Growth and characterization of hydroxyapatite crystals by hydrothermal method

**M. Ashok · S. Narayana Kalkura ·
N. Meenakshi Sundaram · D. Arivuoli**

Abstract Hydroxyapatite crystals were grown by hydrothermal method using dicalcium phosphate dihydrate crystals as a starting material. The grown crystals were found to be free from carbonate inclusion. Two distinct morphologies were obtained by following two different growth methods. Controlled slow growth process and rapid growth process results in hexagonal and whisker like morphologies.

Keywords Hydrothermal · Hydroxyapatite · Whisker

Introduction

Hydroxyapatite (HAP) $Ca_{10}(PO_4)_6(OH)_2$ is one of the major constituents of inorganic components of human hard tissues, bone, teeth and tendon, which is responsible for the stability, harness and function of these organs [1]. Because of the chemical similarity with the biological calcified tissues, all calcium phosphates are remarkably biocompatible and bioactive [2]. This property is widely used in medicine for biomaterials that are either entirely made of or coated with calcium phosphate. Clinically, there is a high demand for synthetic bone substitution materials that mimic the chemical composition of natural hard tissue, as autologous bone is available only in limited amounts [3]. HAP can be synthesized by various methods like wet chemical method, gel diffusion, sol-gel crystallization method, metal organic chemical vapour deposition and hydrothermal-electrochemical [4–8]. By altering the morphology of HAP crystals by controlling the orientation of crystal planes novel properties may be produced [9]. Especially, hexagonal morphology of the HAP may find promising application in dental implants because the human tooth enamel has flattened hexagonal prisms of hydroxyapatite crystals arranged parallel with the organic molecules [10, 11]. In spite of the large amount of preparation procedures only a few have been devoted to synthesis of these apatite compounds with controlled morphology [12]. Among them, the hydrothermal method has been found to be the suitable method to prepare well crystallized and non agglomerated crystals with homogenous in size, shape and composition that can be achieved even at low temperature [13, 14]. HAP crystals with different morphology were grown by hydrothermal method using various starting materials such as, calcite, brushite, monotite [15–17]. Among them, deposition of platy hydroxyapatite on dicalcium phosphate dihydrate (DCPD) single crystals using simulated body fluid by hydrothermal-electrochemical deposition attracted more interest due to their better biomimetic properties [18]. However they have reported that DCPD-HAP phase is having CO_3 inclusion. We have successfully attempted to grow HAP single crystals with two different morphologies by

M. Ashok (✉)
Department of Physics, National Institute of Technology,
Tiruchirappalli 620015, Tamilnadu, India
e-mail: ashok76@gmail.com

S. N. Kalkura · N. M. Sundaram
Crystal Growth Centre, Anna University, Chennai,
Tamilnadu, India

D. Arivuoli
Department of Physics, Anna University, Chennai,
Tamilnadu, India

simple hydrothermal method at relatively low temperature with DCPD ($CaHPO_4 \cdot 2H_2O$) crystals as a starting material. These crystals are proved to be without carbonate inclusion as well as DCPD crystalline phase and the results are presented in this paper.

Experimental methods

Hydrothermal reactions were carried out in an indigenously made autoclave with Teflon lined chamber (Fig. 1). The inner volume capacity of autoclave is 250 cm^3 with wall thickness of 2 cm. The inside chamber temperature was measured by a sensor placed in the chamber. The temperature was maintained throughout the experiment using a heater attached to the outer side wall of the chamber and controlled by a temperature controller with ±1°C. The pressure was measured using an analog pressure gauge (WIKA) with maximum pressure limit of 2,400 psi. DCPD single crystals and Millipore water were used as starting materials for synthesizing HAP crystals. The pH of water was adjusted to 7.5 using NH_4OH solution. Initially, single crystals of DCPD with cell parameters $a = 6.365$(Å), $b = 15.189$ (Å), $c = 5.814$ (Å), were crystallized using 1 M KH_2PO_4 and 1.6 M $Ca(NO_3)_2$ $4H_2O$ in sodium meta silicate gel at the pH of 6.6. The detailed preparation method was reported elsewhere [19]. Hydroxyapatite was crystallized by adopting two methods. In the first method DCPD crystals were kept in a beaker containing water (method A) as shown in Fig. 1a, where as in the second method DCPD crystals were kept in an empty glass beaker (method B) as shown in Fig. 1b. The crystallization processes by both procedures were carried out in an autoclave containing 50 ml water inside the chamber. The autoclave was heated to 220°C to obtain 500 psi of pressure and maintained at
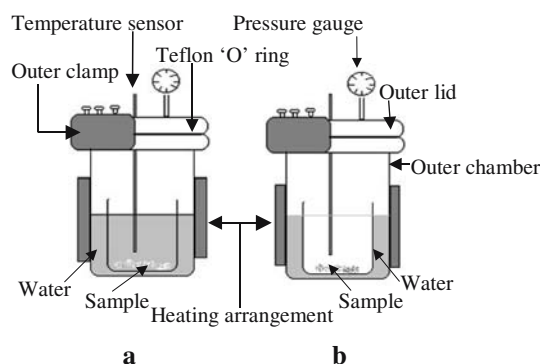
the same condition for 3 h. After brining the autoclave to room temperature, the samples were washed with distilled water and then dried in air at room temperature. These samples were characterized by powder X- ray diffraction (XRD) method using STOE diffractometer with Cu Kα radiation. Spectroscopic analysis of the starting material and products were studied by Fourier Transform Infrared Spectroscopy (FT-IR, Bomen Michelson series) using KBr as standard. Surface morphology of the samples were studied using Leica, scan 420 Scanning Electron Microscopy (SEM).

Results and discussion

XRD pattern of the DCPD crystals and the hydrothermal grown HAP crystals were shown in Fig. 2. Figure 2a–c shows the XRD pattern of DCPD crystal before hydrothermal treatment, hydrothermal grown HAP by method B and hydrothermal grown HAP by method A, respectively. The absence of DCPD peak in Fig. 2b, c clearly indicates the total conversion of DCPD to HAP. Higher peak intensity and the narrow width of the (112) peak in Fig. 2b compared to Fig. 2c might be due to the high crystalline quality of the HAP crystals grown by method B compared to method A.
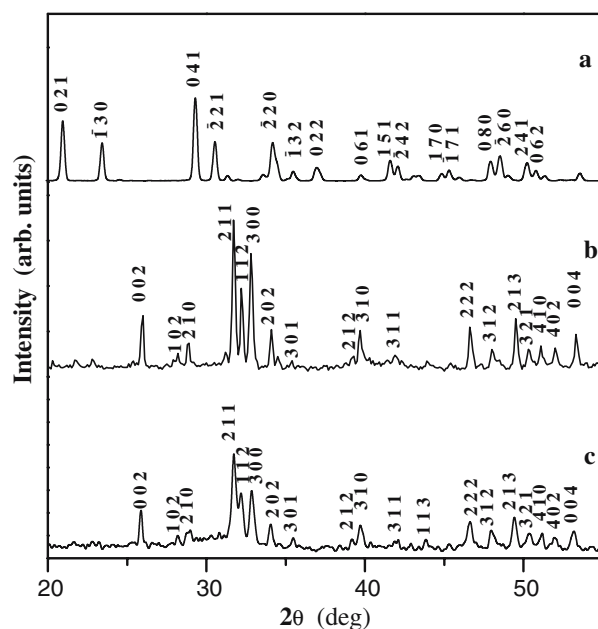

Fig. 1 Schematic diagram of hydrothermal setup adopted by method A and method B

Fig. 2 XRD pattern of (a) DCPD crystal, (b) hydrothermal grown HAP with method B, and (c) hydrothermal grown HAP with method A

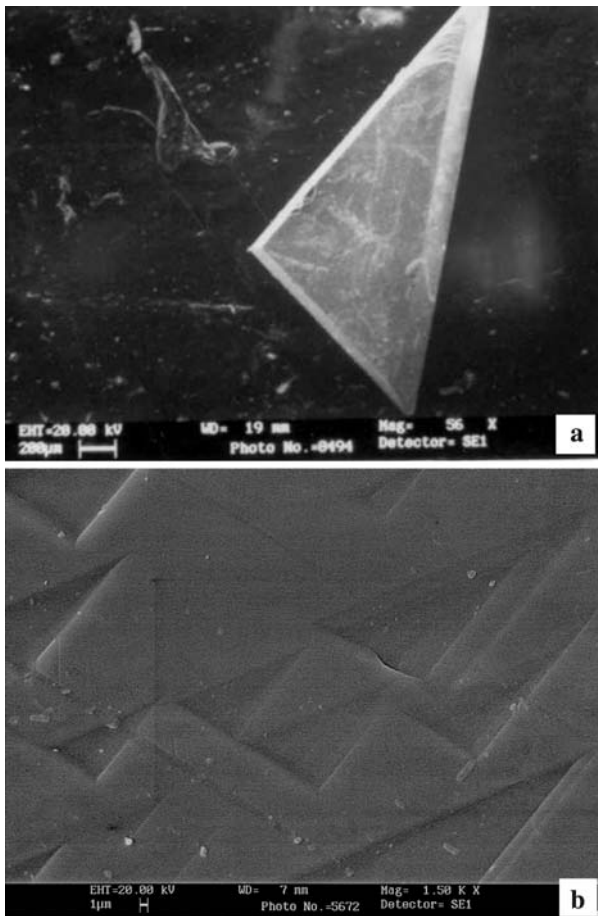**Fig. 3** SEM pictures of (**a**) triangular DCPD crystal and (**b**) surface of DCPD crystal before hydrothermal treatment

The surface morphology of the DCPD crystals before hydrothermal treatment and hydrothermal grown HAP crystals were shown in Figs. 3, 4. A triangular DCPD single crystal which is used as starting material in this method was shown in Fig. 3a. As seen in Fig. 3b, triangular etch bits are observed on DCPD crystals surface. HAP crystals grown by two different methods yielded two distinct morphologies. As shown in Fig. 4a, HAP crystals grown by method A shows the whisker morphology with average size of 4×45 μm, whereas, hydrothermal treatment by method B yields controlled hexagonal morphological HAP crystals with 25 μm in size, as shown in Fig. 4b, c. The physico-chemical process during hydrothermal treatment can be analyzed as follows. When the water is vaporized, the water vapor contains H^+ and OH^- ions, and its content increases with increasing temperature [17]. The resultant OH^- ions in the vapor are expected to react with $CaHPO_4 \cdot 2H_2O$ and convert it into HAP according to the following equilibrium reaction [5].

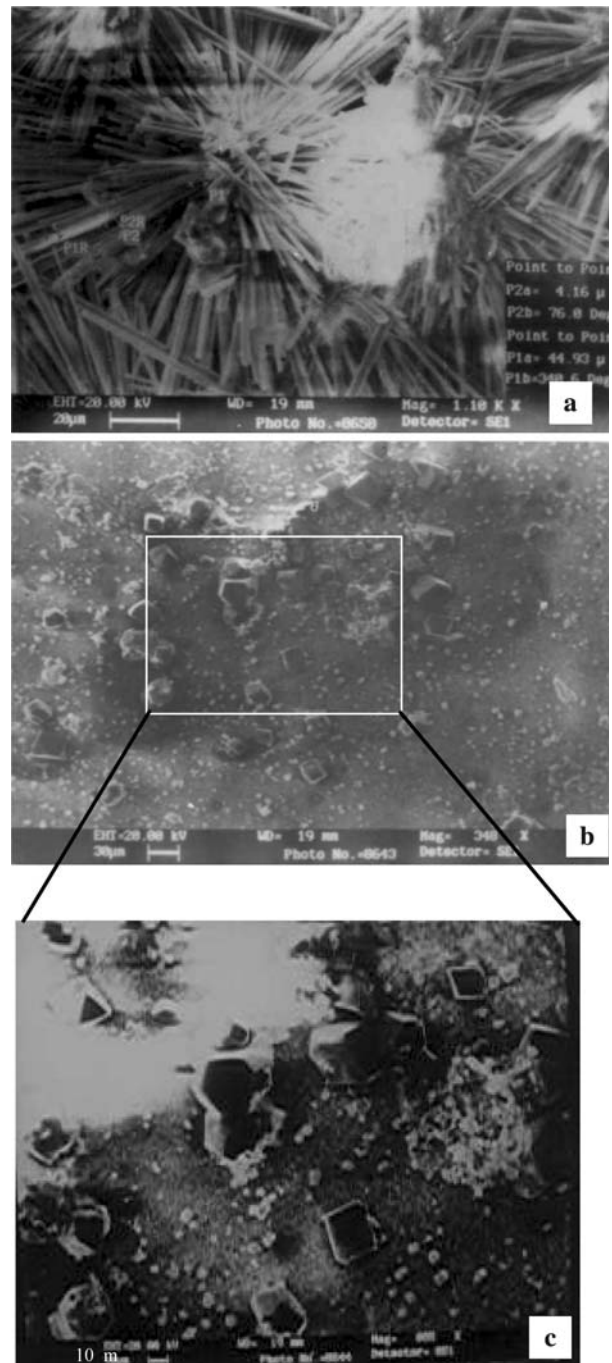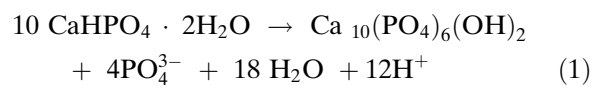**Fig. 4** SEM picture of surface (**a**) after hydrothermal treatment [method A], (**b**) after hydrothermal treatment [method B] and (**c**) magnified image of selected region

$$10\ CaHPO_4 \cdot 2H_2O\ \rightarrow\ Ca_{10}(PO_4)_6(OH)_2 + 4PO_4^{3-} + 18\ H_2O + 12H^+ \qquad (1)$$

The Ca/P ratio of the sample was 1.67, as determined by titration and UV–VIS spectroscopic analysis.
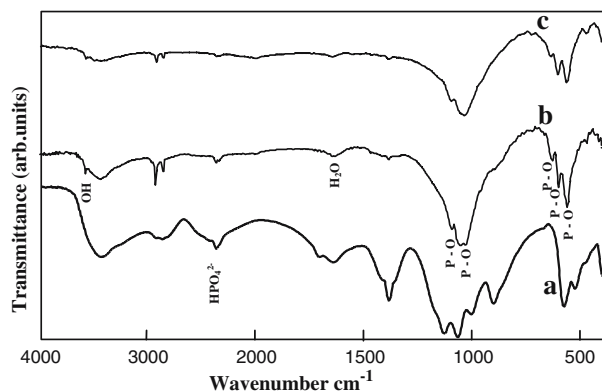
Fig. 5 FT-IR spectrum (**a**) DCPD crystal, (**b**) after hydrothermal treatment [method A], and (**c**) after hydrothermal treatment [method B]

The yield of the product is 93%. The FT-IR spectra of DCPD crystal, and the HAP obtained by method A and method B were shown in Fig. 5. FT-IR spectra show the PO_4^{3-} bending modes at 563 and 600 cm^{-1}. Asymmetric stretching modes were observed at 1033 and 1085 cm^{-1}. The CO_3^{2-} bands were not observed. The peak at 1630 cm^{-1} corresponds to the absorbed water and the broad peak around 3,600–3,000 cm^{-1} corresponds to O–H stretching. Therefore, the presence of 1,630 cm^{-1} peak in HAP crystal grown by method A (Fig. 5b) and its absence by method B (Fig. 5c) suggest that HAP crystals grown by method A might have acquired water inclusion during the growth. It is plausible that water could be included into the HAP aggregates by fast formation and association of crystallites. Whereas, when the crystal formation process is slow, it is less likely that water molecules could be trapped into HAP aggregates [20]. This further suggests that the HAP crystallization by method B could be a controlled and slow growth process.

Conclusion

Hydroxyapatite crystals with well defined morphology were obtained by the hydrothermal treatment of DCPD crystals. The crystals were found to be free from DCPD crystalline phase. Carbonate free HAP crystals with bone like whisker morphology and enamel like hexagonal morphologies were obtained by adopting two different growth methods. We believe that the slow and controlled growth process results in hexagonal morphology whereas rapid growth process results in whisker morphology. It is noteworthy to mention that the hexagonal morphology found in our hydrothermal.

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