ELSEVIER

Biomaterials 21 (2000) 1429–1438

Biomaterials

Synthesis of biomimetic Ca-hydroxyapatite powders at 37°C in synthetic body fluids

A. Cüneyt Tas*

Department of Metallurgical and Materials Engineering, Middle East Technical University, Ankara 06531, Turkey

Received 1 October 1998; accepted 1 September 1999

Abstract

An important inorganic phase for synthetic bone applications, calcium hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), was prepared as a nano-sized (~ 50 nm), homogeneous and high-purity ceramic powder from calcium nitrate tetrahydrate and diammonium hydrogen phosphate salts dissolved in modified synthetic body fluid (SBF) solutions at 37°C and pH of 7.4 using a novel chemical precipitation technique. The synthesized precursors were found to easily reach a phase purity $> 99\%$ after 6 h of calcination in air atmosphere at 900°C, following oven drying at 80°C. There was observed, surprisingly, no decomposition of HA into the undesired β-TCP phase even after heating at 1600°C in air for 6 h. This observation showed the superior high-temperature stability of such 'biomimetic' HA powders as compared to those reported in previous studies. The former powders were also found to contain trace amounts of Na and Mg ions, originating from the use of SBF solutions instead of pure water during their synthesis. Characterization and chemical anaalysis of the synthesized powders were performed by X-ray powder diffraction, energy-dispersive X-ray spectroscopy, Fourier-transform infra-red spectroscopy, scanning electron microscopy, and inductively coupled plasma atomic emission spectroscopy. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Bioceramics; Calcium hydroxyapatite; Biomimetic; Synthetic body fluid

1. Introduction

Calcium hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$), the main inorganic component of the hard tissues in bones, is a member of the 'apatite' family, including compounds with similar structure but not necessarily of identical composition. Biological apatites, which comprise the mineral phases of calcified tissues (enamel, dentin, and bone), differ from pure HA in stoichiometry, composition and crystallinity, and in other physical and mechanical properties. They are usually observed [1] to be carbonate-substituted and calcium-deficient.

Synthetic body fluids (SBF), prepared in accord with the chemical analysis of human body fluid, with ion concentrations nearly equal to those of the inorganic constituents of human blood plasma, were first used by Kokubo et al. [2] to prove the similarity between in vitro and in vivo behavior of certain glass-ceramic compositions. In these studies, the glass-ceramic samples were soaked in SBF solutions and their surfaces were observed to be coated with a poorly crystallized calcium-deficient and carbonate-containing apatite, which was similar to 'bone apatite' [3]. Therefore, the formation of an apatite layer on the glass and glass-ceramic surfaces was confirmed to be an essential condition for the bonding of implants made from such materials to the surrounding living bone in human body. Kokubo et al. [3,4] proposed that the calcium ions, dissolved from the glass-ceramic samples immersed in SBF, increase the degree of supersaturation of the surrounding body fluid with respect to the apatite, and the hydrated silica that forms on the surfaces of the glass-ceramics provides the sites favorable for further apatite nucleation. Once apatite is nucleated, it can grow spontaneously in the body environment [5].

HA powders have generally been synthesized from aqueous solutions for use in bioceramic applications. It is known [6] that calcium hydroxyapatite is the least soluble and the most stable compound of calcium phosphate phases in aqueous solutions at pH values higher than 4.2. However, HA has usually been preferred to be

---

* Correspondence address: Pulvermetallurgisches Laboratorium, Max-Planck-Inst. Metallforschung, Heisenbergstrasse 5, 70569 Stuttgart, Germany. Tel.: + 49-711-686-1233; fax: + 49-711-686-1131.

E-mail address: tas@aldix.mpi-stuttgart.mpg.de (A. Cüneyt Tas).

PII: S0142-9612(00)00019-3

synthesized in highly alkaline media [7–12] to insure the thermal stability and phase purity of the resultant powders at high-temperature (1100–1300°C) sintering. Chemical synthesis of HA powders in neutral and/or slightly acidic aqueous media is known to be a more complicated and difficult task [9,13]. This study [14] focuses on the determination of the experimental parameters of HA synthesis at physiological pH (7.4) and temperature (37°C). The degree of supersaturation, presence of seeds, temperature, pH, and ionic strength are all known to affect the course of synthesis of calcium phosphate bioceramics from aqueous solutions [15–20].

The effect of intentional seeding has played an important role in inducing the formation of phases that would normally not nucleate [16,17]. The process of seeding to precipitate HA and octacalcium phosphate (OCP: $Ca_8H_2(PO_4)_6 \cdot 5H_2O$) was investigated using β-TCP (Whitlockite), dicalcium phosphate dihydrate (DCPD; $CaHPO_4 \cdot 2H_2O$), calcium fluoride, amorphous calcium phosphate (ACP), OCP and HA, itself, as seed materials [15–17,19]. HA and OCP overgrowths in solutions were also studied owing to the biological interest of these phases that appear to precipitate during hard tissue mineralization. It was shown that it is possible to grow apatite on OCP, and vice versa, in the vicinity of the physiological pH value of 7.4 [18].

Electrolytes that were added to alter the ionic strength of the solutions have also been shown to influence the course of precipitation [15–17,19]. NaCl, KCl, or KNO_3 were added to a precipitation solutions as a background electrolyte to maintain the ionic strength constant at a value near to that of human plasma or saliva.

de Groot et al. studied [10] the high-temperature characteristics of synthetic HA and reported that synthetic HA with a Ca/P ratio near to 1.67 was stable only below 1200°C when sintered in a dry or wet air atmosphere. It was claimed that beyond 1200°C, HA loses its OH groups gradually and transforms into oxyapatite ($Ca_{10}(PO_4)_6O$). When heated to 1450°C, oxyapatite was found [10] to dissociate into the phases α-TCP $Ca_2P_2O_7$ and $Ca_4P_2O_9$. On the other hand, Tas et al. recently reported [12] that pure HA powders synthesized in pure water may withstand, without decomposition, temperatures as high as 1300°C when heated in an air atmosphere for 6 h.

Synthetic HA is used mainly in hard tissue replacement applications since it is capable of undergoing bonding osteogenesis and is chemically stable in vivo for long periods of time [21,22]. Studies performed on the sintering of HA showed [23] that the tensile strength of HA dropped drastically corresponding to the decomposition of HA into TCP, and the associated dehydroxylation.

The surface properties and synthesis conditions of calcium hydroxyapatite, prepared by using pure water in highly alkaline medium, have previously been examined using different solutions [6,8,24] including SBF. Metastable synthetic body fluids (SBF) were previously proven [25–31] to incubate and facilitate the spontaneous nucleation and growth of a carbonated and 'bone-like' calcium hydroxyapatite on immersed silica or titania gels, bioglass and titanium samples at physiological pH and temperature. However, the chemical synthesis of HA powders by using SBF solutions maintained at physiological pH and temperature (i.e., 7.4 and 37°C, respectively), instead of pure water, has never been attempted, to the authors' knowledge, by using the starting chemicals calcium nitrate and di-ammonium hydrogen phosphate. The study presented here thus becomes the first systematic step taken in this specific field of 'biomimetic' calcium hydroxyapatite powder synthesis technology.

2. Materials and methods

2.1. Preparation of SBF

SBF is known to be a metastable buffer solutions [3,5], and even a small, undesired variance in both of the preparation steps and the storage temperatures, may drastically affect the phase purity and high-temperature stability of the produced HA powders, as well as the kinetics of the precipitation processes.

Merck-grade NaCl (99.5%), $NaHCO_3$ (99.5%), KCl (99.0%), $Na_2HPO_4 \cdot 2H_2O$ (99.5%), $MgCl_2 \cdot 6H_2O$ (99.0%), Na_2SO_4, $(CH_2OH)_3CNH_2$ (99.5%), $CaCl_2 \cdot H_2O$ (99.0%) and HCl (37 vol%, Carlo-Erba, Rome, Italy) were used in the preparation of the SBF of this study.

SBF solutions [25–29] were prepared by dissolving appropriate quantities of the above chemicals in de-ionized water. Reagents were added, one by one after each reagent was completely dissolved in 700 ml of water, in the order given in Table 1. A total of 40 ml of 1 M HCl solution was consumed for pH adjustments during the preparation of 1 l of SBF solutions. A 15 ml aliquot of this acid solution was added just before the addition of the sixth reagent, viz., $CaCl_2 \cdot 2H_2O$. Otherwise, the solution would display slight turbidity. The remaining part of the HCl solution was used during subsequent titration. Following the addition of the eigth reagent (*tris*(hydroxymethyl)aminomethane), the solution temperature was raised from ambient to 37°C. This solution was then titrated with 1 M HCl to a pH of 7.4 at 37°C. During the titration process, the solution was also continuously diluted with consecutive additions of de-ionized water to make the final volume equal to 1 l. It was observed in this study that the prepared SBF solutions can be stored at 5°C for a month without degradation.

The ion concentrations of the SBF solutions used in this study were matched more closely with those of 'human plasma' than the ones given by Kokubo et al. [2–4,25–29]. The most significant differences of this study, as compared to the previous workers, in terms of

Table 1
Chemical composition of SBF solutions[a]

Order	Reagent	Amount (gpl)
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

[a] *Patent pending*. Turkish Patent Institute, Turkey, *Appl. No.* 99-0037, 11 January 1999.

Table 2
Ion concentrations of SBF solutions and human plasma

Ion	Kokubo et al. (mM)	Present work (mM)	Human plasma (mM)
Na	142.0	142.0	142.0
Cl^-	147.8	125.0	103.0
HCO_3^-	4.2	27.0	27.0
K^+	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5
Ca^{2+}	2.5	2.5	2.5
HPO_4^{2-}	1.0	1.0	1.0
SO_4^{2-}	0.5	0.5	0.5

the 'SBF ion concentrations' may be summarized as follows:

(a) the nominal, initial HCO_3^- ion concentration of SBF was increased from 4.2 mM [2–4,25–29] to 27.0 mM which is exactly the same concentration as that of human plasma,
(b) The Cl^- ion concentration was decreased from 147.8 mM [2–4,25–29] 125.0 mM whereas the Cl^- ion concentration of human plasma is 103.0 mM.

These significant improvements in Cl^- and HCO_3^- concentrations of SBF are shown to be achievable just by changing the starting chemical $K_2HPO_4 \cdot 3H_2O$ [2–4,25–29] to $Na_2HPO_4 \cdot 2H_2O$ during solution preparation, while keeping all remaining chemicals the same. The differences between the ion concentrations of SBF solutions and those of human plasma are compared in Table 2.

2.2. Synthesis of HA powders in SBF

$Ca(NO_3)_2 \cdot 4H_2O$ (99%) and $(NH_4)_2HPO_4$ (99%) solutions were used in the precipitation runs as the calcium and phosphate ion sources, respectively. NH_4OH solutions were used to adjust the pH values of the solutions when necessary.

The required amounts of the starting chemicals $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ were first dissolved in SBF solutions contained in separate beakers at the start of precipitation experiments. As soon as both reagents were dissolved in SBF, a slight turbidity was observed in the beakers due to the immediate formation of fine precipitates, i.e., 'seeds'.

For the study of formation characteristics and phase evaluation of these fine precipitates, 23.615 g of $Ca(NO_3)_2 \cdot 4H_2O$ and 5.154 g of $(NH_4)_2HPO_4$ reagents were dissolved and diluted to a total volume of 250 ml in SBF. The solutions were then left still for a day at room temperature without stirring. Thus formed and aged 'seeds' were removed from the mother liquors by filtration, washed two times with 100 ml of deionized water, dried in an oven at 80°C for a day, and heated (3°C/min) to higher (900–1170°C) temperatures to improve the levels of crystallization attained.

Following the characterization of these seeds by X-ray powder diffraction (XRD), HA powders were synthesized at different pH ranges varying from highly alkaline to physiological media (i.e., SBF).

Two precipitation procedures were studied during the synthesis of HA powders via SBF solutions. The first procedure involved the addition of pre-determined volumes of diluted NH_4OH solutions into the reaction beakers, for pH adjustment purposes, at the beginning of the precipitation runs. These were called the '*initial-addition*' experiments. On the other hand, in the second procedure utilized, the ammonium hydroxide additions were made by '*continuous-addition*' during the precipitation process.

A series of experiments of the '*initial-addition*'-type were performed over the pH range of 11.0–4.5 (at 37°C), ignoring the presence of the seeds in the reactant solutions. $Ca(NO_3)_2 \cdot 4H_2O$ solutions having seven different concentrations were first prepared in the SBF medium. Different volumes of each $Ca(NO_3)_2 \cdot 4H_2O$ solution were placed in 25 ml beakers, placed in a 37°C thermostated water bath, and stirred for 2 h. Experimental parameters pertaining to the reaction mixtures, labeled I1 through I7, are given in Table 3. In a set of experiments, labeled as I8, the ammonia additions were intentionally not made. A typical process flowchart prepared for the powders labeled I7 is given in Fig. 1(a). On the other hand, a typical process flowchart prepared for the synthesis of HA powders (labeled as Cl) by the '*continuous-addition*' of NH_4OH is given in Fig. 1(b).

The powders recovered from their mother liquors (following overnight drying in an oven at 90°C) were lightly ground by hand for 20 min using an agate mortar and pestle, and calcinated at 1200°C (3°C/min) for 6 h in alumina crucibles. Powders I7 and Cl were also calcined in the temperature range 1300–1600°C for 6 h, in

Table 3
Experimental parameters of HA synthesis in SBF solutions

Exp.	$Ca(NO_3)_2 \cdot 4H_2O$ concentration (M)	$Ca(NO_3)_2 \cdot 4H_2O$ volume (ml)	NH_4OH volume (ml)	Initial pH at 37°C	Final pH at 37°C
I1	0.400	54	75	10.7	9.6
I2	0.338	64	65	10.4	9.3
I3	0.230	94	35	10.1	9.0
I4	0.208	104	25	9.8	8.7
I5	0.190	114	15	9.5	8.4
I6	0.182	119	10	9.2	8.0
I7	0.174	124	5	8.7	7.4
I8	0.167	129	—	6.7	5.6
I8A	0.167	129	—	5.6	4.9

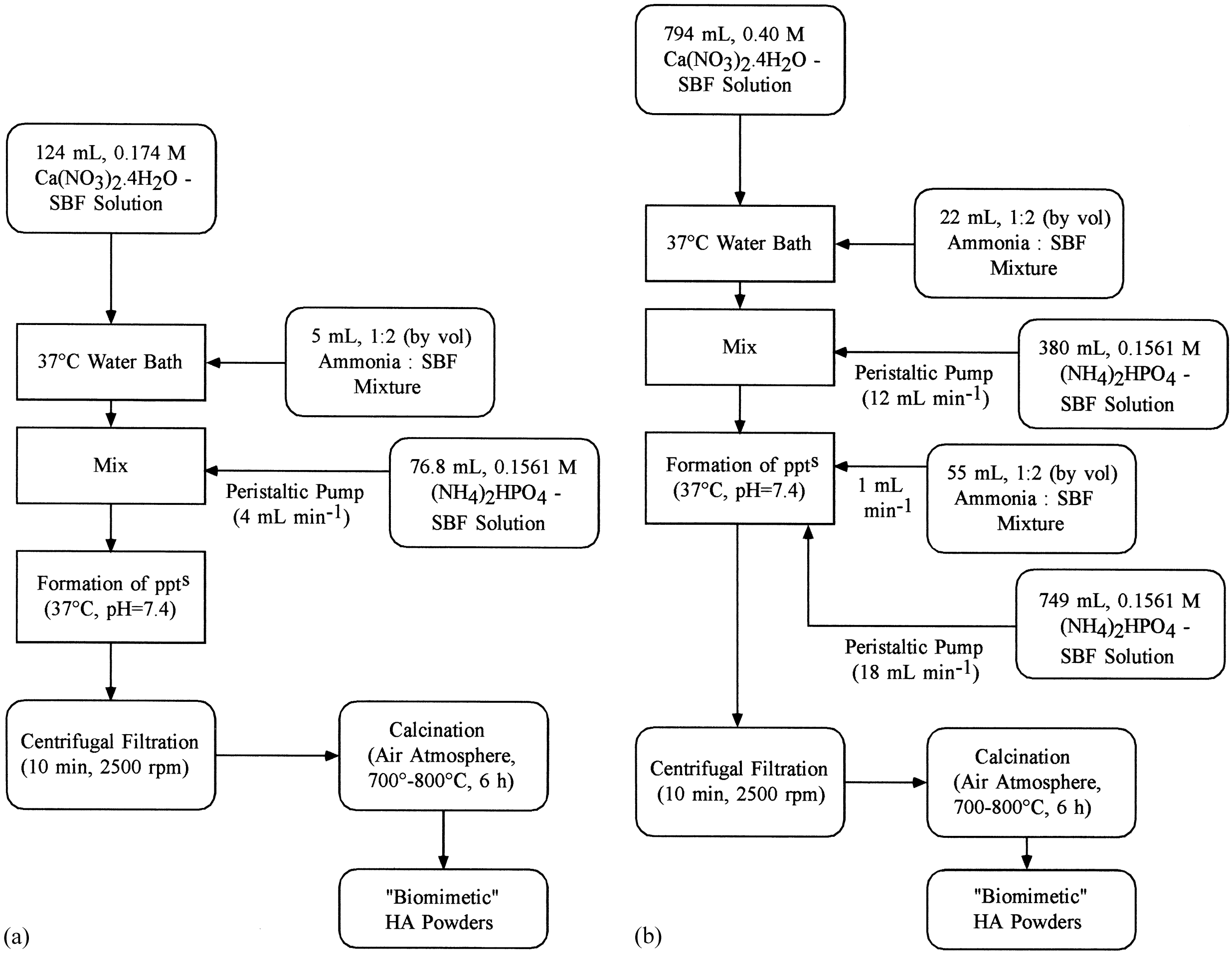

Fig. 1. (a) Process flowchart for HA synthesis by the 'initial addition' technique and (b) process flowchart for HA synthesis by the 'continuous addition' technique.

platinum crucibles in a vertical $MoSi_2$ tube furnace using a stagnant air atmosphere.

2.3. Powder characterization

The phase purity and the levels of crystallinity of the calcined HA powders were studied by X-ray powder diffraction (XRD) at the typical step size of 0.05° 2θ and a count time of 1 s. A Cu K_α tube operated at 40 kV and 20 mA was used for the generation of X-rays. All of the collected X-ray spectra were corrected by using pure silicon (99.95%) as an external standard. A least-squared unit cell refinement algorithm [32] was used for the precise determination of crystallographic parameters of the synthesized powders. Further details of XRD analysis were described elsewhere [33].

Fourier-transform infrared spectroscopy (FT-IR) was used in the wave number range of 4000–400 cm^{-1} to support and/or verify the XRD findings, to differentiate between varying levels of relative crystallinity, and to provide evidence of ionic substitution. Experimental spectra of solid samples were obtained by preparing KBr pellets with a 3 : 100 'sample-to-KBr' ratio.

Morphological and microstructural features of the samples (either in the powder or pellet form were investigated by scanning electron microscopy (SEM). A typical accelerating voltage of 20 kV was used for secondary electron imaging (SEI) of 1500 nm thickness Au–Pd alloy-coated samples. Energy-dispersive X-ray spectroscopy (EDS) was used for obtaining semi-quantitative elemental information of the samples. The EDS information was believed to be accurate to ±3 at%.

2.4. Chemical analysis

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used for the accurate chemical analysis of Na and Mg ions in the SBF-synthesized HA powders (after being dissolved in acid solutions). These ions were believed to originate from the use of 'synthetic body fluids' during the synthesis experiments.

3. Results and discussion

3.1. Formation of seeds in SBF solutions

When appropriate (Fig. 1(a) and (b)) amounts of $Ca(NO_3)_2 \cdot 4H_2O$ were dissolved in SBF, a slight turbidity was observed in the beakers due to the immediate formation of fine precipitates, viz., 'seeds'. The seeds were found to be amorphous (by XRD) in their as-recovered forms. Heating of the precursor seeds in air atmosphere at the temperature range of 900–1170°C caused them to crystallize (Fig. 2a). The observed phases in the heated 'seed' powders were determined by powder XRD to be primarily a mixture of HA (International Centre of Diffraction Data (ICDD), Powder Diffraction File (PDF) No: 9-432), and CaO (PDF No: 4-777, 37-1497). However, only a few peaks in these XRD patterns were found to coincide with those of $Ca(OH)_2$ (PDF No: 4-733) and $CaCO_3$ (PDF No: 29-305).

The FT-IR spectra of these 'Ca-nitrate + SBF' seeds showed the CO_3^{2-} ion absorption peaks (Fig. 2b) at around 1470–1420 and 875 cm^{-1} indicating the presence of $CaCO_3$, together with the strong and sharp O–H stretching peak of $Ca(OH)_2$ observed at 3640 cm^{-1}. The symmetric and anti-symmetric stretching of the PO_4 group (at 1096, 1046, 963, 604 and 576 cm^{-1}) and the stretching and vibrational modes of the O–H group at 3571 and 639 cm^{-1} showed the presence of HA phase in support of the XRD data.

When appropriate amounts of $(NH_4)_2HPO_4$ were dissolved in SBF, the observed 'seeds' were found to display a phase mixture of $(Ca,Mg)_3(PO_4)_2$ (ICDD PDF No: 13-404), $Ca_2P_2O_7$ (PDF No: 20–24), β-TCP (PDF No: 9-169), $Mg_3(PO_4)_2$ (PDF No: 25-1373) and $Ca_7Mg_2(PO_4)_6$ (PDF No: 20-348), following air calcination at 900°C (Fig. 2c). Slow heating and cooling rates (1°C/min) were used in the calcination of these seed powders in order to prevent the formation of an amorphous phase. The orthophosphate and pyrophosphate structures of the seeds were also evident from their FT-IR spectra of Fig. 2d. The FT-IR spectrum of the 'as-dried (80°C) seeds' showed the presence of a broad band of adsorbed water in the range of 3400–3200 cm^{-1}. The water band diminished upon heating the samples at 900°C. The 'dried seeds' also displayed broad NH_4 peaks at ~1500–1420 cm^{-1}, which again disappeared upon heating. The CO_3^{2-} peaks seen in the FT-IR spectrum of 'dried' seeds (1470–1420 and 875 cm^{-1}) also disappeared in the samples heated at 900°C. A similar situation was observed in the FT-IR spectra of the seeds formed upon the dissolution of calcium nitrate powders in SBF. The typical P–O–P vibrational mode of pyrophosphates was found at 740 cm^{-1}, together with the multiple bands of the orthophosphate group at 1180–970, 570 and 562 cm^{-1}.

SBF is known [3,5,27] to be a metastable buffer solution like human plasma. It is saturated with respect to the formation and precipitation of an amorphous calcium phosphate (ACP) phase owing to its relatively high Ca/P ratio of 2.5. The small variations in the temperature of SBF solutions were also known to cause the spontaneous and in situ precipitation of different calcium phosphates as a function of solution pH. Therefore, the external addition of chemical reagents like $Ca(NO_3) \cdot 4H_2O$ and $(NH_4)_2HPO_4$ into the SBF would also be expected to cause precipitaiton. The added chemicals resulted in a variation of the solution's Ca/P ratio and thus the formation of calcium phosphate 'seeds' of different compositions.

The Ca/P ratio of SBF solutions, which was increased by ~40 times during the preparation of $Ca(NO_3) \cdot 4H_2O$–SBF mixtures (Table 2), would then easily allow the precipitation of an ACP by rendering the solution to be extremely rich in calcium. Seeds so formed were found to crystallize out primarily the HA phase upon calcination in air atmosphere at 900–1200°C. The second phase CaO, which appeared in the calcined seed powders (of calcium nitrate–SBF mixtures), was believed to form by the combination of excess Ca^{2+} with the CO_3^{2-} ions present (i.e., coming from one of the SBF constituents, $NaHCO_3$) in the SBF · $CaCO_3$ formed in this way would then decompose into CaO [34] during the calcination of these seed powders.

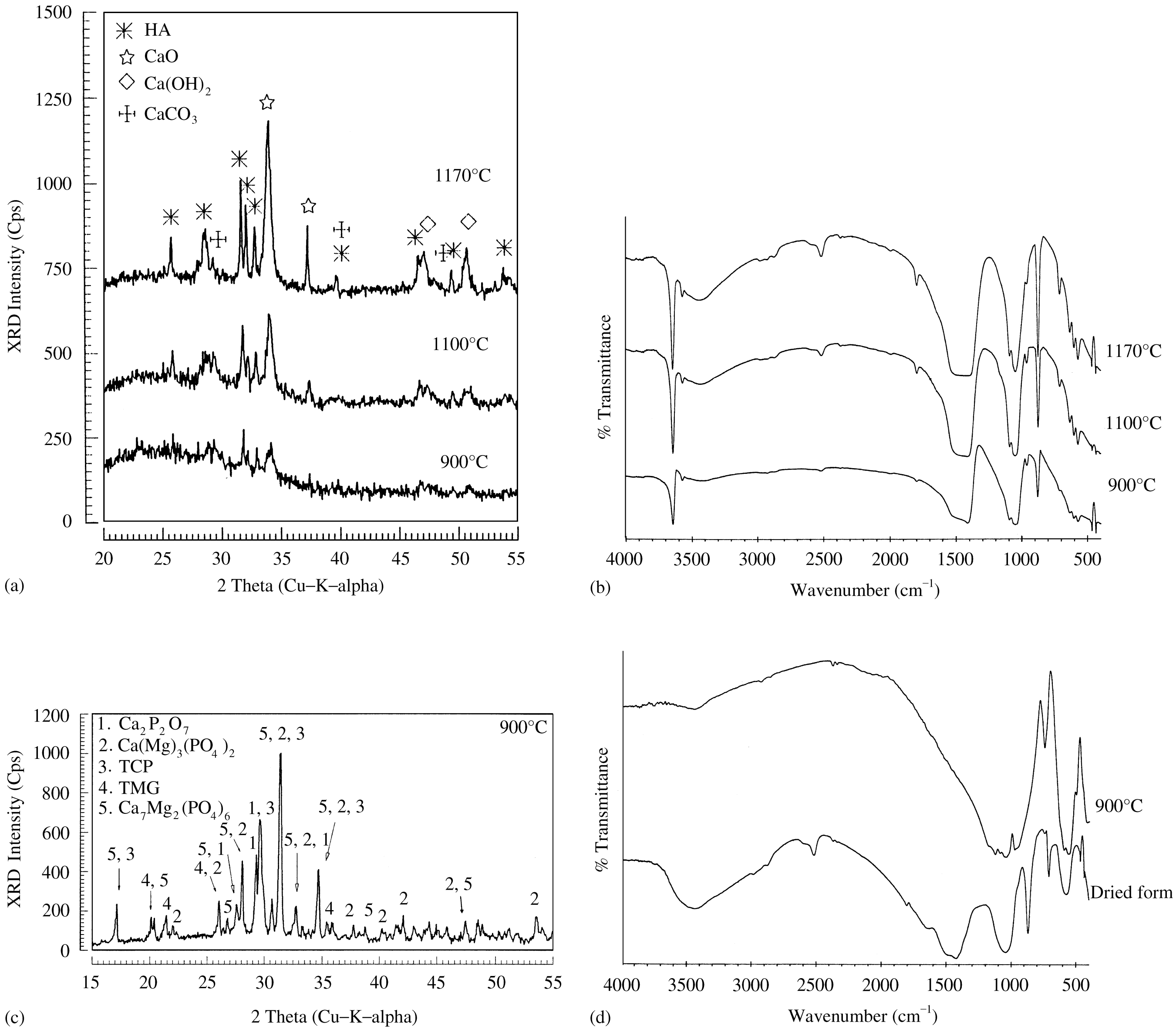

Fig. 2. (a) XRD spectra of seeds obtained from the mixtures of Ca nitrate and SBF solutions, (b) FT-IR spectra of seeds obtained from the mixtures of Ca nitrate and SBF solutions, (c) XRD spectra of seeds obtained from the mixtures of diammonium hydrogen phosphate and SBF solutions, and (d) FT-IR spectra of seeds obtained from the mixtures of di-ammonium hydrogen phosphate and SBF solutions.

The 'seed' precipitates formed upon dissolving $(NH_4)_2HPO_4$ powders in SBF were expected to be TCP-like phases (i.e., pure TCP and/or its Mg-doped relatives), without any HA. This fact was also confirmed. The excess amount of PO_4^{3-} supplied by the added $(NH_4)_2HPO_4$ would reduce the theoretical Ca/P ratio of SBF by $\sim$40 times and this is very much below the required ratio necessary for HA formation. It was considered that the Mg^{2+} ions present in SBF would precipitate in the solid seed particles within the TCP structure (following the consumption of almost all of the Ca^{2+}) since the Mg^{2+} ion has chemical characteristics similar to those of Ca^{2+}. Therefore, the previously claimed [15–17] effect of intentionally added 'seeds' (HA, OCP, TCP, DCPD, or ACP) in 'pure' aqueous precipitation solutions used for HA synthesis was also observed in this study, but here the initial seeds were spontaneously formed in the reaction vessel.

HA powders of this study prepared in SBF solutions, under 'biomimetic' conditions (viz., pH of 7.4 and 37°C) are believed to resemble the 'natural bone structure and mineralogy', more than powders synthesized in 'pure' aqueous media owing to the incorporation of Mg^{2+} and CO_3^{2-} ions by the above-described seed formation mechanisms.

3.2. Formation of HA powders

HA powders were synthesized over a wide pH range extending from 11.0 to the physiological pH of 7.4 in SBF solutions at 37°C. The '*initial-addition*' type HA synthesis experiments (labeled as I1 through I7 in Table 2) all produced pure HA powders. These experiments can be considered as a stepwise process to reduce the pH of 11 to the final physiological pH value of 7.4 in order to obtain and simulate 'biomimetic' conditions. The main purpose of this procedure was to decrease the ammonia content of the medium while increasing the SBF content, simulating in vitro conditions as closely as possible.

HA synthesis was also attempted in the total absence of any added ammonia. However, in these cases, calcium hydroxyapatite was not obtained with precipitation from solution at $\text{pH} < 7.4$. The resultant powders, labeled I8, were found to consist of β-TCP (Whitlockite) (ICDD PDF No: 9-169). Following the commencement of precipitation, the TCP-like precursors were aged for 4 h at higher temperatures (70–80°C) to test the possibility of the conversion of these precursors to HA by hydoxylation [9,10]. These aged powders, labeled I8A, were also found (by XRD) to consist of pure TCP. The FT-IR spectra of these powder samples had the characteristic anti-symmetric P–O stretching modes of the β-TCP phase at 948 and 975 cm^{-1} together with the other multi-bands of the PO_4^{3-} group. The symmetric stretching modes of this phase were present at 610 and 550 cm^{-1}.

Biomimetic conditions were most effectively simulated during the preparation of sample Cl (*continuous-addition*-type). During the precipitation of Cl powders (Fig. 1(b)), the contents of the reaction beaker were titrated closely to the pH value of 7.4. A typical XRD pattern of the pure HA powders obtained from Cl samples is reproduced in Fig. 3. The XRD spectra of all the HA powders synthesized in SBF closely matched with the ICDD PDF No: 9-432 of calcium hydroxyapatite.

Cl powders dried at 80°C were heated over the temperature range of 700–1600°C for 6 h, in a stagnant air atmosphere, in order to investigate the phase evolution characteristics of the precursor powders. The initially obtained amorphous calcium phosphate (ACP) precursor phase completed its crystallization at $\sim$900°C. Crystallization behavior of the HA precursors as a function of temperature (80–1600°C) is given in Fig. 4. There was, surprisingly, no decomposition of HA to β-TCP even after heating at 1600°C for 6 h. This observation confirms the superior high-temperature stability of these biomimetic HA powders compared to those reported in previous studies [7,12,13]. These powders also contained trace amounts of other inorganic ions originating in the SBF. A typical ICP analysis performed on the 1200°C calcined Cl powders indicated that these HA powders had 0.157% Mg and 0.082% Na. These ions, originated from the use of SBF solutions during precipitation, were believed to incorporate themselves in the crystal structure of calcium hydroxyapatite.

The lattice parameters of these powders were measured as $a = 9.4125$ and $c = 6.8765$ Å by powder XRD. The hexagonal unit cell (of the space group $P6_3/m$) volume was equal to 527.60 Å^3. The lattice parameters of pure, stoichiometric hydroxyapatite are known [35] to

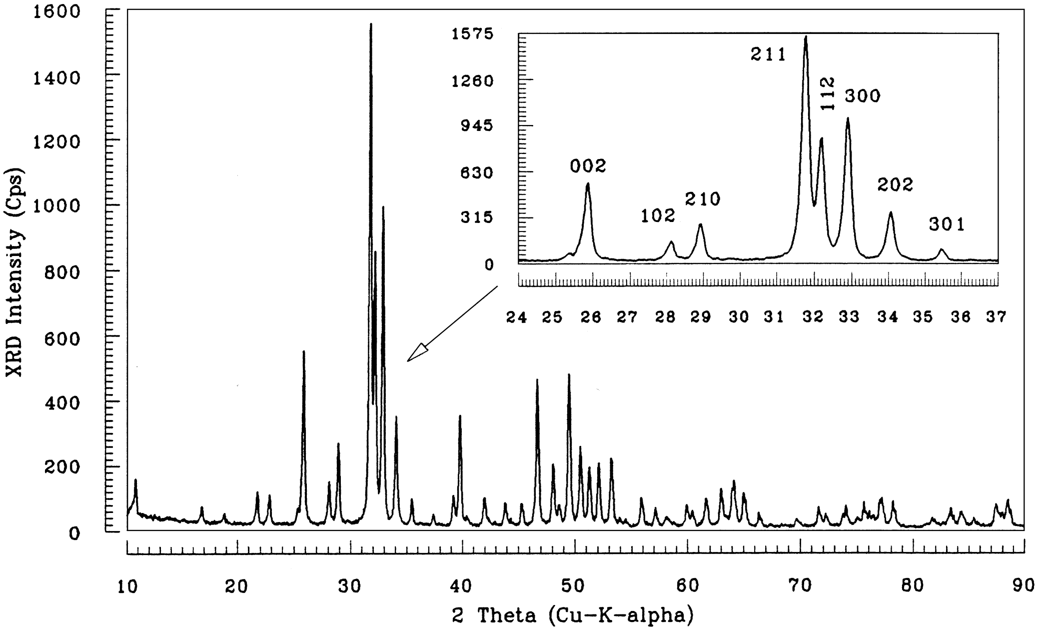

Fig. 3. XRD spectrum of SBF-synthesized HA powders from Cl (1200°C, 6 h, air).

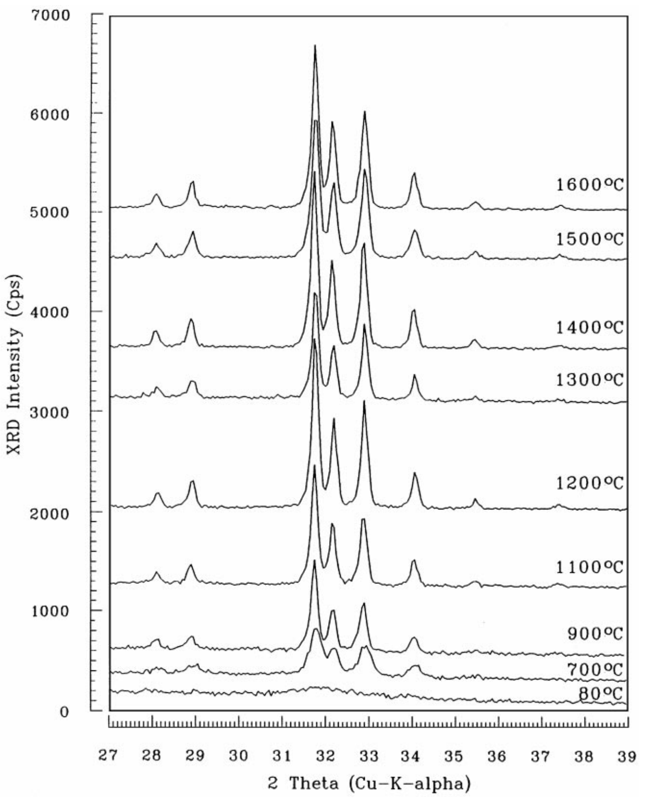

Fig. 4. Crystallization behavior of HA powders from Cl (80–1600°C, air).

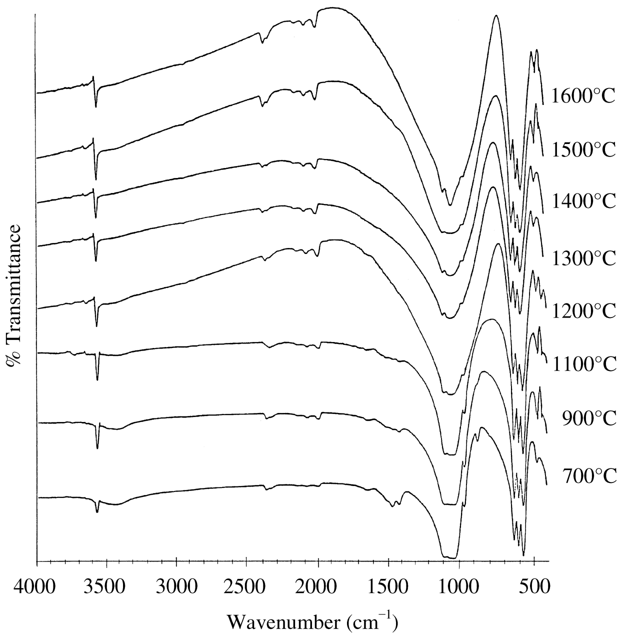

Fig. 5. FT-IR spectra of HA powders from Cl (700–1600°C, air).

be $a = 9.438$ and $c = 6.882$ Å, whereas the lattice parameters of human bones were reported with $a = 9.419$ and $c = 6.880$ Å. LeGeros et al. [35] tabulated that the presence of Mg^{2+} ions in HA would decrease both of the lattice parameters. The presence of CO_3^{2-} ions was also reported, in the same study, to cause a decrease in the a parameter. These indicate that the lattice parameters of our calcined Cl powders (with a measured Ca/P ratio of 1.658) exhibit a close resemblance to those of human bones, rather than those of pure, stoichiometric HA [36].

The CO_3^{2-} ions are known [1] to substitute, in part, the OH^- sites, and again, in part the PO_4^{3-} groups of the HA structure. On the other hand, Na^+ and Mg^{2+} ions were claimed by the previous researchers [1] to replace the Ca^{2+} sites of the HA structure. We also believe that the Na^+ ions might have placed themselves nearby the OH^+ sites (close to the P-bonded oxygens), and hence, caused a reduction in the extent of 'de-hydroxylation' at the significantly high calcination temperatures of this study.

The FT-IR spectra of calcinated Cl powders are given in Fig. 5. The CO_3^{2-} content of these powders can be followed from these spectra. The CO_3^{2-} ion peaks at 1470–1420 and 875 cm^{-1} are notable, especially for the powders calcined at 700 and 900°C. As the calcination temperature increased, the sharp peaks of the CO_3^{2-} ion disappeared. The OH^- stretching vibration was observed at 3571 cm^{-1} and the OH^- bending vibration was at 636 cm^{-1}. The PO_4 bands were recorded at 470 (ν_2), 570 and 603 (ν_4), 962 (ν_1), 1045 and 1096 (ν_3) cm^{-1}.

The microstructural characteristics of the synthesized HA samples were investigated by scanning electron microscopy. The HA powders obtained by the *continuous-addition*-type precipitation process (Cl) had spherical particles with an average diameter in the range of 35–50 nm. To the authors' knowledge, such a small particle size has never been reported or achieved before for synthetic HA manufactured using the calcium nitrate/diammonium hydrogen phosphate route. A typical SEM micrograph of 80°C-dried Cl powders is given in Fig. 6(a). These powders displayed a significant level of agglomeration.

The sintering behavior of these powders was studied for the flat surfaces of the circular pellets (approximately 0.8 cm diameter) heated in the temperature range 900–1200°C for 6 h in air atmosphere. A typical SEM micrograph given in Fig. 6(b) shows the unpolished, surface microstructure of a pellet heated at 1200°C for 6 h. The pellets prepared from these powders (Cl) reached ~96% bulk density (measured by the Archimedes' method). The average grain sizes in the samples heated at 900, 1000, 1100, and 1200°C were determined (by lineal analysis on the micrographs) to be 0.3, 0.4. 0.9 and 1.3 μm, respectively.

It is known that the synthesis of HA in aqueous (i.e., pure water) solutions using the starting chemicals $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ requires high pH values in excess of 10, without a need for solution aging

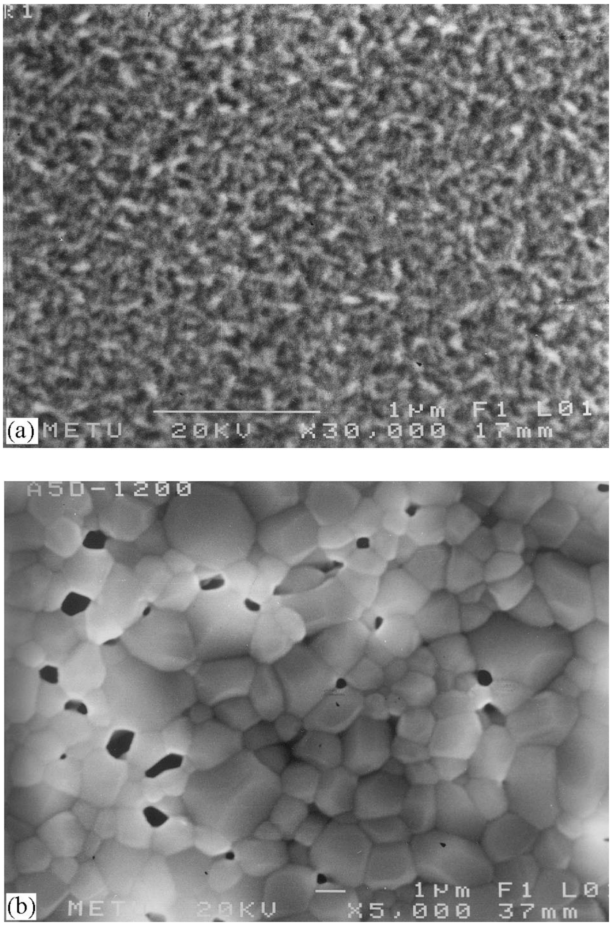

Fig. 6. (a) SEM micrograph of HA powders at 80°C (bar = 1 μm) and (b) SEM micrograph of HA pellet heated at 1200°C (bar = 1 μm).

[7–12]. On the other hand, in the case of using SBF, the pH value needed for HA synthesis decreases considerably. In conjunction with the initial presence of spontaneously formed seeds, the high ionic strength of the precipitation medium provided by SBF plays an important role in inducing the formation of pure calcium hydroxyapatite phase that would otherwise not nucleate [16,17].

4. Conclusions

Nano-sized and chemically homogeneous calcium hydroxyapatite (HA) powders have been synthesized successfully from a modified and novel synthetic body fluid solution, at the physiological and biomimetic conditions of pH 7.4 and 37°C, containing dissolved calcium nitrate and di-ammonium hydrogen phosphate salts in appropriate amounts by a chemical precipitation technique. The produced HA powders were observed to have unprecedented phase stability even when heated at 1600°C for 6 h in an air atmosphere.

The HA powders also contained trace amounts of other inorganic ions, provided and incorporated into the HA structure by the SBF solution used. A typical ICP analysis performed on the 1200°C-calcined powders indicated that the biomimetic HA powders of this study included 0.16% Mg and 0.08% Na.

The sintering behavior of biomimetic, SBF-grown HA powders was studied by observations on the flat surfaces of the circular pellets heated in the temperature range of 900–1200°C, for 6 h, in a dry air atmosphere. The average grain sizes of the sintered samples remained mostly in the sub-micron range. SBF-synthesized HA powders were found to reach about 96% densification when heated at 1200°C for 6 h.

Acknowledgements

This work was supported, in part, by the Turkish State Planning Agency (ODTU-AFP-03-08-DPT-95K-120491), and Turkish Scientific and Technical Research Foundation (TUBITAK/Misag-58).

References

[1] Hench LL, Wilson J. An introduction to bioceramics. London: World Scientific, 1993. p. 8, 146, 331, 335.

[2] Kokubo T. Surface chemistry of bioactive glass ceramics. J Non-Cryst Solids 1990;120:138–51.

[3] Ohtsuki C, Kokubo T, Yamamuro T. Mechanism of HA formation of $CaO–SiO_2–P_2O_5$ glasses in simulated body fluid. J Non-Cryst Solids 1992;143:84–92.

[4] Li P, Ohtsuki C, Kokubo T, Nakanishi K, Soga N. Apatite formation induced by silica gel in simulated body fluid. J Am Ceram Soc 1992;75:2094–7.

[5] Neuman W, Neuman M. The chemical dynamics of bone mineral. Chicago: University of Chicago Press, 1958. p. 34.

[6] Klein CPAT, de Blieck Hogerworst JMA, Wolke JGC, de Groot K. Studies of solubility of different calcium phosphate ceramic particles in vitro. Biomaterials 1990;11:509–12.

[7] Jarcho M, Bolen CH, Thomas MB, Babock J, Kay JF, Doremus RH. Hydroxyapatite synthesis and characterization in dense polycrystalline form. J Mater Sci 1976;11:2027–35.

[8] Asada M, Miura Y, Osaka A, Oukami K, Nakamura S. Hydroxyapatite crystal growth on calcium hydroxyapatite ceramics. J Mater Sci 1988;23:3202–5.

[9] Ebrahimpour E, Johnson M, Richardson CF, Nancollas GH. The characterization of HA precipitation. J Colloid Interface Sci 1993;159:158–63.

[10] Zhou J, Zhang X, Chen J, Zeng S, de Groot K. High temperature characeristics of synthetic hydroxyapatite. J Mater Sci: Mater Med 1993;4:83–5.

[11] Lazic S. Microcrystalline hydroxyapatite formation from alkaline solutions. J Crystal Growth 1995;147:147–54.

[12] Tas AC, Korkusuz F, Timucin M, Akkas N. An investigation of the chemical synthesis and high-temperature sintering behaviour of calcium hydroxyapatite (HA) and tricalcium phosphate (TCP) bioceramics. J Mater Sci: Mater Med 1997;8:91–6.

[13] Madsen HEL, Thodvadarson G. Precipitation of calcium phosphate from moderately acid solutions. J Crystal Growth 1984; 66:369–76.

[14] Simsek FA. Chemical preparation of calcium hydroxyapatite in synthetic body fluids at 37°C and its coating on titanium and stainless steel strips. MSc thesis. Middle East Technical University, Ankara, Turkey, July 1997.

[15] Moreno EC, Varughese K. Crystal growth of calcium apatites from dilute solutions. J Crystal Growth 1981;53:20–30.

[16] Haugbert JC, Zawacki SJ, Nancollas GH. The growth of octacalcium phosphate on β tricalcium phosphate. J Crystal Growth 1983;63:83–90.

[17] Haugbert JC, Nancollas GH. Kinetics of crystallization of octacalcium phosphate. J Phys Chem 1983;88:2478–81.

[18] Nelson DGA, Salimi H, Nancollas GH. Octacalcium phosphate and apatite overgrowths: a crystallographic and kinetic study. J Colloid Interface Sci 1985;110:32–9.

[19] Inskeep WP, Silvertooth JC. Kinetics of hydroxyapatite precipitation at pH 7.4 to 8.4. Geochim Chosmochim Acta 1988; 52:1883–93.

[20] Graham S, Brown PW. The low temperature formation of octacalcium phosphate. J Crystal Growth 1993;132:215–25.

[21] Alliot-Litch B, Gregoire M, Orly I, Menanteau J. Cellular activity of osteoblast in the presence of HA: an in vitro experiment. Biomaterials 1991;12:752–6.

[22] Oguchi H, Ishikawa K, Mizoue K, Seto K, Eguchi G. Long-term histological evaluation of HA ceramics in humans. Biomaterials 1995;16:33–8.

[23] Rusy AJ, Wei M, Sorrell CC, Dickson MR, Brandwood A, Milthorpe BK. Sintering effects on the strength of HA. Biomaterials 1995;16:409–15.

[24] Yubao L, Klein CPAT, Xingdong Z, de Groot K. Formation of a bone apatite-like layer on the surface of porous HA ceramics. Biomaterials 1994;15:835–40.

[25] Li P, Kangasniemi I, de Groot K, Kokubo T. Bone-like hydroxyapatite induction by a gel-derived titania on a titanium substrate. J Am Ceram Soc 1994;77:1307–12.

[26] Li P, Nakanishi K, Kokubo T, de Groot K. Induction and morphology of HA precipitated from metastable simulated body fluids on sol–gel prepared silica. Biomaterials 1993;14:963–8.

[27] Li P, Kangasniemi I, de Groot K, Kokubo T, Yli-Urpo AU. Apatite crystallization from metastable calcium phosphate solution on sol–gel prepared silica. J Non-Cryst Solids 1994; 168:281–6.

[28] Cho SO, Nakanishi K, Kokubo T, Soga N, Ohtsuki C, Nakamura T, Kitsugi T, Yamamuro T. Dependence of apatite formation on silica gel on its structure: effect of heat treatment. J Am Ceram Soc 1995;78:1769–74.

[29] Kokubo T, Miyaji F, Kim HM, Nakamura T. Spontaneous formation of bonelike apatite layer on chemically treated titanium metals. J Am Ceram Soc 1996;79:1127–9.

[30] Jarcho M. Calcium phosphate ceramics as hard tissue prosthetics. Clin Orthop Relat Res 1981;157:259–78.

[31] van Blitterswijk CA, Grote JJ, Kuijpers W, Blok-van Hoek CJG, Daems WT. Bioreactions at the tissue–hydroxyapatite interface. Biomaterials 1985;6:243–51.

[32] Appleman DE, Evans HT. Least-squares and indexing software for XRD data. U.S. Geological Survey, Computer contribution No. 20, U.S. National Technical Information Service, Document PB-216188, 1973.

[33] Taspinar E, Tas AC. Low-temperature chemical synthesis of lanthanum monoaluminate. J Am Ceram Soc 1997;80:133–41.

[34] Pleshka N, Boskey A, Mendelsohn R. Novel infrared spectroscopic method or determination of crystallinity of hydroxyapatite minerals. Biophys J 1991;60:786–93.

[35] LeGeros RZ, LeGeros JP. Dense hydroxyapatite. In: Hench LL, Wilson J, editors. An introduction to bioceramics. London: World Scientific Publishing Co, 1993. p. 144–5.

[36] Kivrak N, Tas AC. Synthesis of calcium hydroxyapatite-tricalcium phosphate (HA-TCP) composite bioceramic powders and their sintering behavior. J Am Ceram Soc 1998;81:2245–52.