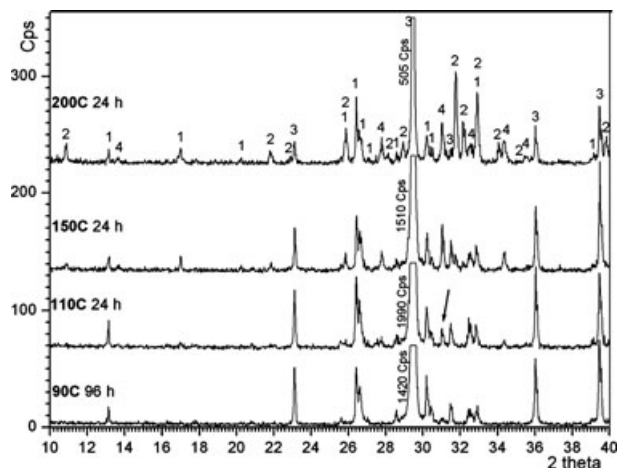


Fig. 6. XRD traces of marble granules heated in a pressure vessel between 90° and 200°C, in a solution prepared by adding NH_4OH into H_3PO_4 . Phases = 1: DCPA, 2: HA, 3: calcite, 4: β -TCP.

shown in Fig. 6, the samples heated in the pressure vessel at 110°, 150° and 200°C crystallized β -TCP besides DCPA, and the samples heated at 150° and 200°C were consisting of four phases: DCPA, β -TCP, HA, and calcite. The appearance of HA and β -TCP phases should be at the expense of calcite and DCPA. Crystallization of β -TCP at a temperature as low as 110°C was noteworthy.²⁵ The decrease observed in the amount of calcite (Fig. 6), with an increase in the temperature of the pressure vessel from 150° to 200°C, implied that a further increase in temperature up above 200°C could have totally consumed the calcite phase. Nevertheless, to prepare granules comprising the phases of HA and β -TCP was not one of our initial purposes, and for this reason we opt not to deliberate much on these multi-phase high temperature-high pressure samples.

The DCPD and OCP granules of this study may be expected to show a high *in vivo* degradability since their cores were not made out of β -TCP as was the case with the previous granule synthesis studies,^{16–18} but were out of calcite, CaCO_3 . Calcite is more soluble than β -TCP and this fact was proved by the *in vitro* cell culture study of Monchau *et al.*,²⁶ which directly compared HA, β -TCP, and CaCO_3 with one another by using both human and rat osteoclasts.

The preparation of biphasic DCPD–OCP granules (in any desired weight percentages) would be as easy as weighing and mixing the DCPD ($\log K_{\text{SP}} = -6.6$) and OCP ($\log K_{\text{SP}} = -72.5$) granules with one another. Such biphasic granule mixtures may thus provide a tool of fine-tuning the desired *in vivo* solubility/resorbability level, between the solubilities of the end members DCPD and OCP, of these biomaterials.

It is obvious that the weakest part of this *proof-of-the-concept* study was the absence of compressive strength measurements on the marble, DCPD, and OCP granules (although it was still impossible to crush the DCPD and OCP granules into a powder by using the fingertips), as well as the *in vitro* and *in vivo* characterization, however, our follow-up study is already focusing right on those aspects.

IV. Conclusions

Marble (calcite, CaCO_3) granules over the size range of 0.9–2 mm were used as the starting material to prepare brushite (DCPD, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) granules, without changing the starting granule sizes. DCPD granule synthesis consisted of soaking the marble granules at room temperature in an acidic (of pH 4) phosphate solution, without stirring, for less than a day. DCPD granules were found to contain small amounts of residual marble in their cores. DCPD granules were then used as the starting material for the preparation of

octacalcium phosphate (OCP, $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$) granules. A non-stirred Tris-buffered solution (of pH 7.4) containing Na^+ , K^+ , Ca^{2+} , Cl^- , and HPO_4^{2-} ions was used in synthesizing OCP granules at 37°C in 1 week. DCPD and OCP granules were containing residual marble (CaCO_3 , calcite) in their cores.

Notes

Certain commercial instruments or materials are identified in this article to foster understanding. Such identification does not imply recommendation or endorsement by the author, nor does it imply that the instruments or materials identified are necessarily the best available for the purpose.

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