Ishraq A. kadhim
Materials Engineering Department, University of Technology , Baghdad, Iraq
130108@uotechnology.edu.iq

Zuhair J. Abdul Ameer
Engineering collage, University of Kerbela, Kerbela, Iraq.

Assel B. Alzubaidi
Materials Engineering Department, University of Technology, Baghdad, Iraq

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Synthesis and Characterization of Chitosan-Polyvinyl alcohol Blend Modified by Genipin and Nanohydroxyapatite for Bone Tissue Engineering

Abstract- *Use nanohydroxyapatite into the polymeric matrix as bioactive material for bone tissue engineering has enormous therapeutic potential because beneficial properties biocompatibility, biodegradability, and consider a major inorganic constituent of the bone matrix. The blended films of Chitosan and Polyvinyl alcohol with Genipin as cross-link agent were studied with and without addition Nanohydroxyapatite. Samples were prepared by solvent casting. The resulting films blended composite were characterized by Fourier transfer infrared (FTIR) spectroscopy, degradation behavior, swelling degree and tensile strength. Degree of swelling, and weight loss of the films blended composite was decreased with an increase of genipin and nanohydroxyapatie concentrations while tensile strength was increased with an increase of genipin and nanohydroxyapaite concentrations. The results showed that the chitosan composite could be used as effective biomaterials for bone regeneration engineering with different degradation rates.*

Keywords- *Chitosan/Polyvinyalcohol blend, Genipin, Nanohydroxyapatite, Bone engineering, Degradation.*

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1. Introduction

Biodegradable polymers have been used as biomaterials for tissue engineering, wound healing, and drug delivery systems. Chitosan is considered biodegradable polymer derivative from chitin by deacetylation process, which is existent in shells of crustacean [1]. Chitosan has unmatched properties such as biodegradability, non-toxicity, bioactivity, good absorption, and sorption, antimicrobial activity, ability to brew gel, and film, these properties reveal it potential for multiple applications [2]. Medically chitosan can be manufacturing as films, which used as a bandage wound dressing and as scaffolds for bone regeneration engineering [3]. Chitosan is an outstanding film-forming material with permeability to nutrient and good mechanical properties [4]. Genipin is a natural material that can be obtained from the plant, Gardenia jasminoides Ellis. Genipin used as cross-linking agent for chitosan, collagen and proteins to improve various properties [5]. Hydroxyapatite (HAp) bioactive substrate [$Ca_{10}(PO_4)_6(OH)_2$] is a major component in bone tissue that play an important role in healing bone [6]. Chitosan blended with different biodegradable materials such as polyvinyl alcohol (PVA) that almost used in applications for tissue engineering to improve mechanical properties and cell attachment [7]. Bone is composite materials consist of organic and inorganic elements. An organic matrix consists of fibrous protein and collagen, and inorganic consists primarily of calcium phosphate and calcium carbonate [8]. The mineral crystals form bioactive ceramics hydroxyapatite, which precipitates around the collagen fibers of the osteoid, which provide the strength to the bone [9].

The addition of hydroxyapatite micro powders and nanopowders to the chitosan and starch blended was investigated by Ai et al. showed that tensile strength was increased in composites containing nano-hydroxyapatite than composites containing microhydroxyapatie. The swelling degree decreased in the composite containing nanohydroxyapatite than the composite containing microhydroxyapatie [10].

Synthesis and properties of biopolymer composite in order to enhance bone regeneration were studied by Maji, and Dasgupta, the chitosan and gelatin reinforced with hydroxyapatite and crosslinking with glutaraldehyde. FTIR indicated the chemical bond formation between chitosan and gelatin. Formation apatite layer from hydroxyapatite on the surface of the sample when immersed in synthetic body fluid (SBF) at 37 ^{o}C for one week [11].

The aim of the present paper is to prepare and characterize of chitosan and PVA cross linking

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2412-0758/University of Technology-Iraq, Baghdad, Iraq

with genipin and reinforced with nanohydroxyapatie as a biomaterial for bone tissue regeneration.

2. Experimental Part

I. Materials

Chitosan powder (CS) with 75 % of degree deacetylation with molecular weight 161 g/mol was imported from Xianm Shaanxi (China), acetic acid (99%) was provided from Chem-lab NV (Belgium), Polyvinyl alcohol with molecular weight (1300-2300)g/mol was imported from CDH (India) ,Genipin was from CN Lab Nutrition, Asian Group (China), nanohydroxyapatite was imported from Xianm Shaanxi (China), phosphate buffer saline (PBS), pH7.2 was imported from HIMEDIA (India). lysozyme (LZ, ≥ 90 % proteins, activity ≥ 40 000 U/mg) CDH was imported from (India).

II. Preparation of films

Blended chitosan/PVA was prepared by dissolved chitosan in acetic acid to obtained 1% w/v and 3% w/v chitosan solution by magnetic stirring for 6h at 50 °C. As well as, the PVA was dissolved in distilled water to obtained 1% w/v by magnetic stirring at 170 °C. Then mixed the two polymer solution at ratio (80:20) to prepare the blend. Genipin and nanohydroxyapatite were added to the blended. Then these solutions were poured in a petri dish at 60 °C for drying and to get the films. Table 1 shows the different concentrations of chitosan and with different concentrations of genipin and hydroxyapatite.

Table 1: Different concentrations of CS: PVA composite films.

Samples No.	Samples Composition (w/v)	CS:PVA ratio	Genipin wt.%	nHAP wt.%
1	1%CS+1%PVA	80:20	-	-
2	3%CS+1%PVA	80:20	-	-
3	1%CS+1%PVA	80:20	0.2% wt.	0.2% wt.
4	3%CS+1%PVA	80:20	0.2% wt.	0.6% wt.
5	1%CS+1%PVA	80:20	0.4% wt.	0.4% wt.
6	3%CS+1%PVA	80:20	0.4% wt.	1.2% wt.
7	1%CS+1%PVA	80:20	0.6% wt.	0.6% wt.
8	3%CS+1%PVA	80:20	0.6% wt.	1.8% wt.

III. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

A Fourier transform infrared spectrometer (Germany) was used to characterize the prepared films blended (CS: PVA) with and without cross-linker and reinforcement with hydroxyapatite. The FTIR spectra were recorded in the wavenumber range of (400 - 4000 cm−1).

IV. Degree of swelling

The degree of swelling of the films was determined according to ASTM D4546-08 [12]. The films Immersion in the solution (PBS phosphate buffer saline pH 7.2 at room temperature for one day). By using a tissue paper, the excess water was removed from the surfaces of the film, and then the weight was registered. The degree of swelling was calculated by the following equation [13]:

$$\text{Degree of swelling } (\%) = \frac{w2-w1}{w1} \times 100 \quad (1)$$

Where, w_1, w_2 are the weights of dried and swollen samples, respectively.

V. In Vitro Degradation study

The degradation test was carried out with the standard ASTM F1635-04a [14]. The solution of degradation is phosphate buffer saline PBS containing lysozyme 0.0001g/L and incubated at 37 °C up to 4week. Every third day the degradation medium was refreshed. After each week the films were dried with distilled water, and the weight of the dried films were recorded as W_d. ,rate of the degradation film was calculated by the equation given below [15]:

$$\text{Weight loss } (\%) = \frac{w0-wd}{w0} \times 100 \quad (2)$$

Where w_0 the dry weight before degradation behavior and w_d the dry weight after degradation behavior of the films.

VI. Tensile test

The tensile test was measured according to the ASTM Standard Method D 882-01[13]. The tests were carried out at room temperature. According to this standard, the samples have length 80 mm, the width of the sample 10mm, the thickness (0.25-0.3 mm), and the crosshead speed was fixed at 1 mm/min.

3. Results and Discussion

I. (FTIR)

Figure 1 showed the FTIR spectra obtained from the samples. The spectrum of chitosan without blended can be shown in Figure 1, A. Peaks located at 3388cm^{-1} and 2924 cm^{-1} can be specified to N-H and C-H stretching, respectively. The peak observed at 1631 cm^{-1} was

specific to N-H stretching in amide II. PVA spectrum is evinced in Figure 1, B. The bands located at 3253cm^{-1} and 2927 cm^{-1} associated with O-H and C-H bond, respectively. CS: PVA blended showed another peek at 1743cm^{-1} indicates the occurrence interaction bet Figure 1, C, and Figure 2 peaks locate in 1251cm^{-1}, 1406 cm^{-1}, 1630 cm^{-1} linked to C-O, CH_3, C=C bonds, respectively [16]. The band at 961cm^{-1} and 1011 cm^{-1} in figure 1D, which has been referred to the interaction between the CS and HAP [17].

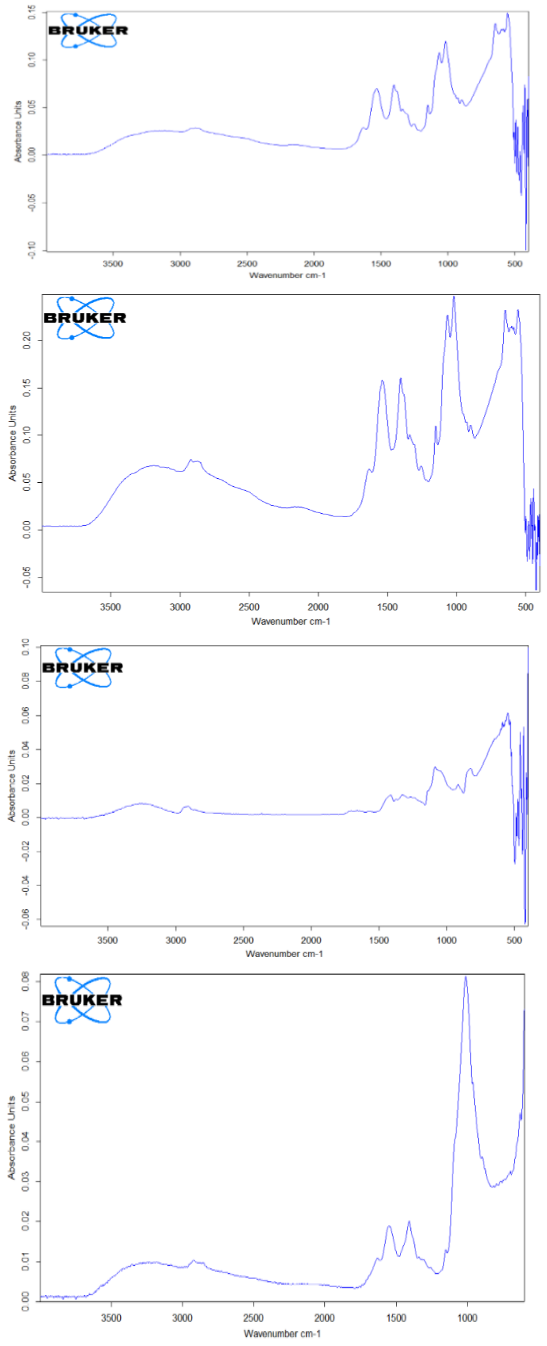

Figure 1: [A(CS),B(PVA),C(CS/PVA),D(CS/PVA/Gn/nHAP)] FTIR of the CS-PVA samples.

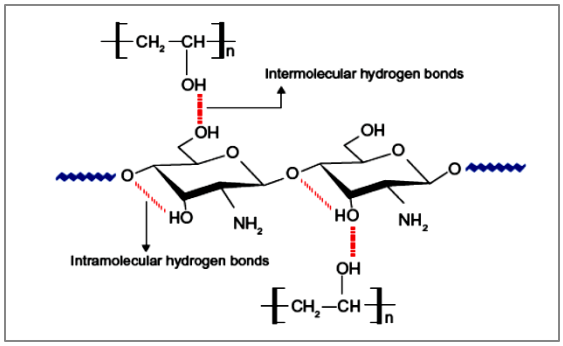

Figure 2: Intermolecular and intramolecular hydrogen bonds of chitosan/ PVA [18]

II. Swelling test

The films to use for biomedical applications, they must absorb the liquid from the body to permit for a suitable allocation of nutrients, metabolites, and growth factor during the extracellular matrix. Figure 3 shows the swelling degree of chitosan blended films shown in Table 1. The swelling degree of CS/PVA blended films decreased with increasing genipin concentrations and nanohydroxyapatite, because of the cross-link bonds between chitosan and genipin. In addition to increasing the polymer chains stability to the incorporation of nanohydroxyapatite with the blended polymer [19, 20].

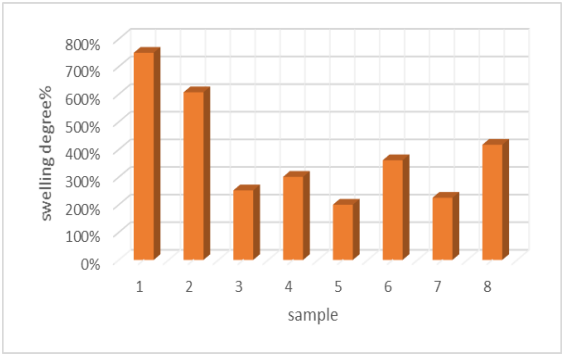

Figure 3: Swelling degree of CS-PVA samples.

III. Degradation test

The degradation rate of biofilms play a significant role of tissue engineering because of the self-healing ability of new tissues corresponding with the degradation rate of the biofilms. To study the degradation rate of the CS; PVA blended films were soaked in phosphate buffer saline (PBS) with lysozyme for four weeks. Figure 4 showed the results of the weight loss of the blended films, as shown in table 1. After 4 weeks of incubation, the degradation rate of films blended composite was increased. With an increased genipin and nanohydroxyapatite concentrations, weight loss of films blended composite was degreased due to limited penetration of lysozyme into the polymer chain [21].

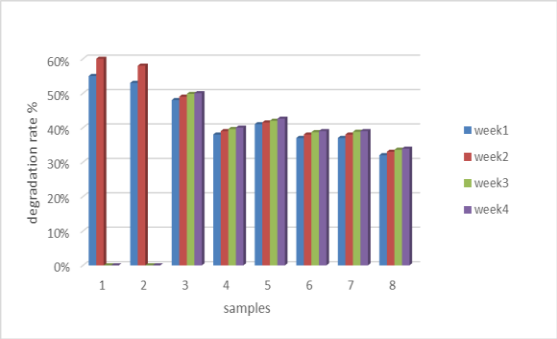

Figure 4: Degradation rate of CS-PVA samples.

IV. Tensile test

Figure 5 showed the results of the tensile strength of the films blended composite shown in table 1. The tensile strength of CS: PVA films blended with different concentrations of genipn and nanohydroxyapatite was determined; the results appeared that the tensile strength of the films blended increased with increased genipin concentrations and nanohydroxyapatie percentage. These results could elucidate the intermolecular hydrogen bonds which formation between the group amine in the chitosan and group carboxymethel to form secondary amid, and because the cross-link density increase with increasing genipin percentage, this is in addition to the excellent distribution of the nanohydrixyapatie in the blended polymer which limits the movement of chains [22].

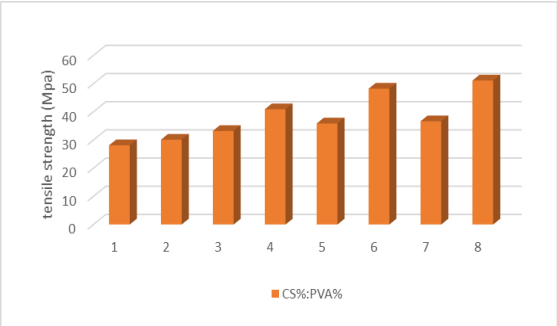

Figure 5: Tensile strength of the CS-PVA samples.

Conclusion

In this study, Chitosan-polyvinyl alcohol (PVA) blends were modified by adding Genipin as a cross-linking agent and by adding Nanohydroxyapatite. The results elucidate that the degree of swelling, and weight loss of films blended composite was decreased and tensile strength was increased with an increase in genipin and nanohydroxyapatie concentrations. The chitosan/ PVA films have been found to exhibit biological properties, which had better meet the requirements for bone tissue engineering.

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