

Preparation and Characterization of Chitosan Composite Membranes Crosslinked by Carboxyl-capped Poly(ethylene glycol)*

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Abstract In this study a series of chemically crosslinked chitosan/poly(ethylene glycol) (CS/PEG) composite membranes were prepared with PEG as a crosslinking reagent other than an additional blend. First, carboxyl-capped poly(ethylene glycol) (HOOC-PEG-COOH) was synthesized. Dense CS/PEG composite membranes were then prepared by casting/evaporation of CS and HOOC-PEG-COOH mixture in acetic acid solution. Chitosan was chemically crosslinked due to the amidation between the carboxyl in HOOC-PEG-COOH and the amino in chitosan under heating, as confirmed by FTIR analysis. The hydrophilicity, water-resistance and mechanical properties of pure and crosslinked chitosan membranes were characterized, respectively. The results of water contact angle and water absorption showed that the hydrophilicity of chitosan membranes could be significantly improved, while no significant difference of weight loss between pure chitosan membranes and crosslinked ones was detected, indicating that composite membranes with amidation crosslinking possess excellent water resistance ability. Moreover, the tensile strength of chitosan membranes could be significantly enhanced with the addition of certain amount of HOOC-PEG-COOH crosslinker, while the elongation at break didn't degrade at the same time. Additionally, the results of swelling behaviors in water at different pH suggested that the composite membranes were pH sensitive.

Keywords: Chitosan; Membrane; Poly(ethylene glycol); Crosslink.

INTRODUCTION

Membranes prepared from naturally occurred chitosan have been explored in many uses such as water-ethanol pervaporation^[1], enzyme immobilization^[2], cationic specimen transportation^[3] and among others^[3–6]. Although hydrophilicity and mechanical properties of chitosan membranes are very important for many applications, such as packaging films^[7], protein separation membranes^[8] and wound dressings^[9], these properties are usually not ideal^[5, 10, 11]. Researchers have explored various modification methods^[12]. Among them, blending chitosan with other polymers, such as PNIPAAm^[13], PVA^[6, 14] and kudzu starch^[15], has been proved to be an effective way^[11]. Besides, poly(ethyl glycol) (PEG), a hydrophilic polymer with flexible molecular chain, is widely accepted as a good alternative^[16–19]. It was often reported that the elasticity of CS/PEG composite membranes was better than that of pure CS membranes, while the tensile strength decreased in most cases^[16, 17, 20]. Moreover, the stability of CS/PEG composite membranes in aqueous environment was unsatisfied. Because of the physical and weak interactions between CS and PEG molecules, the PEG component tends to solubilize in aqueous solution,

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leading to weight loss and worsening of the composite membrane performances^[11, 16].

Crosslinking has been used to improve the water stability of CS/PEG composite membranes^[11]. Additional crosslinking reagents with active groups such as aldehyde groups^[2, 21], epoxy groups^[22] and carboxyl^[23, 24] etc.^[11, 12, 25] were applied. However, the crosslinking reagents are usually toxic, which is disadvantageous in many fields, especially for biomedical applications^[21]. In the case of genipin, a novel biological crosslinker with low toxicity, the hydrophilicity of CS/PEG membranes decreased, and the weight loss in water on the 8th day was still more than 5%^[11]. The basic reason is that the addition of crosslinking reagents can not change the physical interaction between CS and PEG molecules. And the PEG component is still likely to be excluded and dissolves in aqueous environment.

To obtain covalent bonds between CS and PEG molecules, PEG with active ending groups such as epoxy groups and carboxyl was developed and applied in the preparation of CS/PEG composite gels^[22, 26, 27]. In these strategies, functional PEG was synthesized and used as a chemical crosslinker. As the synthetic route of carboxyl-capped PEG is comparatively simpler and greener than that of epoxy-capped PEG, we chose the former to prepare chemically crosslinked dense CS/PEG membranes. In this study, carboxyl-capped PEG was synthesized at first and then blended with CS solution. CS/PEG composite membranes were prepared with the casting/evaporation method. Chemical crosslinking reaction was carried out under heating. The structures of carboxyl-capped PEG and CS/PEG composite membranes were analyzed by ¹H-NMR and FTIR, respectively. The hydrophilicity, water stability and mechanical properties of the composite membranes were characterized and studied.

MATERIALS AND METHODS

Materials

Chitosan was purchased from Chengdu Kelong-Chemical Co. (Chengdu, China) and purified before use by the method described in previous literature^[28]. The viscosity average molecular weight and deacetylation degree of the chitosan were 6.12×10^5 Da and $(85.0 \pm 0.8)\%$, respectively. Other reagents were commercially available and of analytic grade. Water used in this study was double-distilled.

Synthesis of Carboxyl-capped Poly(ethylene glycol) (CT-PEG)

Poly(ethylene glycol) (PEG) of 2000 g/mol was precipitated in cold ether and then dried in vacuum oven at 40 °C. Succinic anhydride (SA) was purified through recrystallization in acetic anhydride. 1,4-Dioxane was treated with Na to remove water. Poly(ethylene glycol) (1 mmol, 2 g), SA (5 mmol, 0.5 g), triethylamine (TEA, 600 μ L) and 4-methylaminopyridine (DMAP, 0.025 g) were dissolved in 1,4-dioxane and reacted for 24 h under stirring and protection in N₂ atmosphere. Most of the solvent was then evaporated followed by addition of excess carbon tetrachloride to the system. Then the reaction mixture was filtered. The product was finally obtained by precipitation of the filtrate in cold ether. The structure of the product was analyzed by ¹H-NMR.

Preparation of Chemically Crosslinked CS/PEG Composite Membranes

Chitosan (CS) (1 g) was dissolved in 75 mL 2 vol% acetic acid. CT-PEG in a mass ratio of 20% (20%CT-PEG/CS), 30% (30%CT-PEG/CS) or 40% (40%CT-PEG/CS) was then added. The mixture was subsequently stirred at room temperature for 24 h. After that, the mixture was cast in a petri dish, which was then placed on an aclinic table at room temperature for 3–5 days. Chitosan membranes were peeled off from the dish and were dried at 35 °C in vacuum for 36 h at first, and then heated at 90 °C for 60 min. After being cooled to room temperature, the membranes were immersed in 1 mol/L NaOH solution for 1 h, and were washed with double distilled water to remove residue acetic acid and CT-PEG. Finally, the membranes were dried at 35 °C in vacuum and kept in desiccator until use.

FTIR Analysis

FTIR analysis was performed on a Nicolet 560 Fourier transform infrared spectrometer with a resolution of 2 cm⁻¹ (64 scans) in transmission mode. The specimens were prepared with KBr-disk method. Briefly,

membranes were cut into small pieces and were then ground. The powder-like samples mixed with KBr were pressed into disks (0.5 mm in thickness) for test.

Water Contact Angle Measurement

Water contact angle of the membranes was measured using the sessile drop method on a contact angle meter (CA-X, Kyowa Interface Science Co., Tokyo, Japan) at 25 °C in atmosphere. The water droplet was limited to about 0.3 mL to prevent gravitational distortion of its spherical profile.

Swelling Experiments in Water

Swelling experiments of the membranes were performed according to the following procedure. Membrane *ca.* 20.0 mg in weight and about 1 cm × 1 cm in size was immersed in a vial containing 20 mL distilled water in a temperature controlled water bath at the desired temperature of 28 °C until equilibrium swelling was achieved (for about 24 h). The equilibrium swelling weight of the sample was measured after removing the excess surface solution with filter paper. The pH response behavior of the membranes was studied in buffer solutions at different pH values. Buffer solutions were prepared by the standard method^[6]. Equilibrium of water absorption (EWA) and equilibrium swelling area (ESA) in water were calculated according to Eq. (1) and Eq. (2), respectively.

$$\text{EWA(g/g)} = \frac{W_e - W_d}{W_d} \times 100\% \quad (1)$$

where W_d is the weight of the dry membranes, and W_e is the weight of the swollen membranes after being immersed in aqueous medium for 24 h.

$$\text{ESA(cm}^2\text{/cm}^2\text{)} = \frac{S_e - S_d}{S_d} \times 100\% \quad (2)$$

where S_d is the area of the dry membranes, and S_e is the area of the swollen membranes after being immersed in aqueous medium for 24 h.

Weight Loss in PBS

Membranes (*ca.* 20.0 mg in weight and *ca.* 1 cm × 1 cm in size) were immersed in a vial containing 20 mL phosphate buffer solution (PBS, pH = 7.4) in a temperature controlled bath at 37 °C. After 8 days, the membranes were taken out and washed with redistilled water. Weight of the membranes was measured after dried in vacuum. Residual weight (RW, %) was calculated according to the following equation.

$$\text{RW(\%)} = \frac{W_t}{W_0} \times 100\% \quad (3)$$

Where W_0 and W_t are the weight of the membranes before and after being immersed in PBS, respectively.

Mechanical Properties

The mechanical properties of the membranes were determined with a tensile strength instrument (AGS-J, Shimadzu, Japan). Before the test, the prepared membranes were cut into a specific dog-bone shape (5 cm long, 8 mm wide at the ends, and 4 mm wide in the middle), and were allowed to equilibrate in ambience overnight. The mechanical analysis was performed at a stretching rate of 25 mm/min with a preload of 0.5 N to determine the maximum load and the elongation at maximum load for each membrane.

RESULTS AND DISCUSSION

Structure Analysis

To initiate chemical reaction between CS and PEG molecules, carboxyl-capped PEG was designed and synthesized at first, because amidation between carboxyls and aminos in CS molecules can easily occur^[24]. The end groups of PEG were transformed from hydroxyl to carboxyl through a simple coupling reaction with

succinic anhydride. $^1\text{H-NMR}$ was used to examine the structure of carboxyl-capped PEG (Fig. 1). Proton signals of the methylene at $\delta = 4.27$ and $\delta = 2.65$, from ester linkage and carbonyl structure, respectively, indicate the success of the coupling reaction. The ratio between the peak area at $\delta = 3.65$ and at $\delta = 4.27$ showed that the conversion of the end group, from hydroxyl to carboxyl, was more than 90%.

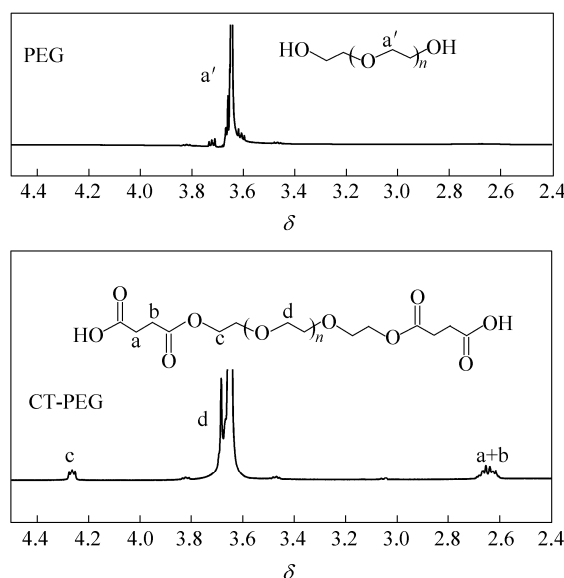


Fig. 1 $^1\text{H-NMR}$ spectra of PEG and carboxyl-capped PEG (CT-PEG)

FTIR spectra of the pure chitosan membranes (CS), the uncrosslinked PEG/CS membranes (Fig. 2, uncrosslinked) and the chemically crosslinked PEG/CS membranes (Fig. 2, crosslinked) were summarized in Fig. 2. The characteristic bands of chitosan at 1655 cm^{-1} (amide I) and at 1595 cm^{-1} (amide II) were clear. Stretching vibrations of N—H and O—H appeared as a wide band at $3200\text{--}3500\text{ cm}^{-1}$. The bands attributing to its saccharide structure were observed at 1154 , 1073 and 1032 cm^{-1} ^[23, 24, 27] (Fig. 2, CS). For the uncrosslinked membranes, peaks around 1240 and 842 cm^{-1} were assigned to PEG^[23], indicating the presence of PEG after blending. The peak around 1730 cm^{-1} was C=O deformations of the carboxylic groups at the end of PEG in the uncrosslinked membranes^[24] (Fig. 2, uncrosslinked). After the uncrosslinked membranes were heated in vacuum, the peak around 1730 cm^{-1} almost disappeared, indicating that the carboxylic groups at the end of PEG participated in the crosslinking reaction. Besides, the peak assigned to PEG (1240 cm^{-1} and 842 cm^{-1}) still existed (Fig. 2, crosslinked). These results showed that carboxyl-capped PEG performed not only as a blending component but also as a chemical crosslinker in the composite membranes. In addition, $-\text{COOH}$ of PEG reacted with $-\text{NH}_2$ other than $-\text{OH}$ in chitosan because the stretching vibration of C=O from ester bond (at around 1730 cm^{-1}) wasn't found as shown in the spectra^[29–31] (Fig. 3).

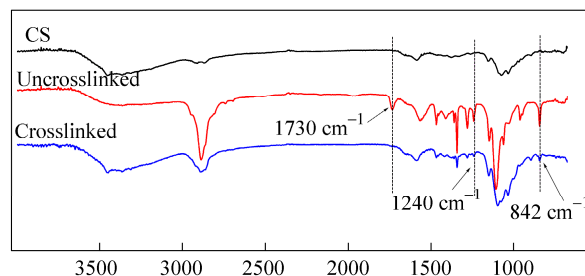


Fig. 2 FTIR of pure chitosan membranes (CS), uncrosslinked CT-PEG/CS membranes (uncrosslinked) and crosslinked CT-PEG/CS membranes (crosslinked)

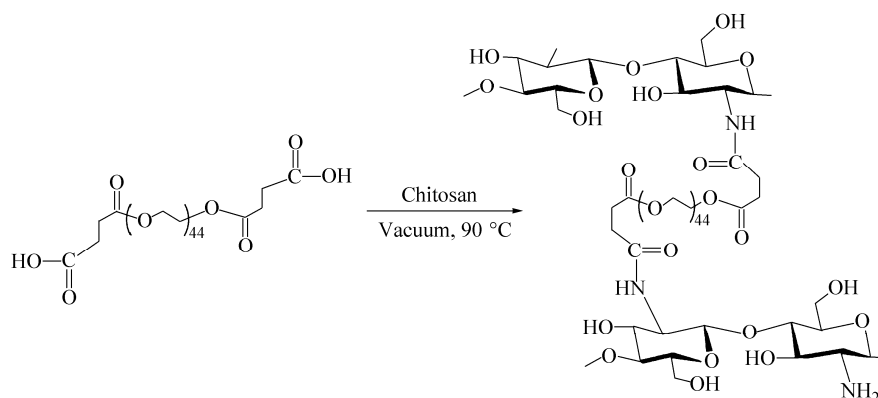


Fig. 3 Proposed crosslinking reaction of the membranes by heating

Hydrophilicity of the Membranes

Hydrophilicity of pure CS and crosslinked membranes was investigated by water contact angle as well as equilibrium of water absorption. The results are summarized in Fig. 4. Water contact angle of pure CS membranes was $(97 \pm 13)^\circ$. After introduction of 20% of PEG or 20%CT-PEG/CS showed lower water contact angle, that is $(80 \pm 2)^\circ$. When PEG increased to 30%, the water contact angle of crosslinked membranes decreased to $(76 \pm 5)^\circ$. These results indicated that introducing PEG could improve the membrane hydrophilicity, which was further confirmed by the measurement of the equilibrium of water absorption (Fig. 4b). Equilibrium of water absorption (EWA) of pure CS membranes was 0.85 ± 0.01 , while that of 20%CT-PEG/CS was 0.98 ± 0.03 . EWA of 30%CT-PEG/CS and 40%CT-PEG/CS was very close to that of 20%CT-PEG/CS. That was to say, there was significant difference between the EWAs of the CT-PEG/CS and pure CS. So, addition of PEG into the chitosan membranes increased the EWA significantly. The improved hydrophilicity of the CT-PEG/CS membranes was due to the addition of hydrophilic PEG. The carboxyl-capped PEG provided the hydrophilicity of the CT-PEG/CS membranes, but also consumed amine groups by crosslink reaction and broke the strong intermolecular and intramolecular hydrogen bonds, resulting in the increase of the hydrophilicity of chitosan chain^[22].

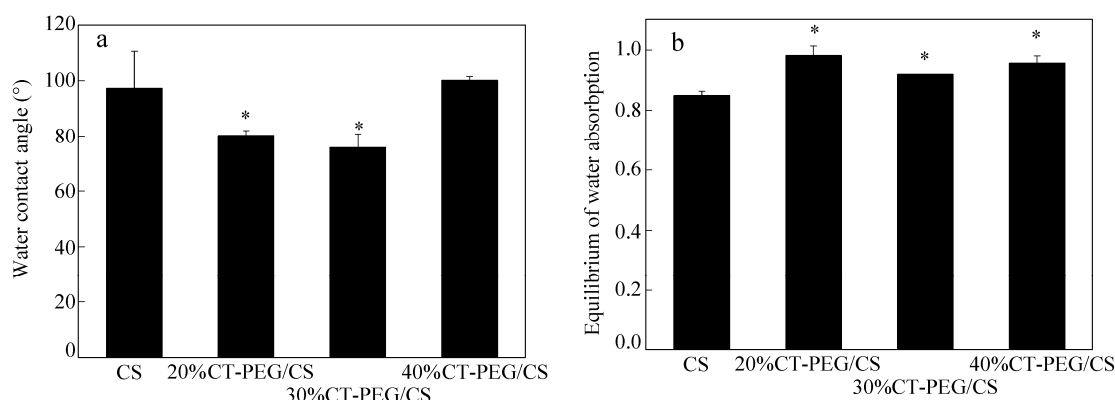


Fig. 4 Hydrophilicity of chitosan and crosslinked membranes: (a) water contact angle and (b) equilibrium swelling degree (ESD) in water ($n = 4$, mean $\pm$ SEM; *, $p < 0.05$, versus CS)

It's probably worth pointing out, however, PEG was used not only as a hydrophilic component but also as a chemical crosslinker. Thus, the PEG's effect on the crosslinked CT-PEG/CS membranes was not only to enhance the hydrophilicity but also to increase the crosslinking degree. For 20%CT-PEG/CS and 30%CT-PEG/CS, hydrophilicity of PEG and breakage of hydrogen bonds in chitosan chains played dominate role, so, water

contact angle of these membranes were smaller than that of pure chitosan membranes. However, for 40%CT-PEG/CS membranes, crosslinking reaction formed dense network and restricted the movement of molecular chains of PEG and chitosan. The dense network tended to decrease the membrane hydrophilicity, resulting in the increase of the water contact angle to the level of pure CS membranes.

Stability in Aqueous Solution

The stability of membranes in aqueous solution was of great importance for some applications^[11], such as protein separation membranes^[8]. Blending membranes with PEG and CS were not stable in aqueous solution and the PEG component was easily excluded. Even with the addition of crosslinker, the weight loss of the modified CS membranes was still serious^[11]. In our study, residual weight of pure and crosslinked membranes after 8 days' immersion in PBS under shaking at 37 °C did not show significant differences (Fig. 5a). These results confirmed that it could be achieved to prevent the loss of PEG and to obtain better water stability through crosslinking chitosan with carboxyl-capped PEG.

Figure 5(b) shows the area change of these membranes in water. When 20% CT-PEG was used, equilibrium swelling area (ESA) increased compared with pure CS membrane. But when CT-PEG increased to 30% and 40%, the EWA was not significantly different from that of pure CS membrane. This is mainly due to two reasons. On one hand, PEG is a hydrophilic polymer and the addition of PEG increases ESA of the membranes^[13], resulting in volume expanding; on the other hand, the crosslinking reaction forms a network, which limits the expanding. As a result, ESA increased at first, but with the adding of more crosslinker, the network became dense, leading to the decrease of ESA. These results showed that the deformation of the crosslinked membranes could be adjusted by controlling the quantity of crosslinker.

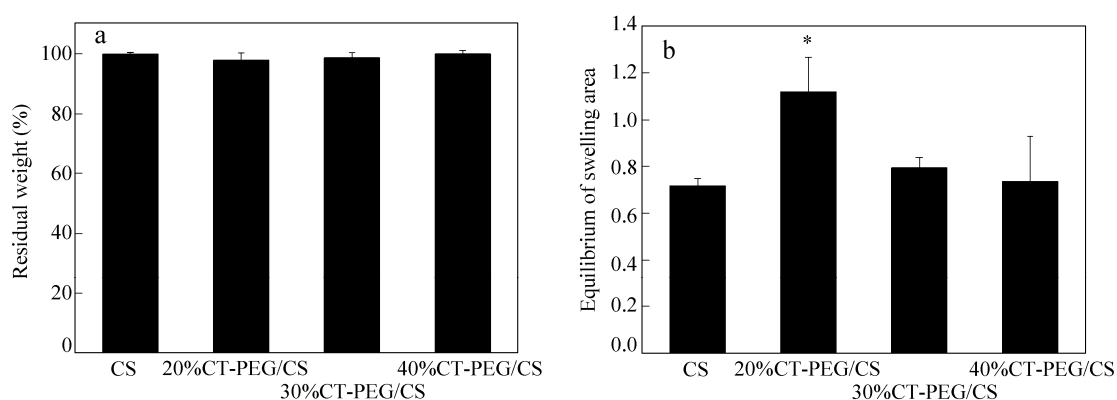


Fig. 5 Stability in aqueous solution of chitosan and crosslinked membranes: (a) residual weight of the chitosan and crosslinked membranes after immersing in PBS at 37 °C for 8 days and (b) equilibrium swelling area (ESA) in water ($n = 4$, mean $\pm$ SEM; *, $p < 0.05$, versus CS)

Mechanical Properties

Mechanical properties were of great importance for many applications^[5, 8–11]. The results of tensile strength and elongation of pure chitosan membranes and crosslinked membranes are shown in Fig. 6. All of these crosslinked membranes got higher mechanical strength than the pure one. Especially with the application of 30 wt% CT-PEG, the mechanical strength went up to 129 ± 11 MPa, nearly twice as much as that of the pure one. As to elongation, slight increase was measured with the addition of CT-PEG compared to the pure one. PEG is usually used to improve the mechanical properties of CS membranes^[16, 18–20, 32]. Normally, the addition of PEG results in improved elongation and lowered tensile strength^[12, 13, 16]. For example, Jin *et al.* prepared blend PEG/CS membranes crosslinked by genipin. Their tensile strength was no more than 70 MPa. With water included, the membrane showed large elongation to over 100%, but the tensile strength decreased obviously. Kiuch *et al.*^[22, 26] synthesized PEG with epoxy groups at the two ends of molecular chains and used them to crosslink chitosan gel films and found that the tensile strength of the crosslinked chitosan membranes decreased, while the elongation

enhanced to 80%, compared to pure chitosan membranes. Another research reported that blending membranes with PEG and CS showed larger elongation and tensile strength compared with pure CS membranes, elongation of the blending membranes was still not larger than 35% and tensile strength remained lower than 12 MPa^[19]. In this study, we use CT-PEG as a crosslinker, and the tensile strength of the crosslinked membranes goes up first, but decreases then with the increase of CT-PEG. A combination of plastic effect from the flexibility of PEG and chemical crosslinking resulted in tensile strength profile of the crosslinked membranes as a function of PEG dosage^[13]. But the tensile strength of the crosslinked membranes was indeed greatly enhanced without sacrificing the elongation when 30% PEG was used.

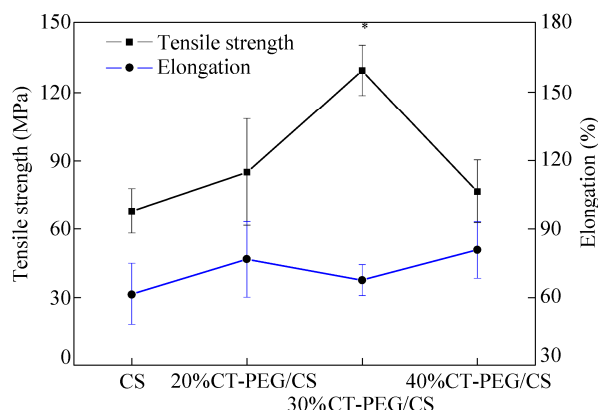


Fig. 6 Mechanical properties of chitosan and crosslinked membranes ($n = 4$, mean $\pm$ SEM; *, $p < 0.05$, versus CS)

Equilibrium of Water Adsorption (EWA) in Different pH Media

Membranes prepared with chitosan usually showed the property of pH response^[11]. These crosslinked membranes did not dissolve in acidic medium and got the ability to swell in different pH media, but pure chitosan membrane would dissolve when the pH was lower than 6.5^[11]. So, in this work the swelling behavior experiment in different pH media was only conducted for crosslinked membranes. EWA in different pH media is shown in Fig. 7. As the pH decreased from 7.4 to 2.0, all EWA of the crosslinked membranes increased, but when the pH rose from 7.4 to 8.3, their EWA increased, too. The effect of pH on the equilibrium swelling degree of the complex membranes could be explained on the basis of protonation of the amino groups in chitosan^[23, 33]. In the acidic medium, the protonation of the amino groups leads to repulsion of the polymer chains, thus allowing more water enter into the network. At higher pH, deprotonation of the amino groups takes place, and repulsion of polymer chains decreases, thus less water is accommodated in the network. Furthermore, within pH 5.0–7.4, we can see that EWA of all the crosslinked CT-PEG/CS membranes changed slightly. In the pH period from 7.4 to 8.3, most amino groups were not capable of protonation, however, some unreacted carboxyl in the complex membranes would turn into carboxylate, resulting in the increase of EWA.

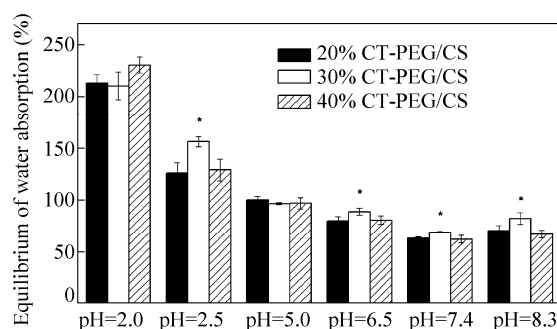


Fig. 7 Equilibrium swelling degree in different pH media for crosslinked membranes ($n = 3$, mean $\pm$ SEM; *, $p < 0.05$, versus the others)

Besides of that the water content in the network mainly depended on the ionic bonds, PEG could also increase the water content of the crosslinked CT-PEG/CS membranes through hydrogen bonds between the PEG molecular chains and the water molecules. Thus 30%CT-PEG/CS got bigger EWA than 20%CT-PEG/CS at most pH. But, the addition of more crosslinker, dicarboxyl-capped PEG, would consume more amino groups and form a dense network, leading to less water in the network. In addition, the presence of PEG might provide a porous structure because of phase separation and of exclusion volume of polymer^[22]. As a result, the network could not hold so much water. These resulted in that the EWA of 40%CT-PEG/CS was less than that of 30%CT-PEG/CS. But, at pH = 2.0 most amino groups of the chitosan protonated, the repulsing force between chitosan chains was likely stronger than the influence of phase separation, thus the porous structure was destroyed, leading to that 40%CT-PEG/CS displayed larger EWA than other two CT-PEG/CS crosslinked membranes.

CONCLUSIONS

Chitosan membranes crosslinked by carboxyl-capped PEG were prepared. The crosslinking structure formed by the reaction between the carboxyl in the crosslinker and amino in chitosan was confirmed by FTIR. The hydrophilicity, water-resistance ability and mechanical properties of pure and crosslinked chitosan membranes were characterized. The results of water contact angle and water absorption showed that the hydrophilicity of chitosan membrane might be affected after being crosslinked by carboxyl-capped PEG, while no significant differences of weight loss between pure chitosan membranes and crosslinked ones were detected, indicating excellent water resistant ability of the composite membranes. Moreover, the tensile strength of chitosan membranes could be significantly enhanced with the addition of 30 wt% carboxyl-capped PEG crosslinker. At this point, the elongation at break didn't degrade evidently. Additionally, the results of swelling behaviors in water at varied pH suggested that the composite membranes were pH sensitive. In brief, chemical crosslinking of chitosan membranes by carboxyl-capped PEG at suitable amount could improve the hydrophilicity, water resistance ability and tensile strength without sacrificing the flexibility of modified chitosan membranes.

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