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## Biomimetic Preparation of HA Powders at 37°C in Urea-and Enzyme Urease-Containing Synthetic Body Fluids

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**Abstract:** An important inorganic phase of synthetic bone applications, calcium hydroxyapatite (HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), was prepared as a single-phase and sub-micron bioceramic powder. Carbonated HA powders were synthesized from calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate salts dissolved in synthetic body fluid (SBF) solutions, containing urea ( $\text{H}_2\text{NCONH}_2$ ) and enzyme urease, under the biomimetic conditions of 37°C and pH 7.4, with a novel chemical precipitation technique. These powders were also found to

contain trace amounts of Na and Mg ions in them, intentionally incorporated by using SBF solutions, instead of pure water, during their synthesis. The characterization and chemical analysis of the synthesized biomimetic HA powders were performed by scanning electron microscopy (SEM), powder X-ray diffraction (XRD), Fourier-transformed infrared spectroscopy (FT-IR), and inductively-coupled plasma atomic emission spectroscopy (ICP-AES).

### Introduction

Calcium hydroxyapatite (HA:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), the main inorganic component of human bones, is a member of the apatite family of compounds. Biological apatites, which comprise the mineral phases of calcified tissues (enamel, dentin, and bone), slightly 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 accordance 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 the 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].

HA powders for bioceramic applications have generally been synthesized by using aqueous solutions. It is known [4] 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 powders synthesized in highly alkaline media [5-10] are recognized by their relatively high thermal stability and phase purity even after high-temperature (1,100°-1,300°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 [7, 11]. This study [12] will focus on the determination of the experimental parameters of HA synthesis at the physiological pH (7.4) and temperature (37°C), in SBF solutions rich in urea and enzyme urease.

K. de Groot et al. [8] studied the high-temperature characteristics of synthetic HA and reported that the synthetic HA with a Ca/P ratio near 1.67 was only stable below 1,200°C, when sintered in a dry or wet air atmosphere. It was claimed that beyond 1,200°C, HA

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loses its OH<sup>-</sup> groups gradually and transforms into oxyapatite (Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>O). When heated to 1,450°C, oxyapatite was found [8] to dissociate into β-TCP, Ca<sub>2</sub>P<sub>2</sub>O<sub>7</sub> and Ca<sub>4</sub>P<sub>2</sub>O<sub>9</sub>. On the other hand, Taş et. al. [10] recently reported that pure HA powders prepared in distilled water may withstand, without decomposition, temperatures as high as 1,300°C when heated in an air atmosphere for 6 h.

The surface properties and synthesis conditions of calcium hydroxyapatite, prepared by using pure water in a highly alkaline medium, has previously been examined in different buffers [4, 6, 13] including SBF; however, there is no published work, to our knowledge, on the precipitation and formation of calcium hydroxyapatite powders in SBF solutions, containing urea and/or enzyme urease, at 37°C and pH 7.4. The study presented here is thus the first systematic step taken in this specific field of biomimetic HA powder synthesis technology.

## Experimental Procedure

### (1) Preparation of Synthetic Body Fluid (SBF)

SBF is known to be a metastable [3] buffer solution, 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 (Darmstadt, Germany)-grade NaCl (99.5 %), NaHCO<sub>3</sub> (99.5 %), KCl (99.0 %), Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O (99.5 %), MgCl<sub>2</sub>·6H<sub>2</sub>O (98.0 %), Na<sub>2</sub>SO<sub>4</sub>·(CH<sub>2</sub>OH)<sub>3</sub>CNH<sub>2</sub> (99.2 %), CaCl<sub>2</sub>·2H<sub>2</sub>O (99.0 %) and HCl (37 vol%, Carlo-Erba, Italy) were used in the preparation of the synthetic body fluids. SBF solutions were prepared [12, 17, 18] by dissolving appropriate quantities of the above chemicals in de-ionized water. Reagents were added one by one after each was completely dissolved in 700 mL of water, in the order given in Table 1.

A total of 40 mL of 1M HCl solution was consumed for pH adjustments during the preparation of SBF solutions. The 15 mL aliquot of this amount was added just before the addition of the 6<sup>th</sup> reagent, i.e., CaCl<sub>2</sub>·2H<sub>2</sub>O. The second portion of the HCl solution was used in the remainder of the titration process. Following the addition of the 8<sup>th</sup> reagent (tris(hydroxymethyl)-aminomethane), the solution temperature was raised from the ambient to 37°C. This solution was then appropriately titrated with 1M HCl to a pH value 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.

### (2) Synthesis of HA Powders in SBF Solutions

Stock solutions of 0.4 M Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O (Riedel de-Haen, 99%, Germany) and 0.16 M (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (Merck, 99%, Germany) were used in the precipitation experiments as the calcium and phosphate ion sources. Urea solutions of 8 to 9.5 M were prepared (by dissolving the urea powder in SBF) and then aged at 85°C overnight in a sealed glass jar, to ensure the decomposition of urea. The pH values of 9.5 M aged urea solutions were found to be as 9.3 at 37°C, prior to their use in powder synthesis. The enzyme urease (Merck, Lot No: 108489, 5 units/mg, Germany) was added, within a concentration range of 2.5 to 10 units/mL, into the precipitation solutions to establish [14] and accelerate the complete decomposition of urea at 37°C.

Two different precipitation schemes were used during our HA powder synthesis experiments. These two routes are given in the flowcharts of Figures 1 and 2. The first scheme of synthesis may be called the initial addition-type, since it involves the addition of the inorganics (calcium nitrate and di-ammonium hydrogen phosphate) at the beginning. The second scheme of synthesis used was called the continuous addition-type due to the continuous addition of a solution of the phosphate salt into that of nitrate with a peristaltic pump.

The pH values of the solutions were maintained [12, 17] at the physiological level of 7.4 throughout the chemical precipitation experiments. The synthesized powders were calcined in an air atmosphere over a temperature range of 1,100°-1,600°C for 6 to 17 hours, following their drying at 80°C.

### (3) Powder Characterization

The phase purity and the levels of crystallinity of the calcined HA powders were studied by powder X-ray

Table 1. Chemical Composition of SBF Solutions\*.

Order	Reagent	Amount (gpl)
1	NaCl	6.547
2	NaHCO <sub>3</sub>	2.268
3	KCl	0.373
4	Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O	0.178
5	MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.305
6	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.368
7	Na <sub>2</sub> SO <sub>4</sub>	0.071
8	(CH <sub>2</sub> OH) <sub>3</sub> CNH <sub>2</sub>	6.057

\* Patent Pending, Turkish Patent Institute, Turkey, Appl. No: 99/00037, January 11, 1999.

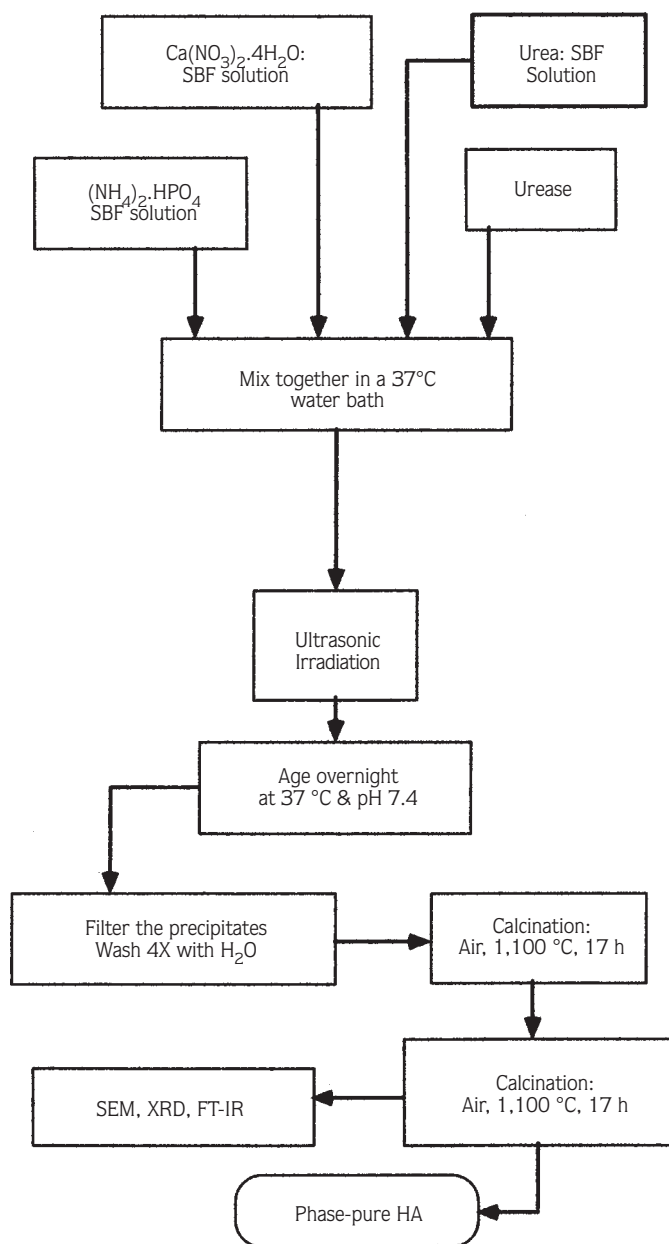


Figure 1. Process flowchart for HA synthesis by the initial addition scheme.

diffraction (Model: D-Max/B, Rigaku Co., Tokyo, Japan) at the typical step size of  $0.05^\circ 2\theta$  and a count time of 1 s. A monochromated  $\text{Cu-K}_\alpha$  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 for the shifts in the d-spacings by using pure silicon (Starck Inc., Germany) as an external standard. A least-squares unit cell refinement program [15] was used for the precise determination of crystallographic parameters of the synthesized powders. Further details of XRD analysis are described elsewhere [16].

Fourier-transformed infra-red spectroscopy (FT-IR) (Model: DX-510, Nicolet Co., WI, USA) was used in the wave number range of  $4000\text{--}400\text{ cm}^{-1}$ . Experimental spectra of solid samples were obtained by preparing KBr pellets at a 3:97 (wt%) sample-to-KBr ratio.

#### (4) Chemical Analysis

Inductively-coupled plasma atomic emission spectroscopy (ICP-AES) (Model Plasma-1000, Perkin Elmer Co., London, UK) was used for the accurate chemical analysis of Na and Mg ions in the SBF-

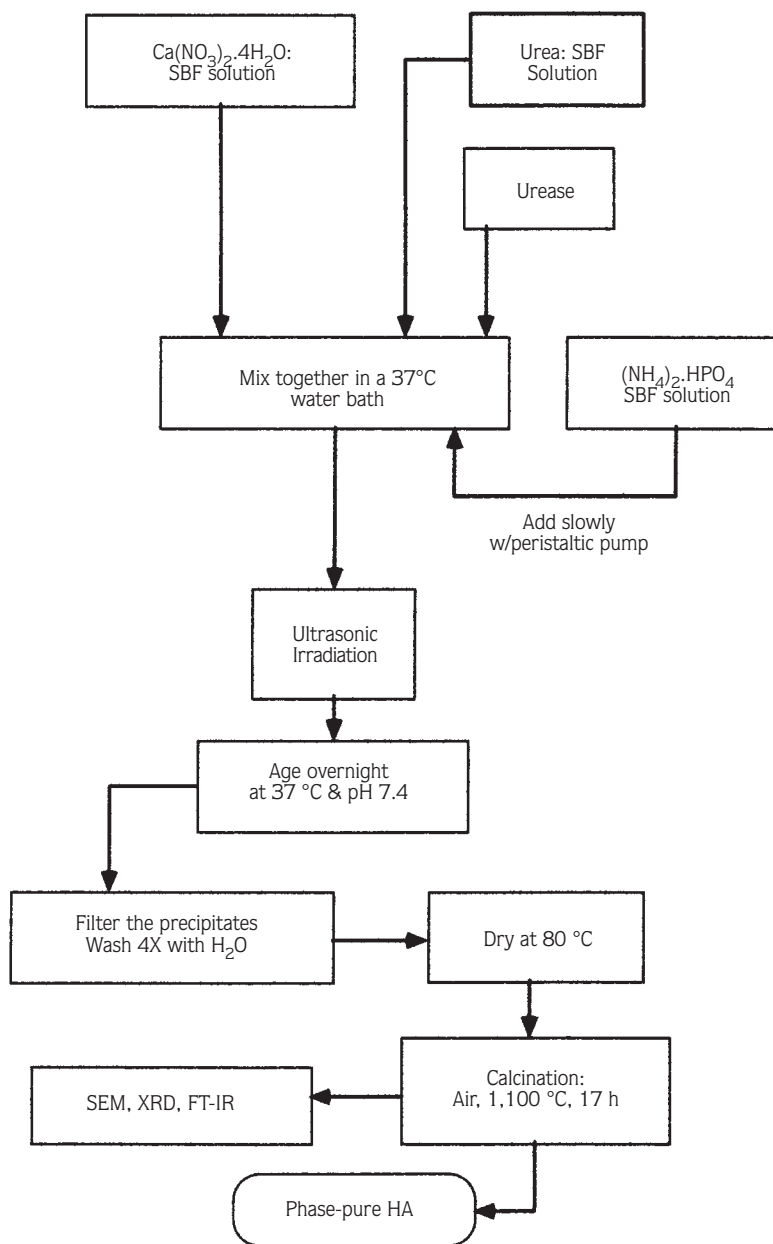


Figure 2. Process flowchart for HA synthesis by the continuous addition scheme.

synthesized HA powders (after dissolution in acid solutions) which were believed to originate from the use of synthetic body fluids during the synthesis experiments.

### Results and Discussion

Biological apatites mainly differ from the synthetically produced calcium hydroxyapatites in terms of their carbonate ion content (i.e., the carbonate ion levels being incomparably lower in the latter). Body fluids may be considered a biomimetic and plausible source for

increasing the carbonate contents of synthetically prepared HA bioceramics. However, the significantly low levels of  $\text{HCO}_3^-$  (i.e., 27 mM) in human plasma or SBF solutions makes it impractical for their economically feasible use within the shorter aging times allowed in powder synthesis technology, as compared to the aging of natural bones of offsprings in the human body for a typical period of about 9 months + 10 days. The pursuit of a means of manufacturing a genuinely carbonated HA powder mainly originated from a clinical demand for the synthesis of an HA powder with improved in vivo

biocompatibility. We have hereby attempted the taking of a first systematic step into the comparison of the carbonate ion incorporation into the resultant hydroxyapatite precipitates, (i) those grown in pure SBF, and (ii) those grown in urea- and enzyme urease-containing SBF solutions, under the physiological conditions of 37°C and pH 7.4.

The nominal, starting ion concentrations of the SBF solutions used in this study [12] were matched more closely with those of human plasma than the ones given by Kokubo and his co-workers [2, 3]. The most significant differences of this study, as compared to previous works, in terms of SBF ion concentrations can be stated as follows:

(a) the nominal, initial  $\text{HCO}_3^-$  ion concentration of SBF was increased from 4.2 mM to 27.0 mM, which thus acquired exactly the same value as that of human plasma;

(b) Cl<sup>-</sup> ion concentration was decreased from 147.8 mM to 125.0 mM in the preparation recipe, whereas the Cl<sup>-</sup> ion concentration of human plasma is 103.0 mM.

This has been achieved [12, 17, 18] solely by changing the 4<sup>th</sup> reagent of the SBF preparation recipe from K-phosphate [2, 3] to Na-phosphate. The differences between the ion concentrations of our SBF solutions and those of human plasma are compared in Table 2.

The as-filtered, dried and nano-sized calcium phosphate precipitates formed at 37°C and pH 7.4 via pure SBF (i.e., without urea) solutions have been found [12, 17, 18] to be amorphous. Upon calcination of these amorphous precursors in a stagnant air atmosphere at 1,100°C for 6 hours, they completely transformed into crystalline, single-phase HA. However, it has long been a well-confirmed experimental fact [6, 8, 10] that synthetically prepared (via aqueous chemical precipitation techniques) HA powders usually decompose (partially) into tri-calcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ), calcium

pyrophosphate ( $\text{Ca}_2\text{P}_2\text{O}_7$ ), tetra calcium phosphate ( $\text{Ca}_4\text{P}_2\text{O}_9$ ), and/or oxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6\text{O}$ ) when heated above 1200°C in air.

The HA precipitates [12, 17, 18] formed in pure SBF solutions had an excellent thermal stability even when heated at 1,600°C for 6 hours in an air atmosphere. Figure 3a shows the XRD traces of pure SBF-synthesized HA precipitates heated at different temperatures over a range of 1300° to 1600°C for 6 hours. The XRD spectra of all samples were closely matched with the ICDD PDF No: 9-432 for calcium hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ). HA powders of high thermal stability are expected to be promising materials, especially for the high-temperature requiring implant manufacturing processes, such as plasma-spraying of ceramic powders onto metals. Figure 3b, on the other hand, shows the crystallization behavior of initially amorphous HA precursor powders synthesized in SBF solutions containing urea and enzyme urease. These powders began crystallization at around 500°C without decomposing to any other compound of the calcium phosphate family at 1,100°C. The HA powders in this study synthesized at 37°C, through the use of urea and enzyme urease-containing SBF solutions, were not calcined at temperatures higher than 1,150°C.

The biomimetic HA powders of the present study [12] also contained trace amounts of other inorganic ions, provided by the use of SBF solutions. ICP-AES analysis performed on representative 1,150°C-calcined samples indicated that these HA powders had 1,500 ppm Mg and 160 ppm Na. The powders did not contain any nitrogen, the organic material volatilized completely during calcination at 500°C. Na<sup>+</sup> and Mg<sup>2+</sup> 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 SBF-grown powders were measured as  $a = 9.417$  and  $c = 6.876$  Å. The hexagonal unit cell (of the space group  $\text{P6}_3/\text{m}$ ) volume was found to be equal to 527.95

Table 2. Ion Concentrations of SBF Solutions and Human Plasma.

Ion	Kokubo, <i>et al.</i> [2,3](mM)	Present work (mM)	Human Plasma(mM)
Na <sup>+</sup>	142.0	142.0	142.0
Cl <sup>-</sup>	147.8	125.0	103.0
$\text{HCO}_3^-$	4.2	27.0	27.0
K <sup>+</sup>	5.0	5.0	5.0
Mg <sup>2+</sup>	1.5	1.5	1.5
Ca <sup>2+</sup>	2.5	2.5	2.5
$\text{HPO}_4^{2-}$	1.0	1.0	1.0
$\text{SO}_4^{2-}$	0.5	0.5	0.5

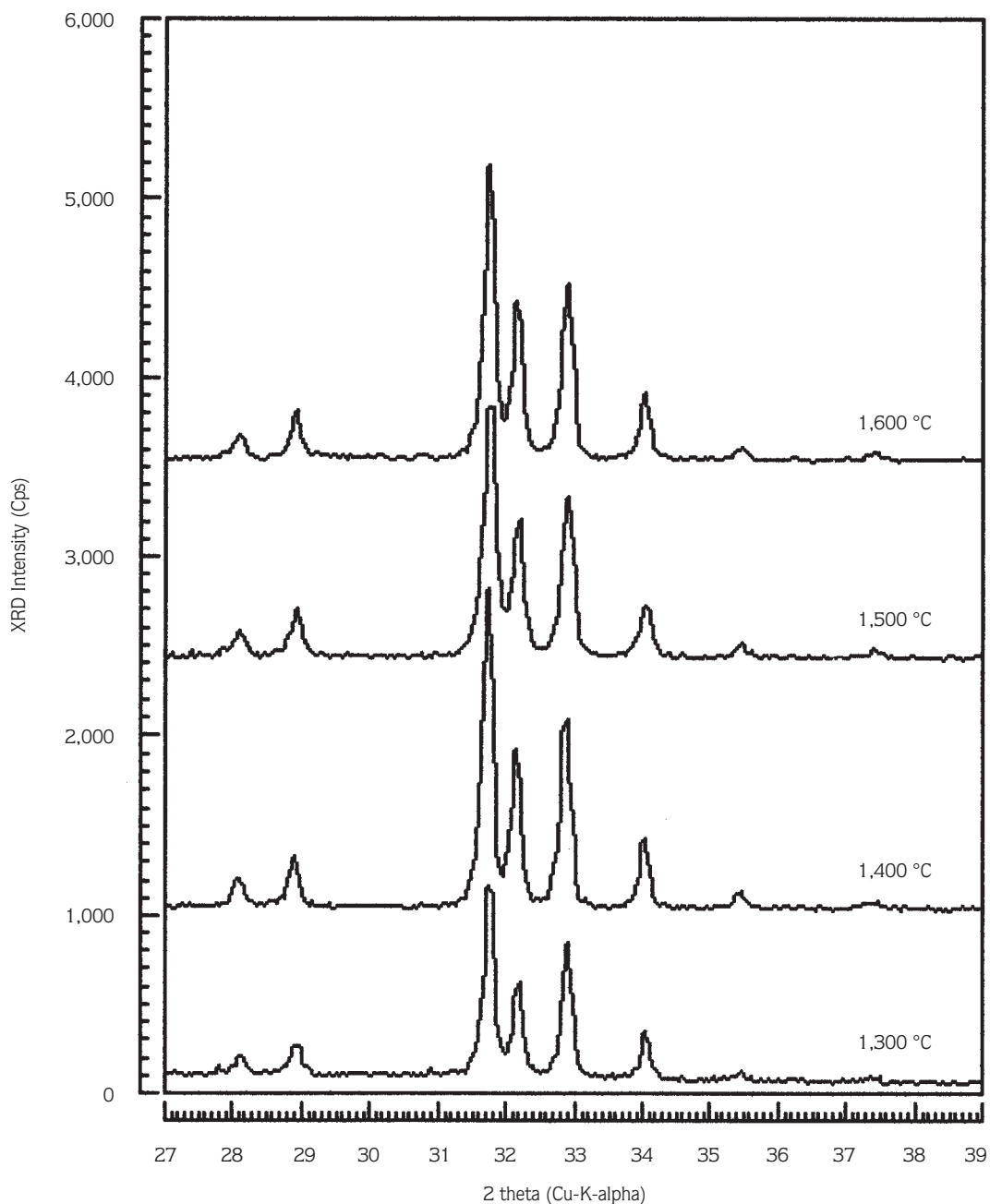


Figure 3a. XRD traces of HA precipitates formed in pure SBF, after heating at different temperatures (1,300°-1,600°C, 6 h, air).

Å<sup>3</sup>. The CO<sub>3</sub><sup>2-</sup> ions are known [1] to substitute, in part, the OH<sup>-</sup> sites, and again in part the PO<sub>4</sub><sup>3-</sup> groups of the HA structure. On the other hand, Mg<sup>2+</sup> ions have been claimed [1] to replace the Ca<sup>2+</sup> sites of the HA structure. We also believe that the Na<sup>+</sup> ions might have placed themselves near the OH<sup>-</sup> 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 SBF-synthesized HA powders are given in Figure 4. The top spectrum of this figure belonged to pure SBF-grown (i.e., SBF solutions without urea/urease) HA precipitates, after 6 hours of calcination at 700°C in air. The CO<sub>3</sub><sup>2-</sup> ion peaks, at 2368-2361

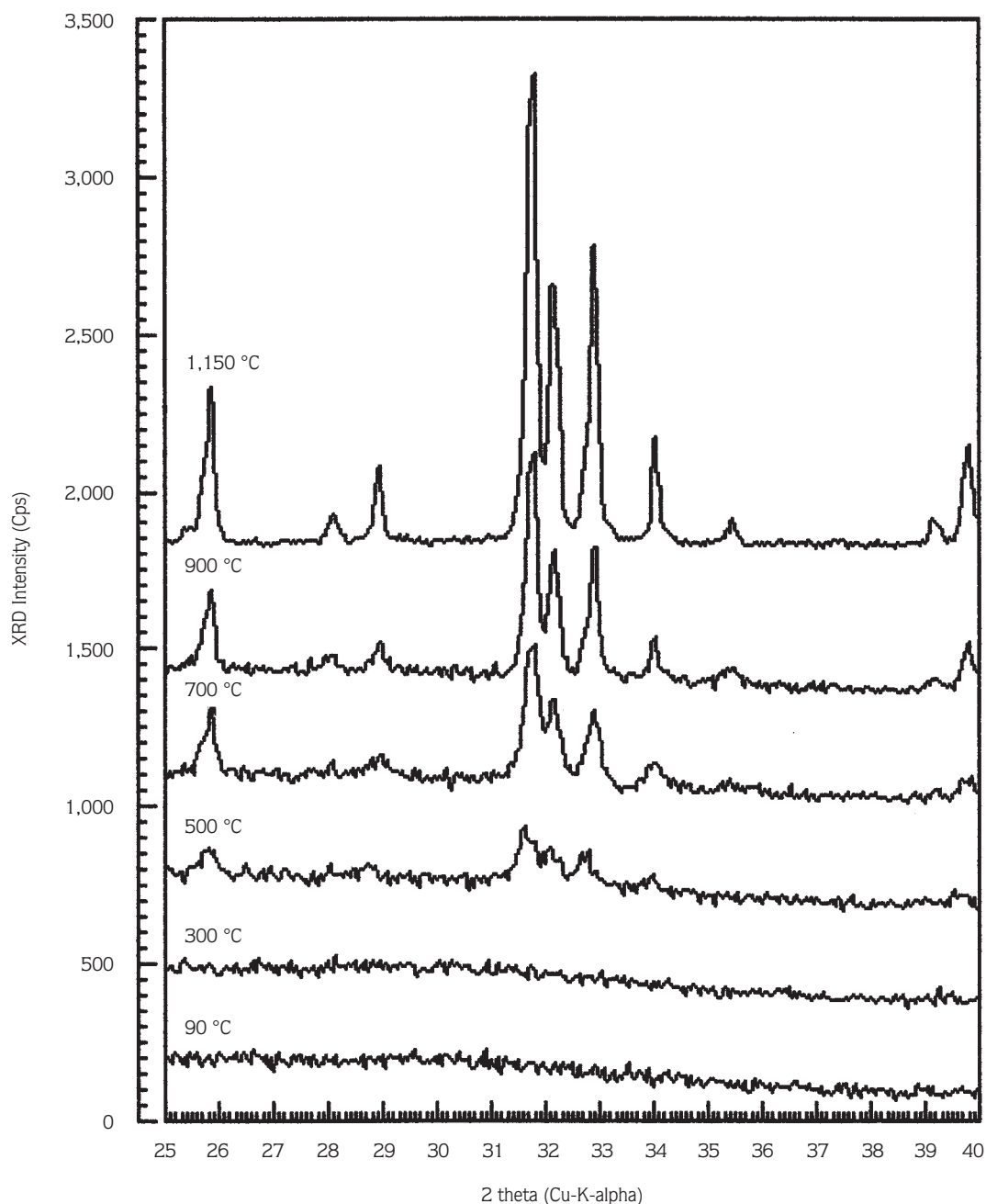


Figure 3b. XRD traces of HA precipitates formed in SBF+Urea+Urease, after heating at different temperatures (90°-1,150°C, 6 h, air).

(combination of  $\nu_2 + \nu_3$ ), 1467-1412 and 878  $\text{cm}^{-1}$ , were clearly visible. As the calcination temperature increased, the FT-IR peaks of the  $\text{CO}_3^{2-}$  totally disappeared. The  $\text{OH}^-$  stretching vibration was observed at 3571  $\text{cm}^{-1}$  and the  $\text{OH}^-$  bending vibration was recorded at 635  $\text{cm}^{-1}$ . The  $\text{PO}_4$  bands [19] were detected at 470 ( $\nu_2$ ), 570 and 603 ( $\nu_4$ ), 962 ( $\nu_1$ ), 1045 and 1096 ( $\nu_3$ )  $\text{cm}^{-1}$ . The bottom FT-IR

spectrum of Figure 4 was obtained from the powder synthesis experiments that used the urea- and enzyme urease-containing SBF solutions. The significant qualitative presence of carbonate ions in HA structure was again clearly apparent.

The morphology of these biomimetic HA powders were observed by the SEM micrographs given in Figure 5.

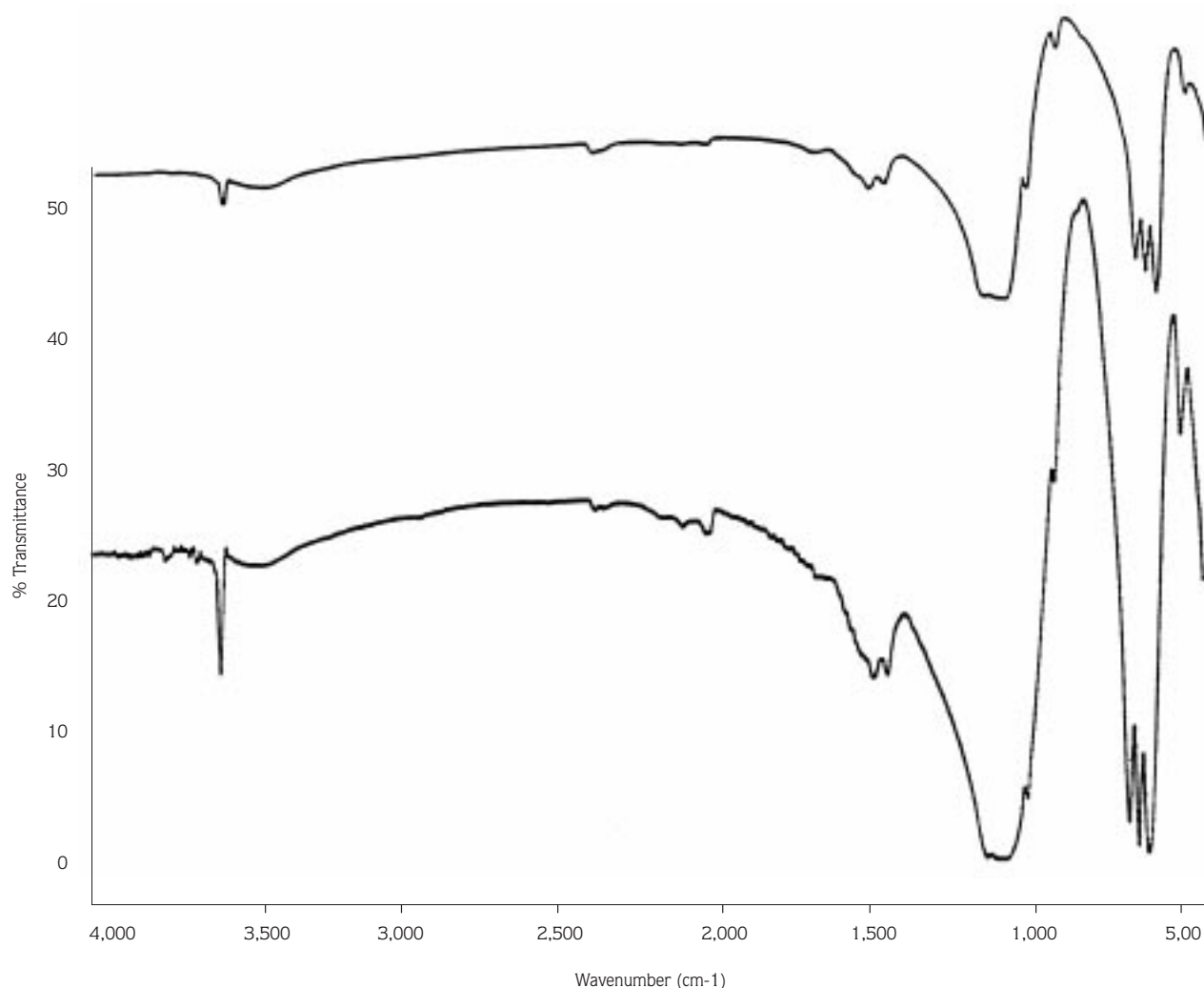


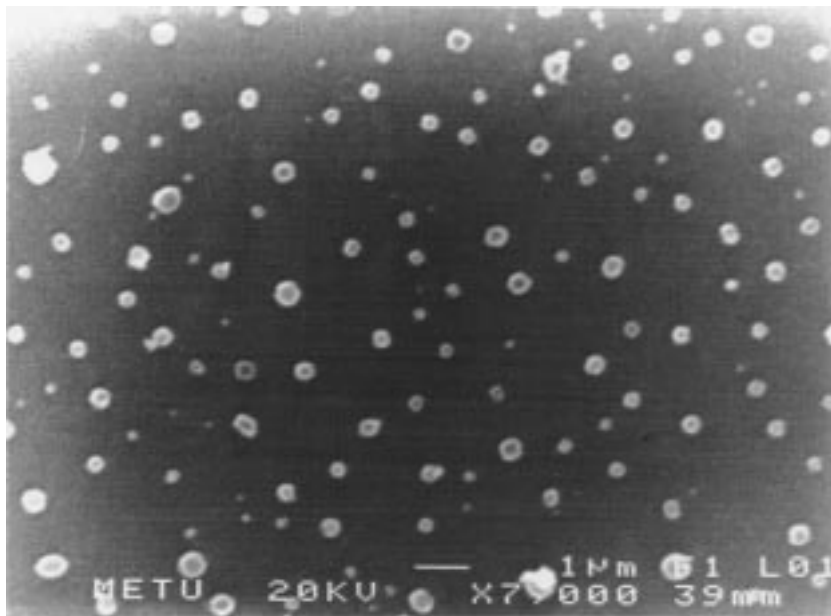
Figure 4. FT-IR spectra of HA powders prepared in SBF solutions (Top: in pure SBF, Bottom: in urea- and enzyme urease-containing SBF, samples heated at 700°C in air for 6 h).

The micrograph given in Figure 5(a) shows the sub-micron (with an average value of 0.25  $\mu\text{m}$ ) particles of as-filtered biomimetic HA powders after drying at 90°C. HA powders synthesized by using distilled water (instead of SBF) had a typical particle size of 0.6-0.7  $\mu\text{m}$  [10]. The particles shown were first ultrasonically dispersed in isopropyl alcohol (5 mg powder in 10 mL alcohol), and then a drop of the formed dispersion was evaporated on a clean glass substrate, prior to SEM study. The micrograph given in Figure 5(b), on the other hand, showed the morphology of the same powders after being heated at 1,150°C for 12 hours, in the form of a loose powder compact in an alumina crucible. Further investigation of the sintering behavior of such powders

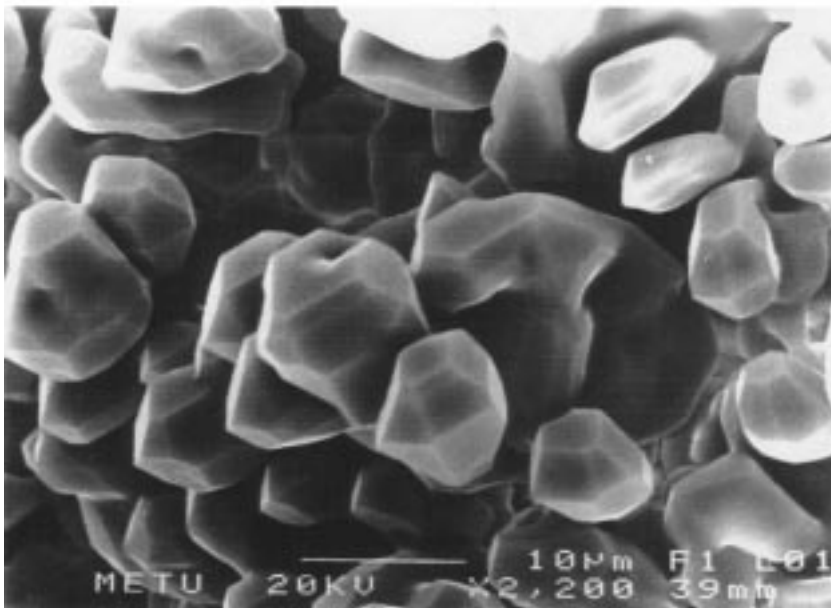
would be important for the production of densified bioceramic prostheses and parts. A further set of decisive carbon analysis would, however, be required for a quantitative determination of the amounts of  $\text{CO}_3^{2-}$  ions present in such samples.

The use of SBF solutions and the addition of enzyme urease to SBF solutions containing urea were both attempted for the first time in the present study for the biomimetic synthesis of carbonated HA powders. In the HA synthesis experiments that used the starting chemicals  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $(\text{NH}_4)_2\text{HPO}_4$ , it was known that if the synthesis medium was pure water, then the solution pH values must be in the alkaline region [6-11]. On the other hand, in the case of using SBF, the pH value needed





A



B

for HA synthesis decreases [12, 17, 18, 20] considerably. In combination with the initial presence of spontaneously formed seed crystals [12, 17] in precipitation solutions, the high ionic strength of the precipitation medium (provided by SBF) played an important role in inducing the formation of a pure calcium hydroxyapatite precursor phase that would otherwise, normally, not nucleate at such a low pH value as 7.4. Following the addition of the

enzyme urease into the precipitation solutions, the pH values changed as shown in Figure 6. (Without the addition of enzyme urease into the same solutions, the plots would look like horizontal straight lines.) When the enzyme concentration was kept at the initial, nominal level of 2.5 units/mL, the synthesized powders consisted of two phases, i.e., a mixture of HA (20%) and  $\text{Ca}_3(\text{PO}_4)_2$  (20%). However, when the enzyme concentration was

Figure 5. SEM micrographs of SBF-synthesized HA powders. (a) 90°C, (b) 1,150°C.

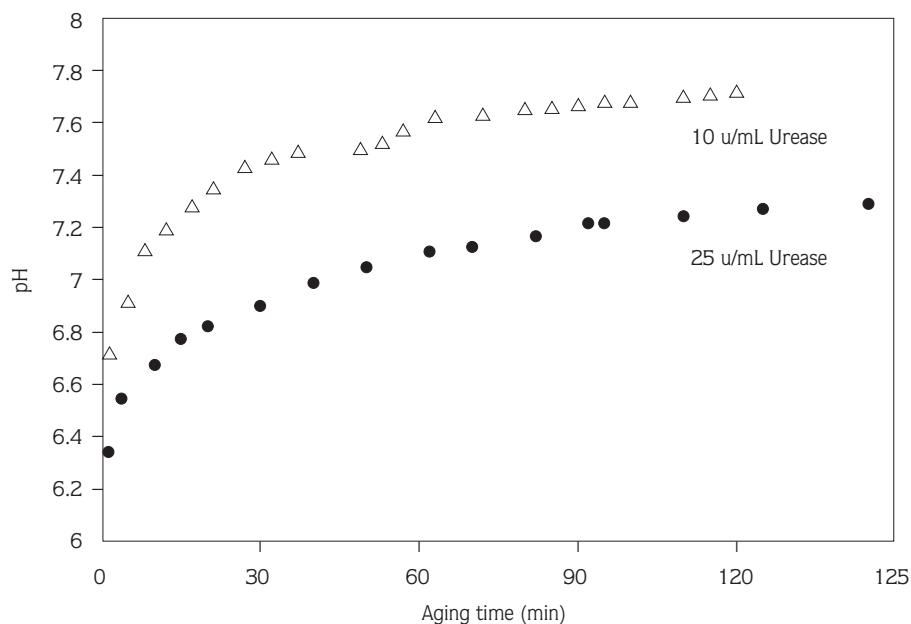


Figure 6. The smooth pH control provided by the enzyme urease (at two different concentrations) in urea-containing SBF solutions during HA powder precipitation at 37°C.

increased to 10 units/mL, the solution pH value was observed to rise to the vicinity of 7.4, in about the first half hour of aging at 37°C. The powders obtained at this concentration of urease were found to be single phase HA after calcination at 1,100°C. The biomimetic pH control (in the neighborhood of pH 7.4) in urea-containing synthetic body fluids has thus been shown to be achievable, at 37°C, by the addition of the enzyme urease in appropriate amounts (8-10 units/mL).

### Conclusions

Chemically homogeneous, single-phase calcium hydroxyapatite (HA) powders were synthesized by a novel chemical precipitation technique via synthetic body fluid solutions (either pure or urea/enzyme urease-containing),

at the physiological conditions of pH 7.4 and 37°C, with dissolved calcium nitrate tetrahydrate and di-ammonium hydrogen phosphate salts in appropriate amounts. The produced HA powders were shown to be carbonated and to have unprecedented phase stability even when heated at 1,600°C, for 6 hours, in a stagnant air atmosphere. Biomimetic HA powders were shown to have small amounts of other inorganic ions, provided and incorporated into the HA structure by the SBF solutions used during synthesis. ICP analysis performed on calcined powders indicated that the HA powders of this study had 1500 ppm Mg and 160 ppm Na. Enzyme urease when added in proper amounts into the urea-containing synthetic body fluids used for HA synthesis was shown to supply a plausible pH control required for the achievement of biomimetic conditions.

### References

- Hench L L, Wilson J, "An Introduction to Bioceramics," World Scientific, London, 1993, pp. 8, 146, 331, 335.
- Kokubo T, "Surface Chemistry of Bioactive Glass Ceramics," *J. Non-Crystal. Sol.*, 120: 138-51, 1990.
- Ohtsuki C, Kokubo T, Yamamuro T, "Mechanism of HA Formation on CaO-SiO<sub>2</sub>-P<sub>2</sub>O<sub>5</sub> Glasses in Simulated Body Fluid," *J. Non-Crystal. Sol.*, 143: 84-92, 1992.
- Klein C P A T, De Blicke-Hogerworst J M A, Wolke J G C, De Groot K, "Studies of Solubility of Different Calcium Phosphate Ceramic Particles In Vitro," *Biomaterials*, 11: 509-12, 1990.
- Jarcho M, Bolen C H, Thomas M B, Babock J, Kay J F, Doremus R H, "Hydroxyapatite Synthesis and Characterization in Dense Polycrystalline Form," *J. Mat. Sci.*, 11: 2027-35, 1976.
- Asada M, Miura Y, Osaka A, Oukami K, Nakamura S, "Hydroxyapatite Crystal Growth on Calcium Hydroxyapatite Ceramics," *J. Mat. Sci.*, 23: 3202-5, 1988.
- Ebrahimpour E, Johnson M, Richardson C F, Nancollas G H, "The Characterization of HA Precipitation," *J. Coll. Int. Sci.*, 159: 158-63, 1993.

8. Zhou J, Zhang X, Chen J, Zeng S, De Groot K, "High Temperature Characteristics of Synthetic Hydroxyapatite," *J. Mat. Sci.: Mat. in Med.*, 4: 83-5, 1993.
9. Lazic S, "Microcrystalline Hydroxyapatite Formation from Alkaline Solutions," *J. Cryst. Growth*, 147: 147-54, 1995.
10. Taş A C, Korkusuz F, Timuçin M, Akkaş N, "An Investigation of the Chemical Synthesis and High-Temperature Sintering Behaviour of Calcium Hydroxyapatite (HA) and Tricalcium Phosphate (TCP) Bioceramics," *J. Mat. Sci.: Mat. in Med.*, 8: 91-6, 1997.
11. Madsen H E L, Thodvarson G, "Precipitation of Calcium Phosphate from Moderately Acid Solutions," *J. Cryst. Growth*, 66: 369-76, 1984.
12. Taş A C, "Biomimetic Synthesis of Carbonated Calcium Hydroxyapatite Powders at 37°C with Urea- or Enzyme Urease-containing Synthetic Body Fluids," Patent Pending, No: 99/00037, Turkish Patent Institute, Ankara, Turkey, January 11, 1999.
13. Yubao L, Klein C P A T, Xingdong Z, De Groot K, "Formation of a Bone Apatite-Like Layer on the Surface of Porous HA Ceramics," *Biomaterials*, 15: 835-40, 1994.
14. Simpson R E, Habeger C, Rabinovich A, Adair J H, "Enzyme-Catalyzed Inorganic Precipitation of Aluminum Basic Sulfate," *J. Am. Ceram. Soc.*, 81: 1377-79, 1998.
15. Appleman D E, Evans H T, "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.
16. Kıvrak N, Taş A C, "Synthesis of Calcium Hydroxyapatite-Tricalcium Phosphate (HA-TCP) Composite Bioceramic Powders and Their Sintering Behavior," *J. Am. Ceram. Soc.*, 81: 2245-52, 1998.
17. Şimşek F A, "Chemical Preparation of Calcium Hydroxyapatite (HA) in Synthetic Body Fluids at 37°C and Its Use in Chemical Coating of Titanium and Stainless Steel Strips," M.Sc. Thesis, (Thesis Supervisors: A. C. Taş and H. Ö. Pamuk), Middle East Technical University, July 1997.
18. Pleshka N, Boskey A, Mendelsohn R, "Novel Infrared Spectroscopic Method on the Determination of Crystallinity of Hydroxyapatite Minerals," *Biophys. J.*, 60: 786-93, 1991.
19. Şimşek F A, Pamuk H Ö, Taş A C, "Synthesis of HA Bioceramic Powders at 37°C by using Synthetic Body Fluids," 99th Annual Meeting of The American Ceramic Society, Bioceramics Symposium, Cincinnati, Ohio, USA, May 1997.