Synthesis of nanocrystalline hydroxyapatite powders in stimulated body fluid

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Hydroxyapatite has been used to manufacture biomaterials for bone tissue implantation because of its biocompatibility and bioactivity as well as for being the most similar material to the inorganic component of the hard tissues in bones [1, 2]. Human bone apatites are not pure HA, containing small amount of CO_3^{2-}, Cl^-, Na^+, K^+, Mg^{2+} [3]. Although HA has been formed by a variety of methods [4–7], such as solid-state reaction, wet precipitation, hydrothermal reaction, sol–gel, most methods require higher pH value (more than 10) and higher sintering temperature (more than 600 °C, even up to 1200 °C) in order to obtain a stoichiometric apatitc structure. Chemical synthesis of HA powders in neutral or slightly acidic aqueous media is more complicated and difficult, furthermore high sintering temperature leads to increasing the size of synthesized HA powders [8].

Simulated body fluid (SBF), with ion concentrations nearly equal to those of the inorganic constitutes of human blood plasma, was used to prove the similarity between *in vitro* and *in vivo* behavior of certain glass–ceramic compositions [9]. Some investigators [10–12] reported that SBF could be used as a tool to synthesize apatite-like powders *in vitro*. A. Cüneyt Tas [11] reported that spherical HA powders with an average diameter in the range of 35–50 nm were synthesized using the calcium nitrate and diammonium hydrogen phosphate as starting materials Because NO_3^- and NH_4^+ are not in SBF and a large amount of NO_3^- and NH_4^+ is placed into SBF, the properties of SBF might change and HA produced might contain the ions not in bones. In our studies, using calcium chlorine and diapotassium hydrogen phosphate as starting materials, platelet-shaped and needle-like nanocrytalline hydroxyapatite (HA) powders were directly synthesized in SBF at 37 °C.

SBF [13–17] was prepared by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl{\cdot}6H_2O$, $CaCl_2$ and Na_2SO_4 in deionized water. Reagent was added, one by one after each reagent was completely dissolved in 2000 ml of deionized water, in the order given in Table I. The concentration of prepared SBF is given in Table II. The most significant differences of this study, as compared to the previous workers, are that pH values were adjusted only by using 1N NaOH, not using HCl and $(CH_2OH)_3CNH_2$. The HA synthesized flow was following as Fig. 1.

The pH value of prepared SBF solution was measured as 8.31, prior to its use, at room temperature. The SBF solution was placed in four glass bottles, 500 ml to each bottle. A measured amount of $CaCl_2$ and $K_2HPO_4{\cdot}3H_2O$ was added to each bottle, under continuous stirring, to produce depositions, the pH values of the SBF solution in each bottle decreased to 7.19, then were adjusted to 7.4 by using 1N NaOH. The bottles were tightly sealed. They were kept at 37 °C for 2, 4, 6 and 8 days, respectively. The depositions in each bottle were filtrated and washed six times with deionized water, then dried at 50 °C for 24 hrs. The dried samples were lightly ground by hand using an agate mortar and pestle to obtain HA powders.

The levels of crystallinity and the phase purity of the HA powders were studied by X-ray powder diffraction (XRD) (Model: D-Max, Rigaku Co., Tokyo, Japan) at the step size of 0.02° 2θ and the speed of 10° 2θ per min. A Cu-K_α tube operated at 40 KV and 80 mA was used for the generation of X-rays. The HA powders obtained at 37 °C for 2, 4, 6 and 8 days were denoted as HA2, HA4, HA6 and HA8, respectively. Four of the samples were determined by powder XRD to be poor crystallinity (Fig. 2). HA2, HA4 and HA6 contained HA (PDF, NO: 9-432) and trace other composition (between 14.4–21.4° 2θ). The trace composition gradually decreased with the day increasing during the observation periods and finally disappeared at 8th day. Only HA existed in HA8.

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TABLE I Chemical composition of SBF solution

Order	1	2	3	4	5	6	7
Reagent	NaCl	$NaHCO_3$	KCl	$K_2HPO_4 \cdot 3H_2O$	$MgCl \cdot 6H_2O$	$CaCl_2$	Na_2SO_4

TABLE II Ion concentrations of SBF solution

Ion	Na^+	Cl^-	HCO_3^-	K^+	Mg^{2+}	Ca^{2+}	HPO_4^{2-}	SO_4^{2-}
Concentration (mM)	142.0	147.8	4.2	5.0	1.5	2.5	1.0	0.5

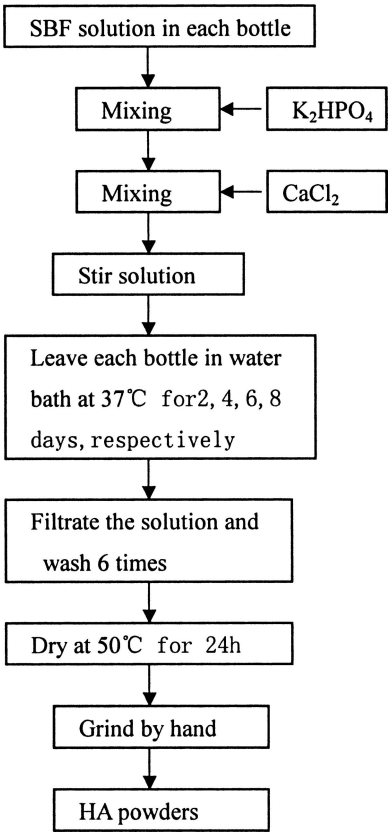


Figure 1 Process flow for HA synthesis by the SBF route.

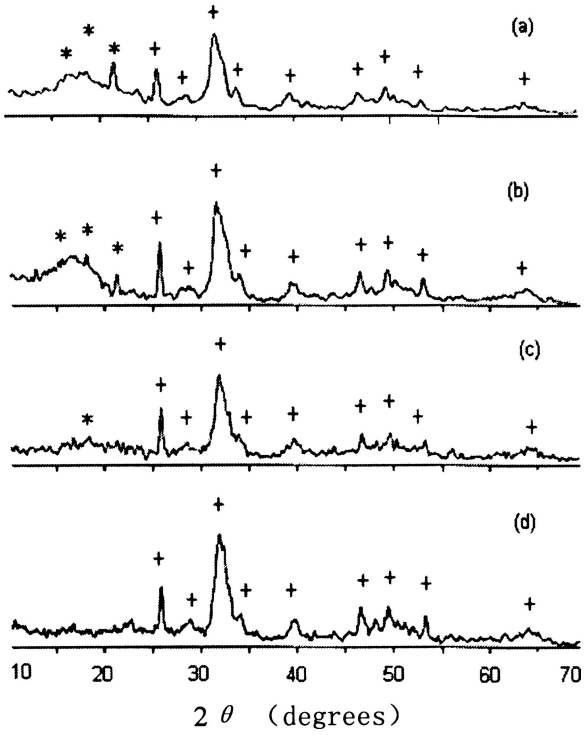


Figure 2 X-ray diffraction pattern of four HA samples.

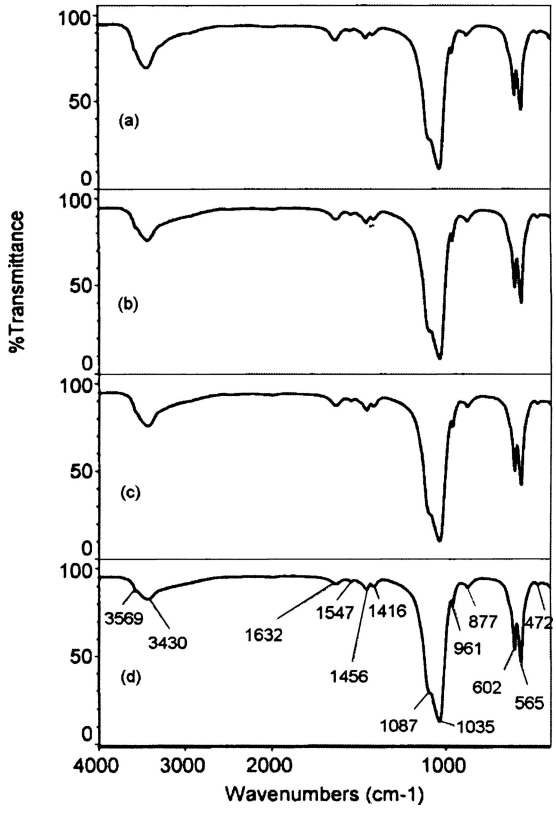
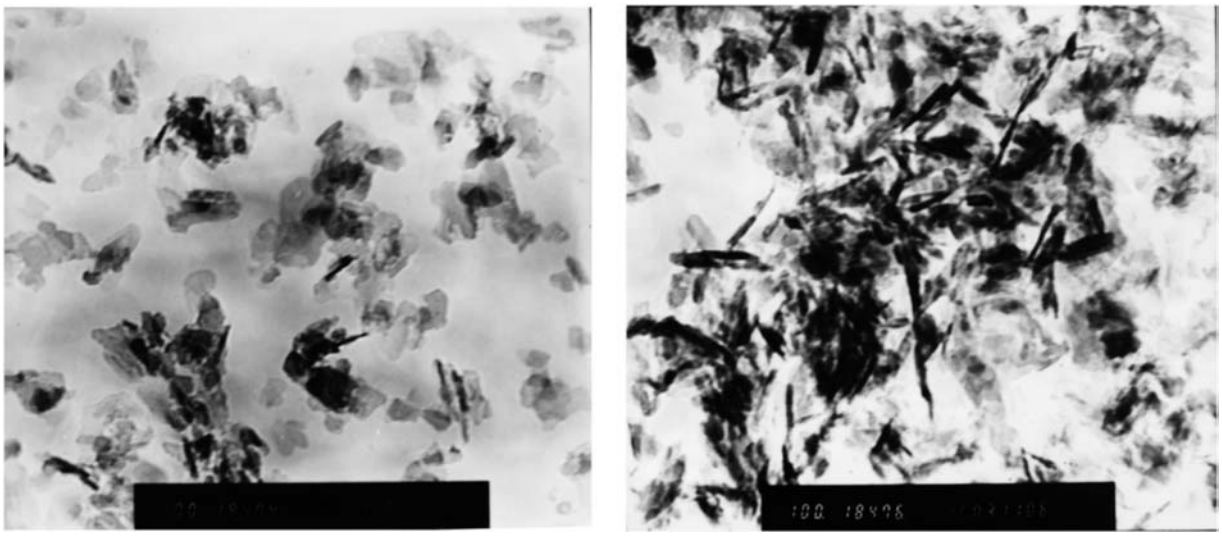


Figure 3 The FTIR spectras of four HA samples.

Furier-transformed infrared spectroscopy (FTIR) (Model: DX-510, Nicolet CO.) was used in the wave number range of 4000–400 cm^{-1}. Experimental spectra of solid samples were obtained by preparing KBr plates with a 100:3 'KBr-to-HA powders' ratio. The FTIR spectras of four HA samples were given in Fig. 3. No marked difference has been observed in the four samples. This is a typical spectrum for stoichiometric HA. The PO_4^{3-} bands were detected at 472 (ν_2), 565 and 602 (ν_4), 961 (ν_1), 1032 and 1087 (ν_3) cm^{-1}. The CO_3^{2-} ion peaks were visible at 1415 (ν_3), 1457 (ν_4 or ν_3), 1547 (ν_4) cm^{-1}. The water associated with HA is present at 3430 and 1632 cm^{-1}. The OH^- stretching vibration was observed at 3569 cm^{-1}, but the OH bending vibration (631 cm^{-1}) was not apparent. It has been reported [18] that the absorption band at 1072–1032 cm^{-1} is attributed to chloride ions in the lattice of HA crystals and the chlorapatite formation results in weakening of the absorption bands assigned to the hydroxyl group at 631 cm^{-1}. We also believe that chloride ions incorporated into HA crystals because the concentrations of chloride ions in the prepared SBF solutions were very high. It has also been reported [3] that the extents of CO_3^{2-} and Cl^- are 5.36 wt% and 0.23 wt% in



(a) (b)

Figure 4 TEM image of HA8 powders.

human bone in the range of 20–30 years, respectively. Therefore, a further study is required for a quantitative determination of the amounts of CO_3^{2-} and Cl^- ions present in such samples.

Morphology and sizes of the HA powders were investigated by Transmission electron micrograph (TEM) (Model: H800, Japan). The morphology and size of HA8 powders were investigated by TEM (Fig. 4). Most HA crystals were platelet-shaped, with irregular edges (Fig. 4a). The mean lengths and widths of crystals were 50 and 30 nm, respectively. A small amount of crystals were needle-like with the 100 nm in length and 10 nm in width (Fig. 4b). The morphology of synthesized HA is very similar to that of HA crystals in human woven bone that were reported [19], but its size is larger. To the authors' knowledge, such nanocrystalline HA powders have never been reported before for synthetic HA powders manufactured using the SBF route, except for the HA produced by the ceramic coating soaking in SBF.

The pH value changes for the four prepared SBF solutions during observation periods are shown in Table III. The pH values of the SBF solutions slowly decreased with time. The measured pH decrease of the solutions may be due to the following chemical reaction Equation 1.

$$2H_2O + 10CaCl_2 + 6K_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12KCl + 8HCl \quad (1)$$

It is known that the synthesis of HA in pure water using the starting chemicals $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$ requires high pH values in excess of 10. Our experimental findings indicate that, in the case of using SBF, the pH values needed for HA synthesis decreases considerably. Thus, we think that ions in SBF may play an important role in inducing the formation of HA. Further work on this field will be required.

Without high-temperature calcification, HA nanocrystalline powders were successfully synthesized in SBF solutions at 37 °C for 8 days, using calcium chlorine and diapotassium hydrogen phosphate as starting materials. The pH values in SBF changed from 7.40 to 6.41 during HA formation. These nano-powders were found to be platelet-shaped and needle-like. The morphology of synthesized HA are very similar to those of HA crystals in human woven bone. Further study will be carried out on reaction mechanism, mechanical properties and bioactivity of the HA synthesized by using the SBF route.

TABLE III pH values of SBF solutions

Days	0	2	4	6	8
pH	7.40	6.51	6.47	6.45	6.41

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References

1. L. L. HENCH, *J. Amer. Ceram. Soc.* **74** (1991) 1487.
2. M. M. A. RAMSELAAR, F. C. M. DRIESSENS, W. KAL, J. D. DE WIJIN and P. J. VAN MULLEN, *J. Mater. Sci. Mater. Med.* **2** (1991) 63.
3. R. G. HANDSCHIN and W. B. STERN, *Bone* **16** (1995) 355s.
4. T. KANAZAWA, T. UMEGAKI, K. YAMASHITA, H. MONMA and T. HIRAMATSU, *J. Mater. Sci.* **26** (1991) 417.
5. D. LIU, T. TROCZYNSKI and W. TSENG, *Biomaterials* **22** (2001) 1721.
6. R. TANG, Z. J. HENNEMAN and G. H. NANCOLLAS, *J. Cryst. Growth* **249** (2003) 614.
7. C. S. CHAI, K. A. GROSS and B. N. BESIM, *Biomaterials* **19** (1998) 2291.
8. E. EBRAHIMPOUR, M. JOHNSON, C. F. RICHARDSON and G. H. NANCOLLAS, *J. Coll. Int. Sci.* **159** (1993) 158.
9. C. OHTSUKI, T. KOKUBO and YAMAMURO, *J. Non-Cryst. Solids* **143** (1992) 84.
10. J. G. LI, H. L. LIAO and M. SJOSTROM, *Biomaterials* **18** (1997) 743.
11. A. CÜNEYT TAS, *ibid.* **21** (2000) 1429.

12. Y. E. GREISH and P. W. BROWN, *J. Biomed. Mater. Res. Part B: Appl. Biomater.* **67B** (2003) 632.
13. P. LI, K. NAKANISHI, T. KOKUBO and K. DE GROOT, *Biomaterials* **14** (1993) 963.
14. P. LI, I. KANGASNIEMI, K. DE GROOT, T. KOKUBO and Y. LI-URPO AU, *J. Non-Cryst. Solids* **168** (1994) 281.
15. P. LI, I. KANGASNIEMI, K. DE GROOT and T. KOKUBO, *J. Amer. Ceram. Soc.* **77** (1994) 1307.
16. T. KOKUBO, F. MIYAYI, H. M. KIM and T. NAKAMURA, *ibid.* **79** (1996) 1127.
17. S. O. CHO, NAKANISHI, T. KOKUBO, N. SOGA, C. OHTSUKI, T. NAKAMURA, T. KITSUGI and T. YAMAMURO, *ibid.* **78** (1995) 1769.
18. R. Z. LEGEROS, *Arch. Oral. Biol.* **20** (1975) 63.
19. X. SU, K. SUN, F. Z. CUI and W. J. LANDIS, *Bone* **32** (2003) 150.

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