

In situ synthesis and characterization of nano-size hydroxyapatite in poly(vinyl alcohol) matrix

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Received 12 March 2006; received in revised form 3 May 2006; accepted 27 June 2006

Available online 11 September 2006

Abstract

Hydroxyapatite (HAp) crystals were prepared via an in situ biomimetic process in the presence of poly(vinyl alcohol) (PVA). The effect of polymer amount and its molecular weight on the physical properties of the HAp crystals were investigated. X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FT-IR) analysis, confirmed the formation of the crystalline HAp at room temperature. Microstructural features such as size and morphology of the resulting HAp samples were characterized using BET, scanning and transmission electron microscopy. The results indicate that the development (size and shape) of the HAp nanocrystals precipitated in an aqueous solution of PVA was influenced by the molecular weight of the polymer in such a way that smallest crystallite size was observed in the case of PVA with the highest molecular weight. It is believed that the HAp formation was initiated through the interaction of Ca^{2+} ions with the negative side groups on the polymer surface. The larger number of reaction sites in the PVA polymer with higher molecular weight led to a higher number of HAp nuclei and therefore smaller crystallite size.

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Keywords: In situ synthesis; Hydroxyapatite; Nano-size; Poly(vinyl alcohol)

1. Introduction

Bone is a natural ceramic-organic composite material with a complex hierarchical structure [1]. It consists mainly of collagen fibrils (20 wt.%) which have been infiltrated by a well arranged nano-size crystalline inorganic phase (69%) and water (9%). The inorganic constituent of the bone resemble synthetic hydroxyapatite (HAp), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ in composition. The HAp crystals in natural bone are needle-like or rod-like in shape 40–60 nm in length, 10–20 nm in width, and 1–3 nm in thickness. The synthesized hydroxyapatite with bone-bonding properties is widely used in hard tissue replacement due to their biocompatibility and osteoconductive properties [1–3]. However, the poor mechanical properties of the synthesized HAp, such as high elastic modulus and low fracture toughness restrict its applications. Thus, HAp is used in composite form (HAp-polymer) to retain useful bioactive properties as well as enhancement in mechanical properties. However, in the

composite materials synthesized based upon a simple physical mixture there is neither a morphological control nor there is any chemical interaction at the organic–inorganic interface. The mechanical properties of the composite systems prepared in this manner are inadequate for bone replacement applications [4–7]. Therefore, in recent years there have been many attempts in the direction of developing materials and techniques to impart suitable biological and mechanical properties to synthetic composites to be used in the replacement of the natural bone. Most promising of these attempts has been a biomimicking approach in the synthesis of artificial bone-like composite material [7]. The essence of the biomimicking process lies in mimicking biological mineralization in which an organic phase provides a template for inorganic crystals to nucleate and grow from supersaturated solution [7,8]. A molecular control on the morphology, size, and anisotropy of the inorganic crystals and their uniform distribution over the organic phase are some of the main goals aimed at in the biomimicking process. Polymers containing polar functional groups such as COOH , CH , CH_2 , PO_4H_2 , and OH have been found to be useful in this regard since these ionizable side groups provide a greater affinity to positive calcium ions and the nucleation of hydroxyapatite

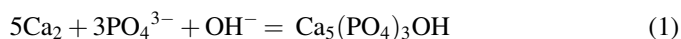
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crystals in the solution [1,5,6,9,10]. Some of the more recent attempts in the in situ mineralization technique have made use of polymeric additives such as poly(lactic acid) (PLA), poly(acrylic acid) (PAAc), collagen, and gelatin due to their calcium binding properties [6–10]. Another polar polymer with potential biomedical application is poly(vinyl alcohol) (PVA). It is a low cost water soluble biocompatible polymer and its potential use in the form of hydro gels as drug carrier, and its use as template for in situ precipitation of nano-sized HAp particles in the fabrication of a porous HAp/gelatin composite has already been reported in the literature [11–13]. In this manuscript, using a biomimetic approach, we report the effect of amount and molecular weights of the poly(vinyl alcohol) on the physical properties of the resulting HAp particles.

2. Experimental procedures

In situ synthesis of hydroxyapatite particles was carried out in the presence of PVA (Merck product). For comparison HA particles were also prepared in the absence of polymer. The effect of polymer molecular weight on powder characteristics was studied by using PVA with different molecular weights (22,000, 88,000, and 145,000). In addition in order to study the effect of the amount, different percentages of PVA namely 20, 40, and 60 wt.% were used in this study (calculations were made based upon an organic–inorganic composite system). The first step in the preparation of HA particles was the preparation of a 0.5 M calcium chloride (Merck) solution and a 0.3 M solution of sodium dihydrogen phosphate (Merck) using double-distilled water. In addition a water based solution of PVA was also prepared separately. In the preparation of HA particles in the presence of polymer, the calcium chloride stock solution was slowly added to polymer solution. The calcium chloride–PVA solution was stirred slowly on a magnetic stirrer for 4–6 h, after which the proper amounts (Ca/P molar ratio of 5:3) of sodium dihydrogen phosphate solution was added gradually to the above mixture. A milky white coloration was observed almost instantaneously after the addition of phosphate solution. However, in the absence of the polymer the appearance of milky white coloration was not observed before PH = 10–11. High values of PH in the solution have been reported to promote HAp nucleation through the following reaction [2]:



A 25 wt.% ammonia–water solution was used to control the PH in the solution. It should be mentioned that the appearance of milky coloration has been associated with the formation of hydroxyapatite in the literature [13]. The solutions obtained in this manner (with and without polymer) were aged for 12 h at room temperature following which the precipitates were decanted and washed thoroughly with deionized water and dried at 60 °C for 12 h. The as dried products were characterized using scanning electron microscope (SEM; Cam Scan MV2 300), transmission electron microscope (TEM; CM 200 Philips), X-ray diffractometry (XRD; Philips expert Pro), Fourier-transform infrared spectroscopy (FT-IR; Shimadzu, IR solution, 8400S), and single point BET method (Gemini 2375 4.02).

3. Results and discussions

The formation of highly organized natural bone mineral in the presence of biological polymer (collagen) is one of the most fascinating processes in nature. In this study an attempt has been made in the application of this so-called biomimetic method in the processing of synthetic hydroxyapatite particles. Specifically the biocompatible PVA polymer has been chosen as templating agent for the in situ precipitation of HA crystals. PVA is a polar, water soluble polymer with CH, CH₂, and OH as side groups. Upon ionization, the negatively charged surface groups can provide binding sites for the Ca ions present in the solvent. This process is followed by the attraction of PO₄ anions towards these uniformly distributed Ca sites, initiating the crystallization process of hydroxyapatite. Such an electrostatic model for the hydroxyapatite formation has been indicated in earlier studies [2–4,14–16]. The binding between calcium ions and the charged groups in the polymer phase at this molecular level has been shown to have a positive effect on the mechanical behavior of the composite systems prepared in this manner [14]. As mentioned already, in this portion of the study the focus has been on investigating the effect of molecular weight and the amount of polymer on the characteristics of the HA crystals. As dried chemically precipitated products (see Section 2) were characterized using XRD for structural determination. The formation of crystalline hydroxyapatite phase both in the presence and in the absence of polymer was verified using this technique. The analysis of the XRD patterns indicated that the change in the polymer molecular weight and/or its amount did not have an observable effect on the peak positions and/or their

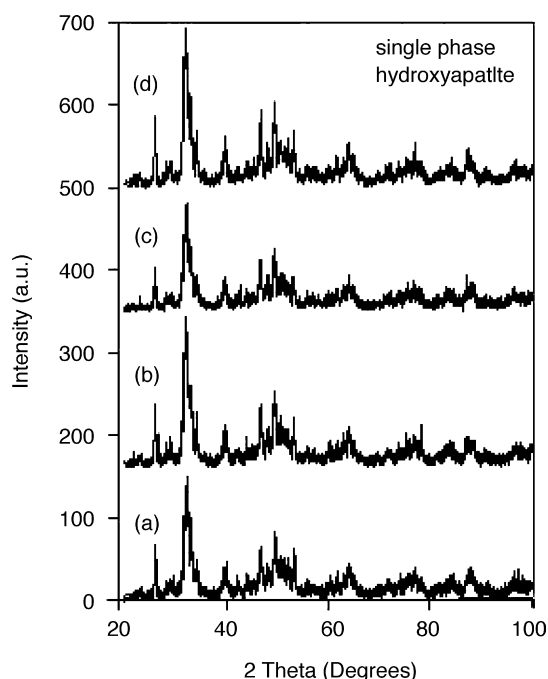


Fig. 1. XRD patterns of HAp samples synthesized: (a) in the absence of polymer, (b) in the presence of 20 wt.% polymer with molecular weight of 145,000, (c) in the presence of 40 wt.% polymer with molecular weight of 145,000, and (d) in the presence of 60 wt.% polymer with molecular weight of 145,000.

intensity. As a representative result, the X-ray diffraction patterns for the samples prepared in the presence of various amounts of PVA polymer with molecular weight of 145,000 are shown in Fig. 1.

For comparison the XRD pattern for the sample prepared in the absence of polymer is also shown in this figure. As shown in this figure, the diffraction peaks shown in each plot correspond to that of a single phase hydroxyapatite. The BET analysis was used to determine the specific surface area and the crystallite size for the HAp samples prepared under different conditions. Samples prepared in the absence of polymer (coded as A) and in the presence of 60 wt.% of PVA polymer with molecular weights of 22,000, 88,000, and 145,000 were chosen for this study (the latter samples were coded as B–D, respectively). In order to eliminate the effect of polymer in the BET experiments, HAp samples prepared both in the presence and in the absence of polymer were calcined at 500 °C for 4 h prior to BET test. The results of specific surface area analysis and the corresponding particle sizes are presented in Table 1.

As shown in this table, regardless of the polymer molecular weight the HAp crystals prepared in the presence of polymer

Table 1

The surface area and equivalent particle size for the HAp samples produced both in the absence and in the presence of polymer

	Sample code			
	A	B	C	D
BET average specific surface area (m ² /g)	36 ± 3	55 ± 3	56 ± 2	90 ± 3
Equivalent particle size (nm)	53 ± 3	35 ± 3	33 ± 2	20 ± 3

show higher specific surface areas. In addition it is seen that with an increase in the molecular weight of the polymer there is also an increase in the HAp surface area. In other words the smallest crystallite size was achieved in the presence of the polymer with the highest molecular weight used in this work. We believe that the decreasing trend observed in the size of HAp crystals with the increase in the molecular weight of the polymer is related to the availability of a higher number of nucleating sites in the polymer with higher molecular weight. As mentioned before, based on the available theory, the ionization of side groups on the organic polymer provides a site

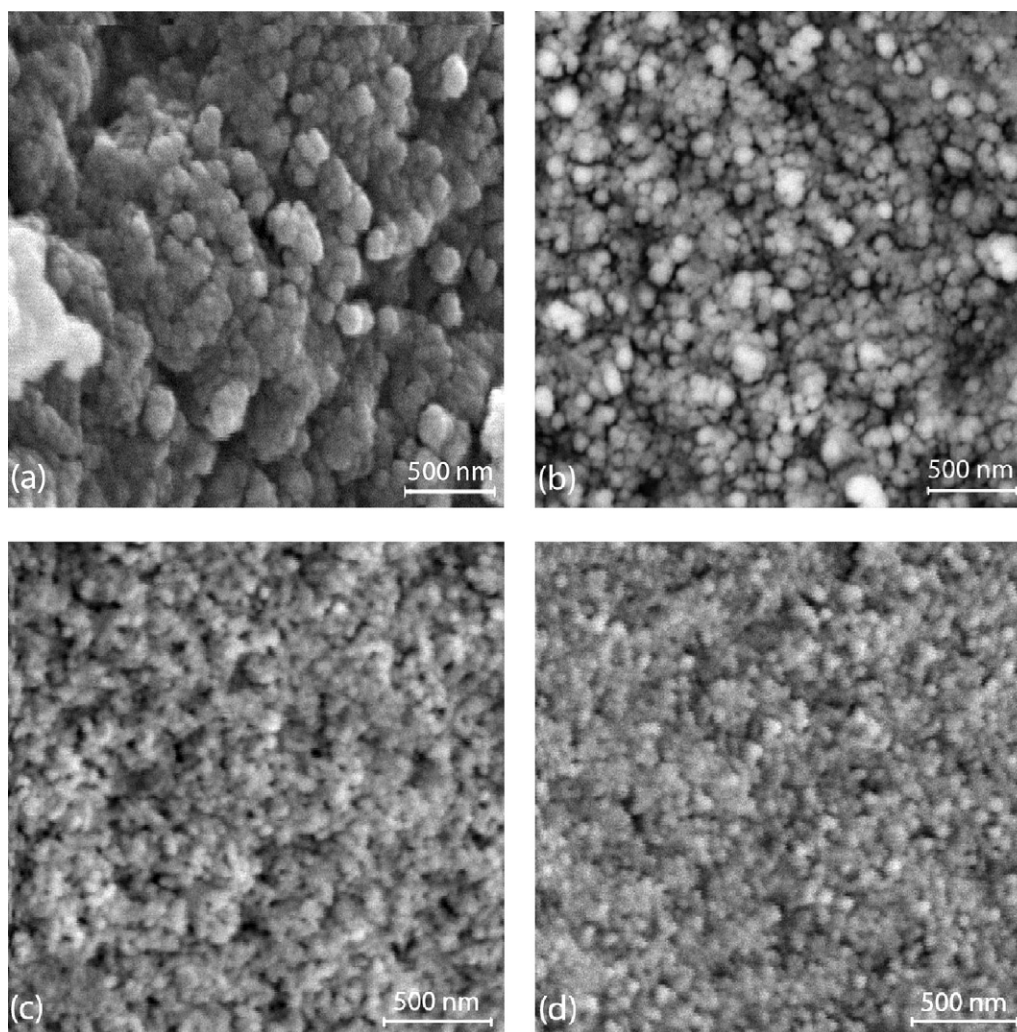


Fig. 2. SEM micrographs of the HAP sample synthesized: (a) in the absence of polymer, (b) in the presence of polymer with molecular weight of 22,000, (c) in the presence of polymer with molecular weight of 88,000, and (d) in the presence of polymer with molecular weight of 145,000.

for the selective adhesion of Ca ions. As the calcium ions accumulate on the surface, the surface gains an overall positive charge. At a later stage, these positively charged surface sites will combine with negatively charged phosphate ions (PO_4^{3-}) initiating the nucleation of nanometer-sized HAp particles on organic polymer. The increase in the molecular weight of polymer leads to the formation of larger number of nuclei and therefore smaller HAp crystallite size. These results demonstrate the importance of the physical characteristics (such as molecular weight) of the polymer used in the in situ synthesis of HAp crystals in determining the properties of the final HAp product. HAp particles prepared with and without the presence of polymer were examined by SEM. Fig. 2a–d is SEM micrographs for the samples prepared in the absence and in the presence of polymer with molecular weights of 22,000, 88,000, and 145,000, respectively.

It is difficult to say anything concrete about the size and the morphology of the HAp crystallite from these micrographs. However, when comparing these figures one sees less

agglomerated, smaller size particles having a more uniform distribution (regular arrangement) for the HAp crystallites prepared in the presence of polymer. These important characteristics are the direct consequence of the interaction and binding between the orderly arranged side groups on the polymer and the nucleating HAp nanocrystals. The formation of HAp particles on the polymer surface was also investigated by FT-IR analysis. Fig. 3a presents the FT-IR spectrum for the pure HAp (HAp particles prepared in the absence of polymer) showing a peak around 3569 cm^{-1} corresponding to stretching vibration of the hydroxyl group [17].

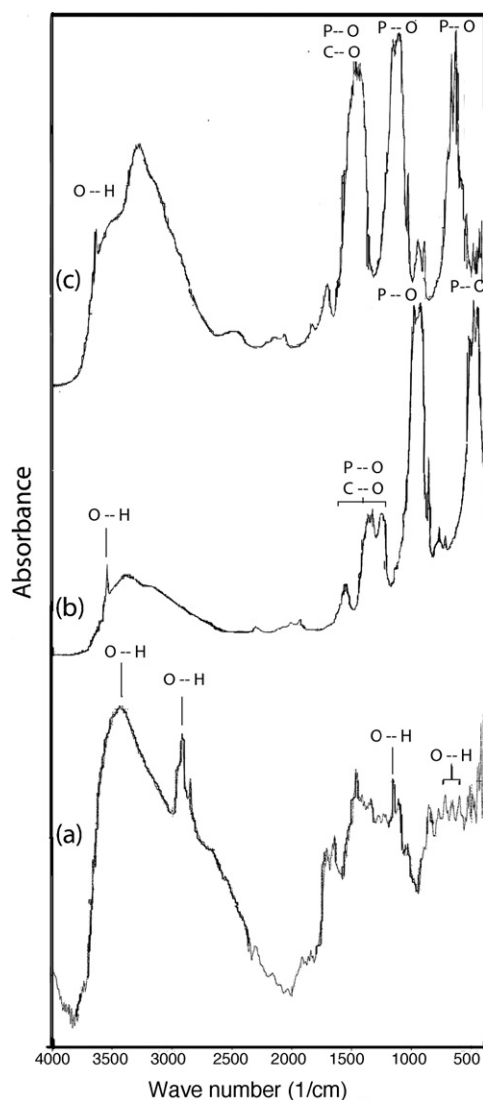


Fig. 3. FT-IR spectra of (a) HAp sample synthesized in the absence of polymer, (b) PVA polymer, and (c) HAp sample prepared in the presence of polymer.

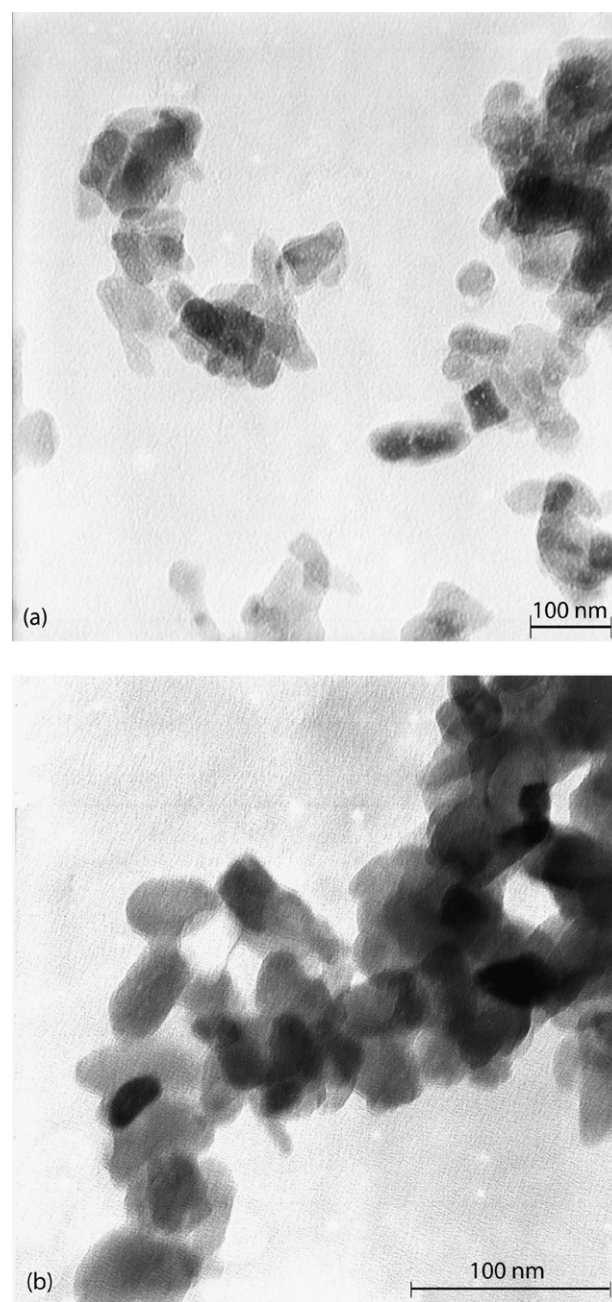


Fig. 4. TEM micrographs of HAp samples synthesized: (a) in the absence of polymer and (b) in the presence of 60 wt.% polymer with molecular weight of 145,000.

Characteristic double split bands at around 600 cm^{-1} and a band around 1049 cm^{-1} are due to the bending and stretching modes of P–O vibrations in the phosphate network, respectively [12,13]. The absorption bands observed in the range of $1300\text{--}1650\text{ cm}^{-1}$ are due to the stretching and bending modes of C–O and P–O bonds and air carbonate (CO_3) $^{2-}$ ions [3,4,17]. The presence of the carbonate peaks in this study could be due to contamination from the air. The FT-IR spectrum for the PVA polymer is displayed in Fig. 3b. The comparison of the FT-IR spectrum for the HAp particles synthesized in the presence of polymer (Fig. 3c) with the spectra shown in Fig. 3a and b shows that there are an increase in the intensity of the absorption bands associated with the OH^- suggesting the contribution from the similar groups in the PVA polymer [13]. There are no obvious broadening of the absorption PO_4^{3-} and OH^- bands in the IR spectra for the polymer mediated synthesized samples, indicating the crystalline nature of the HAp crystallites. The slight shift in the position of absorption bands for the HAp prepared in the presence of polymer is indicative of dissociation and interactions of polymer with the nucleating HAp crystals [2,13,18].

Fig. 4a and b shows transmission electron micrographs of hydroxyapatite prepared in the absence of polymer and in the presence of polymer with the molecular weight of 145,000, respectively.

It is difficult to identify primary particles but mostly particles with angular and irregular morphology are observed in the sample prepared in the absence of polymer (Fig. 4a). As seen in Fig. 4b the particles appear more elongated when the synthesis is done in the presence of polymer. These micrographs also show that HAp particles synthesized in the presence of polymer are smaller than those prepared in the absence of polymer.

4. Conclusions

PVA polymer was used as templating agent in the in situ synthesis of HAp particles. The formation of the HAp crystals at room temperature was confirmed by both the XRD patterns and the presence of characteristic absorption bands in the IR spectra. Experimental results indicate that the molecular weight of the templating polymer is an important factor in determining the particle size in the final HAp product. The in situ synthesized particles were less agglomerated which is believed to be the result of the nucleation of HAp crystallites on the regularly arranged side groups located on polymer chains. In addition to the size, the crystallite shape was also influenced by the presence of polymer as evidenced by TEM micrographs. The in situ synthesis method is expected to lead to nano-composite biomaterials which will be structurally more comparable to the natural bone with closely related mechanical and biological properties. The mechanical and bioactivity of the

in situ fabricated PVA-HAp nano-composites are currently under investigation.

Acknowledgements

The financial support provided by the Ministry of Science, Research, and Technology is highly appreciated by the authors.

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