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Effect of Different Phosphorus Precursors on Biomimetic Hydroxyapatite Powder Properties

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Hydroxyapatite is the main component of natural hard tissues, such as teeth and bone. It has been studied extensively as a candidate biomaterial for its use in prosthetic applications. Hydroxyapatite was formulated as $Ca_{10}(PO_4)_6(OH)_2$ and it has a high stability under physiological conditions. Also hydroxyapatite can be synthesized using different calcium and phosphorus precursors. In this study, biomimetic hydroxyapatite powder has been synthesized simulating physiological conditions. Synthetic body fluids which have the same composition as human blood plasma instead of pure water were used as precipitation media to obtain biological conditions. Recent research involved the effect of different Ca-precursors however aim of this study is to determine the effect of phosphorus resources. In this study, the synthesis of hydroxyapatite powder is carried out by using biomimetic method in synthetic body fluids. Calcium acetate [$Ca(CH_3COO)_2$, CA] and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$, DAHP], ammonium dihydrogen phosphate [$NH_4H_2PO_4$, ADHP], dipotassium hydrogen phosphate [K_2HPO_4, DPHP] and orthophosphoric acid [H_3PO_4, OPA] were used as Ca- and P-precursors. Chemical structures of synthesized powders have been examined by Fourier transform infrared and X-ray diffraction. Results showed that synthesized powders have a pure hydroxyapatite structure. However, ADHP precursors have an unfavorable effect on sintered hydroxyapatite powders. Using ADHP phase transition was caused in pure hydroxyapatite structure and apatite and whitlockite were observed as secondary phases. Their particle size, surface area determination and morphological structures have been characterized by Zeta-Sizer, biomimetic hydroxyapatite the Brunauer–Emmett–Teller analysis and scanning electron microscopy images, respectively. As a result different starting materials have affected the structure, particle size and morphological properties of biomimetic hydroxyapatite.

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1. Introduction

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] has been used for decades as a biomaterial in orthopedic and dental applications due to its biocompatibility and similarity to the inorganic components of bone and teeth in human [1]. Apatite used in biomedical applications mostly has a μm- or nanometer-size. There are a lot of techniques such as precipitation, hydrothermal, sol–gel, crystal conversion, microwave techniques and nanoindentation etc. to synthesize HA. Because it is easy and economic, precipitation method is the most common technique [2]. Also precipitation method can be classified as a biomimetic [3] and chemical precipitation [4]. The main difference between biomimetic and chemical methods in addition to pH and synthesis temperature is preparation media. Water and simulated body fluids were preferred as a precipitation solution for chemical synthesis (CS) and biomimetic synthesis (BS), respectively. Also CS has occurred at highly alkaline media and room temperature compared with BS (7.4 pH and 37 °C). The surface properties and synthesis conditions of calcium hydroxyapatite prepared by using distilled pure water in highly alkaline solution have been studied in different solutions including synthetic body fluids (SBF). Moreover the chemical synthesis of HA powders has been examined by using biomimetic method with the purpose of synthesis of more stable and biocompatible HA powders [3]. However, HA precipitation was carried out in highly alkaline media in all techniques. Furthermore $Ca(NO_3)_2 \cdot 4H_2O$, $Ca(OH)_2$, $(NH_4)_2HPO_4$, and H_3PO_4 are the most common precursors to synthesize HA. Unfortunately, according to experimental results we observed that these precursors cause an increase of the pH of the media.

In the present study as a calcium precursor $Ca(CH_3COO)_2$ and as phosphorus precursors $(NH_4)_2HPO_4$, $NH_4H_2PO_4$, K_2HPO_4 and H_3PO_4 have been used. During the HA synthesis, biomimetic method and physiologic conditions (7.4 pH and 37 °C) have synthesised HA powder. Structural analysis was performed by using X-ray diffraction (XRD) and Fourier transform infrared (FTIR) methods, morphology of the powders were investigated by scanning electron microscopy (SEM) and compared with each other. Also thermal stability, surface area, particle size and density was determined by using thermogravometric/differential thermal analysis (TG/DTA), Brunauer–Emmett–Teller (BET) method, Zeta-Sizer and ultrapicnometer.

2. Materials and methods

The SBF solution prepared by Othsuki et al. [5] offered cleaning procedure to obtain more stabilized SBF solutions. For that purpose each material (such as beaker, flask etc.) were cleaned according to Othsuki's recipient. SBF solutions were prepared according to Tas's SBF solution [6]. The reagents used to prepare one liter of SBF are NaCl (6.547 g/L, 99.5%), $NaHCO_3$ (2.268 g/L, 99.5%), KCl (0.373 g/L, 99.0%), $Na_2HPO_4{\cdot}2H_2O$ (0.178 g/L, 99.5%), $MgCl_2{\cdot}6H_2O$ (0.305 g/L, 99.0%), $CaCl_2{\cdot}H_2O$ (0.355 g/L, 99.0%), Na_2SO_4 (0.355 g/L, 99%), $(CH_2OH)_3CNH_2$ (6.057 g/L, 99.5%), and HCl (37 vol.%). All reagents were purchased from Merck. Reagents were added one by one after each reagent was completely dissolved in water with given order. Also after $MgCl_2{\cdot}6H_2O$ and $(CH_2OH)_3CNH_2$ was dissolved, desired amount of 1 M HCl (37 wt% Merck) solution was added to adjust pH value 7.4 [6].

Calcium acetate [$Ca(CH_3COO)_2$, CA] and diammonium hydrogen phosphate [$(NH_4)_2HPO_4$, DAHP], ammonium dihydrogen phosphate [$NH_4H_2PO_4$, ADHP], dipotassium hydrogen phosphate [K_2HPO_4, DPHP] and orthophosphoric acid [H_3PO_4, OPA] were supplied from Sigma–Aldrich and used as calcium and phosphorus precursors. Firstly, 0.167 M CA and 0.1561 M DAHP solutions were prepared in SBF. Then DAHP solutions were added to CA solution with 4 mL/dk addition ratio. The final suspension was called S1. The same experiments were also carried out using other Ca and P precursors and final suspensions were called S2, S3, and S4, respectively. Ca/P ratio of HA in all suspensions was calculated as 1.67. Finally, these suspensions were aged for a day at 37 °C. Following the aging process precipitates was filtered and washed with ultrapure water. The resulting precipitates were dried at 80 °C for 8 h and then ground using agate mortar. Dried powders were sintered in air atmosphere at 900 °C for 2 h with 10 °C/min heat rate in a kiln oven (Protherm). Heat treated powders was called S1S, S2S, S3S, and S4S, respectively.

Chemical structures of synthesized powders have been examined by FTIR (Perkin–Elmer Spectrum-100) and XRD (Shimadzu, XRD-2000, TUBITAK, MAM). Surface area measurements and morphological structures have been characterized by using BET (Quardrosorb SI, Malvern) method and SEM (JEOL-JSM-6335F, TUBITAK, MAM) analysis, respectively. Also ultrapicnometer (Quardrosorb Ultrapyc 1200e, Malvern) was used to measure the density of dried and sintered powders.

3. Result and discussion

Figure 1 shows the FTIR spectra of dried and sintered powders. OH^- at 3580 cm^{-1} and PO_4^{3-} at 630 cm^{-1} are peaks characteristic of HA structure. However, band position of OH^- was not clearly seen in Fig. 1 because of its low concentration in the structure. Also 1020 cm^{-1}, 960 cm^{-1} and 1093 cm^{-1} peaks are referred to PO_4^{3-} groups in the HA. A very small hump at the wave number 1454 cm^{-1} in this figure arose from vibrations of CO_3^{2-} ions resulting from dissolution of air CO_2 in the hydroxyapatite structure. Also it can be clearly seen that carbonate peaks become insignificant with sintered powders which means that CO_3^{2-} has very weak attractions with the HA structure.

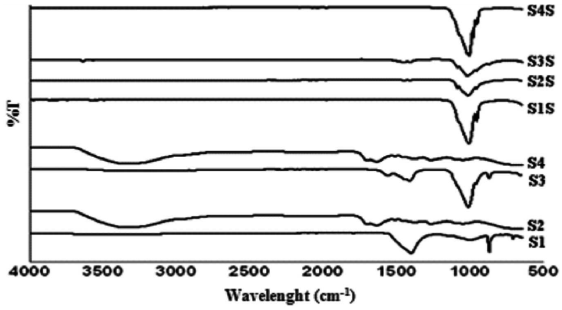

Fig. 1. The FTIR spectrum of dried samples at 80 °C (S1, S2, S3, S4) and sintered samples at 900 °C (S1S, S2S, S3S, S4S).

Figure 2 shows the XRD patterns of HA powders. HA peaks occurred at 2θ of 28° and 32°, which are consistent with the standard XRD peaks for HA. The XRD patterns of all the samples indicate that the main phase is HA. Even if literature said that HA could be synthesized with some impurities at initial pH values higher than 10, our previous study [7] has proved that HA can be synthesized at low pH to compare with the literature [8]. Moreover present study is supported that observation and HA was produced at initial pH 8.0 in a low temperature. Also XRD patterns of synthesized amorphous HA structure could be turned to crystalline phase when it sintered at higher temperature without any or negligible transition. However with the high sintering temperatures, HA structure can be decomposed as seen in Fig. 2. Sample S4 has apatite [$Ca_5(PO_4)_3Cl$] and whitlockite [$Ca_3(PO_4)_2$] as secondary phases. However S1, S2, S3, S4, S1S, S2S and S4S had a pure HA structure. These observations showed that precursors could affected the final purity of synthesized HA at high temperature.

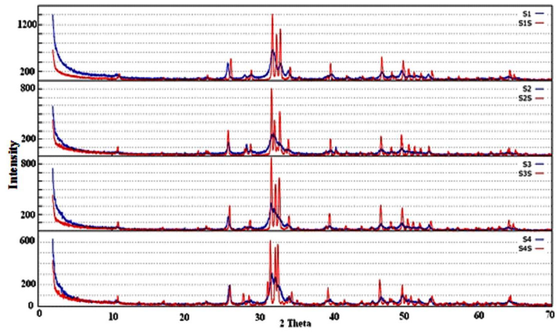

Fig. 2. XRD spectrum of HA samples S1, S2, S3 and S4 at 80 °C-dried and S1S, S2S, S3S and S4S at 900 °C--sintered.

SEM micrographs of 80 °C-dried and 900 °C-sintered samples were given in Fig. 3. These powders showed a significant level of agglomeration without heat treatment at 900 °C. However S1 powder had very fine particles when it was compared with the other powders. On the other hand, S1S (Fig. 3b) and S2S (Fig. 3d) samples had more regular and rod-like structure unlike the irregular structure of S3S (Fig. 3f) powders. Moreover, whitlockite phase in the S4S structure was seen as a worm shape in the SEM image in Fig. 3h.

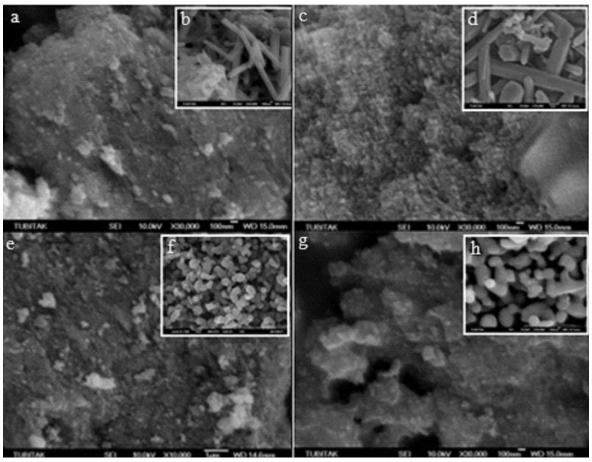

Fig. 3. SEM micrographs of synthesized powders (a) S1 at 80 °C-dried, (b) S1S at 900 °C-sintered, (c) S2 at 80 °C-dried, (d) S2S at 900 °C-sintered, (e) S3 at 80 °C-dried, (f) S3S at 900 °C-sintered, (g) S4 at 80 °C--dried, (h) S4S at 900 °C-sintered.

TABLE

Surface area, density and particle size values of green and sintered samples.

Sample	Surface area [m^2/g]	Density [g/cm^3]	Particle size [nm]
S1	158.134	2.70	12
S2	115.131	2.93	16.5
S3	87.707	2.88	21.7
S4	102.076	2.92	18.6
S1S	3.049	3.07	622.8
S2S	2.895	3.28	655.9
S3S	1.131	3.20	1678.8
S4S	3.727	3.24	509.6

Table shows the specific surface area of the powders. According to these measurements mean size of the HA particles was calculated by the following equation [9]:

$$\text{particle size} = 6/(\rho S_{\text{BET}}), \quad (1)$$

where the ρ is the density of the pure HA sample ($3.16\ g/dm^3$) and S refers to specific surface area of powders obtained from BET method. The results showed that particle size of all dried samples was the nanometer scale and powders could be densification of the powders could be increased with heat treatment at high temperature.

4. Conclusion

Nano-sized and chemically homogeneous hydroxyapatite powders have been precipitated by using calcium acetate with diammonium hydrogen phosphate, ammonium dihydrogen phosphate, dipotassium hydrogen phosphate and orthophosphoric acid in SBF, at the physiological and biomimetic conditions of pH 8.0 and 37 °C. Except of DPHP all P-precursors would be able to produce pure HA structure at high temperature. It was concluded that different phosphorus precursors affect the HA structure and using different Ca- and P-precursor combinations could be a useful method to obtain such samples with appropriate properties. Also this study proved that higher pH values are not necessary to obtain pure HA. In the literature there is any study that is focused on synthesis HA at low pH values. As a result this study has proved that HA powders could be possibly synthesised at low pH values and it is a promising technique to obtain more biocompatible HA structure for biomedical applications. Recent researches will be focused on the studies of mechanical (elasticity modulus, fracture toughness etc.) and biological properties (*in vitro* toxicity, *in vivo* behavior) of HA bioceramics.

Acknowledgments

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