

USE OF VATERITE AND CALCITE IN FORMING CALCIUM PHOSPHATE CEMENT SCAFFOLDS

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ABSTRACT

A series of novel orthopedic calcium phosphate (CaP+CaCO₃) cements have been developed. The common point in these cements was that they all utilized single-phase CaCO₃ (calcite or vaterite) in their powder components. The major phase in the end-product of these cements was carbonated, Ca-deficient, apatitic calcium phosphate, together with some varying amounts of unreacted CaCO₃. Calcite powders used were needle-like or acicular in shape, whereas the vaterite powders were monodisperse, and spherical or ellipsoidal in shape. A new method for synthesizing spheroidal vaterite powders has also been developed. Setting solutions for calcite-based cement scaffolds were prepared by acid-base neutralization of concentrated H₃PO₄ and NaOH. 0.5 M phosphate buffer solution was used to transform precipitated vaterite powders into CaP at 37° to 70°C. Setting solutions possessed pH values ranging from 3 to 7.4 at room temperature. Resultant cements were micro- (5 to 50 micron pores) and macroporous (200 to 700 micron pores). In the macroporous cements, total porosity was variable from 20 to 45%. Setting times of those cements were adjustable over the range of 12 to 25 minutes. Compressive strengths of these cements varied from 2 to 3 MPa, depending on their porosity. CaP+CaCO₃ cements thus obtained had relatively high surface areas (30 to 85 m²/g) whose surfaces were covered with nanocrystallites similar in size to the nanoplatelets found in biological collagen-calcium phosphate composites. Cement scaffolds were characterized by XRD, FTIR, SEM, ICP-AES, surface area and compressive strength measurements.

INTRODUCTION

Calcium carbonate (CaCO₃), being an important material of marine and geological biomineralization processes, has three anhydrous polymorphs; vaterite, aragonite and calcite. Amorphous calcium carbonate may also be added to this list as the fourth form.¹ At the ambient temperature and one atmosphere, calcite is the most stable and common polymorph of calcium carbonate, while vaterite² is known to be the least stable. Owing to its instability, vaterite is rare in nature as it would readily convert into one of the more stable calcium carbonate phases, aragonite or calcite, and possibly to monohydrocalcite (CaCO₃·H₂O). However, Lowenstam and Abbott³ reported that a special type of sea squirts, i.e., *Herdmania momus*, ascidiacea, exhibited vaterite as the mineralization product of the spicules of their skeletons. The remainder of marine organisms (excluding those yet to be discovered) uses aragonite or calcite as the biomineralization phases in forming their hard tissues, due to the scarcity of dissolved phosphorus (< 0.1 ppm) in the sea water.⁴ The thermodynamic instability of vaterite makes it quite suitable for the rapid formation of carbonated and apatitic calcium phosphate scaffolds as synthetic bone substitutes [1]. To the best of our knowledge, the conversion of vaterite to calcium phosphates (CaP) has not been thoroughly studied.

Synthetic, implantable bone-like materials are useful in many different biomedical applications. For example, bone-like scaffolding material can be implanted to fill large bone defects caused by trauma or removal of cancerous or otherwise diseased bone or cysts.⁵ The ideal biomaterial should have a chemical and structural design so as to induce a response similar to that of natural fracture healing when placed in an osseous defect, when invaded by mesenchymal cells, fibroblasts and osteoblasts before new trabeculae of bone infiltrate into the porous structure of the implant from the walls of the defect.⁶⁻¹⁰ Bone mineral consists of small crystals (3 to 5 nm in thickness and 40-100 nm in length, aligned parallel to the collagen fibrils) of CO₃-containing (5.5 wt%), alkali and alkaline earth ion (Na, K, Mg)-doped, Ca-deficient, non-stoichiometric, apatitic calcium phosphate (*Ap-CaP*) with a large and reactive surface area of 100-200 m²/g.^{11, 12} Bone mineral is similar to, but far from being identical with, the mineral Ca-hydroxyapatite (HA: Ca₁₀(PO₄)₆(OH)₂). A synthetic bone substitute should have high porosity dominated by macropores similar to the structure of trabecular bones, with pore sizes ranging from 100 to 700 μm.¹³ Macroporosity in bones allows for vascularization and provides a habitat for the cells, whereas microporosity mainly acts as fluid channels for nutrient supply and transmission.

The development of calcium phosphate cements (CPC) started almost three decades ago with the formulation of in situ-setting calcium phosphate pastes for dental repair and restoration applications.¹⁴⁻¹⁷ Cements provide the surgeon with the unique ability of manufacturing, shaping, and implanting the bioactive bone substitute material on a patient-specific base, in real time, in the surgery room. Moreover, during their preparation in the surgical theatre, such cement pastes can be impregnated with the patient's own bone marrow cells or platelet-rich plasma to enhance their *in vivo* osteogenetic properties.¹⁸ Early CPCs commonly employed either α-tricalcium phosphate (α-TCP: α-Ca₃(PO₄)₂) or tetracalcium phosphate (TTCP: Ca₄(PO₄)₂O), or both, in their powder components. Driskell *et al.*¹⁴ described in 1974 the very first calcium phosphate cement paste, which used α-TCP powders. α-TCP and TTCP do share a useful property through which both undergo in situ hydrolysis into nanocrystalline, non-stoichiometric Ap-CaP or carbonated CDHA (calcium-deficient hydroxyapatite: Ca₉(HPO₄)(PO₄)₅OH), upon contact with aqueous solutions at 20-37°C.¹⁹⁻²² For a comprehensive assessment of commercialized CPC formulations, classified with respect to their end-products (i.e., after setting), the reader is referred to a recent study of Bohner *et al.*²³

CaCO₃ is not yet seen as a major starting material in commercialized CPCs. The only exception to this was seen in the formulations of *Calcibon*[®] and *Norian*[®] ²³, which both employed about 10 to 12% of CaCO₃ in their powders for the purpose of obtaining carbonated apatite as the end-product. Very recently, Combes *et al.*²⁴ reported the very first experimental CaCO₃ cement, which had a biphasic starting material of amorphous calcium carbonate (doped with either strontium or magnesium) and crystalline vaterite. The end-products of these low-compressive strength cements were again calcium carbonate (vaterite + aragonite) because of the use of 0.9% (w/w) NaCl isotonic solution as their cement liquid. This solution was not able to transform CaCO₃ into Ap-CaP. The *in vitro* resorption of CaCO₃ (of calcite form) by the human or rat osteoclasts, in simultaneous comparison with HA and β-TCP bioceramics, has been previously studied by Monchau *et al.*⁷

The purpose of the present study was to develop a low-compressive strength but macroporous and inexpensive CaCO₃+Ap-CaP biphasic scaffold for skeletal repair by using single-phase CaCO₃ (calcite or vaterite) powders. Commercially available calcite or chemically precipitated (*in house*) vaterite powders were used, and the powder components of the cements

did not contain any other additive whatsoever. Cell culture studies on these macroporous cements will be published later.

EXPERIMENTAL PROCEDURE

Vaterite powder

Vaterite powders were synthesized as follows: 12.0 g of $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ (Catalog No: C79-500, 99.9%, Fisher Scientific, Fairlawn, NJ) was first dissolved in 1200 mL of deionized water at room temperature. 12.0 g of gelatin (Catalog No: G7-500, 100 Bloom, FisherSci) was then added to the above solution and dissolved in it by heating the solution to 35°C. Upon dissolving gelatin, the solution was cooled back to room temperature (i.e., $21 \pm 1^\circ\text{C}$). 12.0 g of NaHCO_3 (Catalog No: S233-500, 99.9%, FisherSci) was separately dissolved in 300 mL of water and placed into a glass burette, which was positioned over the Ca-gelatin solution. Ca-gelatin solution was stirred at 500 rpm. NaHCO_3 solution was then fed into the Ca-gelatin solution at the rate of 13 mL/min in a dropwise manner. At the end of NaHCO_3 addition, the solution containing the precipitates was immediately filtered by using a vacuum-assisted Buechner funnel and filter paper (Whatman, No: 41, FisherSci). Precipitates were washed with 2 L of water and then dried overnight at 65°C in air.

Preparation of the 0.5 M phosphate buffer solution for vaterite conversion

800 mL of deionized water was placed in a 1 L-capacity beaker, followed by adding 39.749 g of Na_2HPO_4 (Merck, Catalog No: 1.06585) at room temperature. Upon stirring, one obtained a transparent solution. To this solution 16.559 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (Merck, Catalog No: 1.06346) was added to raise the pH to about 7.4. Solution was then transferred into a 1 L-capacity media bottle and stored in a refrigerator when not in use.

This solution was used to convert the vaterite powders into Ap-CaP. 800 mg of vaterite powders were first placed into a 250 mL-capacity glass media bottle, followed by adding 200 mL of 0.5 M phosphate buffer solution and sealing the bottle. The bottle was kept undisturbed in a 70°C oven for 17 h. The solids were filtered and then washed with deionized water, followed by drying overnight at 70°C.

Calcite powder

The powder component of the macroporous cement of this study consisted of single-phase CaCO_3 (calcite). These cements were initially developed by using the reagent-grade precipitated CaCO_3 powders manufactured by Merck KGaA (Catalog No: 1.02076, Darmstadt, Germany). The optimized cement recipe was also tested by using the precipitated CaCO_3 (calcite) powders supplied by Fisher Scientific (Catalog No: C63-3, 99.8%, Fairlawn, NJ), as well. The cement recipe successfully worked for both reagent-grade calcite powders.

Preparation of the cement liquid for calcite powders

The setting solution was prepared as follows; first a 100 mL aliquot of concentrated H_3PO_4 (Merck, Catalog No: 1.00573, 85%, 14.8323 M) was placed into an unused erlenmeyer flask of 300 mL capacity together with a Teflon[®]-coated magnetic stir bar. This erlenmeyer flask was then placed on a magnetic stirrer plate at room temperature ($21 \pm 1^\circ\text{C}$). A 138 mL portion of concentrated NaOH (Merck, Catalog No: 1.05590, 32%, 10.8 M) was taken into a burette and placed directly on top of the open mouth of the erlenmeyer containing H_3PO_4 . The first 50 mL portion of the NaOH solution in the burette was slowly (in 20 minutes) added in a drop wise

manner, to prevent excessive heating and boiling, with a magnetic stirring rate of about 300 rpm. Upon slowly (ca. 10 min) adding another 25 mL aliquot of NaOH, the pH of the solution in the erlenmeyer rose to 0.4 (at 66-67°C). It should be noted that until now only 75 mL of NaOH was added into the erlenmeyer. The slow (ca. 10 min) addition of another 25 mL portion of NaOH increased the pH to 1.60 (at 75°C). With the following addition of another 25 mL aliquot of NaOH, pH became 2.60 (at 75°C). Finally, after adding (again slowly in about 10 min) the last 13 mL portion of NaOH in the burette to the erlenmeyer, the pH rose to 3.2 (at 45-50°C). The total volume of the viscous liquid thus formed in the erlenmeyer was completed to 250 mL by adding deionized water. The solution was cooled to room temperature under constant stirring, and transferred into a 250 mL-capacity Pyrex[®] media bottle (with a cap) for long-term storage. This solution has been confirmed to preserve its constant pH at 3.2 (at room temperature) for more than two years of storage on the laboratory bench. 1 mL of this solution (called afterwards as “SS” as an abbreviation of “setting solution”) thus contained 0.0059 mol of phosphor.

Cement preparation

A plastic cup of cylindrical geometry (approx. 8 cm tall with a diameter of 6.5 cm) was used to mix the calcite powder with the SS (setting solution) to form the cement paste. CaCO₃ powder of appropriate quantity was first weighed and then placed into this cup. SS of proper volume was measured with a pipette and then placed into a clean glass bottle. At the moment of mixing the CaCO₃ powder and the setting solution, SS in the glass bottle was immediately poured into the plastic cup containing CaCO₃. Mixing was manually performed by using a plastic spatula. Mixing was continued for 80 to 90 seconds in all trials, and at the end of mixing the formed paste was cast into a polyethylene, square-shaped weighing boat. Therefore, upon setting (which took place in 4 minutes), porous cement blocks readily took the shape of those weighing boats. Cement blocks were then soaked in deionized water at room temperature for 3 hours, followed by drying at 24°C in an air-ventilated oven.

Sample characterization

Samples were characterized by powder X-ray diffraction (XRD; Model D5000, Siemens GmbH, Karlsruhe), scanning electron microscopy (SEM; Model 630, Jeol Corp., Tokyo and Model S-3500, Hitachi, Tokyo), Fourier-transform infrared spectroscopy (FTIR; Nicolet 550, Thermo-Nicolet, Woburn, MA), water absorption [63], density measurements (Pycnometer; AccuPyc 1330, Micromeritics, Norcross, GA), inductively-coupled plasma atomic emission spectroscopy, (ICP-AES; Model 61E, Thermo Jarrell Ash, Woburn, MA) and compressive strength measurements (Model 4500, Instron Deutschland GmbH). Percentage water absorption values of the porous cement scaffolds ($\frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100$) were measured in accord with the ASTM standard C20-92.²⁵ Dry weights of the samples were recorded after drying at 24°C for 72 hours, whereas the wet weights were recorded immediately after soaking the cement samples in deionized water (at 21°C) for 3 hours. Pore sizes of the porous scaffolds were determined by using the linear intercept method on the photomicrographs.

To give more details on the characterization runs; samples for XRD analyses were first ground in an agate mortar using an agate pestle and then sprinkled onto ethanol-damped single crystal quartz sample holders to form a thin layer, followed by tapping to remove the excess of powder. The X-ray diffractometer was operated at 40 kV and 30 mA with monochromated Cu K_α radiation. XRD data (over the range of 20 to 50° 2θ) were collected with a step size of 0.03°

and a preset time of 1 sec at each step. FTIR samples were first ground in a mortar, in a manner similar to that used in the preparation of XRD samples, then mixed with KBr powder in a ratio of 1:100, followed by forming a pellet by using a uniaxial cold press. 128 scans were performed at a resolution of 3 cm^{-1} . Powder samples examined with the scanning electron microscope (SEM) were sputter-coated with a thin Au layer, to impart surface conductivity to the samples. The BET surface area of powder samples was determined by applying the standard Brunauer–Emmet–Teller method to the nitrogen adsorption isotherms obtained at -196°C using an ASAP 2020 instrument (Micromeritics Corp., Norcross, GA). Powder samples used in the ICP-AES analyses were first dissolved in nitric acid prior to the measurements. Cylindrical cement samples (1 cm diameter, 2 cm height) were loaded under compression until failure, at a crosshead speed of 1 mm/min, with a 1.5 kN load cell, to determine compressive strength. The ASTM standard C1424-04 was observed in strength measurements.

RESULTS AND DISCUSSION

Vaterite synthesis and conversion

The phase purity of vaterite powders was deduced by using the XRD technique defined by Rao.²⁶ As shown in the XRD trace of Fig. 1a, the formed powders contained around 10% calcite, the rest being vaterite. The IR spectra of the powders also revealed the vaterite structure, with a characteristic band at 744 cm^{-1} (Fig. 1b).²⁷ When precipitated in gelatin solutions as described above, vaterite was always formed with a spheroidal morphology (Figs. 1c and 1d).

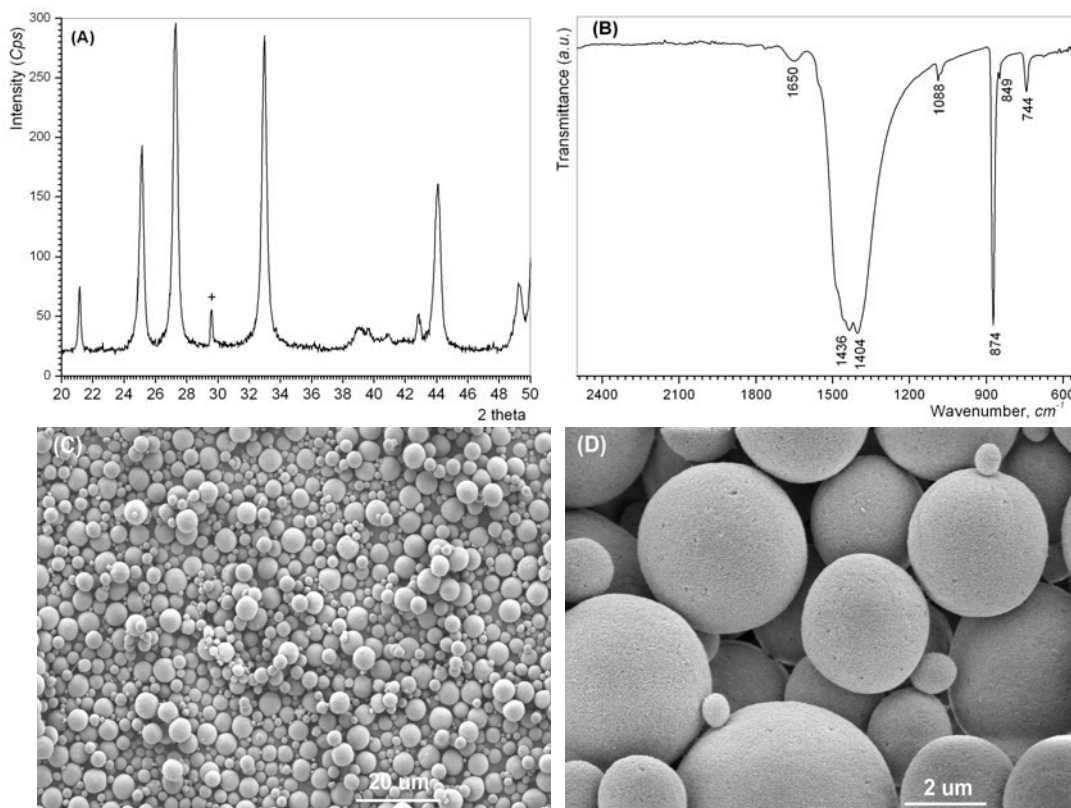


Fig. 1 (a) XRD, (b) FTIR traces, (c) & (d) SEM pictures of precipitated vaterite powders

The only peak denoted in Fig. 1a with + corresponded to calcite, all the other peaks belong to vaterite. ICP-AES analyses performed on these precipitates showed that they had about 700 ppm Na, which originated from the use of NaHCO_3 in their preparation. Although a tremendous amount of literature is available on the synthesis of vaterite particles in the presence of exotic polymers or bizarre organic substances,^{28, 29} gelatin was not a typical choice. If one intends to use vaterite as a biomaterial for skeletal repair, gelatin is logical to use in synthesis since it is simply the denatured collagen. Collagen is the only biopolymer of human bones.

Vaterite powders aged in 0.5 M phosphate buffer solutions at 70°C for 17 h were totally converted into Ap-CaP. The resultant Ap-CaP powders had again spheroidal particles but with an increased surface area. The combined SEM photomicrographs and the BET surface area data presented in Fig. 2 below summarized the conversion of vaterite spheres.

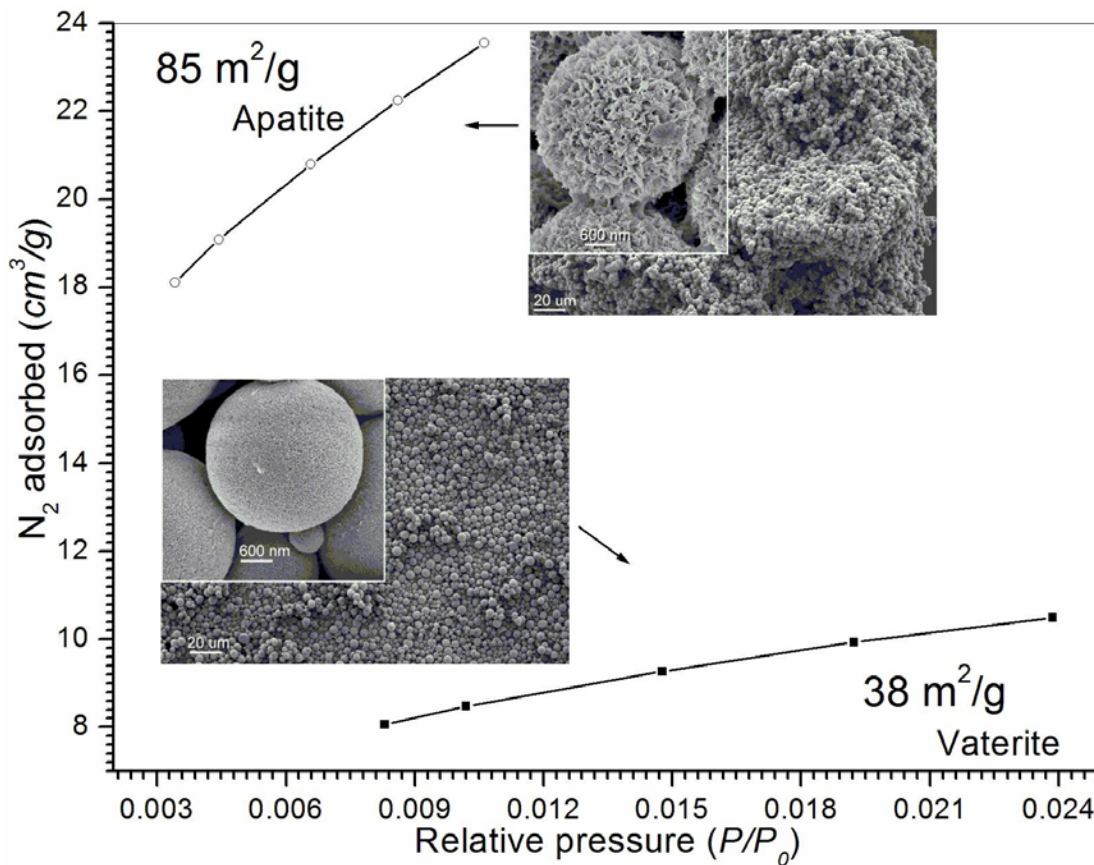


Fig.2 Conversion of ppt^d vaterite into Ap-CaP by ageing in 0.5 M phosphate buffer at 70°C; surface area and SEM morphology before and after the conversion

Porous cement preparation from calcite powder

This cement was designed to have a unique property; calcium was supplied by its powder component, whereas phosphorus was only received from its setting solution. Since this inexpensive, low-strength cement³⁰ had a powder component comprised of single-phase calcite, the development of a self-setting cement out of pure calcite solely relied on the manufacture of a proper cement liquid or the setting solution (SS). If one used concentrated phosphoric acid alone, the reaction of the acid with calcite would be too severe and instantaneous (resulting in the formation of a mixture of acidic phosphates, such as $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$), and the

handling or shaping of the material into a specific shape would be almost impossible. Partial neutralization of phosphoric acid with concentrated sodium hydroxide solution, in forming the SS, thus seemed to be a viable alternative. Pore formation in this biphasic cement proceeded with the conversion of only a certain fraction of CaCO_3 into Ap-CaP upon coming into contact with the mildly acidic SS, and the by-product of this limited conversion reaction was CO_2 gas. The pore cavities were the direct result of physically entrapped bubbles of the evolved CO_2 gas in the setting body.

SEM photomicrographs of Figs. 3a and 3b portrayed the starting morphology of the powder component (i.e., CaCO_3) of this cement. Calcite powders used in this study were commercial powders (supplied by Merck KGaA and Fisher Scientific Corp.) and both of these precipitated chalk powders were morphologically similar to one another. Both calcite powders were found to have a surface area of about $6.15 \pm 0.25 \text{ m}^2/\text{g}$ (BET runs in triplicate on each), and similarly consisted of $0.5 \text{ }\mu\text{m}$ -thick and $1.5 \text{ }\mu\text{m}$ -long spindle-shaped agglomerated particles.

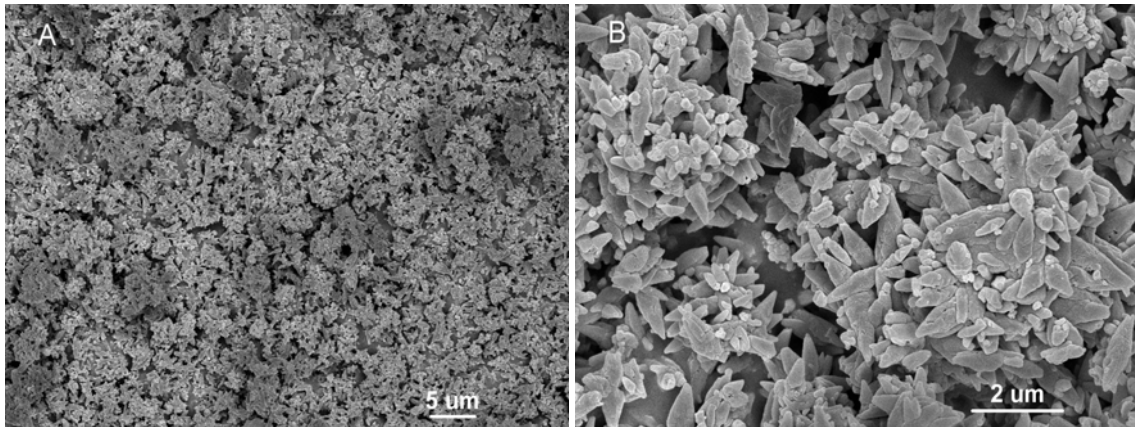


Fig. 3 SEM photomicrographs of starting calcite powders, (a) low, (b) high mag image

Table I listed three optimal recipes developed for the preparation of these cements. The nominal, starting Ca/P molar ratio in these cements could be adjusted either by simply varying the amount of calcite powder or the volume of the SS to be used. However, since the SS developed for calcite powders was so mild that the conversion of CaCO_3 to Ap-CaP would never reach to completion.

Table I. Optimal recipes for the preparation of biphasic (CaCO_3 +Ap-CaP) porous cements by using pure calcite powders

Powder (g)	SS (mL)	SS/P (mL/g)	Mixing time (sec)	Nominal Ca/P (molar)	% CaCO_3 left* (rest Ap-CaP)
29.75	30	1.0084	80	1.67	82 ± 3
26.90	30	1.1152	85	1.51	79 ± 3
22.30	30	1.3453	90	1.25	77 ± 4

* determined by using the ratio of XRD peak intensities of calcite and Ap-CaP phases

It should be apparent from Table I that when one keeps the mixing time limited to between 80 and 90 seconds, there would not be enough time for a complete reaction between calcite and the SS. If one increased the mixing (or agitation) time to about 4 or 7 minutes, more calcite would convert to Ap-CaP, and total porosity and pore sizes would significantly decrease (data not shown). Figures 4a and 4b showed the optical micrographs of the porous cement scaffolds produced at the nominal Ca/P molar ratio of 1.51. Figures 5a through 5d, on the other hand, depicted the SEM photomicrographs of the same. Pores of these scaffolds are big enough to allow the attachment and proliferation of bone cells, i.e., osteoclasts and osteoblasts.

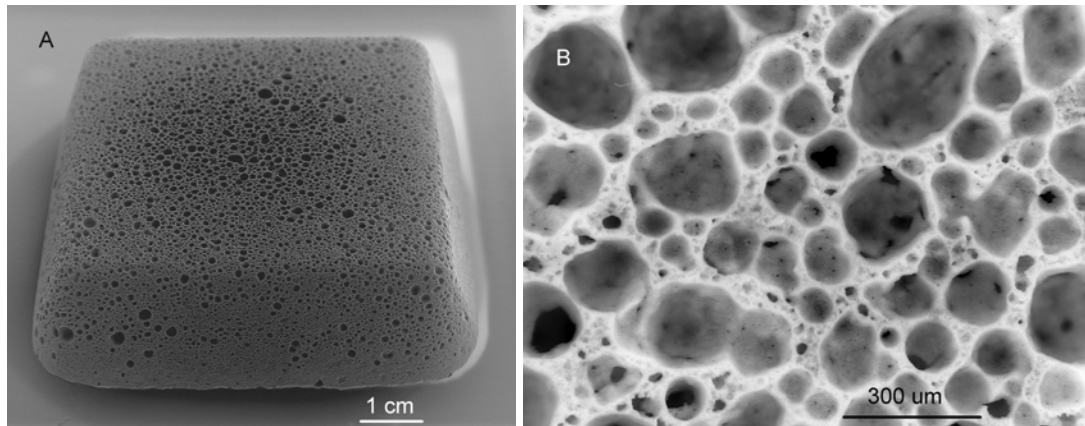


Fig. 4 Optical micrographs of the set cement body (*Table I*; $Ca/P = 1.51$), (a) macro view of the body cast into a large weighing cup, (b) optical microscope image of the same

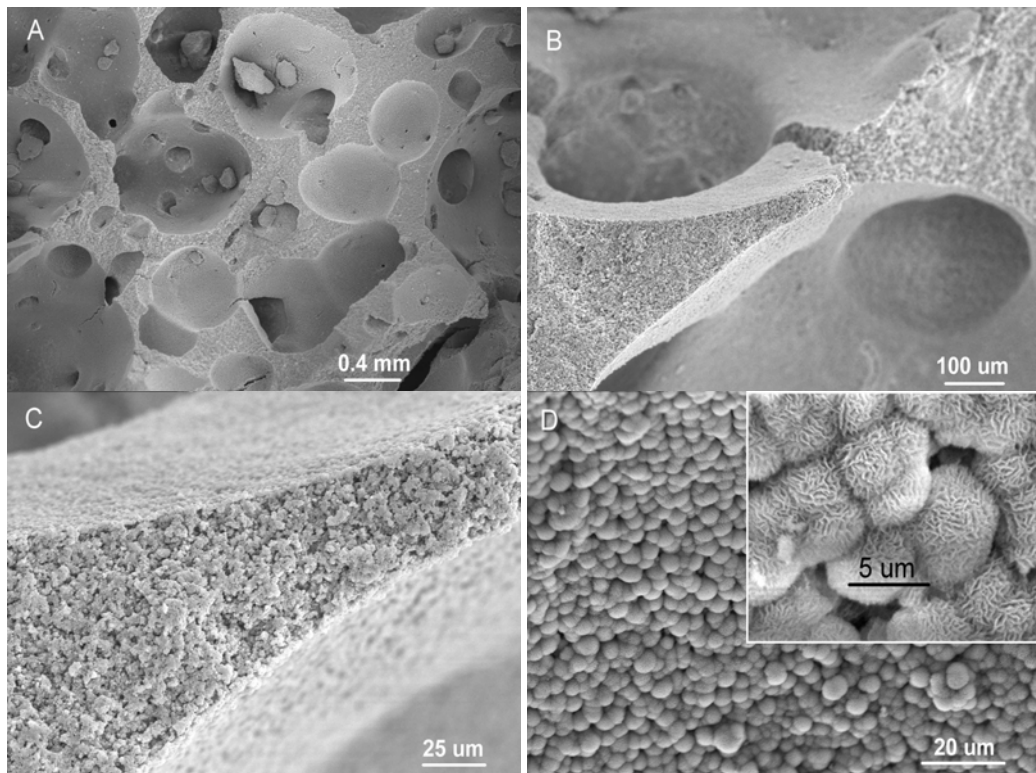


Fig. 5 SEM photomicrographs of set cement body (*Table I*; $Ca/P = 1.51$); (a) to (c) pore morphology of the cement body, (d) close-up image of the Ap-CaP skin

Upon casting the bubbling paste (after 80-90 seconds of agitation/kneading with a spatula) into a plastic weighing boat (Fig. 4a), the paste completed its setting within the next 4 to 5 minutes. After 4 minutes, the cement body was stable and workable. Figures 5b and 5c revealed the skin of Ap-CaP formed along the entire external surfaces. The micromorphology of the Ap-CaP skin shown in the inset of Fig. 5d resembled that of Ap-CaP biomimetically deposited via SBF (synthetic body fluid) solutions onto metals.³¹

The variation in the SS/P ratios was found to have a small but detectable effect on the degree of conversion of calcite to Ap-CaP. On the other hand, the change in SS/P ratios employed did not produce a significant change in percentage water absorption, density and compressive strength of the resultant cement bodies (as shown in Table II), as well as total apparent porosity. Unfortunately, in this study, the percentage porosity was mainly estimated by visual inspection of the photomicrographs, in combination with the water absorption and density measurements. According to the results of measurements by using the linear intercept method, pore sizes in the samples were varying between 20 and 750 μm . Micropores (20 to 80 μm) were typically located around the macropores (90 to 750 μm), and the struts defining the macropores were porous, as well (Figs. 4b and 5c).

Table II. Variation of the physical properties of biphasic (CaCO_3 +Ap-CaP) porous cements

Powder (g)	SS (mL)	Setting time (min)	Water absorption (%)	Density* (g/cm^3)	Compressive strength* (MPa)
29.75	30	4	172 \pm 5	0.60	0.71
26.90	30	4	169 \pm 3	0.63	0.67
22.30	30	4	179 \pm 6	0.59	0.69

* reported as the averages of measurements in triplicate

It should be noted that when a whole cement block (as shown in Fig. 4a) was placed into 150 mL of deionized water (in a 400 mL-capacity beaker) at RT, its pH was recorded as 6.4 \pm 0.2. However, upon changing that water, after 10 minutes of soaking, with a fresh 150 mL aliquot, the pH of the water rose to 6.8 \pm 0.2. After 1 h of soaking, the pH of the water became stabilized at 7.0 \pm 0.1. This soaking step was deemed to be necessary to substantially remove the sodium- H_2PO_4 species originating from the mildly acidic SS.

Conversion of cement scaffolds to Ap-CaP

The bottom trace of Fig. 6 showed one of the characteristic XRD spectra for the porous cement blocks soaked in water for 3 hours, following the setting. It shows the presence of about 80% calcite (ICDD PDF 05-586). The top XRD trace of Fig. 6 confirmed the formation of single-phase Ap-CaP of low crystallinity upon soaking these biphasic cement scaffolds in a 0.5 M phosphate buffer solution at 80°C for 36 h. The XRD trace shown in Fig. 6b was obtained from the agate mortar-ground powders of these 0.5 M phosphate buffer-soaked and washed scaffolds.

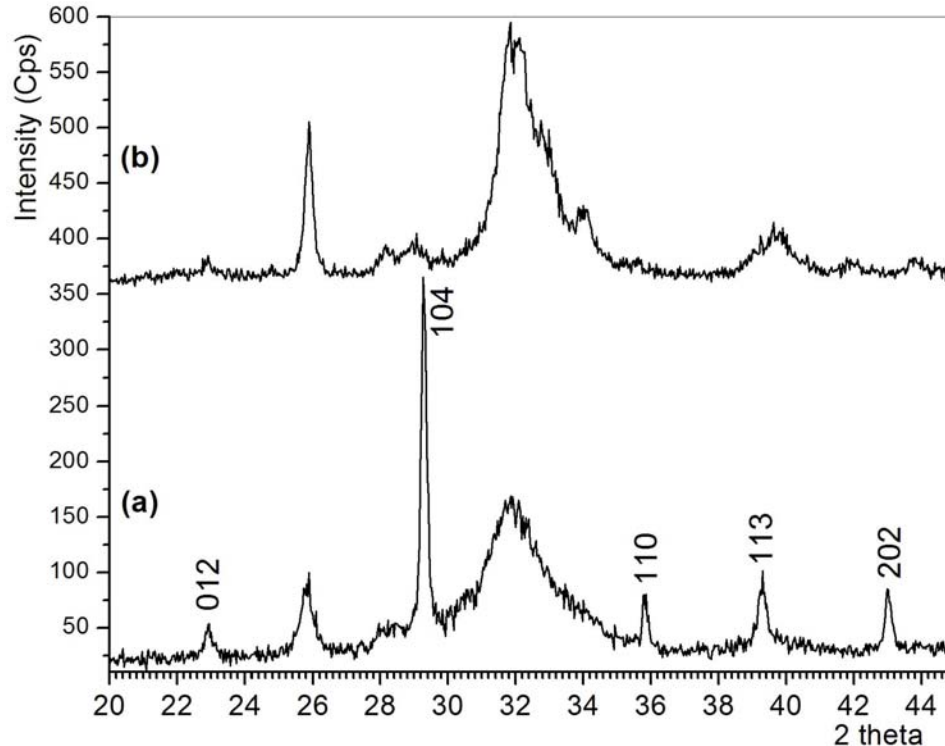


Fig. 6 XRD traces of cement scaffolds (Table I; Ca/P = 1.51 composition); (a) cement scaffold after 3 h of soaking in water at RT, peaks of calcite were indicated by their Miller indices and the rest belonged to Ap-CaP of low crystallinity, (b) cement scaffold after 36 h of soaking in 0.5 M phosphate buffer at 80°C

FTIR spectra of the same samples were plotted in Figure 7, respectively. In Fig. 7a, the bands observed at 3643, 2513, 1797, 1587, 1450, 871, 848, and 713 cm^{-1} were characteristic of calcite. On the other hand, in Fig. 7b, the weak stretch for OH, which was observed at 3571 cm^{-1} , indicated the presence of hydroxyl ions in Ap-CaP. Soaking of the porous cement scaffolds in a 0.5 M phosphate buffer solution at 80°C caused the conversion of calcite into Ap-CaP.

Water absorption and density measurements performed on these converted scaffolds showed that the values given in Table II did not change. However, the compressive strength of the converted scaffolds increased to 2.4 ± 0.4 MPa from the starting value of ca. 0.7 MPa. It should be noted that the compressive strength of trabecular bones is between 2 and 10 MPa, whereas that of cortical bones is in the vicinity of 100 MPa. The weak cement scaffolds of this study can only match the compressive strength of trabecular bones at its lower limit. Many commercial cements could reach compressive strengths in excess of 50 MPa.^{16, 23} However, those high-strength cements, which employed α -TCP or TTCP powders in their formulations (with lesser amounts of CaCO_3), were not able to show any significant overall *in vivo* resorbability even after one year of implantation.

ICP-AES analyses of the agate mortar-ground powders of the converted cement scaffolds (from the samples of Figs. 4(b) and 5(b)) gave a Ca/P molar ratio of 1.56 ± 0.3 , together with a Na concentration of 7700 ± 250 ppm. The reader is referred to the works of Yubao *et al.*³², Ivanova *et al.*³³, and Kasten *et al.*³⁴ for more detailed treatises (including *in vitro* and *in vivo* experiments) on non-stoichiometric Ap-CaP.

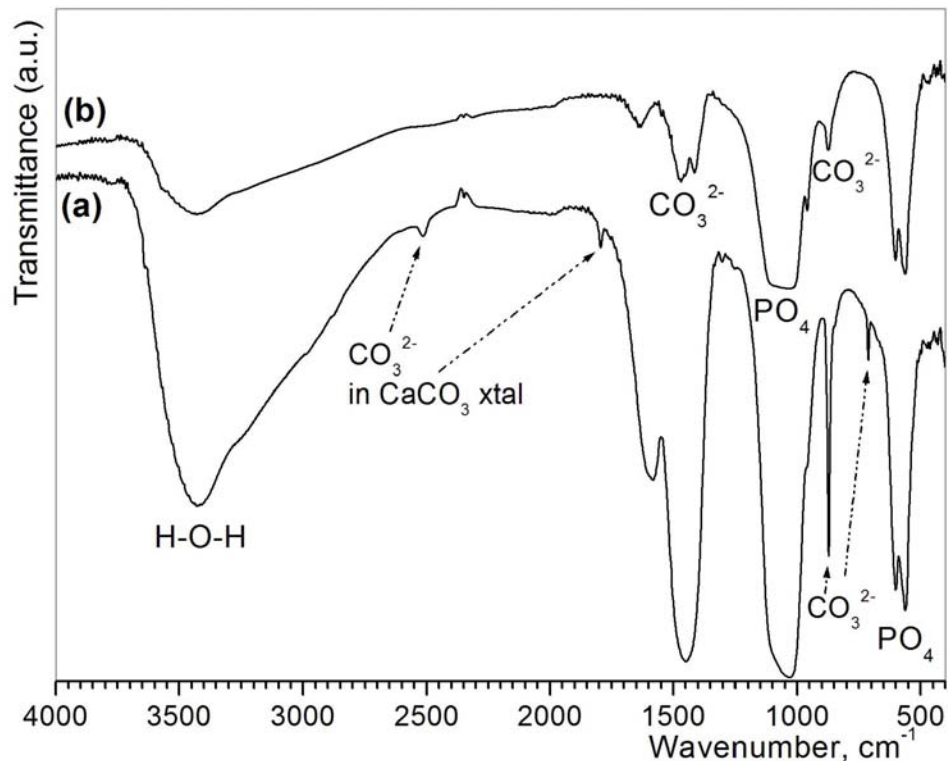


Fig. 7 FTIR traces of cement scaffolds (Table I; Ca/P = 1.51 composition); (a) cement scaffold after 3 h of soaking in water at RT, (b) cement scaffold after 36 h of soaking in 0.5 M phosphate buffer at 80°C

Pure calcite powders (Fisher Sci. Corp., Catalog No: C63-3) could also be converted to Ap-CaP by simple soaking in a dilute phosphate solution at 60 to 80°C. We have previously shown that even the surfaces of calcitic marble blocks would transform to Ap-CaP by using such a soaking procedure at 60°C.³⁵ To transform the calcite powders, this time we used the Soerensen's buffer solution (5.26 g KH_2PO_4 and 8.65 g of Na_2HPO_4 dissolved in 1 L water). Soaking experiments were performed in 250 mL-capacity Pyrex[®] media bottles, which contained 3.1 g calcite powder (whose morphology was given in Fig. 3) and 225 mL of Soerensen's buffer in each. Sealed bottles were first kept in a microprocessor-controlled oven at 60°C for 24 h, and then the solutions were replenished with an unused solution at every 24 h for the next 48 hours period. For the last 48 hours of the total soaking time of 72 h, the temperature of the oven was increased to 80°C. Powders in the media bottles were filtered out, washed with deionized water and dried overnight at 80°C. Resultant powders had the morphology illustrated in Fig. 8a. Characteristic XRD spectra of these Ap-CaP powders were given in Fig. 8b. ICP-AES analyses of these powders returned a Ca/P molar ratio of 1.58 ± 0.2 . BET surface area of these powders were measured as $40 \pm 2 \text{ m}^2/\text{g}$. This procedure was presented here as a robust and economical way of synthesizing Ap-CaP powders of high surface area in bulk form. It also served to confirm the phase nature of the "Ap-CaP skin" observed on our porous cement scaffolds as depicted in Fig. 5d.

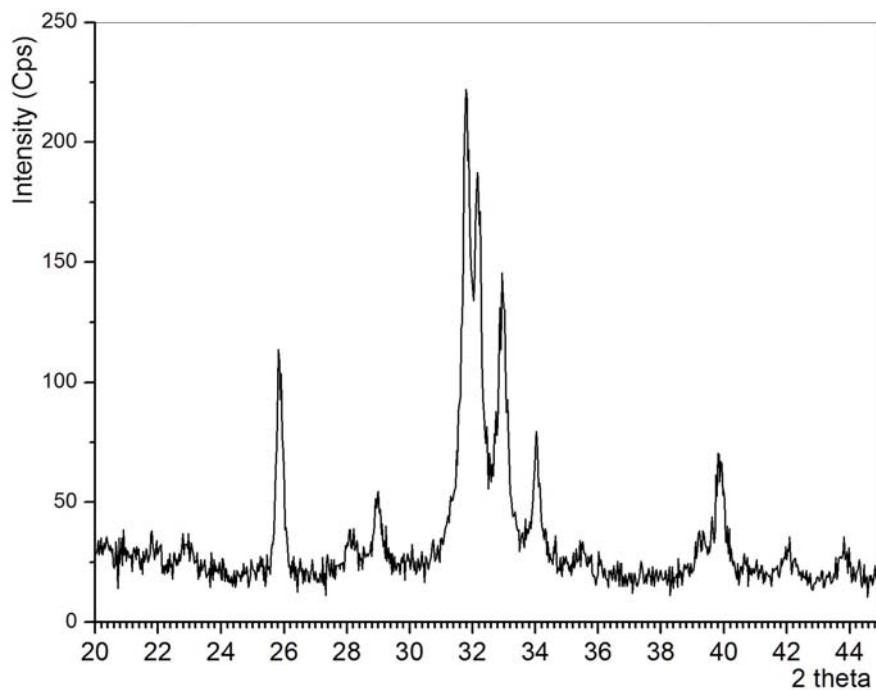
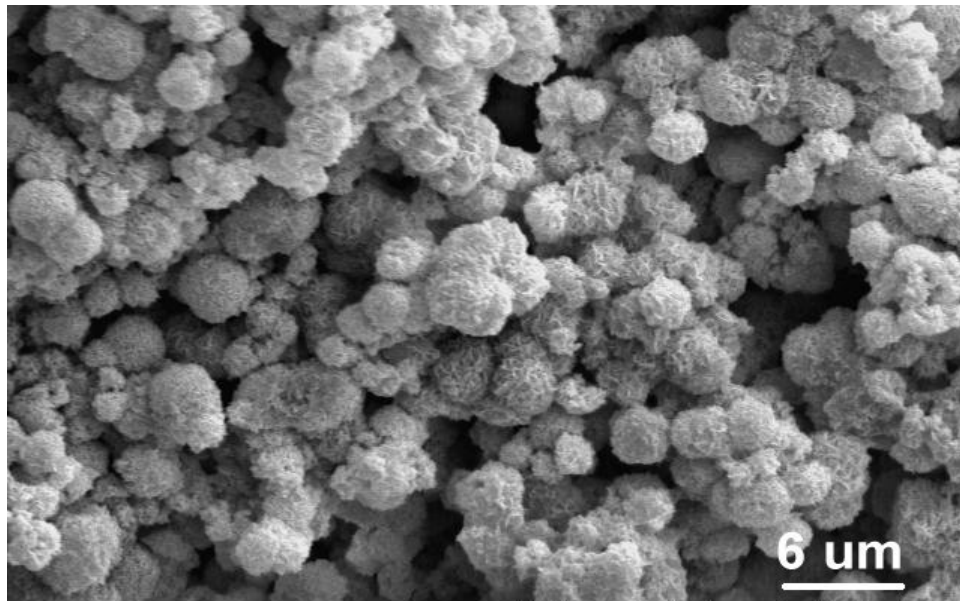


Fig. 8 (a) SEM photomicrograph of Ap-CaP powders synthesized by soaking calcite powders in Soerensen's buffer at 60-80°C for 72 h, (b) XRD trace of the same powder

The interest in the conversion of CaCO_3 into Ap-CaP, to obtain porous biomaterials, started with the pioneering work of Prof. Della M. Roy in the early 70's.³⁶⁻³⁸ In these extremely competent studies, Roy *et al.*^{37, 38} investigated the conversion of natural coral skeletal aragonite (Porites) by using high temperatures (140 to 260°C) and high autoclave pressures (550 to 1055 kg/cm^2) in the presence of acidic (such as, CaHPO_4 and $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$) and basic (such as,

(NH₄)₂HPO₄) phosphates, as well as of sodium or potassium orthophosphates and acetic acid. Hydrothermal reactions were carried out from 12 to 48 h under the above-mentioned experimental conditions. Over the last three decades, the early studies of Roy *et al.*^{36, 38} were duplicated, improved, or inspired by and the scientific literature related to these is immense, but just to name a few proficient examples, the works of Zaremba *et al.*³⁹, Su *et al.*⁴⁰, Ni *et al.*⁴¹, and Jinawath *et al.*⁴² must be cited.

Nowadays, converted coral and other marine skeletal species are also available as commercial porous implant biomaterials.^{43, 44} However, it should be noted that in most coral or marine species the major phase was biological aragonite, and its conversion to Ap-CaP was more difficult than that of calcite or vaterite of this study. This was apparent from the need for higher temperatures and higher autoclave pressures in converting biological aragonite to Ap-CaP. Experimental studies which started only with calcite or vaterite powders and which produced macroporous Ap-CaP biomedical scaffolds from those were quite rare, and this study was conceived to fill this gap.

From now on, vaterite shall be expected to be seen in the powder components of new self-setting, orthopedic CaP cement formulations.

CONCLUSIONS

- (1) Vaterite (CaCO₃) powders were synthesized in mixed solutions of Ca-chloride and gelatin by slowly adding a NaHCO₃ solution to those. Formed vaterite particles had a spheroidal morphology and surface area greater than 35 m²/g.
- (2) Soaking of vaterite powders in 0.5 M phosphate buffer solution at 70°C caused them to completely transform into Ap-CaP. Upon this transformation, the surface area of the samples increased to 85 m²/g.
- (3) Single-phase calcite (CaCO₃) powders were used to form a macroporous, biphasic (calcite+Ap-CaP) and weak cement scaffold.
- (4) Concentrated orthophosphoric acid, partially neutralized by NaOH to pH 3.2, was used as the setting solution of the above-mentioned cement.
- (5) 0.5 M phosphate buffer solutions were able to fully convert the calcite+Ap-CaP biphasic scaffolds into pure Ap-CaP, upon soaking these weak scaffolds in those solutions at 80°C.
- (6) Soerensen's buffer was used to synthesize single-phase Ap-CaP powders, with a Ca/P molar ratio of 1.58 and BET surface area ≥40 m²/g, by soaking pure calcite powders in these solutions at 60 to 80°C.

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