

Genipin Cross-Linked Chitosan / Polyvinyl Alcohol blend for Biomedical Engineering

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Abstract

Chitosan/polyvinyl alcohol cross-linked with genipin at different concentrations were utilized to synthesize films by solvent casting for bone tissue engineering applications. Fourier transform infrared spectroscopy (FTIR), swelling degree, degradation rate, contact angle, tensile strength was investigated. The results revealed a decrease in swelling degree, degradation rate, contact angle with an increased genipin concentration, while tensile strength increased. It can be indicated that cross-linked CS/ PVA with genipin could be applied in application for tissue engineering.

Keyword: chitosan, degradability, lysozyme enzyme.

Introduction

Regenerative medicine is a specialized field for restoring, safeguard and improvement body functions¹. Scaffold materials are a significant component for regenerative medicine because it can mimic with cells of tissue that contribute to the construction of new tissues^{2,3}. Chitosan, a polysaccharide biomaterial which amalgamates adorable group physiochemical and biological properties that authorize to utilized for variety applications⁴. Chitosan can be used in body of human due to biocompatible, biodegradable, and biomedical⁵. Chemical modification of chitosan is required to enhance properties to concur with application⁶. Polyvinylalcohol is hydrophilic material utilized in vitro and vivo medical application due to biocompatibility⁷. PVA blended with many natural polymers such as chitosan, gelatin due to its ability to form film⁸. Genipin is naturally cross linker have been used for biomedical applications due to its susceptibility and biocompatibility to form crosslinked products stable and biocompatible⁹. Physical and mechanical properties for chitosan biomaterials can be improvement by crosslinking remediation because of amino and hydroxyl groups for chitosan have capability to construct amide, ester linkages¹⁰. Bones have capability to renew but existence of obstacles such loss

of bone due to injury or disease may hinder this ability so bone tissue engineering can solve this problem¹¹. The purpose of the present research is improvement the blend of chitosan/poly (vinayl alcohol) films properties crosslinked with different concentrations of genipin. Dimida s. et.al, studied the effect of natural crosslinking genipin with different concentration for chitosan scaffold in (PBS) phosphate buffer saline solution that containing lysozyme enzyme. Rate of degradation of scaffolds was degreased with genipin concentrations¹². Kadh m A. et.al, studied enzymatic degradation of chitosan blend. The weight loss of chitosan blend increased through four weeks. Degradation rate of chitosan improvement by addition of genipin¹³. Bi L.et.al, prepared the scaffolds of chitosan and collagen with various concentration of genipin to investigate the degradation rate of scaffolds from 7 to 21 day. Results shown that, degradation behavior of the scaffolds was decreased when the concentration of genipin increased¹⁴.

Experimental:

Materials

Chitosan (CS) powder with deacetylation degree(75%), molecular weight (161) g/mol importation

from Xianm Shaanxi (china), Acetic acid 99% importation from Chem-lab NV (Belgium), Ethanol (EtOH, 99,9 %) from Scharlau (Spain), polyvinyl alcohol molecular weight (1300-2300) from CDH (India) ,Genipin importation from (CN Lab Nutrition), Asian Group (China), (PBS) pH7.2 from HIMEDIA (India). lysozyme enzyme was (LZ, ≥ 90 % proteins, activity $\geq 40\ 000$ U/mg) from CDH (India).

Prepare of blended Films:

Chitosan (CS) powder was dissolved in acetic acid aqueous solution (2%) via magnetic stirred at 1400 rpm, 50°C for 1hr. polyvinyl alcohol powder was dissolved in distilled water via magnetic stirred at 1400 rpm, 120°C. The blend films was prepared by mixing two polymers at ratio (80:20). Genipin added at concentration (0.2%, 0.4%, 0.6% wt.) to the polymer blend. Table 1 indicated the composition of the polymer blend. The polymer blend solution was casted in petri dish and after 3day the films dried at room temperature.

Fourier transform infrared spectroscopy:

The FTIR spectra was carried out on FTIR microscopy (Bruker, Germany) to determine the vibration of function groups of blended films.

Degree of Swelling:

Swelling degree was utilized to assay the swelling degree of blended films according to ASTM D4546-08¹⁵. The blended films inundation in solution of (PBS) pH 7.2 for 24h at room temperature. Swollen blended films were taken away, then removing the surplus solution by a filter paper. The swelling degree was determined by using the Eq (1).

$$\text{Degree of swelling (\%)} = \times 100 \dots\dots\dots (1)$$

Where, w_1 , w_2 were weights of dried, swollen samples, respectively.

Degradation rate:

To study the enzymatic degradation behavior, blended films were submerged in (PBS) solution that

containing lysozyme enzyme 0.0001 g/L according to ASTM F1635-04a¹⁶. And incubated at 37°C for 6 weeks. The solution was revived every three days. Blended films removed from solution after each week, then washed by phosphate buffer saline solution (PBS), dried and then weight. Degradation rate was determined by the Eq. (2).

$$\text{Weight loss (\%)} = \times 100 \dots\dots\dots (2)$$

Where w_0 dry weight of film before degradation rate and w_d the dry weight of film after degradation rate.

contact angle test:

Knowledge the extreme time of blended films to become more hydrophilic was very significant so as to boost the cell attachment with the blended films. The method consist of measuring contact angle of the water droplet on surface blended films (Young-Laplace fitting method) according to ASTM standard D5946-04¹⁷.

Tensile test:

According ASTM Standard Method D 882-0 the tensile test was determined¹⁸. 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.

Results

FTIR:

Figure 1 illustrated the FTIR of films blended. Peaks at 3388 and 2924 cm^{-1} was linked to C-H and N-H stretching band, respectively. The peak noticed at 1631 cm^{-1} was correlated to N-H stretching in amide II¹⁹. chitosan blended with PVA, peak at 1743 cm^{-1} was noticed illustrated the reaction between O-H to PVA and N-H to chitosan, see figure 1¹². CS: PVA crosslinked with genipin peaks at 1253 cm^{-1} , 1405 cm^{-1} , and 1629 cm^{-1} related to C-O, CH₃, and C=C bonds, respectively. This indicate the reaction between carboxymethyl group of genipine with amine group of chitosan.¹²

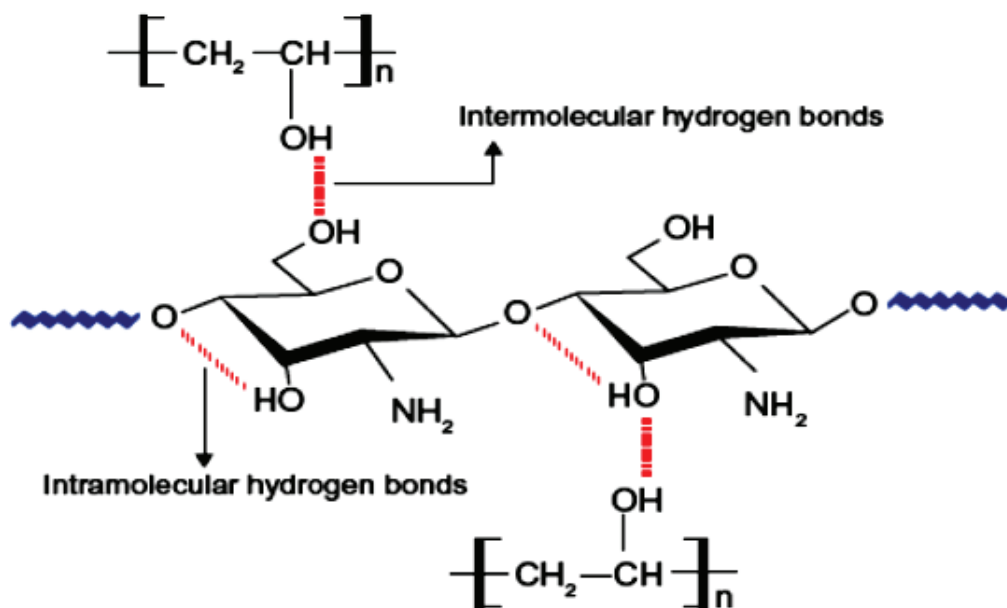


Figure 1. Intermolecular and intramolecular hydrogen bonds of chitosan/ PVA.²⁰

Swelling Degree:

Figure 2 show swelling degree of blended films submerged in phosphate buffer saline solution. The assay indicated the cells can fasten with the blended films. Incorporation of two polymer CS: PVA forming flexible network which give swelling to the blended

films. With an increased genipin concentration, swelling degree decreased due to increase bonding forces between the blended polymers resulting from increase cross-link density¹². This manner may be demonstrate by reaction between the crossliker genipin and -NH₂ that reduces of amino groups concentrations, resulting reduction of reaction with water.

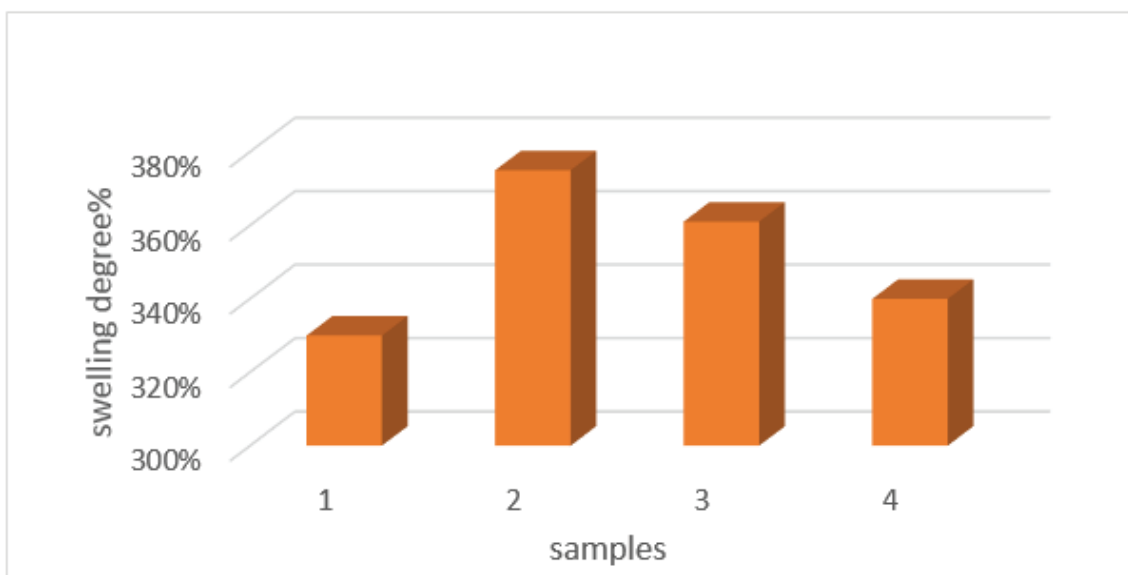


Figure 2. Swelling degree of blended films.

Degradation Rate:

Biodegradability is significant property for films in biomedical application. Enzymatic degradation rate of CS: PVA blended films and CS: PVA cross-linked with natural crosslinker genipin were investigated. Figure 3 show that, weight loss for CS: PVA blended films and CS: PVA cross-linked blended films. The results shows

that the degradation rate of CS: PVA blended films was completely degradation after 3 weeks while CS: PVA cross-linked films was decreased through 6 weeks due to limited breakthrough of lysozyme through the films resulting of the increase cross-like density. However, significant change was indicated between the films with and without cross-linker additions ¹².

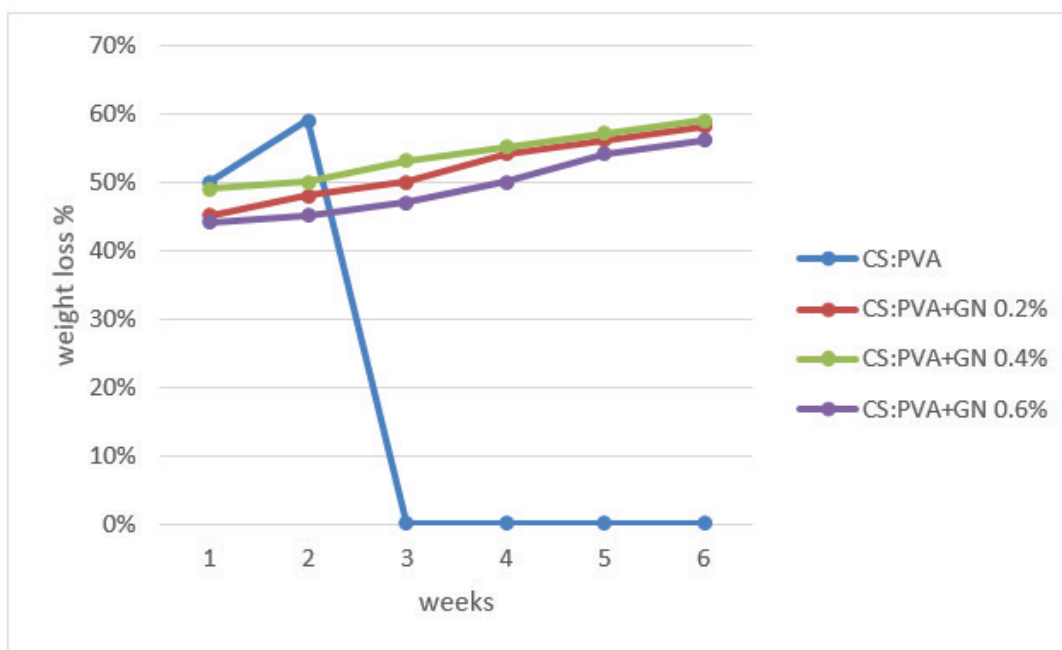


Figure 3 Weight loss of blended films.

Contact Angle:

The wettability of the films was investigated via contact angle analysis. Water contact angle of films are shown in table 2. The average contact angle of CS: PVA films reveals the hydrophilic nature of chitosan. Decrease of average contact angle has been attributed to increase cross linker concentration make it more hydrophilic. It is obvious that genipin addition affected the hydrophilicity of the blended films because its addition decreased contact angle value. This is may be due to the high cognition between molecules of genipin and water considering that both of them are polar. Amendment in hydrophilicity of the films lead to good cells attachment ¹².

Tensile Test:

The tensile strength and elongation (%) of CS: PVA blended films and CS: PVA cross-linked were indicated in figures 4 and 5 .The results investigated that the tensile strength of CS: PVA blended films increased as crosslinker concentration increased while elongation (%) varied thus due to formation intermolecular hydrogen bonds between carboxymethyl group of genipin and amine group of chitosan that form secondary amid ²².

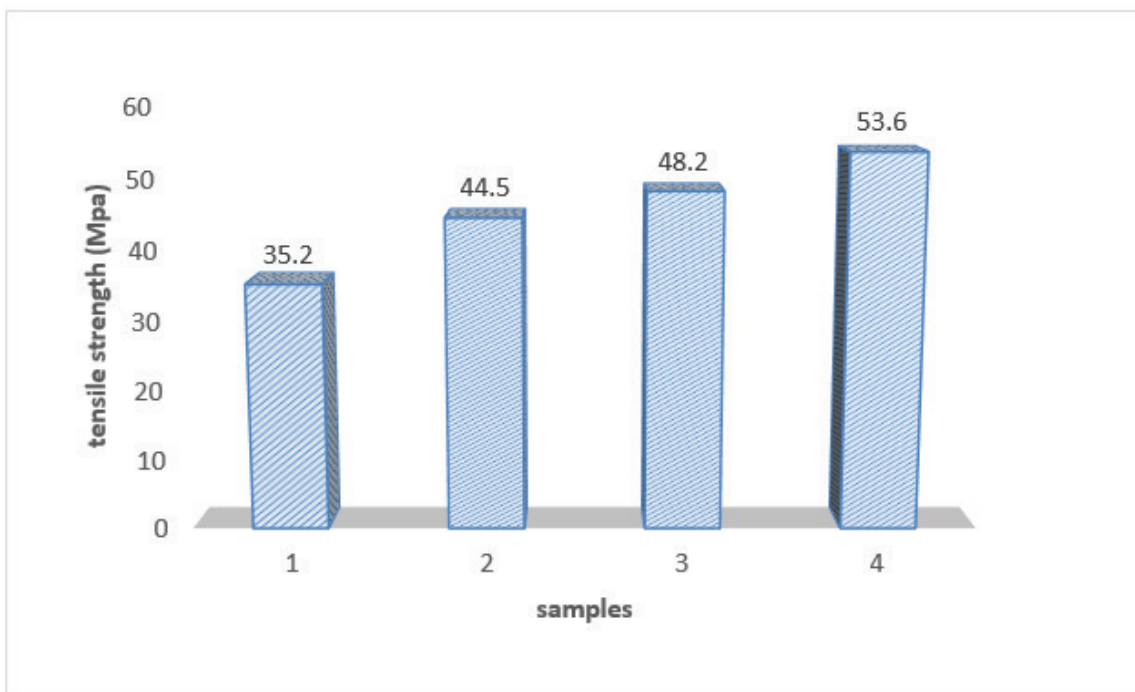


Figure 4 Tensile strength of blended films.

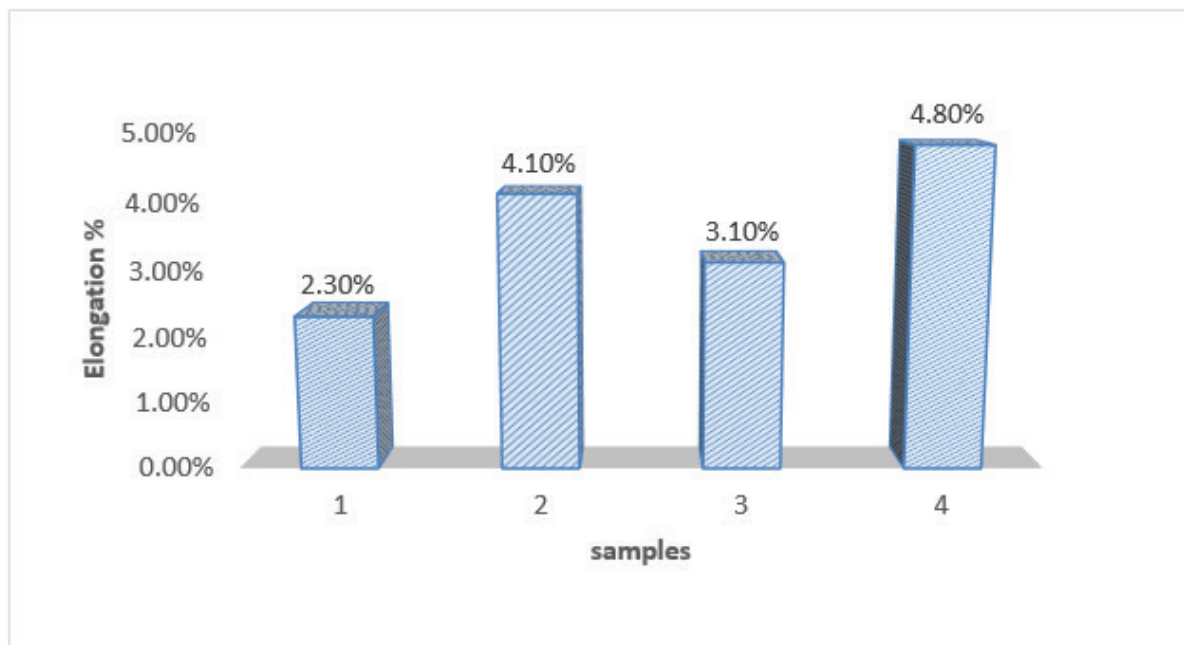


Figure 5. Elongation % of blended films.

Conclusion

Chitosan /Polyvinyl alcohol has been invented with Genipin as natural crosslinker. Crosslinked chitosan/polyvinyl alcohol films by using different concentrations of Genipin enhanced mechanical and physical properties appropriate for application tissue engineering. The

investigation has discovered that combination of genipin to chitosan/polyvinyl alcohol enhanced the swelling degree, degradation rate, contact angle and tensile strength. This study allowed use chitosan/polyvinyl alcohol crosslinked with genipin for bone tissue engineering.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Technology / Materials Engineering Department, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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