

Biomimetic Preparation of Nano Hydroxyapatite in Gelatin-Starch Matrix

M.Meskinfam, M. S. Sadjadi, H.Jazdarreh

Abstract—In this study, we report the synthesis and characterization of nanohydroxyapatite (nHAp) in gelatin-starch matrix via biomimetic method. Characterization of the samples was performed using X-ray diffraction (XRD) and Fourier Transform infrared spectroscopy (FT-IR). The Size and morphology of the nHAp samples were determined using scanning and transmission electron microscopy (SEM and TEM). The results reveal that the shape and morphology of nHAp is influenced by presence of biopolymers as template. Carbonyl and amino groups from gelatin and hydroxyl from starch play crucial roles in HAp formation on the surface of gelatin-starch.

Keywords—Biocomposite, Biomimetic, Nano Hydroxyapatite, Template.

I. INTRODUCTION

THE main composition of the extra cellular matrices of hard tissues are included both organic and inorganic phases. The inorganic phase consist of hydroxyapatite (HAp) crystals and the organic phase consist of type-I collagen and small amounts of glycosaminoglycans, proteoglycans and glycoproteins [1]. So, in recent years, many interests have been attracted by inorganic nanoparticles which are embedded in polymeric matrixes. Various methods have been carried out to prepare this type of composite to obtain required properties and structures. Template - based synthesis is one of the most common methods [2]. This technique is based on the interaction between organic template and the inorganic filler, affecting controlled nucleation and crystal growth of the inorganic part or to the orientation of the organic components to form a higher order hierarchical structure [3]. HAp by $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ formula, which has compositional and biological similarities to the mineral phase of natural bone and biodegradable polymers such as collagen, gelatin, chitosan, starch, etc are the best candidates for preparing biomaterials for use in bone tissue regeneration. Gelatin and starch are natural biopolymers by functional groups such as amino acids and hydroxyl, respectively. Both of them are biodegradable, biocompatible, water soluble and inexpensive in comparison to other biodegradable polymers. Gelatin can be obtained by thermal, physical or chemical denaturation of collagen [2], [4]–[7]. Gelatin due to its hydrophilicity has great affinity

with HAp and can be homogenized well with it in aqueous solution; in other side the polar nature of starch facilitates strong adhesion between the HAp and starch. So, biocomposite of gelatin, starch and HAp expected to give improved bioactive and biocompatible biomaterial for tissue regeneration use as reported before by Sundaram et.al [1]. In this work, synthesis of nano HAp using gelatin and starch as a matrix, the effect of biopolymers concentration alteration on the final structure and characterization of the prepared composites are reported.

II. MATERIAL AND METHODS

A. Material

Food grade gelatin and extra pure water soluble wheat starch were obtained from Merck. All chemicals needed for synthesis of hydroxyl apatite $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, Na_3PO_4 , and NH_4OH were also supplied from Merck and used without any further purification

B. Method

In situ synthesis of nHAp was carried out in the food grade gelatin and starch matrix. Pure nHAp particles were also prepared in the absence of biopolymers for comparison. Different concentration of starch (1.36, 2.05, and 2.75 g of starch in 50 ml double-distilled water) and gelatin (2.8, 4.2, and 5.6 g of gelatin in 50 ml double-distilled water) were prepared separately for studying the effect of the biopolymers concentration on the HAp final structure. At first, above mentioned water based solution of gelatin using a magnetic stirrer at its maximum speed with the simultaneous application of heat about 50 °C were prepared. Then, gelatin solution was added to starch solution during one hour under slowly stirring condition. After stirring the obtained mixture at 40 °C for one hour, the solution of calcium nitrate (40 ml, 0.1M) was slowly added to biopolymers mixture ,pH adjusted about 10, mixed and held at 70°C for 24 h by reflux. Finally, 24ml sodium phosphate 0.1 M was added to the mixture and pH readjusted at 10. nHAp was synthesized in this manner by omission of biopolymers stages. Obtained suspensions (with and without biopolymers) were filtered on buchner funnel, and then washed with double-distilled water. The resultant precipitate was dried overnight at room temperature under vacuum. As dried products were characterized using a Fourier Infrared spectroscopy (FT-IR) Thermo Nicolet Nexus 870, X-ray Powder diffraction (XRD) Seisert Argon 3003 PTC using nickel-filtered XD-3a Cu $K\alpha$ radiations ($\lambda=0.154$ nm), Scanning Electron Microscopy (SEM) Philips, and

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Transmission electron microscope (TEM) Philips operated at 100 kV.

III. RESULT AND DISCUSSIONS

A. Fourier Transform Infrared Spectroscopy (FT-IR)

Fig.1 presents FT-IR spectra of amylopectin-rich starch, gelatin, synthesized HAp at the absence of biopolymer, as well as HAp in the presence of: 2.8- 1.36, 4.2- 2.05, and 5.6- 2.75g gelatin-starch respectively from top to bottom. In the starch spectrum, the wide band observed at 3348 cm^{-1} is attributed to the O-H stretching of the amylopectin and its width ascribed to the formation of inter and intra molecular hydrogen bonds. The bands at 2935 and 2887 cm^{-1} are attributed to the asymmetric stretching of C-H, while the band at 1656 cm^{-1} is due to the adsorbed water. The band at 1015 cm^{-1} is assigned to the C-O alcohol bond and the bands at 1421 and 1357 cm^{-1} may concern to the angular deformation of C-H bonds in starch molecule [8], [9]. The FTIR spectra of gelatin shows peaks at 3450 cm^{-1} and 3423 cm^{-1} due to -NH stretching of secondary amide, C=O stretching at 1700 cm^{-1} and 1640 cm^{-1} , -NH bending between 1550 cm^{-1} and 1500 cm^{-1} , -NH out-of-plane wagging at 670 cm^{-1} , and C-H stretching at 2922 cm^{-1} and 2850 cm^{-1} [10]. In the spectrum of HAp, the characteristic bands of $\nu_4(\text{PO}_4^{3-})$ is observed at $560\text{--}604\text{ cm}^{-1}$. The weak band at 470 cm^{-1} is due to the ν_2 of the phosphate. $\nu_1(\text{PO}_4^{3-})$ can be seen at 960 cm^{-1} and the bands at $1030\text{--}1100\text{ cm}^{-1}$ assigned to the $\nu_3(\text{PO}_4^{3-})$. In phosphate network, bending and stretching modes of P-O vibrations are present as bands around 600 cm^{-1} and 1049 cm^{-1} , respectively.

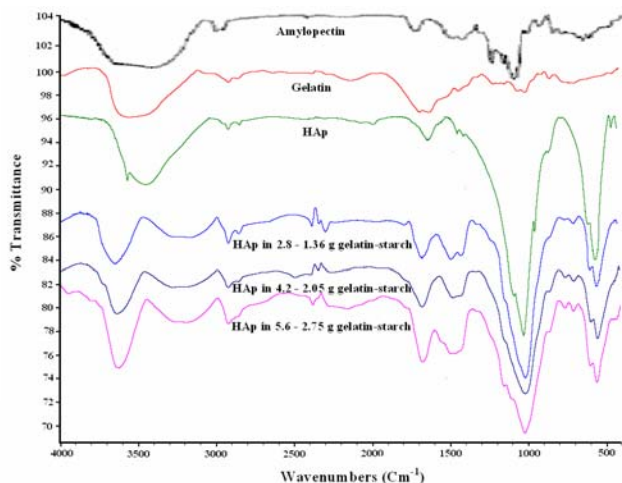


Fig.1 FT- IR spectra of Amylopectin, gelatin, synthesized HAp in the absence of polymer, in the presence of 2.8- 1.36, 4.2- 2.05, and 5.6- 2.75 g gelatin-starch

Besides of these spectra, a broad band concerning to the main vibration of $\nu\text{ OH}^-$ at 3566 cm^{-1} , joined with the bands at 3400 and 1629 cm^{-1} (H-O-H) of water absorption in the products are observed [11,12]. Remained spectra are matched well with the pure synthesized HAp as well as gelatin and

starch spectra. In fact, using biocompatible and biodegradable gelatin - starch facilitates in situ precipitation of the nHAp. There are the slight shifts in the position of absorption bands for the HAp prepared in the presence of polymer is indicative of dissociation and interaction of polymer with the nucleating crystals [5].

B. X-ray Diffraction (XRD)

Fig. 2 represents the X-ray diffraction patterns of the synthesized nano HAp in the presence and absence of biopolymers. As shown in this figure, HAp and biocomposites have similar XRD patterns which the diffraction peaks can be assigned to monophasic low crystalline HAp. Broadening the peaks in XRD pattern implying to small size and low crystallinity of HAp similar to natural bone mineral. The peaks of nanocomposites are slightly broader than pure HAp which can be a sign for decreasing the HAp size and crystallinity in the presence of biopolymers matrix [13].

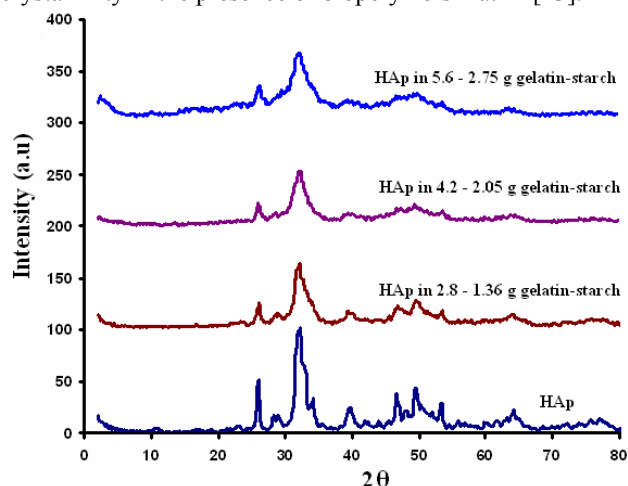


Fig. 2 XRD patterns of synthesized HAp in the presence of 5.6- 2.75 g gelatin-starch, 4.2- 2.05 g gelatin-starch , 2.8- 1.36 g gelatin-starch and in the absence of polymer

C. Scanning Electron Microscopy (SEM)

SEM micrographs of the HAp and HAp in biopolymers matrix are shown in Fig. 3a-d. It seems that the overall morphology of the obtained powders in absence and presence of gelatin-starch matrix is spherical. Micrographs reveal that increasing the gelatin content can be the cause of uniformly distribution of the spherical particles by smaller crystallite sizes due to interaction between the OH^- , RCOO^- , C=O, and amino groups of biopolymers and the Ca^{2+} ions in the solution and on the surface of HAp particles.

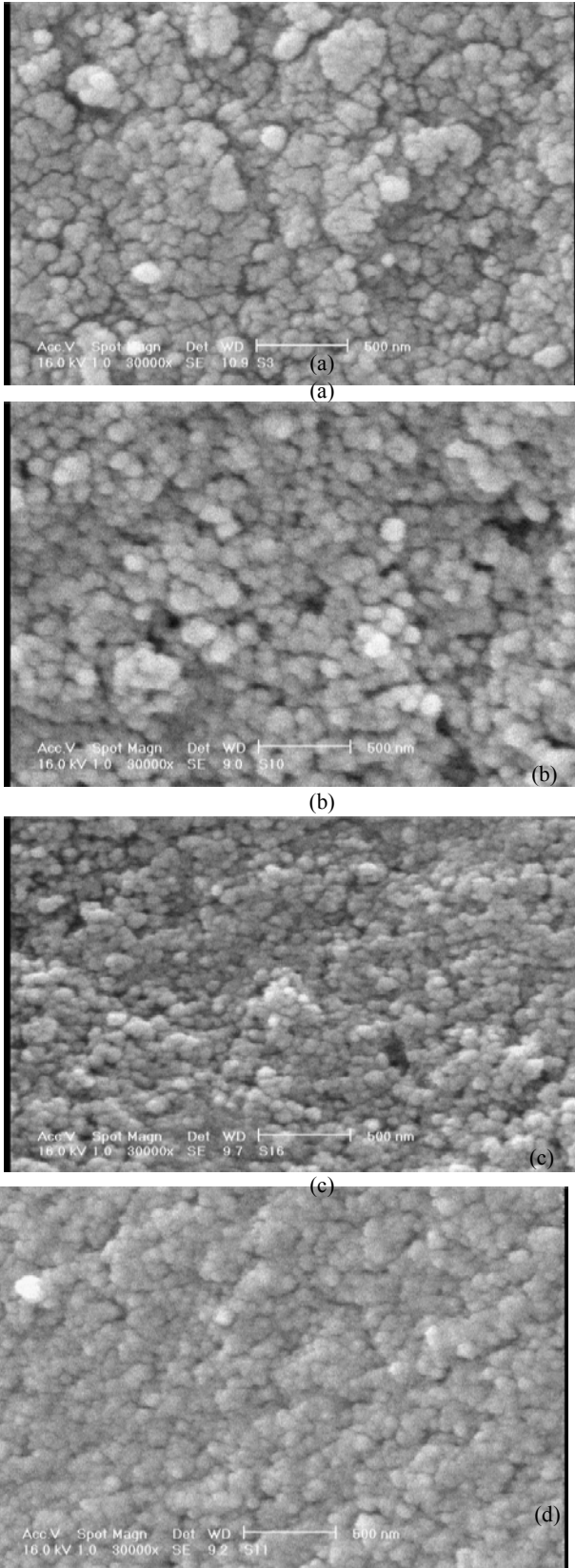


Fig.3. SEM micrographs of synthesized HAp (a) in the absence of polymer, in the presence of (b) 2.8- 1.36 , (c) 4.2- 2.05, and (d) 5.6- 2.75 g gelatin-starch

D. Transmission Electron Microscopy (TEM)

Fig 4a shows the TEM images of HAp prepared at the absence of biopolymers. Primary identification of particles is difficult but mostly particles with irregular rods as well as hexagonal plate can be seen. Fig. 4b shows that HAp particles prepared in the presence of 5.6- 2.75 g gelatin-starch which have hexagonal plate shape with size about 7 nm. This image confirms the template effect of biopolymers on final morphology of prepared nHAp.

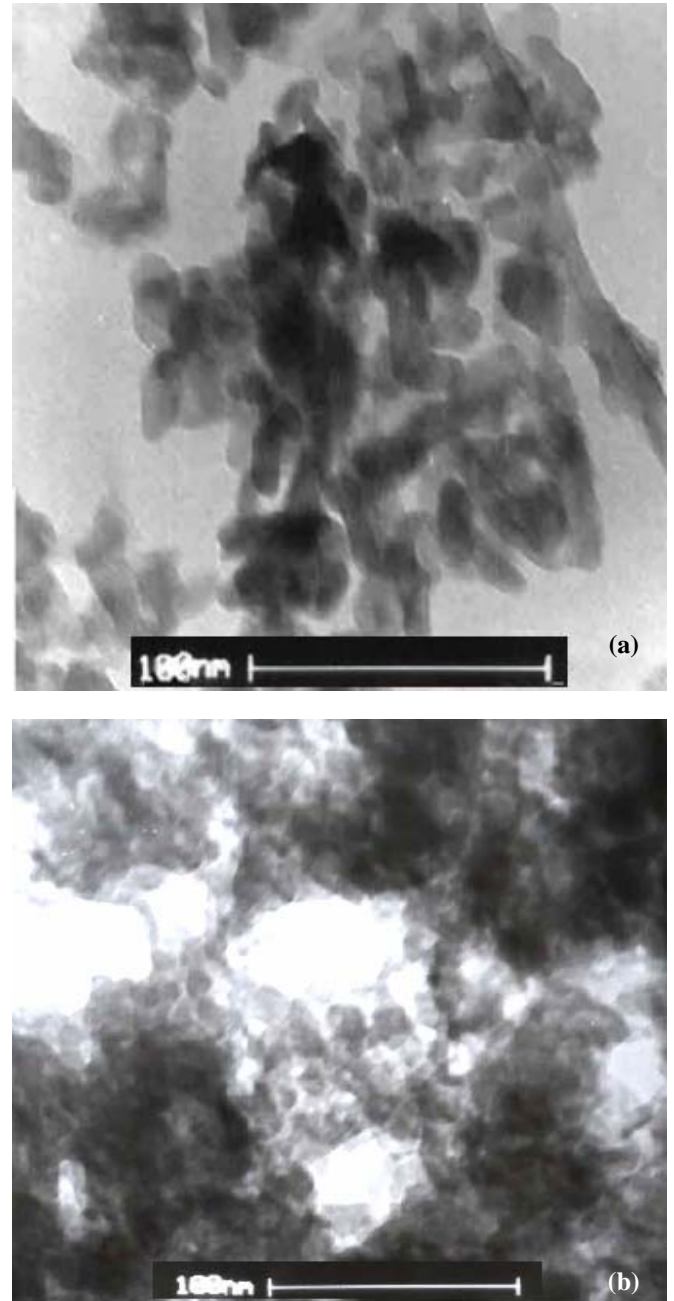


Fig.4. TEM micrographs of synthesized HAp (a) in the absence of polymer, and (b) in the presence of 5.6- 2.75 g gelatin-starch

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IV. CONCLUSION

We conclude that

- nHAp composite synthesis can be performed at room temperature via biomimetic method using gelatin-wheat starch as a template agent.
- XRD patterns and absorption bands in the FTIR spectra confirmed the formation of the nano HA in gelatin-starch matrix
- Controlling of the size and shapes of nHAp to some extent is possible by using gelatin-starch as a template due to HA crystallites nucleation on the regularly arranged side groups of biopolymers chains.

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