SYNTHESIS AND CHARACTERIZATION OF POLY(VINYL ALCOHOL)-HYALURONIC ACID BLENDED HYDROGEL MEMBRANES

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ABSTRACT

Poly(vinyl alcohol)PVA is a hydrophilic polymer and water soluble . It is used in many biomedical and pharmaceutical applications, due to its advantages such as: non-toxic, non-carcinogenic, and biodegradable characteristics with the ease of processing. Physically cross-linked hydrogel membranes composed of different amounts of hyaluronic acid (HA) blend with (PVA) were prepared by freeze–thawing method. This freezing–thawing cycle was repeated for three consecutive cycles. Properties of (PVA–HA) hydrogel membrane such as gel fraction, swelling, mechanical properties(tensile strength, elongation to break),degradation and protein adsorption were investigated. With the increasing of HA content, the gel fraction, the maximum tensile strength and elongation at break(%) of (PVA-HA) hydrogel membranes were decreased. Furthermore, with the increase of HA content, the swelling, the protein adsorption and the hydrolytic degradation of PVA-HA hydrogel membrane were increased.After soaking of hydrogel membrane for three days in phosphate buffer saline (PBS), the maximum weight loss of PVA–HA hydrogel membranes ranged between 18% and 70% according to HA content, this indicates that they are biodegradable.

Key words : Poly(vinyl alcohol),Hyaluronic acid, Hydrogel membranes, Freezing- thawing.

1-Introduction

In recent years, there has been an increasing interest in physically cross-linked gels **(Peppas & Scott, 1992 ;De Jong *et al.*, 2001; Molina *et al.*, 2001; Berger *et al.*, 2004 and Van Tomme *et al.*, 2005)**. The main reason is to avoid the use of chemical cross-linking agents which are not only often toxic compounds but also can affect the integrity of the substances when entrapped (e.g., proteins, cells). To create physically cross-linked gels, there are different methods such as ionic interaction **(Berger *et al.*, 2004 and Van Tomme *et al.*, 2005)**, amphiphilic block and graft copolymers (Molina et al., 2001), stereocomplex formation (De Jong et al., 2001 and Hennink et al.,2004), freezing–thawing **(Yokoyama *et al.*, 1985; Peppas & Scott, 1992 and Hassan & Peppas, 2000)**. PVA holds tremendous promise as a hydrogel-forming polymer via crystallization owing to its non-toxic and hydrophilic nature **(Peppas, 1987).** Different methods have been reported in preparation of PVA-based hydrogels including radiation cross-linking **(Yoshii *et al.*, 1999 and Park & Changnho, 2003;)**, chemical reaction with glyoxal **(Teramoto *et al.*, 2001)**, bifunctional reagents, e.g., glutaraldehyde **(Peppas & Benner, 1980 and Dai & Barbari, 1999)**, or borates **(Korsmeyer & Peppas, 1981)**. Aqueous solution of PVA would be eventually transformed to a low strength gel upon long storage at room temperature however; this does not meet many applications' requirements where mechanical properties are important. In a method, pioneered by **Peppas *et al.* (1975)**, semi-crystalline gels were prepared by exposing PVA aqueous solution to repeated freezing and thawing cycles which induced crystallization and resulted in a network structure with the quasi-permanent crystallites which act as physical cross-linking sites in the network. This method is the preferred route to prepare an

"ultrapure" network without the using any toxic cross-linking agents with tunable mechanical properties **(Yokoyama *et al.*, 1985)**. Mechanical properties of the gel increase with the temperature, number of freezing-thawing cycles, polymer solution concentration and its molecular weight hence; different mechanical properties and erosion time up to six monthes were obtained **(Hassan & Peppas, 2000)**.

PVA as a hydrophilic polymer is water soluble **(Briscoe *et al.*, 2000)**. It is used in many biomedical and pharmaceutical applications, due to its advantages such as: non-toxic, non-carcinogenic, bio-adhesive and biodegradable characteristics with the ease of processing **(Wensheng *et al.*, 2002)**.

Hyaluronic acid (HA) is a naturally-occurring linear polysaccharide comprised of β-1,4-linked D-glucuronic acid (β-1,3) N-acetyl-D-glucosamine disaccharide units, and it is the only non-sulfated glycosaminoglycan (GAG) in the extracellular matrix (ECM) of all higher animals. This polyanionic polymer has unique physicochemical properties and distinctive biological functions **(Laurent *et al.*, 1995)**.

Some recent biomedical applications of HA include ophthalmic surgery, arthritis treatment, scaffolds for wound healing, tissue engineering, drug delivery (Saettone et al 1994) and components for implant materials **(Candy & Sharma,1990; Okamoto *et al.*, 1992 and Hong *et al.*, 1993)**.

Hydrogles being used as basic materials for manufacturing of wound dressings were invented in **1989** by **Rosiak *et al***. However, some of the hydrogel dressings, due to their low strength and elasticity, did not satisfy the ideal dressing requirements, viz, they might stick to the wound surface or crushed under high stresses **(Yoshii *et al.*, 1999)** .

The present work is designed to prepare blended hydrogel membranes (PVA-HA) by freezing-thawing method as membranes with good strength and elasticity to be compatible in biomedical application. This is the first time to prepare these membranes by this method for wound dressing.

2-. EXPERIMENTAL

2.1. Materials

PVA (typically average Mw = 72,000 g/mol; 98.9% hydrolyzed) was obtained from Biochemica, Germany. HA(was purchased from shanghaijiaoyuan industry co, ltd, china), Ascorbic acid(MW=176.13 g/mol; 99% hydrolyzed). Distilled water was used throughout this work.

2.2.Preparation of hydrogel membranes

PVA-HA hydrogel membranes were prepared by freezing- thawing (F-T) cycle according to the reported procedure of **Peppas and Stauffer (1991)**. Briefly, aqueous solution containing 5% (w/v) PVA, 1% (w/v) HA and 0.3% (w/v) of ascorbic acid (AA) were carefully dissolved in distilled water. Different proportions of PVA and HA contents (0%, 10%, 20%, 30%, 40%, and 50%) solutions were mixed, sonicating, and vortexing for one hour. Proper amounts of this mixture were poured in Petri dishes, followed by freezing at -20 °C for 18 hours and thawing for 6 hours at 25 °C for one, two, and three continuous cycles.

2.3- Characterizations

2.3.1-Gel fraction

The pieces of PVA-HA hydrogel membrane samples 2 × 2(cm) were dried for 6 hrs at 50°C in an oven and weighted (*Wo*). They were soaked in distilled water into Petri dishes for 24 hrs up to a constant weight and taken out from Petri dishes in order to remove the soluble parts. The gels were dried again at 50°C in an oven and weighted again (*We*). The gel fraction percentage was calculated by the following equation **(Yoshii *et al.*, 1999 and Ajji *et al.*, 2005)**:

$$Gel\ fraction\ (GF\ \%) = (We/\ W0)\ X\ 100 \qquad (1)$$

Where (W_0) and (*We*) are the weights of hydrogel samples dried for 6 hrs at 50 °C before and after soaking, respectively.

2.3.2-Water uptake

In order to measure the water uptake of PVA-HA hydrogel membranes, the membrane samples were cut into 2x2 (cm) pieces and dried at 50 °C in an oven for 6 hrs, the weight of dried sample were determined (*We*). The dried samples were soaked indistilled water, maintained and incubated at 37 °C, then weighted (*Ws*) at specific interval times. The water uptake of PVA-HA hydrogel membranes was determined using the following Eq. (2)**Yang *et al.* (2008):**

$$Water\ uptake\ (\%) = (Ws-\ We/\ We)\ X\ 100 \qquad (2)$$

Where (*Ws*) is the weight of swelled sample and (*We*) is the weight of dry sample.

2.3.3-Mechanical properties

The maximum tensile strength and the elongation to break of PVA-HA blend hydrogel membranes have been conducted using a tensile test machine (model: AG-I / 50 N – 10 KN,Japan). PVA- HA membranes were cut into specific adog-bone shape (5 cm long, 1.5 cm wide at the ends and 1 cm in the middle). The analysis was performed at stretching rate 10 mm / min. The thickness of membrane samples were measured with an electronic digital micrometer before examination **(Alencar *et al.*, 2003).**

2.3.4-Protein adsorption study

The amount of adsorbed bovine serum albumin (BSA) was detected by UV-visible spectrophotometer . In order to establish the relationship between the visible absorbance of BSA at 630 nm and the concentration of BSA, a calibration curve was drawn for standard solution of BSA ranging from 3.1-60 mg/ml. All standard solutions were prepared with distilled water. From the calibration curve a study was made restricting the curve to the linear part that followed linear part that followed Beer's law

$$A = acL$$

Where A the absorbance, c is the concentration, a is a proportionality constant and L is the path –length which is constant **(Queiroz *et al.*, 2001).**

Pieces of PVA- HA hydrogel membranes cut into 1 x1 (cm) were immersed in 10 ml phosphate buffer saline (PH 7.4), and incubated at 37 °C for 24 hrs until reach to equilibrium swelling weight.

The swollen hydrogel pieces were transferred to buffer solution containing BSA (30 mg/ml) and shacked for 4hrs at 37 °C. After protein adsorption, the hydrogel pieces were gently removed. The protein adsorption for each sample were calculated by the difference

between protein concentrations before and after immersing hydrogel pieces in protein/ phosphate buffer solution using albumin reagent kit (absorbance range at 630 nm), this procedure has been adapted and modified from the procedure of **Lin *et al.* (2006).**

2.3.5- Hydrolytic degradation

The degradation activities were determined by the hydrolytic degradation method **(Xiao and Zhou, 2003).** This method is based on gravimetric determination study of the weight loss % of the gel. Procedure: dry 2 x 2 (cm) membrane samples were weighed and immersed in 10 ml phosphate-buffer saline (PBS) (0.1 M, pH 7.4) at 37°C. The samples were removed at timed intervals, blotted with soft paper to remove surface water, dried at ambient temperature and weighed.

3. RESULTS AND DISCUSSION

3.1-Gel fraction

Fig. (1) Shows the gel fraction of the PVA hydrogels membrane with different HA content. The results clearly indicated that with the increase of HA content, the gel fraction of the hydrogel membrane decreased. During F–T cycles, the cross-linking strength of HA was weaker than that of PVA, even though HA formed a cross-linking bond with PVA in the gel. Generally, as the gel fraction decreased, the strength of the gel was weakened (**Ajji *et al.*, 2005 and Kim *et al.*, 2008).** There fore, HA could be used to control the strength of hydrogel because it reduced the cross-linking reaction and, consequently, the gelation process. This is in agreement with **Maolin *et al.* (2002)** on their study at PVA-starch blended hydrogels, **Long *et al.* (2003)** on PVA-carboxymethylated chitosan blended hydrogels .

3.2-Water uptake

As shown in Fig. (1) the water uptake of PVA hydrogel membrane increases with increasing HA content in hydrogel membranes. This due to, the high hydrophilicity of HA in PVA film which increases the water uptake character of the studied hydrogel. This coincide with **Hwang *et al.* (2010) and Abou El-Enin (2013).** They found that the swelling of PVA-dextran and PVA-alginate blended hydrogel membranes increased with increasing dextran and alginate contentes respectevily.

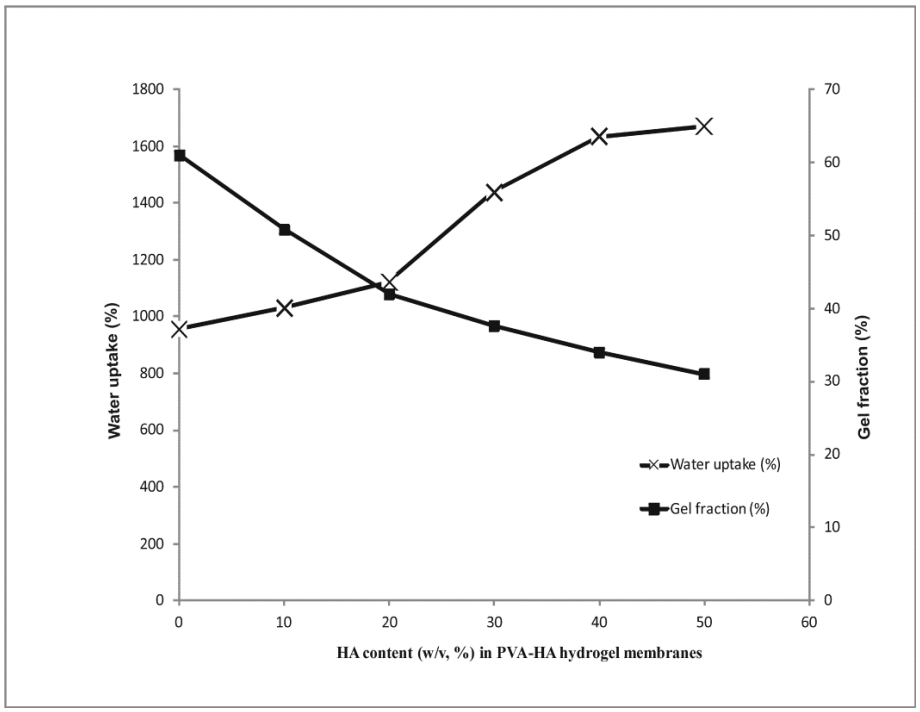

Fig. 1- Effect of HA content in PVA hydrogel membranes on gel fraction and water uptake(%).

3.3-mechanical properties

To investigate the influence of HA on the mechanical properties of the hydrogels, their tensile strength and elongation to break were evaluated (Fig.2).The maximum tensile strength and elongation at break of hydrogel membranes decreases with increasing HA content. As HA was blended with PVA, the cross-linking density of the gel was decreased. These results are coincide with that of **Rosiak *et al.* (2001).** They referred that the maximum tensile strength of PVA hydrogel decreased with increasing blend materials due to decreased cross-linking density. **Hwang *et al.* (2010)** demonstrated that the maximum tensile strength of PVA hydrogel has sharply decreased with increasing dextran portions in the hydrogel and **Abou El-Enin (2013)** who reported that the maximum tensile strength and elongation at break of PVA–HES hydrogel membranes, sharply decreased with increasing hydroxyethylstarch (HES) contents.

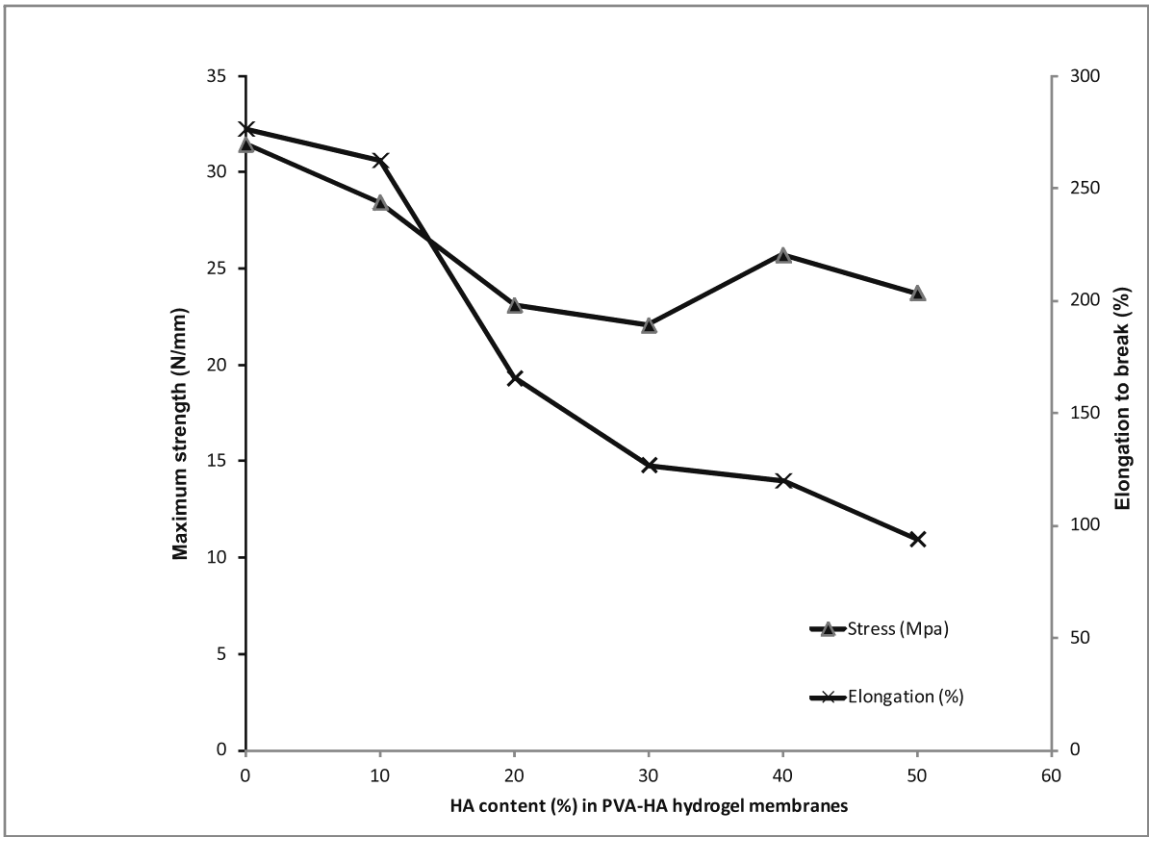

Fig. 2- Effect of hyaluronic acid contentes on mechanical properties of PVA hydrogel membrane.

3.4-Protein adsorption

The protein adsorption onto PVA–HA blend hydrogel membranes has been conducted via in vitro experiments**.** Fig.(3) shows the Protein adsorption of PVA hydrogel membrane at different Hyaluronic acid content in PBS. The results appeared that the Protein adsorption of PVA hydrogel membrane increases with increasing HA content in hydrogel membranes, the highest values of protein adsorption on PVA–HA surface have been cleared with the highest values of hydrophilic surface interaction due to the addition of HA contents. The blood compatibility of the hydrogel was evaluated by the amount of plasma protein adsorbed onto the hydrogel surface. When foreign material was placed in contact with blood, the adsorption of protein onto the surface occurred, leading to platelet adhesion and activation **(Colm *et al.*,1982 and Burkatovskaya *et al.*, 2006)**. Because the albumin adsorption on the synthetic surfaces could inhibit platelet activation, it did not promote clot formation. Generally, as the albumin/fibrinogen adsorption ratio was higher, the number of adhering platelets was lower **(DionI *et al.*, 1993)**. Thus, HA gave less adhesion of platelets to artificial surfaces. This agrees the reported results by **Kim *et al.* (2008) and Hwang *et al.* (2010)**,they revealed that the adsorption of protein increased with increasing blended alginate and dextran respectively in PVA hydrogels.

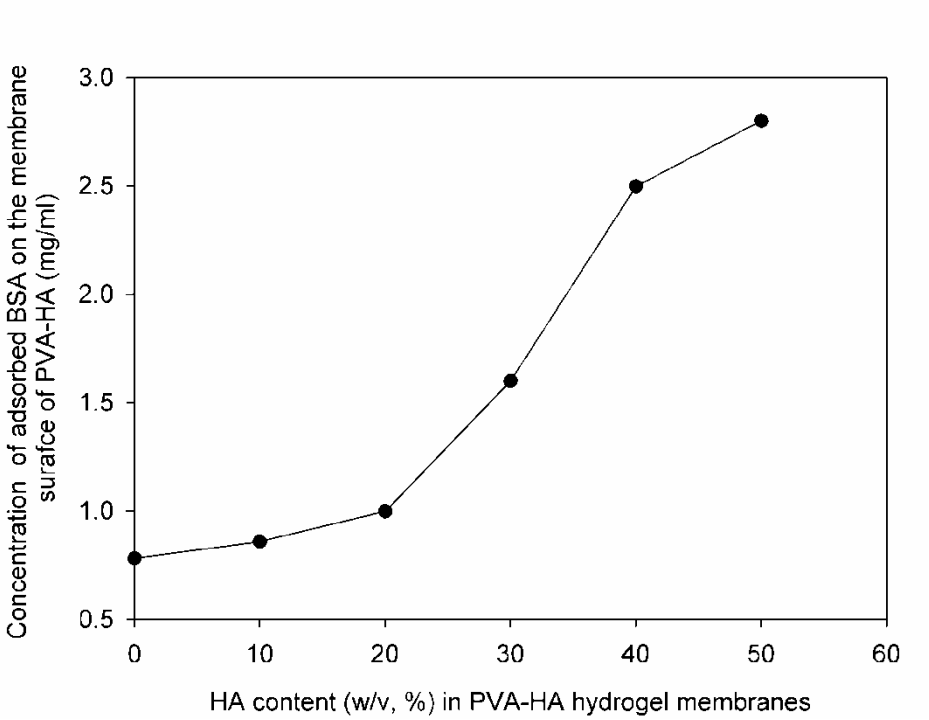

Fig. 3- Effect of Hyaluronic acidcontent on protein adsorption of PVA Hydrogel Membrane.

3.5-Hydrolytic degradation

The hydrolytic degradation of PVA hydrogel membrane at different hyaluronic acid content in Phosphate buffer saline(PBS) is showed in fig.(4). The results indicated that the hydrolytic degradation of PVA hydrogel membrane increases with increasing HA content in hydrogel membranes. This phenomenon can be ascribed to the degradation of PVA–HA hydrogel membranes that are predominantly the cleavage of cross-linking segments of PVA and is consistent with the fact that the degradation of PVA is quite slow **(Takasu *et al.*, 2002)**, whereas the degradation of PVA–HA is quite high. In addition, as PVA and HA are nontoxic, the PVA–HA hydrogel and its degradation by-product might be expected to be nontoxic too.

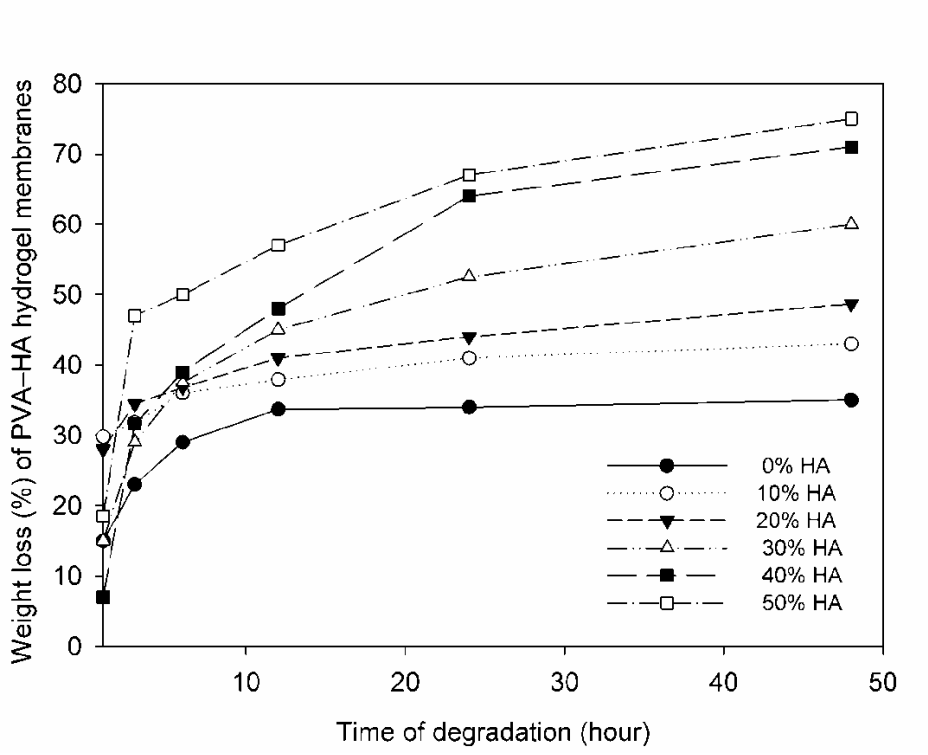

Fig. 4- Effect of HA contents on weight loss of the PVA–HA hydrogel membranes after different degrading times in phosphate buffer saline (PBS) (0.1 M, pH 7.4, at 37 °C).

CONCLUSION

The PVA–HA blended hydrogels have been prepared by freezing–thawing technique as a physical cross-linking method. Each of swelling, hydrolytic degradation and protein adsorpition of PVA–HA hydrogel membranes increased with increasing HA content in hydrogels but both gel fraction and mechanical properties (tensile strength and elongation at break) decreased with increasing HA content in hydrogels . Then the addition of HA to PVA hydrogels is expected to improve the benefit of hydrogel membrane for biomedical applications.

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"توصيف أغشية مخلوطة من عديد الفينيل الكحولى و الهيالورينات المحضرة بطريقة فيزيائية"

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اتجهت الأبحاث العلمية لانتاج أغشية هيدروجيلية من عديد الفينيل الكحولى نظرا لمميزاته العديدة مثل عدم السمية وعدم السرطنة والتحلل الحيوى ، لذا فقد تم ادخاله فى كثير من التطبيقات الحيوية والصيدلانية.

استهدفت هذه الدراسة تحضير وتوصيف أغشية مخلوطة من عديد الفينيل الكحولى والهيالورينات بنسب متافوته وقد تم تحضيره بطريقة فيزيائية عن طريق تجميده عند درجة حرارة 20 درجة مئوية تحت الصفر لمده 18 ساعة وتركه فى درجه حرارة الغرفة لمده 6 ساعات وتكرر هذه العمليه ثلاث مرات متتالية.

أظهرت النتائج أنه بزيادة كمية الهيالورينات فى أغشية الهيدروجل تؤثر على الخصائص الفيزيائية لتلك الآغشية، مثل درجة تشرب الماء ودرجة التشابك والخواص الميكانيكية .حيث تبين أن زيادة كمية الهيالورينات فى أغشية الهيدروجل أدى الى زيادة تشرب الماء بينما انخفضت درجة التشابك والخواص الميكانيكية بشكل ملحوظ.

أوضحت النتائج أيضا أن زيادة كمية الهيالورينات فى محتوى هيدروجل عديد الفينيل الكحولى يعطى ادمصاص أعلى للبروتين وتحلل حيوى أكثر حيث أن الوزن المفقود من الأغشية المخلوطة محل الدراسة يتراوح بين 18الى70 بالمائة بعد ثلاثة أيام من غمره فى محلول ملحى درجة حموضة 7.4.

مما سبق يتضح أن الخصائص الفيزيائية لأغشية عديد الفينيل الكحولى تتحسن بمشاركة الهيالورينات مما يؤهل هذه الأغشية للاستخدامات الحيوية.