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Research Paper

Preparation and Determination of the Characteristics of Hydrogel Membrane of Poly-Vinyl Alcohol, Starch and Chitosan

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ABSTRACT

In this study, a new kind of crosslinking film of polyvinyl alcohol, starch, and chitosan was obtained by alternating freeze-thaw cycles, and its various properties such as swelling rate, water vapor transmission rate, mechanical properties, and morphology were checked using electron microscopy. The results of this study showed that the hydrogel, made by providing a damp environment and the ability to pass water vapor in the range 720-1680 g/m², has acceptable mechanical properties so that the fracture stress in polyvinyl alcohol was equal to 0.642 kgf/ mm², which reached 0.372 kgf/ mm² with the introduction of starch and 0.2475 kgf/ mm² with the addition of chitosan. When we add starch and chitosan to polyvinyl alcohol at the same time, the fracture stress reaches 0.261 kgf/ mm². In the sample containing pure polyvinyl alcohol, the elastic strain is equal to 6.157 and with the addition of starch, this value reaches 4.625 and with the addition of chitosan, this value reaches 5.70. Adding starch and chitosan to polyvinyl alcohol increases the modulus and toughness and decreases the flexibility of the polyvinyl alcohol hydrogel membrane. Images of SEM from a cross-section of hydrogel membrane fracture show that hydrogel membranes containing polyvinyl alcohol are very smooth and monotonous; however, when starch and chitosan are added to polyvinyl alcohol, the porosity will increase.

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1. Introduction

Hydrogels are three-dimensional solids similar to solids that are capable of absorbing large amounts of water while maintaining their dimensional stability [1]. The amount of water adsorbed in the hydrogels is related to the presence of specific groups such as COOH, OH, CONH₂, CONH and SO₃H, while their resistance to dissolution is due to crosslinks between the network chains [2]. Other factors such as capillary effect, pH, osmotic pressure, and temperature also affect the absorption of hydrogel water. The ability of hydrogels to absorb and release water reversibly and their ability to respond to specific environmental stimuli make hydrogels suitable for use in a variety of engineering fields. Hydrogels are classified into two groups based on the nature of the crosslinking: physical or chemical. Physically crosslinked polymers have transient and temporary bonds. In this method, networking begins with pH, temperature, and other physical stimuli. In physically crosslinked hydrogels, a covalent bond is not established between the chains but is networked by physical interactions such as ionic interactions, hydrogen bonds, or hydrophilic interactions [3]. Chemically crosslinked hydrogels have permanent connections. These types of hydrogels are prepared by forming a covalent bond between different polymer chains. Increasing attention to these types of hydrogels is due to their good mechanical strength. Polyvinyl alcohol is a synthetic hydrophilic polymer that can be chemically crosslinked by electron beam, gamma, and glutaraldehyde, or physically crosslinked by the freeze-thaw process. When aqueous solutions of polyvinyl alcohol are kept at room temperature, the gel gradually forms with low mechanical strength. Aqueous solutions of polyvinyl alcohol form a strong and highly elastic gel when exposed to the freezing-thawing process. The properties of the resulting gel depend on the molecular weight of polyvinyl alcohol, its concentration in water, temperature, freezing time, and the number of freezing-thawing cycles. Polyvinyl alcohol, part of which is hydrolyzed, has a lower melting/degradation temperature. Excessive hydrolysis reduces solubility and increases viscosity with increasing molecular weight. Polyvinyl alcohol can be used to make films and coatings that have high tensile strength and flexibility and are resistant to the penetration of oxygen, carbon dioxide and other vapors. Specific applications of polyvinyl alcohol vary with the degree of hydrolysis and viscosity. If the degree of hydrolysis is higher, water resistance will be better. Moisture in polyvinyl alcohol acts as a softener and reduces tensile strength, increases elongation and tear threshold [4-6].

Starch is a plant polysaccharide of the homo polysaccharide type that is stored in the roots, buds and seeds of the plant. Among the types of polysaccharides, starch is of special importance due to its low price and abundance in nature. The starchy powder is odorless, tasteless, soft, and white. Starch grains contain long-chain glucose polymers that are insoluble in water. Starch is a dense sugar that consists of two main components. One of them dissolves in boiled water and makes up about 10 to 20% of the total starch, which is called amylose. The next part, which makes up about 80 to 90% of starch and dissolves in boiling water, is called amylopectin. These compounds are made up of glucose units. Amylose forms a colloidal precipitate in hot water, while amylopectin remains insoluble. In the hydrolysis process, long polysaccharide chains are broken down into smaller carbohydrate chains. The network structure of starch has good biodegradability, high mechanical properties, and chemical stability. Starch films have advantages such as low thickness, flexibility, and transparency, but also have disadvantages, such as poor mechanical properties and its permeability to water vapor. To improve the mechanical properties of starch films, we can mention the creation of cross-links and the combination of starch with other natural polymers. The combination of starch with biodegradable polymers such as polyvinyl alcohol improves its properties such as strength, high thermal stability and flexibility [7-10].

Chitosan has various properties, including non-toxicity, low cost, availability, antimicrobial activity [11], physiological neutrality, significant protein affinity, anticoagulant, anti-tumor [12] and so on. Chitosan is insoluble in aqueous solutions with a pH above 7 but dissolves in dilute acids with a pH below 6 such as glutamic acid, hydrochloric acid, acetic acid, formic acid, and butyric acid. The method of dissolution is that the amino groups of the polymer are protonated in acid and lead to the production of soluble polysaccharides. Solubility is also strongly affected by the addition of salt to the solution. The higher the ionic strength, the lower the solubility. Degradation of chitosan depends on factors such as the degree of deacetylation and the pH of the solution. Structures that are highly deacetylated (DD > 85%) have a relatively slow degradation rate, while structures with a lower degree of deacetylation degrade faster. Also, enzymatic hydrolysis is higher in acidic conditions [11]. Chitosan is a polysaccharide with varying amounts of free amine groups and can participate in reactions by forming hydrogen or ionic bonds. Molecular shape, hydrogen bonding, electrostatic repulsion between the chains adjacent to

chitosan cause the movement of this polyelectrolyte in dilute solutions [12,13].

The aim of this study was to obtain a suitable hydrogel membrane as a wound dressing. Considering that dressings must have the ability to absorb liquid, have good moisture permeability and at the same

time have acceptable mechanical properties, according to the said properties for the three cheap, available and biocompatible materials" polyvinyl alcohol, starch and chitosan "This study was put on the agenda for the first time.

Table 1: Types of hydrogel membranes made in this research

| CS (dry weight of PVA/St) | Starch (g) | Starch (mol) | PVA (g) | PVA (mol) | Hydrogel membranes | Number |
|---------------------------|------------|--------------|---------|-----------|--------------------|--------|
| ---- | ---- | ---- | 15 | 0.34 | PVA | 1 |
| ---- | 2.5 | 0.015 | 15 | 0.34 | PVA/St | 2 |
| 7 % | ---- | ---- | 15 | 0.34 | CS PVA/ | 3 |
| 7 % | 2.5 | 0.015 | 15 | 0.34 | CS PVA/ St/ | 4 |

2. Materials and methods

The raw materials used in this study were polyvinyl alcohol with a molecular weight of 72000 g/mol from the German company Merck, corn starch from the company Sigma Aldrich, chitosan with an average molecular weight of 1224,000 g, and 90 % degree of deacetylation from the company Sigma Aldrich and acetic acid Made by Merck Germany.

2.1. Preparation of polymer membranes

At this stage, polymer solutions with specific concentrations were prepared according to Table 1 and networked using successive freezing and thawing cycles. This method is based on phase separation and crystallite formation in polymer solutions. When a polymer solution is placed at a low temperature, the solvent (water in the case of aqueous solutions) freezes, and during this process, the polymers in the solution accumulate in small areas called "unfrozen liquid microphases." In this region, the local concentration of polymers increases, and this polymer arrangement remains unchanged during melting and the connection points form physical bonds and cause structural strength.

2.1.1. Preparation of polyvinyl alcohol hydrogel

15 g of polyvinyl alcohol (0.34 mol) was mixed in 50 ml of distilled water at 80 ° C and placed on a magnetic stirrer for 6 hours to obtain a uniform solution of polyvinyl alcohol.

2.1.2. Preparation of polyvinyl alcohol/starch hydrogel

15 g of polyvinyl alcohol (0.34 mol) was mixed in 50 ml of distilled water at 80 °C and placed on a magnetic stirrer for 6 hours to obtain a uniform

solution of polyvinyl alcohol. Then 2.5 g of starch (0.015 mol) was mixed in 50 ml of distilled water at 70 ° C and placed on a magnetic stirrer for 4 hours to obtain a uniform solution of starch. The polyvinyl alcohol and starch solutions were then mixed and placed on a magnetic stirrer for 3 h to obtain a uniform solution of polyvinyl alcohol/starch.

2.1.3. Preparation of polyvinyl alcohol/chitosan hydrogel

15 g of polyvinyl alcohol (0.34 mol) was mixed in 50 ml of distilled water at 80 °C and placed on a magnetic stirrer for 6 hours to obtain a uniform solution of polyvinyl alcohol. Then 1 g of chitosan (7 % dry weight of polymer) was dissolved in 50 ml of 1 % acetic acid and then mixed with a solution of polyvinyl alcohol for 24 hours at room temperature using a magnetic stirrer.

2.1.4. Preparation of polyvinyl alcohol/starch/chitosan hydrogel

First, 15 g of polyvinyl alcohol (0.34 mol) was mixed separately in 50 ml of distilled water at 80 ° C and placed on a magnetic stirrer for 6 hours to obtain uniform solutions of polyvinyl alcohol. Then 2.5 g of starch (0.015 mol) was mixed in 50 ml of distilled water at 70 °C and placed on a magnetic stirrer for 4 hours to obtain a uniform solution of starch. The solutions were mixed and placed on a magnetic stirrer for 3 hours to obtain a uniform solution. 7 % dry weight of polymer (polyvinyl alcohol and starch) chitosan was dissolved in 100 ml of acetic acid 1% and then the three solutions obtained (polyvinyl alcohol, starch, and chitosan) were dissolved for 24 hours. They were mixed at room temperature using a magnetic stirrer.

2.1.5. Preparation of hydrogel cross-linked membranes

The resulting hydrogels were poured into Petri dishes made of polystyrene and the bubbles inside the hydrogel were removed using nitrogen gas. Consecutive freeze-thaw cycles were used to crosslink the resulting solution. In this way, first, the obtained hydrogel was frozen at $-20\text{ }^{\circ}\text{C}$ for 18 hours

and then melted at $25\text{ }^{\circ}\text{C}$ for 6 hours. This operation was repeated 3 times. To remove unreacted material, the crosslinked hydrogel was immersed in distilled water for 3 h and changed once every hour. The resulting hydrogel was dried in an incubator at $48\text{ }^{\circ}\text{C}$ for 48 h to a relative humidity of 53%. To maintain the obtained moisture, it was kept in a closed container at $4\text{ }^{\circ}\text{C}$ (Fig. 1) [14-17].

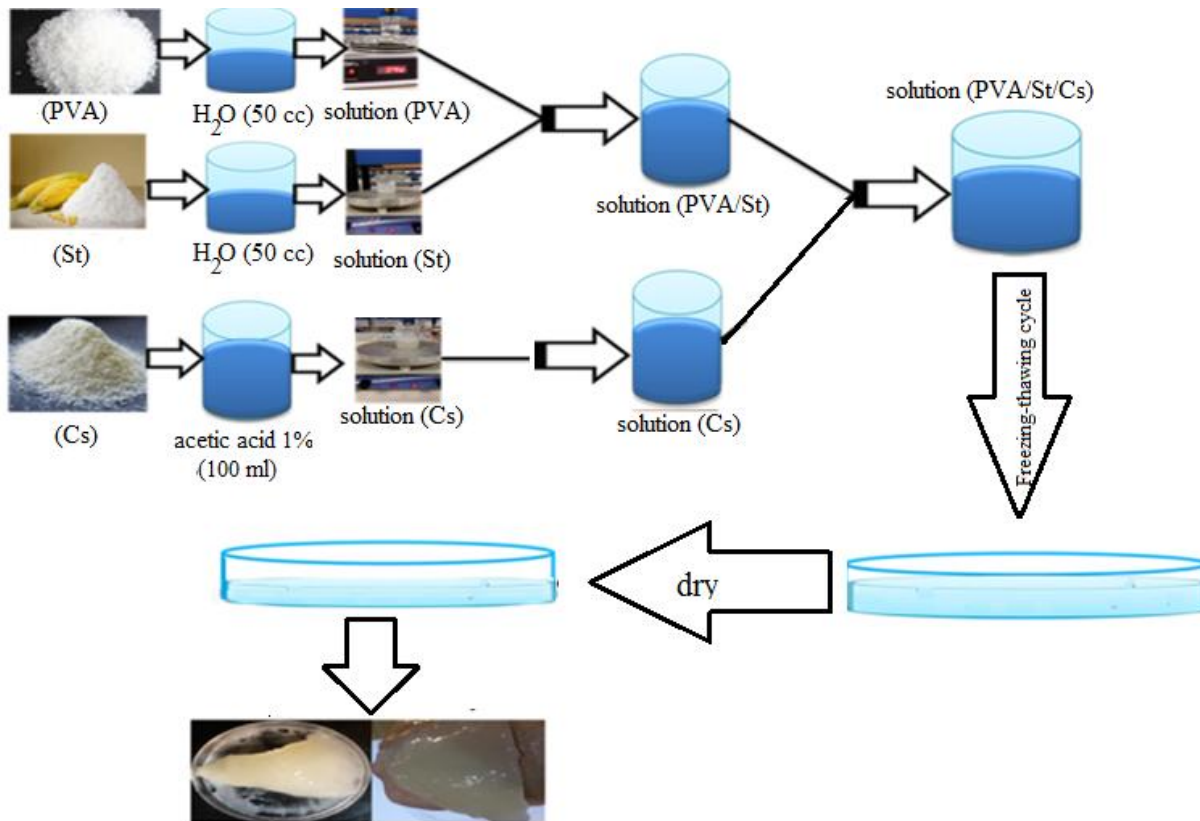


Fig.1. Hydrogel membrane containing PVA/St/CS

3. Investigating the properties of hydrogels

3.1. Equilibrium swelling ratio (ESR)

Samples of hydrogels ($2\text{ cm} \times 2\text{ cm}$ and 2 mm thick) were prepared. The samples were then dried at $60\text{ }^{\circ}\text{C}$ for 12 hours and their weight was measured using a digital scale. The phosphate buffer solution (PBS) was then placed in a laboratory at $37\text{ }^{\circ}\text{C}$ for 24 hours and its weight was measured every hour by removing the hydrogel and drying its surface and returning it to the container. The experiment was performed three times for each sample and the results were reported as average. The following formula was used to calculate the amount of hydrogel swelling [18].

$$\text{ESR} = \frac{W_w - W_d}{W_d} \quad (1)$$

The weight of the hydrogel is in the swollen state (W_w) and the initial weight of the hydrogel is dry (W_d).

3.2. Water Vapor Transmission Rate (WVTR)

Moisture permeability of a hydrogel membrane is possible by measuring the degree of water vapor transfer using materials and using the ASTM standard. The standard for this test is the JIS1099A method. A round sample of hydrogel was placed on the nose of a 7 cm diameter cup containing 50 g of CaCl_2 so that no water vapor could pass through the edges. Teflon tapes were used for this purpose. Both were then placed in an incubator at 90 % relative humidity at $40\text{ }^{\circ}\text{C}$. Water vapor transmission rate (WVTR) is calculated as follows [19].

$$\text{WVTR} \left(\text{g} / \text{m}^2 / \text{day} \right) = \frac{(W_2 - W_1)}{S} \times 24 \quad (2)$$

W_1 and W_2 the total weights of the cup in the first and second hours and 'S' is the surface area of the sample.

3.3. Mechanical test

Tensile strength and elongation of the specimens were determined using a model tensile tester (Instron 4464, UK). After freezing-thawing cycles, the hydrogel membranes were cut in a dumbbell-like manner (6 cm long, 2 cm wide at both ends, and 1 cm wide at the center) and both ends of the sample were fixed with a special clamp. Samples were measured at a speed of 20 mm/min using a cell load of 0.5 N at room temperature to measure tensile strength, elongation, and maximum stress until failure of the samples. After the experiment, the stress-strain diagram was received from the device. The thickness of the sample was measured before the experiment. This test was performed 3 times for each sample and the result was reported as average [19].

3.4. Scanning electron microscope test (SEM)

In a Scanning Electron Microscopy (SEM) (Model VEGAX MU), a relatively high-energy electron beam travels over the sample surface. To perform this experiment, the prepared samples were first placed in liquid nitrogen to be completely frozen. They were then broken up with liquid pliers into the liquid nitrogen to completely preserve their cross-sectional morphology. After preparing the samples, their surface was covered with a thin sheet of gold. In this case, the samples are ready to be viewed with an electron microscope.

4. Results and discussion

In this section, first physical properties such as hydrogel swelling rate, water vapor permeability, and mechanical properties such as tensile strength, yield stress, fracture stress, toughness, and Young's modulus were studied, and then the morphology of hydrogel membrane fracture surfaces was investigated.

4.1. Hydrogel swelling rate

The swelling behavior of the hydrogel depends on the nature of the polymer network such as the

presence of hydrophilic groups, crosslinking density, and the elasticity of the polymer network, pH, and temperature of the swelling environment. Thus, any variation in the structure of the hydrogel network directly affects the swelling behavior. In this study, changes in the swelling capacity of hydrogel membranes over time were investigated. Fig. 2 shows the equilibrium swelling capacity of PVA/CS, PVA/St, and PVA/St/CS hydrogel membranes as a function of time. The increase in the swelling ratio of hydrophilic membranes containing starch and chitosan is due to the hydrophilic nature of the hydroxyl (OH) groups of starch and chitosan molecules in the gel networks. Because the presence of chitosan reduces the density of the polymer network and increases the porosity of the structure, it is obvious that it has higher water absorption than the hydrogel membrane containing starch. Higher bonding within hydrogel membranes limits the infiltration of water for swelling. The porous hydrogel network increases the water holding capacity and this helps to improve the swelling feature of the plates. Figure 3 also shows the equilibrium swelling capacity of PVA/St, PVA/CS, and PVA/St/CS hydrogel membranes at pH of 1.2 and 7.4 in different percentages of polyvinyl alcohol. It is observed that all hydrogel membranes have higher water absorption in an acidic environment than an alkaline environment, which is due to the presence of amine groups. The chitosan amine group contains protons in an acidic environment, resulting in electrostatic repulsion and the separation of hydrogen bonds between the polymer and the release of macromolecular chains, which ultimately leads to an increase in the swelling ratio. In a neutral or alkaline environment, chitosan amino groups become proton-free and electrostatic repulsion is reduced, leading to reduced swelling properties. Changing the pH of the environment causes the volume of holes in the polymer to fluctuate, which affects the swelling behavior of the hydrogel.

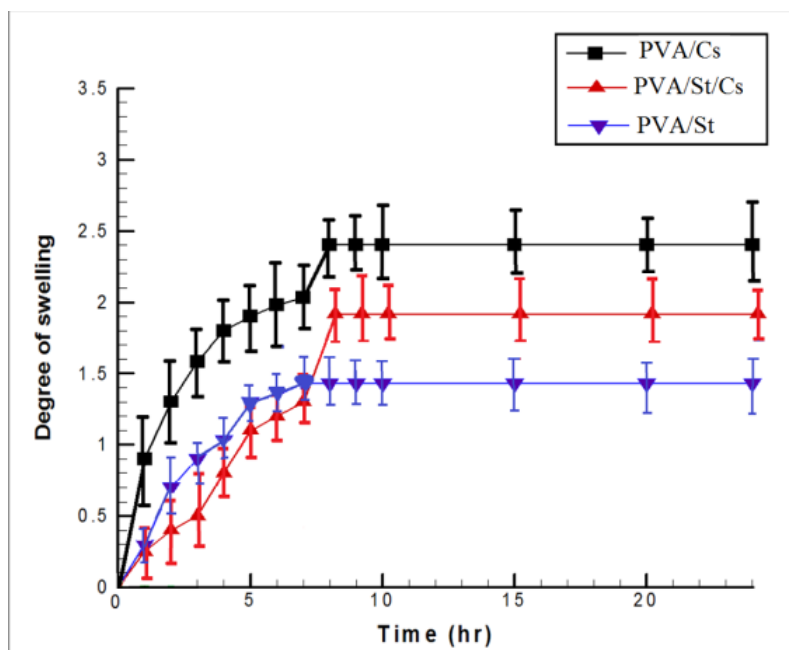


Fig. 2. Equilibrium swelling capacity of PVA/St, PVA/CS, and PVA/St/CS hydrogel membranes in PBS

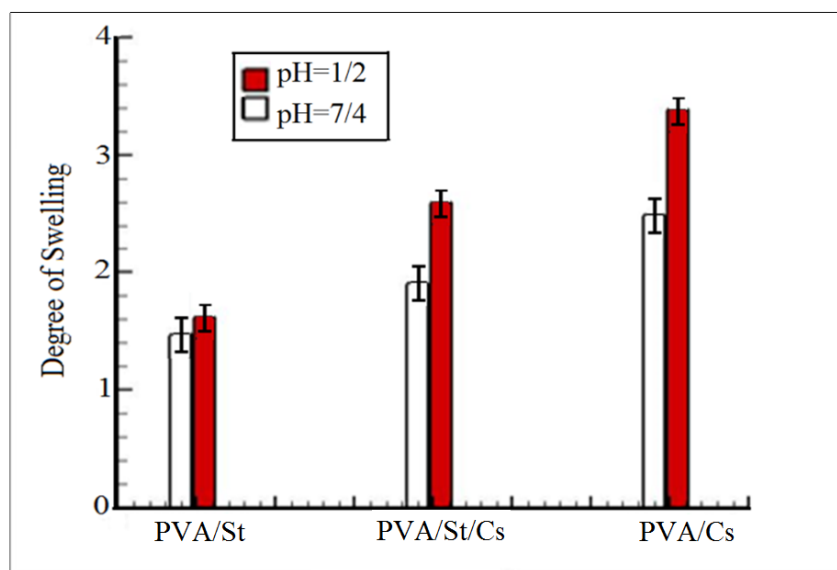


Fig. 3. Equilibrium swelling capacity of PVA/St, PVA/CS, and PVA/St/CS hydrogel membranes at pH = 2.1 and pH = 4.7 in PBS

4.2. Water Vapor Transmission Rate (WVTR)

Figure 4 shows the rate of passage of water vapor on different hydrogel membranes. It is observed that the hydrogel membrane, which contains only polyvinyl alcohol, has the lowest water vapor permeability. Polyvinyl alcohol hydrogel membranes allow less water vapor to pass through due to the greater crosslinking. Higher bonding within the hydrogel

membranes restricts the passage of water vapor. It is observed that the presence of starch increases the passage of water vapor. Starch has poor mechanical properties and high permeability to water vapor, and the use of starch alone to produce a hydrogel membrane limits its application. To improve the mechanical properties of starch films and at the same time increase their resistance to water vapor passage, crosslinking and starch composition with other polymers such as polyvinyl alcohol can be used. By

crosslinking the polyvinyl alcohol/starch hydrogel membrane, we see an increase in equilibrium swelling and water vapor permeability in polyvinyl alcohol. Polyvinyl alcohol/starch mixtures have hydrophilic surfaces that tend to get wet in aqueous media, and the hydrophilic nature of polyvinyl alcohol increases the compatibility with starch and prepares it for preparation as a polymer mixture. As can be seen in Figure 4, by adding chitosan to polyvinyl alcohol, the rate of water vapor passage in the hydrogel membrane reaches its highest level among the other membranes examined. This is due to the hydrophilic nature of the hydroxyl (OH) groups of chitosan molecules in hydrogel networks.

The presence of chitosan reduces the degree of crystallinity of polyvinyl alcohol. Chitosan reduces crosslinks within the polyvinyl alcohol network and creates a porous hydrogel network that has the highest water holding capacity and the highest water vapor transmission capability. This effect can be attributed to the reduction of polymer substrate cohesion in the presence of chitosan. The simultaneous presence of starch and chitosan in the hydrogel membrane fills the voids inside the gel network and increases the density of the network; therefore, the transfer of water vapor to the polyvinyl alcohol/chitosan hydrogel membrane is slightly reduced.

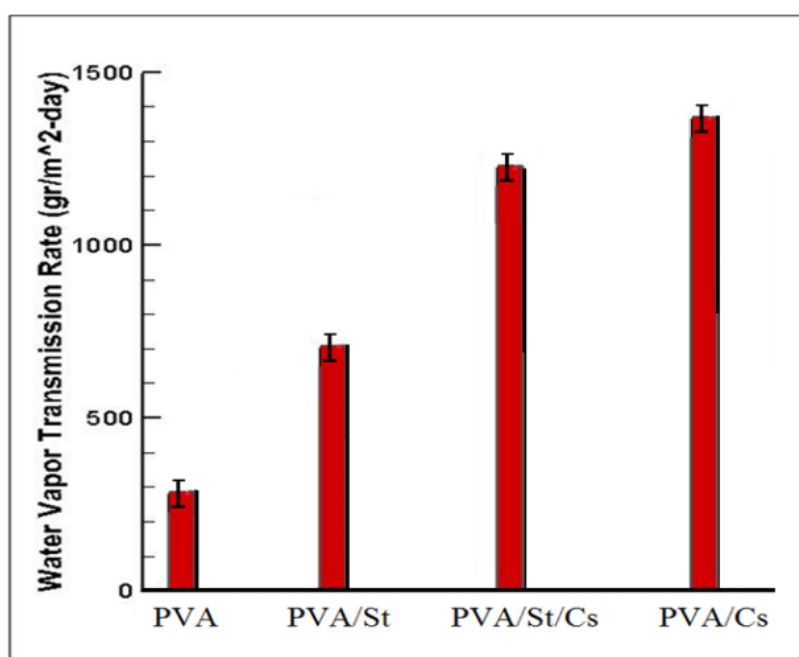


Fig. 4: Water vapor transmission rate on different hydrogel membranes

4.3. Mechanical test

The interaction between different components plays an important role in the properties of hydrogel membranes, especially mechanical properties. There are different interactions between different amounts of polyvinyl alcohol, starch, and chitosan. As a result, different amplitudes of hydrogen bonding occur in the hydrogel membranes, leading to different mechanical behavior in the samples. In this study, polyvinyl alcohol, polyvinyl alcohol/starch, polyvinyl alcohol/chitosan, and polyvinyl alcohol/starch/chitosan hydrogel membranes were synthesized and tested for mechanical properties. The stress-strain diagrams of the polyvinyl alcohol, polyvinyl alcohol/chitosan, polyvinyl alcohol/starch, and polyvinyl alcohol/starch/chitosan hydrogel

membranes are shown in Figure 5. According to the diagrams and Table 2, the fracture stress in polyvinyl alcohol was equal to 0.642 kgf/mm², which reached 0.372 kgf/mm² with the introduction of starch and 0.2475 kgf/mm² with the addition of chitosan. The polyvinyl alcohol hydrogel membrane has the maximum fracture strength among other hydrogel membranes due to the presence of more cross-links. Adding chitosan and starch to polyvinyl alcohol reduces the maximum force required for failure and the specimens change less length before failure. According to Table 2, when we add starch and chitosan to polyvinyl alcohol at the same time, the fracture stress reaches 0.261 kgf/mm². Starch has poor mechanical properties. To increase the strength and flexibility of starch, cross-linking and combining starch with polyvinyl alcohol can be used. The

presence of chitosan also reduces the degree of crystallinity of polyvinyl alcohol. According to Table 2, in the sample containing pure polyvinyl alcohol, the elastic strain is equal to 6.157 and with the addition of starch, this value reaches 4.625 and with the addition of chitosan, this value reaches 5.70. It can also be concluded that due to the stress applied to the polyvinyl alcohol/starch hydrogel membranes, the starch granules disappear as single structures and form a discontinuous membrane such as after swelling and gelatinization. The effect of high pressure on starch granules leads to limited swelling and begins to degrade, leading to failure at less stress and less change in length. Toughness is the amount of energy needed to reach the breaking point. In designing an ideal specimen, we often sacrifice the strength of the material to some extent to increase its toughness. The material is more elastic strain and toughness can be more before the break, pulled high. Adding starch and chitosan to polyvinyl alcohol increases the modulus and toughness and decreases the flexibility of the polyvinyl alcohol hydrogel membrane. Intermolecular interaction between starch, chitosan and polyvinyl alcohol polymers leads to a decrease in the density of crosslinking in polyvinyl alcohol.

Numerous synthetic and natural polymeric materials have been developed to treat burn wounds as antibacterial agents. However, due to poor mechanical

properties as well as low water absorption rate, their application is limited. The interaction between different components plays an important role in the properties of hydrogel membranes, especially mechanical properties. There are different interactions between different amounts of polyvinyl alcohol, starch and chitosan. As a result, different hydrogen bond domains are formed in the hydrogel membranes, leading to different mechanical behavior in the samples. Therefore, a completely homogeneous mixture must be prepared. The polyvinyl alcohol hydrogel membrane has the maximum force required for failure among other hydrogel membranes due to the presence of more crosslinks. Also, the largest change in length to the breaking point is related to polyvinyl alcohol, and by adding chitosan and starch to polyvinyl alcohol, the maximum force required for failure is reduced and the samples change less length before failure. Chitosan creates a porous hydrogel network by reducing the cross-links within the polyvinyl alcohol network, thus reducing the force required for failure and breaking the sample with less change in length. Due to its high tensile strength and flexibility, polyvinyl alcohol hydrogel membranes can be used to make wound dressings. However, adding starch and chitosan increases the density of the polyvinyl alcohol crystal lattice and reduces flexibility by reducing intermolecular distances.

Table 2. Mechanical properties of various types of hydrogel membranes made in this research

| Young module kgf/mm ² | Toughness kgf/mm ² | Fracture stress kgf/mm ² | Yield stress kgf/mm ² | Elastic strain | Hydrogel membranes | No. |
|-------------------------------------|----------------------------------|--|-------------------------------------|----------------|--------------------|-----|
| 0.6056 ± 0.10 | 1.965 ± 0.18 | 0.642 ± 0.11 | 0.075 ± 0.16 | 6.157 ± 0.12 | PVA | 1 |
| 0.7025 ± 0.12 | 0.879 ± 0.12 | 0.372 ± 0.05 | 0.027 ± 0.02 | 4.625 ± 0.08 | PVA/St | 2 |
| 0.7909 ± 0.11 | 1.353 ± 0.21 | 0.475 ± 0.08 | 0.05 ± 0.01 | 5.70 ± 0.10 | CS PVA/ | 3 |
| 0.7932 ± 0.09 | 2.168 ± 0.32 | 0.61 ± 0.12 | 0.05 ± 0.01 | 6.17 ± 0.07 | CS PVA/ St/ | 4 |

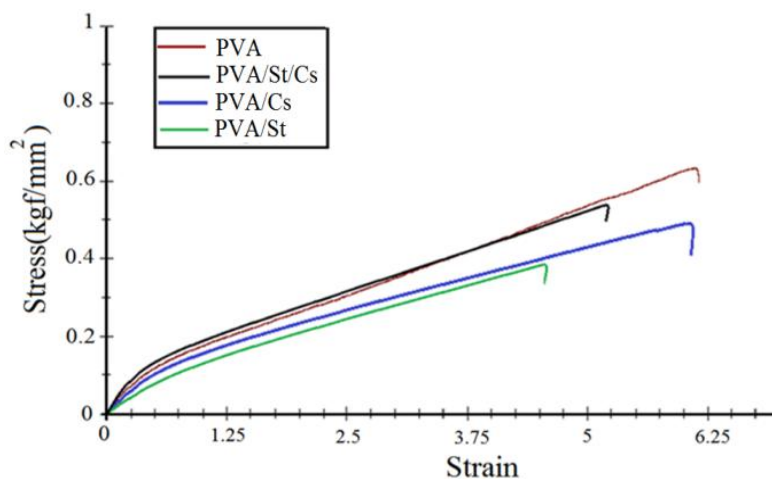


Fig. 5. Stress-strain diagram of PVA, PVA/CS, PVA/St, PVA/St/CS hydrogel membranes

4.4. Electron microscope images

SEM imaging is a promising method for studying the topography of samples that provides important information about the shape and size of the synthesized particles. SEM micrographs taken from the fracture cross-sections of the specimens are shown in Fig. 6. As can be seen, the cross-sectional area of the hydrogel membranes containing polyvinyl alcohol is very smooth and lacks the smallest cavities, and is quite uniform due to the presence of more cross-links. As can be seen, when starch and chitosan are added to the polyvinyl alcohol hydrogel membrane, the number of pores increases, which is consistent with the results of water absorption and water vapor permeability. The

larger the pores, the more hydrogel membranes can hold water and allow more water vapor to pass through. Adding starch and chitosan to polyvinyl alcohol reduces the degree of crystallinity and crosslinking within the polyvinyl alcohol network, resulting in a porous hydrogel network. In addition, the increase in porosity along with the decrease in the atomic distance leads to a decrease in the membrane's ability to stretch and an increase in length to failure. Since hydrogel membranes are synthesized with regular and alternating freeze-thaw cycles based on the presence of polyvinyl alcohol, it is observed that the presence of chitosan and starch in the membrane interferes with the physical bonds of polyvinyl alcohol and as a result, porosity is increased and we no longer see a smooth surface.

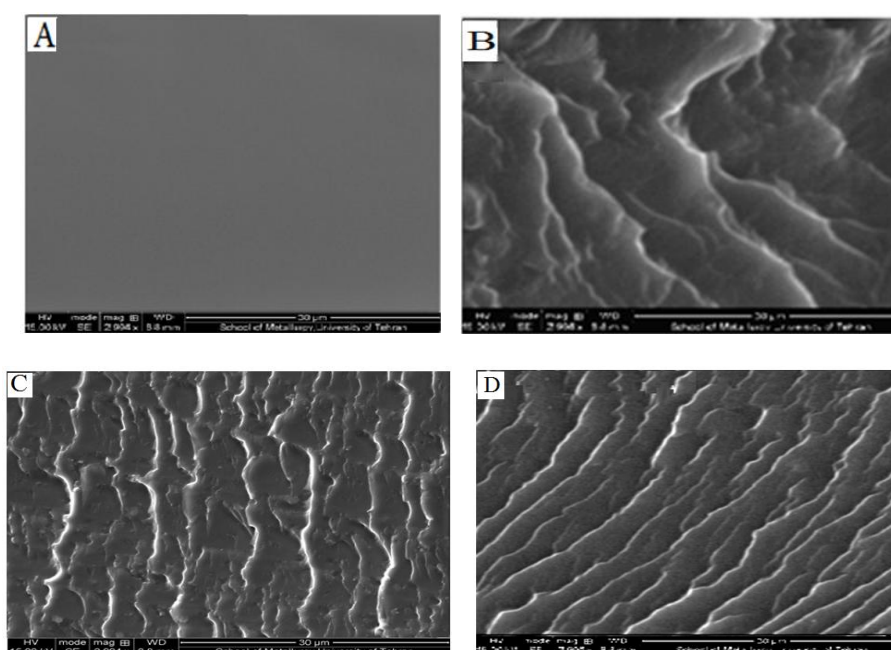


Fig. 6. The cross-sectional SEM image of A) PVA, B) PVA/St, C) PVA/CS, D) PVA/St/CS

5. Conclusion

Polyvinyl alcohol, polyvinyl alcohol/starch, polyvinyl alcohol/chitosan, and polyvinyl alcohol/starch/chitosan membranes were synthesized. The equilibrium swelling capacity of hydrogel membranes was investigated and it was found that all hydrogel membranes have higher water absorption in an acidic environment than alkaline environments due to the presence of amine groups. Also, due to the different amplitudes of hydrogen bonding between different components of hydrogel membranes, we see different mechanical behavior in the samples. The polyvinyl alcohol hydrogel membrane has the maximum fracture strength among other hydrogel membranes due to the presence of more cross-links. Adding chitosan and starch to polyvinyl alcohol reduces the maximum force required for failure and

the specimens change less length before failure. Chitosan creates a porous hydrogel network by reducing cross-links within the polyvinyl alcohol network, thus reducing the force required for failure and breaking the sample with less change in length. Adding starch and chitosan to polyvinyl alcohol increases the modulus and toughness and decreases the flexibility of the polyvinyl alcohol hydrogel membrane. Intermolecular interaction between starch, chitosan, and polyvinyl alcohol polymers leads to a decrease in the density of cross-links in polyvinyl alcohol. In the water vapor transmission rate, it was found that hydrogel membranes containing polyvinyl alcohol allow less water vapor to pass due to the presence of more crosslinks. Also, the presence of chitosan and starch increases the passage of water vapor in the range (720-1680 g/m²). Electron

microscope images were taken from the cross-sectional area of the hydrogel membrane for topographic study and it was observed that the hydrogel membranes containing polyvinyl alcohol were very smooth, without the smallest cavities, and perfectly uniform. When starch and chitosan were added to the polyvinyl alcohol hydrogel membrane, the number of pores is increased and the porosity is increased. As a result, the 3D network density is reduced.

References

- [1] Y. Xiong, X. Zhang, X. Ma, W. Wang, F. Yan, X. Zhao, X. Chu, W. Xu and Ch. Sun, "A review of the properties and applications of bioadhesive hydrogels", *Polymer Chemistry*, Issue 26, 2021, pp. 3721-3739.
- [2] I. A. Khalil, B. Saleh, D. M. Ibrahim, C. Jumelle,^f A. Yung, R. Dana and N. Annabi, "Ciprofloxacin-loaded bioadhesive hydrogels for ocular applications", *Biomater. Sci.*, 2020, 8, 5196-5209.
- [3] M.F. Akhtar, M. Hanif, N.M. Ranjha, "Methods of Synthesis of Hydrogels: A Review", *Saudi Pharm. J.*, Vol. 24, 2016, pp.554-559.
- [4] R. R-Rodríguez, H. E-Andrews, C. V-Martínez and Z. Y. G-Carvajal "Composite hydrogels based on gelatin, chitosan and polyvinyl alcohol to biomedical applications: a review", *International Journal of Polymeric Materials and Polymeric Biomaterials* Volume 69, 2020 - Issue 1
- [5] B. E. Karaogul, E. Altuntas, T. Salan and M. H. Alma "The Effects of Novel Additives Used in PVA/Starch Biohybrid Films", Submitted: July 2nd 2018 Reviewed: September 28th 2018 Published: December 19th 2018 DOI: 10.5772/intechopen.81727.
- [6] A. Jayakumar¹, H. K. V¹, S.T. S¹, M. Joseph¹, Sh. Mathew¹, P. G², I. C Nair³, R. E K⁴ "Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application". *Int J Biol Macromol.* 2019 Sep 1;136:395-403. doi: 10.1016/j.ijbiomac.2019.06.018. Epub 2019 Jun 4.
- [7] Y. Fan, F. Picchioni, "Modification of starch: A review on the application of "green" solvents and controlled functionalization", *Carbohydrate Polymers* Volume 241, 1 August 2020, 116350.
- [8] K. Bashir and M. Aggarwal, Physicochemical, structural and functional properties of native and irradiated starch: a review, *J Food Sci Technol.* 2019 Feb; 56(2): 513–523. Published online 2019 Jan 1. doi: 10.1007/s13197-018-3530-2. PMID: PMC6400748
- [9] Z.H. Xiong, P. Fei, "Physical and chemical modification of starches: A review", Vol. 13, 57(12), 2017, pp.2691-2705.
- [10] U. Shah, A. Gani, B. Ahmad Ashwar, A. Shah, M. Ahmad, A. Gani, A. Idrees, F.A. Wani Masoodi, "A review of the recent advances in starch as active and nanocomposite packaging film", Vol. 1, 2015, pp.1115640-1115649.
- [11] Ch. Sh.Karua, A.Sahoo, "Synthesis and characterization of starch/chitosan composites", *Materials Today*, Volume 33, Part 8, 2020, Pages 5179-5183
- [12] Gh.Sargazi, D.Afzali, A.Mostafavi, A.Shadman, B.Rezaee, P.Zarrintaj, M.Saeb, S.Ramakrishna, M.Mozafari, "Chitosan/polyvinyl alcohol nanofibrous membranes: towards green super-adsorbents for toxic gases", *Materials Science*, Volume 5, Issue 4, April 2019, e01527.
- [13] H M Manal, Different fabrication methods of tissue engineering, *An International Journal of Medicine*, Volume 111, Issue suppl_1, December 2018, hcy200.224.
- [14] J. D. Carrera, D. A. V. Narváez, M. Leon, J. F. Alvarez, "Effect of Starch Oxidation Degree on the Properties of Hydrogels from Dialdehyde Starch and Polyvinyl Alcohol", *Journal A Bimonthly Peer-Review Journal* (ISSN: 2415-6698).
- [15] N. Mallick, D. Pal, and A. B. Soni, "Cornstarch/polyvinyl alcohol bio-composite film for food packaging application", *AIP Conference Proceedings* 2201, 020005 (2019); <https://doi.org/10.1063/1.5141429>.
- [16] Y. Ying, Y. Wu, J. Huang, Preparation and characterization of chitosan/poly(vinyl alcohol)/graphene oxide films and studies on their antibiofilm formation activity. *J Biomed Mater Res A.* 2020 Oct;108(10):2015-2022. doi: 10.1002/jbm.a.36961. Epub 2020 May 4.
- [17] I. P. Merlusca, D. S. Matiut, G. Lisa, M. Sillion, L. Gradinaru, S. Oprea & I. M. Popa, "Preparation and characterization of chitosan-poly(vinyl alcohol)-neomycin sulfate films", *Polymer Bulletin* vol. 75, pages3971–3986 (2018).
- [18] Gh.D.Afzali, A.Mostafavi, A.Shadman, B. Rezaee, P.Zarrintaj, M.Saeb, S.Ramakrishna M.Mozafari "Chitosan/polyvinyl alcohol nanofibrous membranes: towards green super-adsorbents for toxic gases", Volume 5, Issue 4, April 2019, e01527.
- [19] S. Nikzad, B. Hashemi, Z.M. Hassan, H. Mozdarani, "The cell survival of F10B16 melanoma and 4T1 breast adenocarcinoma irradiated to gamma radiation using the MTT assay based on two different calculation methods", *Journal of Biomedical Physics & Engineering*, Vol. 3(2), 2013, pp.29-36.