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Fabrication of Antimicrobial Hydrogel Using Biodegradable Blended Materials for Skin Applications

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Abstract

Biodegradable polymers are very useful polymers in biomedical applications. In this research, several hydrogels were fabricated by using two polymers, Polyvinyl alcohol (PVA) and Chitosan (Chs) by the solvent casting method in order to use them for skin applications. Several tests were carried out on these membranes such as Agar diffusion method to examine their antimicrobial activities, Fourier transform infrared microscopy (FTIR) test to study the differences in their chemical structures. Uniaxial tensile test was performed to examine the mechanical characteristics of these membranes. In addition, the wettability test was used to investigate the hydrophobicity or hydrophilicity of the surfaces. The results showed that all membranes are hydrophilic, of which the contact angles are less than 90°. The membrane manufactured from 75:25 Chs-PVA is more hydrophobic (contact angle is 74°) than other membranes made of 50:50 Chs-PVA and 25:75 Chs-PVA as the contact angles were 59° and 61°, respectively. The tensile test results indicate that the membrane fabricated of the PVA and the membrane that was fabricated by 75% Chs and 25% PVA has the highest tensile strength of 17.9 MPa, 16.2 MPa and Young^' s Modulus of 181.2 MPa and 7.18 MPa, respectively. The highest strain at break was observed by the membrane of 25:75 Chs-PVA which equals to 24.67%. Chitosan membranes showed inhibition zones of about 2.99 cm and 2.75 cm in length, and 75:25 Chs-PVA membranes showed 5.1 and 5.91 cm in length for *E.coli*. To sum up, this copolymer is considered as promising hydrogel for skin applications such as wound dressing.

Keywords: Hydrogels, Wound Healing, Antimicrobial, FTIR, Tensile Test, Wettability.

تصنيع هيدروجيل مضاد للميكروبات باستخدام مواد مخلوطة قابلة للتحلل الحيوي لأغراض تطبيقات الجلد

لينا عبدالله ، آلاء عايد

الخلاصة:

تستخدم البوليمرات القابلة للتحلل على نطاق واسع في التطبيقات الطبية الحيوية. في هذه الدراسة ، تم تصنيع العديد من الأغشية الحيوية باستخدام بوليمرين ، كحول البولي فينيل (PVA) والشيتوزان (Chs) بطريقة صب ألمذيبات من أجل أستخدامها في تطبيقات الجلد. تم إجراء العديد من الأختبارات على هذه الأغشية مثل طريقة إنتشار Agar لفحص أنشطتها المضادة للميكروبات ، وتم إجراء أختبار FTIR الطيفي بالأشعة تحت الحمراء لدراسة الأختلافات في بُنيتها الكيميائية. أيضاً تم تطبيق أختبار شد أحادي المحور لفحص الخصائص الميكانيكية لهذه الأغشية. بالإضافة إلى ذلك ، تم استخدام أختبار قابلية البلل للتحقق من طبيعة الأسطح كمقاومة او محبة للماء. أظهرت النتائج أن جميع أسطح الاغشية كانت محبة للماء ، وزوايا التلامس فيها أقل من ٩٠ درجة. الغشاء المصنوع من ٧٥ : ٢٥ شيتوزان – كحول البولي فينيل أكثر غشاء كاره للماء (زاوية التلامس ٧٤ درجة) مقارنة بالأغشية الأخرى المصنوعة من ٥٠ : ٥٠ و ٢٥ : ٧٥ شيتوزان – كحول البولي فينيل حيث كانت زوايا التلامس ٥٩ درجة و ٦١ درجة ، على التوالي . أشارت نتائج اختبار الشد إلى أن الغشاء المصنوع من كحول البولي فينيل والغشاء المصنوع بنسبة ٧٥ ٪ شيتوزان و ٢٥ ٪ كحول البولي فينيل يتمتع بأعلى مقاومة شد ومعامل Young

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حيث يساوي ١٧.٩ ميگاباسكال ، ١٦.٢ ميگاباسكال ، ١٨١.٢ ميگاباسكال و ٧.١٨ ميگاباسكال على التوالي. لُوحظ أن أعلى إجهاد عند الكسر سُجل بواسطة غشاء ٢٥ : ٧٥ شيتوزان – كحول البولي فينيل والذي يساوي ٢٥٨.٨ ٪ . كانت قابلية الكشف عن البكتيريا أفضل بالنسبة للشيتوزان و غشاء ٧٥ : ٢٥ شيتوزان – كحول البولي فينيل حيث أظهرت مناطق تثبيط يبلغ طولها حوالي ٣.٥ سم و ٣ سم بالنسبة لسلالات *E.coli* و ٢.٣ سم و ٢.٤ سم لسلالات نموذج *S.aureus*. باختصار ، يعتبر هذا البوليمر المشترك بمثابة هيدروجيل واعد لتطبيقات الجلد.

1. Introduction

Biodegradable polymers are used widely in the field of biomedical applications [1]. The natural process when the organic materials in the environment are decomposed by microorganisms to simpler nutrients is known as Biodegradation. Both natural and synthetic polymers have been extensively investigated as biodegradable biomaterials [2]. The development of biocompatible materials that can be disappeared without leaving any trace of foreign materials [3].

Biodegradable hydrogels have been used for medical applications. Escobar-Sierra and Perea-Mesa developed several membranes made of Polyvinyl alcohol (PVA) and Chitosan (Chs), have the ability to absorb the solution of Aloe Vera and regularly released to enhance the process of healing. The membranes were composed of Polyvinyl alcohol and Chitosan at 10% and 5% w/v and executing different relations of PVA/Chs such as 50/50, 70/30 and 30/70 (v/v) embedded them in 2% (v/v) of Aloe Vera solution to make hydrogel membranes.

After the membranes were gained, a scanning electronic microscopy and an Infrared spectroscopy were used to study the feature characteristics. A matrix with regular release of Aloe Vera and rich absorption capacity was allowed by the crosslinking degree between the PVA/Chs membranes, Also the mechanical features and dimensional stability that following the rehydration. Bactericidal tests revealed a protective activity against Aloe Vera and Chitosan. It was concluded that this mixture might be appropriate for the applications of wound healing [4]. In the same manner, Yang et al. used formaldehyde to treat various ratios of PVA/Chs blended membranes to evaluate PVA/Chs blended hydrogels. It was detected that an increase in the content of Chitosan leads to an increase in the content of water and the amounts of Moisture vapor transmission on the fabricated hydrogels. All of the PVA/Chs blended hydrogels have the same antibacterial activity. The cell viability of *aerococcus* on the different PVA/Chs blended hydrogels was around $(2.5 \pm 0.5) \times 10^7$ cells/ml [5]. Jamal Moshtaghian et al. developed hydrogel membranes based on the Nano Zinc oxide (nZnO) together with strach, PVA and Chitosan. The hydrogels were manufactured using the freeze-thaw cycle. The test showed that the hydrogels were more efficient as a wound dressing in the first levels of wound recovering. In the topic applications that require the use of the antibacterial activity in a wide domain, the gel is employed as a pad for wound dressing [6]. In the year of 2021, Zahra Aghababaie et al. physically used the freez-thawing procedure to develop cross-linked hydrogel membranes while added various amounts of honey to the membranes for rushing the recovery time. 2:1:1 (v/v) ratio of Chitosan, Polyvinyl alcohol and gelatin were used respectively to prepare the hydrogel. Also, In vivo tests, the impact of honey concentrations on cell behavior and antibacterial activities were examined in in vivo testing which included rat model wound healing and histological analysis of section tissue samples. The findings showed that the addition of honey to the hydrogels reduced their Young's modulus and ultimate tensile strength while increasing their ultimate strain by nearly two times. Furthermore, as the concentration of honey increasing, the antibacterial properties of the specimens were increased. This increased the hydrogels' cell viability up until an ideal honey content. Moreover, the hydrogel matrix preserved a layer of epidermis that was well-structured and included generated collagen as a result of the honey's incorporation and the rate of wound healing was hastened. A promising method for wound healing may be the 3D Chitosan/PVA/Gelatin hydrogel with honey since it has sufficient mechanical, antibacterial and biocompatibility properties [7]. Pedro Fardim et al. in 2021 employed the freez-thawing mechanism to prepare Polyvinyl alcohol (PVA)/N–succinyl Chitosan (NSCS)/lincomycin hydrogels to use in wound treatment. The swelling behavior, compression strength, antibacterial property, drug release, cytotoxicity and water retention capacity were examined. According to the findings, 0.75 MPa of ideal compression strength with 30% content of NSCS was achieved. In addition, the integration of lincomycin resulted in a notable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Specifically, lincomycin 75 mg/mL inhibited 77.71% of *Staphylococcus aureus*. In extract, this hydrogel demonstrated a promising future for treating wounds [8].

The aims of this study are to: (1) Fabricate a hydrogel from Polyvinyl alcohol/Chitosan (PVA /Chs) polymers at different (w/v) percentage; (2) Test the antimicrobial performance of the manufactured samples; and (3) Study the mechanical and material characteristics of the samples including Young's modulus, tensile strength and strain at break and (4) study the surface Wettability.

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2. Experimental Section

2.1 Materials and Procedures

Chitosan (Chs) was brought from Areej Al-Furat company in Baghdad. The deacetylation level is around 95.7%, and the particle size is approximately 80 nm. Polyvinyl alcohol (PVA) and Distilled water were used the way they received.

2.2 Preparation of Chitosan/Polyvinyl alcohol (Chs/PVA) Membranes

Five samples were prepared using Chitosan (Chs) and Polyvinyl alcohol (PVA) by the solvent casting method. the first sample was manufactured by dissolving 3.5% (0.35 g) of pure Chitosan in 10 ml of distilled water. The second sample was fabricated from dissolving 10% (1 g) of pure PVA in 10 ml of distilled water. The other three samples were prepared as a composite of these two polymers with different concentration each time such as 50:50 Chs-PVA, 25:75 Chs-PVA and 75:25 Chs-PVA, each polymer was melted separately in 10 ml of distilled water and then was mixed with the other polymer. The polymeric powder of all five samples were dissolved under continuous stirring using a magnetic stirrer for different times for each sample until the powder was completely dissolved. Then the solutions were put onto Petri plates and put in the oven to dry at a temperature of 50°-60° for at least 8-9 hours. Thus, the membranes were obtained and tested later.

2.3 Antimicrobial Test

The antibacterial ability of the formulated Samples (B-C-D-E-F) (Figure 1) was tested against *E.coli* using agar well diffusion assay [9, 10]. A Muller-Hinton (MH) agar solution was aseptically cast onto sterile Petri plates in an amount of about 20mL. A sterile wire loop was used to collect the various bacterial species from the cultures in their supply. Using a sterile point, 6 mm-diameter wells were punched onto agar plates after the organisms had been cultured. Several specimen concentrations (B-C-D-E-F) were inserted into the bored wells. At 37 °C, the test organisms and the cultured plates that contains the samples (B-C-D-E-F) were incubated. Then, the average zones of the inhibition diameter were measured [11].

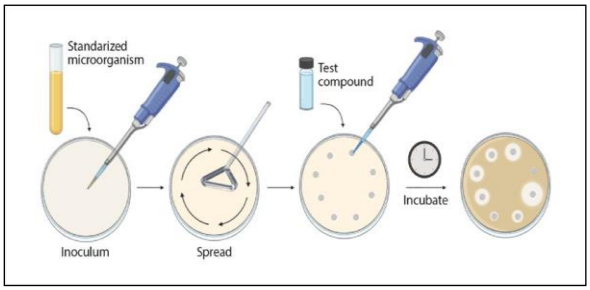


Figure (1): Scheme of the agar disk diffusion method [12].

2.4 Fourier Transform Infrared Spectroscopy Test (FTIR):

A scanning Buker (ALPHA) FTIR spectrometer was used to examine the FT-IR spectra of the PVA, Chitosan and the 50:50 Chitosan/PVA hydrogel membranes at a transmission range of 400 to $4000 cm^{-1}$. Then, the characteristics patterns of the polymeric materials were identificated and analyzed.

2.5 Tensile Test

A Testometric M500 testing device was used to calculate the elastic modulus, tensile strength, and stresses at break of Chs-PVA and PVA tissues. A 3 mm/min speed was chosen for the test. The width, length and thickness of all samples was 3 mm, 9.53 mm and 0.5 mm respectively. All the tested samples were in a regular similar shape as stated by ASTMD-638-V standard shape in Figure 2.

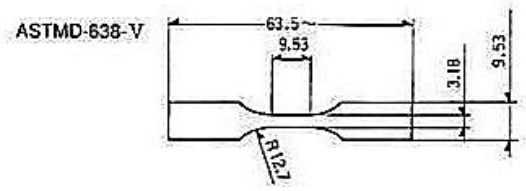


Figure (2): ASTMD-638-V Standard shape for the tensile test.

2.6 Wettability Test

The sessile drop method was performed by dropping a drop of distilled water mixed with red food colouring material perpendicularly onto the specimen's surface as shown in figure 3. The contact angle between the liquid and the membrane was measured on one side of the drop by using the ImageJ program. The procedure was to select the required angle with the cursor and click on it to have the result. If it's value was found to be less than 10°, it reveals that the surface is super-hydrophilic, if it's between 10° and 90°, this indicates that the surface is hydrophilic. If the angle is between 90° and 150°, then the surface is a hydrophobic. In the case of super-hydrophobic the contact angle is larger than 150.

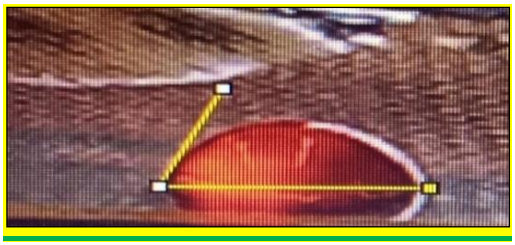


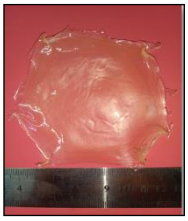
Figure (3): The specimen's dropping to measure the contact angle.

3. Results and Discussion

3.1 Solvent Casting Method

Five types of hydrogels were obtained including pure chitosan, pure PVA, 50:50 Chs-PVA, 25:75 Chs-PVA and 75:25 Chs-PVA. They were all almost similar in the circular diaphanous shape with 0.5 mm thickness as shown in figure 4. Chitosan which was fast affected against heat made it difficult to us to obtain it as a complete solid structure without cracking.

(A): pure PVA hydrogel



(B): 50:50 Chs-PVA hydrogel

(C): 25:75 Chs-PVA hydrogel

(D): 75:25 Chs-PVA hydrogel

Figure (4): The fabricated hydrogels, (A)pure PVA, (B) 50:50 Chs-PVA, (C) 25:75 Chs-PVA, (D) 75:25 Chs-PVA.

3.2 Antimicrobial Activity

To evaluate the antimicrobial effects of the hydrogels, *E. coli* was used as model strains. In figure 5, an inhibition zones were clearly observed around (C-D-E-F). (C: *E. coli* handled with chitosan, D: *E. coli* handled with (25 :75 Chs-PVA), E: *E. coli* handled with (50:50 Chs-PVA), F: E. coli handled with (75: 25 Chs-PVA)). These zones were measured to be found 2.99 cm and 2.75 cm in length for C, 2.71 cm and 3.75 cm for D, 3.25 cm and 3.86 cm for E and 5.1 cm and 5.91 cm for F. It indicates the ability to inhibit the growth of bacteria

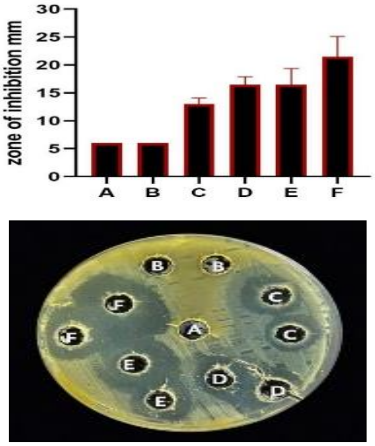


Figure (5): Antibacterial activity of (B, C, D, E, F) against *E. Coli*. A, control. B, *E. Coli* handled with PVA. C, *E. Coli* handled with Chitosan. D, *E. Coli* handled with (25:75 Chs-PVA). E, *E. Coli* handled with (50:50 Chs-PVA). F, *E. Coli* handled with (75:25 Chs-PVA).

3.3 FTIR

The FT-IR spectrum of the PVA, Chs, and Chs-PVA films was tested, as shown in Figure 6. The characteristic of Chitosan peaks which include the -OH, -CH, and -CO amide I and II functional groups appeared at 3240 $\boldsymbol{cm^{-1}}$, 2877 $\boldsymbol{cm^{-1}}$, 1756 $\boldsymbol{cm^{-1}}$, 1620 $\boldsymbol{cm^{-1}}$and 1514 $\boldsymbol{cm^{-1}}$respectively. The high stretching vibration of the -CH2OH of the C6 position of the sugar moiety was also present in the peaks at 1374 $\boldsymbol{cm^{-1}}$, 1148 $\boldsymbol{cm^{-1}}$, 1062 $\boldsymbol{cm^{-1}}$, 1021 $\boldsymbol{cm^{-1}}$, and 553 $\boldsymbol{cm^{-1}}$, as well as other related peaks. The FT-IR of PVA revealed substantial absorption bands for -OH, -CH2, -CO, CH2 (ben), CH (deform) and CO (str) at 3263 $\boldsymbol{cm^{-1}}$, 2906 $\boldsymbol{cm^{-1}}$, 1657 $\boldsymbol{cm^{-1}}$, 1418 $\boldsymbol{cm^{-1}}$, 1323 $\boldsymbol{cm^{-1}}$and 1088 $\boldsymbol{cm^{-1}}$ respectively. Because both polymers were crosslinked, the Chs-PVA blend membrane's FT-IR spectrum differed noticeably from the pure Chs and PVA. The Chs-PVA membrane demonstrated the discrepancies between their respective absorbance bands at 1638 $\boldsymbol{cm^{-1}}$, 1420 $\boldsymbol{cm^{-1}}$, 1372 $\boldsymbol{cm^{-1}}$ and 1023 $\boldsymbol{cm^{-1}}$ suggesting the crosslinking effect between Chs and PVA. As a result, the FT-IR analysis's findings unequivocally demonstrated that the Chs-PVA blend films' interfacial adhesion caused the polyelectrolyte complex to disperse homogeneously and this agrees with previous study [13].

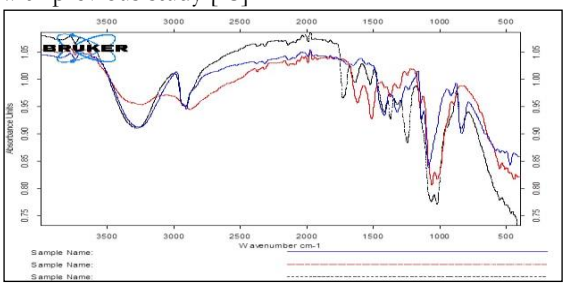


Figure (6): FTIR of Chs (_ _ _), PVA (____) and Chs-PVA composite (-----).

3.4 Wettability Test

The contact angles obtained were equal to 74.8°, 69.6°, 59.3°, 61.9° and 74° for Chs, PVA, 50:50 Chs-PVA, 25:75 Chs-PVA and 75:25 Chs-PVA respectively. The results indicate that the contact angles for all surfaces were less than 90 ° which means the wettability of all the membranes to water is considered hydrophilic. The samples of Chs and 75:25 Chs-PVA were found to be more hydrophilic than other samples. This could increase the membrane inhibition of bacterial growth, this agrees with previous study [14].

3.5 Tensile Test

Uniaxial tensile test was performed to compute the tensile strength, the Young's modulus and strain at break as shown in table 1.

Table (1): The measured parameters for PVA and Chs-PVA composites.

Samples	Young's Modulus **MPa**	Tensile Strength **MPa**	Strain at Break %
PVA	181.2	17.9	155.7
50:50 Chs-PVA	6.22	12.5	169.1
25:75 Chs-PVA	5.82	16.2	258.8
75:25 Chs-PVA	7.18	16.2	182.7

The findings showed that the PVA and the 75:25 Chs-PVA membranes had the highest Young's modulus and tensile strength. Their results were equal

to 181.2 MPa and 17.9 MPa for the PVA respectively and 7.18 MPa and 16.2 MPa for the 50:50 Chs-PVA membrane. The highest strain at break was observed by the membrane of 25% Chs and 75% PVA which is equal to 258.8 %. These findings revealed a good ductility, tensile strength and Young's Modulus of such hydrogels and this agrees with previous studies [7] [15] [16].

4. Conclusion:

Chitosan (Chs) and Polyvinyl Alcohol (PVA) are biodegradable, low cost and water-soluble polymers. The Solvent casting method which used to fabricate five hydrogel samples found to be an easy and effective procedure. However, long time was needed for PVA to dissolve as well as for the samples to dry. Also, Chs was fast affected by heating, made it very difficult to obtain a complete membrane without cracks.

By the agar diffusion method, Chs-PVA with 75-25 % showed a good ability to inhibit the growth of bacteria against *E. coli* which indicates the ability of use for skin applications.

The tensile test showed that the PVA and 75:25 Chs-PVA membranes had the maximum Young's modulus and tensile strength, while the 25:75 Chs-PVA had the highest strain at break value. However, such membranes are good enough to handle the stress. FTIR Spectroscopy test presented the difference in chemical structure between Chs, PVA and Chs-PVA composite.

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