EFFECT OF HEAT TREATMENT ON PHASE DEVELOPMENT, MORPHOLOGY AND PARTICLE SIZE OF FHA/CDHA NANOPARTICLES PREPARED BY SOL-GEL METHOD

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Abstract

Hydroxyapatite [HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] is one of the types of calcium phosphates, which has extensive applications in the healing of bone and tooth, due to biocompatibility and bioactivity and also similar composition to the natural bone. One method to control of stability of HA is substitution of OH- by F- in HA structure and formation of fluor-hydroxyapatite [FHA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH},\text{F})_2$]. Calcium deficient Hydroxyapatite (CDHA) particle is suitable as carriers for drug-delivery systems.

In this study, FHA containing CDHA nanoparticles were synthesized via sol-gel method. Triethyl phosphate, calcium nitrate tetrahydrate and ammonium fluoride were used as P, Ca and F precursors. The synthesized powders were heat-treated at different temperatures. The phase composition, chemical structure, morphology and particle size measurement were performed by using X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Zetasizer measurement. The results of XRD and FT-IR studies proved the presence of fluorapatite (FA), HA and CDHA in the powders heat-treated at 550 °C and above. Also, XRD patterns indicated that the crystallinity of apatite structures gradually became well crystallized with the increasing of the temperature. Heat treatment at higher temperatures led to the improvement in the atomic arrangement of the apatite structure. The average crystallite size and particle size values of heat-treated samples, estimated by Scherer equation and Zetasizer instrument were found to be between 10-20 nm (confirmed also by TEM) and 130-160 nm, respectively.

Keywords: Fluorapatite; Hydroxyapatite; Fluor-hydroxyapatite; Calcium deficient hydroxyapatite; Sol-gel; Nanoparticle.

1. Introduction

Hydroxyapatite [HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] is one of the types of calcium phosphates, which has extensive applications in the healing of bone and tooth, due to biocompatibility and bioactivity and also similar composition to that of natural [1,2,3]. When HA is implanted inside the body, no poisonous effect, swelling or negative response (rejecting HA) is seen from the body [4]. Biocompatibility and good histological response of HA is principally attributed to its structural and chemical similarity to bone [5].

Pure fluorapatite [FA, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$] has more chemical and structural stability in comparison to HA [6, 7] and form the outer layer of tooth [8]. Most new investigations of the fluorine ion effects are focused on healing teeth [7]. In addition, F$^-$ encourages the mineralization and crystallization of calcium phosphates in bone formation process [9, 10]. One method to control the stability of HA is therefore the substitution of OH$^-$ by F$^-$ in hydroxyapatite structure and formation of fluor-hydroxyapatite [FHA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH},\text{F})_2$] [11]. In spite of the great advantages of FA and FHA, few investigations have taken place in the synthesis and characterization of these materials [12-15].
Nonstoichiometric Ca-deficient HA particles (CDHA) has the ratio of Ca/P<1.667 [16]. CDHA seem particularly suitable as carriers for drug-delivery systems [17]. Kasten et al. showed the resorbable ceramic CDHA which characteristically has a high surface area per volume has properties comparable to β-tricalcium phosphates with regard to the support of osteogenic induction of cells. They proved cells can adhere more easily to CDHA than to β -TCP due to its high specific surface area [18]. The degree of non-stoichiometry depends on the synthesis method and is usually characterized by the Ca/P ratio [19].

Some of the synthesis methods of apatite compounds are solid state reaction [20], co-precipitation [21], hydrothermal [22], hydrolysis of other calcium phosphates [23], emulsion and fine emulsion methods [24], electrochemical deposition [25] and sol-gel [26-31]. Sol-gel method exhibit advantages such as high purity, homogeneous composition and low synthesis temperature comparing with other methods [32, 33].

In this study, FHA powders composed of CDHA particles were prepared via sol-gel method and heat-treated at different temperatures (400, 550, 700 and 800 °C). The phase composition, chemical structure and morphological aspects of the powders were investigated.

2. Experimental

In order to prepare the sol, the solutions of (TEP, [(C₂H₅O)₃P]; Merck), (NH₄F; Merck), (Ca(NO₃)₂·4H₂O; Merck) were prepared in ethanol as the precursors of P, F and Ca. First, NH₄F was added to TEP in ethanol and a small amount of distilled water (P/F=6) and in a separate container, calcium nitrate tetrahydrate was dissolved in ethanol. The resulted solutions were vigorously agitated for 24 h. Subsequently, the Ca-containing solution was added to the P-containing one drop wise (Ca/P=1.67). The concentrations of both solutions were 2M. The resulted sol was kept at room temperature for 72 h and then aged at 40 °C for 24 h. The samples were dried at 70 °C for 3 days and heat-treated at 400, 550, 700 and 800 °C for 1 h. The flowchart of our sol-gel process is summarized in Fig. 1. The samples heat-treated at 400, 550, 700 and 800 °C are designated as FHC400, FHC550, FHC700, and FHC800 respectively.

Phase composition and crystallinity were studied by XRD (D4, Siemens, Bruker Co, Germany). The chemical structure was studied with Fourier transform infrared (FT-IR, D4, SIEMENS, Bruker Co, Germany) analysis using KBr as a standard. The crystallite sizes were estimated from XRD patterns. Particle size values were measured by Malvern Zetasizer, model 3000 HAS in which the data were taken on each particle sample in double distilled water. Triton X-100 with a concentration of 0.03 (v/v) was used as dispersant. To investigate morphological characteristics of grains and crystallites, scanning electron microscope (SEM, XL30, Philips, Holland) and transmission electron microscope (HRTEM, CM200FEG, Philips) along with selected area electron diffraction (SAED) were used.

3. Results and discussion

3.1. Chemical structure analysis and phase evaluation

X-ray diffraction patterns of the powders heat-treated at different temperatures are given in Fig 2. The size of the crystallites was estimated by Scherrer equation [34]:

\[ d = \frac{K \lambda}{B \cos \theta} \]

where 
\[ d \] is the crystallite size, 
\[ K \] is a constant (typically 0.94 for randomly oriented crystallites), 
\[ \lambda \] is the wavelength of the X-ray radiation (1.54 Å for Cu Kα radiation), 
\[ B \] is the broadening of the diffraction peak at half maximum height (in radians), and 
\[ \theta \] is the Bragg angle.
The size of the crystallite in Å, \( D \), can be calculated using the Scherrer equation:

\[
D = \frac{K\lambda}{B_{\frac{1}{2}}\cos \theta}
\]  

(1)

where \( K \) is the shape factor of the average crystallite (approximately 0.9), \( \lambda \) is the wavelength of the X-ray, \( \theta \) is the Bragg angle of the diffracted peak, and \( B_{\frac{1}{2}} \) is the peak width at half-maximum.

The parameter \( B_{\frac{1}{2}} \) can be calculated as:

\[
B_{\frac{1}{2}} = (B_m^2 - b_s^2)^{1/2}
\]  

(2)

where \( B_m \) is the peak width at half-maximum of the sample and \( b_s \) is the peak width at half-maximum of the standard sample.

The degree of crystallinity was calculated via equation below [35]:

\[
X_C = 1 - \left( \frac{V_{112/300}}{I_{300}} \right)
\]  

(3)

In this equation, \( X_C \) is the degree of crystallinity, \( V_{112/300} \) is the depth of the valley between the characteristic peaks corresponding to the planes of (112) and (300) and \( I_{300} \) is the intensity of (300) planes.

The characteristic peaks of apatite such as HA, FA and CDHA phases in the range of \( 2\theta = 25-35^\circ \) were identified according to JCPDS File Cards nos. 09-0432, 15-0876 and 46-0905 respectively. The pattern corresponding to the sample FHC400 (Fig. 2 A) revealed that it was often amorphous. The intensity of the characteristic peaks in the sample FHC550 (Fig. 2 B) were higher than those of FHC400. The intensity of these peaks at 700 and 800 °C exhibited even more significant increase (Fig. 2C and 2D). Accordingly, the increasing of the temperature led to the high degree of crystallinity (Fig.3A). XRD analysis of the samples FHC700 and FHC800 showed also the presence of a little \( \beta \)-TCP phase which is considered as a bioreabsorable component of biphasic calcium phosphate (BCP) bodies [36].

The size of the crystallites (Fig. 3B) did not have significant changes with increasing the temperatures from 550 to 800 °C and were found to be in the range of 10 to 20 nm. Considering the fact that this method was not exact, TEM was used to observe directly and measure the crystallite size that will be presented in the next section.

Fourier Transform Infrared spectroscopy (FTIR) spectra are shown in Fig. 4. The typical characteristic P-O bands of apatite structure (550–610 and 950–1100 cm\(^{-1}\)) were identified in all samples. The peaks corresponding to OH group of apatite structure in 632 cm\(^{-1}\) was appeared in all samples whereas its other peak in 3500-3550 (3541) cm\(^{-1}\) was only found in samples FHC700 and FHC800. This latter peak has not been reported by Kim et al [12]. It seems F ions could replace more OH ions with increasing the temperature especially after 550 °C. As reported by Rodrigo [37], the main difference between IR spectra of HA and FA is the bond 3500 - 3650 cm\(^{-1}\) and in the absence of F ions, the peak of OH group should appear in 3573 cm\(^{-1}\)
The P-O peak in 964 cm\(^{-1}\) has been hidden in the broad P-O peak in 1044 cm\(^{-1}\) for the sample FHC400. The absence of P-O peak in 603 cm\(^{-1}\) for the sample FHC400, the sharpening of the peak in 1044 cm\(^{-1}\) for the sample FHC550 and the presence of P-O peaks in 400-500 cm\(^{-1}\) for the samples FHC700 and FHC800 would indicate an improvement in the atomic arrangement of the apatite structure [38] which is confirmed by the variation of crystallinity (Fig. 3A).

In addition, the specified small peak in 866 cm\(^{-1}\) is related to the HPO\(_4^{2-}\) bond [39, 40]. That suggests the synthesized FHA could contain CDHA which is confirmed by Ca/P ratio of 1.59 (sample FHC550) measured by ICP. The incorporation of the carbonate group is believed to be correlated to the non-stoichiometric nature of apatite structure [12].

In spite of the peak in 866 cm\(^{-1}\), absorbing bond in 1360-1460 cm\(^{-1}\), corresponding to the C-O bonds of the carbonate groups, decrease with the temperature. That confirms the sample FHC400 contained carbonate groups within the apatite structure to a higher degree. The peak intensity decrease and sharpening of the C-O bonds with the heat treatment temperature can be also attributed to the liberation of carbonate groups contained in a higher ordering of atomic structure.

The peak in 1638 cm\(^{-1}\) and the large peak in 3435 cm\(^{-1}\) corresponding to the bending modes of H\(_2\)O bond of water decreased significantly with the increasing of temperatures. The bond of 821 cm\(^{-1}\) for the nitrate group in dried gel, disappeared gradually with the increasing of temperature.

### 3.2. Particle size distribution

Fig. 5 shows the distributions of dispersed particles in the Zetasizer experiment conditions for the powders heat-treated at temperatures 550, 700 and 800 °C.

The average particle sizes of the samples FHC550, FHC700 and FHC800 were 136, 156 and 155 nm (Fig. 6). Zetasizer experiments showed that the sample FHC400 contained the particles with sizes ranging from few nanometers to several micrometers. The samples heat-treated at temperatures 550, 700 and 800 °C displayed monomodal distribution. The samples heat-treated at temperatures 700 and 800 °C contained the same average particle sizes which were higher than that heat-treated at 550 °C.

### 3.3. Morphological investigation

The morphology of powder samples was studied by SEM at different magnification. Fig. 7 shows that an important agglomeration has been occurred between the temperatures of 550 and 700 and 800 °C. By comparing the particle size ranges (Fig. 5 and 6) and related SEM images, it seems the agglomerates were sufficiently dispersed in the Zetasizer testing conditions.

Fig. 8 shows the results of TEM studies of FHA/CDHA powders heat-treated at 550, 700 and 800 °C. Some overlapped crystallites with an average size of about 15-20 nm is observed in Fig. 8A. Crystalline planes resulted from the impact of electron beam can be observed in Fig. 8B. Similar to findings of Kim et al. [12], SAED brought in Fig. 8C demonstrates a weak crystalline structure in comparison with pure crystalline structures such as simple metallic or ceramic materials. It can be deduced from Fig. 8 that the increasing of heat treatment temperature led to crystallite growth somewhat. Diffraction patterns were became more intense and the crystallites have been shown to have a tendency to change theirs spherical morphology distributions with the temperature.
4. Conclusion

FHA nanoparticles containing CDHA was successfully synthesized via sol-gel method. The typical characteristic FA, HA and CDHA structures were observed in the FTIR spectra and XRD patterns. The crystallinity and crystallite size increased with the temperature. Heat treatment at higher temperatures led to the improvement in the atomic arrangement of the apatite structure.

It is expected that the samples heat-treated at higher temperatures can exhibit better bioactivity due to their higher degrees of crystallinity. Taking into account the role of chemical composition, choosing the Ca/P ratio of less than 1.67 in the starting materials might be considered as an alternative to investigate in-situ preparation of the powders containing bioactive TCP/CDHA/FHA nanoparticles. The company of chemical composition factor to control the bioactivity and feasibility of preparing very fine particles increases the hope for the production of suitable bioactive powders for dental tissue applications.

5. Acknowledgement

The authors would like to appreciate the cooperation of different laboratories and precious guidance of the professionals of Materials and Energy Research Center, X-ray diffraction laboratory of Geological Survey of Iran, the laboratory complex center of Islamic Azad University (science and research branch) and the supports of the Iranian Nanotechnology Initiative.

6. References:


**Figure Captions:**

Fig. 1. Flowchart of the preparation of fluor-hydroxyapatite via sol-gel

Fig. 2. X-ray diffraction patterns of samples FHC400 (A), FHC550 (B), FHC700 (C) and FHC800 (D).

Fig. 3. Crystallite sizes and degrees of crystallinity for the FHA samples heat treated in different temperatures.

Fig. 4. FTIR spectra of the samples FHC400 (A), FHC550 (B), FHC700 (C), FHC800 (D).

Fig. 5. Particle size distribution of the samples FHC550 (A), FHC700 (B), FHC800 (C).

Fig. 6. The average particle sizes of the samples FHC550, FHC700 and FHC800.

Fig. 7. SEM images of the samples FHC400 (A), FHC550 (B), FHC700 (C), and FHC800 (D).

Fig. 8. TEM images and SAED of the samples FHC550 (A, B), FHC700 (C, D) and FHC800 (E, F).
Fig. 1.

- Solution of Ca(NO$_3$)$_2$.4H$_2$O in ethanol
- Solution of NH$_4$F/(C$_3$H$_5$O)$_3$P in water and ethanol

Mixing Ca and P-containing solutions

Aging

Drying

Heat treatment
Fig. 2.
Fig. 3.

- Top graph: Degree of crystallinity, $X_c$ vs. Heat treatment temperature ($^\circ$C)
  - Degree of crystallinity increases with heat treatment temperature.

- Bottom graph: Crystallite size, $X_s$ [nm] vs. Heat treatment temperature ($^\circ$C)
  - Crystallite size increases with heat treatment temperature.
Fig. 4.
Fig. 5.

Size distribution(s)

Fig. 6.

Average particle size (nm) vs. Temperature ( ºC)
Fig. 7.
Fig. 8.