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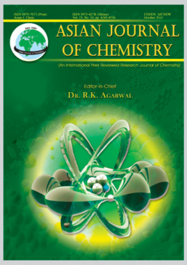

Preparation and Characterization of Genipin-Crosslinked Chitosan/Poly(ethylene glycol) Composites Films

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Chitosan/poly(ethylene glycol) interpenetrating networks were prepared using solution casting technique. A naturally occurring and nontoxic cross-linking agent, genipin, was used to form the genipin-crosslinked chitosan and chitosan/poly(ethylene glycol) blends networks, where four types of poly(ethylene glycol) were used. Genipin overcomes the problem of physiological toxicity inherent in the use of some common synthetic chemicals as cross-linking agents. It was shown that the properties of films made by blending chitosan with four types of poly(ethylene glycol). The swelling ratio increase with the increase of molecule weight of poly(ethylene glycol). The mechanical properties were slightly improved with the proper amount of molecule weight of poly(ethylene glycol). The surface properties of the films were also examined by contact angles. The results showed that in water, the cross-linked chitosan and chitosan/poly(ethylene glycol) blends were more hydrophobic with the decrease of the molecular weight of poly(ethylene glycol).

Key Words: Chitosan, Poly(ethylene glycol), Mechanical properties, Genipin.

INTRODUCTION

There has been a growing interest over the past few years in the development of biomaterials, especially for clinically applicable biomaterial that is to modify the surface of a material that already has excellent bio-functionality and bulk properties[1]. Blends are often used to improve tensile properties and to provide a stronger structural component for separation media that supports the active polymer. The physical properties of a polymer can also be altered by introducing a second polymer that improves the properties of the original polymer in certain aspects.

Chitosan is a cationic biopolymer obtained by a full or partial N-deacetylation of chitin, which is known to be the second most abundant biopolymer in nature and is the major component of the exoskeleton of crustaceans[2]. Chitosan has been evaluated for various uses in food packaging, medical, pharmaceutical, agricultural and chemical industries because of its nontoxic, biocompatible, mucoadhesive and biodegradable properties[3-5]. But chitosan has some drawbacks. It is only soluble in aqueous medium in presence of a small amount of acid such as AcOH and its mechanical properties are not good for some biomedical application. Therefore, many researchers tried to modify its properties.

Poly(ethylene glycol) is frequently used in the production of polymer blends, as it is an amphipathic polymer and has a wide range of molecular weights. Poly(ethylene glycol) can improve flexibility and ductility of the blended films. Poly(ethylene glycol)'s have also been used as a good plasticizer in the polymer industry. Physically cross-linked films have recently received more research attention[6-9]. However, they are not as strong and stable as covalent cross-linked systems and can eventually disintegrate, regardless of whether any film components are biodegradable or not. Genipin is manufactured from geniposide, a glucoside, by using b-glucosidase. It should be added that the production protocol and the quality evaluation have recently been re-assessed and established in modern terms[10]. It has been used over the years in traditional Chinese medicine to treat symptoms of type 2-diabetes[11,12].

Films made from pure chitosan are brittle in nature[13]. Chitosan/poly(ethylene glycol) blend films may provide additional functionality compared with the pure polymer films. Chitosan may improve mechanical properties and decrease water solubility of the poly(ethylene glycol) films, while poly(ethylene glycol) may contribute to the formation of films that are more flexible. Many attempts[13-17] have tried to improve the mechanical properties of chitosan. Modification of chitosan through blending with other polymers[18,19] and cross-linking[20,21] are both convenient and effective in improving its physical properties for practical applications. There have been some reports[22-24] dealing with polyblends of chitosan with other natural or synthetic polymers. Genipin and its related iridoid glucosides have been widely used as an antiphlogistic and cholagogue in herbal medicine[25]. This implied that genipin could form biocompatible cross-linked products. Although the preparation of chitosan/poly(ethylene glycol) blend films have been reported in a few works[26,27], there are no reports in the systematic results on the effect of poly(ethylene glycol) with different molecular weight.

Because poly(ethylene glycol) acted as a plasticizer in the chitosan/poly(ethylene glycol)/genipin films, poly(ethylene glycol) molecule weight is expected to be an important factor that can affect the interactions among the three macromolecular components as well as the crystallization process. In this work, we produced genipin-crosslinked chitosan/poly(ethylene glycol) blends with different molecular weight of poly(ethylene glycol) between blend components. Physico-chemical properties of the genipin-crosslinked chitosan/poly(ethylene glycol) films were investigated using FTIR and scanning electron microscopy. Contact angle, swelling ratio, mechanical properties and stability of cross-linked samples were also measured.

EXPERIMENTAL

Chitosan (> 85 % deacetylated) was purchased from Fluka. Four poly(ethylene glycol) samples, PEG2000 (Mw =2000), PEG4000 (Mw = 4000), PEG8000 (Mw = 8000) and PEG20000 (Mw = 20000) and acetic acid were purchased from Sigma. The buffer solutions of pH 2, 4, 7 and 10 were purchased from Samchun pure Chemical. Genipin was obtained from Aldrich chemicals.

Preparation of chitosan and chitosan/poly(ethylene glycol) films cross-linked by genipin: Chitosan was dissolved in 1 wt % aqueous acetic acid at room temperature overnight to obtain a concentration of 1.5 (w/v). The viscous chitosan solution was filtered through filter paper to remove any undissolved gel. The clear, light yellow chitosan solution was then mixed with a 5 % aqueous solution of poly(ethylene glycol) in different molecular weight (2000, 4000, 8000 and 20000) and the mixtures were stirred overnight at room temperature. The genipin (genipin/chitosan) was dissolved in 3 mL of water and then was added to the chitosan solutions or to the chitosan/poly(ethylene glycol) mixed solutions under stirring at room temperature. After 2 h, the solutions started to turn light blue and became increasingly viscous. The solutions were then immediately cast on glass plates. The cross-linked chitosan and chitosan/poly(ethylene glycol) blends become dark blue after 1 day. For all experimental studies, the samples at the feed ratio of 79.4/20/0.6 were first treated to remove the residual acetic acid. After drying the solutions at room temperature, both non-cross-linked and cross-linked chitosan membranes were peeled off and neutralized in a 0.1 M NaOH solution for about 10 min, washed thoroughly with distilled water and dried again.

Infrared analysis: Fourier transform infrared attenuated total reflectance spectroscopy (FTS 175C, Bio-Rad) spectra of pure chitosan, poly(ethylene glycol) and genipin-crosslinked chitosan/poly(ethylene glycol) films spectra were recorded at room temperature in the range 4000-500 cm^{-1}.

Swelling tests: The swelling properties of all films were gravimetrically studied. The known weight of film sample was immersed in distilled water at both room temperature and 37 ºC. The swollen films were removed at predetermined intervals and weighed after wiping off the excess water on the film surface with a wet filter paper. The swelling ratio is defined and calculated as below:

$$\text{Swelling ratio (\%)} = (W_t - W_0)/W_0 \times 100\ \%$$

here, the W_t and W_0 represent the weights of swollen and dry state samples respectively.

Contact angle analysis: Contact angle goniometry (DSA100, Krüss GmbH, Germany) was carried out using a computerized system, fitted with an automatic image capture system. Sessile drop method was applied, using a 5 µL double distilled water droplet. For each angle reported, at least five measurements on different surface locations were averaged.

Water solubility: The dried films were immersed in 40 mL methanol for 24 h to remove acetic acid existing in the films and then were taken out and dried in the vacuum oven for 24 h at room temperature. The dried films were weighed (W_1), immersed in distilled water for 8 h, dried in a vacuum oven for 24 h at room temperature and then were stored in a desiccator for 5 h for weight balance. The equilibrium weights of the films were weighed (W_2). Water solubility was calculated with the following equation:

$$Ws = (W_1 - W_2)/W_1 \times 100\ \%$$

Mechanical strength: Tensile strength and percentage elongation were measured on film strips using instrument. The specimens were cut into 6 mm wide, 20 mm length. The extension rate was 2 mm/min. The tensile strength, the toughness and the strain at failure were calculated from the load extension curves. To examine the physical properties of the films as a function of moisture content, they were placed in a relative humidity chamber over distilled water (pH 7).

RESULTS AND DISCUSSION

Characterization of genipin-crosslinked chitosan/poly(ethylene glycol) films: Fig. 1 shows the spectra of individual polymer components at the feed ratio of genipin-crosslinked chitosan/poly(ethylene glycol) 79.40/20/0.6 with different molecule weight of poly(ethylene glycol). The IR peaks at 840 cm^{-1} and 1340 cm^{-1} are the characteristic peaks of poly(ethylene glycol) and are from its crystalline regions[28].

Fig. 1 shows that the relative intensity of the poly(ethylene glycol) crystalline absorbance peaks at 840 and 1,340 cm^{-1} increased as the poly(ethylene glycol) molecule weight increased from spectrum c (genipin-crosslinked chitosan/PEG 2000) to spectrum f (genipin-crosslinked chitosan/PEG 20000). Poly(ethylene glycol) in genipin-crosslinked chitosan/poly(ethylene glycol) 2000 showed no distinct crystalline peaks, which is consistent with previous study[23]. However, the poly(ethylene glycol) crystalline peaks increased dramatically when poly(ethylene glycol) molecule weight increased to 20000. Since the intensity of these two peaks is directly proportional to the degree of the crystallinity of poly(ethylene glycol), an increase in the relative intensity of these two peaks indicated an increase in poly(ethylene glycol) crystallinity.

Low molecular weight poly(ethylene glycol) interacts with both chitosan and genipin components in the chitosan/poly(ethylene glycol)/genipin blends and weakens the physical cross-linking, thus improving the properties of the hydrogels at the expense of retarded chain mobility of poly(ethylene glycol) molecules. When poly(ethylene glycol) molecule weight is high enough, due to the relatively smaller numbers of polar hydroxyl end groups, the interaction between a higher molecular weight poly(ethylene glycol) with chitosan macromolecules would be lesser and the free poly(ethylene glycol) chain segments could be present in the blend, which would help poly(ethylene glycol) chains to reorganize themselves relatively more freely to crystallize as evidenced by the higher poly(ethylene glycol) crystallinity in the genipin-crosslinked chitosan/poly(ethylene glycol) 20000 blend.

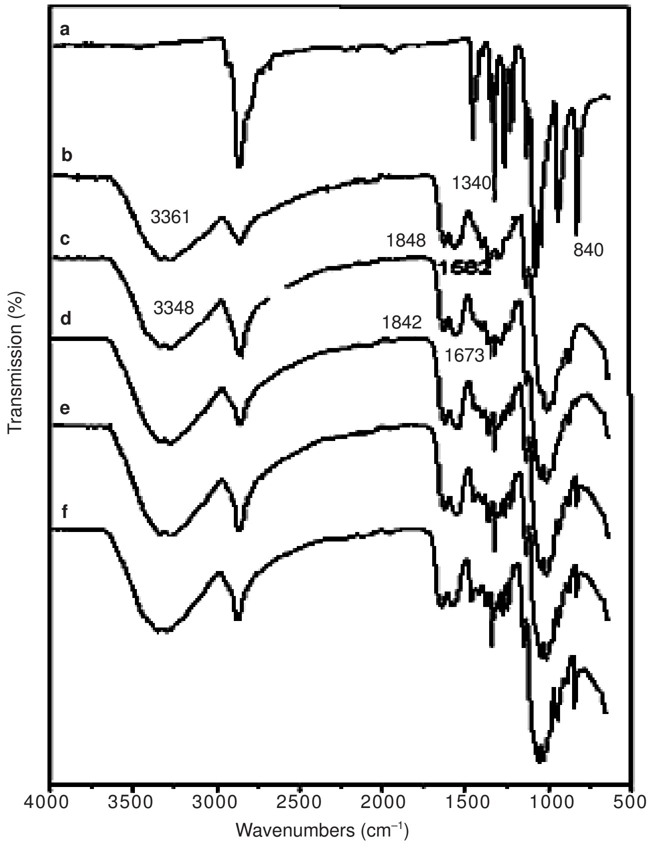

Fig. 1. IR spectra with different poly(ethylene glycol) (PEG) molecular weight (a) PEG; (b) chitosan; (c) GC/PEG2000; (d) GC/PEG 4000; (e) GC/PEG 8000; (f) GC/PEG 20000

Swelling tests: Fig. 2 shows the result of the swelling test for the films with different molecular weight of poly(ethylene glycol) but with the same poly(ethylene glycol) content. It is obvious that the higher the molecular weight of poly(ethylene glycol) is, the higher is the water content of the film. It is suggested that the length of poly(ethylene glycol) chain in the film also affects the swelling behaviour. On the other hand, as the level of crystallinity of the genipin-crosslinked chitosan/poly(ethylene glycol) film decreased, a lesser crystalline structure would be available to provide physical cross-linking of the network structure, leading to a more open and porous network with a higher swelling ratio.

Effect of poly(ethylene glycol) on the mechanical property of the genipin-crosslinked chitosan/poly(ethylene glycol) films: The effects of the molecular weight of poly(ethylene glycol) on the mechanical property of the physical hydrogels are shown in Fig. 3. The data in these figures show that the stress, strain and toughness of the genipin-crosslinked chitosan/poly(ethylene glycol) films first increased with an increase in poly(ethylene glycol) molecule weight, then deteriorated with a further increase in the molecule weight of poly(ethylene glycol). For example, when poly(ethylene glycol) molecule weight increased from 2000 to 8000, the stress and strain increased by about 31.1 % (30.2 to 39.6 MPa) and 86.6 % (21.7 to 40.5 MPa), respectively. However, with the further increase of poly(ethylene glycol) molecular weight, *i.e.*, 8000-20,000, the stress and strain dropped by about 37.1 % (39.6 to 24.9 MPa) and 12.1 % (40.5 to 35.6 MPa), respectively.

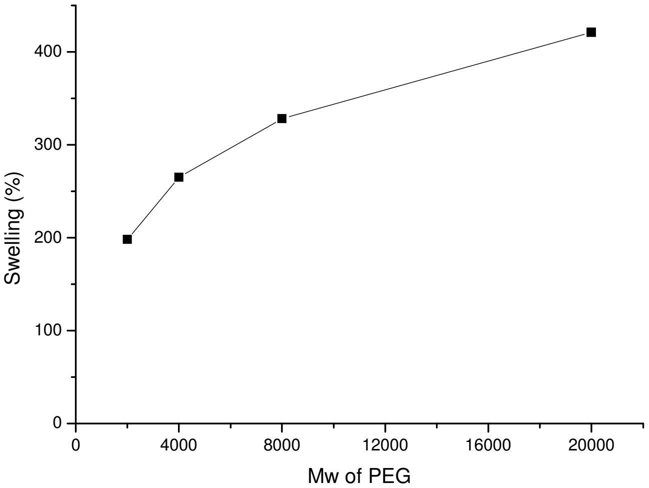

Fig. 2. Effect of poly(ethylene glycol) (PEG) on the equilibrium swelling of the genipin-crosslinked chitosan/PEG hydrogels as a function of PEG molecular weight

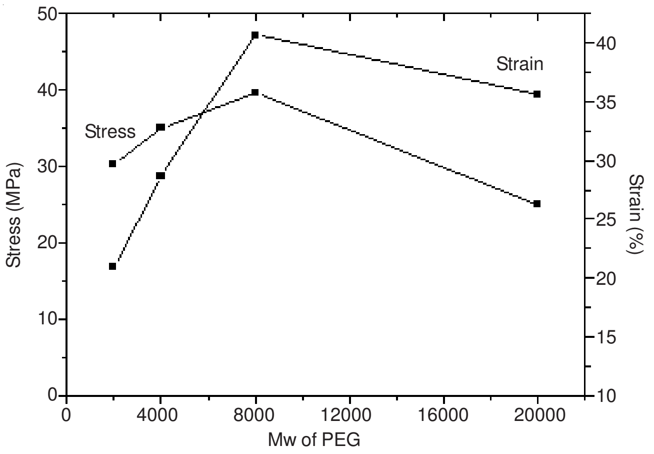

Fig. 3. Effect of poly(ethylene glycol) (PEG) on the stress and strain of genipin-crosslinked chitosan/PEG films as a function of PEG molecular weight

There are two factors that contribute to the mechanical property of these films *i.e.*, the level of crystallinity and the intermolecular force. In the case of film genipin-crosslinked chitosan/poly(ethylene glycol) 2000, it has a higher crystallinity, but intermolecular forces, like van der Waals force and entanglement, between poly(ethylene glycol) and chitosan/genipin are weak because of the shorter poly(ethylene glycol) chain. As the molecular weight of poly(ethylene glycol) increases to 8000, intermolecular forces between poly(ethylene glycol) and other molecules are strengthened without noticeable expenses of crystalline domains and this combined effect resulted in an increase in film mechanical property. However, as the molecular weight of poly(ethylene glycol) reached 20000 in genipin-crosslinked chitosan/poly(ethylene glycol) 20000 film, the intermolecular forces and molecular entanglement would reach the level that could retard the uncoiling of macromolecular conformation under force.

From Fig. 4, it is found that the addition molecular weight of poly(ethylene glycol) in the genipin-crosslinked chitosan/poly(ethylene glycol) blend could lead to the enhancement of toughness when the molecular weight of poly(ethylene glycol) was less than 8000. Fig. 4 shows toughness drops dramatically. It's known that toughness is inversely proportional to brittleness and a decrease of toughness means an increase in brittleness. As the content of poly(ethylene glycol) is constant in the blending, the increase of molecule weight of poly(ethylene glycol) means the decrease in the number of poly(ethylene glycol) end groups in the blending. The number of end groups is an important factor for polar plasticizer and such reduction led to low plastic efficiency. Therefore, films increase in brittleness.

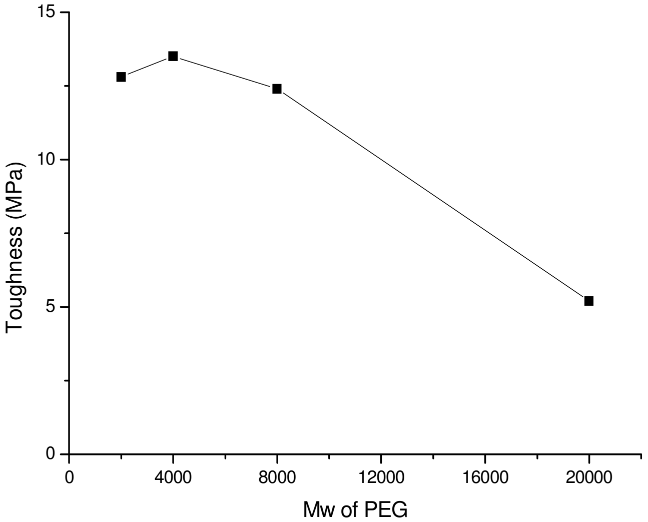

Fig. 4. Effect of poly(ethylene glycol) (PEG) on the toughness of genipin-crosslinked chitosan/PEG films as a function of PEG molecular weight

Contact angle analysis: The surface properties of the chitosan films and the blends genipin-crosslinked chitosan/poly(ethylene glycol) films samples were investigated by contact angle analysis. Fig. 5 shows the changes in the contact angles of the pure chitosan and the genipin-crosslinked chitosan/poly(ethylene glycol) films using water with different molecular weights of poly(ethylene glycol). As shown in Fig. 5, the contact angle on the surface of pure chitosan film is 76.2º and the contact angles on the surface of genipin-crosslinked chitosan/poly(ethylene glycol) 2000 and genipin-crosslinked chitosan/poly(ethylene glycol) 4000 films are 63.6 and 59.4º, respectively. The pure chitosan was presented the highest degree indicating that the surface is hydrophobic and genipin-crosslinked chitosan/poly(ethylene glycol) 2000 film was decreased by the contact angle of approximately 12.6º compared with pure chitosan film. The contact angle on the surface of genipin-crosslinked chitosan/poly(ethylene glycol) 8000 films was 51.1º and markedly decreased by about 25.1º compared with chitosan of 76.2º. It was confirmed that the contact angles on the surfaces of the films were greatly decreased with increasing molecular weights of poly(ethylene glycol). The contact angles for all of the genipin-crosslinked chitosan/poly(ethylene glycol) blends in all different molecular weight of poly(ethylene glycol). On the whole, the contact angle analysis was helpful in aiding the understanding of the surface properties of the blends.

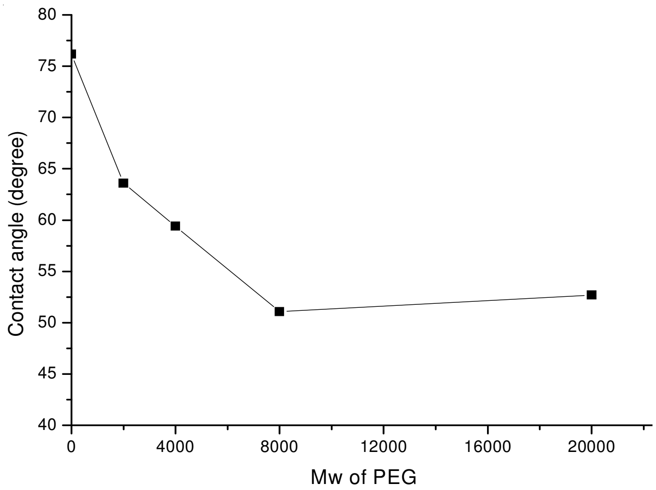

Fig. 5. Contact angles of genipin-crosslinked chitosan/PEG films using water with different molecular weight of poly(ethylene glycol) (PEG)

Stability of cross-linked blend films in water: The results for water solubility of genipin-crosslinked chitosan/poly(ethylene glycol) blend films are shown in Fig. 6. Chitosan is soluble in dilute aqueous acidic solution (pH < 6.5), but insoluble in pure water, while poly(ethylene glycol) is water soluble polymer. Although all the films maintained the original shape after being immersed in water for 8 h at room temperature, the 40/60 films dissolved the most, from 76.4 to 57.2 %. However, with the increase of molecular weight of poly(ethylene glycol), the water solubility significantly increased. A comparison shows that the genipin-crosslinked chitosan/poly(ethylene glycol) film of lower molecular weight poly(ethylene glycol) is more stable than the higher molecule weight film, with the latter being unstable which is because the miscibility of lower molecule weight of poly(ethylene glycol) with chitosan is higher than that of higher molecule weight of poly(ethylene glycol) with chitosan.

Although high water resistance of plastic films may be desired for many industrial applications, controlled solubility of biodegradable films offers advantages for use in the food, pharmaceutical and agricultural industries. For example, it has been reported that microporous genipin-crosslinked chitosan/poly(ethylene glycol) films can control the rate of drug release by stepwise solubilization of poly(ethylene glycol) in water[29].

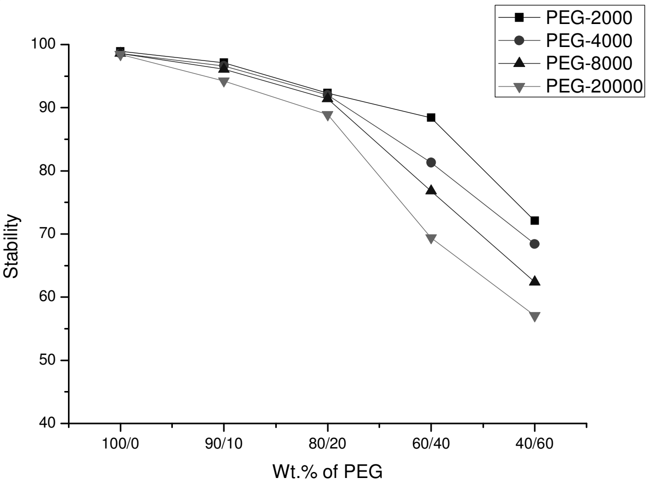

Fig. 6. Effect of genipin-crosslinked chitosan/PEG blend ratio from 100/0 to 40/60 on water solubility of the corresponding films

Conclusion

In this study, the effect of molecular weight of poly(ethylene glycol) on the properties of genipin-crosslinked chitosan/poly(ethylene glycol) films was investigated. The FTIR analysis revealed that the low molecular weight poly(ethylene glycol) lost the crystallization ability, while the high molecular weight poly(ethylene glycol) still had some crystallinity in the genipin-crosslinked chitosan/poly(ethylene glycol) films. The crystallization change of physical gels had an immediate effect on the swelling property of the hydrogels and the swelling ratio of the hydrogels increased due to the reduction of crystal cross-links. The mechanical property is the results of counter balance between crystallization and the intermolecular force. With the increase of the molecular weight of poly(ethylene glycol), there were also noticeable increase in the stability of the genipin-crosslinked chitosan/poly(ethylene glycol) blends. In a word, the molecular weight of poly(ethylene glycol) plays a key role in the property of the films. Consequently, the choice of appropriate molecular weight of poly(ethylene glycol) is important in the formulation of the films.

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